



ISTANBUL MEDENİYET UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
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Particle Detection and Signal Analysis in Nanopores

PhD Thesis

Dürdane Yılmaz

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STATEMENTS

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Declaration of Originality

I hereby declare that all information in this dissertation has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conducts, I have fully cited and referenced all material and results that are not original to this work.

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Dürdane Yılmaz

GENİŞ ÖZET

Nanogözeneklerde Parçacık Algılama ve Sinyal Analizi

Yılmaz, Dürdane

Doktora Tezi, Nanobilim ve Nanomühendislik Anabilim Dalı

Danışman: Doç. Dr. Ali Dinler

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Nanoteknoloji, benzeri görülmemiş özelliklere ve işlevlere sahip yeni malzemelerin, cihazların ve sistemlerin geliştirilmesi yoluyla endüstride çok sayıda devrim yaratma potansiyeliyle son yıllarda hızla büyüyen bir alan hâline gelmiştir. Bu alandaki araştırma yöntemleri içinde en önemli ve uygulamaları hızla genişleyen tekniklerden birisi de nanogözeneklerle sinyal algılamadır.

Nanogözenekler, moleküler veya atomik ölçekte, silindirik, konik veya düzensiz şekillerde farklı geometrilere sahip, tipik olarak (1-100) nanometre ölçeğinde (çapında), son derece küçük delikler veya kanallardır. Bu gözenekler biyolojik membranlar, katı hâl malzemeler veya sentetik polimerler gibi çeşitli malzemelerde bulunabilir. Bunlar genellikle nanoteknoloji uygulamalarında algılama, filtreleme ve molekülleri manipüle etme gibi çeşitli amaçlar için kullanılır. Öte yandan, kullanılan nanogözenek türü spesifik uygulamaya ve üzerinde çalışılan moleküllerin özelliklerine bağlıdır. Katı hâl nanogözenekler, biyolojik nanogözenekler ve proteinle tasarlanmış nanogözenekler olarak da adlandırılan hibrit nanogözenekler de dâhil olmak üzere birkaç farklı nanogözenek türü vardır. Katı hâl nanogözenekler, tekrar üretilebilirlikleri, kimyasal ve termal olarak sağlam olmaları gibi kararlı yapıları nedeniyle dirençli darbe algılama (resistive pulse sensing-RPS) için tercih edilen özelliklere sahiptirler. Bununla birlikte biyolojik nanogözenekler de belirli şekle ve boyutlara sahip olmaları, aynı boyutla üretilebilmeleri, biyo-uyumlulukları, yüksek hassasiyetleri ve versatil yapılarıyla katı hâl gözeneklere göre çeşitli avantajlar sunar. Bu özellikler onları çeşitli alanlarda çok farklı uygulamalar için cazip hâle getirmektedir. Özellikle tekli moleküllerin yüksek hassasiyet ve seçicilikle algılanmasına, analiz edilmesine olanak tanıyan olağanüstü algılama yetenekleriyle bilinen nanogözenekler, DNA,

RNA, proteinler ve diğerk küçük molekülleri tanımlamak ve analiz etmek için kullanılır. Nanogözeneklerle algılama yöntemi olarak adlandırılan bu metod nano ölçekte küçük gözenekler oluşturunanın gelişmiş bir uygulamasıdır. Bu gelişmiş teknik, nanometre ölçeğinde cihazlar ve yapılar üretmeye odaklanan nanofabrikasyon ilkelerine dayanmaktadır.

Dirençli darbe algılama (resistive pulse sensing) metodu üzerine işleyen sistem, tipik olarak birkaç nanometreden birkaç on nanometreye kadar değişen boyutlarda nano gözenekleri kullanan ve tek moleküllerin etiketsiz bir şekilde algılanmasını, analiz edilmesini sağlayan nano-sensör platformlarının bir varyasyonudur. Dirençli darbe algılamanın çalışma metodu, partiküllerin veya moleküllerin yalıtkan bir membrana gömülü bir nanogözenekten geçerken elektrik akımındaki geçici değişikliklerin tespit edilmesine dayanır. Parçacıklar içinden geçerken bir nanogözenegın elektrik direncindeki değişikliklerin ölçülmesini içeren bir tür algılama yöntemidir.

Coulter Counter prensibine dayanan sistem, ince bir membranda tipik olarak birkaç nanometre çapında küçük bir gözenek kullanılmasını içerir. Membran ve nano gözenek boyunca bir elektrik akımı uygulanır ve bu da akım akışına karşı ölçülebilir bir dirence neden olur. Gözenegın direnci, gözenegın boyutu ve şekli ile belirlenir. Bir parçacık veya molekül nanogözenekten geçtiğinde kanalı kısmen tıkararak elektrik akımında "dirençli darbe" olarak adlandırılan geçici bir düşüşe neden olur. Akımdaki bu azalma tespit edilip kaydedilerek partikülün boyutu, şekli ve konsantrasyonu hakkında bilgi sağlar. Direnç darbesinin büyüklüğü ve süresi, gözenekten geçen parçacık veya molekülün boyutuna ve şekline bağlıdır.

Bu teknik, virüslerin, bakterilerin, nanopartiküllerin boyutunu ve şeklini incelemenin yanı sıra DNA, RNA ve proteinler gibi biyomolekülleri tespit ve karakterize etmek için yaygın olarak kullanılmaktadır.

Algılama araçlarında nanogözeneklerin kullanılması analitlerin olağanüstü hassasiyet ve duyarlılıkla tespit edilmesini mümkün kıldığı gibi karmaşık molekül karışımlarını da analiz edebilir. Bununla beraber sistemin potansiyeli ve ortaya koyacağı performansı açısından en önemli unsur seçilen parametrelerin optimizasyonudur. Gözenek boyutunun analiz edilecek parçacığa göre seçimi, yapılacak uygulama için kritik öneme sahiptir. Nanogözenegın boyutu, yüzey

kimyası, seçiciliği, hassasiyet ve geçirgenliği çeşitli fiziksel ve kimyasal özellikleri etkileyebilir. Örneğin, daha küçük gözenek boyutlarına sahip nanogözenekli malzemeler daha yüksek yüzey alanına ve artan aktif bölge sayısı nedeniyle daha yüksek reaktiviteye sahip olabilirken daha büyük gözenek boyutları daha yüksek geçirgenlik ve daha hızlı kütle taşınımı ile sonuçlanabilir. Bununla beraber konik gözenek şekli cihazın hassasiyetini, silindirik geometri ise doğruluğunu artırabilir.

Gözenek boyutunun yanı sıra malzeme seçimi de kararlılık, dayanıklılık, deformasyon veya kırılmaya karşı direnç oluşturmak için önemli bir parametredir. Nanogözenekler silikon, cam, metal ve polimerler dâhil olmak üzere çok çeşitli malzemelerden üretilebilir ve farklı boyutlar, şekiller ve yüzey kimyaları ile tasarlanabilir. Bu da onları bir dizi uygulama için çok yönlü hâle getirir. Malzemeden kaynaklanan gözenek yüzeyinin kimyasal özellikleri, moleküllerle olan etkileşimlerini etkileyebilir ve yüzeydeki modifikasyonlar bu etkileşimleri geliştirmek veya kontrol etmek için kullanılabilir. Nanogözenek malzemesinin kararlılığı ve dayanıklılığı, özellikle uzun süreli kullanım veya zorlu ortamlara maruz kalmayı gerektiren uygulamalar için önemli faktörlerdir.

Cihazın doğruluğunu ve hassasiyetini etkileyecek diğer etkenler hedef analiti nanogözenekten geçirmek için kullanılan voltaj ve akımdır. Uygulanan voltaj, gözenekten geçen moleküllerin hızını ve frekansını etkiler. Voltaj çok yüksekse moleküllerin gözenekten çok hızlı geçmesine yol açabilir, bu da zayıf sinyal-gürültü oranına ve doğruluğun azalmasına neden olur. Öte yandan, voltaj çok düşükse moleküller gözenekten hiç geçmeyebilir veya çok yavaş geçebilir, bu da düşük hassasiyet ve düşük verimlilikle sonuçlanır.

Nanogözenek platformunun performansını etkileyebilecek bir diğer unsur da sistemin sıcaklığıdır. Düşük sıcaklıklar stabiliteyi ve hassasiyeti artırabilirken yüksek sıcaklıklar analit geçiş hızını artırabilir.

Tuz konsantrasyonunun da çözeltinin iyonik gücü, iletkenliği ve stabilitesi üzerinde büyük etkisi vardır. Düşük tuz konsantrasyonlarında, elektrostatik etkileşimler daha zayıftır, bu da daha yavaş translokasyon sürelerine ve hassasiyetin azalmasına neden olur. Aksine, yüksek tuz konsantrasyonlarında elektrostatik etkileşimler daha güçlü hâle gelir, bu da daha hızlı translokasyon süreleri ve artan hassasiyetle sonuçlanır. Ayrıca tuz konsantrasyonu nanogözenek

iç stabilitesini de etkiler. Yüksek tuz konsantrasyonları iyonik gücün ve iletkenliğin artmasına yol açar, bu durum gözeneğin dengesizleşmesine ve bozulmasına sebep olur.

Bu parametrelerin etkilerini değerlendirmek ve optimize etmek, çok çeşitli biyolojik ve biyoteknolojik uygulamalarda etkili nanogözenek cihazları tasarlamak ve kullanmak için gereklidir. Bununla beraber nanogözeneklerin benzersiz içsel özellikleri de sistemin potansiyelini önemli ölçüde etkiler. Nanogözeneklerin fiziksel özelliklerinden biri olan iletkenlik, gözenekten geçen molekülleri algılamak veya tespit etmek için kullanılabilen elektrik akımını iletir. Nanogözeneklerin diğer temel özellikleri seçicilik ve hassasiyettir. Nanogözenekler, boyutlarına, yüklerine veya diğer özelliklerine bağlı olarak belirli molekül türleri için seçici olacak şekilde tasarlanabilir ve sıcaklık, pH veya belirli moleküllerin varlığı gibi çevredeki değişikliklere karşı oldukça hassas olabilir. Ayrıca moleküllerin yüksek çözünürlüklü analizini sağlayarak özelliklerinin ve davranışlarının ayrıntılı olarak incelenmesine olanak tanır.

Bu niteliksel özellikler nanogözenekleri çeşitli uygulamalar için umut verici araçlar hâline getirmektedir. Örneğin, nanogözeneklerin en heyecan verici uygulamalarından biri DNA analizinde devrim yaratma potansiyeline sahip olan DNA dizilemedir. Bu yöntem DNA'nın yapısal analizi için hızlı, hassas ve yüksek verimlilikte sonuç sağlar. DNA bir nanogözenekten geçirilirken her bir nükleotid geçişi sırasında meydana gelen elektrik akımındaki değişiklikler ölçülebilir, bu da hızlı ve doğru DNA dizilemesine olanak tanır. Bu teknoloji, genomik araştırmalarda devrim yaratma potansiyeline sahiptir ve hâlihazırda taşınabilir DNA dizileme cihazlarının geliştirilmesinde kullanılmaktadır. Boyuta dayalı ayırma özelliği sayesinde nanogözenekler molekülleri boyutlarına göre seçici olarak filtreleyebilir ve ayırabilir, bu da tek bir cihazla DNA'nın hızlı ve doğru bir şekilde dizilenmesini sağlar.

Nanogözenekler protein analizinde de potansiyele sahiptir. Bireysel proteinlerin elektriksel özelliklerini ölçmek için nanogözenekler kullanılarak elektrik sinyalindeki değişiklikler proteini boyutuna, şekline ve yüküne göre tanımlamak için kullanılabilir. Bu şekilde araştırmacılar, ilaç keşfi ve geliştirilmesi için değerli olabilecek protein yapısı ve işlevi hakkında fikir edinebilir. Bir proteini gözenekten

geçirerek her bir amino asitin gözenekten geçişi sırasında oluşturduğu elektrik sinyalindeki değişikliklere dayanarak proteinin amino asit dizisi belirlenebilir. Nanogözenekler ayrıca proteinlerde meydana gelen konformasyonel değişiklikleri analiz etmek için de kullanılabilir. Nanogözenek, proteinin belirli bölgelerine seçici olarak bağlanabilen ligandlarla modifiye edilerek elektrik sinyalindeki değişiklikler, protein ligandlarla etkileşime girdikçe meydana gelen yapısal değişiklikleri izlemek için kullanılabilir.

Genel olarak zorluklarına rağmen bu teknoloji, tanımlama, miktar belirleme, sıralama ve konformasyonel analiz için hızlı, hassas ve yüksek verimli bir yöntem sağlayarak DNA ve protein analizinde devrim yaratma potansiyeline sahiptir.

Bununla birlikte, umut verici potansiyellerine rağmen, nano gözenek algılama, bu teknolojinin yeteneklerinden tam olarak yararlanmak için daha fazla araştırma gerektiren sinyal-gürültü oranı, gözenek üretimi ve veri analizi gibi çeşitli zorluklarla karşı karşıyadır. Bu zorlukları aşmak için kullanılan metotlardan biri sayısal yöntemler ve simülasyon uygulamalarıdır. Sayısal yöntemler, deneyler yoluyla kolayca incelenemeyen karmaşık olayların daha ayrıntılı bir analizini sağlar. Bu uygulamalar, nanogözenek algılama alanını ilerletmek ve potansiyelini tam olarak ortaya çıkarabilmek için temel araçlardır.

Sayısal yöntemler ve simülasyon uygulamaları, nanogözeneklerin içindeki moleküllerin ve iyonların davranışını modellemek ve simüle etmek için bir yöntem sağlayarak nanogözenek algılamanın zorluklarının üstesinden gelmede kritik bir rol oynamaktadır. Modelleme ve simülasyon teknikleri, deneysel yeteneklerin ötesinde, karmaşık olayların daha ayrıntılı bir analizini sağlar. Bu uygulamalar gözenek boyutu, elektrolit çözeltisi ve uygulanan voltaj gibi faktörleri ayarlayarak nanogözeneklerle algılamanın bazı zorluklarının üstesinden gelmeye yardımcı olabilecek değişkenlerin ve parametrelerin kontrollü manipülasyonuna izin verirler ve böylece çeşitli parametrelerin nanogözeneklerin algılama performansı üzerindeki etkisinin ortaya koyulmasına yardımcı olurlar. Bu, deneysel parametrelerin optimizasyonunu mümkün kılar ve nanogözenek algılamada yer alan karmaşık olaylara ilişkin iç görüler ortaya koyar. Ayrıca, simülasyon tabanlı yaklaşımlar, yeni nanogözenek tasarımlarını keşfetmek ve yeni algılama platformlarını? geliştirmek için de yararlıdır.

Sinyal-gürültü oranı ve gözenek üretimi gibi nano gözenek algılamanın bazı zorlukları, gözenek boyutu, elektrolit çözeltisi, gözenek/parçacık yüzey yükü ve gözenek boyunca uygulanan voltaj gibi algılama sürecine dâhil olan parametrelerin simülasyon ortamında modellenerek optimize edilmesiyle aşılabilir.

Bu doktora tezinde, sayısal yöntemlerden sonlu elemanlar metodu kullanılarak seçilen parametrelerin hem katı hâller hem de biyolojik nanogözenekler açısından tek molekül algılama, ssDNA ve protein geçişleri için nanogözeneklerin hassasiyetini ve seçiciliğini nasıl etkilediği araştırılmaktadır. Özellikle gözenek geometrisi ve yüzey yükü değişimlerinin parçacık duyarlılığı üzerindeki etkileri incelenmiştir. Ayrıca nanogözenek algılamanın hassasiyetini ve doğruluğunu artırmak için yeni yaklaşımlar ele alınmaktadır.

Bu çalışma temel olarak hem katı hâl hem de biyolojik nanogözenekler üzerine sayısal modelleme çalışmalarını içermekle birlikte deneysel validasyonlar sunmaktadır. Literatüre genel bir girişten sonra (Bölüm 1), katı hâl ve biyolojik nanogözeneklerin molekül algılama potansiyelleri üç ayrı bölümde ele alınmaktadır.

Daha sonra (Bölüm 2) sentetik gözenekler olarak da adlandırılan katı hâl nanogözenekler üzerinden modelleme yapılarak nanogözenek geometrisinin gözenek seçiciliği ve hassasiyeti üzerindeki etkileri açıklanmaktadır. Bu bölüm, gözenek geometrisinin hem sinyal şekli hem de darbe büyüklüğü üzerinde önemli bir rol oynadığını ortaya koymaktadır. Konik, puro ve kum saati olmak üzere modellenen üç gözenek geometrisinin karşılaştırılması, geometrinin hem büyüklük hem de dalga biçimi (yani dirençli/bifazik) açısından ayırt edici direnç sinyallerinin üretilmesi üzerinde derin etkileri olduğunu ortaya koymaktadır. Parçacık boyutu ayırt etme kabiliyetini değerlendirmek amacıyla 120 nm, 260 nm ve 360 nm çaplı parçacıklar kullanılırken; duyarlılık analizi için benzer boyutlarda 240 nm, 260 nm ve 280 nm çaplı parçacıklar incelenmiştir. Parçacık yükünün darbe büyüklüğü üzerinde önemli bir etkisi olduğundan, nanogözeneklerin parçacık yükündeki değişikliklere verdiği tepkiler de araştırılmıştır.

Sunulan simülasyon sonuçlarına dayanarak sinyal dalga biçimlerinin gözenek geometrisine bağlı olduğu açıkça tanımlanmıştır; örneğin konik ve puro gözenekleri bifazik sinyaller üretirken; kum saati gözeneği yalnızca dirençli darbe

retmektedir. Sonular, sinyallerin partikllere verdiđi tepkilerin algılama blgesinin konumu, elektrik alanının yođunluđu ve u (algılama blgesi) kenarına yakın iyonik yođunluktaki kademeli deđişikliklerle iliřkili olduđunu ortaya koymaktadır. Ayrıca, bulgular iletken darbelerin nispeten kk partikllerin tespiti iin deđerli bir bilgi kaynađı olabileceđini de gstermektedir. Ayrıca, seilen geometriler dhilinde gzenek geometrisi konikten kum saati veya puro řekline dnřtke hassasiyet ve seicilikte bir artıř gzlemlenmiřtir. Bununla birlikte farklı paracık ykleri ile yapılan modellemelerde, zellikle iletken darbeler dikkate alındıđında, paracık yknn paracıđın yanlıřlıkla fazla veya eksik boyutlandırılmasına yol aabileceđi grlmřtir.

Paracıkların yrngesi de nano gzenek algılamada sinyal znrlklerini etkileyebilecek bir diđer nemli faktr olarak ele alınmıřtır. Paracıđın eksen dıřı geiřinin konik ve puro řekillerinde darbe byklklerini artırabilmesine rađmen, kum saati gzeneđindeki paracık boyutunun znrlk kapasitesini azaltacak kadar byk olmayabileceđi sonucuna varılmıřtır.

Ardından (Blm 3) konik řekle sahip bir PET nanogzenekten geen DNA hareketinin, bu sreteki farklı paracık yk yođunlukları ve nanogzeneđin yzey yk yođunlukları dikkate alınarak teorik bir incelemesi sunulmaktadır. Simlasyonların sonuları, aynı kořullar altında gerekleřtirilen deneylerle elde edilen verilerle tutarlıdır. Simlasyon sonuları, gzenek yk yođunluđu dřk olduđuunda retilen elektrik akımı darbelerinin dzgn olduđuunu ve akım artıřı olmadan keskin bir ani ykseliře benzediđini ortaya koymuřtur. te yandan, gzenek ve DNA'nın yzey yklerinin yksek olması durumunda, sinyallerdeki bozulmalar daha belirgin hle gelir. Bu da elektrik yk dađılımında bir dengesizlik olduđuunu ve ykl paracık ile gzenek yzeyi arasında bir etkileřim olduđuunu gstermektedir. Sonular hem DNA'nın hem de nanogzenek yzeyinin yk yođunluklarının akım darbelerinin řekli zerindeki etkisini anlamak iin bir yntem sunmuřtur.

Son olarak (Blm 4) biyolojik nano gzeneklerin (AeL, α -HL, MspA, CsgG) sinyal darbe byklkleri zerindeki yzey yk etkileri aısından karřılařtırmalı bir alıřması anlatılmaktadır. Paracık translokasyonu sırasında mutasyonların biyolojik nanogzeneklerdeki etkisi sayısal analiz kullanılarak deđerlendirilmiřtir.

Moleköl geiři sırasında üretilen sinyalleri arařtırmak için farklı řekillere sahip üç moleköl tipi yani ubuk řeklindeki tek sarmallı DNA (gerilmiş DNA'yı temsil eder), katlanmış řekli (sarmal), tek sarmallı DNA ve protein řeklindeki paracıklar modellenmiřtir.

Yüzey yük yoğunluęunun farklı deęerlerinden elde edilen sonuçlar, yüzey yükü deęerinin belirli bir noktaya kadar gözenek hassasiyeti üzerinde belirleyici bir etkiye sahip olmasıyla açıklanmaktadır. Sonuçlar ayrıca mutasyonun yapılıř řeklinin seilen gözenek geometrisinden daha önemli olabileceęini göstermektedir. Bunun yanı bulgular, yüzey yüküne baęlı olarak daha küçük aplı gözeneklerin biraz daha duyarlı olabileceęini düşündürmektedir. Bu gözlem, Bölüm 4'ün tüm bölümlerinde tutarlı olan $AeL > MspA > CsgG > \alpha\text{-HL}$ řeklindeki gözeneklerin hassasiyet sırasını potansiyel olarak netleřtirebilir. Ayrıca, gözeneęin yüzey yükü yoğunluęu, eřik etkisi olarak adlandırılan belirli bir noktaya kadar, řekillerinden baęımsız olarak, geen paracıkların büyüklükleri üzerinde önemli bir etkiye sahiptir. Elektrostatik etkiler sadece bu sınıra kadar etkili olduęundan gözeneęin performansını artırmak için yüzey yük yoğunluęunun belirli bir eřik deęerine ulaşması gerektięi sonucuna varılabilir.

Buna ek olarak, seilen dört biyolojik nano gözenek, paracıkları uzunluklarına göre ayırt etme kabiliyetleri açısından da karřılařtırılmıştır. Bu, farklı veya benzer nükleotid uzunlukları için doęru ve güvenilir DNA dizilimi için ok önemlidir. Ayrıca bu, daha hassas ve güvenilir dizileme teknolojileri geliřtirmek amacıyla nükleik asitlerdeki yapısal varyasyonların tespiti için bir yöntem sunmaktadır. Sonuçlara göre, dört nanogözenek de özellikle en küçük nanoparacık için ayırt edilebilir sinyaller üretmektedir. Önceki bulgularla uyumlu olarak, AeL nanogözenek hem büyük hem de küçük partiküller için en yüksek sinyal büyüklüklerini göstermektedir. Öte yandan, dört nanogözenekten her birinin farklı uzunluklardaki paracıklar için benzersiz sinyaller ürettięi açıktır.

Sonuç olarak, alıřmada öncelikle nanogözenek geometrisinin gözenek seicilięi ve hassasiyeti üzerindeki etkileri ortaya koyulmak üzere konik, puro ve kum saati olarak üç farklı gözenek geometrisi modellenerek gözenek geometrisinin hem sinyal řekli hem de darbe büyüklüęü üzerinde ayırt edici bir rol oynadıęı, yüzey yük deęiřimlerinin ve paracığın geiř yörüngesinin de sinyal genlikleri üzerindeki

etkileri ortaya koyulmuştur. Daha sonra aynı parametrelere sahip izli aşındırılmış PET membran gözenekle sonlu elemanlar yöntemiyle oluşturulan model gözenekten DNA geçişi sırasında elde edilen sinyallerin tutarlı olduğu gösterilmiştir. Bu yolla yüklü parçacık ile gözenek yüzeyi arasında meydana gelen etkileşim ortaya koyulmuş hem DNA'nın hem de nanogözenek yüzeyinin yük yoğunluklarının akım darbelerinin şekli üzerindeki etkisini anlamak için bir yöntem sunulmuştur. Son olarak dört yaygın kullanılan biyolojik gözenek (AeL, α -HL, MspA, CsgG) DNA geçişi ve protein geçişi sırasında üretilen sinyal darbe büyüklükleri üzerindeki yüzey yükü etkileri mutasyon uygulaması açısından karşılaştırmalı olarak değerlendirilmiştir. Literatürle tutarlı olarak ve deneysel çalışmaların bir doğrulaması olarak AeL gözenegin daha iyi algılama yaptığı, MspA' in ise daha iyi hassasiyete sahip olduğu ortaya konulmuştur. Ayrıca sonuçlar mutasyonun yapılış şeklinin seçilen gözenek geometrisinden daha önemli olabileceği gösterilmiştir.

Bu doktora çalışmasından elde edilen bilimsel veriler çeşitli basılı akademik ortamlarda yer bulmuştur. Çalışmada ilk olarak ele alınan *“nanogözenek geometrisinin gözenek seçiciliği ve hassasiyeti üzerindeki etkileri”* nin incelendiği bölüm *Chemistry Select* dergisine konu olmuştur. Kimya bölümüyle iş birliği yapılarak sonuçlandırılan *“konik şekle sahip bir PET nanogözenekte, parçacık ve nanogözenek için farklı yüzey yük yoğunluk durumunun DNA geçiş hareketi üzerine etkisi”* nin teorik ve deneysel incelemesi *IEEE* dergisinde yer almıştır. Tezin son bölümünde yer alan *“seçili biyolojik nanogözeneklerde (AeL, α -HL, MspA, CsgG) DNA ve protein geçişi sırasında üretilen sinyal darbe büyüklükleri üzerindeki yüzey yükü etkilerinin mutasyon uygulaması açısından karşılaştırmalı olarak değerlendirilmesi”* ni içeren çalışma da *European Biophysics Journal*'a başvuru aşamasındadır.

Anahtar Kelimeler: Dirençli Darbe Analizi, Tekli Molekül Algılama, Katı Hâl Nanogözenekler, Biyolojik Nanogözenekler, Sonlu Elemanlar Yöntemi.

ABSTRACT

Particle Detection and Signal Analysis in Nanopores

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Nanopore sensing is a rapidly growing field that enables the detection and analysis of single molecules in a label-free manner. However, despite their promising potential, nanopore sensing faces several challenges, such as signal-to-noise ratio and pore fabrication, which require further research to fully exploit the capabilities of this technology. Numerical methods provide a more detailed analysis of complex phenomena beyond experimental capabilities.

In this thesis, using the numerical finite element and resistive pulse analyses methods, we investigate how the selected parameters affect the sensitivity and selectivity of nanopores for single molecule sensing, both in terms of solid state and biological membranes.

In the first section, the effects of nanopore geometry and the size of the particle to be detected on particle detection are analyzed. Particle surface charge and trajectory are also discussed to analyze their influence on nanopore sensitivity. In the second chapter surface charge effect on DNA translocation in track-etched nanopores is discussed in terms of signal wave-shapes and electric potential. In chapter three, the effects of surface charges on the sensing capabilities are examined for widely used four biological nanopores (i.e., Aerolysin (AeL), alpha-hemolysin (α -HL), MspA, and CsgG). In this case, translocated particles are simulated in the form of a rigid (rod) ssDNA, folded (coiled) ssDNA, and a 3-node protein. This chapter also includes a comprehensive overview of biological nanopores.

The thesis results provide a basis for optimizing nanopore sensing parameters to make it a routine tool for high-throughput, label-free, and real-time single molecule analysis in various applications.

Keywords: Resistive Pulse Analyses, Single Molecule Sensing, Solid-State Nanopores, Biological Nanopores, Finite Element Method.



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Table of Contents

	Page
List of Figures	IV
List of Tables	VII
List of Symbols	VIII
List of Abbreviations.....	IX
CHAPTER 1.....	1
1. Introduction	1
1.1. Nanopores.....	2
1.1.1. Solid-State Nanopores.....	5
1.1.1.1. Track-Etched Nanopores	6
1.1.2. Biological Nanopores.....	7
1.1.2.1. AeL (Aerolysin)	8
1.1.2.2. α -HL (Alpha Hemolysin).....	10
1.1.2.3. MspA (porin A from Mycobacterium smegmatis).....	12
1.1.2.4. CsgG (Curli specific genes G).....	14
1.1.3. Hybrid Nanopores	16
1.2. Resistive Pulse Sensing	16
1.3. Finite Element Method	18
1.4. Effective forces.....	20
1.4.1. EDL	20
1.4.2. Electrophoresis	21
1.4.3. Electroosmosis	22
1.4.4. Drag forces	22
1.5. General Approach to the Method and Governing Equations of the Simulations....	23
CHAPTER 2.....	26
2. Role of Nanopore Geometry on Particle Detection and Discrimination.....	26
2.1. Introduction	27
2.2. Method and Simulation	28
2.3. Results	30
2.3.1. Comparison of Nanopore Capabilities for Discriminating Particle Size Variation.....	30
2.3.2. Evaluation of Ionic Distributions and Electric Field Strengths.....	36

2.3.3. Comparison of Nanopore Capabilities for Discriminating Particle Size Change with Charge Variation.....	37
2.3.4. Transition Analysis in an Symmetry-Axis vs Off-Axis	40
CHAPTER 3.....	43
3. Surface Charge Effect on DNA Translocation in Track-Etched Nanopores	43
3.1. Introduction	43
3.2. Method and Simulation	44
3.3. Results	45
3.3.1. Surface Charge Density Effects on Resistive Signal Wave-shapes	45
3.3.2. Effect of Electric Potential	47
CHAPTER 4.....	50
4. Surface Charge Effects on Sensing Features of AeL, α -HL, MspA and CsgG Biological Nanopores Sensors	50
4.1. Introduction	50
4.2. Method and Simulation	53
4.3. Results	55
4.3.1. Uniformly Varied Pore SCD relative to the Rigid (rod) ssDNA Translocation.....	56
4.3.2. Residual Varied Pore SCD relative to the Rigid (rod) ssDNA Translocation.....	58
4.3.3. Uniformly Varied Pore SCD relative to the Folded (coiled) ssDNA Translocation.....	61
4.3.4. Uniformly Varied Pore SCD relative to the Protein Translocation	64
4.3.5. Comparison of Nucleotide Transitions of Different Lengths.....	67
Summary	71
Appendix A	74
A.1 Boundary Conditions in Simulations.....	74
A.2 ΔI and $\Delta I/I_0$ Data.....	75
A.2.1 ΔI and $\Delta I/I_0$ magnitudes	75
A.3 Validation of the Axially Symmetric Model	78
A.4 Mesh Independence Test for Axially Symmetric Simulation.....	80
A.5 Mesh Independence Test for 2D Simulations.....	81
Appendix B	82
B.1 Mesh and Boundary Conditions in Simulations	82

B.2	Base Diameter of PET Membrane	82
B.3	Mesh Independence Test for Simulations.....	83
Appendix C		84
C.1	Finite Element Method	84
C.1.1	Finite Element Simulation Details (COMSOL Multiphysics) Geometry, Boundary Conditions, System Parameters	85
C.1.2	COMSOL Model Report.....	87
C.2	Mesh Distribution and Boundary Conditions	90
C.3	Validation	92
C.3.1	Validation pore models as an electrostatic potential distribution	92
C.3.2	Validation (Confirmation) of pore models for the mutant case relative to the WT	92
C.4	Mesh Independence Test	97
C.5	ΔI Histogram Distributions of Pores for Different Particle Translocations	98
C.5.1	Uniformly varied pore SCD relative to rigid (rod) ssDNA translocation	98
C.5.2	Residual varied pore SCD relative to rigid (rod) ssDNA translocation.....	98
C.5.3	Uniformly varied pore SCD relative to folded (coiled) ssDNA translocation	99
C.5.4	Uniformly varied pore SCD relative to protein translocation.....	99
C.5.5	Comparison of Nucleotide Transitions of Different Lengths.....	100
References		101
List of Publications		114

List of Figures

	Page
Figure 1	Illustration of four biological nanopores.....7
Figure 2	Structure of the wild type aerolysin 8
Figure 3.	Structure of the wild type α -HL 10
Figure 4.	Structure of the wild type MspA.....13
Figure 5.	Structure of the wild type CsgG.....14
Figure 6.	Illustration of resistive pulse method based on Coulter Counter Principle 17
Figure 7.	Modeled pore geometries. (a) Conical, (b) cigar and (c) hourglass nanopore.... 28
Figure 8.	Current pulse shapes of conical, cigar, hourglass pores for particles of 120 nm, 260 nm, and 360 nm diameters 32
Figure 9.	Resistive and conductive pulse magnitudes for 120 nm, 260 nm, and 360 nm particles 33
Figure 10	Current pulse shapes of conical, cigar and hourglass nanopores for particles of 240 nm, 260 nm and 280 nm diameters.....35
Figure 11	Resistive and conductive pulse magnitudes, $\Delta I/I_0$, for 240 nm, 260 nm, and 280 nm particles. 35
Figure 12	Electric field strengths obtained around the tip edge of the conical pore, upper tip of the cigar pore, bottom tip of the cigar pore, hourglass pore.....36
Figure 13	Pulse magnitudes, $\Delta I/I_0$, obtained for 360 nm diameter particle for varying particle surface charges.....39
Figure 14	Pulse magnitudes, $\Delta I/I_0$, obtained for 260 nm diameter particle for varying particle surface charges.....40
Figure 15	Pulse magnitudes, $\Delta I/I_0$, obtained for 120 nm diameter particle for varying particle surface charges 40
Figure 16	$\Delta I/I_0$ values obtained for 360 nm, 260 nm and 120 nm diameter particles translocating through the center of the pore and near the pore edge (off-axis) 41
Figure 17	Differences between $\Delta I/I_0$ values for 120 nm, 260 nm, and 360 nm diameter particles 42
Figure 18	Simulated resistive-pulse signals of 100 bp DNA with different charge densities.....46

Figure 19	Experimental resistive-pulse signals of 100 bp DNA in 1M KCl.....	47
Figure 20	Simulation of current-pulse shape at $\sigma_{\text{DNA}} = -0.44 \text{ C/ m}^2$ (100 bp)	48
Figure 21	Experimentally obtained resistive-pulse signals with different shapes as a function of potential in 1M KCl.....	49
Figure 22	2D view of modeled four protein nanopore geometries	53
Figure 23	Demonstration of particle types along the different nanopores according to the application of charge density to different pore regions	55
Figure 24	Histogram distribution of $\Delta I/I$ values of Rigid (rod) ssDNA translocation relative to uniformly varied pore SCD.....	58
Figure 25	Histogram distribution of $\Delta I/I$ values of Residual Charge (Site Directed Mutagenesis) Effects on the Signal Generation and Detection	60
Figure 26	Histogram distribution of $\Delta I/I$ values of Folded (coiled) ssDNA translocation relative uniformly varied pore SCD.....	63
Figure 27	Histogram distribution of $\Delta I/I$ values of protein translocation relative uniformly varied pore SCD.....	65
Figure 28	Transition signals of particles with different lengths.....	69
Figure 29	Biological nanopores' discrimination capability for particles with different length.....	70
Figure A.1	Simulation boundary conditions.....	74
Figure A.2	Signals obtained from our simulations for varying values of the voltage using same parameters at identical conditions of the results in Figure SI2 of Lan et al	78
Figure A.3	Signals wave shapes are obtained from our simulations for varying values of particle surface charge densities	79
Figure A.4	Deviation from the baseline current of the most refined mesh as the number of mesh elements increases	80
Figure A.5	Deviation from the baseline current of the most refined mesh as the number of mesh elements increases	81
Figure B.1	Simulation parameters for DNA translocation	82
Figure B.2	SEM image of multipore PET membrane with 520 nm base diameter	82
Figure B.3	Deviation from the baseline current of the most refined mesh as the number of mesh elements increases	84
Figure C.1	3D-view of the four biological nanopore geometry models when the target particle at the system reservoir	86

Figure C.2	Axial symmetry view of the simulation model represented by the AeL biological nanopore.....	88
Figure C.3	Electrostatic module boundary conditions to perform Poisson Equation.....	88
Figure C.4	Transport of Diluted Species (chds) Module boundaries for ionic salt concentration to perform Nernst-Planck Equation	89
Figure C.5	Creeping Flow module boundary for electroosmotic flow to perform Navier-Stoke Equation	89
Figure C.6	Mesh distribution and boundary conditions of considered four protein nanopore	91
Figure C.7	Validation of pore models as a electrostatic potential distribution.....	92
Figure C.8	Simulation validation result corresponding to the experimental study.....	93
Figure C.9	Current blockage change of biological pores relative to WT vs Mutant states	94
Figure C.10	Experimental site-directed mutagenesis study of ssDNA translocation through α HL pores	95
Figure C.11	Experimental study involves construction of two MspA mutants and demonstration their capabilities for single-molecule detection of ssDNA	96
Figure C.12	Deviation from the baseline current of the most refined mesh as the number of mesh elements increases	97
Figure C.13	Histogram distribution of ΔI values of Rigid (rod) ssDNA translocation relative to uniformly varied pore SCD.....	98
Figure C.14	Histogram distribution of ΔI values of Residual Charge (Site Directed Mutagenesis) Effects on the Signal Generation and Detection	98
Figure C.15	Histogram distribution of ΔI values of Folded ssDNA translocation relative uniformly varied pore SCD.....	99
Figure C.16	Histogram distribution of protein translocation relative uniformly varied pore SCD.....	99
Figure C.17	Transition signals of particles with different lengths.....	100

List of Tables

	Page
Table 1	The maximum blocking amounts of pores corresponding to ΔI and $\Delta I/I$ for the rigid (rod) ssDNA translocation relative to uniformly varied pore SCD.....58
Table 2	The maximum blocking amounts of pores corresponding to ΔI and $\Delta I/I$ for Rigid (rod) ssDNA translocation relative to residual varied pore SCD.....61
Table 3	The maximum blocking amounts of pores corresponding to ΔI and $\Delta I/I$ for folded (coiled) ssDNA translocation relative to uniformly varied pore SCD 63
Table 4	The maximum blocking amounts of pores corresponding to ΔI and $\Delta I/I$ for protein translocation relative to uniformly varied pore SCD.....66
Table 5	Comparison of threshold surface charge densities of four biological nanopores obtained under different pore surface charge states and different shaped particle translocation.....66
Table A.1	Adjusted simulation parameters.....74
Table A.2	Constant simulation parameters.....75
Table A.3	Numerical data of Figure 9 for different-sized particles 120 nm, 260 nm, and 360 nm 75
Table A.4	Numerical data of Figure 11 for similar-sized particles 240 nm, 260 nm, and 280 nm 76
Table A.5	Numerical data of Figure 13 for 360 nm particle 76
Table A.6	Numerical data of Figure 14 for 260 nm particle 77
Table A.7	Numerical data of Figure 15 for 120 nm particle.....77
Table B.1	Parameters and Values Used in Calculations.....83
Table C.1	Parameters Module in COMSOL.....87
Table C.2	Mesh 1 Module in COMSOL 90
Table C.3	Parametric Sweep Module in COMSOL 90
Table C.4.	Dimension of four pore models 91

List of Symbols

E	Electric potential (mV)
ρ	Electric charge density (mC/m ²)
ϵ_r	Relative permittivity (F/m)
ϵ_0	Vacuum permittivity (F/m)
F_a	Faraday constant (C/mol)
z_i	Valence of the i^{th} ionic species
c_i	Concentration of the i^{th} ionic species (mol/m ³)
D_i	Diffusivity of the i^{th} ionic species (m ² /s)
R	Gas constant (J/(mol*K))
T	Absolute temperature (K)
	Velocity vector (m/s)
$\mathbf{n}$	Normal vector to the surface
k_B	Boltzmann constant
η	Deionized water viscosity (Pa*s)
P	Fluid pressure (mmHg)
I_0	Base line current (Open pore current) (nA)
I	Blockade current (nA)
A	Adenine nucleotide
T	Thymine
G	Guanine
C	Cytosine

List of Abbreviations

AeL	Aerolysin
bp	Base pair
CsgG	Curli specific genes G
DNA	Deoxyribonucleic acid
EDL	Electric Double Layer
EOF	Electroosmotic Flow
FEM	Finite Element Method
fwhm	Full-width half-maximum
HCOOH	Formic acid
KCl	Potassium Chloride
LiCl	Lithium chloride
MPSA	Mercapto-propanesulfonate
MspA	porin A from Mycobacterium smegmatis
MUMPS	Multifrontal massively parallel sparse direct solver
nA	Nano-Ampere
NaOH	Sodium hydroxide
nm	Nanometer
nt	Nucleotide
ONT	Oxford Nanopore Technologies
PC	Polycarbonate
PDBID	Protein Data Bank Identification Number
PDE	Partial Differential Equation
PET	Poly(ethylene terephthalate)
PI	Polyimide
PNP	Poisson-Nernst-Planck
RPS	Resistive Pulse Sensing
SCD	Surface Charge Density
SEM	Scanning Electron Microscopy
SNR	signal-to-noise ratio
ssDNA	single strand Deoxyribonucleic acid
um	micrometer
WT	Wild-Type
α -HL	Alpha Hemolysin

CHAPTER 1

1. Introduction

Nanotechnology has recently become an expeditiously evolving discipline, with the capability to thoroughly transform a lot of industries through the development of new materials, devices, and systems with unprecedented properties and functionalities.

