UC San Diego

UC San Diego Previously Published Works

Title

Frequency-Based Analysis of Gramicidin A Nanopores Enabling Detection of Small Molecules with Picomolar Sensitivity.

Permalink

<https://escholarship.org/uc/item/46w8c77q>

Journal

Analytical chemistry, 90(3)

ISSN

0003-2700

Authors

Kim, Young Hun
Hang, Leibniz
Cifelli, Jessica L
et al.

Publication Date

2018-02-01

DOI

10.1021/acs.analchem.7b02961

Peer reviewed

Frequency-Based Analysis of Gramicidin A Nanopores Enabling Detection of Small Molecules with Picomolar Sensitivity

Young Hun Kim,^{†,‡} Leibniz Hang,[‡] Jessica L. Cifelli,[‡] David Sept,[#] Michael Mayer,[¶] and Jerry Yang^{*,†,‡,¶}[†]Materials Science and Engineering Program and [‡]Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman, MC 0358, La Jolla, California 92093-0358, United States[#]Department of Biomedical Engineering, University of Michigan, Ann Arbor, Michigan 48109-2110, United States[¶]Adolphe Merkle Institute, University of Fribourg, Chemin des Verdiers 4, 1700 Fribourg, Switzerland

Supporting Information

ABSTRACT: Methods to detect low concentrations of small molecules are useful for a wide range of analytical problems including the development of clinical assays, the study of complex biological systems, and the detection of biological warfare agents. This paper describes a semisynthetic ion channel platform capable of detecting small molecule analytes with picomolar sensitivity. Our methodology exploits the transient nature of ion channels formed from gramicidin A (gA) nanopores and the frequency of observed single channel events as a function of concentration of free gA molecules that reversibly dimerize in a bilayer membrane. We initially use a protein (here, a monoclonal antibody) to sequester the ion channel activity of a C-terminally modified gA derivative. When a small molecule analyte is introduced to the electrical recording medium, it competitively binds to the protein and liberates the gA derivative, restoring its single ion channel activity. We found that monitoring the frequency of gA channel events makes it possible to detect picomolar concentrations of small molecule in solution. In part, due to the digital on/off nature of frequency-based analysis, this approach is 10^3 times more sensitive than measuring macroscopic membrane ion flux through gA channels as a basis for detection. This novel methodology, therefore, significantly improves the limit of detection of nanopore-based sensors for small molecule analytes, which has the potential for incorporation into miniaturized and low cost devices that could complement current established assays.

Methods to detect small molecular analytes (<600–700 molecular weight¹) have found important use in applications including the development of new pharmaceuticals,² the elucidation of complex pathways in biological systems,^{3,4} and the detection of biological warfare agents.⁵ For example, methods to monitor the presence of small molecules involved in bacterial signaling pathways, biofilm formation, and virulence⁶ or related to diseases such as heart failure, cancer, and neurodegenerative disorders^{7,8} could provide valuable insight into understanding their function in a biological system.⁹ Additionally, the detection of small molecule analytes could serve a vital role for identifying biological species (i.e., bacteria, viruses, or spores) in potential contaminated areas (i.e., air, water, food, or even human).^{10,11} While ELISA,^{12,13} electrochemical,^{3,14,15} and surface spectroscopy-based^{16,17} methods have been developed to detect the presence of small molecules in various types of samples, new sensing platforms are still needed to improve sensitivity, selectivity, portability, and cost.

To date, very few examples utilizing the insertion of transmembrane ion channels into lipid bilayers have been reported for the detection of small molecules, with most examples employing the use of natural nanopores formed by α -

hemolysin as the channel sensing element for detection of, e.g., cocaine,¹⁸ antibiotics,¹⁹ ATP-binding aptamers,²⁰ and second messenger inositol (IP₃).^{21,22} For example, Bayley and co-workers reported a method for detecting multiple neurotransmitters²³ simultaneously in real-time as well as enantiomers of drug molecules²⁴ using natural α -hemolysin. Once incorporated in the membrane, α -hemolysin forms a permanently open pore and detection of analytes is achieved through modulation of ion flux through the channel. We^{25,26} and others,^{27–29} on the other hand, have previously reported the use of gramicidin A (gA) peptides to detect molecular analytes in solution through modulation of macroscopic transmembrane current flux or through sensitized photo-inactivation³⁰ of gA channels. As opposed to permanently open nanopore channels like α -hemolysin,^{31,32} gA partitions strongly into bilayers^{33,34} and reversibly dimerizes to form transient nanopores. The frequency and conductance of gA channel events depends on various environmental parameters and can be quantified using single channel recordings.^{25,35}

