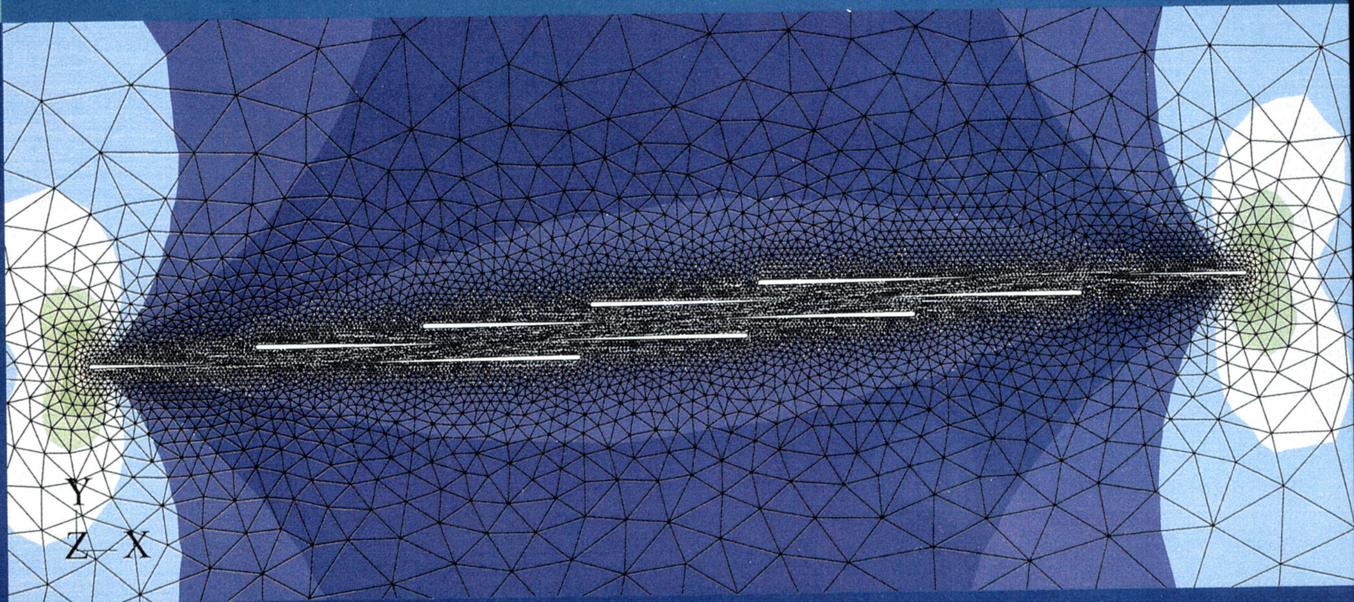
Friedrich G. Barth · Joseph A. C. Humphrey
Mandyam V. Srinivasan *Editors*

Frontiers in Sensing

From Biology to Engineering



Single-molecule detection of proteins using nanopores

Liviu Movileanu

Contents

Abstract 363
1. Introduction 363
2. Watching polypeptide translocation 366
3. Probing protein-ligand interactions 370
4. Detection of proteins with synthetic nanopores 370
5. Exploring protein folding and stability 372
6. Probing protein-nucleic acid complexes with nanopores 374
Concluding remarks 376
Acknowledgments 377
References 377

Abstract

Recently, protein and synthetic nanopores have been employed extensively as single-molecule probes to illuminate the functional features of proteins, including their binding affinity to different ligands, backbone flexibility, enzymatic activity and folding state. In this chapter, I present a brief overview in this emerging area of biosensing. The underlying principle of detection is that the device is based upon a single nanopore drilled into an insulating membrane, which is immersed in a symmetric chamber containing electrolyte solution. The application of a transmembrane potential across the membrane will enable the recording of a well-defined electric current due to the flow of ions crossing the nanopore. The partitioning of single proteins into the interior of the nanopore is detected by discrete current fluctuations that depend upon the interaction between the proteins and the nanopore. The detection mechanisms include chemical modification and genetic engineering of protein nanopores, electrophoretic capture of proteins via movable nucleic acid arms, and functionalization of the inner surface of synthetic nanopores. This approach holds promise for the exploration of proteins at high temporal and spatial resolution. Moreover, nanopore probe techniques can be employed in high-throughput devices used in biomedical molecular diagnosis and environmental monitoring.

Liviu Movileanu
Syracuse University, Department of Physics
201 Physics Building
Syracuse, NY 13244-1130, USA
e-mail: lmovilea@physics.syr.edu

1. Introduction

There is an unprecedented need for novel methodologies to detect a broad range of substances, from small organic molecules to macromolecules, bacteria and viruses. In this chapter, we discuss the power, versatility

and tractability of the nanopore technique as a single-molecule probe that holds potential to reveal several important features of polypeptides and proteins. The underlying principle of nanopore probe techniques is simple: the application of a voltage bias across an electrically insulated membrane enables the measurement of a tiny picoamp-scale transmembrane current through a single nanopore. Each molecule, translocating through the nanopore, produces a distinctive current blockade, the nature of which depends on its biophysical properties as well as the molecule-nanopore interaction. Such an approach proves to be quite powerful, because single small molecules and biopolymers are examined at very high spatial and temporal resolutions (Bayley 1999; Bayley et al. 2000, 2004; Bayley and Martin 2000; Bezrukov 2000; Deamer and Akeson 2000; Bayley and Cremer 2001; Deamer and Branton 2002; Meller 2003; Bayley and Jayasinghe 2004; Dekker 2007; Martin and Siwy 2007; Howorka and Siwy 2008; Howorka and Siwy 2009; Movileanu 2008, 2009). Here, we also discuss recent studies that provided a mechanistic understanding of the forces that drive protein translocation through a nanopore. These measurements facilitate the detection and exploration of the conformational fluctuations of single molecules and the energetic requirements for their transition from one state to another. We present recent strategies for engineering new functional nanopores, in organic and silicon-based materials, and with properties that are not encountered in nature. From a practical point of view, this methodology shows promise for the integration of engineered nanopores into nanofluidic devices, which would provide a new generation of research tools in nanomedicine and high-throughput instruments for molecular biomedical diagnosis and environmental monitoring.

Stochastic biosensors represent a unique class of single-molecule detectors that is based upon the alteration of electrical current by distinct molecules that interact either transiently or permanently with an engineered recognition group located within a nanopore (Martin and Siwy 2007; Howorka and Siwy 2008; Movileanu 2009) (Fig. 1A). Each transient interaction of a single molecule with the engineered recognition group produces a well-defined current blockade, the duration of which depends upon several parameters, including the size, charge and shape of the interacting molecule, the accessibility of the engineered recognition group (e.g., the diameter of the inner surface of the nanopore; the position of the recognition group), and the strength of the analyte-reactive group interaction (Fig. 1B) (Bayley and Martin 2000; Bayley and Cremer 2001). This current blockade occurs as a result of the interacting molecule-induced obstruction of the ionic flow through the nanopore. Therefore, the partitioning of a single molecule into the interior of the nanopore is probed by the resistive-pulse technique in real time (Bezrukov 2000).

In the past decade, research in the area of nanopores has been stimulated by the keen interest of this technique in single-molecule sequencing of nucleic acids (Kasianowicz et al. 1996; Akeson et al. 1999; Henrickson et al. 2000; Meller et al. 2000; Howorka et al. 2001a,b; Howorka and Bayley 2002; Kasianowicz 2004; Meller et al. 2005; Bayley 2006; Kim et al. 2006; Butler et al. 2007; Rhee and Burns 2007; Branton et al. 2008; Butler et al. 2008; Howorka and Siwy 2008; McNally et al. 2008; Wanunu et al. 2008 Comer et al. 2009; Dorvel et al. 2009). Despite numerous obvious challenges in using nanopores for nucleic acid sequencing (Branton et al. 2008), recent studies have demonstrated that nanopores represent versatile single-molecule probes for a broad range of molecules and their assemblies (Dekker 2007; Griffiths 2008; Hayden 2008). An extensive research on biosensing has been carried out with the