Within the field of nanotechnology, nanopore sensing has emerged as an effective and promising instrument to detect and analyze the target biomolecules (1), offering high sensitivity, selectivity, and speed, as well as the ability to operate in a label-free and non-destructive manner (2). The commonly used method for particle analysis with nanopore sensing is resistive pulse sensing (3). This is a widely accepted approach in nanopore sensing and involves the measurement of changes in electrical current as biomolecules pass through a nanopore. The distinctive features of the resistive pulse signal (RPS) such as frequency, shape, magnitude, and duration are examined during the analysis process.

To fully understand and optimize resistive pulse sensing in nanopore sensing, numerical simulations and theoretical frameworks have been examined to investigate the underlying physical mechanisms and improve the precision and stability of data analysis (4). Numerical studies have confirmed that it is a strong device in understanding the complex physics of nanopore sensing, enabling it to simulate the behavior of molecules in nanopores and provide insights into the experimental observations that would be difficult to obtain otherwise (5)(6).

This PhD thesis aims to contribute to the further development and optimization of this technology via the numerical analysis method. Through the theoretical modeling and experimental validation, this work focuses on providing new insights into the underlying physics of nanopore-based sensing and demonstrates the potential of this technology for applications in ssDNA and protein detection. The findings have important implications for the advancement of biosensing tools that have the potential to revolutionize the field of biomolecule characterization and sequencing.

In Chapter 1, nanopore types are introduced generally in terms of solid-state nanopores, biological nanopores and hybrid nanopores. Then, the details of the FEM used for the numerical analysis are discussed. Subsequently, the effective forces to be considered for modeling are mentioned.

The geometry of nanopores is a critical factor in resistive pulse sensing, as it can substantially affect the sensitivity, resolution, and precision of the measurements, making it

essential to carefully design and control the geometry of nanopore devices. From this point of view, in Chapter 2, the effect of nanopore geometry on signal generation and pore sensing and sensitivity is extensively discussed. Three different nanopore geometries are introduced. The effects of geometry on the sensing of particles of different sizes and surface charges, and on the passage of particles through different trajectories are studied.

In Chapter 3, the movement of DNA molecules through the nanopore is discussed theoretically and compared with the experimental outcomes of tracked nanopores. The impacts of surface charge density and applied potential on the waveforms of resistive signals are investigated.

After the investigations on solid-state nanopores presented in the previous chapters, the final chapter of this thesis focuses on biological nanopores. In Chapter 4, the sensing potentials of four commonly used AeL, α -HL, MspA and CsgG biological nanopores are comparatively discussed. Simulations are run for two cases of pore surface charges, i.e., uniformly varied, and residually varied pore surface charges, under different applied potentials. Translocated ssDNA molecules are modeled in the form of rigid (rod) ssDNA, folded (coiled) ssDNA, and protein. The separation potentials of the pores for different lengths of nucleotide transitions are also compared.

The thesis concluded with a brief overview of all chapters.

1.1. Nanopores

Nanopores are extremely small holes or channels on the nanometer scale, typically in the range of 1-100 nanometers in diameter (7) with different shapes and geometries, such as cylindrical (8), conical (9), or irregular (10), which can influence their interaction with target particles on a molecular or atomic scale. These pores can be found in a variety of materials, such as biological membranes (11), solid-state materials (12), and synthetic polymers (13), are often used in nanotechnology applications for various purposes, such as sensing (14), filtering (15), and manipulating molecules (16).

Nanopore sensing include flowing current through a nanopore (typically a few nanometers in diameter) and detecting alterations in the current that arise when molecules pass through the pore. The settings for various factors such as potential, ion concentration, temperature, and pore size affect the behavior of the system and can significantly impact the quality of the results. Choosing the right parameter settings is crucial for the success of

nanopore sensing, and careful consideration of these settings can significantly improve the quality of the results.

The choice of pore size is critical for the specific application (17), as different sizes may be more suitable for different molecules or analytes. Nanopore size can influence a variety of physical and chemical properties, including surface chemistry (18), selectivity (19), sensitivity (20), and permeability (21). For instance, nanoporous materials with smaller pore sizes can have a higher surface area and higher reactivity due to their increased number of active sites, while larger pore sizes can result in higher permeability and faster mass transport.

The choice of material is also an important parameter for stability (22), durability (23), resistance to deformation or breaking (24). Nanopores can be constructed using a wide variety of materials. In particular, they can be built on glass, metals, silicon and polymers and designed in different sizes, shapes and surface chemistries, making them versatile for a range of applications. The chemical characteristics of the pore surface arising from the material can affect its interactions with molecules, and modifications to the surface can be used to enhance or control these interactions. The material and shape of the nanopore have a critical effect on its performance. For instance, a conical nanopore shape can enhance the sensitivity of the device, while a cylindrical shape can increase its accuracy (20). Similarly, the choice of materials can affect the device's durability, stability, and sensitivity. The stability and durability of the nanopore material are important factors, especially for applications that require long-term use or exposure to harsh environments.

The voltage and current used to drive the analyte through the nanopore can also affect the accuracy and sensitivity of the device (25)(26). The potential affects the speed and frequency of the particle passing through the pore (27) If the voltage is too high, it can cause the molecules to pass through the pore too quickly, resulting in reduced SNR (i.e., signal-to-noise ratio) and reduced accuracy. But then, if the electric potential is too low, the molecules may not pass through the pore at all or may pass through too slowly, resulting in low sensitivity and poor efficiency.

System temperature gradients can also affect the behavior of the nanopore device (28). Lower temperatures can improve the stability and sensitivity while higher temperatures can enhance the speed of the analyte passage.

The salt concentration has also great impact on the solution ionic strength, ionic dispersion conductivity, and nanopore stability (29). At low salt concentrations, the electrostatic interactions are weaker, which can result in slower translocation times and

decreased sensitivity. Conversely, at high ionic salt concentrations, fundamental electrostatic interactions can become stronger, resulting in faster translocation times and increased sensitivity. Additionally, the salt concentration can also impact the stability of the nanopore itself (30). High salt concentrations can lead to increased ionic strength and conductivity, which can cause the nanopore to become destabilized and prone to breakdown.

Understanding and optimizing these parameters is essential for designing and using effective nanopore devices for numerous biological and biotechnological applications. Unique intrinsic properties of nanopores can influence the potential of the system significantly. Conductivity, which is one of the physical characteristics of the nanopores, can conduct electrical current, which can be used for sensing or detecting the passing particles (31). The other key features of nanopores are selectivity and sensitivity (32). Nanopores can be designed to be selective for certain types of molecules, based on their size, charge, or other properties and can be excessively sensitive to variances in the surrounding medium, such as modifications in temperature, pH, or the presence of specific molecules. Also provides high-resolution analysis of molecules, allowing for detailed examination of their properties and behavior.

These qualitative properties make nanopores promising tools for various implementations. One of the most exciting one is DNA sequencing (33) which has the potential to revolutionize DNA analysis by providing a fast, sensitive, and high-throughput method for DNA sequencing (34), epigenetic analysis (35), DNA methylation analysis (36), and structural analysis of DNA (37). By threading DNA through a nanopore, fast and accurate DNA sequencing is possible by measuring disturbances in the electric current as each nucleotide passes through. This technology promises to fundamentally transform genomic research and has already been used in the development of portable DNA sequencers (38).

Owing to their size-based separation feature, nanopores can selectively filter and separate molecules based on their size. This allows for rapid and accurate sequencing of DNA with a single instrument, which has the potential to revolutionize personalized medicine, genetic research, and diagnostics.

Nanopores also have potential in protein analysis (39). By using nanopores to quantify the electrical features of a protein, the variations in the electrical pulse can be used to identify the protein based on its size, shape, and charge. In this way, researchers can gain insight into protein structure and function, which could be valuable for drug discovery and development. One of the most exciting implementations of this technology in protein analysis is protein

sequencing (40). By passing a protein through a nanopore, a series of amino acids can be resolved based on the changes in the electrical signal as each amino acid passes through the pore. Nanopores also be used to analyze the configurational deterioration that occurs in proteins (41). By modifying the nanopore with ligands that can selectively bind to specific regions of the protein, changes in the electrical signal can be used to monitor the structural changes that occur as the protein interacts with the ligands. Nanopore-based protein analysis has several advantages over other methods, including its capability to analyze individual molecules and its potential for high-throughput analysis. However, there are also significant technical challenges associated with the development and use of nanopore-based protein analysis, including the need for high-resolution and sensitive instrumentation, and the difficulty of controlling the motion and behaviour of the target protein as it passes across the nanopore.

Overall, despite the challenges, this technology has the potential to revolutionize DNA and protein analysis by providing a fast, sensitive, and high-throughput method for the identification, quantification, sequencing, and conformational analysis.

Nanopores could also be used to deliver drugs directly to specific cells or tissues (42). By controlling the size and shape of the nanopores, researchers could design delivery systems that selectively target cancer cells or other diseased tissues, minimizing side effects and improving treatment outcomes.

On the other hand, the choice of nanopore hinges on the specific application and the properties of the molecules being studied. There are several different types of nanopores. These include solid-state nanopores, biological nanopores and hybrid nanopores, also called protein engineering nanopores. In the following sections these pores are reviewed separately.

1.1.1. Solid-State Nanopores

Solid-state nanopores are artificial nanopores that are created by etching or drilling nanometer-scale holes in a thin substrate (membrane) made of a solid state substance, such as glass, graphene, or silicon (43). These nanopores have a range of diameters typically ranging from 1-100 nanometers, which can be precisely controlled during fabrication and can have a range of geometries, including cylindrical or conical shapes.

In sensing using solid-state nanopores, by applying a field effect across the membrane, molecules are moved through the nanopore. As molecules pass across the pore's sensing zone, they cause disturbances in the fluid flows along the pore, and these disturbances can be detected and analyzed. This enables individual particles such as biomolecules, viruses,

nucleic acids, proteins, and small molecules to be sensed and characterized with high definition.

The synthetic nanopores have unique properties. These are high mechanical and chemical stability, tunability, and the integration capability with micro- and nano-electronics which also known for their robustness, and longevity, making them suitable for long-term use in different usage areas. These properties make them particularly valuable for applications as diverse as single-molecule sensing, DNA sequencing, and protein identification. They can provide analysis of molecules, allowing for detailed examination of their properties and behavior. Overall, solid-state nanopores are an important instrument for studying the properties of nanoscale materials and have the potential to revolutionize many fields of science and engineering.

1.1.1.1. Track-Etched Nanopores

Track-etched nanopores are tiny, cylindrical pores that are formed by etching a thin membrane, typically made of a polymer material, with ionizing radiation. These pores can have diameters in a wide range from nanometers to micrometers, and can be implemented in many applications, for instance, biological sensing, single molecule analysis, and nanoparticle detection. One of the superiorities of track-etched nanopores is that they can be precisely engineered to have specific sizes and geometries, which makes them useful for studying molecules of different sizes and shapes. They can also be functionalized with various surface chemistries to enhance their selectivity and sensitivity for specific analytes.

The process of track-etching involves the chemical etching of substrates that are exposed to accelerated heavy ions. Chemical etching results in the formation of small pores in the invisible traces created by the heavy ions. This technique makes it possible to create membranes with either a single or multiple pores by using films that are irradiated with one or more heavy ions.

Polyimide (PI), polycarbonate (PC) and Poly(ethylene terephthalate) (PET) are widely utilized polymer substrates in the process of track-etching (44). Among these, PET is often favored as negative carboxylate groups are formed on its walls as a result of etching (45). PET is an attractive option for numerous potentials uses due to its capacity to vary the surface charge by regulating the medium pH, as well as its straightforward surface modification.

1.1.2. Biological Nanopores

Biological nanopores are naturally occurring channels in biological membranes that allow the translocation of ions, molecules, and even macromolecules across the membrane. These channels have diameters on the order of nanometers and are made up of protein or lipid molecules. Recently biological nanopores have drawn considerable interest for their promising potential applications in biosensing, protein analysis, and DNA sequencing. They are typically formed by proteins, such as ion channels or porins, and can selectively allow specific molecules or ions to migrate through the membrane while excluding others. In recent years, researchers have become interested in using biological nanopores as tools for sensing and analysis. For example, nanopore-based sequencing has evolved as a powerful tool for DNA sequencing, where a single DNA strand is passed through a nanopore. Then, resulting electrical signals are used to determine the sequence of nucleotides. Other potential usage areas for biological nanopores include the identification of proteins, viruses, and other biomolecules, as well as the development of nanofluidic devices for drug delivery or chemical synthesis.

Below, the biological nanopores will be described in detail with their structural characteristics and areas of use. Since in Chapter 4, in the sensitivity analysis of biological nanopores, the four most widely used nanopores (i.e., AeL, α -HL, MspA, CsgG depicted in Figure 1) are discussed, only these four pores are favored to be reviewed here.

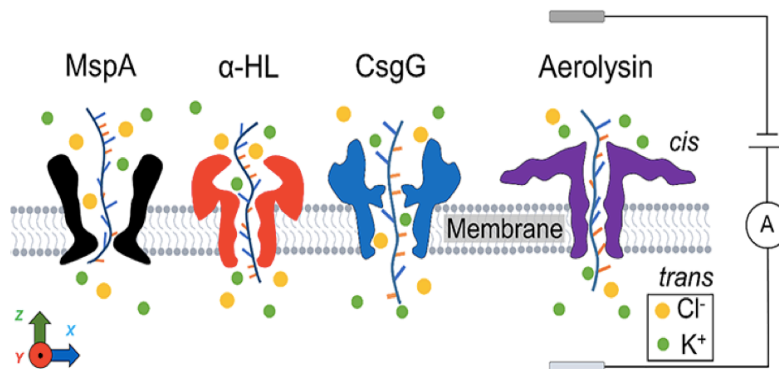


Figure 1. Illustration of four biological nanopores (courtesy of ref. (46))

Chapter 4 of the research study aims to compare the potentials of biological pores, including AeL, α -HL, MspA, and CsgG, under the effect of surface charge/mutation, which

has not been explored previously. Thus, this section provides a detailed review of the four pores based on experimental studies and their usage areas. The study primarily focuses on mutant studies and the structural features of the pores, such as recognition sites, to conduct the comparison. As indicated by previous research, various factors contribute to the effectiveness of the pores, and their advantages over each other vary depending on the operating conditions and mutant applications.

1.1.2.1. AeL (Aerolysin)

Wild type Aerolysin (WT AeL) is a pore-forming toxin that has a long β -barrel channel (10 nm) secreted by the *Aeromonas hydrophilia* bacterium (47) which was first introduced with a debut study, translocation of α -helical peptides (48).

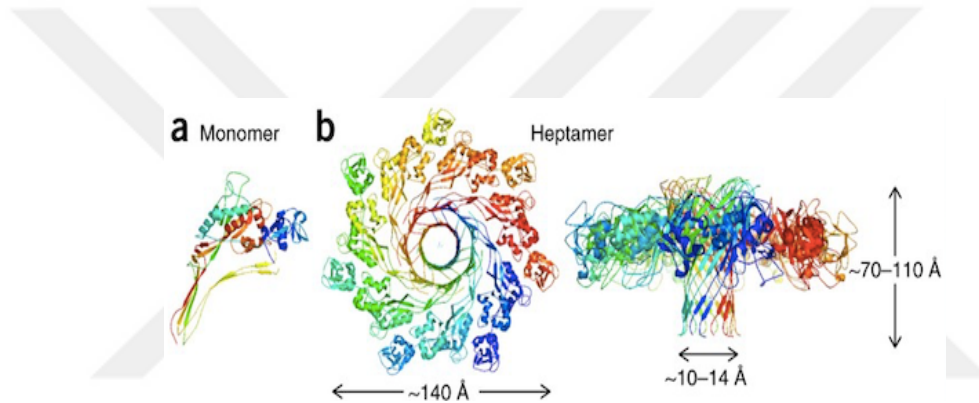


Figure 2. Structure of the wild type aerolysin (courtesy of ref. (49)).

This rivet-shaped pore with a narrow constriction ~ 1.0 - 1.4 nm (50)(51) inside the long channel and lacks the vestibule (Figure 2) has a wide application area such as peptide detection (52)(53), direct nucleic acid sensing and nucleotide discrimination (54)(55) and unfolded/folded protein translocation (56)(57) while its prominent feature appears in the discrimination of quite short oligonucleotides (58)(59). AeL shows distinct sensitivity as small as dinucleotides while α -HL cannot detect the polydeoxyadenines (dA_n) for $n < 5$ (60). To work with this sensitivity, an ideal pore must provide both detection and discrimination very well thus it is critical to determine residue positions to reveal the sensitivity potential of the pore (61). The fact that AeL provides better signal generation and longer dwell time than α -HL and MspA is due to its narrow lumen and too many SCDs, confirming that pore shape and internal charge density distribution are critical factors (61). Although it is difficult to reveal the crystal structure of this pore yet, it is revealed there is two recognition sites, with the assumption that the upper is responsible for detection and the bottom is responsible

for discrimination of molecules (60). This default theory is predicated and proved by single site mutagenesis (62). Results also show that the mutant AeL pore provides higher sensitivity than the WT even though WT AeL pore has a high resolution capability as small as a single amino acid without any modification and labelling (63)(64). On the other hand, the vestibule-free morphology and very narrow internal structure of AeL make it very difficult to capture longer ssDNA (>10 bases) (65) which is tried to overcome by increasing the concentration strength to 3.0 M KCl (66) and performing the translocation in Li^+Cl^- rather K^+Cl^- (67) to increase the capture rate.

The second step after particle capture is recognizing the particle. AeL recognition points are mapped by the WT protein pore using ssDNA of length 14-base nucleotide that completely blocks the pore lumen. By increasing applied potential, which reduces the dwell time, ssDNA overcomes the energy barrier and makes the transition. Duration of dA_{14} between the $n=10-20$ shows longer transition time indicated intense interplay between the pore and dA_{14} attributed to the dA_{14} fully fit into the pore. Alongside demonstrating the recognition sites coincide to the R220 and K238 amino acids their detection capabilities are also demonstrated using abasic sited free oligonucleotides. Results exhibit that the R220 residue, locates at the most constricted region, yields highest discrimination capacity for each of the four nucleotides (66). Besides, it is shown that the K238 site can slow down the passing molecule up to 7-fold, thereby prolonging the residence time, when the residual amino acids in that region are regulated (61). Inconsistent normalized blockade currents obtained from a WT and two mutants states show the size of side chain of the residues be like determinant in surface interactions and blocked ionic flow. The obtained signal is the most direct data to understand the system dynamics. The signal has two components, one of which is the signal width which reflects the translocation time. A longer translocation time derived from the mutated pore by neutral amino acid without a side chain, shows that the pore shape is an important factor as well as chemical properties for the precise discrimination ability. Pulse magnitude, the other component of the signal corresponds to the volume exclusion, is the amount of blocking current due to the occupied pore by the translocated particle. However, it is shown that volume exclusion is not always be sufficient to explain the blocked current magnitude (68). Experimental work using short peptide has showed that modification of residue regions improves interactions between analyte and protein surfaces and produces deeper blockades than WT. It is concluded that by modulating the ionic conductivity through mutation, it is possible to control ionic blocking with enhanced interactions and molecular conformations where the volume exclusion effect remains small.

1.1.2.2. α -HL (Alpha Hemolysin)

Wild type Alpha Hemolysin (WT α -HL) is a pore-forming toxin natively secreted by the *Staphylococcus aureus* bacterium (69). This toxin is the most studied protein channel and continues to be most frequently adapting pore. This heptameric protein pore is a self-assembled in a lipid bilayer to form an aqueous channel across the lipid membrane with a large central-constriction (inner restriction/tip_region) diameter compared to some other pores. α -HL composes of two morphological domains separated by a positively charged narrow aperture of 1.4 nm diameter: vestibule of 4.8 nm long with a cap domain extends to 7 nm with rims at the cis-side and β -barrel of 5.2 nm long at the trans-entrance where total pore long is 10 nm (69) (Figure 3).

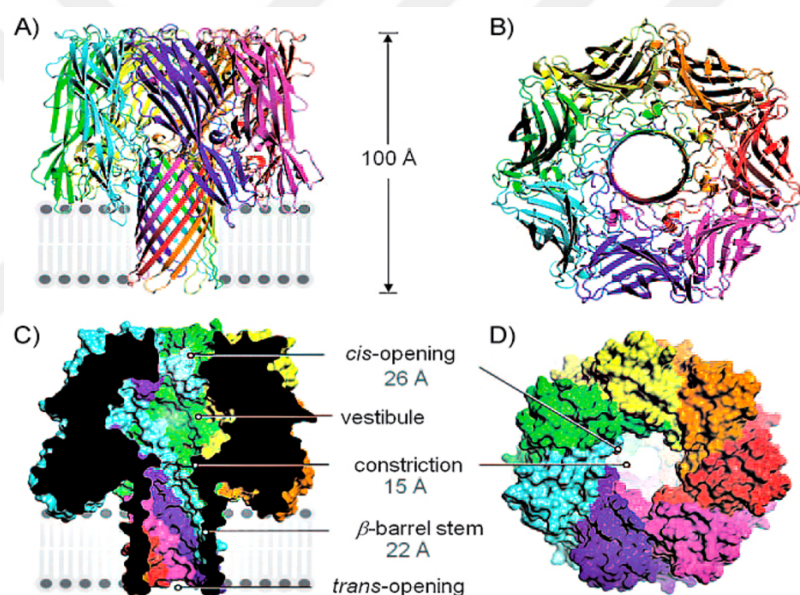


Figure 3. Wild type α -HL crystal structure (courtesy of ref. (70)).

Unlike the mushroom-like-shape of α -HL, in continuum models the pore is modeled as a cylinder (71). Owing to the earlier unveiled crystal structure (PDBID: 7AHL), nucleic acids detection by protein pore is first achieved by using α -HL (69)(72) and potential of α -HL exhibited how precisely recognize the nucleotides, individually (73)(74), even in modified form (75). Due to the very narrow constriction only a molecule as large as single-strand DNA (ssDNA) can pass through this mushroom-shaped pore, while the wide vestibule with a wide entrances allows to capture and keep in larger analytes, such as PEG particles or nucleic acid duplexes (76)(77). It is shown by using the MPSA-coated-gold nanoparticle

how α -HL pore enables to determine the characteristics of a macromolecules through identifying the size, charge and surface qualities by grasping hold of the particle in the cap domain (78). Besides, the reentrant form of this pore yields the entrance is responsible for catching/detecting the molecules (79) while the β -barrel, including the narrow constriction, responsible for the translocating/sensing of target particles with three recognition sites (74)(80).

While WT- α -HL pore has a great potential as a nanoparticle-based biosensor, chemical modifications by engineering mutagenesis provide implementation to further improve the obtained results and wider the application cases (81). Due to the structural limitations of the sensing region to correctly refer to the bases from the obtained signal it is displayed that mutated pores by charge decorations outperform the WT α -HL (82). Previous findings have described how the inner surface of α -HL can increase the rate of DNA capture by manipulating surface charges (83). As demonstrated in many papers α -HL pore has three recognition sites which are located at the β -barrel (80)(84). It is clarified in detail how mutant and WT α -HL pores interact with these recognition sites (84). WT α -HL distinguishes the bases only at the R1 position which is corresponding to the narrow constriction point while 2N-aHL (E111/K147) mutant produces higher blockade current by getting the recognition from nothing but R2-R3 points. Nevertheless, 2NM113Y(E11N/m113Y/K147N) mutant pore gives the best result from the R1 site while each mutant is able to identify 4 bases (84). Integrating additional charges into the pore generates strong interactions between the channel interface and molecule which enhances the sensitivity and selectivity. The anion selective feature of α -HL narrow region, which provides the ionic selection, is the point of modeling and experimental (85) studies that are currently examined at various ionic mediums. Physical dynamics between the oppositely charged DNA and protein (i.e., negatively and positively charged, respectively) channel residues enable to control the velocity of oligonucleotide by engineered site directed mutagenesis (86). It is demonstrated DNA, near the charge decorations, coils more than normal, resulting in decelerated molecule provides more ion blocking. Also, due to the structural feature of α -HL, wreath form of DNA coils in the wide-cap domain (87). Compacted DNA confines the gap to allow the ionic transportation and refuses the ionic charges. Reduced ionic conductivity due to the restricted aperture results in higher blockade while the stretched molecule led more ionic transport thus higher ionic flow (88) indicates the coil formation of DNA induces the deep blockade generation (87). In addition, during the β -barrel transition, the nonuniform velocity of DNA

shows an inconsistent transition with an increasing and then decreasing acceleration, implies the dwell time is mostly dependent on residual position rather than charge amount. Decreasing rate of ionic mobility also points out the recognition site of this channel (89). Surface charge density rearranged by pH regulation has also remarkable effects on electrostatic interactions between interfaces of particles or pore-particle even may have reverse the binding/affiliation strength (90). Residues located nearby the narrow tip zone are directly relevant to the pore selectivity and the resultant electroosmotic flow (71). With the pH alteration amino acids locate at the sensing residual region can be neutralized (acids i.e., glutamic acid, aspartic acid) or evolve to opposite charge (basics i.e., lysine, histidine, arginine). Decreasing the pH value makes the pore surface becomes more positive and strengthen the EOF. As a result of the repulsing of positive ionic flow by the pore surface charges, instability between the positive-negative ionic flow occurs in the pore. Resultant phenomenon of EOF excites to exert possible applications such as particle discrimination (91) and particle catching (92). The consequence of which also investigated in the manner of dependency of trapping and dwell time of macromolecules to the applied voltage under the EOF effect (93), (94). Since previously the pattern and position of the WT- α -HL' s charged residues are revealed precisely (69) the pH effect on the current blockade was also validated by applying the site-directed mutagenesis (95).

1.1.2.3. MspA (porin A from *Mycobacterium smegmatis*)

The other ideal biological nanopore for ssDNA sequencing is MspA (porin A from *Mycobacterium smegmatis*). Its structure allows the pore to be highly sensitive to current fluctuations by even a couple of nucleotides with its short (~0.5 nm long) and confined (~1 nm in diameter) sensing region (96)(97). 9.6 nm long pore has two openings of 4.9 nm and 8.8 nm, at trans side and cis side respectively (Figure 4).

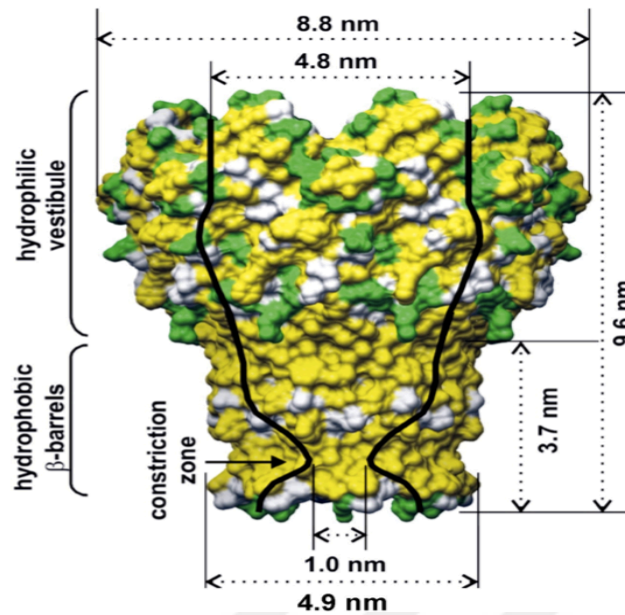


Figure 4. Structure of the wild type MspA (courtesy of ref. (98)).

This intrinsically conical shaped homo-octameric pore is highly stable with its rigid β -barrel structure (96) which also provides long sequencing as a single nucleotide resolution level (99) and can discriminate each nucleotide (100) with its typical lumen form. DNA translocation speed is a difficult situation encountered for sequencing. To overcome this difficulty highly negatively charged pore was mutated by neutralizing the residual charges in the inner constriction (97) which is the leading work that demonstrated the MspA sensing ability for the ssDNA. This mutant form promotes the DNA to migrate under the applied electric field through the pore while imparting positive charges to the vestibule and entrance increase the DNA trapping into the pore and exhibiting extraordinarily high pore-DNA interaction rates. The positive charge effect in the recognition site is also supported by showing that the added arginine amino acids slow down the DNA migration rate without dispose of current blockades (101). To decelerate the DNA speed, it is also demonstrated the combination of the formerly engineered mutant MspA (97) with phi29 DNA polymerase provides highly distinct and consistent signals by controlling the molecule transition (102). Besides, immobilization of ssDNA was examined to provide a long duration time of DNA transition (103). Holding the ssDNA by NeutrAvidin in the constriction of the pore, the nucleotide discrimination potential of the mutant MspA (neutralized negative charges) is revealed. Interpreting the current fluctuations are also important to associate the current blockade to the translocated particle's features. By trapping (instead of complete transition) the ssDNA-NeutrAvidin conjugate in the engineered MspA, obtained current change with

different fluctuation levels are correlated with the effective charges, diffusion constants, and current magnitudes (104). MspA is also released as a precursor commercialized product under the series name R7 by Oxford Nanopore Technologies (ONT) (105) with several mutated versions.

1.1.2.4. CsgG (Curli specific genes G)

Although it is known that amyloids are in part some diseases due to the protein misfolding and accumulation (106), (107), bacterial curli are effective amyloids to develop biofilms, for host cell adhesion and colonization of stationary planes (108). CsgG (Curli specific genes G) is a channel that secretes the functional amyloid curli which formed over the outer membrane of the bacteria. This nonameric protein channel is composed of the highly negatively charged transmembrane β -barrel of 3.5-3.8 nm, the narrow constriction of 2 nm and the periplasmic lumen of 3.8-4 nm respectively, with 8.5-9.6 nm overall length (109)(110). The entrances' diameters are 5-5.2 nm at the periplasmic side and 4-5 nm at the transmembrane β -barrel domain while the internal narrow region is 1.2 nm (Figure 5).

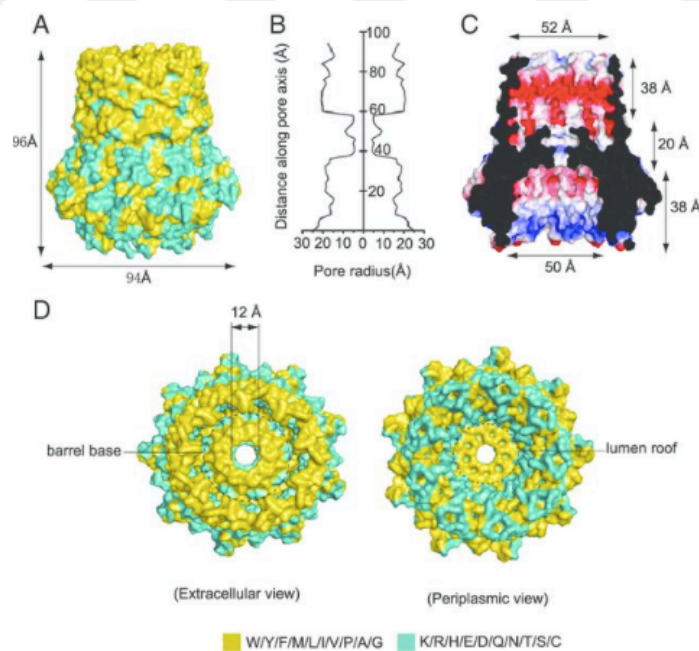


Figure 5. Structure of the wild type MspA (courtesy of ref. (110)).

This pore's barrel is known as the widest with 36 β -strands among others. Recorded currents from a single pore rebuild in the phospholipid membrane under relatively low

applied potential (+50mV or -50mV) yield consistent results, suggesting that CsgG can be utilized as a passive diffusion-based line (109)(111). However, it is disputable whether the reconstructed pores meet real physical conditions. Moreover, it is shown three functional residues, Tyr51, Asn55 and Phe56, at the narrow constriction are responsible for the selective secretion of curli subunits such as, depending on the engineered mutations, severe reduction may occur in the curli product while wild type secretion level can be maintained (109)(110). WT CsgG protein has been mutated to enhance the sensitivity qualifications. Studies demonstrate CsgG-CsgE combined form reduces the ion conductance (109) and CsgG^{F56Q} modified pore generates increased baseline current with amplified blockade signal pulse for the ssDNA capture (112). Besides, adapting the periplasmic accessory protein CsgF to CsgG generates a second constriction (dual constriction) inside the channel. This assembled complex (CsgG^{F56Q} and CsgG^{F56Q}-FCP) demonstrates individual conductance rise for each oligonucleotide. Hence, the dual-constriction model suggests the translocated nucleotide causes distinct conductance from each narrow region. Besides, CsgG is commercialized by ONT under the series name R9 (113). The mutant version of this curli amyloid channel, R9.4 series, was released with high throughput accuracy which enables high-speed DNA translocation. Among 1200 modified versions (114) final variant was selected but the mutant structure has not been reported.

