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Direct Detection of Membrane Channels from Gels Using Water-in-Oil Droplet Bilayers

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Abstract: We form planar lipid bilayers between an aqueous droplet and a hydrogel support immersed in a lipid−oil solution. By scanning the bilayer over the surface of an SDS−PAGE gel, we are able to directly detect membrane proteins from gels using single-channel recording. Using this technique, we are able to examine low levels of endogenous protein from cell extracts without the need for over-expression. We also use droplet bilayers to detect small molecules from hydrogels. The bilayers show enhanced stability compared to conventional planar lipid bilayers, and both bilayer size and position can be controlled during an experiment. Hydrogel scanning with droplet bilayers provides a new method for the discovery and characterization of ion channels with the potential for high-throughput screening.

Introduction

Artificial planar lipid bilayers serve as simplified models of biological membranes and are widely used for the electrical characterization of ion channels and protein pores. Ion channels are a diverse group of membrane proteins that selectively control the movement of specific ions across cell membranes, establishing voltage and electrochemical gradients that are fundamental to a wide variety of biological processes. In humans, ion channels regulate everything from heartbeat and muscle contraction to hormone secretion and the release of neurotransmitters.[1] Defective ion channel function has been linked to a growing list of disorders, including cardiac arrhythmia, periodic paralysis, epilepsy, and diabetes.[1−3] Protein pores are nonspecific channels that pass molecules across cell membranes and are exploited for applications such as molecular sensing[4,5] and DNA sequencing.[6−8]

Single-channel recording (SCR) of individual proteins is a powerful means of studying channel protein function.[9] SCR measures changes in ion current through single protein channels and can examine voltage dependence, gating behavior, ligand binding affinity, and ion selectivity at the single-molecule level. Consequently, SCR can help determine the molecular basis of an ion channel disease. It is also an important technique for the development of new drugs specifically targeting ion channels and for screening other medicines for unwanted side effects.[2,10] Advances in these areas require much higher throughput assays of channel behavior than are currently available.

SCR typically uses either patch-clamping[11] or synthetic planar lipid bilayers.[12,13] Patch-clamping of whole cells is a versatile and sensitive means of examining channels, but it is time-consuming and often complicated by the heterogeneous nature of cell membranes. In contrast, synthetic planar lipid bilayers control the constituents of the system and can study purified proteins. Planar lipid bilayers are usually formed either by painting, where a solution of lipid in an organic solvent is directly applied to an aperture separating two aqueous compartments,[12,13] or by variants of the Langmuir−Blodgett technique, where two air/water monolayers are raised past an aperture.[14] Although widely used, planar bilayers are difficult to prepare, and short lifetimes prohibit their use in many situations.

Alternative emulsion-based approaches to forming bilayers have also been reported,[15] where bilayers are created between aqueous surfaces immersed in a solution of lipid in oil. When immersed in an immiscible lipid/oil solution, aqueous surfaces spontaneously self-assemble a lipid monolayer,[16,17] and when monolayers from two aqueous components are brought into contact they can "zip" together to form a bilayer.[18,19] Although this method was first described four decades ago,[15] recent studies

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have shown that microfluidic flows[20,21] and droplets[21,22] can be contacted in a lipid/oil solution to create bilayers suitable for SCR experiments.

We have adapted this approach, showing that bilayers can also be formed between droplets and semi-solid hydrated supports such as hydrogels. These droplet-on-hydrated-support bilayers (DHBs) are also well suited for SCR studies of channel proteins. DHBs show considerably improved bilayer stability and longevity compared to other methods. We are also able to move the bilayer and control bilayer size. We exploit the properties of DHBs to scan hydrogels containing membrane proteins separated by gel electrophoresis. Using this method, we can detect and characterize natural levels of membrane channels from SDS−PAGE purified cell extracts without the need for over-expression. This has potential applications in proteomics, for example, the discovery of new ion channels or the high-throughput screening of membrane proteins against a target library for drug discovery.

Materials and Methods

General. 1,2-Diphytanoyl-*sn*-glycero-3-phosphocholine (DPhPC, Avanti Polar Lipids), hexadecane (Sigma-Aldrich), β-cyclodextrin (Sigma-Aldrich), γ-cyclodextrin (Sigma-Aldrich), and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (Cyclolab) were used without further purification.