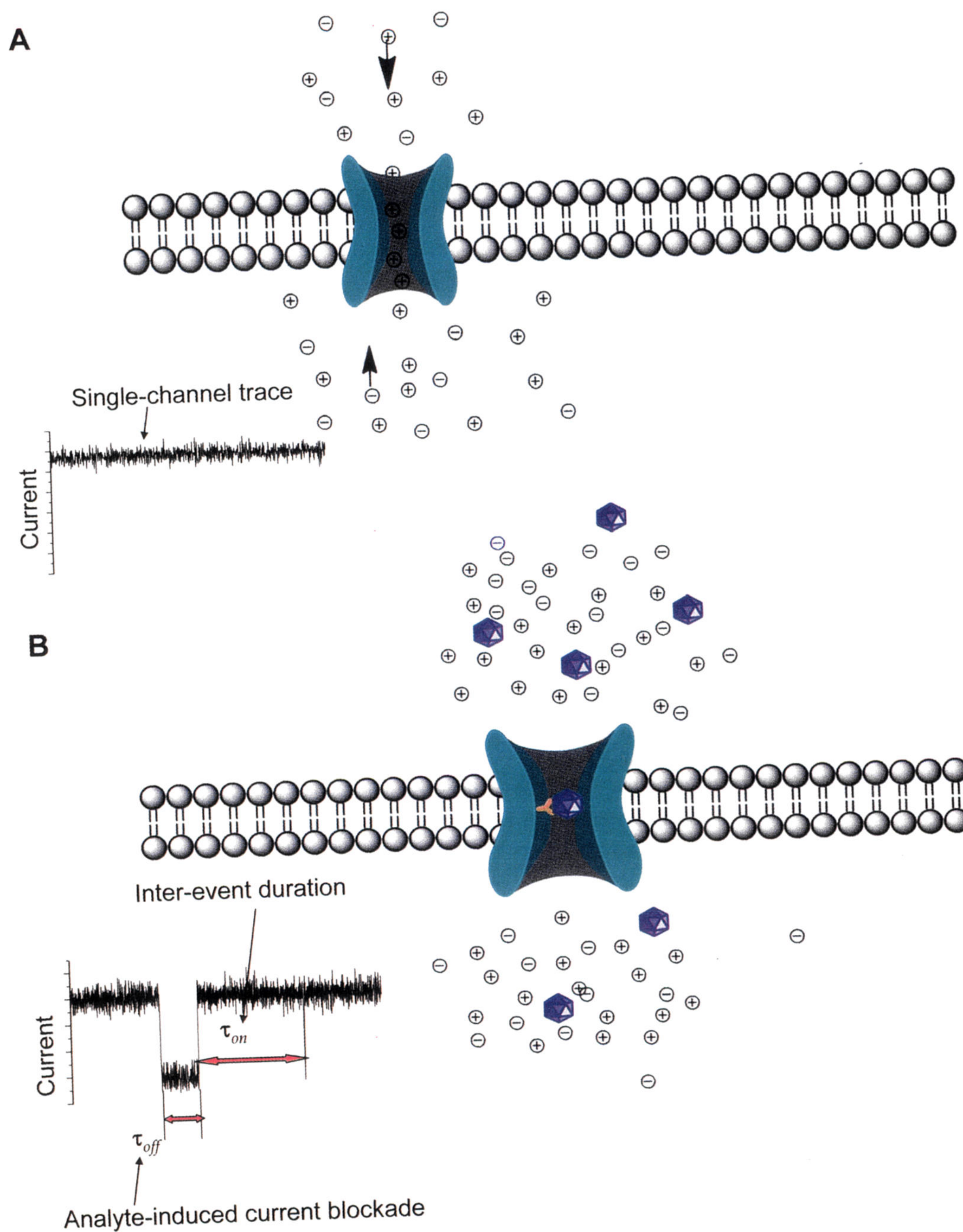


Fig. 1 Principle of stochastic sensing with a permanently open protein nanopore. An open protein nanopore represents the permeation pathway for transmembrane transport of small ions. **A** When a transmembrane potential is applied, a single-channel electrical current is readily recorded. **B** The nanopore interior is either genetically engineered or chemically modified by covalent attachment of a functional reactive group. Analytes are added to one of the chambers and transiently interact with the functional reactive group located within the pore interior. This transient interaction results in a well-defined single-channel current blockade, the nature of which depends on the strength of the interaction between the analyte molecule and the functional reactive group

staphylococcal α-hemolysin (αHL) protein. αHL forms a mushroom-shaped heptameric transmembrane pore in planar lipid bilayers (Fig. 2A) (Song et al. 1996). This protein is a highly tractable β-barrel-type pore because of the following unique combination of attributes: (i) the availability of its high-resolution crystal structure (Song et al. 1996), (ii) the ease of its genetic engineering (Bayley and Cremer 2001; Movileanu et al. 2001), (iii) its large single-channel conductance (Bezrukov et al. 1996; Kasianowicz et al. 1996), which facilitates high time-resolution single-channel electrical recordings, (iv) its high thermal stability (Kang et al. 2005), (v) the pore remains open indefinitely under a wide range of experimental conditions, such as pH (Bezrukov and Kasianowicz 1993; Kasianowicz and Bezrukov 1995), ionic strength (Krasilnikov et al. 2006; Rodrigues et al. 2008), temperature (Kang et al. 2005; Jung et al. 2006), transmembrane potential (Movileanu et al. 2005) and osmolytes (Bezrukov et al. 1996; Krasilnikov and Bezrukov 2004). These attributes made the αHL protein a suitable nanopore platform for single-molecule stochastic sensing of small molecules and large biomolecules (Bayley and Martin 2000; Bayley and Cremer 2001; Howorka et al. 2001b; Bayley 2006; Rhee and Burns 2007; Branton et al. 2008; Griffiths 2008; Hayden 2008; Howorka and Siwy 2008, 2009; Movileanu 2008).

2. Watching polypeptide translocation

In the last decade, several groups have extensively examined the translocation of polypeptides through protein pores using single-channel electrical recordings. These studies, which employed cyclic petides (Sanchez-Quesada et al. 2000), and α-helical (Movileanu et al. 2005; Sutherland et al. 2005; Stefureac et al. 2006) and β-hairpin (Goodrich et al. 2007) polypeptides, revealed specific kinetic signatures of polypeptide-pore interactions. These kinetic signatures depended upon biophysical features of the translocating polypeptides, such as their charge, length, folding and stability. In general, the single-channel electrical recordings showed very short-lived current blockades assigned to bumpings of the polypeptide into the pore entrance and long-lived current blockades assigned to major partitionings of the polypeptide into the interior of the pore. One simple model of the interaction between a polypeptide and a protein pore can be illustrated by a two-barrier, single-well free-energy lansdcape (Movileanu et al. 2005; Wolfe et al. 2007; Movileanu 2008). This kinetic scheme was observed with α-helical peptides (Movileanu et al. 2005; Wolfe et al. 2007). In contrast, other studies showed different kinetic schemes. For example, in a recent study, we combined single-molecule electrical recordings and Langevin dynamics simulations to explore the partitioning of β-hairpin peptides into the αHL protein pore (Fig. 2) (Goodrich et al. 2007). The dwell time derived from event histograms showed a single exponential decay-dependence on the applied transmembrane potential (Fig. 3). This finding suggested that the β-hairpin-pore interaction undergoes a single-barrier free energy lanscape. Moreover, we showed that highly unfolded β-hairpin polypeptides entered the pore in an extended conformation, producing fast single-file translocation events. This result contrasted with that of the translocation of structured β-hairpin polypeptides, which occurred more slowly, producing long-lived current blockades (Goodrich et al. 2007). In Fig. 3, we illustrate that more strutured β-hairpins, K41 and G41, translocate more slowly than the unstructured polypep-

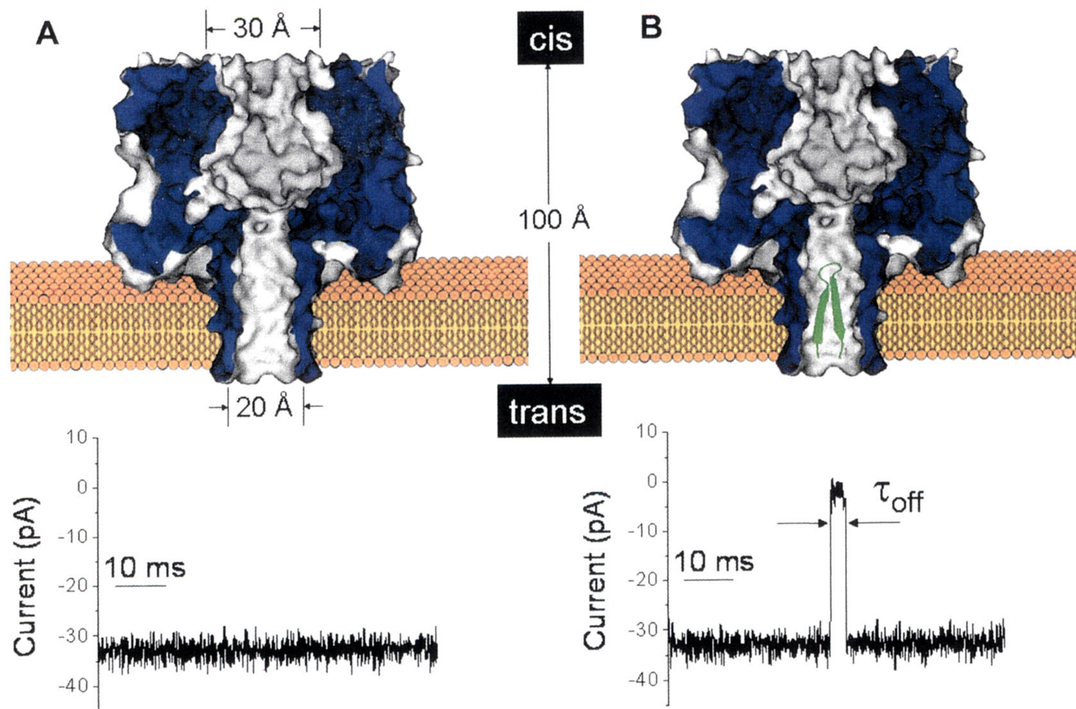


Fig. 2 The interaction of β-hairpin peptides with an aHL pore produces transient current blockades: **A** αHL forms a pore that remains open for long periods. **B** The translocation of a β-hairpin peptide through an αHL pore produces a transient current blockade. Reproduced, with permission, from Goodrich et al. (2007)

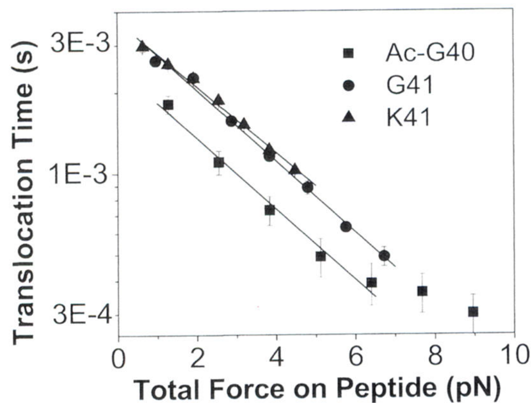


Fig. 3 The dependence of the dwell time of the current blockades produced by the **β**-hairpin peptides on the electric force. The electric force was derived as $F = qV/l$, where the effective charge q is half of the peptide charge, V is the transmembrane potential, and l = 50 Å is the length of the β-barrel lumen. Reproduced, with permission, from Goodrich et al. (2007)

tide Ac-G40. This finding is presented by an increased translocation time measured for more structured peptides regardless of the applied total force on peptide (Fig. 3).