Based on the previous studies (63)(83)(97)(110), it seems that mutagenesis has enhanced the sensing ability of all four biological pores when compared. Therefore, one can draw the conclusion that there has been an improvement in the sensing ability of these pores through mutagenesis. Furthermore, the assessment of pore shape indicates that a geometric structure that is narrower and without a vestibule is more effective in detecting short oligonucleotides (60). In contrast, it is widely recognized that conical pores offer superior detection capability compared to cylindrical pores (20).

The α -HL pore, resembling a mushroom in shape, can prolong the time DNA molecules spend within it by regulating its pH and manipulating the electrical charge through site-mutagenesis. On the other hand, MspA exhibits a conical form with a short and narrow sensing zone, making it an ideal pore for detecting DNA molecules (96).

Nevertheless, as reported by Wang et.al. (61), the fact that cylindrical-like AeL outperforms both MspA and α -HL suggests that mutant structure and internal dynamics could be more crucial factors than geometry in determining pore performance. In addition, the process of selecting the most appropriate CsgG mutant for R9, which involved testing 1200 different models (114), highlights the crucial role of inner surface charge distributions.

The outcomes of these comparisons can provide a response to the inquiry of whether mutating or altering the pore is feasible. Given that each pore geometry has its distinct advantages, the results indicate that modifying the pore through mutation may be a more effective means of improving sensing ability than changing the geometry altogether. To clarify this issue, the effects of surface charges and mutation on sensing in biological nanopores is discussed in Chapter 4 extensively.

1.1.3. Hybrid Nanopores

Hybrid nanopores are a type of nanopore that is created by combining synthetic materials with biological nanopores. Hybrid nanopores merge the superiority of biological nanopores, i.e., high selectivity and sensitivity, with the advantages of synthetic materials, such as tunability and stability.

The integration of synthetic materials with biological nanopores can be realized by a multitude of techniques, including chemical modification, surface coating, and the use of nanofabrication technologies. Their unique properties such as tunability, stability, versatility, and compatibility make them useful in many applications areas. Hybrid nanopores have shown promise in numerous applications, such as specialized molecule identification, DNA sequencing, and nanoparticle analysis, due to their unique properties and tunability. In general, hybrid nanopores offer a versatile platform that is adaptable and tailorable for a variety of applications, including the fields of biomedicine and environmental monitoring.

1.2. Resistive Pulse Sensing

The resistive pulse sensing (RPS) is a method used in the field of biosensors to detect and measure particles such as cells, DNA, viruses, and nanoparticles in solution based on their size and electrical properties.

The basic setup of this technique, fundamentally modeled on the Coulter Counter Principle (Figure 6), involves two electrodes positioned on both sides of a small pore or channel. The solution containing the particles to be measured is introduced into the system. A small pore or channel is used to pass the particles through, one by one. The pore or channel size must be carefully chosen to allow only one particle to pass through at a time, creating a characteristic transformation in ionic current.

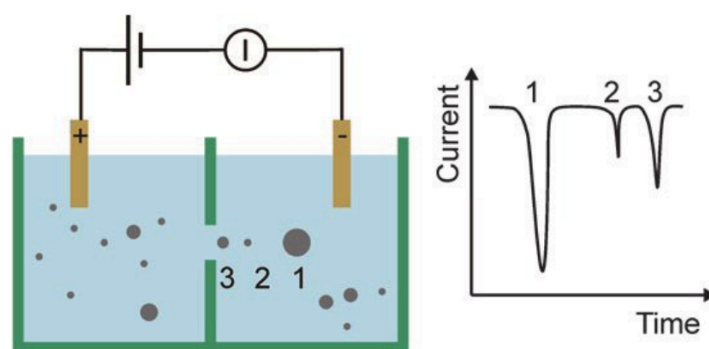


Figure 6. Illustration of resistive pulse method based on Coulter Counter Principle (courtesy of ref. (115)).

The method involves passing a solution containing the particles through a small pore or channel in an isolated membrane, which is held in the middle of two reservoirs filled with an ionic solution. Applying a potential crosswise the membrane with electrodes, with particle passage, a transient current drop occurs. This reduction in the fluid flow is recognized as "pulses" in the electric current.

By examining the pulse characteristics, such as amplitude, dwell time, and frequency, it is possible to determine the volume, form/configuration, and concentration of the molecules, with their surface charge and other electrical properties.

In addition to these basic components, there are several conditions require to be conducted cautiously to obtain precise and repeatable evaluations. These include the type of electrode material, the size and configuration of the pore or channel, the sample solution flow rate, concentration, and particle size distribution.

However, since the system is on the nanometer-micrometer scale, the experimental environment may not allow for the optimization of parameters and manipulation of environmental conditions as desired.

To overcome these difficulties, numerical studies, i.e., simulation tools, are important to control the conditions before the experiment and adjust the right parameter settings.

Simulation allows to explore a wide range of experimental parameters such as pore size, surface chemistry, applied voltage, and buffer conditions before performing experiments. This helps in optimizing the experimental conditions and reducing the number of experiments needed to obtain accurate and reliable results.

Numerical methods also provides a means to understand the fundamental principles and mechanisms underlying nanopore sensing. Simulation of the particle transportation through

the nanopore can give an insight into the parameters influencing the signal and noise of the system. This understanding can then be used to optimize the construction and operation of the system.

Simulation methods can also be used to develop data analysis algorithms for nanopore sensing. By simulating the response of the system to different particle sizes and concentrations, algorithms can be developed that accurately interpret experimental data and extract the desired information.

One of the most powerful numerical techniques for simulating the behavior of nanopores and understanding their nanometer-scale properties is the Finite Element Method.

1.2.1. Finite Element Method

Finite Element Method (FEM) is a quantitative approach used to solve partial differential equations (PDEs) in many areas of applied disciplines, including nanotechnology. Particularly, FEM is a powerful tool in nanopore sensing that allows for the simulation and optimization of nanopore sensors for specific applications. It can also provide insight into the underlying physics of the nanopore sensing mechanism and aid in the advancement of novel and improved sensing technologies.

FEM can be applied to optimize the design of nanopore sensing tools for particular applications. For instance, the shape and size of the nanopore can be varied to optimize sensitivity or selectivity for a particular type of molecule. Additionally, it can be also used to explore the influence of different factors, including temperature or pH, on the sensing performance of the nanopore.

Another application of FEM in nanopore simulation is in modeling the mechanical behavior of nanopores. Nanopores can be stretched or compressed by external forces, and FEM can be used to simulate these deformations and predict the mechanical response of the nanopore. This can help design more robust nanopore-based devices for various applications, such as biosensing and DNA sequencing.

The FEM involves dividing the solution domain into smaller sub-domains, known as finite elements. Each element is connected to its neighboring elements through nodes, and the solution is approximated at these nodes.

In nanopore simulation, FEM is employed to represent the properties and processes of a nanopore by dividing it into small elements, each with its own set of equations, and the equations are solved concurrently for each element.

The electrostatic potential and ion concentration are then interpolated between the elements to obtain a continuous solution.

FEM describes the electrostatic potential, motion of fluid flow in a medium and the ionic mobility in a nanopore by solving the Poisson equation, the Nernst-Planck equation, and the Navier-Stokes equation, respectively. These equations are interdependent and must be applied concurrently. The boundary conditions rely upon the nanopore geometry and the experimental setup.

FEM can be used to represent the cross-transportation of the molecule through the nanopore and corresponding changes in flow. Through solving Poisson's and continuity equations the electrical potential and current density distribution can be determined. In this way, it is possible to simulate an electrical signal generated by a molecule as it passes through a nanopore.

The Poisson equation is applied to consider the electrostatic potential, which determines the driving force for ion transport across the nanopore. The boundary conditions for this equation are typically the voltage and charges on the nanopore interface. The FEM method uses a set of linear equations to solve the potential distribution.

Another application of FEM in nanopore simulation is to study ionic mobility or molecular transport. The Nernst-Planck equation is a fundamental partial differential equation used in nanopore sensing to describe the transportation of charged species subjected to the field effects and concentration gradients. The equation considers the diffusion, drift, and electrostatic interactions of the ions and is based on the principle of conservation of mass.

In nanopore sensing, the electric potential in and around the nanopore is described by governing the Nernst-Planck and Poisson equations (called as PNP equation). This can help to understand the components that have an impact on the rate at which charged species are transported through a nanopore, such as the nanopore dimension, the charge dispersion on its walls, and electric field strength. The specific nanopore geometry and experimental setup define the boundary conditions of the Nernst-Planck equation that can include bulk concentrations of ions, potential, and the nanopore surface charges.

To describe the motion of fluid flow in a medium FEM solves the Navier-Stokes equation. To solve this equation the nanopore region is divided into small elements, and the velocity and pressure fields are approximated using shape functions. The governing equations are solved for each element, and the solutions are interpolated between the elements to obtain a continuous velocity and pressure field.

The specific nanopore geometry and the experimental setup specify the boundary conditions of the Navier-Stokes equation in nanopore sensing. The most common boundary condition is a no-slip boundary condition, where the fluidic velocity is zero in nanopore walls. Other boundary conditions may include a constant pressure gradient, an applied electric field, or an external force.

Once the FEM equations are solved, the fluid flow through the nanopore are evaluated by integrating the fluid velocity over the nanopore cross-section. The fluid flow can then be related to the experimental variables such as the nanopore size, applied pressure or voltage, and fluid viscosity. This allows to extract information about biomolecules in transition.

Overall, FEM provides a powerful tool to solve these equations and predict the electrostatic ionic current variations, which is a key parameter in nanopore sensing.

As widely used in the field of nanotechnology, FEM is an emerging instrument for simulating the behavior of nanopores and understanding their properties. Ongoing research continues to improve and advance the capabilities of FEM for nanopore simulation.

1.2.2. Effective Forces

Effective forces are very important and crucial parameters in nanopore sensing. They should be included in FEM analyses as they determine the speed and direction of the analyte as it passes through the nanopore and can be used to obtain information on properties such as the volume, charge, and structure of the analyte.

The effective force in nanopore sensing can be generated in many ways, depending on the specific system being used. For example, in solid-state nanopores, the effective force is created using the electric field. This force creates an electrophoretic force that drives the particle through the pore. On the other hand, in biological nanopores, the effective force is generated by the electrostatic potential difference.

The factors that constitute effective force are given below.

1.2.2.1. EDL

The electric double layer (EDL) arises as a result of the mutual effects between charged species on the nanopore surface and in the electrolyte solution. When the nanopore surface is charged, opposite charges are attracted to the pore wall from the solution. This results in the presence of an ion layer adjacent to the pore wall. The EDL comprises two regions: the Stern layer and the diffuse layer. The Stern layer is tightly bound to the pore surface and

consists of ions that adsorb strongly to the surface. The diffuse layer extends out from the Stern layer and into the bulk solution and consists of ions that are less strongly bound to the surface.

The EDL thickness depends on the size and charge of the ions in the electrolyte solution, as well as the amount of the charged species on the nanopore. As the surface charge increases, the thickness of the EDL increases, and the concentration of ions in the EDL decreases.

The EDL affects nanopore sensing in several ways. First, the EDL affects the ionic motion through the nanopore, which can impact the sensitivity and precision of the detection. The EDL can also affect the interaction between the target analyte and the pore wall, which can impact the capture and translocation of the molecule through the pore.

Secondly, EDL also has an impact on the nanopore sensing stability. The EDL formation can lead to the adsorption of charged particles on the pore surface, which can modify the pore properties and alter the sensitivity and stability of the sensing system.

To mitigate the effects of EDL on nanopore sensing, various strategies have been developed. This includes modifying the pore surface with functional groups, regulating the ionic strength and solution pH, and using different materials for the nanopore. Therefore, understanding the formation and properties of the EDL is crucial for developing accurate and sensitive nanopore sensing systems.

1.2.2.2. Electrophoresis

Electrophoresis is a widely used method for separating charged molecules corresponding to their size, shape, and charge. It involves the movement of charged molecules along an electric field that causes them to migrate towards the oppositely charged electrode, depending on their charge and size.

In nanopore sensing, a voltage is applied crosswise a nanopore in a thin membrane. The molecules of interest are derived from the nanopore by the generated electric field by the applied potential.

By applying an electric field across the nanopore, a potential difference is generated causing the charged molecules in the electrolyte solution to move towards the nanopore. As the charged molecules approach the nanopore, they can interact with the charged surface of the nanopore, creating a diffuse layer of charge named as the electrical double layer. This diffuse layer of charge can then interact with the bulk solution, generating a flow of solvent

molecules towards or away from the nanopore, corresponding to the electric field direction. The Passage of charged molecules causes an ionic current change. The Analysis is performed by detecting and discriminating the distortions. The ionic current magnitude and dwell time variation are dependent on several factors. Some of them are the size, shape, and charge of the molecules, as well as the strength and orientation of the electric field.

Electrophoresis can be used in nanopore sensing for the separation and analysis of charged molecules corresponding to their size, shape, and charge. By varying the electric field and membrane properties, it is possible to conduct the speed and direct the charged molecules, enhancing the sensitivity and selectivity of the measurement. However, other factors such as electroosmosis can also affect the movement of the molecules and need to be taken into account when interpreting the data.

1.2.2.3. Electroosmosis

Electroosmosis can be generated during nanopore sensing by a potential applied across the nanopore. The resulting electric field creates a difference in potential crosswise the nanopore. This causes the ionic motion in the electrolyte solution towards the nanopore. As the ions approach the nanopore, they can interact with the charged surface of the nanopore, creating a diffuse layer of charge named as the electrical double layer. This diffuse layer of charge can then interact with the bulk solution, generating a flow of solvent molecules towards or away from the nanopore. This flow of solvent molecules is called electroosmosis, and it can be measured as a change in the current flow.

Several factors determine the direction and magnitude of electroosmotic flow, such as nanopore surface charges, pH of the electrolyte solution, and ionic strength. Electroosmosis can sometimes interfere with the detection of analytes, but it can also be exploited to conduct the movement of analytes across the nanopore and enhance the sensitivity of the measurement.

1.2.2.4. Drag Forces

In nanopore sensing, drag forces refer to the forces that resist the movement of particles. These forces can have a critical effect on the movement and detection of biomolecules in nanopore sensing experiments and include several types of forces such as viscous drag, electrostatic drag, Brownian motion, and hydrodynamic interactions. Viscous drag is the resistance encountered by a molecule as it moves through a fluid. It is in direct proportion to

the fluid viscosity, volume, and shape of the target analyte. On the other hand, electrostatic drag is the force that a charged molecule encounters as it moves through a charged medium, such as an electrical double layer near the pore wall. Electrostatic drag is in direct proportion to the charge and size of the molecule, as well as the magnitude and direction of the electric field.

Brownian motion is the random motion of particles in a fluid due to thermal fluctuations. Brownian motion can cause a molecule to deviate from its intended path and can contribute to the overall drag force on the molecule. Besides, hydrodynamic interaction is the interaction between molecules and the surrounding fluid, which can affect the drag force experienced by the molecule. Hydrodynamic interaction is influenced by the factors of size, shape, molecular concentration, and properties of the fluid.

In nanopore sensing experiments, these drag forces can affect the rate and direction of movement of molecules through the nanopore, as well as the pulse amplitude and dwell time. Therefore, it is important to consider the effects of these drag forces when designing and interpreting nanopore sensing experiments.

1.2.3. General Approach to the Method and Governing Equations of the Simulations

Problems in fluid dynamics profound comprehension of the physical phenomena involved and the ability to use mathematical models and computational tools to simulate and analyze these phenomena.

The FEM is a powerful numerical method that has an application in a wide range of problems consisting structural mechanics, fluid dynamics, heat transfer, and hydrodynamics, among others. To employ the potential of this method it needs into integrate to an exclusive software programme.

COMSOL Multiphysics is a strong software tool that combines the FEM with the ability to simulate multiple physical phenomena simultaneously. The software uses advanced numerical algorithms to solve the resulting system of equations, which can involve many thousands of degrees of freedom.

The software uses a modular approach, where each physical phenomenon is represented by a separate module. These modules are then coupled together to simulate the interactions between the different physical phenomena. Here, the potentials of COMSOL and FEM methods are investigated for the nanopore/nanoparticle dynamics problems, and external forces in accordance with the concept of this study.

In nanopore sensing problems, COMSOL uses PDEs to model the physical phenomena involved in nanopore sensing, and then solves these PDEs using the FEM to obtain accurate and detailed results for the system under investigation.

To analyze the geometry effect and resistive signal pulse analysis with full accuracy, the system dynamics and effective forces must be precisely defined.

Electroosmosis and electrophoresis (drag force and pressure can also be included) are the forces governing the system, trying to dominate each other during ionic flow. These forces control the particle motion and fluid flow by coupling with the pore/particle surface charges, ionic concentration and applied potential.

The Poisson, Nernst-Planck and Navier-Stokes equations are governed and incorporate the above-mentioned forces into the system.

The electric potential is associated with the dispersion of charged species, and it is defined and formulated by the Poisson equation as:

$$\nabla^2 E = -\frac{\rho}{\epsilon_r \epsilon_0} = -\frac{Fa}{\epsilon_r \epsilon_0} \sum_i z_i c_i \quad (1)$$

Here, E , ρ , ϵ_r , ϵ_0 , Fa , z_i , and c_i are the variables representing the electric potential, electric charge density, relative permittivity, vacuum permittivity, Faraday constant, valence of ionic species, and concentration of the i^{th} ionic species, respectively.

Then, interactions of ionic concentration and electric potential are solved by the governing PNP equation (i.e., Poisson-Nernst-Planck):

$$\nabla \cdot \left(-D_i \nabla c_i - z_i \frac{D_i}{RT} Fa \nabla E \right) = -\vec{u} \cdot \nabla c_i \quad (2)$$

Here, D_i , c_i , R , T , $\vec{u}$ are the variables representing the diffusivity, concentration of the i^{th} ionic species, gas constant, absolute temperature, and velocity vector, respectively. Also, the equality $D_i = RT$ refers mobility.

The solution to Equation (2) is obtained by considering the boundary condition applied to each wall, where $\mathbf{n}$ represents the normal vector to the surface:

$$\mathbf{n} \cdot \left(-D_i \nabla c_i - z_i \frac{D_i}{RT} Fa \nabla E \right) = 0 \quad (3)$$

The Debye length is defined as

$$\lambda = \left(\frac{\epsilon_r \epsilon_0 k_B T}{2 c_0 F a} \right)^{1/2} \quad (4)$$

Here, k_B is referring the Boltzmann constant.

Finally, the Navier-Stokes, Poisson and Nernst-Planck equations are solved concurrently and incorporate the pressure difference and electro-osmotic flow at the pore surface.

The Navier-Stokes equation is:

$$\eta \nabla^2 \vec{u} - \nabla p - Fa \sum_i z_i c_i \nabla E = 0 \quad (5)$$

Here, η and p are the variables representing the deionized water viscosity and fluid pressure, respectively.

Three main equations given above solved using FEM and COMSOL offer valuable insights into the transport of ions and molecules through nanopores. This allows to understand and predict the behavior of charged particles in a wide range of applications critical to the development of sensitive and accurate nanopore sensing.

CHAPTER 2

2. Role of Nanopore Geometry, Particle Size and Trajectory on Particle Detection and Discrimination

Nanopore signal detection is an emerging technology that stands out for its potential in single particle characterization (i.e., detection, analysis, and separation) of biomolecules or synthetic particles. This electric potential-assisted technique generates resistive pulse signals that can accurately identify individual molecules of various types without labeling. As mentioned in Chapter 1, many parameters affect sensitivity during detection, such as the salt concentration of the medium, applied potential, surface charges, etc., one of the most important of which is the pore geometry. Pore geometry has been relatively little investigated, although it has a significant impact on the signals generated. In this chapter, the nanopore geometry effect on resistance pulses is discussed extensively to address this gap in the literature to some extent.

In the present numerical study, to investigate the geometry effects, three different nanopore geometries, i.e., conical, cigar, and hourglass-shaped, are considered. By running simulations, these pore geometries are compared under previously reported considerations in which both decreasing and increasing pulses occur in the conical pore (116).

The particle to be detected is assumed to be spherical and the pulse magnitudes are analyzed as a function of particle size and charge variations.

The pore geometries are also compared for two different transition axes, one where the particles pass through the pore symmetry axis, the other for an off-axis track, i.e., a path near the pore surface.

The findings show a direct relationship between geometry and pore sensitivity. It is suggested that changing the pore shape from conical to hourglass or cigar-shape can increase the system sensitivity. The signal shape analysis shows that under the same conditions, unlike the other two geometries, the hourglass pore generates only decreased current signal, namely resistive pulses. When compared under these conditions, i.e., without the presence of a conductive signal, the hourglass pore is not much affected by changes in particle charge and particle trajectory. These results suggest that the hourglass pore may provide optimal effectiveness in separation based on particle size. However, it is already known that conductive pulses, especially particle charge-induced, can be crucial for particle detection and separation. Therefore, when signals in the presence of conductive pulses are evaluated

in charged particle detection, the conductive signal may enable the cigar pore to outperform other geometries.

2.1. Introduction

Solid-state nanopores, also called synthetic pores, are sensors that allow production with the desired features such as size, geometry, and charge. They are also preferable to biological nanopores for RPS due to their stable characteristics (117)(44).

Advances in technology are facilitating the engineering of synthetic nanopores for different application areas for different purposes, such as ion gating (118), energy conversion (119), water desalination (120) and particle detection (121).

Different chemical etching methods allow solid-state nanopores to be produced in the desired geometry (122). Cylindrical (123), hourglass (124), cigar (125) and conical (126) geometries are widely used models. In addition, shapes such as bullets (127) and asymmetric hourglass (128) can be obtained as different configurations by adjusting the existing geometries. The cylindrical shape is the most common and most studied pore model in the literature, while conic geometry is also rapidly becoming widespread.

That being said, studies on the effect of pore geometry on particle sensing and detection are not sufficient to fill the gap in the field and more research is needed.

Studies have demonstrated that conical nanopores exhibit superior DNA sequencing (20), and ion gating (129) performance compared to cylindrical pores because of their enhanced detection capacity. Pore geometry is expected to affect both ion and electric field distributions (44)(127)(130). Furthermore, the pulse magnitude is also expected to increase as the gap between the molecule and the constriction region (tip) of the nanopore decreases. When the target particle is charged, electrostatic interactions between particle-pore surfaces, which will be distributed according to the pore geometry, significantly affect the signal generation. Besides the particle-pore radius ratio, the pore length and taper (cone) angle are also important features anticipated to impact the signal shapes. The taper angle can be defined as the diameter ratios of the narrow and wide regions. According to our earlier experimental study, a higher taper angle can alleviate the negative impact of the electro-osmotic flow by facilitating particle motion. As a result, this creates an expanded capture region (44) that can be attributed to the intense field-effect near the narrow region.

Inspired by the experimental works implementing the track-etched PET membranes (117)(44), the significance of geometry on signal responses is examined through the finite element method. Hence, this chapter deals with the comparative evaluation of resistive-pulse

signals for the selected geometries (Figure 7). Three pore geometries are selected to span most of the practical configurations. The variations in the order of resistive and conductive signal magnitudes are discussed from the point of separation of different sized particles. The resistive-pulse signals of these shapes are compared in order to identify an optimum pore shape for a particular detection or discrimination task. The particle to be analyzed is considered as a smooth spherical shape.

The structure of this chapter is summarized as follows; in Section 2.2 method and simulation details are given. In Section 2.3 the generation of pulse shapes and the variation in signal magnitudes are investigated in consideration of different pore geometries and particle sizes. Considering the influence of geometry on ion distribution, the electric field variation during particle passage and its dependence on geometry is also discussed. In Section 2.4 particle charge effects on signal magnitudes are examined. In Section 2.5, the translocation axes are discussed as particle trajectory along the nanopore and the effects on the signal magnitudes are compared for three pore geometries.

2.2. Method and Simulation

Three selected pore features, conical (Figure 7a), cigar (Figure 7b) and hourglass (Figure 7c), are simulated through the COMSOL *Multiphysics* (131) to investigate the configuration effect on signal pulse generation and nanopore particle detection ability. Size parameters are chosen the same for each pore. Each has an identical tip diameter (430 nm), base diameter (7.27 μm) and pore length (20 μm).

Numerical simulations' results are valid for the current case models shown in Figure 7. Model adjustments are detailed in Appendix Figure A.1.

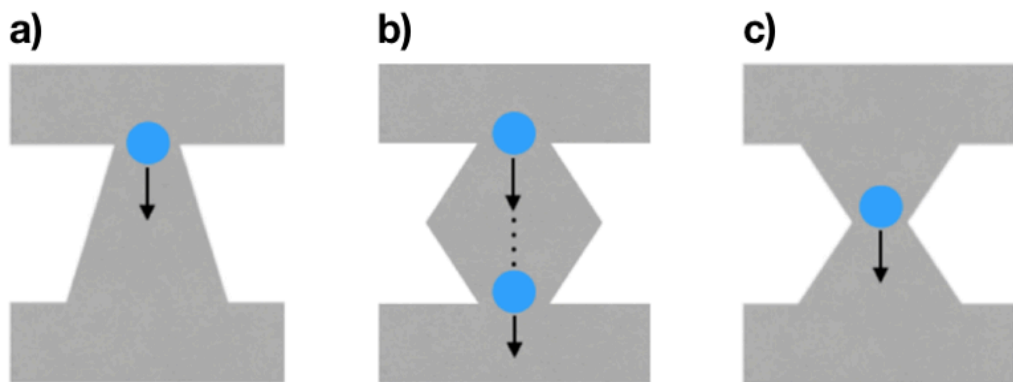


Figure 7. COMSOL nanopore models. (a) Conic shape, (b) Cigar shape, (c) Hourglass shape.

Simulations are performed by applying the finite element method. The solutions and analyses of the models are obtained using *COMSOL Multiphysics v.4.4* with MUMPS (multifrontal massively parallel sparse) direct solver. Positions of particles are executed with parametric sweep mode. Particles are displaced from the upper region to the lower reservoir and translocated in tip-to-base direction. Spherical particles are placed at the center of the symmetry axis in all cases. Exceptionally, they are placed at different locations in simulations to measure trajectory effects. An extremely fine mesh is implemented through a pore model using 161,221 mesh elements. Free triangular elements in the mesh are distributed between 0.4 nm and 134 nm for the minimum and maximum elements, respectively. Additionally, verification of the independence of the results with respect to mesh resolution is also given in Appendix Figure A.4 and Figure A.5. (More detailed simulation conditions can be found in ref. (132) and Appendix A.)

Nanopore models can be described as a pore embedded in a membrane placed in the middle of two reservoirs. Under a voltage of 0.4 V, 4 mmHg pressure is applied in 10 mM KCl solution. Pore surface charge density is assumed to be -5 mC/m^2 .

The system settings are tuned to confirm the formation of biphasic (i.e., contains both up and down signals) wave shapes (133)(116)(134) and comparisons are made based on these values.

Nanopore features are assessed by scaling the pulse magnitudes ($\Delta I/I_0$), i.e., the average signal magnitude. ΔI implies the pulse magnitude is obtained by subtracting the amount of current drop from the base current I_0 .

Simulations are done based on two different dimension models, namely axially symmetric and 2D.

Initially, the geometry is used axisymmetric to measure the discrimination and sensitivity capabilities of the pores. Hence, the system fields, i.e., flow and electric fields, and ionic concentration distribution are obtained in the half-plane.

In the second case to determine current responses to abaxial, i.e., off-axis, positions, a two-dimensional model is constructed. During this analysis, the particle size is varied while the gap between the particle and pore wall is kept constant. At this stage inertia is not included in the system, meaning that hydrodynamic effects are excluded in the trajectory estimation. All variables used in the model are remained identical and are given in Appendix Figure A.1 and Appendix Table A.1-A.2.

Nanopore geometry effect on signal generation are examined through system dynamics generated by the translocated particle. Particles of different sizes are selected according to

the ratio between tip-particle diameters (133)(135)(136). To investigate the nanopores' discrimination potential to the size resolution the transition of particles is generated with 120 - 260 - 360 nm diameters, corresponding to small - medium - large size. Furthermore, to determine the pore sensitivity, a group of particles having diameters of 240 - 260 - 280 nm are considered.

Furthermore, simulation models are confirmed through validation with previously reported cases, and the outcomes are presented in Appendix A.3.

It is important to acknowledge that the simulations are performed without considering the effect of time (run time-independently). The current-position and current-time curves' amplitudes are equivalent, simulation based on time makes no difference. Moreover, the simulations assume stationary particle displacements. Also, the particle moves incrementally moving along the z-axis within the pore.

2.3. Results

2.3.1. Comparison of Nanopores Capability for Discriminating Particle Size Variation

Here to investigate the dependence of the nanopore separation capability on the pore shape, the signals generated by the selected pore geometries for different sized particles are analyzed. The geometrical characteristics, including the pore long, tip and base diameters, and taper (cone) angle of the nanopores, remain the same across the three different geometries.

Obtained open-pore, namely baseline, current differences indicate the geometry effect on the ionic resistance. Three nanopores are produced the following baseline currents; 5.95 nA obtained from the conic pore, 4.64 nA obtained from the cigar pore, and 6.62 nA obtained from the hourglass pore. Referring to the cigar shape's baseline current value, 4.64 nA, it can be suggested that a geometry with two narrow regions (tips) gives rise to higher resistance to ionic flow.

Having said that, while investigating the influence of geometry on nanopore response signals, it is also observed signal wave shapes are significantly distinctive to pore shape, Figure 8. As it is known, resistive pulse signals can be in different forms such as resistive, conductive and biphasic (133)(116). It has been shown that conical geometry can produce a biphasic signal which consists of conductive and resistive peaks, corresponding to the surface charge and size of the particle.

In Figure 8a it is shown the obtained signal shapes agree with previous reports (133)(116). Examination of the counter ion distribution during particle movement in the conical pore reveals an increased concentration of ions around the tip corner as the particle is closer to the capture zone. An increase of ions is generated around the tip corner as the counter ions surrounding the particle migrate with the particle into the pore. This is associated with the formation of the conductive pulse that forms immediately before the particle gets into the pore. The results reveal that the concentration of oppositely charged ions tends to decrease in the vicinity of the tip corner and the signal converts to a resistive pulse. In other words, and to put it briefly, biphasic signals are derived from the inherently dissimilar ionic accumulation/discharge equilibrium in each bottom of the conical nanopores.

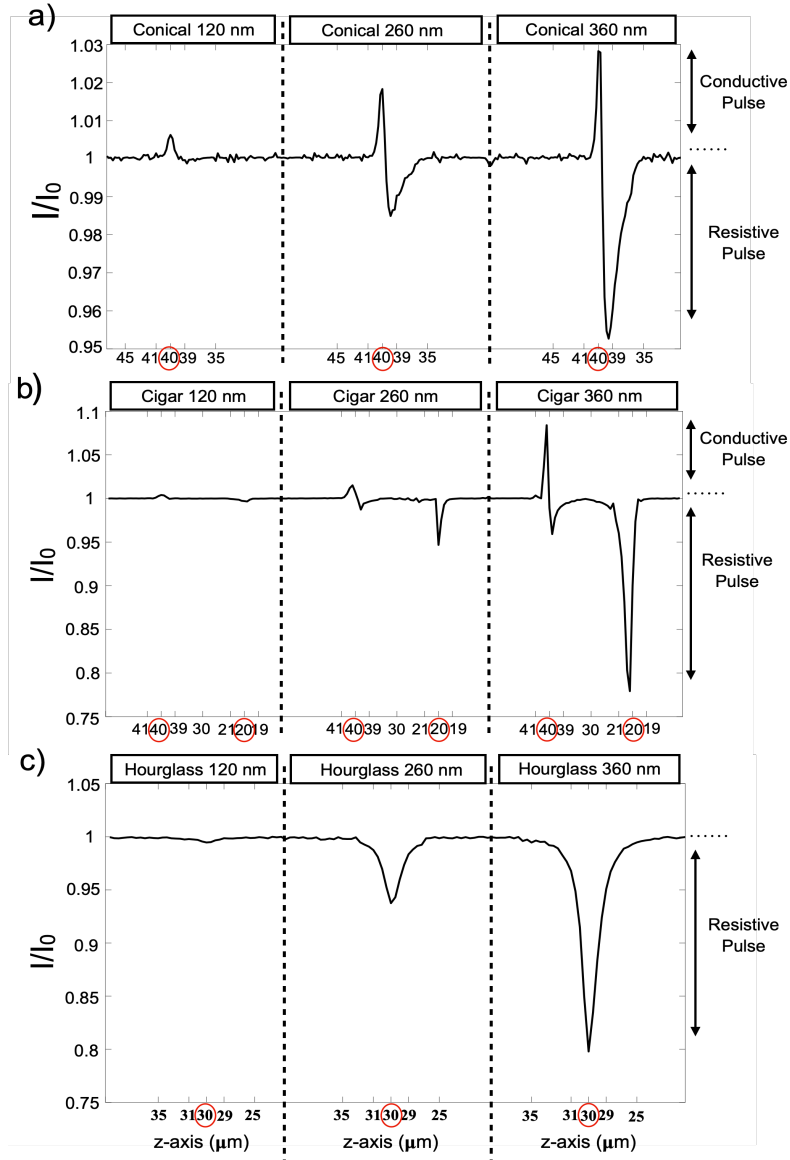


Figure 8. Current pulse shapes of **(a)** conical pore, **(b)** cigar pore, **(c)** hourglass pore geometries for the 120 - 260 - 360 nm diameter spherical particles. Surface charge densities: -5 and -15 mC/m² for pore and for particle, respectively. Magnitudes are normalized to the corresponding open pore current (I_0) to facilitate pore comparison. The vertical arrows indicate the magnitudes of the pulses ($\Delta I/I_0$). The red circles represent the particle positions at the sensing region.

In contrast to the conical pore, the cigar geometry, with both bottoms consisting of a narrow zone, generates two consecutive signals as shown in Figure 8b. The results show that a biphasic signal at the upper tip and then a resistive pulse at the lower tip appears. Analysis of the counterion distribution during translocation reveals the correlation of the conical shaped ionic distribution at the upper constriction of the nanopores and biphasic signal formation. Aside from the shapes, the order of resistive signal magnitudes generated from the upper narrow region is smaller than the downward side.

Figure 8c demonstrates that the hourglass pore's recognition zone is in the nanopore center. Therefore, it can be interpreted that the desired ion transfer for the conductive signal cannot be developed. Numerical models demonstrate that the concentration of oppositely charged ions is particularly high around the tip corner associated with strong electric field. As the particle moves toward the tip, no ionic upward trend is observed surrounding the tip corner and this is linked to the lack of conductive pulses. Accordingly, only the resistive pulse signal is observed.

Considering that conductive pulses can contribute notable data about particle and system dynamics, the amount of changes in current are also examined by taking into account both resistive and conductive signal magnitudes for the responsive signal calculation.

Frequency, magnitude, and duration are the fundamental factors used to characterize RPS pulses. Having said that, to interpret the geometry dependence of nanopore selectivity potential, the yielded signal responses are evaluated using the ratio of resistive pulse to base current, $\Delta I/I_0$.

Here in Figure 9, the discrimination potential of the modeled geometries, i.e., conical, cigar, and hourglass, is discussed by evaluating the signals, given in Figure 8, generated during the passage of particles of different sizes (120 nm-260 nm-360 nm).

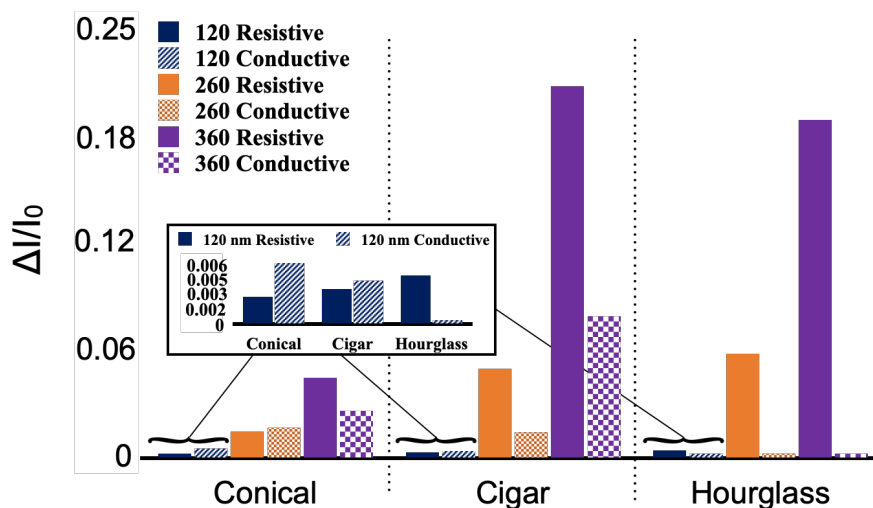


Figure 9. Normalized resistive and conductive magnitudes of 120 - 260 - 360 nm spherical particles. Inner framework depicts the $\Delta I/I_0$ values of 120 nm particle.