Creating Droplet on Hydrated Support Bilayers (DHBs). We use 10 mM DPhPC in hexadecane (C_{16}) as the lipid/oil solution in all our experiments. Aqueous volumes immersed in this solution spontaneously self-assemble a DPhPC monolayer, and when the monolayers of two components are brought into contact they spontaneously form a bilayer.[15,20−22] We form DHBs by contacting aqueous droplets with porous hydrated substrates such as hydrogels (Figure 1a). A stabilization period of at least 15 min is required before contacting monolayers to prevent fusion. After this period, we observe bilayer formation with almost 100% efficiency within a few seconds to a minute of contact of a water droplet with the hydrated support. We observe the DHB bilayers on an inverted microscope (Figure 1b), using this to monitor the position of a bilayer during experiments.

The bilayers are electrically accessed by inserting a 100 μm diameter Ag/AgCl electrode into the droplets (Figure 1a) using a micromanipulator. With a corresponding Ag/AgCl ground electrode in the hydrated support, we are able to carry out electrical measurements across the bilayer. We find that the bilayers are typically able to withstand voltages up to ~300 mV while retaining seals in excess of 100 GΩ. Electrical noise levels are typically of the order of $\pm$0.5 pA rms, with a 1 kHz recording bandwidth. This reflects the limitations of our apparatus and not the inherent noise in DHBs.

Synchronous optical measurements of bilayer area (Figure 1b) in conjunction with capacitance measurements (Figure 1c) yield a specific capacitance of 0.65 μF cm^{-2} at 22 °C for the DPhPC bilayers in our system. This agrees well with other reported values (0.4−0.8 μF cm^{-2}),[14,19,21] indicating that the bilayers are similar in thickness to their planar bilayer counterparts.

***In Vitro* Transcription and Translation of Proteins.** α-Hemolysin wild-type (αHL-WT), αHL-M113F-D8[23] (RL2 background[24] and a C-terminal D8 extension to produce a gel shift relative to αH-WT), and the viral potassium channel Kcv were prepared from genes cloned in the pT7.SC1[25] vector using a coupled *in vitro* transcription−translation (IVTT) kit (Promega Corp.) as previously described.[26] ^{35}S-Methionine was incorporated into the proteins for visualization by autoradiography.

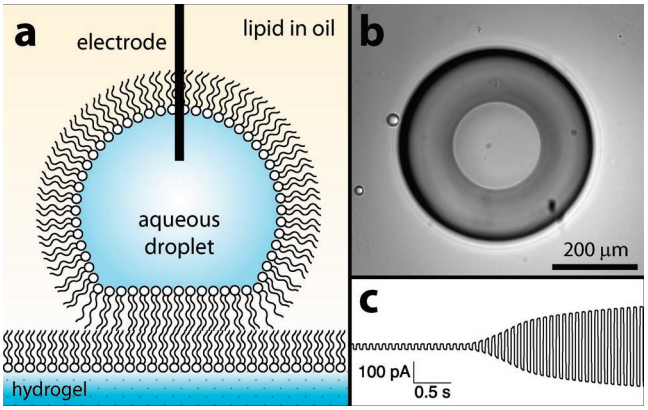

Figure 1. Droplet on hydrated support bilayers (DHBs). (a) Diagram of a DHB. Lipid monolayers spontaneously form on aqueous surfaces when immersed in a solution of lipid in hydrophobic oil. When the monolayers of two components are brought into contact, they zip together to form a bilayer. (b) Droplet bilayers are visualized from below on an inverted microscope. The image shows a droplet without electrode supported on a hydrogel surface. The single continuous bilayer area in the center of the droplet is easily seen due to the high contact angle at the interface. (c) The bilayer between the droplet and hydrated support is electrically accessed via electrodes inserted into each component. The electrical capacitance trace shows the formation of a bilayer. Bilayer capacitance is determined by applying a triangular potential waveform to the bilayer and measuring the square wave peak-to-peak current response. Membrane proteins can insert into the bilayer from either interface.