Extensive research on polypeptide sensing with nanopores has been pursued by the group of Jeremy Lee (Sutherland et al. 2005; Stefureac et al. 2006, 2008; Stefureac and Lee 2008). They showed that the αHL protein pore can be employed to discriminate several polypeptides based upon their size and conformation (Sutherland et al. 2005; Stefureac et al. 2006). In addition, these studies revealed that the electrical recordings hold potential for discovering the presence of intermediate conformations of the polypeptides, which were not apparent from the circular dichroism (CD) spectra (Stefureac et al. 2008; Stefureac and Lee 2008).

One way to alter the kinetics of the

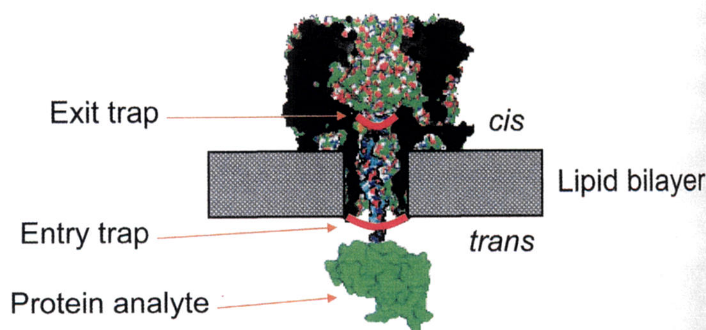


Fig. 4 The αHL protein pore is equipped with electrostatic traps engineered at the entry and exit of the channel. A single electrostatic trap consists of an iris of seven negatively charged aspartic acid residues. Reproduced, with permission, from Mohammad et al. (2008)

polypeptide-pore interactions is to engineer attractive or repulsive groups within the pore interior. These engineered groups might impact the free energy lansdcape of the polypeptide-pore interactions. Recently, we were able to engineer attractive groups, simply called traps, within the interior of the αHL protein pore. Two irises of negatively charged traps have been engineered at the entry and exit of the β-barrel part of the αHL protein pore (Fig. 4) (Wolfe et al. 2007). The engineered traps consisted of aspartic acid residues. In a first study, we examined the translocation of short positively charged polypeptides (~25 residues in length) through αHL protein pores equipped with engineered traps (Wolfe et al. 2007). The label-free single-channel recordings enabled the simultaneous determination of the rate constants of association (k_{on}) and dissociation (k_{off}) (Mohammad et al. 2008; Mohammad and Movileanu 2008). k_{on} is defined as $1/(c\tau_{on})$, where τ_{on} is the inter-event interval, and c is the concentration of the substrate in the bilayer chamber. k_{off} is defined as $1/\tau_{off}$, where τ_{off} is the mean blocked time of the transient substrate-induced current blockades (Fig. 1B). The dissociation constant is given by $K_d = k_{off}/k_{on}$, and the association constant is given by $K_a = 1/K_d$, allowing for the calculation of the standard free energy $\Delta G^o = -RT \ln K_a$. The electrostatic trap engineered at the entry of the pore had a major impact on the rate constant of association (Wolfe et al. 2007). By contrast, the rate constant of dissociation was only marginally altered. Surprisingly, when the entry and exit traps were concurrently engineered within the interior of the nanopore, significant increase in the rate constants of association and dissociation were observed. By analogy with ion-conducting protein channels, these negatively charged traps acted as binding sites for the positively charged polypeptide analyte. The results of this study indicated that the two traps together catalyze the net flow of polypeptides from one side of the membrane to the other, as compared with either the wild type-αHL pore or a single trap-containing αHL protein pore.

In a subsequent study, a similar experimental design was used for examining the polypeptide–pore interactions, but with a much larger interacting protein. In this case, the analyte was a fusion protein (pb_2-Ba) that consisted of a positively charged presequence polypeptide of cytochrome b_2 (pb_2) fused to the folded ribonuclease barnase (Fig. 4) (Mohammad et al. 2008). The results were significantly different from those obtained with short polypeptides. For example, the addition of nanomolar concentrations of pb_2-Ba protein to the chamber produced infrequent and short-lived current blockades with the WT-αHL protein pore (Fig. 5A). This finding was interpreted in terms of a large energetic penalty of the folded protein to

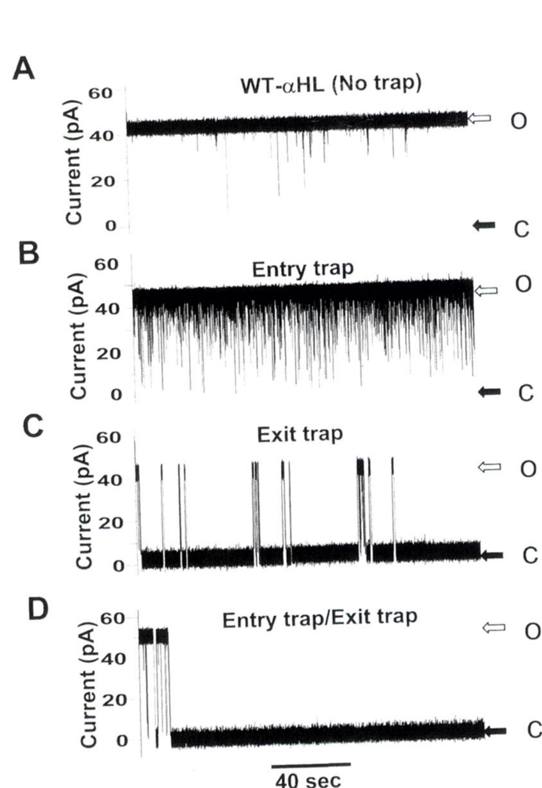


Fig. 5 The effect of the barnase protein on the single-channel electrical recordings performed with the wild-type (WT) and electrostatic trap-containing αHL pores. Representative single-channel electrical recordings with the WT-αHL (**A**), K131 D_7 (**B**), K147 D_7 (**C**) and K131 D_7/K147 D_7 (**D**) pores in the presence of 200 nM pb_2(95)-Ba added to the chamber. The white and black arrows indicate the open and closed states, respectively. Reproduced, with permission, from Mohammad et al. (2008)

partition into the interior of the trap-free αHL protein pore. In contrast, single-channel recordings with an αHL protein pore, which contained a single trap engineered at the entry of the pore, revealed a dramatically increased frequency of the current blockades (Fig. 5B). The event frequency increased by almost two orders of magnitude, as compared with the WT-αHL pore. This finding was consistent with an effective recruitment of the small folded proteins by the engineered electrostatic trap, which was exposed to the aqueous phase. Interestingly, when the electrostatic trap was engineered at the exit of the β-barrel (Fig. 4), away from the aqueous phase, the transient current blockades became long lived, with duration in the range of tens of seconds to minutes (Fig. 5C). The different results from experiments in which the trap was engineered either at the entry or at the exit of the pore illuminated a significantly altered kinetics of the folded protein-pore interactions. Remarkably, the observed transient current blockades became permanent when the pb_2-Ba protein interacted with double trap-containing αHL protein pore (Fig. 5D). These results demonstrate that protein design is instrumental in obtaining major alterations in the single-channel electrical signature as a result of minute changes within the interior of the pore.

Using a similar approach, Guan and colleagues have designed αHL protein pores with hydrophobic traps engineered within the interior of the pore (Zhao et al. 2009b). They studied the translocation of polypeptides containing hydrophobic aminoacids through such hydrophobic traps-containing αHL protein pores. As expected, they found increased interactions between hydrophobic residues-containing peptides and a trap-containing αHL protein pore. Increasing lengths of the translocating peptide produced decreased rate constants of association and dissociation. On the other hand, additional engineering of the αHL protein pore, in the form of many aromatic binding sites, resulted in a stronger binding affinity between the translocating peptide and the hydrophobic trap-containing αHL protein pore. This study is also important, because Guan and colleagues were able to discriminate among polypeptides that differed by a single residue. Therefore, it was suggested that the stochastic biosensing with engineered protein pores holds great promise for peptide and protein sequencing.