Detailed data of $\Delta I/I_0$ for the selected pore models and particles are shown in Appendix Table A.3. From the findings it appears that the hourglass and cigar pores produce deeper signals relative to the conic shape as the particle diameter becomes relatively large. Since the nanopore narrow tip region of each geometry is assumed to be 430 nm, the ratios of relatively large particles (260 - 360 nm) to the pore tip diameter are 0.60 and 0.84, respectively.

When the particle size effect is evaluated by taking the particle surface charge into account, it is found that the cigar pore shows the best performance in the passage of large particles of 360 nm with high surface charge of -15 mC/m^2 (both resistive and conductive parts are included). Hence, it can be suggested that the transformation from a conic shape to an hourglass or cigar, i.e., concave, or convex geometry, can significantly affect the signal magnitudes for particle detection and separation.

It is mentioned above that pulses with upward trend can carry significant data for the identification and characterization of molecules. Considering this statement, when the signals are analyzed by evaluating only the conductive pulses, i.e., upward, parts of the signals, the signal magnitudes of the conical pore are distinguishable for the particles of 120 - 260 nm diameters than from the cigar shape. That having been said, in other surface charge cases, the conical pore shows worse detection capability for large particle where cigar pore outperformed it. This will be addressed in the following part.

When $\Delta I/I_0$ ratio is evaluated to compare the nanopore geometries with respect to the discrimination of particles of different sizes, the signal differences for cigar pores the cigar pore that exceed the geometry of an hourglass. As displayed in Figure 9 and Appendix Table A.3, it can obviously generalize that a cigar pore has the most efficient separation capability for different sized particles within all three pores. Alongside of cigar pore the hourglass pore also provides good separation. The reason why it lags behind the cigar pore can be attributed to the fact that it produces only a resistant pulse. On the other hand, the conical pore generates large conductive pulses but shows smaller signal variation for different size particle transitions.

In addition to the particle separation measurement, a sensitivity assessment for the three modelled pores is also performed and for this purpose three comparable size particles are selected, i.e., 240 - 260 - 280 nm, and the results are given in Figure 10.

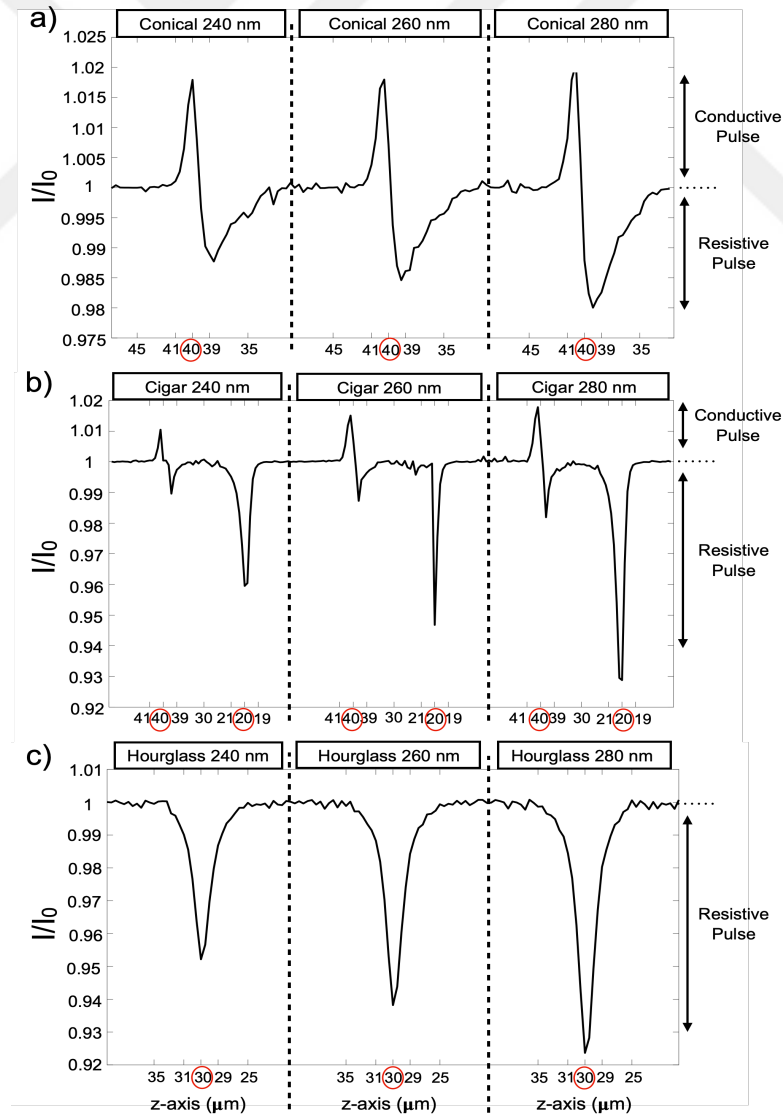


Figure 10. Current pulse shapes of (a) conical shape, (b) cigar shape and (c) hourglass shape for particles of 240 – 260 - 280 nm diameters. Surface charge densities: -5 and -15 mC/m² for pore and particle, respectively. The red circles represent the particle positions at the sensing region.

At first stage sensitivity is discussed by taking only the resistive pulses into account. For this approach, $\Delta I/I_0$ data demonstrates the hourglass pores provide the strongest signals. To evaluate the cigar pore performance, both resistive and conductive pulses are counted in which states cigar pore gives the better responses. The $\Delta I/I_0$ differences of 240 - 260 nm particles are obtained as 0.0032 nA from the conical, 0.0174 nA from the cigar, and 0.0140 nA from the hourglass pore, respectively. On the other hand, differences of 260 - 280 nm are determined as 0.0072 nA from the conical, 0.0207 nA from the cigar, and 0.0145 nA from the hourglass pore, respectively.

It can be clearly concluded from Appendix Table A.4 hourglass and cigar shape pores have very close discrimination precision, namely sensitivity. According to this perspective, results indicate that the cigar pore provides higher precision to close size particle transition than the hourglass pore, followed by the conical pore.

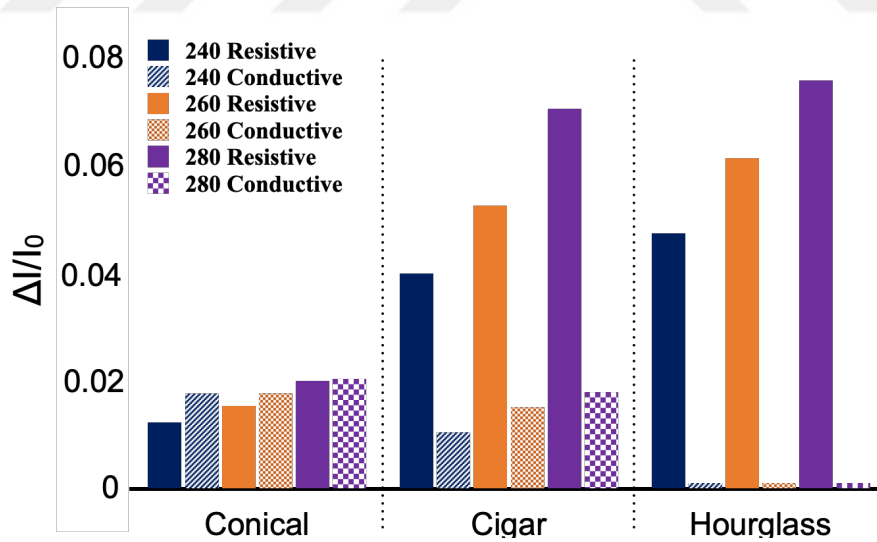


Figure 11. Normalized currents values of downward (resistive) and upward (conductive) pulses, $\Delta I/I_0$, for 240 - 260 nm - 280 nm particles.

2.3.2. Evaluation of Ionic Distributions and Electric Field Strengths

When the particles and pore walls are in charged state, ion dispersion leads to a distortion of the signal waveform from resistive to biphasic pulses (116)(137) where geometry has also inducing effects. Here, the investigation is focuses on the role of pore geometry on the distribution of ions and strength of the electric field without changing the simulation settings. Particle size is kept at 360 nm in diameter and -15 mC/m^2 is set for its surface charge. -5 mC/m^2 is specified for the pore walls.

In this section, pore geometries are compared with respect to the distribution of counterions during particle movement. Considering the migration of a particle through a conical pore ionic enhancement is identified around the edge of the narrow region when the particle is accessed to the recognition zone of the conical pore. Counterions dragged by the particle are moved into the pore and cause ionic accumulation at the pore edge. This observation also explains the formation of the conductive pulse.

According to the simulation images, as the particle continues to move into the pore, the enhanced ion effect disappears and is replaced by a decreasing signal, i.e., the volume exclusion effect.

Due to the similar geometry, the upper tip of the cigar pore produces similar signal formation to the conical pore. However, as described in the above section, the hourglass geometry does not show an increasing signal. Therefore, the ionic enhancement that occurs during particle migration in the other two geometries does not occur here.

As with the ion distribution, pore parameters such as taper (cone) angles, pore long, wide and narrow regions' diameters are kept the same for electric field comparisons.

The electric field strength achieves its peak value at the tip edges of all three pores (Figure 12). Value of $\sim 7.6 \times 10^6 \text{ V/m}$ is observed from the conical pore and $\sim 6.4 \times 10^6 \text{ V/m}$ from the hourglass pore. Cigar shape pore yields two successive electric field measurements as $\sim 9.2 \times 10^6 \text{ V/m}$ from the upper and $\sim 7.9 \times 10^6 \text{ V/m}$ from the bottom tip. Clearly the cigar pore has larger field strength than conical and hourglass. It can also be deduced from the contour lines confirming the result that the highest field effect is higher in the tip zone of the cigar shape than in the hourglass and conical pores. Field strengths at a distance 30 nm from the narrow edge are measured as 3.4×10^6 for conical pore, 3.0×10^6 for hourglass. The cigar pore gives the value of 2.7×10^6 for the upper tip and $2.8 \times 10^6 \text{ V/m}$ for the lower tip, respectively.

As the electric field intensity expands deeper towards the reservoir, the charged particle is drawn towards the nanopore electrophoretically, resulting in a larger capture zone. Comparing the capture region surrounding the upper halves of the cigar and conic-shaped nanopores, it is observed that the conical nanopore has a larger capture zone than the cigar nanopore.

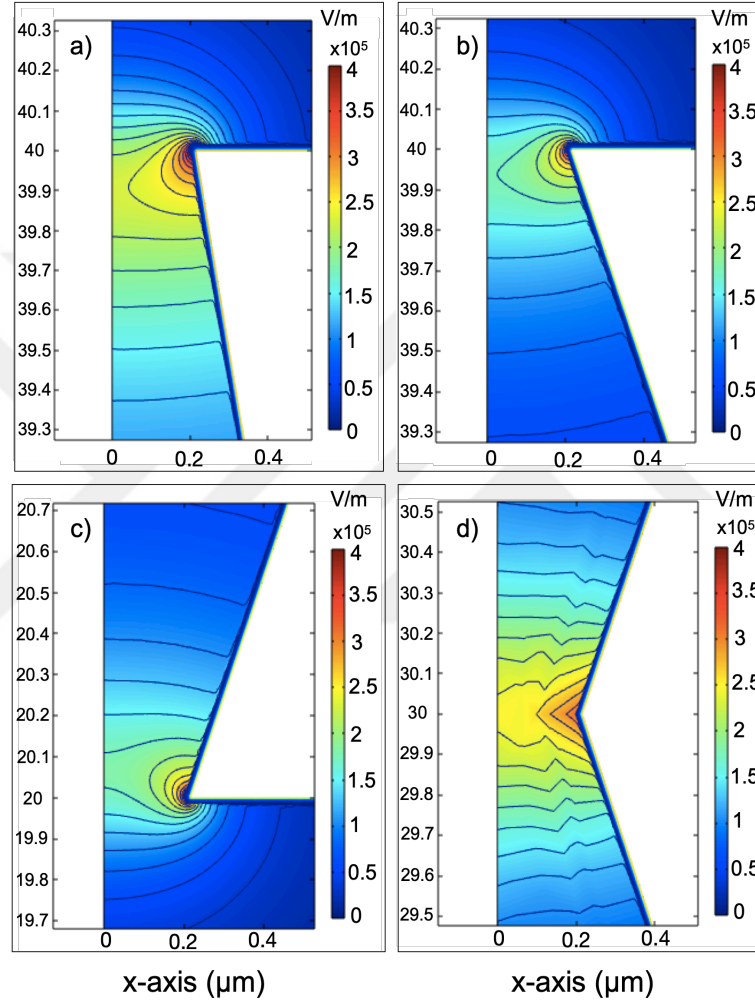


Figure 12. Demonstration of electric field strengths. Values are measured around the narrow region of the **a)** conical pore **b)** upper tip of the cigar pore **c)** bottom tip of the cigar pore **d)** hourglass pore.

2.3.3. Comparison of Nanopores Capability for Discriminating Particle Size Change with Charge Variation

Particle charge is one of the effective factors that can change the resistive pulse magnitude. This relation has been shown for cylindrical pores (138). Having said that, especially when conductive signal forms are considered, charge of particle can lead to the particle being

mistakenly over- or under-sized. Therefore, the size resolution of particles analyzed by means of pulse magnitudes can be weakened by their electrical charge.

In this section, in the existence of different sized particles, the sensitivity of different pore shapes to particle charge alteration is investigated. The analysis is performed for particles of 360 - 260 - 120 nm in diameter with the adjusted surface charge densities, -15, -7, -1 mC/m², respectively. The nanopore surface charge density is chosen as -5 mC/m².

Ionic charge effects carried by the particle to the nanopore detection output are displayed in Figures 13-15 and detailed are given in Appendix Tables A.5-7.

The effect of charge density on the signal intensifies with increasing particle size. As particle size decreases, the sensitivity to particle charge variation decreases.

For the conical pore, biphasic signal generation occurs during translocation of highly charged (-15 mC/m²) particles, regardless of particle size. If the charge decreases towards -1 mC/m² only resistance pulses are generated.

As mentioned in the previous section, conductive signals may carry important information about the particle. It is therefore worth noting that for surface charge analysis, biphasic signals can be used for the detection of higher surface charge in the conical nanopore. It is already mentioned above that the conical pore's and the upper tip of the cigar pore's recognition regions have similar features. Therefore, these results also apply to the cigar's upper sensing zone. But there is only a different situation for the cigar pore. Although the upper tip resembles a conical pore, no such signal shape is exhibited, and the large particle produces a biphasic signal in all charge states.

As aforementioned, hourglass pore yields only resistive pulses and no change in signal shape is observed in any state change.

It is also found that in the conical pore the signal magnitude decreases as the particle charge increases (except for 120 nm particle) while the opposite is the case for the cigar and hourglass nanopores.

Evaluation of particle charge effects in terms of pulse magnitude, hourglass pore provides better signal response except for the large particle with high surface charge, i.e., 360 nm and -15 mC/m². On the other hand, in cigar pore, enhanced conductive pulses are observed with the increased particle charge. In addition, this pore shape yields the best charge sensing ability for large and medium sized particles. The weakest responds are found for the conical pore. In addition, conductive pulses are becoming more dominant with the increased particle charge than the resistive signal.

On the other hand, particle charge can lead to an overestimation of size during the discrimination analyzes. As emphasized above, while conductive pulses can provide worthful data for charge determination of large and medium-sized particles, such as in a cigar pore, they are only useful for detection enhancement involving small particles. Furthermore, the sensitivity of hourglass nanopores to different particle loading conditions increases with decreasing particle size. Additionally, the hourglass nanopore is found to have relatively high resistance to charge variations, except for small particles, as the amplitudes are less affected.

Data also demonstrate that the cigar pore produces consistently larger pulses than the conical, therefore it can be concluded that the cigar pore outperforms other pores in detection of smaller particle regardless of its charge.

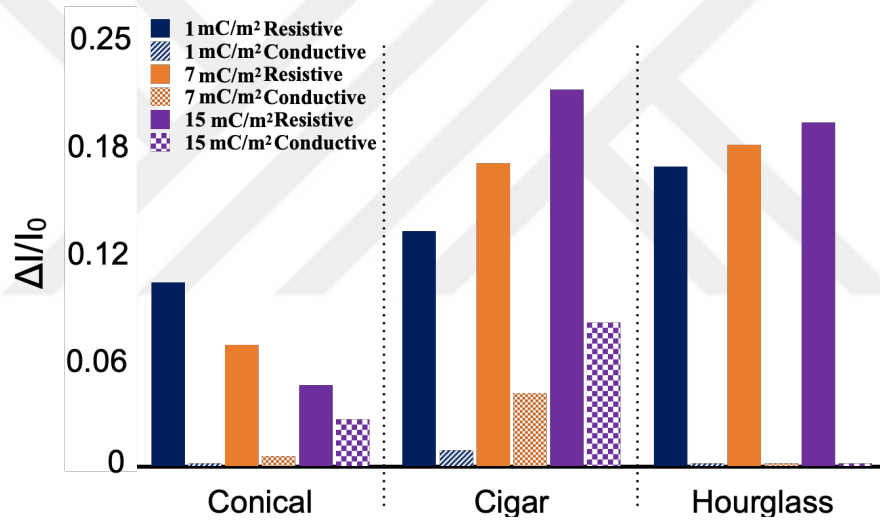


Figure 13. Normalized current values, $\Delta I/I_0$, produced by a large (360 nm) particle. Surface charges: -1, -7, -15 C/m².

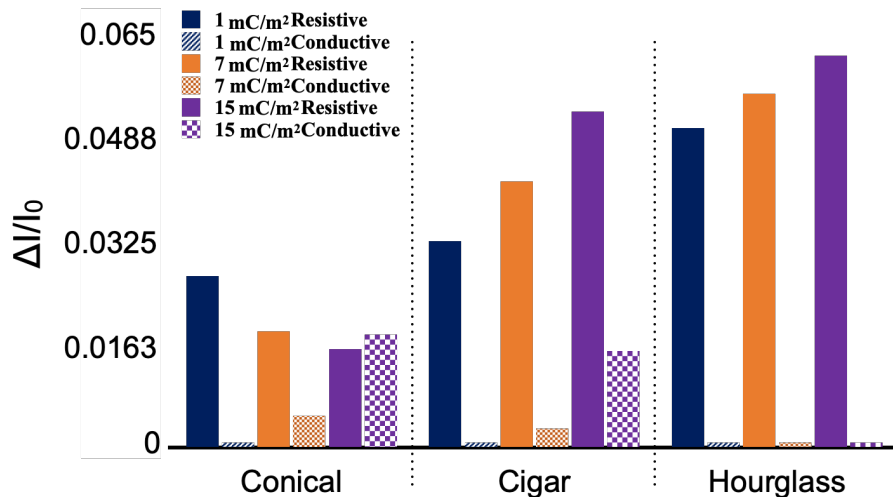


Figure 14. Normalized current values, $\Delta I/I_0$, produced by a medium (260 nm) particle. Surface charges: -1, -7, -15 C/m².

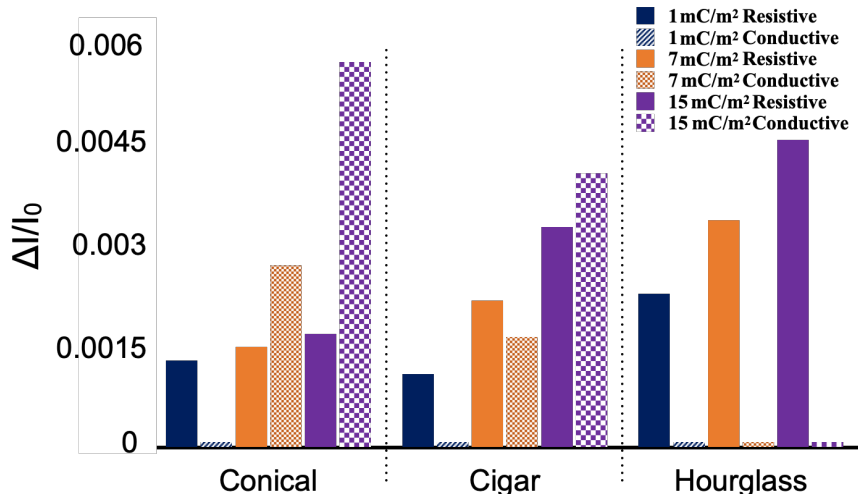


Figure 15. Normalized current values, $\Delta I/I_0$, produced by a small (120 nm) particle. Surface charges: -1, -7, -15 C/m².

2.3.4. Transition Analysis in an Symmetry-Axis vs Off-Axis

In addition to the factors discussed above, another factor affecting the pulse size is the particle transition trajectory. Formerly it is suggested that the signal depth increases with the particle proximity to the pore edge (139). To address this point, in this section simulations are performed using 2D modeling. For the comparison of the signals generated according to the particle passage line along the pore two cases is considered. Particle is positioned at the central axis and 20 nm far from the pore boundary (off-axis).

Off-axis translocations of particles are examined by keeping all boundary conditions and variables same as in Section 2.3.1 and 2.3.2.

As can be observed in Figure 16 a-c, a comparison of the generated signal magnitudes depending on the axis of particle passage reveals that particles do not induce stronger signals as they translocate closer to the pore edge.

When the trajectory dependent comparison is made in terms of geometries, conical pore is identified as the weakest one while the cigar shape is determined as the best sensitive.

On the other hand, it is also found that during the small particle translocation close to the pore wall produces reduced response. Besides, as shown in Figure 17, the trajectory passage close to the wall strengthens the signal pulse generated by the cigar pore. The near-wall passage contributes to a better detection of the small particle for the cigar pore.

In contrast, the sensing capability of conical and hourglass pores for the smallest particle can be reduced by an off-axis trajectory. Figure 17 indicate that passing near the wall of the pore mostly has the effect of increasing the pulse magnitude. However, this amplification is not always directly linked to particle size.

The degree of sliding towards the pore boundary and the degree of fluid obstruction are other significant factors to consider. According to Figure 17, deviation from the trajectory may cause changes in pulse magnitudes. However, in an hourglass pore, it may not be significant to affect resolution sufficiently, but it might not be significant sufficiently to distort the hourglass pore's size resolution capability. According to these results, the trajectory has less effects on the signal formation of the hourglass pore than the cigar pore. This suggests that the former is more robust in terms of particle size resolution.

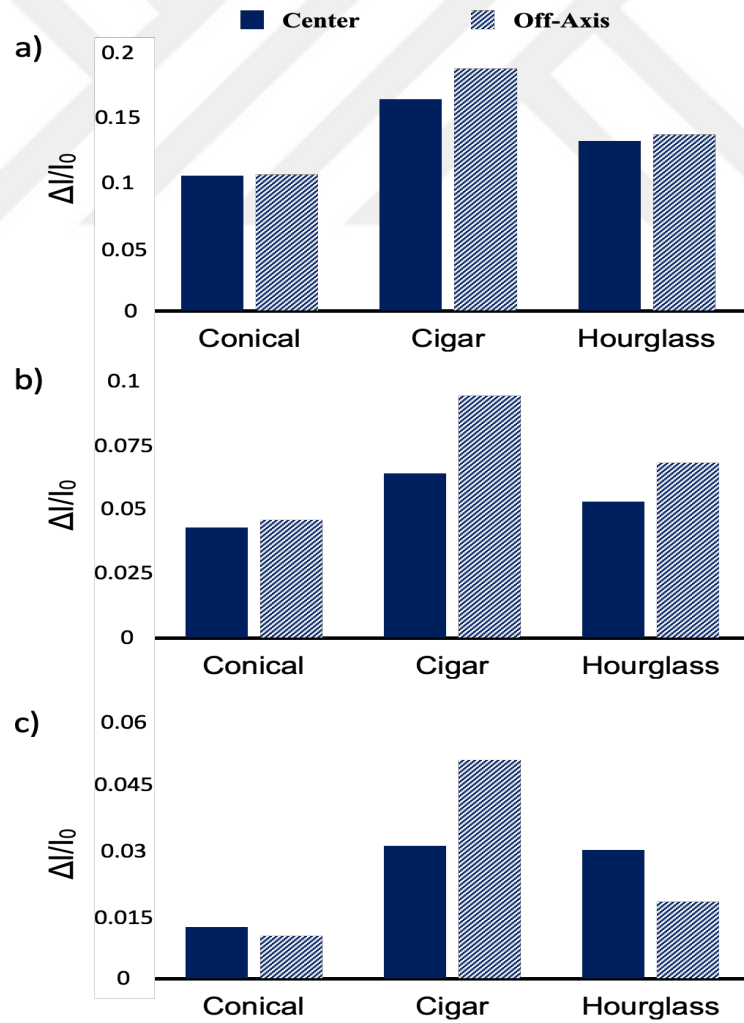


Figure 16. Normalized current values, $\Delta I/I_0$, obtained for a) large (360 nm), b) medium (260 nm) and c) small (120 nm) particles corresponding to trajectory (center axis vs off-axis).

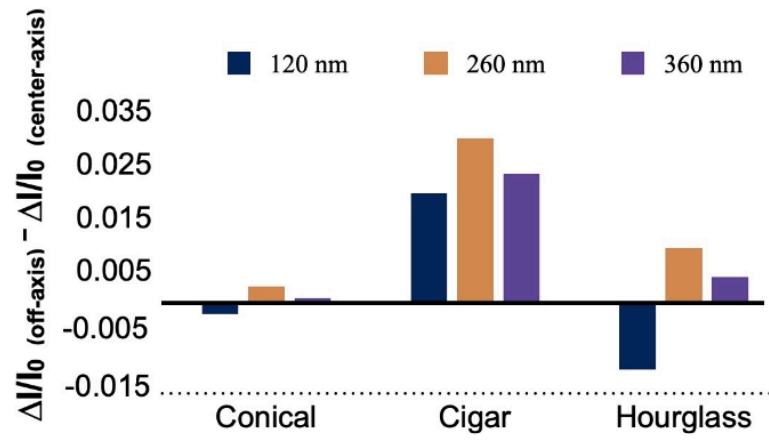


Figure 17. Normalized current differences of center-axis vs off-axis. $\Delta I/I_0$ values are achieved for 120 - 260 - 360 nm diameter particles.

CHAPTER 3

3. Surface Charge Effect on DNA Translocation in Track-Etched Nanopores

In this chapter, a theoretical analysis of DNA molecule displacement is performed. Then, findings are compared with experimental data to comprehend and improve the displacement behaviour of DNA in nanopore-based sensors. First, the conical shaped pore is produced through a track-etched polymer (e.g., PET) membrane. The model is then developed considering the physical features of this fabricated pore. The displacement behavior of a particle (i.e., DNA) and the impact on the current-pulse shape are investigated through simulations, which involved the manipulation of various parameters, such as surface charge densities of the nanopore and particle, particle placement, and potential.

Outcomes revealed that alterations in the charge density of the surface and DNA resulted in conductive distortions, providing evidence for a DNA-surface interaction. Besides, the study verified the charge density variation of DNA and nanopore during translocation, departing from their initial states.

3.1. Introduction

This chapter presented the outcomes of numerical simulations that analyzed the shapes of current pulses concerning surface charge and particle charge, specifically DNA. Additionally, these simulations are compared with the results obtained from experiments (140). Gaining a comprehensive understanding of the changes in local surface charges of nanopores and DNA charge is made possible through an in-depth investigation of the shape of the current pulse. The focus of this chapter is on analyzing alterations in current pulse signals, which are indicative of immediate modifications in both DNA and surface charges.

Since this thesis deals with numerical investigation, not all experimental details are included in this chapter. A part of fundamental experimental details are given in Appendix B. More implementation details can be found in (140).

Concisely, reservoir chambers are typically filled with electrolyte solution. Pore membrane (chemically etched) is placed in the middle of a conductivity cell and divide into two sections. The ds-DNA molecules are put in the cell from the tip. The electrodes used to generate the potential difference are placed in the conductivity cell to be at both sides. Applied potential is selected in the range of -1, +1 V (50 mV). side. Current responses

(events) are quantified by using the Ag/AgCl electrodes. Resistive pulses are realized with nanopores. To determine the tip diameter (d_{tip}) equation (4) is used which gives the resistance. Here, R is the inverse of the current-potential slope (I-V) (117).

$$R = \frac{4\rho I}{\pi d_{tip} d_{base}} \quad (6)$$

Three consecutive sample measurements is taken and utilized for the calculations. The electrolyte conductivity is recorded with a conductivity meter. To specify the signal response, the amplitude (Δi) statement is used. Δi describes the difference from the deepest blocking point to the base current.

3.2. Method and Simulation

To obtain a more precise and measurable understanding of the resistive pulse results from experiments, steady-state finite-element models are conducted. This involves solving the PNP equation implemented in COMSOL Multiphysics v.4.4 software. To conduct the simulations, axially symmetric geometry is used. The pore surface charge density is set to three different values: -25, -50, and -100 mC/m².

Conductive cells are modeled in a cylindrical shape with a thickness of 6 μm and a conical nanotube filled with electrolyte is inserted between the reservoirs (Appendix Figure B.1). Nanopore thickness, tip diameter, and base diameter are kept constant at 12 μm , 20 nm and 520 nm (experimentally observed), respectively, (Appendix Figure B.1). The cylindrically modeled DNA is constructed with a 1.1 nm base radius and 33 nm long (corresponds to 100 bp). Surface charge density of DNA is varied by setting it to -0.11, -0.22, and -0.44 C/m², and it is translocated from up to bottom side. An extremely fine mesh is implemented through a pore model using 865,415 mesh elements. Free triangular elements in the mesh are distributed between 0.1 nm and 0.168 μm for the minimum and maximum elements, respectively. To ensure the accuracy of the simulation, a mesh independence test is conducted and presented in Appendix Figure B.3. The test demonstrated that the deviation from the most refined mesh, which had 2,355,473 elements, is less than 0.5%. Appendix-Table B.1 contains a list of the remaining constants and implemented parameters. More detailed simulation conditions can be found in ref. (140) and Appendix B.)

3.3. Results

3.3.1. Surface Charge Density Effects on Resistive Signal Wave-shapes

A particle that moves through a medium can have two main impacts on the flow of ions. The first effect is commonly referred to as the volume exclusion, which arises from the obstruction of the fluid flow by the moving particle. Ionic current induced reduction caused by this effect is of a mechanical nature and does not rely on the potential (133). The other impact is attributed to the surface charge of the moving particle. This is one of the major functions in defining the ionic distribution close to the pore through electrostatic interactions. This highlights the importance of the particle's surface charge (116). At positive potentials, if a oppositely charged (i.e., negative) particle gets into the pore via a conical pore's tip, the reduction in ions becomes more significant. This leads to a decrease in conductivity and ionic current, ultimately producing resistive signals (133). The resistive current pulse signal's shape and magnitude are influenced by both effects. Previous research has shown that there is a correlation between the signal shape and the particle or pore surface charge. This provides a potential technique for measuring surface charge (116).

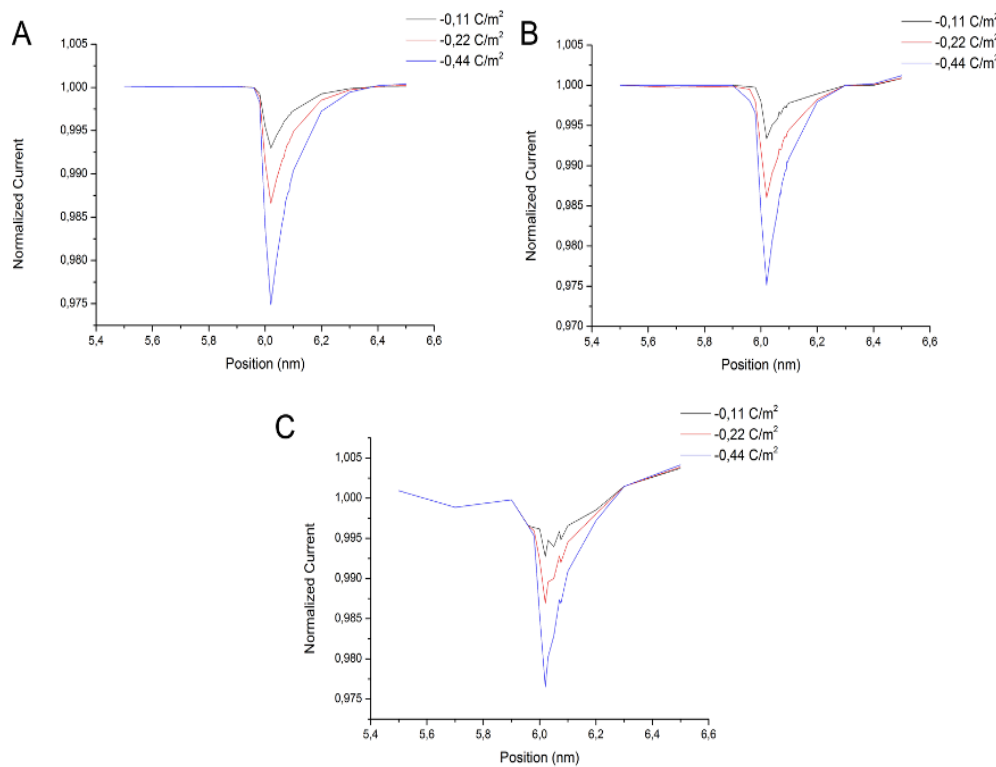


Figure 18. Simulation current responses obtained by the 100 bp DNA for different surface charges (σ_{DNA}) in 1000 mM KCl at 1 V ($\sigma_{\text{pore}} = -25 \text{ mC/m}^2$, (A); -50 mC/m^2 (B); -100 mC/m^2 (C)) $d_{\text{tip}} = 20 \text{ nm}$.

Initially, simulations are conducted for the individual DNA through a conical nanopore. Investigations are focused on the impact of charge density on both the pore (σ_{pore}) and 100-bp DNA (σ_{DNA}). Figure 18 illustrates that when there is a relatively low pore charge (i.e., -0.025 C/m^2) with high DNA charge (i.e., -0.44 C/m^2), the resulting signal exhibits a regular waveform with a sharp tip and does not show a rising current trend indicative of conductivity. Such waveforms have also been reported in previous experimental investigations using track-etched membranes (9)(117). As the charge density of the DNA molecule decreased, the conductive distortions in the current-pulse shape became more pronounced, as depicted in Figure 18. In both Figure 18B and 18C, the current-pulse shape exhibited conductivity even at high σ_{DNA} levels, and this trend persisted even as the σ_{pore} is raised to -0.1 C/m^2 (as shown in Figure 18C). Upon analyzing the peak shapes, various stages of resistance are identified, which are evident from the low Δi values and signal distortions. Across all simulations, the percentage drop in amplitude of the current-pulse signal (Δi) is approximately 2.5% under the 1000mV potential.

The simulation outcomes are compared to experimental findings using an identical size pore. Figure 19 depicts the current-pulse signals acquired experimentally from 100 bp DNA. The observed signals are of two types: blockade with a sharp tip and conductive breakdowns. Figure 19A displays a wave shape corresponding to the low σ_{pore} and high σ_{DNA} states, as seen in Figure 18A. In contrast, in Figure 19B and 19C, the conductive distortions are more evident and indicate a shift in instantaneous σ_{pore} and σ_{DNA} values. The absence of distortion in the spike-shaped current-pulse observed in Figure 19A suggests that the charge density of the DNA molecule is similar to its inherent state (1 e^- charge per phosphate group $\approx -0.44 \text{ C/m}^2$) and there are relatively few counter ions in the solution surrounded it. On the contrary, the presence of distortions in the current-pulse signals depicted in Figures 19B and 19C implies that the DNA charge densities are lower, approximately half of their inherent state (0.5 e^- charge per phosphate group).