Fifty-microliter IVTT reactions of the αHL proteins were oligomerized as described previously[26] and then pelleted and resuspended (20 μL, 10 mM MOPS buffer, pH 7.4, 150 mM NaCl, 1 mg mL^{-1} BSA). Prior to electrophoresis, the 20 μL resuspended oligomer samples were mixed with 5 μL of 5× SDS-containing Laemmli buffer (final concentration: 10% (v:v) glycerol, 5% 2-mercaptoethanol, 2.3% (w:v) SDS, 0.0625 M Tris, pH 7.5).

One hundred-microliter IVTT reactions were performed for Kcv, and the products were subsequently separated in a 10% Tris-HCl gel by electrophoresis. The gel was dried onto paper under vacuum at room temperature and then imaged by autoradiography. The band corresponding to the Kcv tetramer was cut from the gel and rehydrated (300 μL, 10 mM Hepes, pH 7.4). The rehydrated gel was crushed and transferred to a 0.2 μm cellulose acetate microfiltration tube (Rainin) and centrifuged at 25000*g* for 30 min to recover the solubilized protein.

Electrophoresis of IVTT Proteins. Five-microliter aliquots of the gel-purified Kcv tetramers were loaded into a 8.5% Tris−acetate polyacrylamide gel and subjected to electrophoresis (200 V, 20 min) in TBE buffer (8.9 mM Tris, pH 8.3, 8.9 mM boric acid, 200 μM EDTA, 0.1% (w:v) SDS) to separate the protein bands. The gel tank was then refilled with SDS-free TBE buffer, and electrophoresis was continued (50 V, 2 h) to reduce the SDS concentration in the gel and improve DHB stability. This step can be omitted when running gels under native detergent-free conditions.

Five-microliter aliquots of the IVTT αHL oligomers were loaded into 7% Tris−acetate polyacrylamide gels (XT Criterion, Bio-Rad Laboratories Inc.) and subjected to electrophoresis (200 V, 1 h) in XT Tricine buffer (Bio-Rad Laboratories Inc.) to separate the protein bands. The gel tank was then refilled with SDS-free Laemmli buffer, and electrophoresis was continued (100 V, 2 h).

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All gels were run with a pre-stained marker lane (SeeBlue Plus2, Invitrogen). Following DHB gel scanning, the gels were imaged by autoradiography.

***Escherichia coli* Crude Extraction and Electrophoresis.** Competent *E. coli* JM109(DE3) cells (Promega Corp.) were transformed by heat-shock with pT7-plasmids encoding either αHL-WT or αHL-M113F-D8. Single colony transformants were picked and cultured for 16 h in 2 mL of LB medium containing 50 μg mL^{-1} ampicillin. The cells were then centrifuged at 2500*g* for 20 min and resuspended (200 μL, 25 mM MOPS, pH 7.4, 150 mM NaCl, 0.5% (w:v) SDS, 500 ng of DNase 1). Following 30 min of incubation on ice, the 200 μL samples were mixed with 50 μL of 5× SDS-containing Laemmli buffer (final concentration: 10% (v:v) glycerol, 5% 2-mercaptoethanol, 2.3% (w:v) SDS, 0.0625 M Tris, pH 7.5). Forty-five microliters of this solution was then loaded into 10% Bis-Tris polyacrylamide gels (XT Criterion, Bio-Rad Laboratories Inc.) and subjected to electrophoresis (200 V, 30 min) in XT MOPS buffer (Bio-Rad Laboratories Inc.). The gel tank was then refilled with SDS-free buffer (50 mM MOPS, 50 mM Bis-Tris, pH 7.0), and electrophoresis was continued (100 V, 2 h) to reduce SDS.

Following DHB gel scanning, the gels were stained with Coomassie Brilliant Blue (Sigma-Aldrich).

DHB Gel Scanning. After electrophoresis, the Kcv gel was immersed in 10 mM Hepes buffer (pH 7.0) containing 500 mM KCl for at least 30 min. The αHL and *E. coli* gels were immersed in 10 mM Na_iPO_4 (pH 7.0) buffer containing 1 M KCl. After dialysis, the gels were left in 10 mM DPhPC/C_{16} solution for 15 min and then scanned with ~200 nL droplets of the same buffer as that in the gel. Droplets were moved about the surface of the hydrogels with the inserted Ag/AgCl electrode attached to a dxdydz micromanipulator (NMN-21, Narishige).

