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Electrostatic Interactions between OmpG Nanopore and Analyte Protein Surface Can Distinguish between Glycosylated Isoforms

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Abstract

The flexible loops decorating the entrance of OmpG nanopore move dynamically during ionic current recording. The gating caused by these flexible loops changes when a target protein is bound. The gating is characterized by parameters including frequency, duration and open-pore current and these features combine to reveal the identity of a specific analyte protein. Here, we show that OmpG nanopore equipped with a biotin ligand can distinguish glycosylated and deglycosylated isoforms of avidin by their differences in surface charge. Our studies demonstrate that the direct interaction between the nanopore and analyte surface, induced by the electrostatic attraction between the two molecules, are essential for protein isoform detection. Our technique is remarkably sensitive to the analyte surface, which may provide a useful tool for glycoprotein profiling.

Graphical Abstract

Nanopore sensors are based on the modulation of ionic current flowing through a nano-scale pore as an analyte translocates through the opening.^{1, 2} Protein nanopores are usually derived from pore-forming proteins with well-defined structures at atomic precision. Solid-state nanopores that are fabricated from synthetic material, have the advantage of being

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robust and easily integrated into microelectronics. Although the technique has been extensively explored for the detection of nucleic acids and single-molecule DNA sequencing,^{3–5} nanopores are also particularly useful for detecting protein molecules.^{6, 7} Nanopore-based detection of protein analytes has used various strategies, including: i) translocation of native analytes through unmodified⁸ and modified pores;^{9, 10} ii) binding of native proteins to nanopores either directly or via tethered high-affinity binding sites such as aptamers,^{11–13} ligands^{14, 15} and peptide binding sequences¹⁶ and iii) binding of proteins to an auxiliary polymer in solution which alters the translocation of polymers through nanopore.^{17–19} As a single molecule analytical tool, nanopores have been used to investigate the biochemical and biophysical properties of proteins, e.g. folding²⁰ and unfolding,^{21–23} protein aggregation,²⁴ ubiquitin linkage type,²⁵ and enzymatic activity^{17, 26}. Recently, the unfolding and threading of a protein through a nanopore by an unfoldase demonstrated the unidirectional and processive translocation of a linearized peptide strand which opens the possibility for nanopore-based protein sequencing.^{27, 28} Another study used a nanopore to identify phosphorylation sites by threading.²⁹

Despite these advances, it remains a challenge to detect a single protein in a complex analyte mixture. Bell *et al.* have recently addressed this problem by introducing DNA carriers containing precisely positioned protein binding sites along a double stranded DNA.¹⁸ Binding of target proteins to the DNA carrier altered the current signature when it translocated through a glass solid-state nanopore. Using this approach, they demonstrated that a single protein species can be identified in a protein mixture. Recently, we have explored an alternate nanopore sensing scheme based on outer membrane protein G (OmpG).³⁰ OmpG from *Escherichia coli* (*E. coli*) is a β -barrel protein with 14 strands connected by seven short turns on the periplasmic side and seven long loops on the extracellular side (Figure 1a).^{31–33} Loop 6 moves dynamically, switching the protein between open and closed conformations resulting in gating signal in the current recording (Figure 1b).^{34–36} Previously we showed that the flexible architecture of the OmpG nanopore can be used to resolve between structurally homologous protein analytes in mixtures.³⁰ Specifically, upon binding to a biotin group tethered to loop 6, a mixture of anti-biotin antibodies raised from different host species was clearly distinguished. Each antibody species was identified by its unique gating fingerprint and no interference from the other antibodies was observed. So far, no other nanopore has shown the ability to differentiate among a mixture of proteins that bind to the same high affinity ligand and share similar structural organization. The principle behind such high selectivity in OmpG is intriguing. It was previously speculated that the antibodies not only bind the biotin ligand but also non-specifically interact with the OmpG loops.³⁰ The latter plays an essential role in distinguishing among similar analytes.

Here, we probe the nature of non-specific interactions between OmpG and target proteins. To do so, we interrogate the interaction between the loops of OmpG and three homologous biotin binding proteins, streptavidin, avidin and extravidin. Our results reveal that electrostatic forces may be the dominating inter-molecular interaction between the OmpG nanopore and the target protein. Such interactions between the positively charged target protein surface and the negatively charged loops of the OmpG sensor are essential for discriminating among protein homologous and isoforms.