3. Probing protein-ligand interactions

Probably, the most important limitation of polypeptide translocation through nanopores is the inability to capture all kinetic information of the polypeptides in the aqueous phase. This limitation has been overcome by attaching a flexible linker, a poly(ethylene glycol) (PEG) chain, to the interior of the nanopore, within the large vestibule of the αHL protein pore (Howorka et al. 2000; Movileanu et al. 2000). A biotinyl ligand was permanently attached to the untethered end of the PEG linker. The biotinyl group was able to move across the interior of the nanopore from one side of the membrane to the other, a distance of ~100 Å. If a low-affinity streptavidin mutant was added to the chamber, a distinctive alteration in the single-channel current occurred (Movileanu et al. 2000). This experimental design demonstrated that the single-molecule stochastic sensing of proteins can be fundamentally expanded into the aqueous phase. Inspired by this experimental design, Kong and Muthukumar (2005) performed Langevin molecular dynamics simulations and Poisson-Nernst-Planck calculations to obtain the current fluctuations produced by a single PEG covalently anchored within the large vestibule of the αHL protein pore (Kong and Muthukumar 2005). This computational work revealed the dynamics of the single-molecule captures of the binding protein in the aqueous phase.

A similar approach was employed to examine the binding kinetics of a lectin protein to one or more ligands (Howorka et al. 2004). These experiments showed the ability to detect monovalent and divalent binding events directly. Moreover, a genetically encoded stochastic sensor element has been used for sensing proteins at the single-molecule level (Cheley et al. 2006). In this investigation, an ezymatic peptide inhibitor was engineered in one subunit of the αHL protein nanopore near the pore entrance. Individual binding events of the peptide inhibitor to the catalytic subunit of the cAMP-dependent protein kinase (PKA) were detected through the modulation of the single-channel electrical current. This methodology provided detailed kinetic and thermodynamic information of the peptide–enzyme interactions. This aproach holds potential for rapid screening of kinase inhibitors.

Guan and colleagues have employed an engineered αHL protein pore that contained an iris of seven phenylalanine residues to study the protease activity at the single-molecule level. The hydrophobic trap within the interior of the nanopore functioned as a binding site for hydrophobic residue-containing amyloid-β peptide (Zhao et al. 2009a). Therefore, long-lived current blockades were recorded when this peptide was added to the chamber. Remarkably, the frequency of amyloid-β peptide-induced current blockade was significantly reduced in the presence of trypsin, a serine protease that cleaves peptide bonds after arginine and lysine. Moreover, the frequency of events was also dependent on time, suggesting that this experimental design might be used to obtain a quantitative chemical kinetic information on the enzymatic process. Again, these experiments have proven that the engineered αHL protein pore might be used to examine the enzyme-protein interactions.

4. Detection of proteins with synthetic nanopores

Single-molecule detection of proteins with protein nanopores proves to have a number

of disadvantages and limitations, such as the inability to tune the diameter of the nanopore and limited stability of the protein nanopore-lipid bilayer system under harsh experimental conditions of salt concentration, pH, applied transmembrane potential and other osmotic stress. This daunting challenges might be overcome by the fabrication of solid-state nanopores, which exhibit obvious advantages, such as the accurate control over the inner diameter and the length of the nanopore, as well as a significantly enhanced stability for long periods (Li et al. 2001; Storm et al. 2003; Dekker 2007). Therefore, a recent natural expansion of the single-molecule studies of polypeptides with protein nanopores was the initiation of experiments with synthetic nanopores (Siwy et al. 2005; Han et al. 2006, 2008; Fologea et al. 2007; Sexton et al. 2007; Talaga and Li 2009). In 2005, the first protein biosensor, based on a synthetic nanopore, was designed by Charles Martin's group (Siwy et al. 2005). In this case, the nanopore was a conically shaped gold nanotube reconstituted into a 12-µm-thick poly-ethylene terephthalate membrane. The strategy was to functionalize the small diameter of the conical nanopore with a reactive group. The polymeric membrane was coated with a single layer of Au that functioned as a chemically reactive group for the permanent attachment of a molecular-recognition agent (MRA). Remarkably, such a detector was versatile to a broad range of MRA applications, including protein–ligand (e.g. streptavidin–biotin) and antibody-binding protein (e.g. immunoglobulin IgG–protein-G) complexes. A permanent current blockade was recorded upon the binding of each analyte with the corresponding MRA. The same research team expanded their ability to probe selectively protein analytes with synthetic nanopores (Sexton et al. 2007). In this case, the nanopore element was a PEG-functionalized gold conical nanotube. Bovine serum albumin (BSA) and a Fab fragment from a BSA-polyclonal antibody were detected from the distinctive signature of the current blockades made by the free BSA and the BSA–Fab complex. The Fab fragment was used as an anti-BSA interacting molecule with high binding affinity for the BSA protein. The sensing element was also tested for proteins that do not bind to Fab. It would be interesting to design an experimental protocol that employs proteins with varying binding affinity, so they produce distinctive transient current blockades.

Several research teams pursued single-molecule detection of proteins with single nanopores based on silicon nitride membranes (Han et al. 2006, 2008; Fologea et al. 2007; Talaga and Li 2009). The use of synthetic nanopores has an array of advantageous features, including a greater robustness of the solid-state membranes, the ability to tune the diameter of the nanopore, and the potential for either integration into a *lab-on-a-chip* platform or parallelization for high-throughput devices. Therefore, it is conceivable that the single-molecule studies performed with protein nanopores might be expanded to incorporate a much wider spectrum of applications and harsh conditions of experimentation. For example, temperature-dependence experiments with synthetic nanopores would enable the measurement of kinetic and thermodynamic enthalpies and entropies of the complex formation between a protein and a ligand, revealing information about which process (e.g. enthalpic or entropic) in the ligand-binding protein interactions is dominant. On the other hand, the use of solid-state nanopores has limitations (Griffiths 2008). One obvious challenge is to attach a single functional reactive group within a strategic position of the interior of the synthetic nanopore. This approach would allow transient bindings of a protein analyte to a ligand engineered within a synthetic nanopore.

5. Exploring protein folding and stability

Protein pores and solid-state nanopores were also used as sensor elements for folding and stability features of single proteins. We engineered an exogenous peptide loop within the large vestibule of the aHL protein pore, a cavity with a volume of ~39 500 Å^3 (Jung et al. 2006). The exogenous peptide was an elastine-like-polypeptide (ELP) that undergoes an inverse temperature transition, and with the amino acid sequence $(VPGGG)_5$. It is known that ELPs dehydrate and hydrophobically collapse at greater temperatures, above the inverse transition temperature, and unfold at temperatures below the inverse transition temperature (Urry 1997; Manno et al. 2001; Meyer and Chilkoti 2002, 2004; Urry et al. 2002; Hyun et al. 2004; Zhang et al. 2006). A comprehensive model that shows the conformational fluctuations of the engineered ELP within the large vestibule of the αHL protein pore is depicted in Fig. 6. We probed the hydrophobic collapse of the engineered ELP loop via significant alterations in the temperature-dependent amplitude of the ELP-induced current blockades. For example, at temperatures below its transition temperature, the ELP loop was fully expanded, so it blocked the pore completely, but reversibly (Fig. 7A) (Jung et al. 2006). In contrast, at temperatures above the transition temperature, the ELP excursions into the narrowest region of the interior of the nanopore did not produce a full blockade (Fig. 7B, C). In this case, a substantial flow of ions occurred, because of the hydrophobic collapse of the ELP. These charateristics of the transient ELP-induced

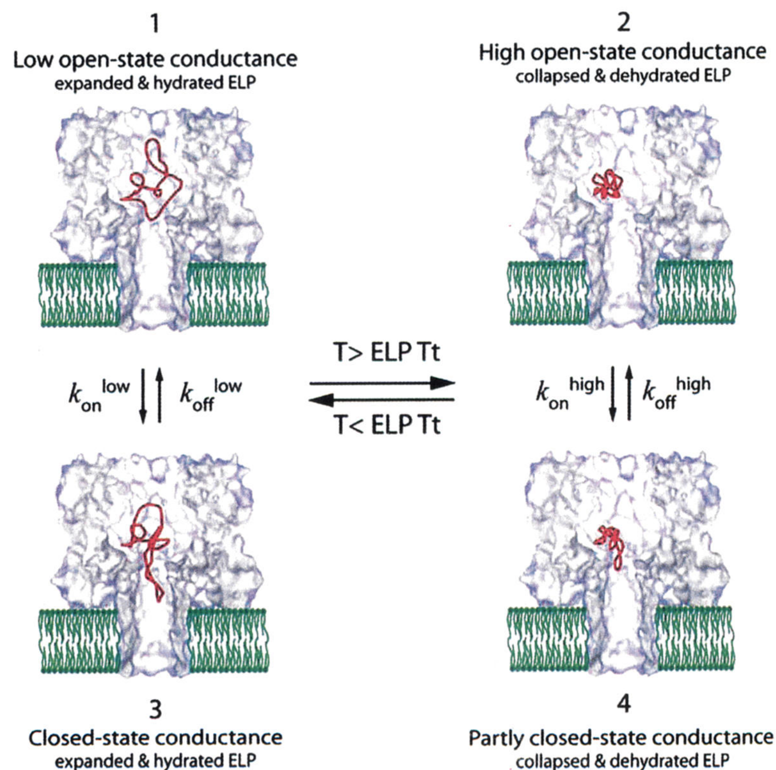


Fig. 6 Model for the temperature-dependent conformational fluctuations of a single ELE loop-containing αHL protein pore. Reproduced, with permission, from Jung et al. (2006)