Electrical Measurements and Bilayer Imaging. One hundred-micrometer-diameter Ag/AgCl wire electrodes were used to electrically access the droplets and gels. Currents were recorded with a patch clamp amplifier (Axopatch 200B, Axon Instruments) and digitized at 1 kHz (MiniDigi-1A, Axon Instruments). Electrical traces were filtered post-acquisition (100 Hz low-pass Gaussian filter) and analyzed using pClamp 9.0 software (Axon Instruments). The gel scanning apparatus and amplifying headstage were enclosed in a Faraday cage attached to an inverted microscope (TE-2000, Nikon Instruments UK) equipped with a camera (DS-1QM, Nikon) for imaging of the DHB bilayers.

Results

DHB Properties. In general, we find that DHBs are stable under the same conditions under which other artificial bilayers are stable. However, in contrast to conventional planar bilayers with a typical lifetime of a few hours,[27] we find DHBs are considerably more robust and typically last for several weeks under working conditions. Figure 2 illustrates this, showing the capacitance of a DHB subjected to a continuous alternating +100 mV/−100 mV capacitive load for 72 h (at which point the experiment was terminated). DHBs can also withstand the shock of repeated drops from heights of 1 m without bursting.

The stability of DHBs enables the droplets to be manipulated and translated about the surface of the hydrated support without breaking the bilayer (moving at speeds of up to 1 mm s^{-1}). Bilayers can also be repeatedly re-formed by removing the droplets from the hydrated surface and then returning them (see Supporting Information). Experiments with solutions containing fluorescent dye suggest that resetting the bilayer in this manner does not result in mixing between the two solutions (data not shown).

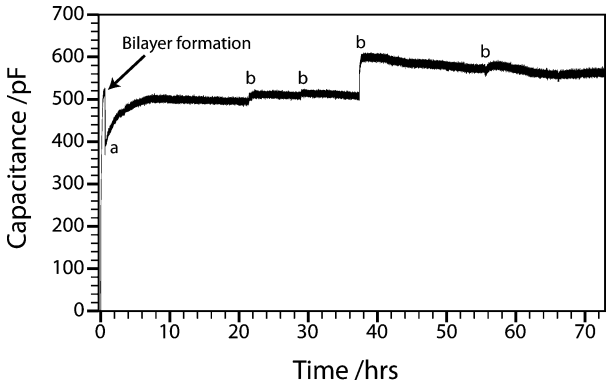

Figure 2. 72 h test of DHB stability. A single 100 nL DHB was electrically accessed and subjected to a continuous triangular potential waveform (2.5 Hz, +100 mV to −100 mV) to measure capacitance. The graph shows the capacitance changes resulting from changes in bilayer size over the course of 72 h. At initial bilayer formation, the bilayer expands quickly up to 500 pF as indicated. Immediately after this, the DHB position was adjusted by means of the inserted electrode, and the bilayer size slightly reduced (a). After this, the bilayer slowly increased in size as the DHB relaxed. The subsequent marked changes in capacitance (b) also result from adjusting the DHB. After 72 h, the experiment was ended with the DHB in an intact state.

Manipulating the vertical height of a droplet also allows the size of the bilayer area to be controlled. The size range is primarily dependent on the volume of the droplet, but for a 200 nL droplet we can adjust the bilayer diameter from ~30 μm to 300 μm in a few seconds by moving the inserted 100 μm diameter electrode (see Supporting Information). Bilayers up to 1 mm in diameter have been created with larger volume droplets. It is also possible to create much smaller bilayers with smaller volume droplets; however, our existing apparatus is limited by the use of 100 μm diameter electrodes and the larger droplet volumes this necessitates. Hence, we are unable to access bilayers of less than 30 μm in electrical experiments.

Gel Scanning with DHBs. We scanned a droplet bilayer across polyacrylamide gels containing the viral potassium channel Kcv[28,29] and two mutants of the staphylococcal pore-forming toxin α-hemolysin[30] (αHL) (Figure 3a,b). When DHBs were positioned over regions of the gels containing channel proteins, we were able to detect stepwise changes in ion current resulting from the spontaneous insertion of channels. Figure 3c shows typical examples of electrical traces acquired when scanning the respective regions of the gels containing Kcv, αHL-WT, and αHL-M113F-D8. Kcv behavior is characterized by stepwise bursts of current as the channels transiently open and close (Figure 3c, top). αHL-WT pores remain open, resulting in stepwise increases in current for each insertion event (Figure 3c, middle). To demonstrate the ability to differentiate between the two α-hemolysin mutants, we also scanned the αHL gels with droplets containing β-cyclodextrin (βCD). βCD acts as a non-covalent blocker that lodges inside the β-barrel of αHL, which is observed as a reversible stepwise change in current in an electrical recording.[31,32] αHL-WT does not bind βCD