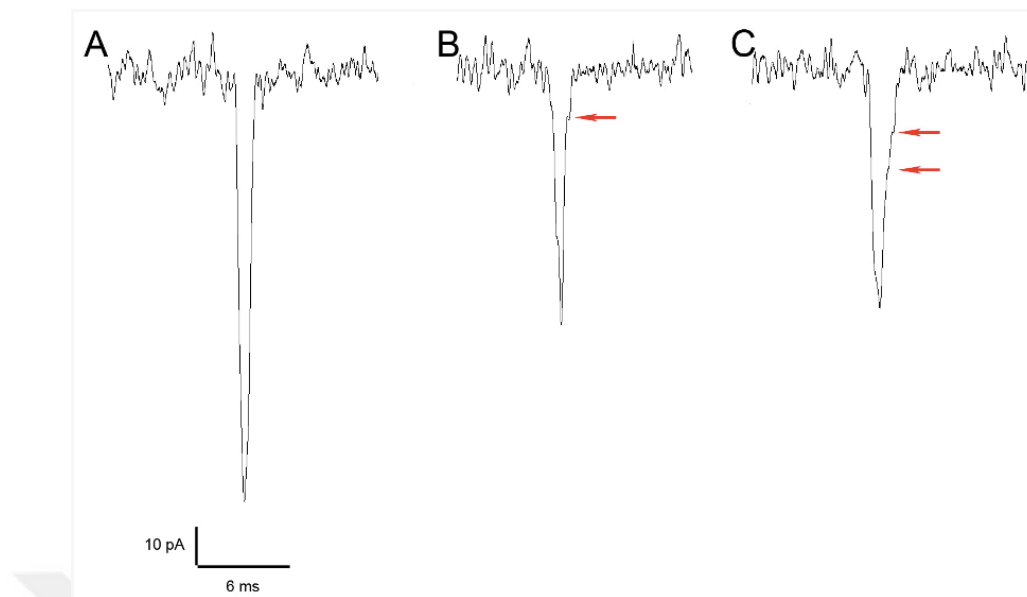


Figure 19. Experimental current responses obtained by the 100 bp DNA in 1000 mM KCl under 1 V potential. $d_{tip} = 20\text{nm}$.

The current-pulse signal may experience changes in conductivity, which can be caused by several factors such as an uneven distribution of particle and pore charges, a non-uniform electric field, and the particles' diffusion (27). There is a contention that the uneven distribution of electrical charge on the surface of nanopores made of Al_2O_3 greatly influenced the speed at which DNA passed through them (141).

Also it is suggested when a double-stranded DNA molecule moves into a nanopore, the ionic flows of potassium (K^+) and chloride (Cl^-) are reduced because of the resistive pulses caused by the volume exclusion effect (142). However, because DNA carries a negative charge on its surface, it will bring additional K^+ ions into the pore, resulting in a lower surface charge density with respect to its original state. The addition of further positive charges is realized as an upward pulse. The signal patterns shown in Figure 19B-C resemble those in Figure 18B, which depicts an increase in σ_{pore} and a decrease in σ_{DNA} . Therefore, by comparing experimental and simulation outcomes, it is suggested that DNA in a solution possess varying charge densities.

3.3.2. Effect of Electric Potential

A second process is done to study the quantitative relevance of the amount of applied voltage and the movement of the 100-bp DNA molecule through a nanopore. As shown in Figure

20A, the findings of the simulation indicated that even at low potentials, the impact of potential is not very noteworthy for low σ_{pore} values. At σ_{pore} values of -0.05 , -0.1 C/m^2 , the conductive impact on the signal became more notable across all potential values. The distortion caused by the alteration in charge distribution is especially noticeable at low potentials and high σ_{pore} . Figure 20 illustrates that the magnitude of the current pulse decreased by almost 2.5% relative to the normalized baseline current.

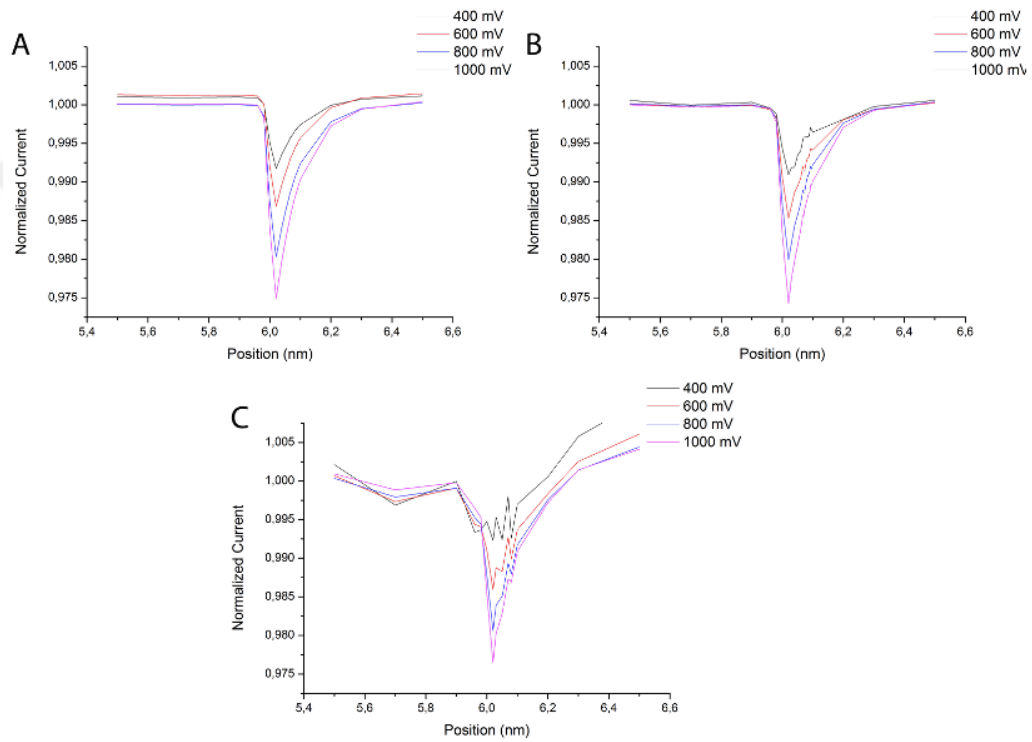


Figure 20. Simulation current response shapes at $\sigma_{\text{DNA}} = -0.44 \text{ C/m}^2$ (100 bp) and $\sigma_{\text{pore}} = -25 \text{ mC/m}^2$ (A), -50 mC/m^2 (B), -100 mC/m^2 (C) (1000 mM KCl).

To demonstrate the experimental-simulation consistency, the signal waves obtained through the experiments are also given as shown in Figure 21.

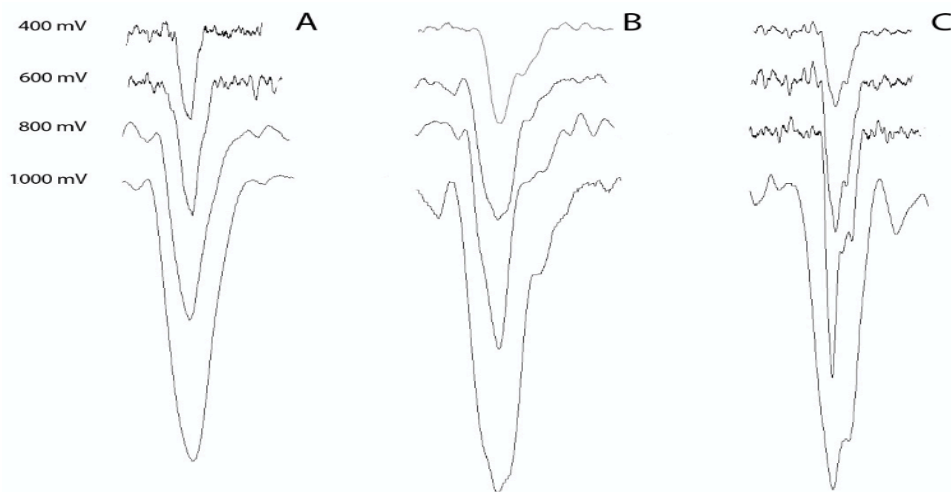


Figure 21. Experimental current responses with different wave shapes corresponding to varied voltages in 1000 mM KCl. $d_{tip} = 20$ nm.

These signals are categorized based on their shapes at different applied potentials in the range of 400 - 1000 mV. Results represent the DNA translocation through a PET membrane. 100-bp DNA is derived under identical experimental conditions. The pulse shapes of the experimental signals produced similar results to those obtained from simulation. There is an increasing trend for all values of current amplitudes at increasing potential.

Figure 21A displayed data that is categorized with the same current-pulse shape as Figure 20A, indicating that these specific current-pulse signals denote the DNA translocation with its original charge. Figure 21B and 21C exhibited signal shapes that contained conductive abnormalities due to an imbalanced distribution of charges on the DNA and pore surface. In Figure 21B, the translocation of the event is captured under two conditions: moderate σ_{pore} (i.e., -0.05 C/m²) and high σ_{pore} (i.e., -0.1 C/m²), which highlighted the increased effect of conductivity on signal shape at higher σ_{pore} values. Additionally, the experimentally obtained current-pulse signals are examined, and comparable signal wave shapes are detected.

The fundamental interaction between the surface and the molecule is another determining factor. According to the results, when subjected to identical experimental conditions, every DNA molecule in the solution exhibited a distinct charge density, which could be attributed to the varying quantity of counter-ions in its vicinity and their uneven distribution in the nanoscale medium. Electrostatic interactions are responsible for the ionic distribution on the particle (DNA) surface. As previously mentioned (133), particle-derived ionic redistribution is primarily reliant on the electrical double-layer associated with the nanopore surface charge, the applied potential, concentration, particularly in conical geometries.

CHAPTER 4

4. Surface Charge Effects on Sensing Features of AeL, α -HL, MspA and CsgG Biological Nanopores Sensors

Biological nanopores, as well as solid-state pores, are high-performance sensors for recognition of various types of biomolecules. Various factors are influential during the detection process, among which pore geometry and surface charges are arguably the most decisive. Surface charge implementation, namely the mutation, is a functional way to enhance these pores. To elucidate how much the mutation affects the pore sensitivity, this numerical work comparatively investigates the sensitivity responses of widely used four protein nanopores (AeL, α -HL, MspA, CsgG) to the different surface charge states. Transpassing biomolecules are modelled as rod-shaped ssDNA, folded-shaped (coiled) ssDNA and protein-shaped. It is found for both the adjusted pH-regulated and site-directed-regulated mutation states that the surface charge is determinant up to a threshold value and thereafter this effect fades away slowly. Under different applied electrical potential (120 mV-180 mV-300 mV) and high salt concentration (1 M), when the pores are ordered according to the surface charge variation effects on their sensitivities, the pores are order as AeL>MspA>CsgG> α -HL in terms of $\Delta I/I$ performance. Furthermore, the comparison suggests that the mutation might be more decisive than the pore geometry on recognition in practical surface charge range, which is between -0.1, +0.1 C/m².

4.1. Introduction

Biological nanopores are the prominent sensor platforms with their precise recognition ability at a single base level (80). Owing to their very narrow structure, they become a good choice to detect and distinguish the target molecule and provide amplified signal (143). Depending on the unique feature of each pore, constriction region length and residue numbers are suggesting as the most decisive arguments that identify the pore ability for the sensing precision (114).

However, some limitations such as rapid passage velocity of DNA or repulsive inner residual charge distribution of channel cause a restrictive effect on resolution of signal magnitudes. It is known that the mutation of biological pores is used to overcome the inherent deficiencies by affecting and improving molecular transition, pore sensitivity and

ionic flux. To enhance the sensing ability and stability, various protein engineering methods such as site-directed mutagenesis (144), add/drop residual amino-acids (145), pore-motor protein integration (146) and adjusting the hydrophilic/hydrophobic properties (147) of pore have been employed. On the other hand, despite the improvements made, the same desired result may not be obtained from every membrane pore. In such circumstances, modeling methods get involved in adjusting the pores to the parameters which can give the desired result in optimizing these parameters.

Even disputable, besides molecular dynamics (MD) methods numerical modeling is a powerful way to understand molecular dynamics, counterion distributions and electrostatic interactions. Biological pores have been investigated by MD studies so far from different aspects such as mutations(101)(148)(149), molecular interactions (150), nucleic acid analyses(87)(151), ionic permeation pathway (152), ionic selectivity (153) and conductance (154). On the other hand, reported numerical modeling studies of protein nanopores, especially handled by COMSOL *Multiphysics*, mostly focuses on the ion transport (155), electrostatic profiles (156) and general nanofluidic properties (157) rather than surface charges effects, for a single protein channel. Having said that, the effect of surface charge/residual charged amino acids seems to be less understood, although it is one of the most determining factors in signal formation and pore sensitivity.

Another shortcoming in the literature is the lack of comparative analysis of the pores with respect to each other. As will be explained in the next section, the commonly used biological pores possesses advantages over each other depending on applied conditions. In literature, the protein pores have been investigated individually (82)(114), such as AeL vs α -HL (158)(159) or MspA vs CsgG (105), in a confined perspective. An MD study compares four pores, same with this work, under different parameters from the potential profile and blocked current aspects for the relatively longer oligonucleotides transition and study on only MspA mutants (46). On the other hand, to the best of our knowledge comparative analysis of different biological pore geometries with different surface charges is seen as incomplete in the literature and such study by COMSOL *Multiphysics* has not been conducted so far.

The present study fills the lack of a comparative numerical study of mutation effects on biological pores. It has under two main sections: comprehensive review and comparative research. We first reviewed extensively literature. Four common protein nanochannel types, AeL, α -HL, MspA and CsgG are discussed with a broad perspective according to recent

research and various usage approaches. Their geometrical and mutation aspects are paid attention.

After the extensive review the research section is given under several headings. In this section, to identify the surface charge effect on the pore structure's translocation control ability, widely used protein pores, namely AeL, α -HL, MspA and CsgG, are compared. Three molecule configurations are modelled, corresponding to the stretched ssDNA, folded (coiled) ssDNA, protein shapes, and their translocation through the simulated pores in different surface charge states under the varied applied potential and high ionic concentration. Since the mutations are expected to apply with small charge steps in practice, the surface charge range is adjusted between the -0.1 , $+0.1$ C/m² with 0.2 increments and it is selected according to the preparatory simulation results that provides the consistent inclination at each case. By slightly altering the charge on the sensing regions, the sensitivity of the pores for pores monitored as if it turned from wild type to mutant or vice versa. To verify the obtained results' accuracy, before performing the simulations, model is validated through the MD study's electrostatic potential profile (46) and the results are given in Appendix C.3. The analysis are conducted under the four parts: rigid (rod) ssDNA translocation relative to uniformly varied pore SCD (pH-regulated charge regulation), rigid (rod) ssDNA translocation relative to residual varied pore SCD (site-directed charge regulation), folded (coiled) ssDNA translocation relative to uniformly varied pore SCD, protein translocation relative to uniformly varied pore SCD.

Biological nanopores are high-performance sensors for recognition of various types of molecules under different conditions. To examine their prominent sensing features in detail, we discuss the structurally and behaviorally most complex biomolecules', namely ssDNA and protein, transition through the mutated protein pores. To perform the numerical simulations, four pores are selected among most studied biological nanopores in the mutant aspects, Aerolysin (AeL), alpha-hemolysin (α -HL), MspA and CsgG. In addition, three different molecule types, rod-like ssDNA, folded (coiled) ssDNA, and protein-like particles, are considered to evaluate the biological nanopores' responses in different charge states according to different setting conditions. Presented molecular particles are patterned on the reported models in the literature (see Methods and Simulations). It must be noted that the present work is one of the first numerical analysis (finite element) methods performed to compare and analyze the recognition ability of the protein pores.

4.2. Method and Simulation

Here, the four modeled biological pores are examined, namely, Aerolysin (AeL), Alpha-Hemolysin (α -HL), porin A from *Mycobacterium smegmatis* (MspA) and bacterial curli specific genes G (CsgG), to evaluate the resultant mutant effects on biological nanochannels' sensing ability (Figure 22).

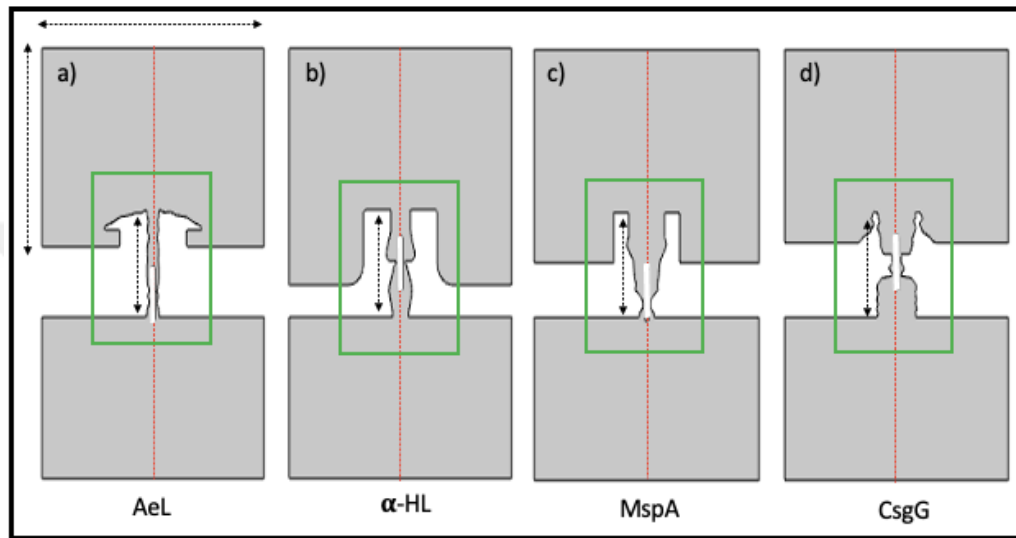


Figure 22. 2D view of modeled four protein nanopore geometries. Figures in green frames display the embedded biological nanopores in membranes **a)** rivet shaped AeL nanochannel, **b)** mushroom-like shaped α -HL nanochannel, **c)** conical shaped MspA nanochannel **d)** hourglass shaped CsgG nanochannel (Green frames are drawn only to highlight the biological nanopores). Parts outside the green frame represent the reservoirs that full with the ionic concentration. Dashed black lines with arrows at both ends indicate the reservoirs and pores' length sizes. Red dashed lines present the axially symmetry of the nanopores. Short DNA fragments are represented as white rods while passing through the constricted points of each nanopore. Although models are shown in 2D here, simulations are performed as axially symmetric (**Appendix Figure C.2-C.6**). For ease of explanation, 3D representations are also given in **Appendix C.1**. Detailed dimensions' parameters are given in **Appendix Table C.4**.

For the blockade signal evaluation two different translocated particle species, namely ssDNA and protein, are considered in three different configurations as rod-shaped ssDNA, folded-shaped (coiled) ssDNA, protein-shaped particle. Each particle is modelled corresponding to the reported cases; rod-shape to represents the stretched phase of ssDNA (88) corresponding to the shallow blockade configuration (87), folded-shape (coiled) ssDNA modeled corresponding to the deep blockade configuration (87) for the folded (coiled) form with two lobules integrated on the rigid DNA's top and middle points, respectively, and

protein-shape modelled as a trinodal to represents the combination of the theoretical PEG model (160), the estimated ellipsoidal (161) and approximated spheroidal (162) shapes (**Fig. 2**). ssDNA is chosen because the protein pores are too narrow for dsDNA to pass through. Therefore, the diameter is valued as 1.1 nm in agreement with literature (163). Also, it is handled as a short oligonucleotide (5 nm in length). While the ssDNA considered as a rod of charge diffusing (104), protein adjusted as a trinodal shaped particle to the maximum size that could pass through the narrowest point of the related pore. Particles translocate from cis to trans side. Signal magnitudes are compared by measuring both ΔI (blocked current magnitude) and $\Delta I/I_0$ (blockade magnitude ratio to the baseline current). ΔI represents the difference between blocked current, I_b , and open pore current, I_0 .

Simulation settings are kept the same for all models. Numerical analyses are examined by the COMSOL *Multiphysics*® v.5.1 (131) programme based on finite element method using the MUMPS with parametric sweep.

Pores are modelled by embedded in reservoirs at cis and trans sides. The medium is filled with the 1000 mM KCl concentration, and no pressure is applied. Applied potential varied as 120 mV, 180 mV and 300 mV (Initially the potential is applied between 80 mV-600 mV but according to the same observed trends, only data obtained from 120 – 180 - 300 mV are shown). In experiments, the mutations are expected to apply with small ranges of values. Therefore, the selected surface charges are kept in narrow. The charge density range is selected between the -0.1 and 0.1 C/m² terminals according to the observed preliminary simulation results that the values show the same increasing /decreasing tendency each time same as the presented figures in this section.

Configuring the ssDNA dynamics is a big challenge due to their very flexible nature. Thus, ssDNA surface charge density is selected same as the dsDNA, -0.136 C/m², for the simulations (164). To the best of our knowledge, since there has not been such a study in the literature before, the selected parameters have been derived from the values in the literature by reaching a common view.

As we know the signal to position tables are agree with the signal to time case, thus simulated models are run in time independent. Translocated particles, namely ssDNA and protein, are passed the pore stationary and stepwise across the z-axis. Signals are obtained from different points according to the location of the constriction regions of each pore. In simulations pores are considered as spatially rigid and particles are always at the central axis (Figure 23). The particle-pore surface interactions are ignored.

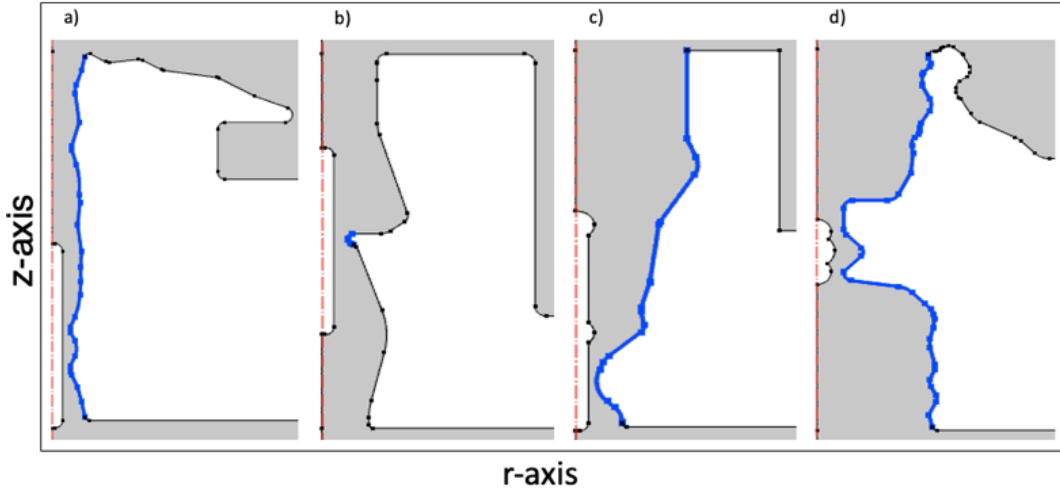


Figure 23. Translocated particle types along different nanopores according to the application of charge density to different pore regions. **a)** rigid (rod) ssDNA in AeL nanochannel with uniformly varying SCD, **b)** rigid (rod) ssDNA in α -HL nanochannel with residual varying SCD, **c)** folded (coiled) ssDNA in MspA nanochannel with uniformly varying SCD, **d)** protein in CsgG nanochannel with uniformly varying SCD. SCD application is performed for each pore both at the constriction (b) and across the pore (a, c, d). Simulation is performed as 2D axially symmetric; therefore, the axes are represented as r-axis and z-axis.

Employed mesh distributions are set to physics-controlled normal mesh to avoid the time consuming. Number of elements for the mesh distributions of AeL, α -HL, MspA and CsgG are 7269, 5435, 4548, 8115 with average element quality 0.907, 0.9077, 0.9085 and 0.9079 respectively. The average growth rate is 1.348 for AeL, 1.351 for α -HL, 1.325 for MspA and 1.346 for CsgG. No slip boundary used, . Detailed presented mesh distribution data and boundary conditions are given in Appendix C, mesh independence test is performed as shown in Appendix C.4. For the most refining mesh the deviation is found less than 0.3% ($\sim 0.27\%$). The element number is found to be 238,287. To verify the our numerical results, model is validated through the MD study's electrostatic potential profile (46). Compatible outcomes are given in Appendix C. Histograms are extracted from the COMSOL data and redrawn by OriginLab graphing and data analyzing software (165).

4.3. Results

It has been already known that the biological pores' sensing ability can be improved by the mutation. Even the pore surface can be differentiated as an anion/cation selective, which is manipulated by various techniques, the mutation application varies between pores to increase the sensitivity. The following sections are aimed to clarify the mutation effects to

the current blockade in a specific charge interval. Furthermore, the effect of particles of different shapes on signal amplitudes is also discussed.

4.3.1. Uniformly Varied Pore SCD relative to the Rigid (rod) ssDNA Translocation

DNA is expected to form two configurations, i.e., stretching and folding (coiling), in its random medium. Rod/stretched DNA is modeled as previously demonstrated in experimental signal detection of stretched ssDNA (88). Additionally, the ssDNA also presented as a charged diffusing rod (104). Therefore, modeling of the ssDNA as a rod is not controversial. In the following two sections, stretched DNA configuration handled as a rod-shape. We should also add that it might be reasonable to simulate the stretched form at first before making some additions to the model particle to get the folded (coiled) form. Besides, the mutation effect is examined by varying the surface charges of the entire pore in an expected small range (-0.1 to 0.1 C/m² with 0.02 increments) under the assumption of experimental applications. This uniformly SCD variation corresponds to pH change of the experimental medium. The mutation effect results also allow us to draw conclusions about the pore geometries.

It is known adjusting the pore's surface charge as anion selective it generates more blockade and longer dwell-time (145). However, our results show that, as can be deduced from the following figures at each section, ionic blockades increase to a certain extent up to a definite surface charge value whereafter the signal magnitude starts to decrease. As it is known, ion exchange, namely ionic accumulation/depletion, is the principal factor in the formation of signal magnitude. Resulting from this, we attributed the signal degradation after a point to the loss of effectiveness of surface charges. We called this resultant case as a threshold surface charge effect.

Within the scope of this study, we consider pores one by one to compare them, as demonstrated in the following figures and tables. The effects of geometry and surface charge are ascertained from the signal shapes, as expected (132). Scaled pulses by base line currents of relative signals provide to classify the modeled pores according to their responses to medium conditions which are, in this study, surface charge density and applied voltage variations. However, it is worth noting that in some cases the shift occurs in the positions where the signal is obtained with the SCD change.

As mentioned above, the threshold value is determined as the turning point of the signal magnitude to an increasing/decreasing tendency. Resulting from this suggestion threshold

charge values are deduced from Figure 24 ($\Delta I/I$) for the rigid (rod) ssDNA translocation as 0.04, 0.02, 0.02, 0.04 C/m² for AeL, α -HL, MspA and CsgG, respectively. Moreover, when the SCD changes from - 0.04 to - 0.02 C/m², a significant increase is seen in blockage, in all pores. Each pore shows an increasing blockage rate towards the positive charge side and reach their maximum blockade amount at the threshold point. Also, as can be interpreted quantitatively from Table 1, all pores generate sufficiently distinct maximum blockage magnitudes. It follows that AeL gives better current magnitude as approximately one and half times higher than to the close value of MspA, two and half times than CsgG and four-times in terms of $\Delta I/I$ under the medium potential. Above findings yield that the AeL outperforms other three pores in line to the previous reports (60)(61) owing to its narrow lumen as mentioned in the above review section.

It must be also note that, although the signal magnitudes start to decrease after a certain point, still the obtained blockade rates are higher than the negative charge side, Figure 24 - Appendix C.13. Considering the -0.1 and 0.1 C/m² terminal charge values, the blocking values for negative charges are around ~0.05 nA, while the lowest current value obtained as ~0.3 nA at positive side of 0.1 C/m², in each case. Therefore, it can be suggested that mutating the AeL, α -HL, MspA and CsgG pores towards more positive charges enhances the blockade magnitudes rather than the negative charges, for the rod-shaped particle translocation. On the other hand, Figure 23 and Appendix Figure C.13. shows higher applied potential enhances the magnitudes. With increasing potential, a linear increase occurs in the blockage of all pores, while relatively high potential (300 mV) creates an approximately 2-times increase in α -HL relative to low potential (120 mV).

In the light of these results, according to ΔI value (Appendix Figure C.13) MspA has the best detection ability in rod/stretched ssDNA transition and the weakest is found to be α -HL. In normalized current value ($\Delta I/I$), as demonstrated in Fig. 3 and Table 1, while AeL is the most sensitive, again α -HL shows the weakest. Thus, depending on the results of this section, pores can be ordered according to their sensitivities as AeL>MspA>CsgG> α -HL. These results confirm the findings, for short oligonucleotides, AeL exhibits higher sensitivity in current and time compared to α -HL and MspA (66). Consistent with the results in the Chapter 2, it can also be deduced from the results that the cigar-like pores (AeL), namely two sensing regions at the two entrances of the pore, can outperform the conic/mushroom-like pores for the short particle detection.

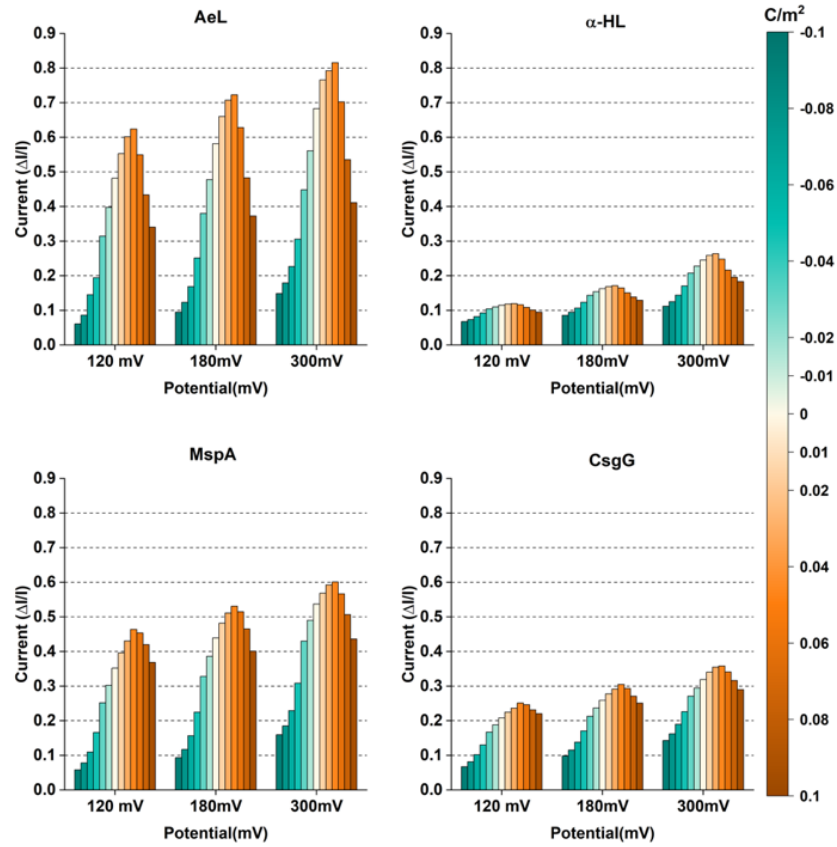


Figure 24. Histogram distribution of Rigid (rod) ssDNA translocation relative to uniformly varied pore SCD **a)** $\Delta I/I$ value of AeL **b)** $\Delta I/I$ value of α -HL **c)** $\Delta I/I$ value of MspA **d)** $\Delta I/I$ value of CsgG. Color map indicates the surface charge density values in accordance with the bars. Labels are corresponded to the bars from left to right.

Table 1 The maximum blocking amounts of pores corresponding to ΔI and $\Delta I/I$ for the rigid (rod) ssDNA translocation relative to uniformly varied pore SCD.

	120 mV		180 mV		300 mV	
	ΔI (nA)	$\Delta I/I$	ΔI (nA)	$\Delta I/I$	ΔI (nA)	$\Delta I/I$
AeL	0.168	0.623	0.299	0.722	0.589	0.816
α -HL	0.076	0.119	0.163	0.171	0.423	0.263
MspA	0.278	0.464	0.487	0.531	0.912	0.601
CsgG	0.181	0.251	0.328	0.305	0.654	0.358

4.3.2. Residual Varied Pore SCD relative to the Rigid (rod) ssDNA Translocation

Apart from the pH regulation mentioned above, another case is engineering mutation, namely site-directed mutagenesis, which is also a widely used method for the molecular

detection to enhance the pore sensing ability (144)(62)(97)(166). In this section engineered mutation state tested by changing the only narrow constriction' s (sensing region) charge density and the rest is kept neutral. The tests are continued to be done with rod/stretched DNA. Potential is applied as 120 mV, 180 mV and 300 mV. SCD at residual regions is varied in the range of -0.1 and 0.1 C/m² with 0.02 increments.

Comparing with the previous section results (Figure24-Appendix C.13), signal magnitudes obtained through the residual charge variation (Figure 25- Appendix C.14) are not higher than the uniformly varied pore SCD. It is seen that responses of pores to the residual SCD variation are not yield a sharp difference between the blockade currents. It may suggest that the site-directed mutagenesis does not boost the ionic blockades. On the other hand, pores yield highly different blockade profiles from each other and other sections. Although the passing particle is the same as in the previous section (rod-shaped), the signal distribution is quite different, indicating the importance of the region where the mutation is applied rather than the translocated particle (will be explained in the following sections). On the other hand, we can still observe the threshold charge effect with small blockade differences, which is the main argument of this study. It is seen that AeL shows linearly increasing profile with increasing potential up to 0.08 C/m² (threshold point) whereafter (at 0.1 C/m²) a relative decreasing starts, as can be identified from Figure 25. According to the maximum blockade rates as shown in Table 2, produced blockade ratios of AeL is nearly one and half times higher than MspA, two and half times than CsgG and 3-times than the α -HL. Besides, the signal profiles of MspA and CsgG show similar trends as in the previous pH regulated SCD variation section and the threshold effect for both occurs at 0.04 C/m². Current magnitudes show a downward trend from these values as consistent with above results while the signal magnitudes decrease with a small difference compared to uniformly SCD variation model. On the other hand, threshold charge effect is more distinct in MspA relative to AeL, α -HL and CsgG meanwhile the threshold effect in CsgG is clearer than the AeL and α -HL.

By contrast, an interesting histogram is obtained from α -HL with an unsimilar blockade distribution to other pores and sections, as can be noticed in Figure 25 with respect to all other histograms. Signal magnitude trends lead a parabolic shape, decrease toward the neutral values then increase toward 0.1 C/m². The threshold SCD value displays as a turning point to increasing direction in contrast to the other pores. It can be said that this behavior of α -HL also confirms the determinative effect of the mutation site on the signal, since the regional application is limited to this part only and this distribution is not observed in other

cases of SCD with uniform variation. Although α -HL has a great potential to be a sensor, it shows a weak performance among four pores. It gives nearly one and half times lower magnitude than the CsgG and nearly 2-times lower than the MspA. On the other hand, if compare the α -HL with itself, it generates a slightly larger current magnitude than the uniformly SCD variation test. As referred at the above review section (83), this result confirms that the mutation improves the sensing ability of the α -HL.

According to these results as can be deduced from the Figure 25 and Table 2, four modeled biological pores' sensitivity ability are found to be sort in the order of AeL>MspA>CsgG> α -HL, for the residual charge variation. Since the simulations are performed under the mutant model unique to this paper, without considering chemical bonds or interactions, order of pore sensitivities according to our results may not show the perfect match with some paper in literature. On the other hand, except for the ordering, it is verified that the sensitivity results are consistent.

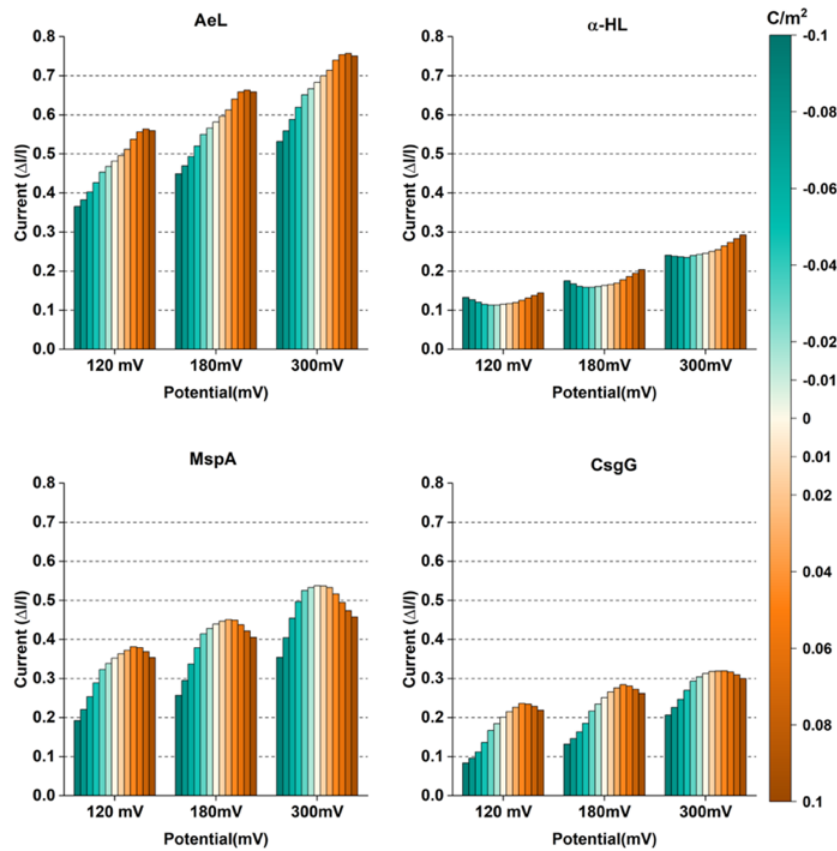


Figure 25. Histogram distribution of Residual Charge (Site Directed Mutagenesis) Effects on the Signal Generation and Detection a) $\Delta I/I$ value of AeL b) $\Delta I/I$ value of α -HL c) $\Delta I/I$ value of

MspA **d)** $\Delta I/I$ value of CsgG. Color map indicates the surface charge density values in accordance with the bars. Labels are corresponded to the bars from left to right.