Received: July 26, 2017

Accepted: December 21, 2017

Published: December 21, 2017

Figure 1. Schematic for the detection of small molecule analytes (red filled circles) using engineered ion channel-forming peptides derived from gramicidin A (gA). Introduction of an antibody that binds to small molecules attached covalently to gA (orange filled circles) sequesters ion flux across a membrane. Upon introduction of a small molecule that competes with the gA derivative for binding to the antibody, two channel properties can be measured: (a) the total ion flux (i.e., the total transported charge per time interval) and (b) the frequency of observed single channel events; the magnitude of both of these channel properties is dependent on the concentration of gramicidin derivatives able to form conducting ion channels.

Furthermore, gA is commercially available in gram scale quantities and synthetic modifications can be made to the C-terminus while retaining its ion channel properties.^{36–41} Gramicidin A can, therefore, be readily customized for detection of a variety of specific analytes.^{36,42–44}

We previously reported that single channel recordings of engineered gA derivatives²⁵ could be used to detect the presence of enzymes such as alkaline phosphatase (AP) and anthrax lethal factor (LF) by monitoring the enzymatic conversion of substrates that were covalently attached near the opening of a gA nanopore.^{42,45} We further demonstrated that high concentrations of proteins or protein inhibitors could be used to modulate macroscopic ionic currents across bilayers through synthetic gA nanopores.^{9,30,36} Here, we build upon these studies and report a novel method for the sensitive detection of small molecule analytes based on monitoring the frequency of single ion channel events from modified gramicidin channels. We exploit antibody–antigen interactions to suppress the ion channel activity of a derivative of gA that is modified with the antigen (i.e., the target small molecule analyte) at its C-terminus. Introduction of a sample of the free analyte in solution to a standard bilayer setup containing the gA–antibody complex restores ion channel activity upon competition of the analyte with the gA derivative for binding to the antibody. Detection of the analyte is accomplished using two methods: (1) measuring the total flux of charge transported across the membrane or (2) measuring the frequency of single ion channel events as a function of concentration of analyte.

This ion channel-based platform offers several advantages for the detection of small molecules compared to methods that are not based on ion channels: (i) this system utilizes the amplification characteristics of ion flux through a modified single ion channel to achieve high sensitivity,^{27,42,46,47} (ii) this approach employs high affinity antibody–antigen interactions to tailor detection toward a targeted small molecule analyte, (iii) the ion channel-based platform requires small volumes (μL to mL) and quantities (picomolar to nanomolar concentrations) of ion channel peptides to achieve low cost,⁴⁸ and (iv) the bilayer sensor setup is amenable to miniaturization making it possible to incorporate this small molecule detection assay into portable devices.^{49–54}

■ EXPERIMENTAL SECTION

Synthesis of gA-Fluorescein. A detailed description of the synthesis and characterization of gA-fluorescein is provided in the [Supporting Information](#).

Formation of Planar Lipid Bilayers. Planar lipid bilayers were formed by the “folding technique” over an aperture with a diameter of $\sim 110\ \mu\text{m}$ in a Teflon film as described previously.²⁰ The recording electrolyte was 100 mM CsCl buffered with 1 mM HEPES at pH 7.0. Briefly, a solution containing 25 mg mL^{-1} 1,2-diphytanoyl-*sn*-glycero-3-phosphatidylcholine (Di-PhyPC) in pentane was spread at the air–water interface of the electrolyte solution in both compartments of the bilayer setup. Three mL of the total volume of 4 mL of electrolyte solution was aspirated in each bilayer compartment into a