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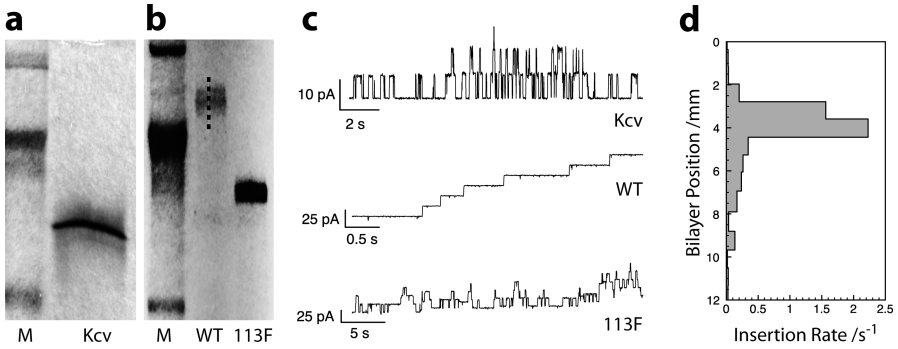

Figure 3. Scanning proteins in hydrogels using DHBs. (a,b) Composite images of polyacrylamide gels after DHB scanning, created by overlaying an image of the dried gel (containing visible pre-stained maker lane M) with autoradiographs to visualize the radio-labeled protein bands. Marker lane (M) bands correspond to molecular weights of approximately 210, 111, and 71 kDa. (a) SDS−PAGE gel containing the potassium channel Kcv. (b) SDS−PAGE gel containing αHL-WT (WT) and αHL-M113F-D8 (113F). After immersion in DPhPC/C_{16} solution, the gels were scanned with 200 nL droplets. Protein insertion and binding was monitored via patch-clamp amplified electrical recordings as a function of droplet−bilayer position on the gel surfaces. (c) Typical electrical recordings from the regions of the gels containing Kcv (+20 mV, 500 mM KCl, 10 mM Hepes, pH 7.0), αHL-WT, and αHL-M113F-D8 (+10 mV, 1 M KCl, 10 mM Na_iPO_4, pH 7.0). The αHL channels were scanned with droplets containing 10 μM β-cyclodextrin (βCD) to differentiate between the two mutants. (d) Channel-protein insertion was observed only in localized regions about the separated protein bands. This is illustrated by a 12 mm linear scan across the αHL-WT band (dotted line marked on gel b), which shows the rate of channel-protein insertion as a function of bilayer position.

strongly,[31,32] whereas the αHL-M113F-D8 mutant binds βCD strongly with a voltage-dependent mean dwell time of ~10 s.[23] Without βCD, the electrical characteristics of the two αHL variants are essentially identical. With βCD, the αHL-M113F-D8 channels are easily distinguishable by the βCD binding events overlaying the stepwise increases in conductance (Figure 3c, bottom).

During gel scanning, the proteins do not appear to diffuse, and insertion events were observed only in highly localized regions about the focused bands in the gel. This is illustrated quantitatively in Figure 3d, which shows protein insertion rate in a linear scan across the wild-type αHL band using a droplet with a bilayer of ~200 μm diameter. We do not know the precise mechanism of protein insertion in these experiments. Additionally, SCR measurements show that all inserted transmembrane proteins are lost when a droplet bilayer is removed and re-formed. No proteins re-insert if the bilayer is re-formed in a protein-free region. This indicates that the proteins are removed from the bilayer or become denatured by the disconnection process. However, if the bilayer is re-formed in a region containing fresh protein, new channels can insert into the bilayer once again (see Supporting Information).