Table 2 The maximum blocking amounts of pores corresponding to ΔI and $\Delta I/I$ for Rigid (rod) ssDNA translocation relative to residual varied pore SCD.

	120 mV		180 mV		300 mV	
	ΔI (nA)	$\Delta I/I$	ΔI (nA)	$\Delta I/I$	ΔI (nA)	$\Delta I/I$
AeL	0.126	0.563	0.218	0.663	0.406	0.750
α -HL	0.088	0.144	0.184	0.203	0.431	0.292
MspA	0.222	0.381	0.394	0.450	0.757	0.537
CsgG	0.165	0.236	0.292	0.283	0.538	0.319

4.3.3. Uniformly Varied Pore SCD relative to the Folded (coiled) ssDNA Translocation

Besides the stretched form of DNA as we described in the above two sections, folding (coiling) of ssDNA is the other formal phenomenon need to explain. Although the DNA coiling is expected, its effects are not fully known and need to explain. It is known the DNA near the charged decoration is more coiled than normal, thus creating more ion blocking (86). But to elaborate further, physical dynamics in this subject is more complex and computational quantification is a challenging. Even so, translocation of ssDNA cannot be explained with rod/stretched form alone. Therefore, in this section, we continue the simulation analyses with the folded (coiled) configuration of ssDNA.

In this part folded (coiled) ssDNA is simulated with two bumps integrated on the rod DNA. Simulation conditions are kept same as applied above models. To simplify the model physical interactions of residual side chains are neglected. Under these numerical conditions observed results from Figure 26 are not far from the findings as above Figure 24. Although the blocked current histograms exhibit nonuniform distributions, it does not affect the consistency of our results. On the other hand, it seems that the folded (coiled) ssDNA transition (in comparison to the rod-shape as shown previous sections) causes serious deviations on the AeL signals independent of the mutation. But, compared with previous sections, the mutation site appears to be effective rather than the passing particle for the other three pores, as explained at the above section.

Although AeL exhibits a fluctuating signal distribution, as can be noticed from Figure 26, except the signals at -0.1 C/m^2 under all potentials, values are at acceptable ranges. Since

the outcomes obtained from the - 0.1 C/m² charge are not conclusive, these data are not shown (This could be suspected due to the accuracy of the resolution of simulation, but it is rechecked, and the results conclusively show that the excessive blocking signals at the - 0.1 C/m² occurs mesh-independently. Besides, mesh independence test for the general model is given in Appendix C.12). As being said it is seen that there is a serious difference in the signal distribution by changing the shape of the passing particle as demonstrated in Figure 26- Appendix C.15. When the threshold value is examined for AeL, since there are three different threshold charge values observed at each potential, one can infer the potential effect is also increase in parallel with the change of particle shape. Threshold values are change between the 0.02, 0.04, 0.01 C/m² at 120 mV, 180 mV, 300 mV, respectively, while the increasing then decreasing trend can be still observed. Even the AeL has the highest magnitudes, differences with other pores are not too much distinct. As shown in Table 3, AeL only higher 1.17-times, 1.31-times, 1.57-times than the MspA, CsgG and α -HL, respectively.

α -HL signal distribution is also in oscillation form, but the threshold value is same under each potential, in contrast to AeL. In each case maximum blockade current occurs at 0.04 C/m² thereafter signals turn to decrease. Although it produced lower signals, the magnitudes are lower only 1.34-times than MspA and 1.21-times than the CsgG. In contrast to the previous section, although the particle shape changes, the signals show the same distribution as in the first section.

MspA produces same histogram profile as linearly increase then decrease magnitudes which confirms its robust structure to all conditions. Nevertheless, the threshold current is not same in all parameters. It is 0.06 C/m² at 120 mV-180 mV and 0.04 C/m² at the 300 mV. Though, it shows very close sensitivity to the narrowest pore, AeL. CsgG also presents linearly increase then decrease trend except at 300 mV. Though, the SCD threshold effect is easily distinguishable at 0.04 C/m², at all potentials. Hence this pore can also be estimate as a robust structure.

By comparing the Table 1-2-3-4 it is observed each pore generate its highest blockade magnitude at the folded (coiled) ssDNA case. In the light of above findings, it can be concluded as according to the increased blocking levels in all cases, this study confirms that folded (coiled) DNA produced more blockade in consistency with the literature (86). On the other hand, the signal magnitudes differences of each pore are closer than previous sections. This section also shows that while AeL is affected by particle shape, the other three pores are affected by the site where the mutation is applied rather than the particle shape.

In addition, the sensitivity order of the pores does not change and same as previous sections: AeL>MspA>CsgG> α -HL.

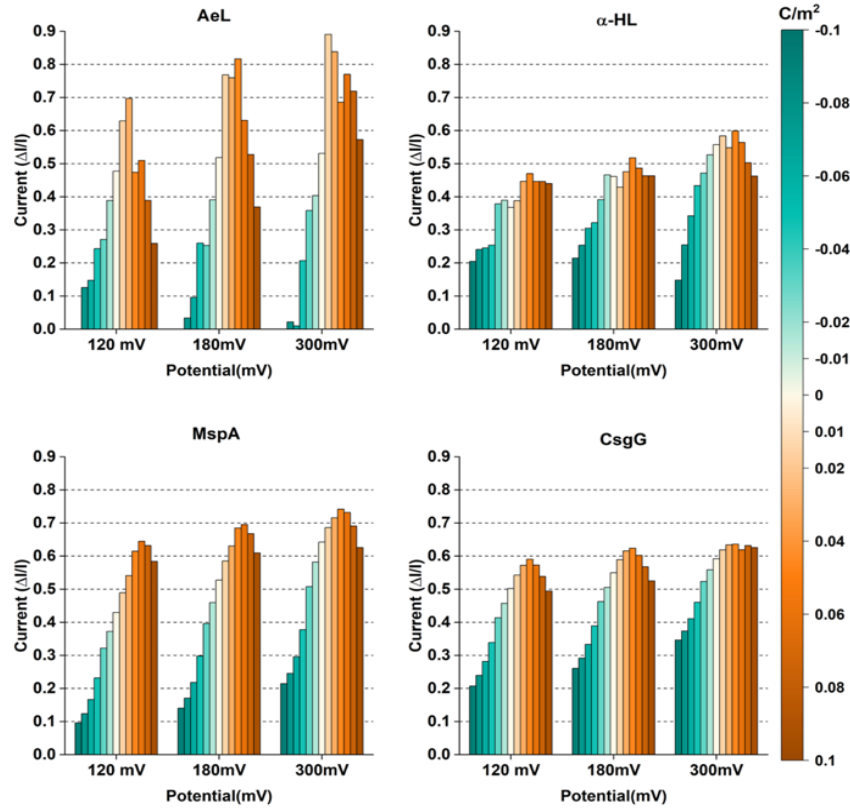


Figure 26. Histogram distribution of Folded (coiled) ssDNA translocation relative uniformly varied pore SCD **a)** $\Delta I/I$ value of AeL **b)** $\Delta I/I$ value of α -HL **c)** $\Delta I/I$ value of MspA **d)** $\Delta I/I$ value of CsgG. Color map indicates the surface charge density values in accordance with the bars. Labels are corresponded to the bars from left to right.

Table 3 The maximum blocking amounts of pores corresponding to ΔI and $\Delta I/I$ for folded (coiled) ssDNA translocation relative to uniformly varied pore SCD.

	120 mV		180 mV		300 mV	
	ΔI (nA)	$\Delta I/I$	ΔI (nA)	$\Delta I/I$	ΔI (nA)	$\Delta I/I$
AeL	0.164	0.697	0.289	0.816	0.552	0.890
α -HL	0.326	0.469	0.537	0.517	1.092	0.599
MspA	0.413	0.644	0.674	0.695	1.189	0.742
CsgG	0.421	0.591	0.675	0.624	1.382	0.636

4.3.4. Uniformly Varied Pore SCD relative to the Protein Translocation

Aside from the above molecular demonstrations two different ssDNA configurations, in this part we are discussing the mutation effect for the protein-like molecular translocation. Protein detection through the nanochannels is another challenge due to their high molecular weight and unpredictable folding structure. Nevertheless, we also performed protein modeling in accordance with the literature to comprehensively consider the effect of particle types on the biological pores' signal generation. In this section protein discrimination response of biologic pores under different charge and potential conditions are represented. To combine the reported theoretical PEG model (160), the estimated ellipsoidal(161) and approximated spheroidal (162) shapes a protein is modeled as a trinodal with a radius of 0.4 nm at the widest point and 1.04 nm long. Simulation conditions are kept same as above.

With the modeled protein translocation protein blocked current magnitude histograms show similar distribution to rod ssDNA's. Each pore, except α -HL, shows highly distinct magnitudes as demonstrated in Figure 27-Appendix C.16. On the other hand, threshold points are not consistent under all potentials. For instance, threshold charge values of AeL occur at 0.02 C/m² under 180 mV-300 mV while it is 0.04 C/m² at 120 mV. Although the blockade currents are close to values produced during the rod DNA translocation through the pore which SCD uniformly varied, there is little to choose between the AeL and MspA, namely 1.07-times, similarly CsgG is 1.94-times lower than AeL. On the other hand, maximum difference occurs between the AeL and α -HL with 6.26-times. Interestingly, in contrast to other three pores, α -HL produces the lowest blockade rates with protein-like shape among three molecule models. Threshold charge density value is 0.04 C/m² for 120 mV and 180 mV while 0.02 C/m² at 300 mV for Δ I, as deduced from the Appendix Figure C.16. The threshold value becomes 0.02 C/m² at 120 mV and 180 mV for Δ I/I while 0.01 C/m² at 300 mV. It has distinct lower signals than other three pores as: 6.26-times lower than AeL, 5.84-times lower than MspA and 3.21-times lower than CsgG. Besides, MspA, as AeL, generates highly selectable distinct signals, as can be inferred from Figure 27. Linearly increase magnitudes turn to decrease a little late than before, from the 0.08 C/m² for Δ I. On the other hand, for Δ I/I, threshold values are 0.06 C/m² at 120 mV-180 mV and 0.04 C/m², as before. MspA produces higher blockade rate for the protein passage than rod ssDNA only as 0.051 amount lower than AeL as although the radius of the protein is smaller. CsgG shows enhanced signal generation for protein rather than rod ssDNA like AeL and MspA. The threshold charge value observed as 0.06 C/m², under all applied potentials. For Δ I/I,

maximum blocked current magnitudes are obtained at 0.04 C/m² for 120 mV-180 mV while it is 0.02 C/m²

For 300 mV. Although it shows the third best precision, it is higher than the lowest sensitive pore α -HL about 0.266.

As a result, in agreement with the above findings from the given rod ssDNA and folded (coiled) ssDNA, the order of pore sensitivity does not change and is ordered as follows; AeL>MspA>CsgG> α -HL which can be observed quantitatively from Table 4. Also, a linear blocking increases towards positive charges, later a relative decrease arises in current from a certain point, consistent with other results.

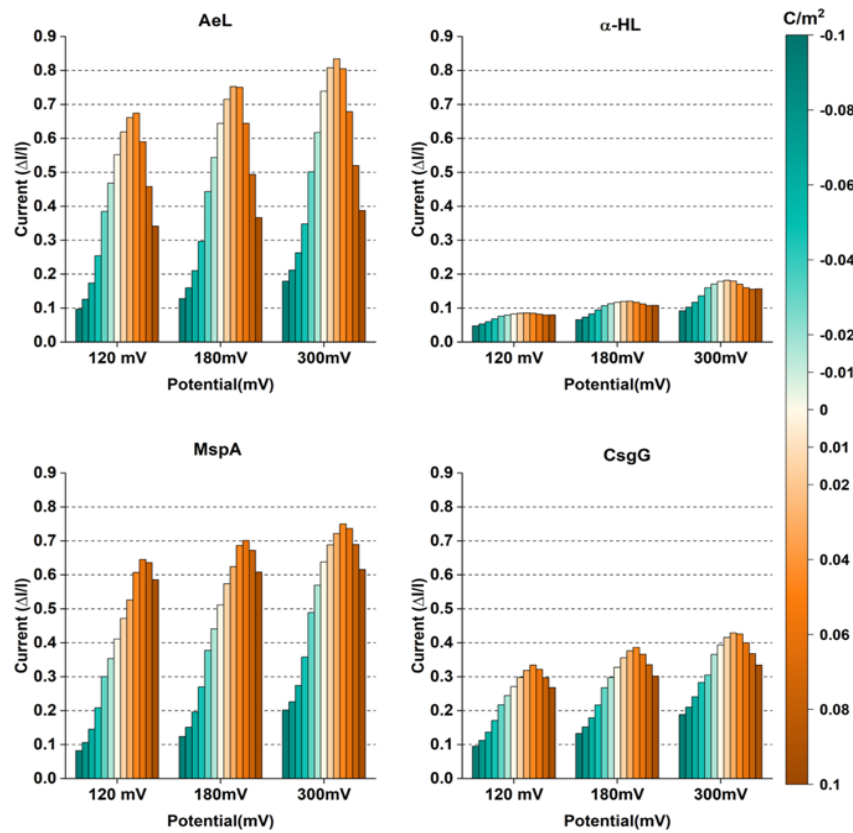


Figure 27. Histogram distribution of protein translocation relative uniformly varied pore SCD **a)** $\Delta I/I$ value of AeL **b)** $\Delta I/I$ value of α -HL **c)** $\Delta I/I$ value of MspA **d)** $\Delta I/I$ value of CsgG. Color map indicates the surface charge density values in accordance with the bars. Labels are corresponded to the bars from left to right.

Table 4 The maximum blocking amounts of pores corresponding to ΔI and $\Delta I/I$ for protein translocation relative to uniformly varied pore SCD.

	120 mV		180 mV		300 mV	
	ΔI (nA)	$\Delta I/I$	ΔI (nA)	$\Delta I/I$	ΔI (nA)	$\Delta I/I$
AeL	0.182	0.674	0.310	0.752	0.581	0.834
α -HL	0.055	0.085	0.115	0.120	0.289	0.181
MspA	0.415	0.645	0.679	0.701	1.187	0.750
CsgG	0.236	0.322	0.410	0.386	0.766	0.429

Table 5. Comparison of threshold surface charge densities of four biological nanopores obtained under different pore surface charge states and different shaped particle translocation.

	AeL	α -HL	MspA	CsgG
Uniformly Varied Pore SCD Rigid (rod) ssDNA	0.04 C/m ²	0.02 C/m ²	0.02 C/m ²	0.04 C/m ²
Residual Varied Pore SCD Rigid (rod) ssDNA	0.08 C/m ²	-0.02 C/m ²	0.04 C/m ²	0.04 C/m ²
Uniformly Varied Pore SCD Folded (coiled) ssDNA	0.02 C/m ² 0.04 C/m ² 0.01 C/m ²	0.04 C/m ²	0.06 C/m ² 0.04 C/m ²	0.04 C/m ²
Uniformly Varied Pore SCD Protein Translocation	0.04 C/m ²	0.02 C/m ² 0.06 C/m ²	0.06 C/m ² 0.02 C/m ²	0.04 C/m ²

The results obtained from different values of the surface charge density and different application sites show that the surface charge value has a decisive influence on the pore sensitivity up to a certain point. The results suggest that the way the mutation is performed

may be more important than the chosen pore geometry. In addition, the findings suggest that smaller diameter pores may be more susceptible depending on the surface charge. In Chapter 2, the best performance is obtained from the cigar pore geometry with two sensing regions compared to the conical and hourglass geometries. In Chapter 4, where biological pores are compared, it is seen that the best performance among the 4 pores is obtained from AeL, which is similar in geometry to the cigar of solid-state pores with two sensing zones at the inlet and outlet. The results obtained from the geometries we have chosen as solid state and naturally occurring biological pore give the same trends shows the consistency of our simulations and measurements. Furthermore, as shown in Figure 28, alpha hemolysin is the weakest pore, showing the same threshold value in only 2 out of 4 different cases. Besides, Although MspA provides the best performance for non-normalized blocking signals (ΔI), it can be interpreted that it does not provide a stable structure within the overall results of this study as it has 3 different threshold values in 4 different cases. On the other hand, CsgG showed the greatest stability by having the same threshold value in 4 different cases. This stability of CsgG, one of the least studied biological pores in the literature, is one of our most important findings in terms of revealing why it is preferred by a commercial company (ONT) in its latest version. This result also shows that our simulations are a powerful tool to determine the performance of a biological pore of unknown structure.

4.3.5. Comparison of Nucleotide Transitions of Different Lengths

In this part, nucleotides of different lengths are analyzed to demonstrate the ability of four biological nanopores to separate particles of different lengths. This comparison can reveal the ability of biological pores to distinguish various types of nucleotides.

The pores in which the separation ability will be evaluated are the same as in this chapter, namely AeL, α -HL, MspA, CsgG. Translocated particle is modeled as ssDNA and are simulated in the lengths between with 1 nm (for comparison; $0.34 \text{ nm} * 3 \text{ nt} = 1.02 \text{ nm*nt}$) to 9 nm (for comparison; $0.34 \text{ nm} * 27 \text{ nt} = 9.18 \text{ nm*nt}$). Applied potential is chosen as 300 mV. Since the surface charges of all pores are naturally different, we assume here that the surface charges are neutralized. All other parameters are kept same as above simulation models.

First, when the signals obtained from particle transitions are analyzed, it is observed that all four pores are able to produce sufficiently discrete signals for particles of all sizes. The

same trend is observed both in axial symmetry (Appendix Figure C.17) and in 2D (Figure 28). Furthermore, it is clearly implicated that the resistive pulses become narrower as the length of the particle becomes shorter. Having said that, conductive pulse formation is also observed in each case. The other point, it can be observed that the peak of the resistive signal shifts towards the pore center with the shortening of the particle length.

For 9 nm particle, full-width half-maximum(fwhm) point is observed at ~ 0.057 nA from the AeL nanopore. As the particle length shortens to 1 nm, the fwhm point shifts to ~ 0.067 nA, indicating that the amount of blockage decreases with shortening length. In addition, blockade signal rate almost same from 9 nm to the 3 nm, a notable decrease is observed at 2 nm and 1 nm particle signals. On the other hand, the decrease of conductive pulses is observed from the 5 nm particle transition.

For α -HL, the fwhm point shifts from ~ 0.205 (for 9 nm) to ~ 0.218 (for 1 nm). Moreover, from the passage of 4 nm particles and smaller than, the signals started to distort, resulting in two-peak waveforms. On the other hand, conductive pulses are decreases gradually as the particle size decreases and the pulse shapes turns into valley shape from the sharp peaks.

The resistive blocking rates from MspA nanopore are not very different for different particle lengths from 9 nm to 1 nm. However, it is also observed that the signal amplitudes narrowed with decreasing particle size (from 9 nm to 1 nm). Up to a particle size of 4 nm the pulses are shaped as an asymmetric triangle, from this size the waveforms change to a regular triangle.

Unlike other pores, maximum conductive pulse value is obtained from 7 nm particle. For lengths from 9 nm to 7 nm there is an increase in the conductive signal and for lengths smaller than 7 nm there is a decrease in the conductive signal. The amount of blocking of resistive signals is approximately the same for lengths up to 1 nm, while the decrease is seen only in the 1 nm long particle.

Fwhm points of CsgG pore is obtained at ~ 0.208 for 9 nm particle while it shifts to 0.217 during the 1 nm particle transition. Deepest point of the blockade signals are almost same for particle 9 nm to 5 nm. For particles 4 nm and smaller, there is less resistance and blockade amounts are reduced. Conductive pulses are same for 9 nm-8 nm particle and started to decrease from 7 nm.

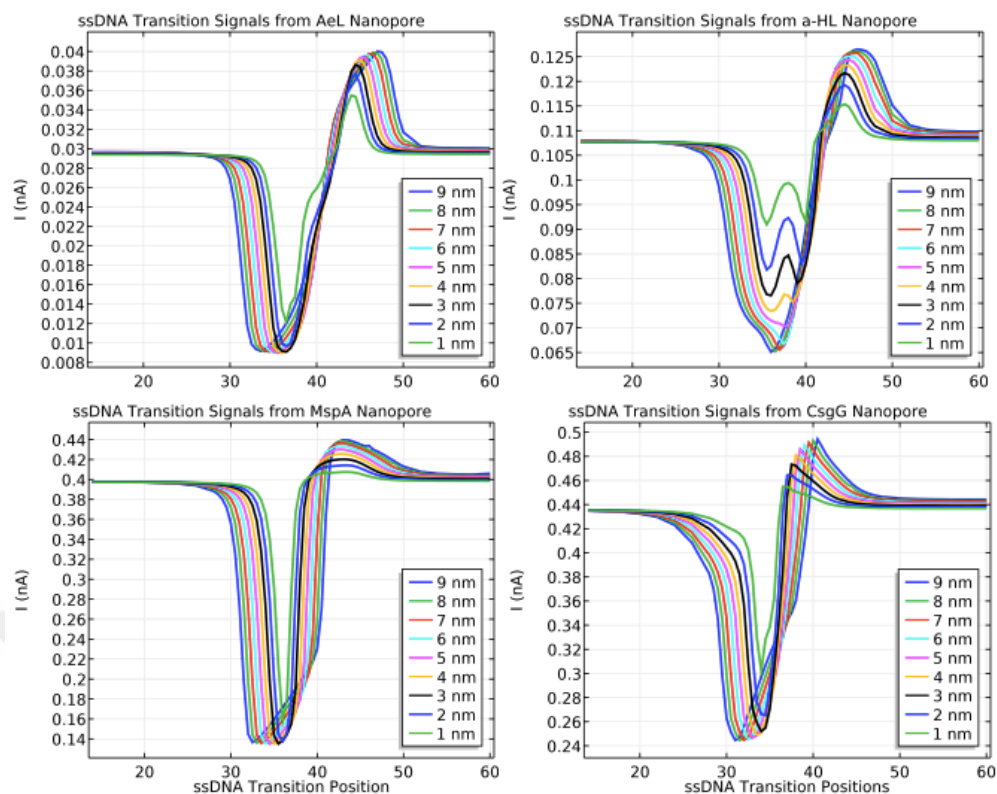


Figure 28. Transition signals of particles with different lengths. Particles lengths are arranged between 9 nm to 1 nm. Signal shapes are generated by 2D model. (Although the trends and blocking ratios are approximately the same with axial symmetry model, the plots of the 2D model are used because it produces a smoother signal.)

Having said that, as shown in Figure 28, the four biological nanopores are evaluated based on their particle discrimination ability for particles with different lengths in terms of axially symmetric geometry. Consistent with the above results, AeL nanopore provides highest blockade rate both for large and smallest particles. Furthermore, in accordance with the discrimination analyzes given in the above section, MspA nanopore has the best differentiation ability with the highest blockade difference between 9 nm and 1 nm lengths. Nevertheless, it can be clearly understood from Figure 29 that each of the four nanopores provides clearly separate signals for each length of the particles.

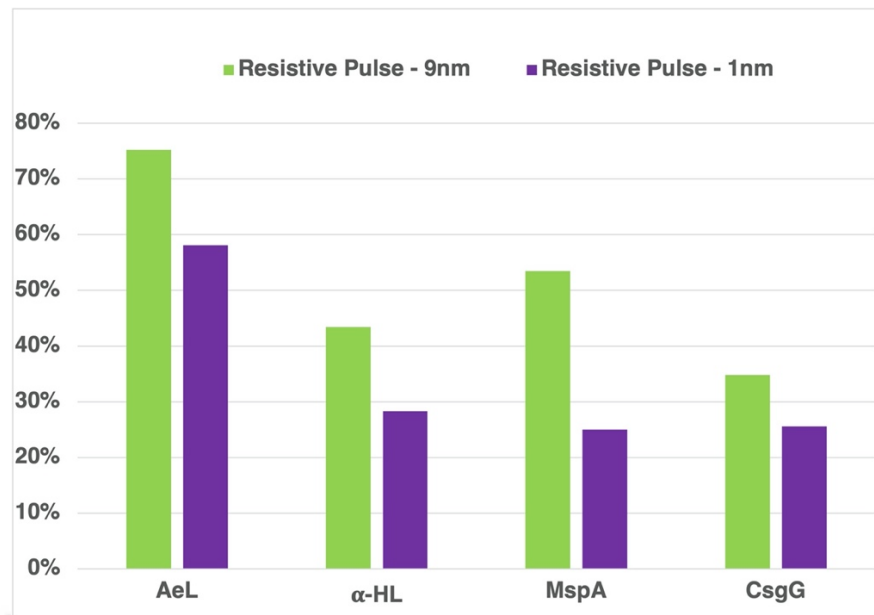


Figure 29. Biological nanopores' discrimination capability for particles with different length. Blockade current percentages are given for 9 nm vs 1 nm particles.

Shorter nucleotides are expected to pass faster with less resistance. This potentially leads to lower accuracy in sequencing. Thus, the results are particularly important in that all four nanopores produce selectable signals, especially for the smallest nanoparticle.

Summary

This thesis mainly presents numerical studies on both solid-state and biological nanopores. After a general introduction to the nanopore literature (Chapter 1), nanopore sensing is discussed in three separate sections for solid state and biological pores.

Chapter 2 describes the nanopore geometry effects on pore selectivity and sensitivity. This section reveals that pore geometry plays a major role in both the waveform and pulse magnitude. Comparison of the three modeled geometries, namely conical, cigar and hourglass, reveals that geometry significantly affects the generation of distinctive resistivity signals, both in terms of magnitude and waveform (i.e., resistive/biphasic). To evaluate the particle size discrimination capability, distinctive sized particles, i.e., of 120 - 260 - 360 nm, are used. On the other hand, similar sized particles are examined for the sensitivity assessment, i.e., of 240 - 260 - 280 nm. Since the particle charge has significant effects on pulse magnitude, responses of the nanopores to changes in the particle charge also were investigated.

According to the simulations presented, it is clearly defined that the signal waveforms depend on the pore geometry. This is evidenced by the generation of biphasic signals from the conical and cigar pores and just blocking signals from the hourglass pore. The results reveal that the signal responses to particle motion depend on certain conditions. These are the sensing zone location, electric field intensity, and gradual changes in ionic density near the tip edge. Besides, findings also suggest that conductive pulses provide valuable information to detect relatively small particles. Moreover, within the selected geometries, an increase in sensitivity and selectivity is observed as the pore geometry distorted from conical to hourglass or cigar shape.

The trajectory of the particles is also studied as another important factor that can affect their resolution in nanopore sensing. It is concluded that although the deviation from the trajectory during the particle passage leads to increased pulse magnitudes in the conical and cigar shapes, the particle size in the hourglass pore may not be large enough to reduce the resolution capacity.

Chapter 3 presents a theoretical investigation of the DNA movement through a conically etched PET nanopore with taking into account different particle charge densities and nanopore surface charge densities in this process. The simulation outcomes are consistent with the data obtained through experimentation carried out under identical conditions. Simulation results revealed that when the pore charge density is low, the generated electric

current pulses are smooth and resemble a sharp spike without current increase. On the other hand, in the case of high surface charges of the pore and DNA, distortions in the signals become more pronounced. This indicates that there is an imbalance in the distribution of electrical charge and particle-pore surface interaction. The results offered a method to comprehend the charge effects on the wave shapes.

Chapter 4 describes a comparative study of biological nanopores (AeL, α -HL, MspA, CsgG) in terms of the surface charge effects on signal pulse magnitudes. The effect of mutations on biological nanopores during particle translocation is evaluated using numerical analysis. To investigate the generated signals during the molecule transition, three molecule types with different shapes, i.e., rod-shaped single-stranded DNA (which represents stretched DNA), folded-shaped (coiled) single-stranded DNA, and protein-shaped particles, are modeled.

The results obtained from different surface charge densities are explained by the fact that the surface charge value has a decisive influence on the pore sensitivity up to a certain point. The results also demonstrate that the way the mutation is made may be more significant than the chosen pore geometry. Additionally, the findings suggest that pores with smaller diameters may be somewhat more responsive, based on the surface charge. This observation could potentially clarify the order of sensitivity of the pores, namely AeL>MspA>CsgG> α -HL which is consistent across all sections of Chapter 4. Furthermore, pore surface charge density has significant impacts on the signal produced by the passing particles, regardless of their shape, until a certain point referred to as the threshold effect. It can be concluded that the surface charge density must reach a specific threshold value to enhance the performance of the pore, as the electrostatic effects are only influential up to this limit. Furthermore, a comparison of the threshold surface charges obtained in the case of four different applied surface charges and displacements of different shaped particles revealed that CsgG, one of the least studied biological pores in the literature, is the most stable structure among all geometries. This result also demonstrates that our simulations are a powerful tool for determining the performance of a biological pore of unknown structure. Moreover, the selected four biological nanopores are also compared in terms of their ability to distinguish particles according to their length. This is crucial for accurate and reliable DNA sequencing for different or similar nucleotide lengths. Furthermore, this offers a method for the detection of structural variations in nucleic acids to develop more sensitive and reliable sequencing technologies. According to the results, all four nanopores generate distinguishable signals, particularly for the smallest nanoparticle. In accordance with the previous findings, for both

large and small particles, the AeL nanopore demonstrates the highest signal magnitudes. Moreover, it is evident that each of the four nanopores generates unique signals for particles of varying lengths.



APPENDIX A

A.1 Boundary Conditions in Simulations

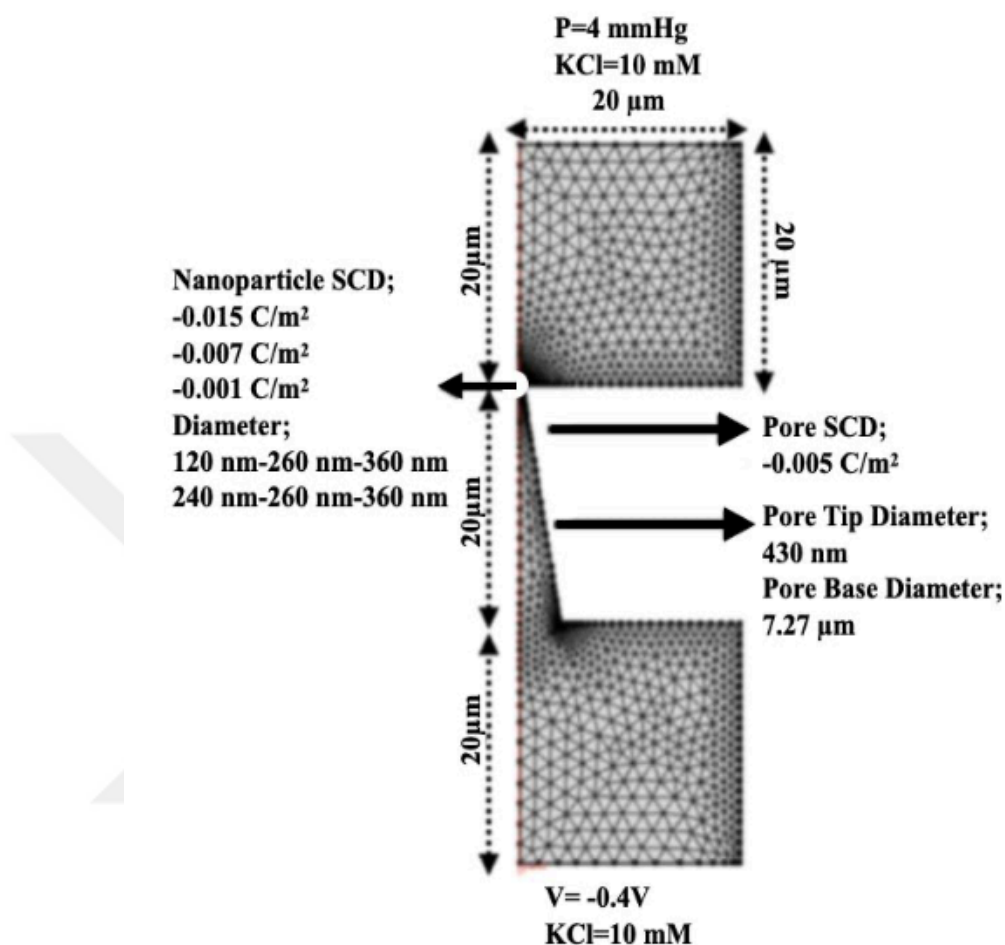


Figure A.1 Simulation boundary conditions. Identical conditions are employed in three pore geometries. (SCD: Surface charge density).

Table A.1 Adjusted simulation parameters.

Concentration	Pressure	Potential	Pore Length
10 mM	4 mmHg	-0.4 V	20 μm
Pore Tip Diameter	Pore Base Diameter	Particle Diameter	Particle Surface Charge Density (SCD)
430 nm	7.27 μm	*120nm-260nm-360nm **240nm-260nm-280nm	-15 mC/m ² -7 mC/m ² -1 mC/m ²

*120 - 260 - 360 nm particles are used to evaluate discrimination capability of the different pore geometries. These particles are also used in the particle charge analysis.

**240 - 260 - 280 nm particles are used to evaluate sensitivity capability of the different pore geometries.

Table A.2 Constant simulation parameters.

ϵ_r (Relative Permittivity)	80
ϵ_0 (Vacuum Permittivity)	8.854×10^{-12} F/m
Fa (Faraday Constant)	96485 C/mol
Diffusion constant of K^+	1.957×10^{-9} m ² /s
Diffusion constant of Cl^-	2.03×10^{-9} m ² /s
c_0 (bulk concentration)	10 mM

A.2 ΔI and $\Delta I/I_0$ Data

A.2.1 ΔI and $\Delta I/I_0$ magnitudes

The pulse magnitude is denoted as ΔI , which is an increase or decrease relative to the open pore current (I_0). $\Delta I/I_0$ values are obtained by scaling the pulse magnitude with the corresponding pore's I_0 . Tables below list the data of bar plots in Figure 9, 11, 13-15. Tables also give the differences between the pulses magnitudes to compare the pores' ability for particle discrimination. Each value in the table is rounded to three significant digits.

Table A.3 Numerical data of Figure 9. Changes in pulse magnitudes, in terms of ΔI and $\Delta I/I_0$, obtained by comparing different-sized particles (120 - 260 - 360 nm). The particle surface charge density is -15 mC/m². Table also gives the differences between pulse magnitudes for increasing particle size in order to analyze the particle size resolution.

Conical	Resistive Pulse		Conductive Pulse		Total	
	ΔI (nA)	$\Delta I/I_0$	ΔI (nA)	$\Delta I/I_0$	ΔI (nA)	$\Delta I/I_0$
120 nm	0.0104	0.00175	0.0343	0.00576	0.0447	0.00750
Difference	0.0821	0.0138	0.0718	0.0120	0.154	0.0259
260 nm	0.0925	0.0155	0.106	0.0178	0.199	0.0334
Difference	0.192	0.0323	0.0593	0.0100	0.251	0.0422
360 nm	0.284	0.0478	0.165	0.0278	0.449	0.0756
Cigar						
120 nm	0.0152	0.00328	0.0192	0.00413	0.0344	0.00740
Difference	0.231	0.0497	0.0515	0.0111	0.282	0.0609
260 nm	0.246	0.0530	0.0707	0.0152	0.317	0.0683
Difference	0.774	0.168	0.319	0.0689	1.09	0.237
360 nm	1.02	0.221	0.390	0.0841	1.41	0.305
Hourglass						
120 nm	0.0306	0.00462	-	-	0.0306	0.00462
Difference	0.378	0.0572	-	-	0.378	0.0572
260 nm	0.409	0.0618	-	-	0.409	0.0618
Difference	0.921	0.139	-	-	0.921	0.139
360 nm	1.33	0.201	-	-	1.33	0.201

Table A.4 Numerical data of Figure 11. Changes in pulse magnitudes, in terms of ΔI and $\Delta I/I_0$, are given for similar-sized particles (240 - 260 - 280 nm).