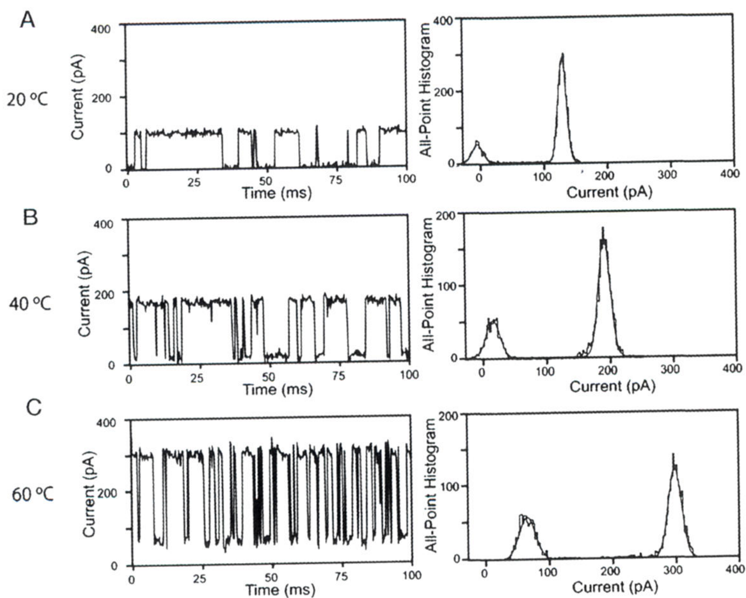


Fig. 7 Single-channel electrical recordings with a single ELP-containing αHL protein pore recorded at different temperatures. Temperature-dependence of the single-channel current through the temperature-responsive pore $E_{10}W_6$, an αHL protein containing a 50-residue long ELP loop, is recorded at (**A**) 20, (**B**) 40 and (**C**) 60 °C. Reproduced, with permission, from Jung et al. (2006)

current blockades were also dependent on the ELP sequence. A test peptide loop with the same length (~50 residues), which was rich in glycine and serine residues, produced a different single-channel electrical signature comprised of highly frequent and very short-lived current spikes. We interpreted that the current spikes were consistent with a substantially increased peptide flexibility because of the glycine residues that have less bulky side chains. These experiments demonstrate our ability to probe conformational fluctuations and flexibilities of the polypeptides engineered within the large vestibule of the nanopore interior. This approach might be expanded to other applications. For example, other engineered αHL pores with responsive polypeptides might be developed. Protein pores with stimulus-activated (e.g., photo-responsive peptides) gating mechanisms might be useful both for basic science studies and the design of new engineered nanopore-based biosensor platforms.

Loic Auvray and colleagues used the αHL protein pore to explore the folding properties of proteins (Oukhaled et al. 2007). These studies were pursued under naturing and denaturing conditions. The protein unfolding was induced by elevated concentrations of guanidinium hydrochloride (Gdm-HCl), a chemical denaturant. The protein under investigation was the 370-residue maltose binding protein of *Escherichia coli* (MBP). The single-channel electrical recordings were performed at different concentrations of Gdm-HCl. In the absence of the denaturing agent, no current blockades were recorded, indicating a large energetic penalty of the protein to interact with the αHL pore. Interestingly, they found protein-induced current blockades in the presence of increased concentrations of Gdm-HCl, suggesting that the MBP protein unfolds at elevated concentrations of chemical denaturant in the chamber. Moreover, the frequency and duration of the protein-induced current blockades was dependent on the concentration of Gdm-HCl in the chamber. This finding was consistent with a clear correlation between the partioning of the MBP protein into the nanopore interior and the folding state of the MBP protein in the aqueous phase.

The thermodynamic stability of a single protein might be altered by a chemical denaturant, temperature or pH. Alternatively, thermodynamic unfolding induced by a chemical denaturant or temperature might be replaced by spontaneous unfolding via single-site mutagenesis within a critical site of the protein. Jeremy Lee and colleagues used the αHL protein pore to demonstrate that the translocation of a protein analyte, a histidine-containing protein (HPr), is strongly dependent upon single-site mutations that modify its folding state (Stefureac et al. 2008). HPr exhibits a low activation free-energy of unfolding HPr (~5 kcal/mol) and this is consistent with the fragile folding state of HPr, as illuminated by single-channel electrical recordings (Stefureac et al. 2008).

Very recently, Talaga and Li (2009) employed silicon nitride-based nanopores to discriminate among folded, partially unfolded and unfolded single proteins (Talaga and Li 2009). The proteins under investigation were β-lactoglobulin and histidine-containing phosphocarrier protein. Interestingly, their findings demonstrated that the proteins translocated through silicon nitride-based nanopores under linear or looped conformation and the applied electrical force can contribute to the protein unfolding during transit.

6. Probing protein-nucleic acid complexes with nanopores

In the previous sections of this chapter, we showed that nanopore research has been employed in the examination of recognition templates, such as small ligand–protein (Movileanu et al. 2000; Howorka et al. 2004; Mayer et al. 2008), peptide–protein (Xie et al. 2005; Cheley et al. 2006) and antibody–protein (Siwy et al. 2005; Sexton et al. 2007; Han et al. 2008) complexes. However, several groups have started to investigate the subtle interactions of the binding proteins with their nucleic acid targets (Astier et al. 2007; Benner et al. 2007; Hornblower et al. 2007; Zhao et al. 2007; Smeets et al. 2009). Nucleic acid-protein interaction is fundamental and ubiquitous in modern biology and medical biotechnology. Therefore, with more adaptation of the nanopore probe techniques, parallel nanopore-based arrays might be used in the future for high-throughput devices in protein sensing and proteomics. Li-Qun Gu's group used nanopores to investigate aptamers and aptamer-binding protein complexes (Shim and Gu 2008; Shim et al. 2009). Aptamers are short nucleic acid sequences that bind with enhanced binding affinity to small organic molecules, polypeptides, and proteins. Li-Qun Gu and colleagues examined the single-molecule detection of thrombin-binding aptamer (TBA), a G-quadruplex nucleic acid, in the presence of divalent cations (Shim and Gu 2008). In this case, the large vestibule of the pore was used to trap single thrombin molecules through the electrophoretic insertion of a single G-quadruplex oligonucleotide. This study demonstrates a remarkable power of the nanopore detector for sensing the DNA/RNA aptamers. There are numerous advantages of the DNA/RNA aptamers as molecular recognition elements in biosensing when compared with traditional detection methodologies using enzymes and antibodies (Song et al. 2008). These include the following: i) enhanced specificity, selectivity, and affinity from small molecules to folded protein domains, enabling the design of high-performance aptamer-based biosensors; ii) extraordinary chemical stability, thereby offering exceptional flexibility and convenience in their design; iii) conformational changes upon target binding, conferring a great flexibility in designing novel

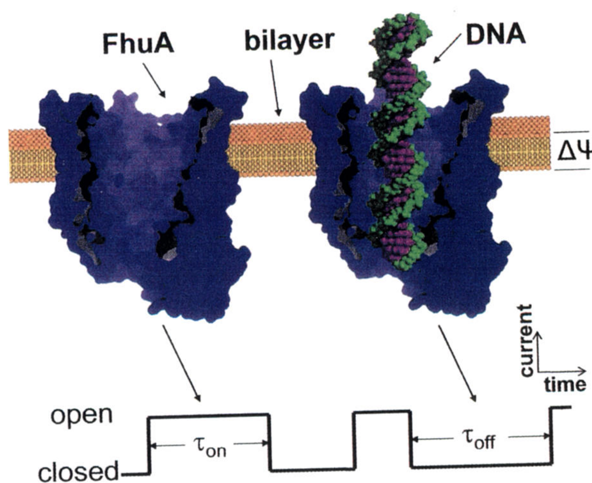


Fig. 8 Translocation of double-stranded DNA (dsDNA) through a single cork-free FhuA-based nanopore placed in a planar lipid bilayer. dsDNA is electrically driven by the transmembrane potential ($\Delta\psi$). The frequency of occurrence ($\sim 1/\tau_{on}$) of the events reveals the concentration of dsDNA in the bulk aqueous phase, whereas the current signature (the mean duration τ_{off} and amplitude of the transient current blockades) reveals its identity (length and structure, respectively). Stochastic sensing of dsDNA provides useful kinetic data that are difficult to obtain with other traditional techniques. In a simple equilibrium, $\tau_{off} = 1/k_{off}$, where k_{off} is the dissociation rate constant, and $\tau_{on} = 1/k_{on}[C_{DNA}]$, where k_{on} is the association rate constant and $[C_{DNA}]$ is the DNA concentration in the aqueous phase

biosensors with unprecedented detection sensitivity.