We extended the gel scanning experiments to SDS−PAGE purified cell extracts. Figure 4 shows the results of scanning an SDS−PAGE gel (Figure 4a) containing crude extracts from *E. coli*, transformed to produce αHL-WT (lane 1) and αHL-M113F-D8 (lane 2) through leaky expression. As with the previous gel example in Figure 3, we were able to electrically characterize these proteins (Figure 4b) from the expected regions of the gel, as shown by subsequent Coomassie staining. Channel insertion rates were higher than observed from the IVTT gels, reflecting the substantially higher concentrations of protein produced from expression in *E. coli*. Surprisingly, in addition to αHL channels, we also detect a number of other channel proteins with markedly different behavior (e.g., Figure 4b, bottom), again localized to specific areas of the gel. The channels typically insert in multiples of three and show substantial voltage-dependent gating behavior. Without further analysis, it is impossible to uniquely classify these channels; however, they exhibit both an electrophoretic mobility and channel behavior characteristic of outer-membrane porins

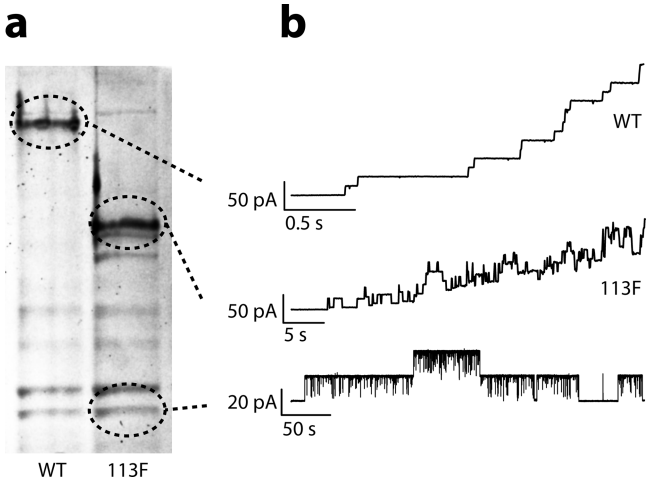

Figure 4. Scanning cell extracts in gels using DHBs. (a) Coomassie-stained polyacrylamide gel after DHB scanning, showing SDS−PAGE-purified *E. coli* cell extracts. The *E. coli* cell lines were separately transformed to produce αHL-WT (lane 1) and αHL-M113F-D8 (lane 2) through leaky expression. After immersion in DPhPC/C_{16} solution, the gel was scanned with 200 nL droplets containing 10 μM β-cyclodextrin. Protein insertion and binding was monitored via patch-clamp amplified electrical recordings as a function of droplet−bilayer position on the gel surface. (b) Typical electrical recordings are shown from scans of the regions marked on the gel. Large numbers of αHL-WT (top) and αHL-M113F-D8 (middle) channels inserted from the regions indicated. Small numbers of unidentified porin-like channels (bottom) were found in the lower region of the gel.

(OMPs) from *E. coli*. Such channels could not be detected in a porin-free strain of *E. coli* (see Supporting Information). While we do not attempt to further characterize these channels, this experiment illustrates that, with our approach, we can discover channels from extracts of cells without the need for overexpression.

Extended immersion in the lipid/oil solution during gel scanning does not appear to result in any substantial loss of protein from the gel matrix. Similarly, extended immersion in electrolyte buffer during the dialysis step does not noticeably deplete the proteins in the gel, judged by the rate of insertion from SCR. As a result, individual gels can be re-used in many consecutive gel scanning experiments, and the gel buffer conditions can be varied as required. Furthermore, the scanning procedure does not affect our ability to subsequently stain or

image the gel, or to recover specific proteins from the gel for further analysis.

Analyte Detection with DHBs. Essentially reversing our gel scanning experiment, we have also been able to use protein channels in DHBs as molecular sensors to scan different analytes within hydrogels. We doped 2% polyacrylamide gels with ~10 μM γ-cyclodextrin (γCD) and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (hβCD) in two regions spaced ~10 mm apart (Figure 5a). Following a 30 min immersion of the gel in a DPhPC/C_{16} solution, we scanned the gel between the two cyclodextrin regions with a 200 nL droplet containing αHL-WT. Under our experimental conditions, γCD binding to αHL-WT[31] produces a current block of 68%, and hβCD binding to αHL-WT[22] produces a current block of 95%. These different binding amplitudes permit positive identification of both analytes with αHL-WT (Figure 5b).