Figure 3. Detection of free small molecule (here, 5(6)-carboxyfluorescein as a representative analyte) in solution based on measurement of total transported charge through gA-fluorescein channels over a 20 min time interval. (a) Representative current traces of gA-fluorescein at +100 mV applied voltage. (i) Recorded trace of 1.1 nM gA-fluorescein reveals multiple, simultaneous channel events in the membrane. (ii) Introduction of 1.6 nM anti-fluorescein antibody suppresses ion channel activity of gA-fluorescein. (iii) After external addition of 22 nM free 5(6)-carboxyfluorescein, ion flux through gA-fluorescein channels in the membrane was fully recovered. (b) Recorded current traces of native gA at +100 mV applied voltage as a control. (i) Macroscopic ion currents are observable from 1.3 nM native gA in the membrane. (ii) Introduction of 1.6 nM anti-fluorescein antibody did not affect ion channel activity of native gA. (c) Graph showing the total transported charge by 1.1 nM gA-fluorescein (in the presence of 1.6 nM anti-fluorescein antibody) within a 20 min interval after addition of increasing concentrations of free 5(6)-carboxyfluorescein. Analysis of the total charge transport (Q) as a function of external free 5(6)-carboxyfluorescein concentration provides an estimate of the EC_{50} (eq S1) for detection of 9.4 nM 5(6)-carboxyfluorescein. All recordings were performed in 100 mM CsCl, 1 mM HEPES, pH 7, in DiPhyPC lipid membranes at an applied potential of +100 mV.

fluorescein retained single ion channel activity that is similar to native gA. Analysis of current versus voltage (I – V) curves of gA and gA-fluorescein revealed single channel conductance (γ) of 14.7 ± 0.3 pS for gA-fluorescein and 17.3 ± 0.1 pS for native gA in a recording electrolyte containing 0.1 M CsCl.

We next examined the effect of addition of an anti-fluorescein antibody on the ion channel activity of gA-fluorescein in bilayer recordings. Figure 2C shows representative current versus time traces of gA-fluorescein in a bilayer before and after addition of the anti-fluorescein antibody to both aqueous chambers of the bilayer setup. These traces revealed that addition of the anti-fluorescein antibody resulted in complete sequestration of ion channel activity of gA-fluorescein. However, subsequent addition of free 5(6)-carboxyfluorescein to the aqueous chambers resulted in complete recovery of ion channel activity. These experiments demonstrate that this gA-fluorescein/anti-fluorescein system could serve as a model to test the limit of detection of an ion channel-based method for sensing the presence of specific small molecule analytes in solution.

Quantifying the Detection Limit for Sensing a Small Molecule Analyte Using Total Transported Charge Recovery from gA-Based Nanopores.

In order to test if a gA-based assay could be adapted for quantifying the detection of small molecules, we sequestered gA-fluorescein (1.1 nM) channel activity in a planar lipid bilayer setup by addition of 1.6 nM anti-fluorescein (Figure 3A). Subsequently, we added increasing concentrations of 5(6)-carboxyfluorescein (0–22 nM final concentrations) to both aqueous compartments of the bilayer setup and monitored channel activity over a 20 min period for each concentration of 5(6)-carboxyfluorescein. Integration of the observed ionic current (I) over this time window provides an estimate of the total transported charge as a function of the concentration of free 5(6)-carboxyfluorescein (see Figure S2). We then normalized this data to the maximum expected total transported charge (i.e., the total transported charge from the same concentration of gA-fluorescein in the absence of antibody or free 5(6)-carboxyfluorescein). Fitting the data to a previously reported dose–response curve⁹ (see eq S1) revealed an EC_{50} of 9.4 nM for the detection of free 5(6)-

carboxyfluorescein using this total transported charge recovery method (Figure 3B). As a control, we added the same concentration of unmodified, native gA into the bilayer setup and did not observe any effect of ion channel activity of gA upon addition of the anti-fluorescein antibody (Figure 3C). This control experiment supports that the change in total transported charge shown in Figure 3B as a function of free 5(6)-carboxyfluorescein in solution is specifically due to competition of the free 5(6)-carboxyfluorescein with the gA-fluorescein for binding to the anti-fluorescein antibody.

Quantifying the Detection Limit for Sensing a Small Molecule Analyte Using Ion Channel Event Frequency of gA-Based Nanopores. In order to explore another method of analysis for detecting small molecules using gA nanopores, we examined the frequency of single ion channel events observed over a window of time. For this assay, we used a 1.1 pM concentration of gA-fluorescein in the bilayer recording experiments to observe single channel events. We found that a 1.6 pM concentration of the anti-fluorescein antibody was sufficient to suppress the single ion channel activity of the gA-fluorescein (see Figure S3). As a control, addition of a 1.6 pM concentration of the anti-fluorescein antibody did not affect the single ion channel activity of native gA (see Figures S3 and S4), again supporting that the effects of the antibody on the single ion channel activity of gA-fluorescein was due to specific binding between the antibody and the nanopore.