Other groups used protein and solid-state nanopores for quantitative explorations of the biophysical and biochemical characteristics of the complexes between proteins and nucleic acids, such as the voltage threshold for complex dissociation (Zhao et al. 2007), the sequence-specific sensitivity detection of individual DNA polymerase (Benner et al. 2007), the underlying equilibrium kinetics and thermodynamics of protein–nucleic acid interactions (Hornblower et al. 2007), the motor activity of RNA-processing enzymes (Astier et al. 2007), the transit time of the protein–nucleic acid complex within the nanopore (Smeets et al. 2009), and DNA polymerase activity (Cockroft et al. 2008). In general, these complexes are much bulkier than single-stranded DNA (ssDNA) or unfolded polypeptides. Therefore, nanopores designed for these applications should have

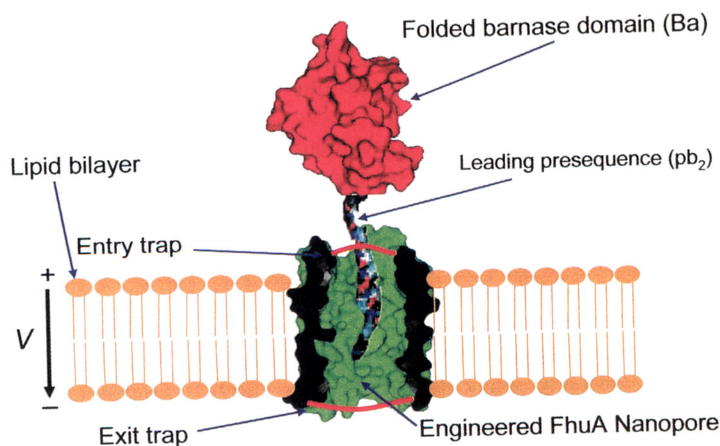


Fig. 9 Clamping a single pb_2-Ba protein with electrostatic traps. The two electrostatic traps (in red), made from aspartic acids, are shown on the entry and exit of the cork-free FhuA-based nanopore. Partitioning of a single pb_2-Ba protein into the nanopore lumen should be observed by a transient current blockade. Both proteins are represented at the same scale

a larger internal diameter, in the range of several nanometers. For example, ferric hydroxamate uptake component A (FhuA) (Ferguson et al. 1998; Locher et al. 1998), a monomeric β-barrel protein might be used for stochastic sensing of double-stranded DNA (dsDNA) (Fig. 8) and proteins (Fig. 9). FhuA is a member of the superfamily of bacterial outer membrane proteins. The principles underlying the functionality of this sensor element are inspired by natural processes: translocation of phage DNA (Letellier et al. 1999; Gurnev et al. 2006) and folded proteins (Hill et al. 1998; Hinnah et al. 2002; Muro et al. 2003; Becker et al. 2005) through a β-barrel protein channel.

Concluding remarks

In this chapter, we discussed a number of ways by which short polypeptides and small folded proteins might be examined using single-molecule nanopore probe techniques and stochastic sensing. The underlying detection principle can be applied to both natural and synthetic nanopores. The protein pores have the advantageous feature of site-directed functionalization and chemical modification within strategic positions of the nanopore interior. In analogy to their biological counterparts, synthetic nanopores are used as stochastic sensors to probe single proteins and their complexes with interacting ligands (Han et al. 2006, 2008; Talaga and Li 2009). The detection mechanisms of polypeptides and folded proteins include genetic engineering and chemical modification of protein nanopores, functionalization of the inner surface of solid-state nanopores and electrophoretic capture through movable nucleic acid arms. Critically important, nanopore probe techniques enable exploration of specific features of proteins such as their enzymatic activity (Xie et al. 2005; Cheley et al. 2006) and binding affinity (Movileanu et al. 2000; Howorka et al. 2004) at the single-molecule level. It is worth mentioning that this methodology enables the determination of these features without resorting to labeling of the interacting molecules (Movileanu 2009), avoiding several disadvantages of the detection using fluorescent dyes.

On the other hand, coupling a nanopore probe technique to an optical or fluorescence platform is expected to enhance our knowledge about the underlying mechanisms of protein translocation. The translocation of proteins through a single protein nanopore represents a critical process in protein unfolding through proteosome channel and protein traffic across mitochondrial cell membranes (Matouschek and Glick 2001; Huang et al. 2002; Matouschek 2003; Prakash and Matouschek 2004; Shariff et al. 2004; Wilcox et al. 2005; Mohammad et al. 2008; Inobe et al. 2008; Prakash et al. 2009). However, in these processes the translocation and unfolding of the protein are two intimately related mechanisms. We anticipate that a biophysical instrument that consists of a solid-state nanopore and an optical trap (Keyser et al. 2006; Dekker 2007) has the potential to illuminate the balance of driving and unfolding forces that act on a protein during its translocation in a linear manner through a nanopore. Such efforts will certainly lead to a better understanding of how proteins undergo conformational transitions when they traverse a nanopore. The experimental studies devoted to protein translocations will inspire new theoretical and computational biophysics studies on protein translocation through nanopores (Li and Makarov 2003, 2004a,b; Kirmizialtin et al. 2004, 2005, 2006; Huang et al. 2005; Tian and Andricioaei 2005; Goodrich et al. 2007; Huang and Makarov 2008a,b; Li et al. 2006; Makarov 2007, 2008).
Moreover, nanopore probe techniques are successfully used to examine the folding state and the flexibility of single proteins (Goodrich et al. 2007; Oukhaled et al. 2007). Despite these recent advances in using nanopores for the exploration of single proteins, more experimentation is needed to better understand the subtle interactions of single proteins with the interior of the nanopores. For example, it is not clearly understood how the flexibility and stability of the protein is altered upon its partitioning into the nanopore interior. How do the proteins interact with the slightly hydrophobic surface of the silicon nitride-based nanopores? What is the magnitude of the electrostatic force between polypeptides and the surface of the protein nanopores? Do the proteins traverse a nanopore in unfolded or partly unstructured conformation? What is the time scale of the

protein unfolding in the interior of the nanopore? It is likely that more experimental work and future advances of nanopore technology will be employed to tackle numerous questions that are pertinent to protein translocation.

Acknowledgements

I am grateful to researchers in my group, Carl P. Goodrich, Aaron J. Wolfe, Mohammad M. Mohammad, David J. Niedzwiecki, Khalil R. Howard, and Robert Bikwemu for their work performed at Syracuse University. I also thank my colleagues Dmitrii Makarov (The University of Texas at Austin, Austin, TX), Andreas Matouschek (Northwestern University, Evanston, IL) and Anatoly Kolomeisky (Rice University, Houston, TX) for stimulating discussions. This work was funded in part by grants from the US National Science Foundation (DMR-0706517 and DMR-1006332), the National Institutes of Health (GM088403-01) and by the Syracuse Biomaterials Institute (SBI).

References

Akeson M, Branton D, Kasianowicz JJ, Brandin E, Deamer DW (1999) Microsecond time-scale discrimination among polycytidylic acid, polyadenylic acid, and polyuridylic acid as homopolymers or as segments within single RNA molecules. Biophys J 77: 3227–3233

Astier Y, Kainov DE, Bayley H, Tuma R, Howorka S (2007) Stochastic detection of motor protein-RNA complexes by single-channel current recording. Chemphyschem 8: 2189–2194

Bayley H (1999) Designed membrane channels and pores. Curr Opin Biotechnol 10: 94–103

Bayley H (2006) Sequencing single molecules of DNA. Curr Opin Chem Biol 10: 628–637

Bayley H, Braha O, Cheley S, Gu LQ (2004) Engineered nanopores. In: Niemeyer CMaMCA (ed) NanoBiotechnology, pp 93–112. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Bayley H, Braha O, Gu LQ (2000) Stochastic sensing with protein pores. Adv Mater 12: 139–142

Bayley H, Cremer PS (2001) Stochastic sensors inspired by biology. Nature 413: 226–230

Bayley H, Jayasinghe L (2004) Functional engineered channels and pores (Review). Mol Membr Biol 21: 209–220

Bayley H, Martin CR (2000) Resistive-pulse sensing – From microbes to molecules. Chem Rev 100: 2575–2594

Becker L, Bannwarth M, Meisinger C, Hill K, Model K, Krimmer T, Casadio R, Truscott KN, Schulz GE, Pfanner N, Wagner R (2005) Preprotein translocase of the outer mitochondrial membrane: reconstituted tom40 forms a characteristic TOM Pore. J Mol Biol 353: 1011–1020

Benner S, Chen RJ, Wilson NA, Abu-Shumays R, Hurt N, Lieberman KR, Deamer DW, Dunbar WB, Akeson M (2007) Sequence-specific detection of individual DNA polymerase complexes in real time using a nanopore. Nat Nanotechnol 2: 718–724

Bezrukov SM (2000) Ion channels as molecular Coulter counters to probe metabolite transport. J Membr Biol 174: 1–13

Bezrukov SM, Kasianowicz JJ (1993) Current noise reveals protonation kinetics and number of ionizable sites in an open protein ion channel. Phys Rev Lett 70: 2352–2355

Bezrukov SM, Vodyanoy I, Brutyan RA, Kasianowicz JJ (1996) Dynamics and free energy of polymers partitioning into a nanoscale pore. Macromolecules 29: 8517–8522

Branton D, Deamer DW, Marziali A, Bayley H, Benner SA, Butler T, Di Ventra M, Garaj S, Hibbs A, Huang X, Jovanovich SB, Krstic PS, Lindsay S, Ling XS, Mastrangelo CH, Meller A, Oliver JS, Pershin YV, Ramsey JM, Riehn R, Soni GV, Tabard-Cossa V, Wanunu M, Wiggin M Schloss JA (2008) The potential and challenges of nanopore sequencing. Nat Biotechnol 26: 1146–1153

Butler TZ, Gundlach JH, Troll M (2007) Ionic current blockades from DNA and RNA molecules in the alpha-hemolysin nanopore. Biophys J 93: 3229–3240

Butler TZ, Pavlenok M, Derrington I, Niederweis M, Gundlach JH (2008) Single-molecule DNA detection with an engineered MspA protein nanopore. Proc Natl Acad Sci U S A 105: 20647–20652

Cheley S, Xie H, Bayley H (2006) A genetically encoded pore for the stochastic detection of a protein kinase. Chembiochem 7: 1923–1927