In this experiment, the droplet was translated across the hydrogel without removing the bilayer from the surface, retaining the αHL-WT channels in the bilayer throughout the scan. The position of the bilayer was recorded by imaging its position on an inverted microscope, and cyclodextrin binding events were observed electrically. Cyclodextrin binding frequency was determined by dividing the total number of events by the number of αHL-WT channels in the bilayer. Figure 4c shows the diffusion-limited localized binding of the two cyclodextrin analytes, plotting γCD and hβCD binding frequency in a scan between the two regions.

Discussion

DHBs possess a number of unique properties: (i) The bilayers can be moved across the surface of a hydrated support. (ii) DHBs provide a wider range of bilayer sizes (<1 μm to >1 mm) than possible using alternative techniques. (iii) The size of the bilayer can be adjusted during an experiment. For example, we use this to control the number of inserted proteins in SCR experiments, where the bilayer is initially enlarged to increase the probability of protein insertion and then rapidly reduced once a single protein has inserted to minimize the chances of further insertions. Reducing the bilayer size can also be used to concentrate transmembrane proteins inserted in a bilayer. We are currently exploring the use of this as an alternative means to crystallize membrane proteins and for improving the probability of observing protein−protein interactions. (iv) The bilayer can be removed and re-formed many times without mixing the droplet and hydrogel solutions. The fact that no contents mixing occurs is important for experiments where cross-contamination is an issue.

The sensitivity of DHB gel scanning allows us to directly study low levels of endogenous protein from cell extracts without the need for over-expression. In contrast, examining proteins without over-expression using either traditional patch-clamp techniques or planar lipid bilayers is difficult. Although whole-cell patch-clamping can examine low levels of endogenous protein, it is often necessary to compensate for other constituents of the system due to the heterogeneous nature of cell membranes.[33,34] It is possible to circumvent this problem using artificial lipid bilayers; however, it is difficult to extract