RESULTS

Discrimination among Avidin Homologues

The OmpG-PEG₂-biotin nanopore was constructed as previously described (Figure 1).³⁰ First, we investigated the ability of OmpG nanopore to detect the three biotin-binding protein homologues. Streptavidin from *Streptomyces avidinii* and avidin from egg white share ~45% sequence similarity. They have very similar structures, molecular weights and both exhibit high affinity (dissociation constant $\sim 10^{-15}$ M) for the biotin ligand.³⁷ Avidin has a basic isoelectric point (pI~10.5) due to its N-linked glycan composed largely of mannose and N-acetylglucosamine.³⁸ Streptavidin is a non-glycosylated protein with a pI of ~6.3. Extravidin is a commercially available deglycosylated form of avidin with a pI ~6.4, similar to that of streptavidin.

As a control, all three biotin binding proteins did not produce any noticeable change in the wild type OmpG behavior (Figure S1, Supporting information). As observed previously,²³ streptavidin binding to OmpG-PEG₂-biotin resulted in an irreversible decrease in OmpG's gating frequency (Figure 2a). By contrast, avidin increased the gating frequency from 160 ± 24 to 227 ± 26 (Table 1). More importantly, avidin binding resulted in a permanent decrease in the open pore current from 25.8 ± 2.7 to 13.8 ± 1.1 (n=3) (Figure 2b). Extravidin addition resulted in three populations that were categorized by their effect on OmpG gating. The majority (10 of 19 trials) of extravidin binding events caused a gating behavior that was similar to that of streptavidin (Figure 2c): the gating frequency decreased from 160 ± 24 to 30 ± 9 and the open-pore current was not affected. We refer to this population of extravidin as E1. Two minor populations, termed as E2 (6 of 19 trials) and E3 (3 of 19 trials), caused a decrease in the open pore current with E3 showing an effect similar to avidin (Figure S2, Supporting information). The E2 population induced a slightly decreased open pore current but reduced OmpG gating in a fashion similar to that of streptavidin. We surmise that the extravidin gating data may reflect sub-populations in the protein sample that have different levels of deglycosylation. E3 for example seems quite similar to fully intact, fully glycosylated avidin. E1 was most similar to streptavidin and would therefore be fully deglycosylated while E2 may represent a partially deglycosylated form. Because of the variability of extravidin's gating patterns, we focused mainly on data that represent the E1 characteristics for the following experiments. Noise spectral density analysis showed that streptavidin and extravidin had similar spectra while avidin binding induced a lower noise amplitude which was due to its reduced open pore current (Figure 2d). We analyzed five parameters including: open probability, gating frequency, inter-event duration, event duration and open pore current (Table 1, Figure S3, Supporting information)²³. The relative change of these parameters after analyte binding determined the fingerprint of the protein. Clearly, avidin shows a distinct fingerprint relative to the other two biotin-binding proteins (Figure 2e). To our knowledge, this is the first report that demonstrates the discrimination of glycosylated isoforms using protein nanopores.

Because avidin has an identical amino acid sequence to extravidin and is homologous to streptavidin, their key difference lies in their unique surface charges. At pH 6.0, the zeta potential of avidin is 7.8 ± 0.9 mV while streptavidin and extravidin are negatively charged

(Table 2). Our experiments suggest that positive charge on the avidin surface may be the key factor that caused the reduced open-pore current. The seven extracellular loops on OmpG consist of many charged residues including 17 ASP and 8 GLU and 5 ARG and 4 LYS. These create a surface rich in negative charge (Figure S4, Supporting information). We hypothesize that the negative charges on OmpG pull strongly on the positively charged avidin after binding. Thus, the avidin might closely approach the surface of the pore, causing a reduction in open pore current. In addition, the close proximity of avidin might confine the oscillations of loop 6 to a smaller space. This restricted movement might explain why avidin binding causes a higher gating frequency.

Influence of Ionic Strength on Binding

If the reduction of open pore current is dependent on electrostatic interactions with the bound avidin, then we might expect this dependence to be reduced under charge screening conditions. Therefore, we investigated avidin binding at concentrations of KCl ranging from 0.3 M to 4.0 M. We found that the gating pattern of OmpG-PEG₂-biotin after avidin binding was influenced by the salt concentration. Specifically, as the salt concentration increased, avidin binding induced less reduction in the open pore current (Figure 3). The relative open pore current (of the avidin-bound state) increased from 0.6 ± 0.07 pA to 0.98 ± 0.18 pA with increasing salt concentration (Figure 3a, b, c, d, e). At 4.0 M KCl, there was no difference in the open pore current between the bound and unbound states (Figure 3e, f). Thus, our data suggests that electrostatic interactions govern the bound-state open pore conductance because they can be screened with excess salt.