Cockroft SL, Chu J, Amorin M, Ghadiri MR (2008) A single-molecule nanopore device detects DNA polymerase activity with single-nucleotide resolution. J Am Chem Soc 130: 818–820

Comer J, Dimitrov V, Zhao Q, Timp G, Aksimentiev A (2009) Microscopic mechanics of hairpin DNA translocation through synthetic nanopores. Biophys J 96: 593–608

Deamer DW, Akeson M (2000) Nanopores and nucleic acids: prospects for ultrarapid sequencing. Trends Biotechnol 18: 147–151
Deamer DW, Branton D (2002) Characterization of nucleic acids by nanopore analysis. Acc Chem Res 35: 817–825
Dekker C (2007) Solid-state nanopores. Nature Nanotechnology 2: 209–215
Dorvel B, Sigalov G, Zhao Q, Comer J, Dimitrov V, Mirsaidov U, Aksimentiev A, Timp G (2009) Analyzing the forces binding a restriction endonuclease to DNA using a synthetic nanopore. Nucleic Acids Res 37: 4170–4179
Ferguson AD, Hofmann E, Coulton JW, Diederichs K, Welte W (1998) Siderophore-mediated iron transport: crystal structure of FhuA with bound lipopolysaccharide. Science 282: 2215–2220
Fologea D, Ledden B, McNabb DS, Li J (2007) Electrical characterization of protein molecules by a solid-state nanopore. Appl Phys Lett 91: 053901
Goodrich CP, Kirmizialtin S, Huyghues-Despointes BM, Zhu AP, Scholtz, J. M., Makarov DE, Movileanu L (2007) Single-molecule electrophoresis of beta-hairpin peptides by electrical recordings and Langevin dynamics simulations. J Phys Chem B 111: 3332–3335
Griffiths J (2008) The realm of the nanopore: interest in nanoscale research has skyrocketed, and the humble pore has become a king. Anal Chem 80: 23–27
Gurnev PA, Oppenheim AB, Winterhalter M, Bezrukov SM (2006) Docking of a single phage lambda to its membrane receptor maltoporin as a time-resolved event. J Mol Biol 359: 1447–1455
Han A, Creus M, Schurmann G, Linder V, Ward TR, de Rooij NF, Staufer U (2008) Label-free detection of single protein molecules and protein-protein interactions using synthetic nanopores. Anal Chem 80: 4651–4658
Han A, Schurmann G, Monding G, Bitterli RA, de Rooij NF, Staufer U (2006) Sensing protein molecules using nanofabricated pores. Appl Phys Lett 88: 093901
Hayden T (2008) Threading proteins through a nanopore needle. Anal Chem 80: 3955
Henrickson SE, Misakian M, Robertson B, Kasianowicz JJ (2000) Driven DNA transport into an asymmetric nanometer-scale pore. Phys Rev Lett 85: 3057–3060
Hill K, Model K, Ryan MT, Dietmeier K, Martin F, Wagner R, Pfanner N (1998) Tom40 forms the hydrophilic channel of the mitochondrial import pore for preproteins. Nature 395: 516–521
Hinnah SC, Wagner R, Sveshnikova N, Harrer R, Soll J (2002) The chloroplast protein import channel Toc75: Pore properties and interaction with transit peptides. Biophys J 83: 899–911
Hornblower B, Coombs A, Whitaker RD, Kolomeisky A, Picone SJ, Meller A, Akeson M (2007) Single-molecule analysis of DNA-protein complexes using nanopores. Nat Methods 4: 315–317
Howorka S, Bayley H (2002) Probing distance and electrical potential within a protein pore with tethered DNA. Biophys J 83: 3202–3210
Howorka S, Cheley S, Bayley H (2001a) Sequence-specific detection of individual DNA strands using engineered nanopores. Nat Biotechnol 19: 636–639
Howorka S, Movileanu L, Braha O, Bayley H (2001b) Kinetics of duplex formation for individual DNA strands within a single protein nanopore. Proc Natl Acad Sci U S A 98: 12996–13001
Howorka S, Movileanu L, Lu XF, Magnon M, Cheley S, Braha O, Bayley H (2000) A protein pore with a single polymer chain tethered within the lumen. J Am Chem Soc 122: 2411–2416
Howorka S, Nam J, Bayley H, Kahne D (2004) Stochastic detection of monovalent and bivalent protein-ligand interactions. Angew Chem Int Ed Engl 43: 842–846
Howorka S, Siwy Z (2008) Nanopores: generation, engineering and single-molecule applications. In: Hinterdorfer P (ed) Handbook of single-molecule biophysics, pp In press. Springer-Verlag, Heidelberg, New York
Howorka S, Siwy Z (2009) Nanopore analytics: sensing of single molecules. Chem Soc Rev 38: 2360–2384
Huang L, Kirmizialtin S, Makarov DE (2005) Computer simulations of the translocation and unfolding of a protein pulled mechanically through a pore. J Chem Phys 123: 124903
Huang L, Makarov DE (2008a) The rate constant of polymer reversal inside a pore. J Chem Phys 128: 114903
Huang L, Makarov DE (2008b) Translocation of a knotted polypeptide through a pore. J Chem Phys 129: 121107
Huang S, Ratliff KS, Matouschek A (2002) Protein unfolding by the mitochondrial membrane potential. Nat Struct Biol 9: 301–307
Hyun J, Lee WK, Nath N, Chilkoti A, Zauscher S (2004) Capture and release of proteins on the

nanoscale by stimuli-responsive elastin-like polypeptide "switches". J Am Chem Soc 126: 7330–7335
Inobe T, Kraut DA, Matouschek A (2008) How to pick a protein and pull at it. Nat Struct Mol Biol 15: 1135–1136
Jung Y, Bayley H, Movileanu L (2006) Temperature-responsive protein pores. J Am Chem Soc 128: 15332–15340
Kang XF, Gu LQ, Cheley S, Bayley H (2005) Single protein pores containing molecular adapters at high temperatures. Angew Chem Int Ed Engl 44: 1495–1499
Kasianowicz JJ (2004) Nanopores: flossing with DNA. Nat Mater 3: 355–356
Kasianowicz JJ, Bezrukov SM (1995) Protonation dynamics of the alpha-toxin ion-channel from spectral-analysis of ph-dependent current fluctuations. Biophys J 69: 94–105
Kasianowicz JJ, Brandin E, Branton D, Deamer DW (1996) Characterization of individual polynucleotide molecules using a membrane channel. Proc Natl Acad Sci U S A 93: 13770–13773
Keyser UF, Koeleman BN, van Dorp S, Krapf D, Smeets RMM, Lemay SG, Dekker NH, Dekker C (2006) Direct force measurements on DNA in a solid-state nanopore. Nature Physics 2: 473–477
Kim M-J, Wanunu M, Bell CD, Meller A (2006) Rapid fabrication of uniform size nanopores and nanopore arrays for parallel DNA analysis. Adv Mater 18: 3149–3153
Kirmizialtin S, Ganesan V, Makarov DE (2004) Translocation of a beta-hairpin-forming peptide through a cylindrical tunnel. J Chem Phys 121: 10268–10277
Kirmizialtin S, Huang L, Makarov DE (2005) Topography of the free-energy landscape probed via mechanical unfolding of proteins. J Chem Phys 122: 234915
Kirmizialtin S, Huang L, Makarov DE (2006) Computer simulations of protein translocation. Phys Stat Sol (b) 243: 2038–2047
Kong CY, Muthukumar M (2005) Simulations of stochastic sensing of proteins. J Am Chem Soc 127: 18252–18261
Krasilnikov OV, Bezrukov SM (2004) Polymer partitioning from nonideal solutions into protein voids. Macromolecules 37: 2650–2657
Krasilnikov OV, Rodrigues CG, Bezrukov SM (2006) Single polymer molecules in a protein nanopore in the limit of a strong polymer-pore attraction. Phys Rev Lett 97: 018301
Letellier L, Plancon L, Bonhivers M, Boulanger P (1999) Phage DNA transport across membranes. Res Microbiol 150: 499–505
Li J, Stein D, McMullan C, Branton D, Aziz MJ, Golovchenko JA (2001) Ion-beam sculpting at nanometre length scales. Nature 412: 166–169
Li PC, Huang L, Makarov DE (2006) Mechanical unfolding of segment-swapped protein G dimer: results from replica exchange molecular dynamics simulations. J Phys Chem B Condens Matter Mater Surf Interfaces Biophys 110: 14469–14474
Li PC, Makarov DE (2003) Theoretical studies of the mechanical unfolding of the muscle protein titin: Bridging the time-scale gap between simulation and experiment. J Chem Phys 119: 9260–9268
Li PC, Makarov DE (2004a) Simulation of the mechanical unfolding of ubiquitin: probing different unfolding reaction coordinates by changing the pulling geometry. J Chem Phys 121: 4826–4832
Li PC, Makarov DE (2004b) Ubiquitin-like protein domains show high resistance to mechanical unfolding similar to that of the 127 domain in titin: Evidence from simulations. J Phys Chem B 108: 745–749
Locher KP, Rees B, Koebnik R, Mitschler A, Moulinier L, Rosenbusch JP, Moras D (1998) Transmembrane signaling across the ligand-gated FhuA receptor: crystal structures of free and ferrichrome-bound states reveal allosteric changes. Cell 95: 771–778
Makarov DE (2007) Unraveling individual molecules by mechanical forces: theory meets experiment. Biophys J 92: 4135–4136
Makarov DE (2008) Computer simulations and theory of protein translocation. Acc Chem Res 42: 281–289
Manno M, Emanuele A, Martorana V, San Biagio PL, Bulone D, Palma-Vittorelli MB, McPherson DT, Xu J, Parker TM, Urry DW (2001) Interaction of processes on different length scales in a bioelastomer capable of performing energy conversion. Biopolymers 59: 51–64
Martin CR, Siwy ZS (2007) Chemistry. Learning nature's way: biosensing with synthetic nanopores. Science 317: 331–332
Mathe J, Aksimentiev A, Nelson DR, Schulten K, Meller A (2005) Orientation discrimination of single-stranded DNA inside the alpha-hemolysin membrane channel. Proc Natl Acad Sci U S A 102: 12377–12382
Matouschek A (2003) Protein unfolding – an im-