Next, we added increasing concentrations of 5(6)-carboxyfluorescein (0–50 pM final concentrations) to both aqueous chambers of the bilayer setup and monitored single ion channel activity of the gA-fluorescein over 30 min for each concentration of free 5(6)-carboxyfluorescein added. Here, we observed an increase in the number of single ion channel events in the recording time window with increasing concentration of free 5(6)-carboxyfluorescein. Figure 4 plots the frequency (i.e., the total number of single channel events divided by the recording time) of observed single channel events⁴¹ versus the concentration of added free 5(6)-carboxyfluorescein to the recording buffer in the gA-fluorescein/anti-fluorescein experimental setup. While each data point reflects the average of at least 3 independent experiments, we attribute the error in these measurements to experimental factors (e.g., concentration of reagents and analyte or membrane thickness) and environmental factors (e.g., vibrational noise and temperature) that are difficult to control between each experiment; these sources of error are expected to disproportionately affect conditions where there are relatively few observed single channel events (i.e., where small differences in the number of channel events can significantly affect estimation of frequency over a finite window of time) as opposed to the extreme conditions where almost no events are observed (i.e., at very low concentrations of 5(6)-carboxyfluorescein) or where the frequency of single channel events is high (i.e., at high concentrations of 5(6)-carboxyfluorescein). Fitting the data in Figure 4 to a dose–response curve⁹ (see eq S1) revealed an EC_{50} of 2.1 pM. Analysis of the ion channel lifetime and conductance of gA-fluorescein before the addition of anti-fluorescein or after recovery of ion channel events upon addition of free 5(6)-carboxyfluorescein to the gA-fluorescein/anti-fluorescein complex revealed no significant difference in the function of the gA-fluorescein (Figure S5), supporting that the data shown in Figure 4 is the result of competition between the free added 5(6)-carboxyfluorescein and the gA-fluorescein for binding to

Figure 4. Detection of free 5(6)-carboxyfluorescein based on recovery of single ion channel event frequency of gA-fluorescein in the presence of an anti-fluorescein antibody. The graph shows the frequency of observed single channel events by 1.1 pM gA-fluorescein (in the presence of 1.6 pM anti-fluorescein antibody) as determined within a 30 min interval after addition of increasing concentrations of free 5(6)-carboxyfluorescein. Analysis of channel frequency as a function of external free 5(6)-carboxyfluorescein concentration provides an estimate of the EC_{50} (eq S1) for sensitivity of detection of the small molecule of 2.1 pM. All recordings were performed in 100 mM CsCl, 1 mM HEPES, pH 7, in DiPhyPC lipid membranes at applied potential of +100 mV. Each data point represents the average frequency of observed channel events over a 30 min time window. Error bars represent standard deviation. All data points reflect an average of at least three independent measurements.

the anti-fluorescein antibody. As a control, when we added 50 pM Rhodamine B (a molecule with similar backbone chemical structure as 5(6)-carboxyfluorescein) to the bilayer setup containing 1.1 pM gA-fluorescein and 1.6 pM anti-fluorescein antibody, we did not observe any single ion channel events over a 30 min time window (Figure S6), demonstrating good selectivity of this ion channel platform for detecting the analyte of interest.

This frequency-based method for detection of small molecules provides a significant advantage in sensitivity compared to the total charge transport method. The digital on/off nature of gA assures that every transient channel event is counted in our frequency-based analysis of unbound gA derivatives, since (1) no events are observed when the gA derivatives are bound by the antibody (Figure 2C) and (2) the single channel conductance of each gA nanopore is large enough to clearly exceed the baseline current noise. In contrast, the detection limit of small molecules using the total charge transport method is dependent on the noise from the background current (i.e., the observed current across the membrane in the absence of ion channels). Under conditions where there are very few, short-lived ion channel events, this background membrane current can dominate over the observed total current from the ion channels, making it difficult to detect the presence of low concentrations of small molecules through measurement of total charge transport alone (see Figure S7). While measurement of total charge transport over longer time windows could help mitigate issues with signal-to-noise, the stability of the underlying lipid membrane using commercial lipids and suspended lipid membranes limits the practicality of such long ion channel recordings. Taken together, our results