Clearly, other gating characteristics were also strongly influenced by the salt concentration. However, as opposed to the open pore current, the relative changes in these parameters of OmpG gating after avidin binding did not show a strong correlation with increasing ionic strength (Figure S5, Table S1&S2, Supporting information).

Effect of pH on Binding

To further support the idea that electrostatic interactions influence the binding, we investigated the effect of pH on gating. Specifically, by protonating or deprotonating residues at the pore-protein interface, we expect the loop dynamics to change in response to the gain or loss of charges. First, the binding effect of the three analytes to OmpG nanopore was recorded at pH 4.0. For these experiments, we used 1.0 M KCl because the open-pore current of OmpG in buffer pH 4.0, 300 mM KCl was on average 10pA or less which was too small to allow for precise analysis of the gating parameters (Figure S6, Supporting information). At pH 4.0, all three proteins have a net positive charge on their surfaces as indicated by zeta-potential measurements (Table 2). All proteins shared similar gating features (Figure 4a, b, c): (i) a decreased open-pore current albeit to a different level and (ii) a significantly reduced open probability due to elongated closure events (Figure 4e) (Table S3, Table S4, Supporting information). As a control, we verified that none of the proteins bind to the wild type nanopore under any pH condition. (Figure S2, Supporting information). At low pH, streptavidin and extravidin acquire positive charge and, like avidin at pH 6.0, are able to reduce the open pore current when bound (Figure 4). As a result, the fingerprints of these three proteins are almost the same, suggesting that the OmpG nanopore

is not able to distinguish among these protein analytes under acidic conditions (Figure 4d, e).

At pH 10.0, OmpG-PEG₂-biotin pore fluctuates between a fully open (31.5 ± 3.4 pA, O1), partially open (23.8 ± 3.6 pA, O2) states and gating states (Figure 5a, b, c). We define the open probability as the probability of the pore staying at either fully open or partially open state. Power spectral analysis of OmpG-PEG₂-biotin pore does not show a significant change in the noise of the unbound and analyte bound states (Figure 5d) at pH 10. However, streptavidin and extravidin binding slightly increased the open probability due to a 50% reduction in gating frequency and event duration (Figure 5e, Table S5&S6, Supporting information). The fingerprint pattern of streptavidin and extravidin binding are similar at pH 6.0 and pH 10.0. This is not surprising as the two proteins are negatively charged at both pH conditions. In contrast, when avidin is bound to OmpG-PEG₂-biotin the gating frequency increased by three fold and the open probability decreased slightly. The open pore current was reduced from 31.5 ± 3.4 pA to 16.4 ± 4.1 pA at +50mV. The zeta-potential measurement at pH 10 shows the charge on the avidin surface approaches zero (Table 2). Since the avidin still induced a strong reduction in open pore current at pH 10.0, we still expect an electrostatic attraction to be present. One possibility is that some of the positively charged residues on the OmpG loops are deprotonated at pH 10.0. For example, there are 4 lysine residues in the loops of OmpG and when all are deprotonated, the net result is a more negatively charged surface. Thus, the loss of positive charge on avidin at high pH is compensated by a gain of negative charge on OmpG's surface. Alternatively, the local charge on avidin might still be positive near the biotin binding site even though the total charge on avidin approaches zero. Indeed the glycosylation site of avidin is found on residue Asn-17, adjacent to residue Ser-16 which comprises part of the biotin binding pocket³² (Figure S7, Supporting information) and could potentially interact directly with the loops of the OmpG. Finally, when all proteins were tested at pH 10.0 in the presence of charge screening conditions (4.0 M KCl), the gating pattern of bound avidin was indistinguishable from streptavidin or extravidin (Figure 5f, g, h). The open probability of the OmpG-PEG₂-biotin increased slightly from 0.87 ± 0.05 to around 0.97 ± 0.02 when bound to any of the three protein analytes (Table S7, Supporting information). In summary, pH, ionic strength or both can be used to modulate the gating behavior between the loops of OmpG and bound target proteins. These findings clearly illustrate the role that electrostatic interactions play in OmpG's ability to differentiate between very similar protein analytes.