portant process in vivo? Curr Opin Struct Biol 13: 98–109
Matouschek A, Glick BS (2001) Barreling through the outer membrane. Nat Struct Biol 8: 284–286
Mayer M, Semetey V, Gitlin I, Yang J, Whitesides GM (2008) Using ion channel-forming peptides to quantify protein-ligand interactions. J Am Chem Soc 130: 1453–1465
McNally B, Wanunu M, Meller A (2008) Electromechanical unzipping of individual DNA molecules using synthetic sub-2 nm pores. Nano Lett 8: 3418–3422
Meller A (2003) Dynamics of polynucleotide transport through nanometre-scale pores. J Phys Condens Matter 15: R581-R607
Meller A, Nivon L, Brandin E, Golovchenko J, Branton D (2000) Rapid nanopore discrimination between single polynucleotide molecules. Proc Natl Acad Sci U S A 97: 1079–1084
Meyer DE, Chilkoti A (2002) Genetically encoded synthesis of protein-based polymers with precisely specified molecular weight and sequence by recursive directional ligation: Examples from the elastin-like polypeptide system. Biomacromolecules 3: 357–367
Meyer DE, Chilkoti A (2004) Quantification of the effects of chain length and concentration on the thermal behavior of elastin-like polypeptides. Biomacromolecules 5: 846–851
Mohammad MM, Movileanu L (2008) Excursion of a single polypeptide into a protein pore: simple physics, but complicated biology. Eur Biophys J 37: 913–925
Mohammad MM, Prakash S, Matouschek A, Movileanu L (2008) Controlling a single protein in a nanopore through electrostatic traps. J Am Chem Soc 130: 4081–4088
Movileanu L (2008) Squeezing a single polypeptide through a nanopore. Soft Matter 4: 925–931
Movileanu L (2009) Interrogating single proteins through nanopores: challenges and opportunities. Trends Biotechnol 27: 333–341
Movileanu L, Cheley S, Howorka S, Braha O, Bayley H (2001) Location of a constriction in the lumen of a transmembrane pore by targeted covalent attachment of polymer molecules. J Gen Physiol 117: 239–251
Movileanu L, Howorka S, Braha O, Bayley H (2000) Detecting protein analytes that modulate transmembrane movement of a polymer chain within a single protein pore. Nat Biotechnol 18: 1091–1095
Movileanu L, Schmittschmitt JP, Scholtz JM, Bayley H (2005) Interactions of the peptides with a protein pore. Biophys J 89: 1030–1045
Muro C, Grigoriev SM, Pietkiewicz D, Kinnally KW, Campo ML (2003) Comparison of the TIM and TOM channel activities of the mitochondrial protein import complexes. Biophys J 84: 2981–2989
Oukhaled G, Mathe J, Biance A-L, Bacri L, Betton J-M, Lairez D, Pelta J, Auvray L (2007) Unfolding of proteins and long transient conformations detected by single nanopore recording. Phys Rev Lett 98: 158101
Prakash S, Inobe T, Hatch AJ, Matouschek A (2009) Substrate selection by the proteasome during degradation of protein complexes. Nat Chem Biol 5: 29–36
Prakash S, Matouschek A (2004) Protein unfolding in the cell. Trends Biochem Sci 29: 593–600
Rhee M, Burns MA (2007) Nanopore sequencing technology: nanopore preparations. Trends Biotechnol 25: 174–181
Rodrigues CG, Machado DC, Chevtchenko SF, Krasilnikov OV (2008) Mechanism of KCl enhancement in detection of nonionic polymers by nanopore sensors. Biophys J 95: 5186–5192
Sanchez-Quesada J, Ghadiri MR, Bayley H, Braha O (2000) Cyclic peptides as molecular adapters for a pore-forming protein. J Am Chem Soc 122: 11757–11766
Sexton LT, Horne LP, Sherrill SA, Bishop GW, Baker LA, Martin CR (2007) Resistive-pulse studies of proteins and protein/antibody complexes using a conical nanotube sensor. J Am Chem Soc 129: 13144–13152
Shariff K, Ghosal S, Matouschek A (2004) The force exerted by the membrane potential during protein import into the mitochondrial matrix. Biophys J 86: 3647–3652
Shim JW, Gu LQ (2008) Encapsulating a single g-quadruplex aptamer in a protein nanocavity. J Phys Chem B 112: 8354–8360
Shim JW, Tan Q, Gu LQ (2009) Single-molecule detection of folding and unfolding of the G-quadruplex aptamer in a nanopore nanocavity. Nucleic Acids Res 37: 972–982
Siwy Z, Trofin L, Kohli P, Baker LA, Trautmann C, Martin CR (2005) Protein biosensors based on biofunctionalized conical gold nanotubes. J Am Chem Soc 127: 5000–5001
Smeets RM, Kowalczyk SW, Hall AR, Dekker NH, Dekker C (2009) Translocation of RecA-Coated

Double-Stranded DNA through Solid-State Nanopores. Nano Lett 9: 3089–3096

Song LZ, Hobaugh MR, Shustak C, Cheley S, Bayley H, Gouaux JE (1996) Structure of staphylococcal alpha-hemolysin, a heptameric transmembrane pore. Science 274: 1859–1

Song S, Wang L, Li J, Zhao J, Fan C (2008) Aptamer-based biosensor. Trends Anal Chem 27: 108–117

Stefureac R, Long YT, Kraatz HB, Howard P, Lee JS (2006) Transport of alpha-helical peptides through alpha-hemolysin and aerolysin pores. Biochemistry 45: 9172–9179

Stefureac R, Waldner L, Howard P, Lee JS (2008) Nanopore analysis of a small 86-residue protein. Small 4: 59–63

Stefureac RI, Lee JS (2008) Nanopore analysis of the folding of zinc fingers. Small 4: 1646–1650

Storm AJ, Chen JH, Ling XS, Zandbergen HW, Dekker C (2003) Fabrication of solid-state nanopores with single-nanometre precision. Nat Mater 2: 537–540

Sutherland T.C., Long YT, Stefureac RI, Bediako-Amoa I, Kraatz HB, Lee JS (2005) Structure of peptides investigated by nanopore analysis. Nano Lett 4: 1273–1277

Talaga DS, Li J (2009) Single-molecule protein unfolding in solid state nanopores. J Am Chem Soc 131: 9287–9297

Tian P, Andricioaei I (2005) Repetitive pulling catalyzes co-translocational unfolding of barnase during import through a mitochondrial pore. J Mol Biol 350: 1017–1034

Urry DW (1997) Physical chemistry of biological free energy transduction as demonstrated by elastic protein-based polymers. J Phys Chem B 101: 11007–11028

Urry DW, Hugel T, Seitz M, Gaub HE, Sheiba L, Dea J, Xu J, Parker T (2002) Elastin: a representative ideal protein elastomer. Phil Trans R Soc Lond, B, Biol Sci 357: 169–184

Wanunu M, Sutin J, McNally B, Chow A, Meller A (2008) DNA translocation governed by interactions with solid-state nanopores. Biophys J 95: 4716–4725

Wilcox AJ, Choy J, Bustamante C, Matouschek A (2005) Effect of protein structure on mitochondrial import. Proc Natl Acad Sci U S A 102: 15435–15440

Wolfe AJ, Mohammad MM, Cheley S, Bayley H, Movileanu L (2007) Catalyzing the translocation of polypeptides through attractive interactions. J Am Chem Soc 129: 14034–14041

Xie H, Braha O, Gu LQ, Cheley S, Bayley H (2005) Single-molecule observation of the catalytic subunit of cAMP-dependent protein kinase binding to an inhibitor peptide. Chem Biol 12: 109–120

Zhang Y, Trabbic-Carlson K, Albertorio F, Chilkoti A, Cremer PS (2006) Aqueous two-phase system formation kinetics for elastin-like polypeptides of varying chain length. Biomacromolecules 7: 2192–2199

Zhao Q, de Zoysa RS, Wang D, Jayawardhana DA, Guan X (2009a) Real-time monitoring of peptide cleavage using a nanopore probe. J Am Chem Soc 131: 6324–6325

Zhao Q, Jayawardhana DA, Wang D, Guan X (2009b) Study of peptide transport through engineered protein channels. J Phys Chem B 113: 3572–3578

Zhao Q, Sigalov G, Dimitrov V, Dorvel B, Mirsaidov U, Sligar S, Aksimentiev A, Timp G (2007) Detecting SNPs using a synthetic nanopore. Nano Lett 7: 1680–1685