Conical	Resistive Pulse		Conductive Pulse		Total	
	ΔI (nA)	$\Delta I/I_0$	ΔI (nA)	$\Delta I/I_0$	ΔI (nA)	$\Delta I/I_0$
240 nm	0.0740	0.0124	0.106	0.0178	0.180	0.0302
Difference	0.0185	0.00310	0.0003	0.00005	0.0180	0.00320
260 nm	0.0925	0.0155	0.106	0.0178	0.199	0.0334
Difference	0.0265	0.00460	0.0160	0.0027	0.0420	0.00720
280 nm	0.119	0.0201	0.122	0.0205	0.241	0.0406
Cigar						
240 nm	0.187	0.0403	0.0493	0.0106	0.236	0.0509
Difference	0.0590	0.0127	0.0214	0.00460	0.0810	0.0174
260 nm	0.246	0.0530	0.0707	0.0152	0.317	0.0683
Difference	0.0830	0.0181	0.0127	0.00280	0.0960	0.0207
280 nm	0.329	0.0711	0.0834	0.0180	0.413	0.0890
Hourglass						
240 nm	0.317	0.0478	-	-	0.317	0.0478
Difference	0.0920	0.0140	-	-	0.0920	0.0140
260 nm	0.409	0.0618	-	-	0.409	0.0618
Difference	0.0960	0.0145	-	-	0.0960	0.0145
280 nm	0.505	0.0763	-	-	0.505	0.0763

Table A.5 Numerical data of Figure 13. Changes in pulse magnitudes, in terms of ΔI and $\Delta I/I_0$, are given for 360 nm particle relative to the varied surface charge densities: -15, -7, -1 mC/m².

Conical	Resistive Pulse		Conductive Pulse		Total	
	ΔI (nA)	$\Delta I/I_0$	ΔI (nA)	$\Delta I/I_0$	ΔI (nA)	$\Delta I/I_0$
-1 mC/m ²	0.640	0.108	-	-	0.640	0.108
Difference	0.217	0.0370	-	-	0.217	0.0370
-7 mC/m ²	0.423	0.0710	0.00141	0.000237	0.423	0.0710
Difference	0.139	0.0232	0.164	0.0276	0.0260	0.00440
-15 mC/m ²	0.284	0.0478	0.165	0.0278	0.450	0.0756
Cigar						
-1 mC/m ²	0.639	0.138	0.0428	0.00922	0.682	0.147
Difference	0.184	0.0390	0.156	0.0338	0.338	0.0730
-7 mC/m ²	0.823	0.177	0.199	0.0430	1.02	0.220
Difference	0.197	0.0440	0.191	0.0411	0.390	0.0850
-15 mC/m ²	1.02	0.221	0.390	0.0841	1.41	0.305
Hourglass						
-1 mC/m ²	1.16	0.176	-	-	1.16	0.176
Difference	0.0900	0.0120	-	-	0.0900	0.0120
-7 mC/m ²	1.25	0.188	-	-	1.25	0.188
Difference	0.0800	0.0130	-	-	0.0800	0.0130
-15 mC/m ²	1.33	0.201	-	-	1.33	0.201

Table A.6 Numerical data of Figure 14. Changes in pulse magnitudes, in terms of ΔI and $\Delta I/I_0$, are given for 260 nm particle relative to the varied surface charge densities: -15, -7, -1 mC/m².

Conical	Resistive Pulse		Conductive Pulse		Total	
	ΔI (nA)	$\Delta I/I_0$	ΔI (nA)	$\Delta I/I_0$	ΔI (nA)	$\Delta I/I_0$
-1 mC/m ²	0.161	0.0270	-	-	0.161	0.0270
Difference	0.0520	0.00870	-	-	0.0290	0.0049
-7 mC/m ²	0.109	0.0183	0.0228	0.00383	0.132	0.0221
Difference	0.0165	0.00280	0.0833	0.0140	0.0670	0.0113
-15 mC/m ²	0.0925	0.0155	0.106	0.0178	0.199	0.0334
Cigar						
-1 mC/m ²	0.151	0.0325			0.151	0.0325
Difference	0.0440	0.00940			0.0480	0.0103
-7 mC/m ²	0.195	0.0419	0.00424	0.000914	0.199	0.0428
Difference	0.0510	0.0111	0.0665	0.0143	0.118	0.0255
-15 mC/m ²	0.246	0.0530	0.0707	0.0152	0.317	0.0683
Hourglass						
-1 mC/m ²	0.333	0.0504	-	-	0.333	0.0504
Difference	0.0360	0.00540	-	-	0.0360	0.00540
-7 mC/m ²	0.369	0.0558	-	-	0.369	0.0558
Difference	0.0400	0.00600	-	-	0.0400	0.00600
-15 mC/m ²	0.409	0.0618	-	-	0.409	0.0618

Table A.7 Numerical data of Figure 15. Changes in pulse magnitudes, in terms of ΔI and $\Delta I/I_0$, are given for 120 nm particle relative to the varied surface charge densities :-15, -7, -1 mC/m².

Conical	Resistive Pulse		Conductive Pulse		Total	
	ΔI (nA)	$\Delta I/I_0$	ΔI (nA)	$\Delta I/I_0$	ΔI (nA)	$\Delta I/I_0$
-1 mC/m ²	0.00750	0.00130	-	-	0.00750	0.00130
Difference	0.00140	0.000200	-	-	0.0176	0.00290
-7 mC/m ²	0.00890	0.00150	0.0162	0.00272	0.0251	0.00420
Difference	0.00150	0.000200	0.0181	0.00304	0.0196	0.00330
-15 mC/m ²	0.0104	0.00170	0.0343	0.00576	0.0447	0.00750
Cigar						
-1 mC/m ²	0.00530	0.00110	-	-	0.00530	0.00110
Difference	0.00480	0.00110	-	-	0.0125	0.00270
-7 mC/m ²	0.0101	0.00220	0.00767	0.00165	0.0178	0.00380
Difference	0.00510	0.00110	0.0115	0.00250	0.0166	0.00360
-15 mC/m ²	0.0152	0.00330	0.0192	0.00410	0.0344	0.00740
Hourglass						
-1 mC/m ²	0.0150	0.00230	-	-	0.0150	0.00230
Difference	0.00770	0.00110	-	-	0.00770	0.00110
-7 mC/m ²	0.0227	0.00340	-	-	0.0227	0.00340
Difference	0.00790	0.00120	-	-	0.00790	0.00120
-15 mC/m ²	0.0306	0.00460	-	-	0.0306	0.00460

A.3 Validation of the Axially Symmetric Model

The present study investigates the pulses magnitudes (in upward or inward trend) for particle resolution and examines the transition between wave shapes depending on the pore geometry. Therefore, our simulation model has been validated at the conditions in which biphasic regime (downward and upward signals) is observed experimentally and theoretically by Lan et al. (167) for the conical pore.

Our simulations use the complete model of the Poisson-Nernst-Planck and the Navier-Stokes equations by taking into account the convection effects and the electroosmosis, improving the simulation model in (167). Figure A.2 gives our simulation results, showing a signal wave shape transforms to a biphasic regime around -0.3 V, which is in agreement with the experiments in (167). The voltages are specified as -0.2 V, -0.3 V and -0.4 V. The surface charges are given as -0.015 C/m^2 and -0.005 C/m^2 for particle and pore, respectively. Figure A.3 (a) gives our simulation results in comparison to simulation results in (167) showing same trend. In Figure A.3 (a), the pore SCD is -0.005 C/m^2 , particle SCD is altered between 0.01 and -0.025 C/m^2 . Additionally, in Figure A.3 (b), our modelling approach is validated with the simulation result in Figure 2b of (134).

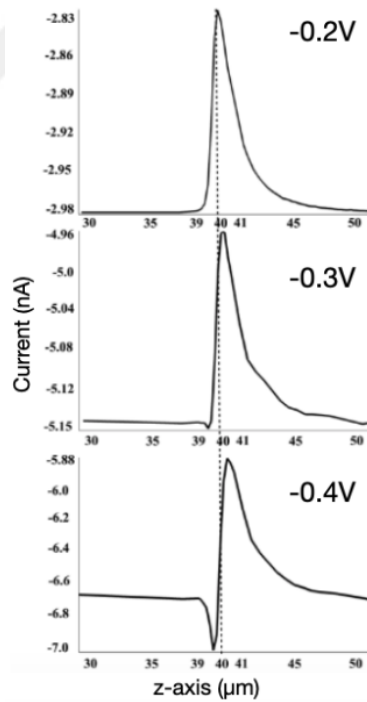


Figure A.2 Signals obtained from our simulations for varying values of the voltage using same parameters at identical conditions of the results in Figure SI2 of Lan et al. (167). The results showing a signal wave shape transforms to a biphasic regime around -0.3 V which is in agreement with the experiments in (167).

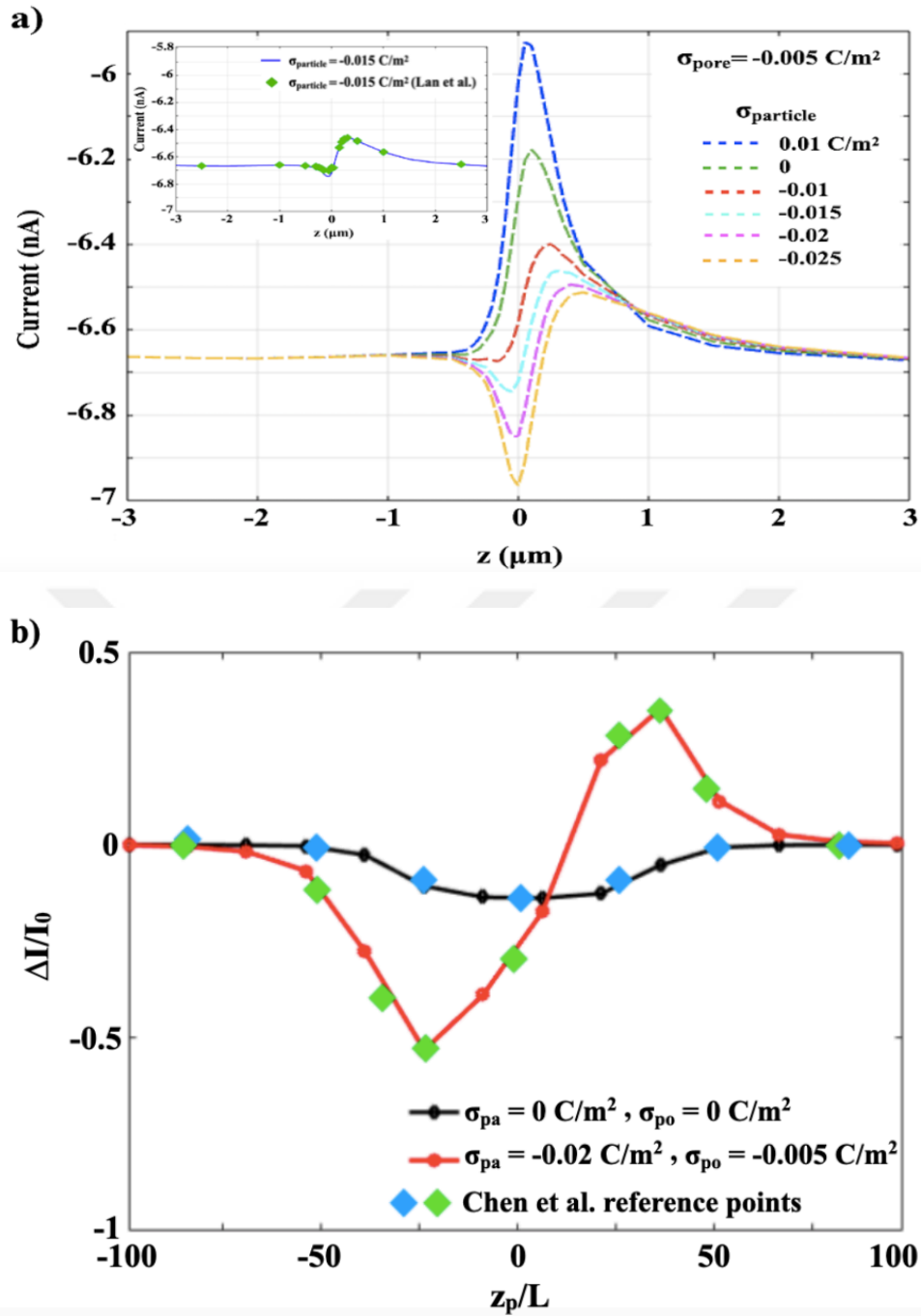


Figure A.3 Signals wave shapes are obtained from our simulations for varying values of particle surface charge densities (σ). **(a)** The simulations are validated by comparing them with the simulation results in Figure 6a of (167) by using their simulation parameters and conditions. The position of the tip is normalized to zero for comparison reasons. The results show that as the particle charge increases, the signal shape gradually turns into a conductive pulse for the conical pore. This transition occurs at particle surface charge density values between -0.015 and -0.02 C/m², in agreement with (167). In the present study, since the surface charge is also set to -0.015 C/m², the embedded figure compares the result for -0.015 C/m². Additionally, in **(b)** our modelling approach is validated with the simulation result in Figure 2b of (134) or cylindrical pore at identical geometry and same conditions.

A.4 Mesh Independence Test for Axially Symmetric Simulation

A mesh independence test has also been conducted for axially symmetric (half-plane) case. The simulations are run in a workstation with a 3.5 GHz 6-core Intel Xeon E5 processor and 32 GB RAM. An extremely fine mesh is implemented through a pore model using 161,221 mesh elements with the maximum growth rate of 1.05. Free triangular elements in the mesh are distributed between 0.4 nm and 134 nm. Most refined mesh is used as the reference mesh, which contains 923,486 elements. In addition, the meshes are refined at both tip and pore walls. With a stretching factor of 1.2, the boundary layers on the pore walls are determined to be 20. Meshes have an average element quality between 0.88 and 0.92. Figure A.4 shows that the deviation is less than 0.34% in the case when the number of elements is 161,221.

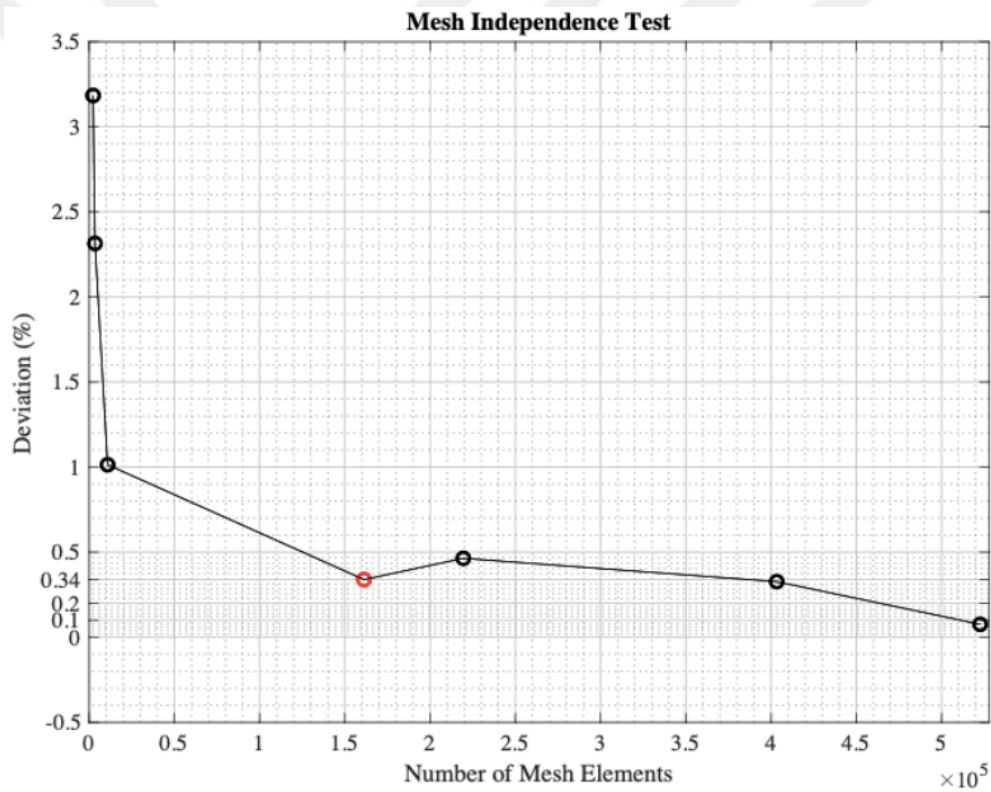


Figure A.4 Deviation from the baseline current of the most refined mesh as the number of mesh elements increases. The deviation is evaluated by $(I-I_0)/I_0 \times 100$ in percentage, where I_0 is the baseline current of the reference (most refined) mesh with 923,486 elements. The mesh that the signals have been generated is circled by red, showing a deviation less than 0.34%.

A.5 Mesh Independence Test for 2D Simulations

The study runs simulations to compare pores with respect to the translocation trajectory. For this purpose, a 2D geometry has been built. All boundary conditions and parameters are set same as the axially symmetric geometry. 2D geometry requires much larger number of mesh elements, therefore a mesh independence test has been performed.

Figure A.5 shows that the deviation is less than 0.5%, when the number of elements is 544,182. All signals obtained from each pore have been generated with 544,182 elements where the maximum growth rate is 1.03. For this mesh, free triangular elements in the mesh are distributed between 1 nm and 250 nm for the minimum and maximum element sizes, respectively. The mesh refinement is applied at both tip and pore walls. With a stretching factor of 1.2, the boundary layers on the pore walls are determined to be 10. This most refined mesh is used as the reference mesh, which contains 1,220,811 elements having an average element quality of 0.9049.

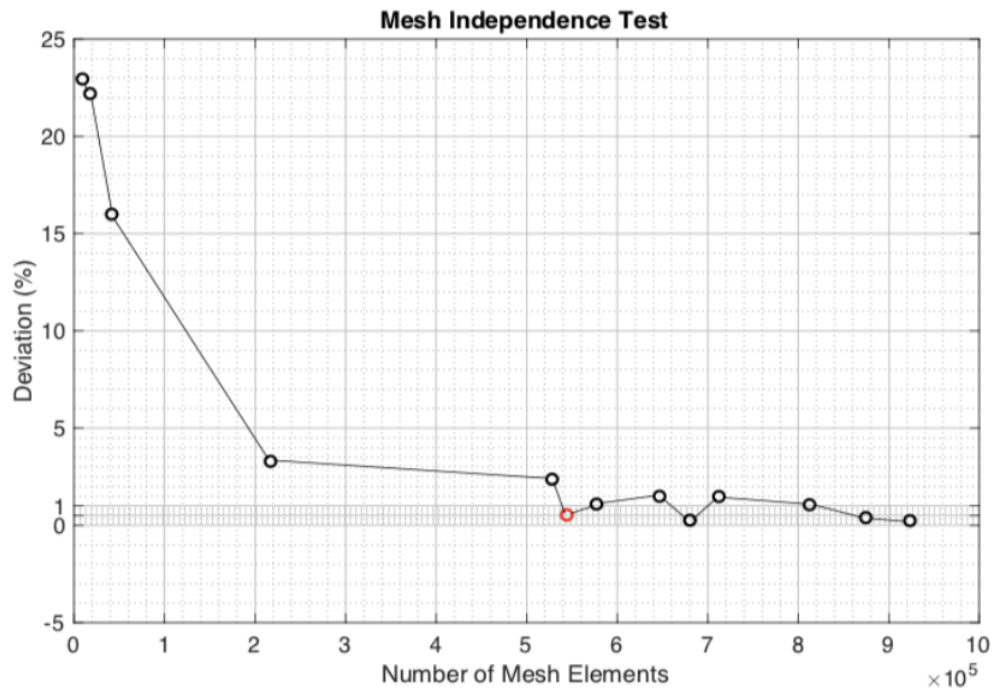


Figure A.5 Deviation from the baseline current of the most refined mesh as the number of mesh elements increases. The deviation is evaluated by $(I-I_0)/I_0 \times 100$ in percentage, where I_0 is the baseline current of the reference (most refined) mesh. The mesh that the signals have been generated is circled by red.

APPENDIX B

B.1 Mesh and Boundary Conditions in Simulations

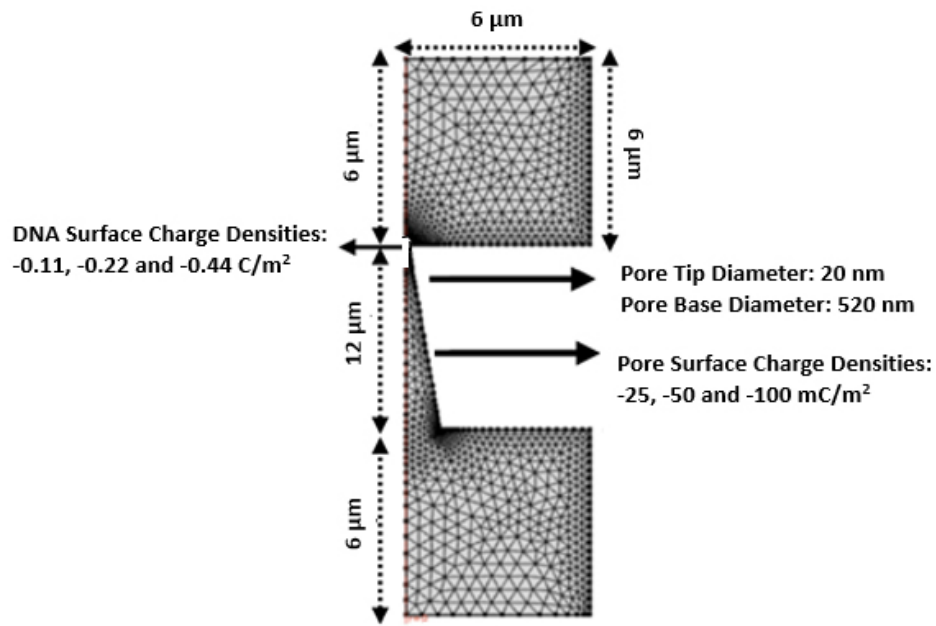


Figure B.1 Simulation parameters for DNA translocation.

B.2 Base Diameter of PET Membrane

For the base diameter determination, SEM images of multipore (10^8 nanopores/ cm^2) membranes are used. Average of minimum 20 pores are taken into account for the calculations. The base diameter of the conical membranes etched for 2 hours in 9 M NaOH is found to be 520 ± 26 nm.

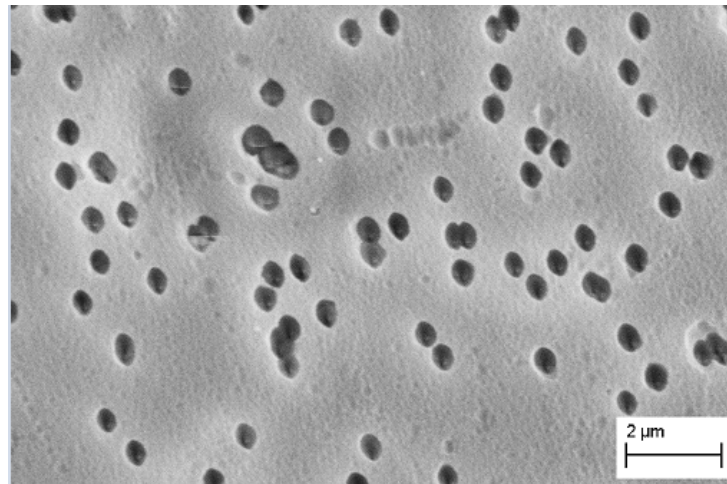


Figure B.2 SEM image of multipore PET membrane with 520 nm base diameter (Obtained at x 20.00 K magnification).

Table B.1 Parameters and Values Used in Calculations

Parameters	Values
a (DNA radius)	1.1 (nm)
L _p (DNA length)	33 (nm) for 100 bp
r _{tip} (nanopore tip radius)	10 (nm)
r _{base} (nanopore base radius)	260 (nm)
L _N (nanopore length)	12000 (nm)
R _R (reservoir radius)	6000 (nm)
L _R (reservoir length)	6000 (nm)
ε _w (relative permittivity of water)	80
ζ (zeta potential of electro-osmotic boundary)	-0.015 (V)
F (Faraday constant)	96490 (C/mol)
η (fluid viscosity)	0.001 (kg/sm)
ρ (fluid density)	1000 (kg/m ³)
ε ₀ (vacuum permittivity)	8.854 × 10 ⁻¹² (C/Vm)
c ₀ (bulk ionic concentration)	1000 (mM)
□ _p (charge density on nanopore wall)	-0.025, -0.05, -0.1 (C/m ²)
□ _d (charge density on DNA surface)	-0.11, -0.22, -0.44 (C/m ²)
T (absolute temperature)	300 (K)
R (universal gas constant)	8.31 (1/mol.K)
E (strength of applied electric field)	-0.4, -0.6, -0.8, -1 (kV)
D ₁ (diffusion coefficient of cations)	1.957 × 10 ⁻⁹ (m ² /s)
D ₂ (diffusion coefficient of anions)	2.032 × 10 ⁻⁹ (m ² /s)
p (strength of applied pressure)	0 (Pa)

B.3 Mesh Independence Test for Simulations

Figure B.3 shows the mesh independence test results. Deviation of the baseline current from the most refined mesh's baseline current is given as the mesh element numbers increase. The baseline current of the most refined mesh is denoted by I_{Ref} . The most refined mesh contains 2,355,473 mesh elements with the 0.911 average element quality. The maximum growth rate is 1.03. For the most refined mesh element sizes are distributed between 0.1 nm and 0.0402 μm for the minimum and maximum element sizes, respectively. In addition, mesh refinement is applied at both tip and base edges. With a stretching factor of 1.2, the boundary layers on the pore walls are determined to be 10.

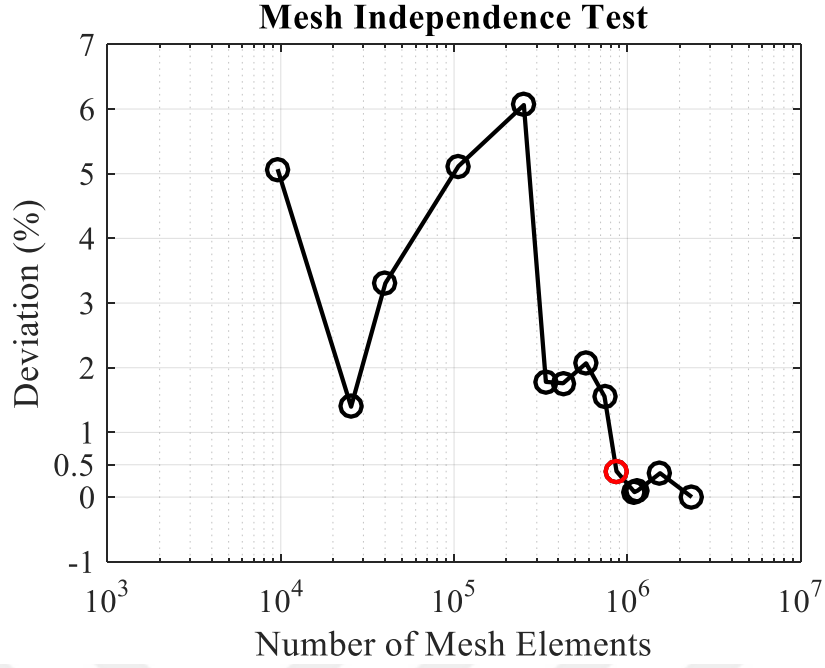


Figure B.3 Deviation from the baseline current of the most refined mesh as the number of mesh elements increases. The deviation is evaluated by $(I - I_{\text{Ref}})/I_{\text{Ref}} \times 100$ in percentage, where I_{Ref} is the baseline current obtained from the reference mesh. The mesh set for the generation of signals is circled by red.

Figure B.3 shows that the deviation is less than 0.5% in the case the number of elements is 865,415 with the maximum growth rate of 1.05. All signals have been generated using this mesh. For the most refined mesh, the element sizes are distributed between 0.1 nm and 0.168 μm for the minimum and maximum element sizes, respectively. In addition, mesh refinement is applied at both tip and base edges. Meshes at the DNA corners are also refined. With a stretching factor of 1.2, the boundary layers on the pore walls are determined to be 10 and to be 5 at the DNA boundary.

APPENDIX C

C.1 Finite Element Method

The complex geometric problem posed by factors such as fluid dynamics and surface interactions in the medium during particle passage across a nanopore, the problem is difficult to address analytically, and it is often required to resort to numerical methods. The finite element method (FEM) has been shown to accurately reproduce current effects in nanopore systems (168). It is used to solve electrokinetic equations in complex geometries. Challenges are analyzed by solving the coupled system of electrokinetic (Poisson (for electrostatics), Nernst–Planck (for ion motion) and incompressible Navier Stokes) equations with suitable boundary conditions. Coupling of these equations by COMSOL Multiphysics is able to reveal the force interactions and fluid flow behaviors that occur across the nanopore and around the nanoparticle.

Therefore, investigation of a short oligonucleotide fragment movement through the biological nanopores, which has not been examined widely in that scope, is carried out by using the FEM.

In the Finite Element Method (FEM) models are partitioned into a finite set of elements. These elements are linked to one another at specific points, these points are called nodes. The simulation process goes as follows.

First, the geometric shape of the part to be analyzed is drawn (Figure C.1-C.2).

Then the material/medium properties such as permittivity, density are entered (Table S1). And so, the properties of the elements to be used for the drawn model are determined.

In the next step, conditions in the boundary regions of the model and, if any, other effects on the model are specified to the program (Fig.S3-S4-S5).

After the element properties are determined, the model is divided into smaller elements. Namely, the model is meshed (Fig.S6). Mesh formation can be defined as the division of a physical domain into smaller regions (elements). The purpose here is to facilitate the solution of a differential equation. What is important here is how to better divide the model into smaller pieces using the selected element. Mesh should be high enough to achieve the accuracy, but it should also not increase the computational cost.

And last, the problem is solved according to the previously entered parameters.

C.1.1 Finite Element Simulation Details (COMSOL Multiphysics)

Geometry, Boundary Conditions, System Parameters

Based on the finite element simulation properties described above, the 2D axisymmetric geometries (cylindrical axis) of biological nanopores, boundary settings, and mesh distribution are modeled to predict the pulse from/blockage amount produced by a translocated short oligonucleotide.

As in experiments, the target molecule moves from one reservoir to the other under the influence of the electric field created by the potential difference applied by the electrodes.

As the particle passes through the biological nanopore, a change in ionic current flow occurs due to the narrow pore occupation by the particle volume and the chemical interaction that occurs between the particle and the pore surfaces. This ionic change allows the particle to be characterized.

Here Figure C.2 displays an axially symmetric system that biological nanopore embedded membrane separated an ionic salt concentration contained reservoir into two (corresponding to Coulter Counter model). The schematics of a short oligonucleotide fragment translocation through four biological nanopores are modeled based on the given nanopores' dimensions in Table S4.

Although the geometry is used in symmetry axis in the model as shown in Figure S2, 3D versions of each pore model displayed to clarify the visualization (Figure C.1).

Due to the axially symmetric geometry (Figure C.2), cylindrical coordinates r and axial z are considered in the result analyzes step. The translocation of an oligonucleotide is considered move along the symmetry axis of the pore.

All system arguments such as physical parameters, salt concentration, imposed electric field, as well as geometric parameters and electrode placements are applied according to those given in Table C.1 (Parameters) by the Component module (Figure C.2-C.3-C.4).

The electrokinetic equations are also coupled under the Component module of COMSOL through three sub-modules of Electrostatics (Poisson Equation), Transport of Diluted Species (Nernst-Planck Equation) and Creeping Flow (Navier-Stoke Equation) as given below in the COMSOL Model Report section. (The equations are explained in detail in the main text.)

The next important step for the simulation accuracy is meshing (Figure C.6). As mentioned above highly finer mesh and adaptive mesh refinement to critical points of the geometry might improve the precision. On the other hand, as shown in the Mesh Independence Test section (Figure C.12), in our simulation mesh element compactness does not extremely affect our results. Namely, in our results signal generation is independent of mesh distribution. Therefore, mesh element is adjusted to Normal Mesh and no refinement is made which also decrease our computation cost (Mesh Module Section and Mesh Distribution and Boundary Conditions, below).

Moreover, the main issue of the simulation model is moving the particle. We use the parametric sweep module to do this action. By adjusting the axially position range, the selected particle is moved incrementally (step by step) in the selected range. Since it is programme controlled, it is not necessary to repeat the process unlike experiments. It is enough to simulate once unless the parameters are changed.

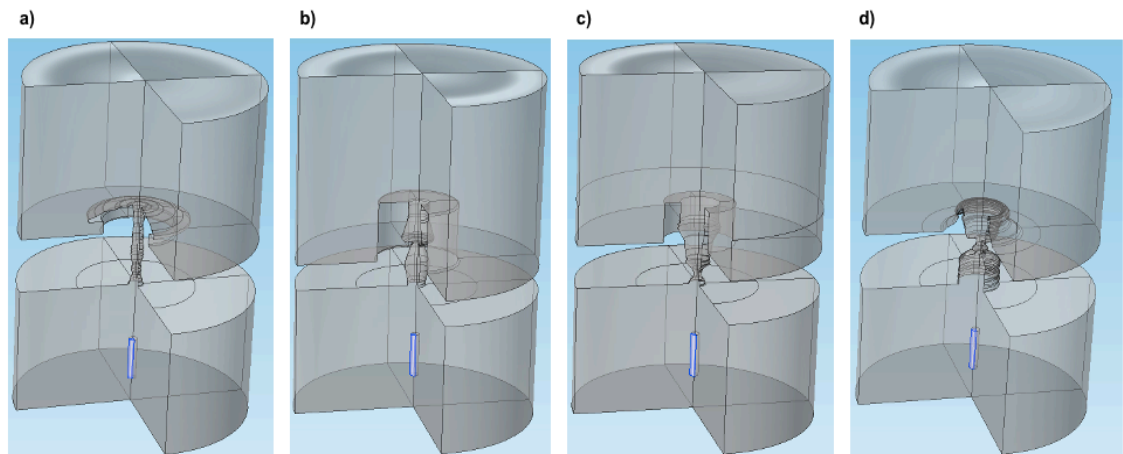


Figure C.1 3D-view of the four biological nanopore geometry models when the target particle at the system reservoir (by COMSOL5.1[®]). a) 3-D view of AeL, b) 3-D view of α -HL, c) 3-D view of MspA, d) 3-D view of CsgG.

C.1.2 COMSOL Model Report

In this section, simulation modeling through COMSOL modules is briefly explained.

1 Global Definitions

1.1 Parameters 1

Table C.1 Parameters Module. This module keep the model organized which get properties for efficient parameter control. (Following data are given according to this study.)

Name	Expression
Potential	-300 [mV]
ssDNA_pos	22.5 [nm]
Concentration	1000 [mol/m ³]
Pressure	0 [mmHg]
ssDNA_length	5 [nm]
ssDNA_dia	1.1 [nm]
ssDNA_surface_charge	-0.136 [C/m ²]
Pore_long	10 [nm]
Pore_tip_diameter	1 [nm]
Pore_base_diameter	1.7 [nm]
Pore_surface_charge	-0.06 [C/m ²]
Reservoir_thick	15 [nm]
Reservoir_width	15 [nm]
Fa (Faraday constant)	96485 [C/mol]
eps0 (Permittivity)	8.854e-12 [F/m]
R0 (Gas constant)	8.3145 [J/K/mol]
D1	1.957e-9 [m ² /s]
D2	2.03e-9 [m ² /s]
Tempreture	300 [K]
z1	1
z2	-1

2 Component 1 (comp1) Module.

Under this module one can adjust the whole model setup

2.1 Geometry 1

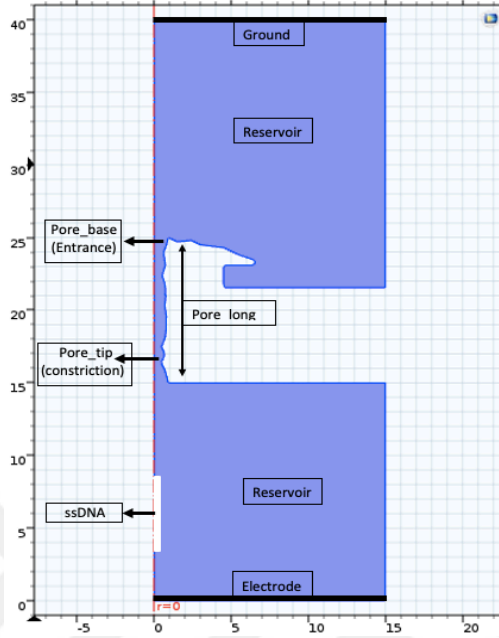


Figure C.2 Axial symmetry view of the simulation model represented by the AeL biological nanopore. All four biological pores are modeled identically except for their respective sizes.