Influence of Applied Voltage on Bound-State Gating Behavior

We investigated how the applied potential affects the gating characteristic of OmpG nanopore during the analyte bound state by varying the applied potential from -75 mV to $+75$ mV. Here, we define the polarity of the potential relative to the orientation of OmpG's loops. A negative potential is when OmpG's loops are on the negative side of the membrane relative to the positive side (Figure 6a). A positive voltage is the opposite scenario (Figure 6b). The most significant effect was observed using the open pore current of the avidin bound state which decreased linearly with increasing voltage (Figure 6 d, g) (Figure S8, Table S8&S9, Supporting information). Also, the gating frequency was higher at positive voltages than at negative voltages (Figure S8 c, d, Supporting information). Other avidin-

induced changes in the gating characteristics, *e.g.* open probability, inter-event duration and event duration, appeared to be less dependent on applied voltage. In contrast to avidin, there was no direct correlation between the gating behavior of OmpG in the streptavidin and extravidin bound states at different applied voltages (Figure 6c, e, f, h) (Figure S8, Supporting information). We expect that these phenomena could explain the dependence of the avidin bound state on applied potential: electroosmosis, electrophoresis and electrostatics. Molecular dynamics simulations suggest that the electric field is strongest in the center of nanopore and weak at the entrance and is essentially negligible outside the pore^{39, 40}. Although the avidin molecule captured by the biotin ligand could be as far as 30 Å away from the entrance, electrophoretic forces are likely negligible in comparison with electroosmotic effects. Thus, a net electroosmotic flow from the loop side to the periplasmic side might drive the avidin towards the nanopore. At the entrance, electrophoresis would bring the analyte closer to the OmpG loops, thus promoting the electrostatic interaction between the two molecules. The three effects combine to force OmpG and avidin together and further decrease the open pore current. Conversely, a negative potential pushes avidin away from the loops. The electroosmotic and electrophoretic effects counteract the electrostatics between avidin and OmpG. As a consequence, avidin blocks the pore to a lesser degree under a negative potential.

While the gating behavior of the nanopore with bound avidin was relatively straightforward to understand in terms of electrostatic interactions, our voltage dependent studies using extravidin revealed more subtle information. When we examined the E1 form of extravidin, the gating behavior was not affected by the voltage polarity (Figure 6e, h). However, the E2 and E3 forms of extravidin showed open pore currents that did respond to voltage. Namely, the open pore current of OmpG-PEG₂-biotin, upon binding to E2 or E3, decreased linearly with increasing voltage from -75 mV to 75 mV (Figure S9, Supporting information). This observation suggests that E2 and E3 population bear net positive charges due to an incomplete removal of glycan from the surface. Notably, the voltage induced a current reduction to similar levels in E3 and avidin while to a lesser extent in E2. This result supports our speculation that E3 was intact avidin and E2 was the intermediate product of the deglycosylation reaction. Taken together, the voltage can influence the binding signal of a charged analyte by either strengthening or weakening the interaction between the OmpG nanopore and the analyte protein.

DISCUSSION

We have demonstrated that analyte proteins with positively charged surfaces are attracted to the negatively charged loops of OmpG. The interaction leads to a reduction of the open-pore current through the nanopore. In control experiments, we showed that our proteins did not induce any significant current change when using wild type OmpG, indicating the interaction of the avidin species only occurs after they bind to the biotin ligand. Therefore, these results strongly support a two-step mechanism for OmpG nanopore sensing (Figure 7). The first step is the capture where the protein binds the ligand on OmpG. The capture of an analyte protein brings it in close proximity to the nanopore opening which then facilitates electrostatic attraction between the two surfaces. In the second step, the analyte interacts directly with the loops of OmpG to obstruct ionic flow through the pore and alter the

dynamic movement of the loops. In essence, the flexible loops of OmpG sense and communicate information about the surface of the analyte protein. This process is termed “sampling.”

Though all three avidin species were clearly captured by the biotin ligand, some did not undergo the sampling step. For example, streptavidin and extravidin at pH 6.0 do not induce a change in the open pore current which indicates that the loops and target surface are repelled by their like charges. At high salt concentrations (4.0 M KCl), charges were screened and avidin also did not cause a change in open pore current. In scenarios where sampling did not occur, the OmpG nanopore could not distinguish between biotin-binding species. Therefore, system conditions that favor sampling are essential for sensitive analyte discrimination.