Figure 5. Detection of free 5(6)-carboxyfluorescein based on recovery of single ion channel frequency of gA-fluorescein in the presence of an antifluorescein Fab fragment. (a) Schematic diagram and representative recorded current traces of 1.1 pM gA-fluorescein (in the presence of 1.6 pM an antifluorescein Fab fragment) before and after addition of 4.0 nM external 5(6)-carboxyfluorescein. (b) Graph showing the frequency of observed single channel events from 1.1 pM gA-fluorescein within a 30 min interval after addition of increasing concentrations of free 5(6)-carboxyfluorescein. Recovered event frequency as a function of externally added 5(6)-carboxyfluorescein concentration reveals an estimate of EC₅₀ of 5.1 pM. All recordings were performed in 100 mM CsCl, 1 mM HEPES, pH 7, in DiPhyPC lipid membranes at an applied potential of +100 mV.

demonstrate that monitoring the *frequency* of channel events makes it possible to detect a small molecule analyte with substantially increased sensitivity compared with measurement of total transported charge recovery under typical conditions used to record ion channel activity of gA derivatives in suspended lipid bilayer assays.

Estimation of Binding Constants of the gA-Fluorescein/Antifluorescein Complex. The remarkable sensitivity for detection of small molecules using frequency-based ion channel analysis prompted us to examine whether the divalency of the antibody played a role in sequestering the ion channel activity of the gA derivative. We hypothesized that, since multiple fluorescein moieties would be present on the membrane surface when gA-fluorescein was inserted in the membrane, it is possible that the antifluorescein IgG antibody could bind divalently to the membrane surface. Previous studies provide evidence for the divalent binding of antibodies to the surface of membranes containing derivatives of gA.³⁰ Such divalent binding would be expected to create a strong

association of the antifluorescein antibody to a membrane containing gA-fluorescein (in the case of the monovalent antifluorescein antibody used here, the reported K_d for binding to free 5-carboxyfluorescein is 48 fM⁵⁵), which could at least partially account for the very low concentrations of antibody required to sequester ion channel activity in this nanopore assay. Analysis of the data in Figure 4 in a cooperative binding model with 2 ligand sites (see Figure S8) revealed a dissociation constant of the first binding event between gA-fluorescein and the antifluorescein antibody (K_1) of 48 fM and the second binding event (K_2) of 38 fM. This result suggests that there is a modest amount of cooperativity⁵⁷ ($\beta = 1.28$) for binding of the second gA-fluorescein to the antibody.

To further investigate the role of divalency in the detection of small molecules in this nanopore assay, we also generated a monovalent Fab fragment from the antifluorescein antibody (see the Supporting Information for details). We then used this Fab fragment as the ion channel sequestration protein in a frequency-based single ion channel analysis for detection of free

5(6)-carboxyfluorescein using gA-fluorescein (Figures 5A and S9). Similar to experiments with the fully intact anti-fluorescein antibody, addition of a 1.6 pM final concentration of the anti-fluorescein Fab fragment to both chambers of a bilayer setup resulted in complete suppression of the ion channel activity of gA-fluorescein (1.1 pM final concentration) (Figure 5A). We subsequently added increasing concentrations of free 5(6)-carboxyfluorescein (0–250 pM final concentration) to the bilayer setup and monitored ion channel activity during a 30 min recording period for each concentration of added free 5(6)-carboxyfluorescein (Figure 5B). This experiment revealed an EC_{50} of 5.1 pM by fitting to a dose–response curve⁹ (see eq S1). This result demonstrates that using a monovalent Fab fragment as an initial suppressor of ion channel activity of gA-fluorescein shows approximately the same level of sensitivity for detection of a small molecule compared to using a fully intact divalent antibody (which we estimated has a dissociation constant for the first binding event (K_1) of 48 fM in the gA-fluorescein/anti-fluorescein complex). This result suggests that divalent binding of an antibody is not required for high sensitivity in the frequency-based gA nanopore detection assay.