2.2 Electrostatics (es) Module

Surface Charge Density Boundaries

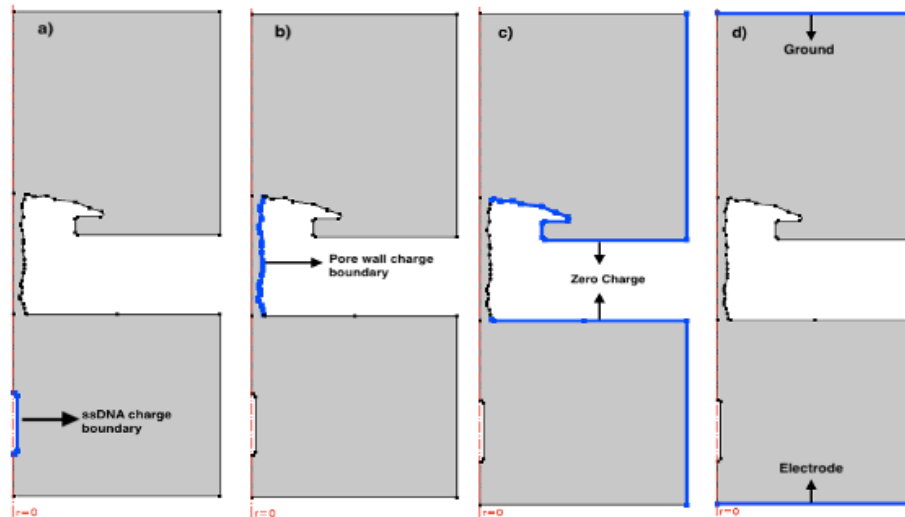


Figure C.3 Electrostatic module boundary conditions to perform Poisson Equation. a) ssDNA surface charge density boundaries, b) Nanopore surface charge density boundaries, c) zero charge boundaries, d) electrode placements where upper reservoir adjusted as Ground and Lower reservoir as Electrode. AeL biological nanopore is used to represent the boundary conditions, but it is applied in the same way for all pores.

2.3 Transport of Diluted Species (chds) Module

Concentration

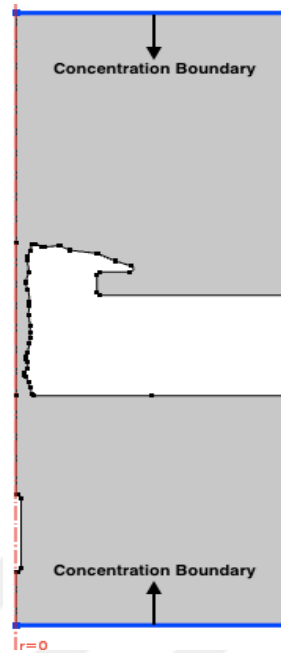


Figure C.4 Transport of Diluted Species (chds) Module boundaries for ionic salt concentration to perform Nernst-Planck Equation at both upper and bottom reservoirs.

2.4 Creeping Flow 1 Module

Electroosmotic Boundary

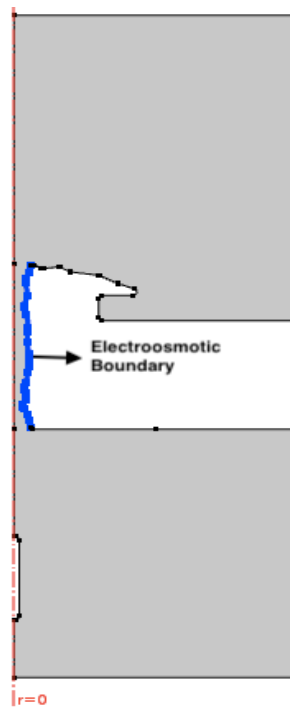


Figure C.5 Creeping Flow module boundary for electroosmotic flow to perform Navier-Stoke Equation. This module is actually referred to Laminar Flow. By selecting the

“neglect the inertial term (Stokes flow)” it is transformed to the creeping flow where Navier-Stoke Equation is performed.

Mesh 1 Module

It is displayed in detail in the Mesh Distribution and Boundary Conditions Section (Figure C.6) and Mesh Independence Test (Figure C.12) sections below.

Table C.2 Mesh 1 Module. Default element values can be used according to the coarse or fine mesh density to be applied under the Physics-controlled mesh sequence type, or manual adjustments can be made with user-controlled mesh sequence type. According to the mesh independence test results (Figure C.12), default normal element size is found sufficient for this study.

Mesh Settings	
Sequence Type	Physics-controlled mesh
Element Size	Normal

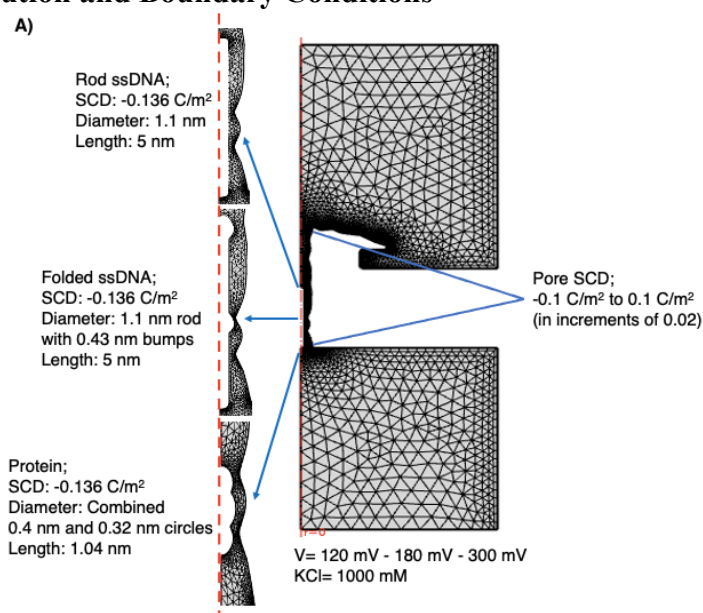
3 Study 1

3.1 Parametric Sweep

Table C.3 Parametric Sweep Module. Particle translocation step ranges are arranged under this module. The intended process can be implemented by selecting Stationary or Time-Dependent steps.

Sweep Type	Specified Combinations
Parameter Name	Parameter Value List
ssDNA_position	range(32,-2,30) range(20,-0.5,12) range(8,-2,6)

C.2 Mesh Distribution and Boundary Conditions



B)

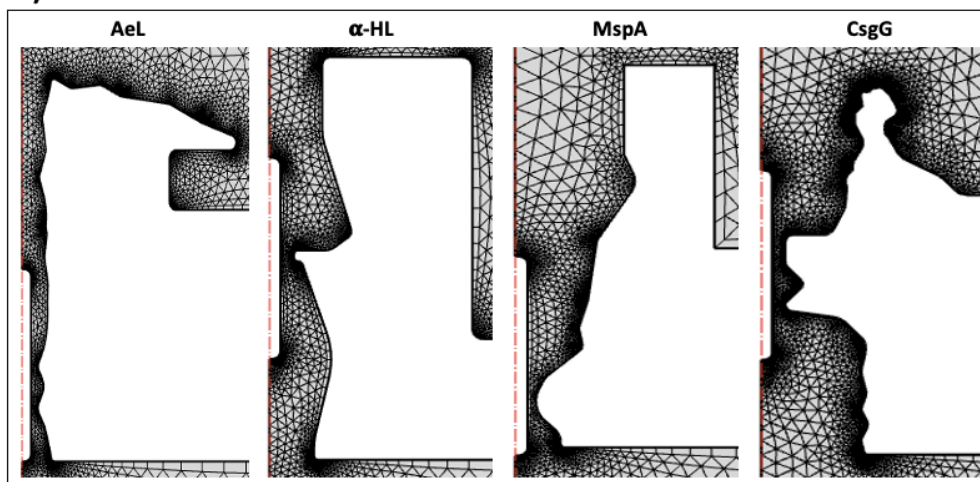


Figure C.6 Mesh distribution and boundary conditions of considered four protein nanopore. A) Representation of mesh distribution and boundary conditions on three different molecule models (rod-shaped ssDNA, folded DNA, protein molecule) and AeL pore. Boundary conditions are used in four pore models. B) Mesh distribution of four biological nanopores across their own recognition channel. Presented pores are Aerolysin, α -Hemolysin, MspA, and CsgG, respectively. Mesh distribution performed by the COMSOL *Multiphysics* programme. While particles of the same diameter and length are displaced across the four pores, each pore has its own internal dimension.

Table C.4. Dimension of four pore models

	Pore Length	Narrow Constriction Diameter	Base Diameter
Aerolysin	10 nm	0.91 nm (trans-side) 1.03 nm (cis side)	1.83 nm (trans & cis side)
α -Hemolysin	10 nm	1.4 nm (in the middle)	2.2 nm (trans-side) 2.6 (cis side)
MspA	9.6 nm	1.2 nm (trans-side)	2.4 nm (trans-side) 4.8 nm (cis side)
CsgG	9.6 nm	1.2 nm (in the middle)	5.2 nm (trans-side) 5 nm (cis side)

C.3 Validation

C.3.1 Validation pore models as an electrostatic potential distribution

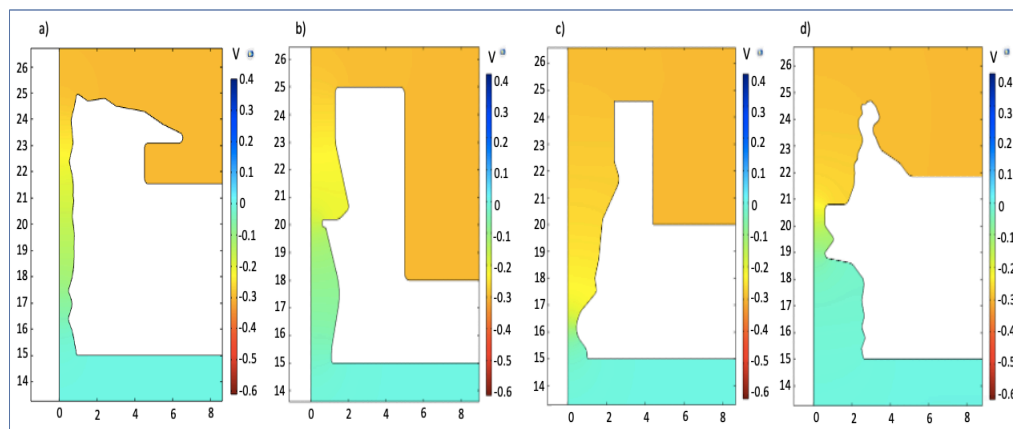


Figure C.7 Validation of pore models as a electrostatic potential distribution. To verify the simulation precision, pore models are verified with a MD study under the 300 mV applied potential (46). a) Aerolysin pore, b) α -Hemolysin, c) MspA, and d) CsgG. Consistent with the reference study electric potential distribution changes along the centerline of each pore and the effective region for the contour pattern is the pore constriction at each pore. While the potential is approximately at -0.1 V at the trans-side, it starts to increase to the -0.2 V, keeps going being in this value along the narrow region of each pore, then becomes stable nearly -0.4 V at the cis side.

C.3.2 Validation (Confirmation) of pore models for the mutant case relative to the WT

The present study numerically investigates the comparison of widely used four biological nanopores, namely, AeL, α -HL MspA, CsgG, to ascertain the surface charge effects on signal pulse magnitudes in terms of $\Delta I/I$.

To ascertain the signal differences among selected pores, three different shaped molecules (i.e., rod-shaped ssDNA (corresponds to stretched ssDNA), folded-shaped (coiled) ssDNA, protein-shaped particle) are modelled and translocated through four considered biological nanopores.

As can be find from the literature, there are selected biomolecules in a wide range of sizes for identification and characterization. In our work, besides the protein, we considered a short passing nucleotide through the pore which represented as a rod shape, and we examined the signal generation of biological nanopores according to the short biomolecule translocations. To verify our simulation precision model has been validated with experimental study results (60).

Since we examined the short particle transition in our study, we verified our model with the experimental study for the short particle transition. Moreover, we chose AeL biological nanopore for validation as our results suggested that AeL is the best performing pore for the short nucleotide recognition. Here, by presenting this validation we show the consistency of our results with the literature.

In the reference study (60) normalized ionic current (I/I_0) calculated in a wide range of size, namely, dA₁₀, dA₅, dA₄, dA₃, dA₂, particles. However, since we dealt with a 5 nm long particle in our main study, here considered only the shortest dA₂.

Simulation is performed under the following parameters corresponding to the reference study; 120 mV applied potential, 1M KCl ionic concentration, -0.05 and -0.136 C/m² for pore and particle surface charge, respectively.

Figure C.8 gives our simulation result corresponding to the reference experimental study. Presented signal shows a resistive pulse wave shape generated by AeL pore for the modeled dA₂ transition. As it is clearly seen in the following figure, our result agrees with the experiment's outcomes (shown in the inset of the figure, signed by red dashed arrows). Signal magnitude of the simulated signal is ~0.03 nA. Also, I/I_0 is obtained as ~0.88 very close to the experimental data of ~%85.

Our simulation result consistency indicates how our model robust for particle recognition as small as dA₂. This is verify our main study's whole outcomes consistency.

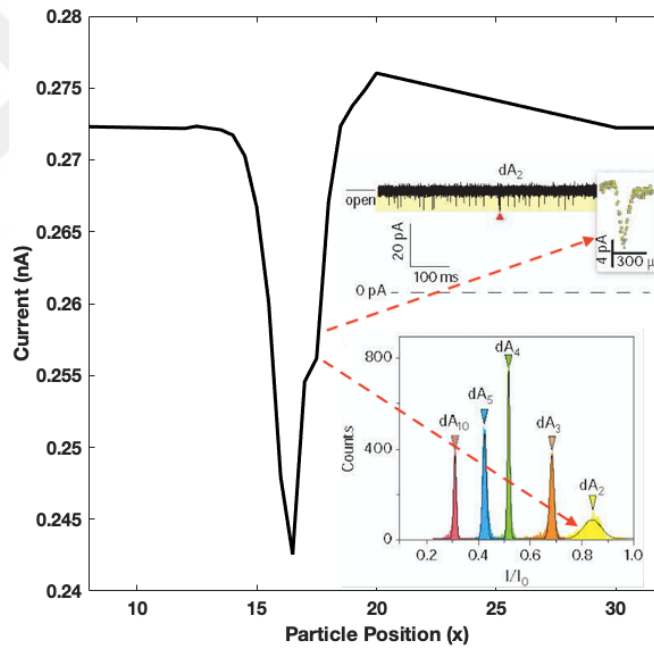


Figure C.8 Simulation validation result corresponding to the experimental study. Signal from the AeL biological nanopore for the dA₂ nucleotide transition. Experimental results are given as inset of the figure by courtesy of Cao et.al (60).

Besides the signal shape and normalized current verification through AeL (shown at the above Figure 1), we also presented the mutant state confirmation by means of other three pores, namely, α -HL, MspA, CsgG.

Each biological pore has distinct inner atomic construction. This yields surface charge distribution unique to each one. Thus, to validate the mutation performance versus wild-type manner we validated each of them through separate studies in the literature. Since we are conducting this study to validate

our own working model, we will focus on demonstrating the fit of our model's results to the literature, not simulating reference studies. Therefore, we only showed the mutation effect changes as trend compliance. Furthermore, since it is hard to predict the inner charge values, we replaced the reported regions by 0.1 C/m^2 to mutate each WT pore.

At first, we validated the α -HL mutant case signal performance relative WT state. We examined this situation by considering Maglia et.al. work (83) and performed the cases under the same parameters with the reference work as 120 mV applied potential and 1M KCl ionic concentration. Inner surface charges of WT α -HL are mainly distributed at the middle constriction region and at the entrance of the cap region. Mutation applications are varied through different molecular adaptations. Here we dealt with the M113R-WT mutant case in which basic (positively charged) amino acid chains additionally inserted along the pore lumen. In the reference study (83) investigation is set on 92-nt long particle. However, since in our main manuscript we modeled the short type of ssDNA molecule, in the validation case we preferred to hold the short molecule to show how the mutation of the pore enhance the sensitivity. This attempt also offers that the sensitivity enhancement through surface charge manipulation is independent of particle size.

As can be deduced from Figure C.9 (simulated validation graph) and Figure C.10 (reference experimental study), mutation clearly improves the sensitivity of the α -HL pore. We observed approximately 4% higher blockade rate from M113R-WT than WT α -HL. It is given as the fractional current blockade in the experimental data (83) as 0.04 for M113R-WT and 0.11 for WT α -HL. Both the quantitative results and graphs indicates the treatment of the inner surface charges improves the α -HL recognition ability and confirms our simulation model.

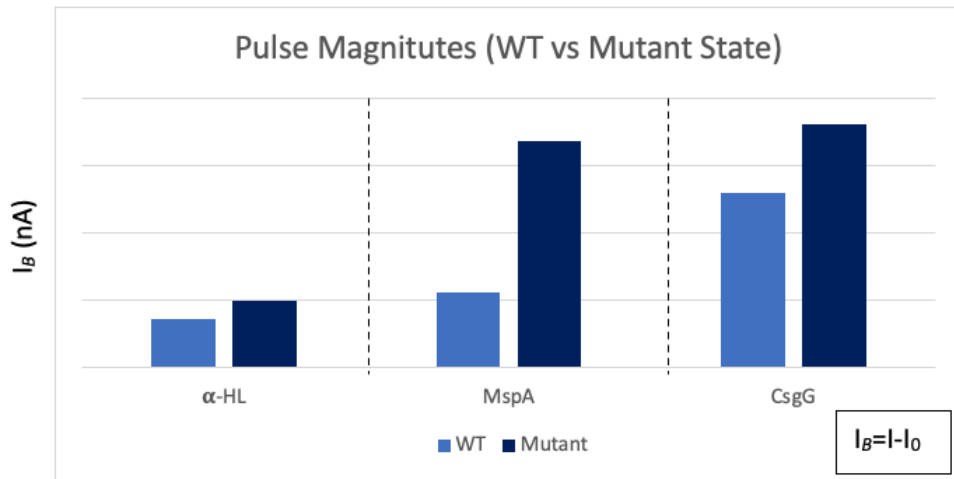


Figure C.9 Current blockade change of biological pores relative to WT vs Mutant states. Light blue indicates the WT cases and dark blue indicates the mutant versions. I_B implies the current blockade magnitude, I_0 and I are point open pore current and blocked current, respectively.

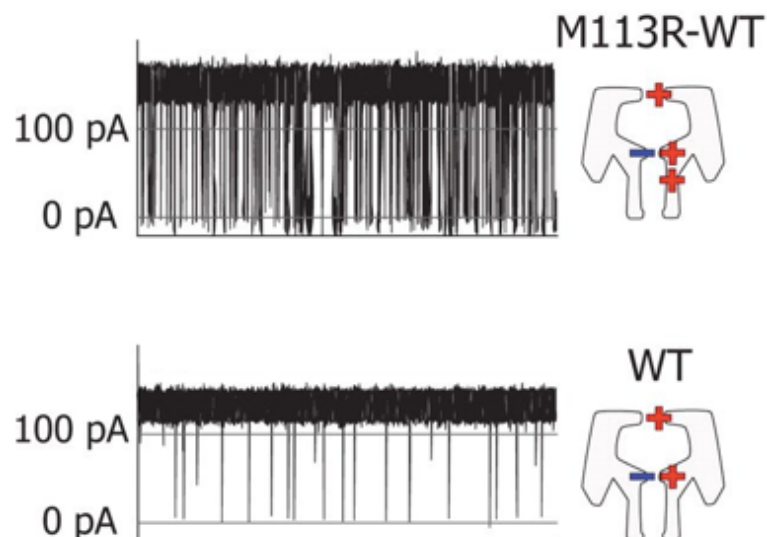


Figure C.10 Experimental site-directed mutagenesis study of ssDNA translocation through α HL pores (by courtesy of Maglia et.al (83)).

Secondly, we took in hand the MspA pore to reveal the mutation effect corresponding to the Butler et.al. study (97). As shown in Figure C.11A WT-MspA has highly negative inner surface charge distribution. In the reference study WT MspA is mutated to M1MspA type by removing the negative amino acid chains at the bottom by replacing with neutral ones. A mutant version of M2MspA is then obtained by replacing all negative residues with positive amino acids. It is reported that the conductivity decreased by a factor of 2-3 with the mutated pore compared to the wild type (Figure C.11C) and deep blockade rate is given by the ionic current dropped $<50\%$ of the unblocked level. We agreed with these results, as shown in Figure C.9 with ionic current drop 45% of the unblocked level. Furthermore, according to our simulation mutated pore produced better pulse magnitude with approximately 3-fold than WT-MspA pore. This consistency testifies to the accuracy of our model as α -HL.

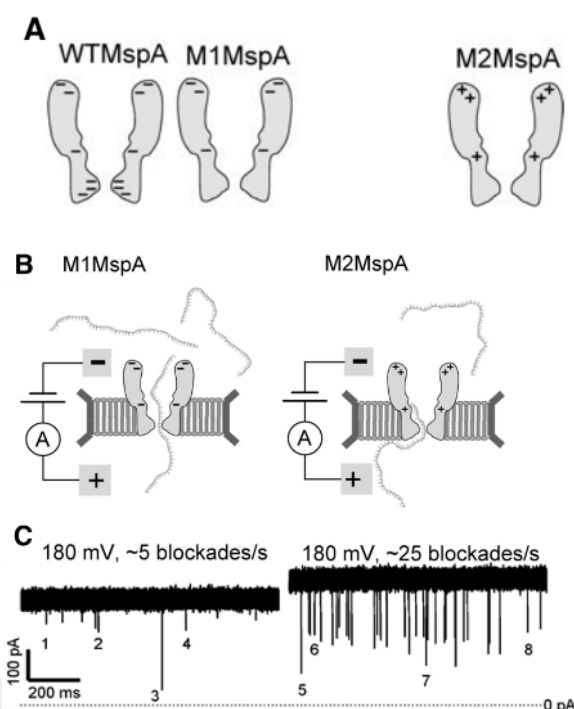


Figure C.11 Experimental study involves construction of two MspA mutants and demonstration of ssDNA. (by courtesy of Butler et.al (97).

Third validation is done for CsgG. Structural, functional and electrophysiological analyses indicate that CsgG is a gateless, non-selective protein secretory channel that is expected to have a diffusion-based, entropy-driven transport mechanism (109). CsgG is extensively mutated by forming a pore complex with other secretion proteins, CsgE, CsgF. On the other hand the mutant state is well known to increase sensitivity which is also reported currently yielding the highest accuracies and pore stability (169). However, it is quite challenging to model the internal dynamics of biological systems exactly. Moreover, CsgG mutants are not publicly revealed. Hence, as we have noted above, we presented the current trend of change in a way that does not contradict and is compatible with the literature. As introduced a non-selective protein secretion channel, we considered the inner surface charge of WT-CsgG state as neutral. As can be seen in **Figure C.9**, even WT-CsgG showed quite high blockage confirming the literature, provides more than 2-fold higher blockage than the WT-MspA. Then we change the surface charge to make a mutant CsgG, it generates approximately 15% higher current blockade rate than WT-CsgG.

According to the above results we can order the selected three pores according to the differences created by the mutant states with respect to WT as MspA>CsgG> α -HL consistently our main work. Although our study and the studies selected for comparison are done in completely different frameworks, we can suggest our results show good agreement with the literature.

C.4 Mesh Independence Test

A mesh independence test has been conducted for axially symmetric (half-plane) case. The simulations are run in a workstation with a 3.5 GHz 6-core Intel Xeon E5 processor and 32 GB RAM. A physics-controlled mesh is set resulting in 4014 mesh elements. Most refined mesh is used as the reference mesh, which contains 238,287 elements. Meshes have an average element quality between 0.91 and 0.97. **Figure C.12** shows that the deviation is less than 0.3% ($\sim 0.27\%$) in the case when the number of elements is 4014.

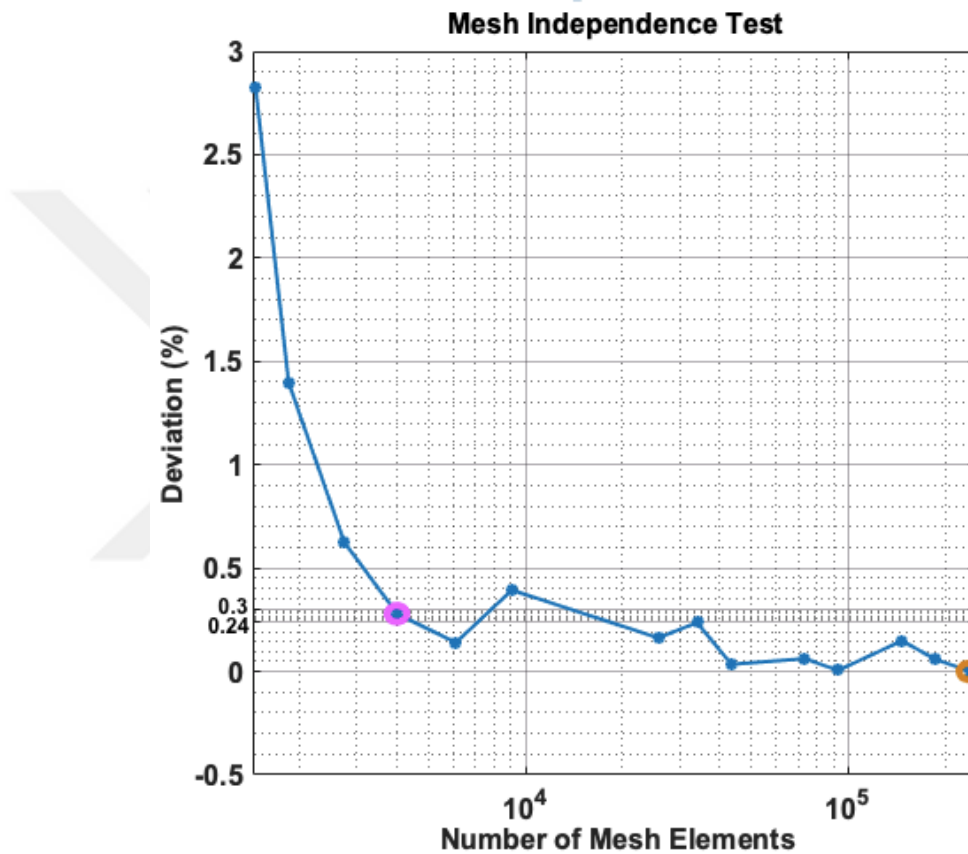


Figure C.12 Deviation from the baseline current of the most refined mesh as the number of mesh elements increases. The deviation is evaluated by $(I-I_0)/I_0 \times 100$ in percentage, where I_0 is the baseline current of the reference (most refined) mesh, circled by orange. The mesh that the signals have been generated is circled by purple.

C.5 ΔI Histogram Distributions of Pores for Different Particle Translocations

C.5.1 Uniformly varied pore SCD relative to rigid (rod) ssDNA translocation

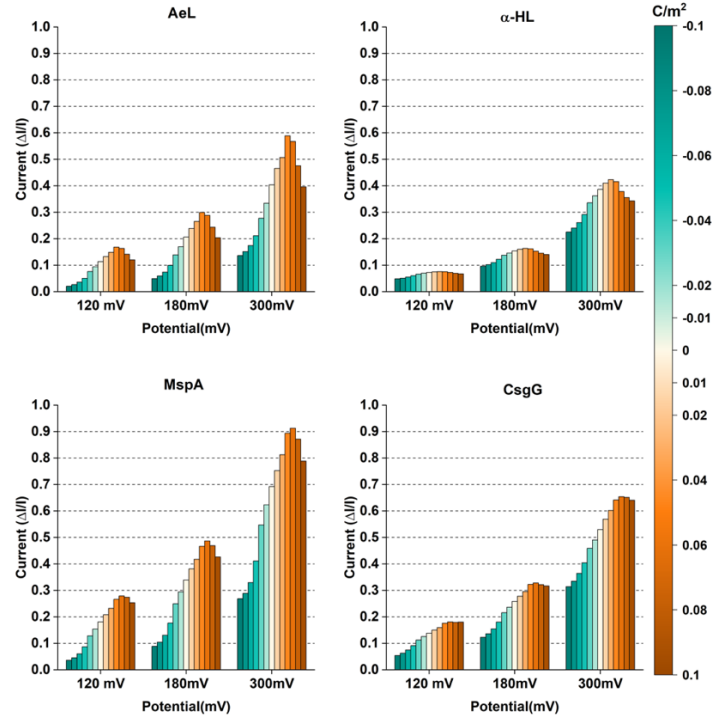


Figure C.13 Histogram distribution of Rigid (rod) ssDNA translocation relative to uniformly varied pore SCD a) ΔI value of AeL b) ΔI value of α -HL c) ΔI value of MspA d) ΔI value of CsgG.

C.5.2 Residual varied pore SCD relative to rigid (rod) ssDNA translocation

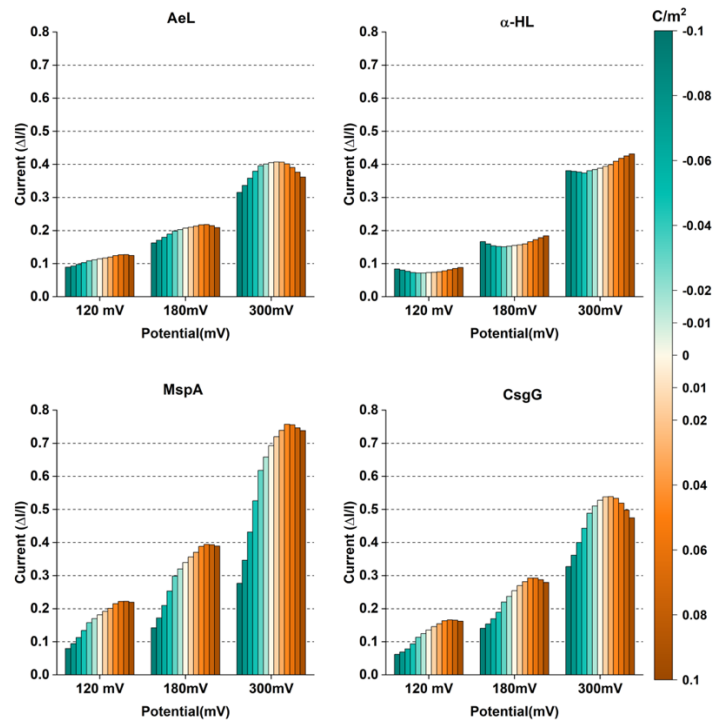


Figure C.14 Histogram distribution of Residual Charge (Site Directed Mutagenesis) Effects on the Signal Generation and Detection **a)** ΔI value of AeL **b)** ΔI value of α -HL **c)** ΔI value of MspA **d)** ΔI value of CsgG.

C.5.3 Uniformly varied pore SCD relative to folded (coiled) ssDNA translocation

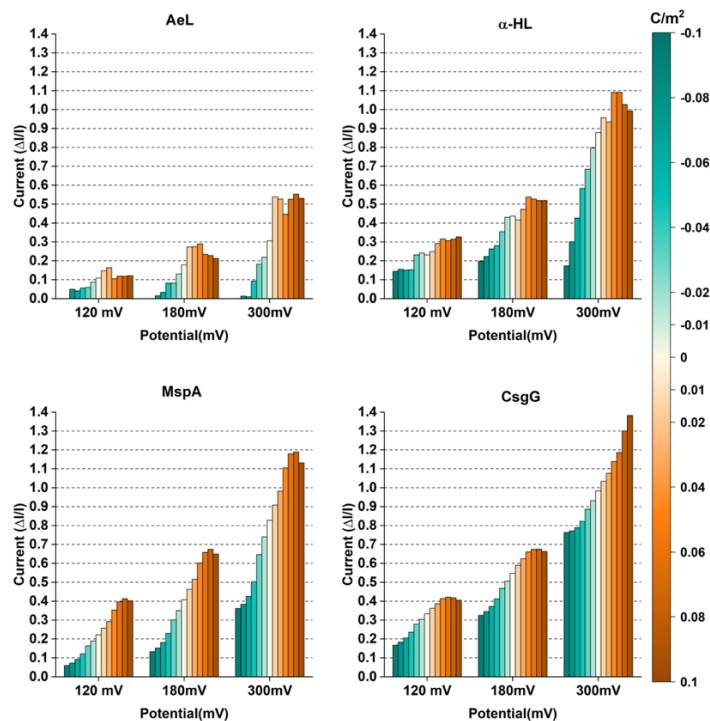


Figure C.15 Histogram distribution of Folded ssDNA translocation relative uniformly varied pore SCD **a)** ΔI value of AeL **b)** ΔI value of α -HL **c)** ΔI value of MspA **d)** ΔI value of CsgG.

C.5.4 Uniformly varied pore SCD relative to protein translocation

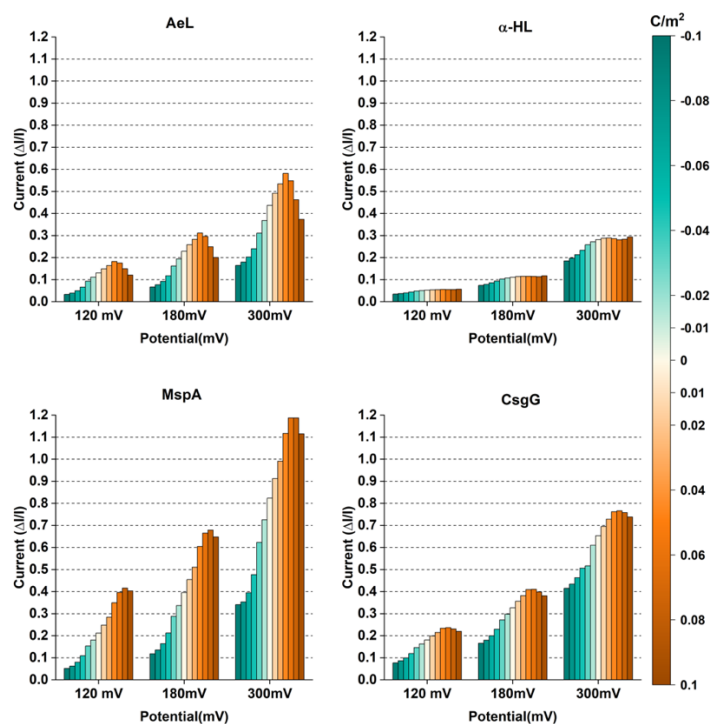


Figure C.16 Histogram distribution of protein translocation relative uniformly varied pore SCD
a) ΔI value of AeL b) ΔI value of α -HL c) ΔI value of MspA d) ΔI value of CsgG.

C.5.5 Comparison of Nucleotide Transitions of Different Lengths

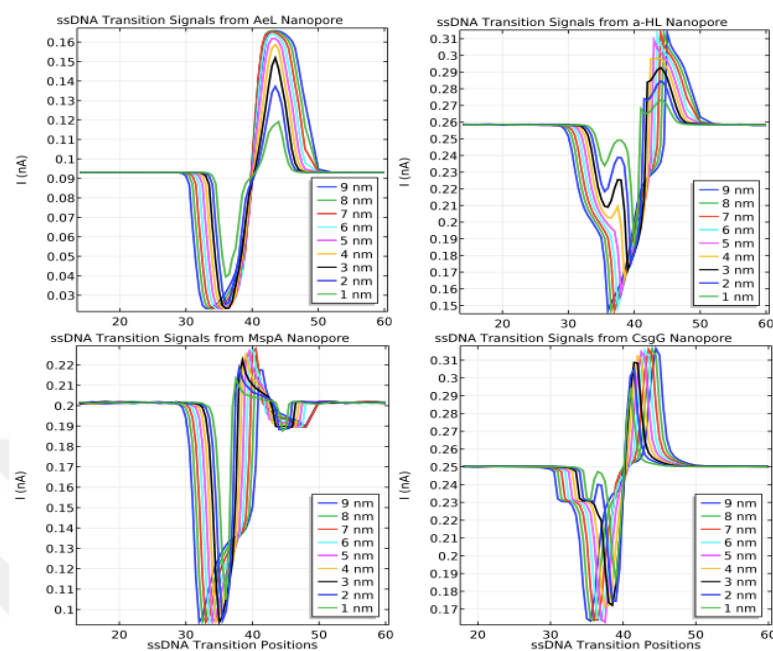


Figure C.17 Transition signals of particles with different lengths. Particles lengths are arranged between 9 nm to 1 nm. Signal shapes are generated by axially symmetric model.

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