Importantly, protein analytes that are sampled by the OmpG loops could induce distinct gating signal. For example, at pH 6.0 in low salt (0.3 M KCl), avidin-OmpG loop interactions increased the gating frequency, indicating that loop 6 oscillates between the closed and open state more rapidly. However, it caused a reduction in the gating event duration by 30%. The effect of bound avidin on the loops was remarkably different from the effect of a mouse monoclonal anti-biotin antibody (mAb) used in a previous study.³⁰ Under identical conditions, mAb binding increased both the gating frequency and event duration, which led to a decrease in open probability. Furthermore, one polyclonal anti-biotin antibody eliminated all gating events and resulted in a constantly open pore.³⁰ The differences in loop interactions between the monoclonal and polyclonal antibodies and the avidin clearly demonstrate that the gating induced by OmpG's loops depends on the unique interaction with the analyte interface. In nature, protein-protein interactions are typically mediated by weak intermolecular forces such as hydrophobic, hydrogen-bonding and electrostatic forces. The OmpG nanopore so far seems predominantly sensitive to attractive electrostatic interactions. Indeed, we note that conditions that leave an avidin species nearly neutral or negatively charged (*i.e.*, conditions that prevent sampling) abolish OmpG's discrimination abilities. The current version of the OmpG nanopore sensor is nearly identical to the wild type save the addition of the biotin ligand. We speculate that further modifications to the loops might allow the use of hydrophobic or hydrogen bond interactions for enhanced sampling of neutral and negative analytes.

We also show that experimental conditions, such as pH, salt concentration and voltage can be tuned to facilitate detection. Sampling process is the key to OmpG's discrimination ability, so parameters that modulate sampling may also be used to enhance sensitivity. This can be accomplished by two general strategies. First, the electrostatic interaction between the analyte and nanopore can be tuned by altering the surface charge of both analyte protein and nanopore. Second, we also showed that voltage could either enhance or weaken the interaction by electrophoretic forces that move the protein closer to or away from the pore entrance. Thus, by optimizing the conditions, we can maximize the relative difference in the fingerprint analysis to generate unambiguous detection patterns. These strategies may also be particularly useful to ameliorate the effect of interference from other species in complex samples.

Post-translational modifications (PMTs) have an important impact on the physical properties and biological functions of proteins. Altered or missing modifications have profound effects on cellular function and are often associated with human diseases,^{41,42} such as neurodegenerative disease,⁴³ cancer⁴⁴ and inflammatory diseases⁴⁵. Mass spectrometry, as the key technology for identifying PMTs, has contributed tremendously our understanding of PMTs in cell.^{46,47, 48} However, analysis of PMTs by mass spectrometry can be challenging due to the instability of the modifications during the MS analysis or the effect of the modification of the peptide's ionization efficiency. Single-molecule strategies using nanopore for detecting modifications are especially appealing because it can identify modifications and variants within an intact protein. Along these lines, Rosen *et al.* recently demonstrated the use of α HL in detecting changes in the phosphorylation of thioredoxin.²² Here we showed OmpG nanopores can recognize changes in glycosylation patterns of proteins through their differences in surface charge. This is especially important, as many forms of glycosylation *e.g.* sialylation and acetylglucosaminylation add negative or positive charge, respectively. Current glycoprotein profiling methods using mass spectrometry often run in tandem with a separation technique, such as liquid chromatography or gel electrophoresis. Equipped with a specific ligand for the protein of interest, OmpG nanopore could be used for differential profiling of cellular glycoproteins in native states without prior separation procedure or liberation of glycan.

CONCLUSION

We demonstrated that an engineered OmpG nanopore can distinguish between protein homologues and post-translationally modified isoforms through their differences in surface charge. This is achieved by the electrostatic interaction between the nanopore loops and analyte proteins and yields a gating fingerprint readout for the analyte.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Schematic diagram of the OmpG nanopore sensor

(a) OmpG-PEG₂-biotin pore. A biotin was tethered to a cysteine residue located on loop 6 (highlighted in blue) via a polyethylene glycol linker that could extend the biotin away from the nanopore by 30 Å. (b) A representative current trace of OmpG-PEG₂-biotin nanopore. Buffer used was 10mM sodium phosphate, pH 6.0, 300mM KCl.