CONCLUSION

Here, we demonstrated that an ion channel platform based on chemically modified gA derivatives can be employed to detect small molecule analytes with high sensitivity by utilizing antibody–antigen interactions. Two different methods of analysis can be used to detect small molecules using this ion channel-based approach: (1) a total transported charge recovery method and (2) a frequency-based ion channel recovery method. We demonstrated that a gA-based nanopore platform could detect the presence of pico- to nanomolar concentrations of small molecules in an analyte sample using similarly low concentrations (pico- to nanomolar) of the nanopore and antibody. While this proof-of-concept study focused on the detection of 5(6)-carboxyfluorescein, the ability to rapidly prepare C-terminally modified derivatives of gA⁵⁸ makes it possible to extend this ion channel-based sensing platform to the detection of other chemical analytes based on protein–ligand interactions,⁵⁹ as proteins other than antibodies can also be used to sequester the ion channel activity of gramicidin derivatives for these nanopore-based assays.³⁶ Furthermore, while a conventional planar lipid bilayer setup was utilized for this research, future work is aimed at minimizing the setup size into a portable sensing system by exploiting microscale chip-based ion channel recording systems that contain stable membranes using lipid-coated hydrogels⁶⁰ or droplet interface bilayers.⁶¹ Further advances in improving membrane leakiness and robustness^{62–64} may also help advance the sensitivity for detection of small molecule analytes in nanopore sensing platforms.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.7b02961.

Details for the synthesis and characterization of the gA-fluorescein, bilayer recordings, and preparation of anti-fluorescein Fab fragment (PDF)

AUTHOR INFORMATION

Corresponding Author

*Tel: 858-534-6006. Fax: 858-534-4554. E-mail: jerryyang@ucsd.edu.

ORCID

Jerry Yang: 0000-0002-8423-7376

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the Air Force Office of Scientific Research (FA9550-12-1-0435).

REFERENCES

- (1) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555–600.
- (2) Zhang, J.; Yang, P. L.; Gray, N. S. *Nat. Rev. Cancer* **2009**, *9*, 28–39.
- (3) Wang, W. U.; Chen, C.; Lin, K.; Fang, Y.; Lieber, C. M. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 3208–3212.
- (4) Strausberg, R. L. *Science* **2003**, *300*, 294–295.
- (5) Rao, S. S.; Mohan, K. V. K.; Atreya, C. D. *J. Microbiol. Methods* **2010**, *82*, 1–10.
- (6) Camilli, A.; Bassler, B. L. *Science* **2006**, *311*, 1113–1116.
- (7) Kenakin, T.; Christopoulos, A. *Nat. Rev. Drug Discovery* **2013**, *12*, 205–216.
- (8) Holliger, P.; Hudson, P. J. *Nat. Biotechnol.* **2005**, *23*, 1126–1136.
- (9) Mayer, M.; Semetey, V.; Gitlin, I.; Yang, J.; Whitesides, G. M. *J. Am. Chem. Soc.* **2008**, *130*, 1453–1465.
- (10) Gooding, J. J. *Anal. Chim. Acta* **2006**, *559*, 137–151.
- (11) Fitch, J. P.; Raber, E.; Imbro, D. R. *Science* **2003**, *302*, 1350–1354.
- (12) Schon, M.; Bong, A. B.; Drewniok, C.; Herz, J.; Geilen, C. C.; Reifemberger, J.; Benninghoff, B.; Slade, H. B.; Gollnick, H.; Schon, M. P. *J. Natl. Cancer Inst.* **2003**, *95*, 1138–1149.
- (13) Li, D.; Ying, Y.; Wu, J.; Niessner, R.; Knopp, D. *Microchim. Acta* **2013**, *180*, 711–717.
- (14) Baker, B. R.; Lai, R. Y.; Wood, M. S.; Doctor, E. H.; Heeger, A. J.; Plaxco, K. W. *J. Am. Chem. Soc.* **2006**, *128*, 3138–3139.
- (15) Lai, L. M. H.; Goon, I. Y.; Chuah, K.; Lim, M.; Braet, F.; Amal, R.; Gooding, J. J. *Angew. Chem., Int. Ed.* **2012**, *51*, 6456–6459.
- (16) Shankaran, D. R.; Gobi, K. V.; Miura, N. *Sens. Actuators, B* **2007**, *121*, 158–177.
- (17) Matsui, J.; Akamatsu, K.; Hara, N.; Miyoshi, D.; Nawafune, H.; Tamaki, K.; Sugimoto, N. *Anal. Chem.* **2005**, *77*, 4282–4285.
- (18) Kawano, R.; Osaki, T.; Sasaki, H.; Takinoue, M.; Yoshizawa, S.; Takeuchi, S. *J. Am. Chem. Soc.* **2011**, *133*, 8474–8477.
- (19) Asandei, A.; Apetrei, A.; Luchian, T. *J. Mol. Recognit.* **2011**, *24*, 199–207.
- (20) Ying, Y.-L.; Wang, H.-Y.; Sutherland, T. C.; Long, Y.-T. *Small* **2011**, *7*, 87–94.
- (21) Kang, X.; Cheley, S.; Rice-Ficht, A. C.; Bayley, H. *J. Am. Chem. Soc.* **2007**, *129*, 4701–4705.
- (22) Cheley, S.; Gu, L.-Q.; Bayley, H. *Chem. Biol.* **2002**, *9* (7), 829–838.
- (23) Boersma, A. J.; Brain, K. L.; Bayley, H. *ACS Nano* **2012**, *6*, 5304–5308.
- (24) Kang, X. F.; Cheley, S.; Guan, X.; Bayley, H. *J. Am. Chem. Soc.* **2006**, *128*, 10684–10685.
- (25) Macrae, M. X.; Blake, S.; Jiang, X.; Capone, R.; Estes, D. J.; Mayer, M.; Yang, J. *ACS Nano* **2009**, *3*, 3567–3580.
- (26) Majd, S.; Yusko, E. C.; Yang, J.; Sept, D.; Mayer, M. *Biophys. J.* **2013**, *105*, 146–153.
- (27) Futaki, S.; Zhang, Y.; Kiwada, T.; Nakase, I.; Yagami, T.; Oiki, S.; Sugiura, Y. *Bioorg. Med. Chem.* **2004**, *12*, 1343–1350.
- (28) Antonenko, Y. N.; Rokitskaya, T. I.; Kotova, E. A.; Reznik, G. O.; Sano, T.; Cantor, C. R. *Biochemistry* **2004**, *43*, 4575–4582.