Figure 2. Detection of biotin binding protein homologues by OmpG-PEG₂-biotin nanopore (a, b, c) Typical current recording traces of streptavidin (a), avidin (b) and extravidin (c) bound to OmpG-PEG₂-biotin at +50mV in 10mM sodium phosphate, pH 6.0, 300mM KCl buffer. Red and green dashed line indicates the open pore current before and after analyte protein binding. The relative open pore current of analyte bound state to unbound state is indicated above the dashed line. (d) Power spectra and fingerprint analysis (e) of before and after target bound to OmpG-PEG₂-biotin.

Figure 3. Effect of ionic strength on the gating characteristic of OmpG nanopore induced by avidin binding

(a, b, c, d, e) Typical current recording traces of OmpG-PEG₂-biotin before and after avidin binding in buffers containing various KCl concentrations. Relative change in open pore current of OmpG at avidin bound state (green line) to unbound state (red line) is indicated in the histogram. Experiments were performed at +50 mV in 10mM sodium phosphate at pH 6.0 containing KCl, the concentration of which is indicated in figures. (f) Fingerprint of avidin binding at various KCl concentration.

Figure 4. Effect of pH on gating signal of analyte proteins

(a, b, c) Typical current recording traces and corresponding histograms of OmpG-PEG₂-biotin nanopore before and after binding of streptavidin (a) avidin (b) and extravidin (c). Relative open pore current of OmpG at avidin bound state (green line) to unbound state (red line) is indicated in the histogram. Experiments were performed in 10mM sodium acetate pH 4.0, 1.0M KCl at +50 mV. (d) Power spectra and fingerprint analysis (e) of before and after target bound to OmpG-PEG₂-biotin.

Figure 5. Binding of analyte proteins at pH 10

(a, b, c) Typical current recording traces and corresponding histograms of OmpG-PEG₂-biotin nanopore before and after binding of streptavidin (a) avidin (b) and extravidin (c).

Relative open pore current of OmpG at avidin bound state (green line) to unbound state (red line) is indicated in the histogram. Experiments were performed in buffer 10mM CAPS pH 10.0, 300mM KCl at +50 mV. (d) Power spectrum and (e) Fingerprint analysis of the three biotin binding proteins on OmpG-PEG₂-biotin behavior. (f, g, h) Binding of analyte proteins at high salt concentration. Typical current recording traces of OmpG-PEG₂-biotin nanopore and corresponding histograms before and after binding of streptavidin (f) avidin (g) and extravidin (h). Experiments were performed in buffer 10mM CAPS pH 10.0, 4.0 M KCl at +50 mV.

Figure 6. Effect of voltage on the gating characteristics of OmpG at analyte bound state
 (a, b) Schematic illustration of analyte binding at negative (a) and positive potential (b). (c, d, e) Single channel recording traces of OmpG-PEG₂-biontin nanopore at the unbound (grey traces) and analyte bound state (black traces). Data were acquired in buffer 10 mM sodium phosphate, pH 6.0, 300 mM KCl at various applied potentials. (f, g, h) Voltage dependence of the open pore current in the streptavidin, avidin and extravidin bound states respectively.

Figure 7.
Schematic presentation of the mechanism for OmpG nanopore sensing.

Table 1

Gating characteristics for streptavidin, avidin and extravidin.

	Open probability	Gating frequency (s ⁻¹)	Inter-event duration (ms)	Event duration (ms)	Open pore conductance (pA)
No binding	0.83±0.08	106±24	8.5±2.1	0.83±0.18	24.8±4.4
Streptavidin	0.95±0.03	56±6	27.6±7.8	0.32±0.01	23.6±3.5
Avidin	0.89±0.08	227±26	7.5±2.1	0.46±0.09	14.6±1.7
Extravidin	0.95±0.03	30±9	29.1±0.4	0.29±0.06	21.7±5.7

Values were calculated from at least three independent experiments that were carried out in buffer 10mM sodium phosphate pH 6.0, 300 mM KCl at +50 mV. The errors indicate the standard deviation.

Table 2

Zeta potential of analyte proteins

pH	Zeta potential value (mV)		
	Streptavidin	Avidin	Extravidin
4	2.5±0.8	7.8±1.5	1.0±0.5
6	-7.0±0.5	7.8±0.9	-8.0±1.9
10	-18.3±5.0	-0.2±0.4	-13.3±3.0

Values were calculated from at least three independent experiments. The errors indicate the standard deviation.