- (29) Borisenko, V.; Zhang, Z.; Woolley, G. A. *Biochim. Biophys. Acta, Biomembr.* **2002**, 1558, 26–33.
- (30) Rokitskaya, T. I.; Macrae, M. X.; Blake, S.; Egorova, N. S.; Kotova, E. A.; Yang, J.; Antonenko, Y. N. *J. Phys.: Condens. Matter* **2010**, 22, 454118.
- (31) Astier, Y.; Braha, O.; Bayley, H. J. *Am. Chem. Soc.* **2006**, 128, 1705–1710.
- (32) Wang, G.; Wang, L.; Han, Y.; Zhou, S.; Guan, X. *Acc. Chem. Res.* **2013**, 46, 2867–2877.
- (33) Hladky, S. B.; Haydon, D. A. *Biochim. Biophys. Acta, Biomembr.* **1972**, 274, 294–312.
- (34) Finkelstein, A.; Andersen, O. S. *J. Membr. Biol.* **1981**, 59, 155–171.
- (35) Lundbæk, J. A. *J. Phys.: Condens. Matter* **2006**, 18, S1305–44.
- (36) Blake, S.; Capone, R.; Mayer, M.; Yang, J. *Bioconjugate Chem.* **2008**, 19, 1614–1624.
- (37) Bali, D.; King, L.; Kim, S. *Aust. J. Chem.* **2003**, 56, 293–300.
- (38) Antonenko, Y. N.; Gluhov, G. S.; Firsov, A. M.; Pogozheva, I. D.; Kovalchuk, S. I.; Pechnikova, E. V.; Kotova, E. A.; Sokolova, O. S. *Phys. Chem. Chem. Phys.* **2015**, 17, 17461–17470.
- (39) Fields, G. B.; Fields, C. G.; Petefish, J.; Van Wart, H. E.; Cross, T. A. *Proc. Natl. Acad. Sci. U. S. A.* **1988**, 85, 1384–1388.
- (40) Macrae, M. X.; Blake, S.; Mayer, M.; Yang, J. *J. Am. Chem. Soc.* **2010**, 132, 1766–1767.
- (41) Macrae, M. X.; Schlamadinger, D.; Kim, J. E.; Mayer, M.; Yang, J. *Small* **2011**, 7, 2016–2020.
- (42) Capone, R.; Blake, S.; Restrepo, M. R.; Yang, J.; Mayer, M. J. *Am. Chem. Soc.* **2007**, 129, 9737–9745.
- (43) Suarez, E.; De, E.; Molle, G.; Lazaro, R.; Viallefont, P. J. *Pept. Sci.* **1998**, 4, 371–377.
- (44) Cornell, B. A.; Braach-Maksyutis, V. L.; King, L. G.; Osman, P. D.; Raguse, B.; Wieczorek, L.; Pace, R. J. *Nature* **1997**, 387, 580–583.
- (45) Blake, S.; Mayer, T.; Mayer, M.; Yang, J. *ChemBioChem* **2006**, 7, 433–435.
- (46) Sakai, N.; Matile, S. *Langmuir* **2013**, 29, 9031–9040.
- (47) Xin, P.; Zhu, P.; Su, P.; Hou, J. L.; Li, Z. T. *J. Am. Chem. Soc.* **2014**, 136, 13078–13081.
- (48) Tonooka, T.; Sato, K.; Osaki, T.; Kawano, R.; Takeuchi, S. *Small* **2014**, 10, 3275–3282.
- (49) Bayley, H.; Cremer, P. S. *Nature* **2001**, 413, 226–230.
- (50) White, R. J.; Ervin, E. N.; Yang, T.; Chen, X.; Daniel, S.; Cremer, P. S.; White, H. S. *J. Am. Chem. Soc.* **2007**, 129, 11766–11775.
- (51) Chun, K.-Y.; Son, Y. J.; Han, C.-S. *ACS Nano* **2016**, 10, 4550–4558.
- (52) Rosenstein, J. K.; Ramakrishnan, S.; Roseman, J.; Shepard, K. L. *Nano Lett.* **2013**, 13, 2682–2686.
- (53) Del Rio Martinez, J. M.; Zaitseva, E.; Petersen, S.; Baaken, G.; Behrends, J. C. *Small* **2015**, 11 (1), 119–125.
- (54) Funakoshi, K.; Suzuki, H.; Takeuchi, S. *Anal. Chem.* **2006**, 78, 8169–8174.
- (55) Boder, E. T.; Midelfort, K. S.; Wittrup, K. D. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, 97, 10701–10705.
- (56) Macrae, M. X.; Blake, S.; Mayer, T.; Mayer, M.; Yang, J. *Proc. SPIE* **2009**, 7397, 739709–739711.
- (57) Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, 37, 2754–2794.
- (58) Mayer, M.; Yang, J. *Acc. Chem. Res.* **2013**, 46, 2998–3008.
- (59) Goodacre, R.; Shann, B.; Gilbert, R. J.; Timmins, E. M.; McGovern, A. C.; Alsberg, B. K.; Kell, D. B.; Logan, N. A. *Anal. Chem.* **2000**, 72, 119–127.
- (60) Sapra, K. T.; Bayley, H. *Sci. Rep.* **2012**, 2, 848.
- (61) Czekalska, M. A.; Kaminski, T. S.; Jakiela, S.; Tanuj Sapra, K.; Bayley, H.; Garstecki, P. *Lab Chip* **2015**, 15, 541–548.
- (62) Koyanagi, T.; Leriche, G.; Onofrei, D.; Holland, G. P.; Mayer, M.; Yang, J. *Angew. Chem., Int. Ed.* **2016**, 55, 1890–1893.
- (63) Koyanagi, T.; Leriche, G.; Yep, A.; Onofrei, D.; Holland, G. P.; Mayer, M.; Yang, J. *Chem. - Eur. J.* **2016**, 22, 8074–8077.
- (64) Koyanagi, T.; Cao, K. J.; Leriche, G.; Onofrei, D.; Holland, G. P.; Mayer, M.; Sept, D.; Yang, J. *Chem. - Eur. J.* **2017**, 23, 6757–6762.