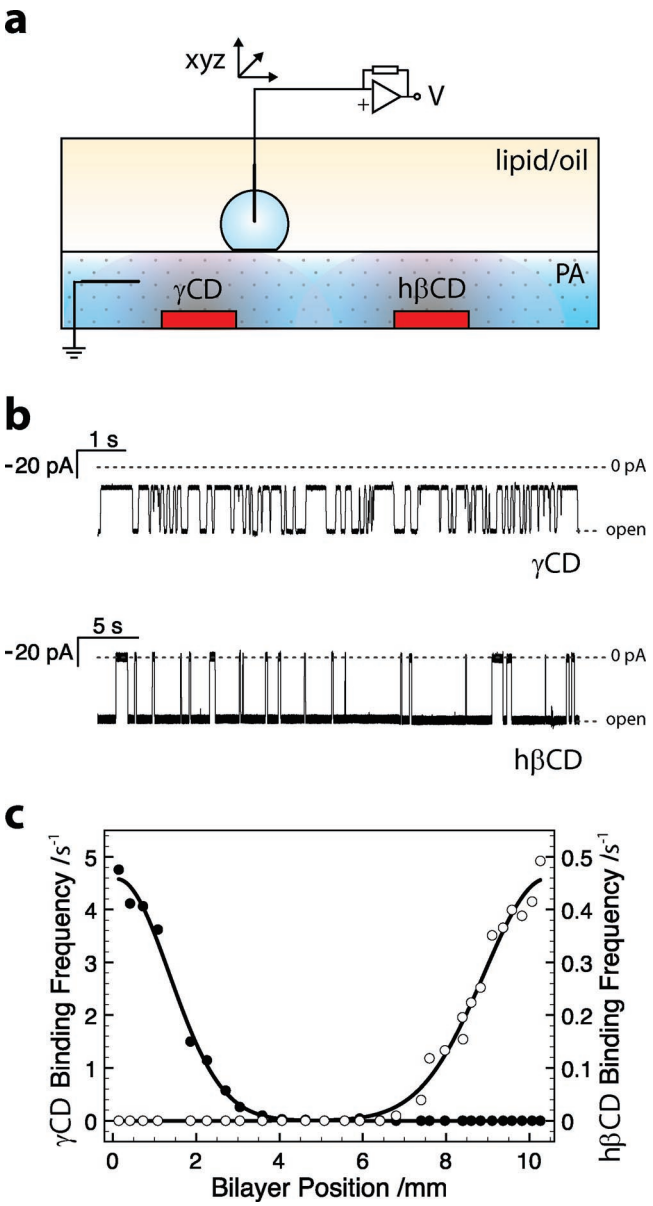

Figure 5. Scanning cyclodextrins in gels using DHBs. (a) Schematic of the experimental approach employed to scan molecules doped into polyacrylamide gels. γ-Cyclodextrin (γCD) and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (hβCD) were introduced to the bottom of the gel ~10 mm apart. After immersion and stabilization in the lipid/oil solution, the gel was scanned with droplets containing αHL-WT channels. Cyclodextrin binding to αHL-WT channels in the droplet bilayer was monitored via patch-clamp amplified electrical recordings as a function of droplet−bilayer position on the gel surface. (b) The binding characteristics of γCD (top, 68% block) and hβCD (bottom, 95% block) to αHL-WT are clearly distinguishable in electrical recordings (−50 mV, 1 M KCl, 10 mM Na_iPO_4, pH 7.0). (c) A plot of the binding frequency of γCD (●) and hβCD (○) as a function of distance in a scan between the two doped locations. Lines indicate Gaussian fits to the measured binding frequency.

and concentrate protein in sufficient quantities to successfully reconstitute in these bilayers.[27,34]

Although it is not shown here, we have also successfully inserted a wide variety of other membrane proteins into DHBs, including peptides (gramicidin), α-helix bundles (bacteriorhodopsin, K^+ channels), and β-barrels (α-hemolysin, *E. coli* porins). We found no difference in electrical recording from unsupported lipid bilayers for any of these proteins (see Supporting Information for data on Kcv). In all cases, proteins

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can insert into the bilayer from either the droplet or the hydrated support. Proteins can also be inserted into DHBs through fusion of protein-loaded vesicles contained within the droplet. Although we exclusively show DHB gel scanning of SDS−PAGE gels due to the superior band resolution, we also scanned gels run under native detergent-free conditions. At present, however, DHB gel scanning is limited to proteins that both retain their activity after gel electrophoresis and spontaneously insert into lipid bilayers. It is likely that other, more complex proteins may require vesicle fusion to effect incorporation into the bilayer.

DHBs can be formed on a wide range of hydrated substrates, including agarose, polyacrylamide, cross-linked polyethylene glycol, nitro-cellulose, polycarbonate, and even glass. The stability of DHBs also enables other droplets to be fused into an existing DHB without the bilayer rupturing. This permits the delivery of reagents (e.g., proteins, vesicles, analytes) to an experiment already in progress. We have also successfully injected reagents directly into DHBs using micropipettes, in a manner akin to existing microinjection techniques.

DHB gel scanning might ultimately be combined with existing proteomic methods that rely upon 2D gel electrophoresis to separate complex mixtures of cellular components for the discovery and characterization of new proteins.[35,36] DHB gel scanning could be used to identify channel proteins in these 2D gels, provided electrophoretic conditions can be found that do not denature the proteins or inhibit insertion. Furthermore, as DHB gel scanning does not appear to affect the proteins within the gel matrix, individual gels could be repeatedly scanned under varying conditions and subsequently analyzed with conventional proteomic techniques (e.g., mass spectrometry).

Many aspects of DHBs suggest that they might provide a new platform for high-throughput studies of ion channels. In particular, the requirement for only nanoliter volumes permits the application of many established emulsion-based technologies[37−40] for scaling-up and automating DHBs. For example, we are currently investigating the use of microfluidics[41] to create thousands of droplets with a controlled size. Large numbers of DHBs might also be manipulated in an automated fashion with existing microfluidic techniques that can create and sort nanoliter droplets in oil.[38,39] The ability to image DHB bilayers also allows the incorporation of fluorescence techniques. SCR experiments have provided a wealth of functional detail on many ion channels, but it is difficult to link this to dynamic changes in protein structure. Single-molecule fluorescence of labeled proteins is one possible method of providing additional structural and dynamic information. Moreover, DHBs could, in the future, be used for simultaneous optical and electrical measurements, which have the potential to uncover new aspects of channel function that cannot be elucidated with the individual techniques alone.[42−47]

Conclusions

The combination of enhanced stability, the ability to manipulate the bilayer, electrical access, and imaging suggest that DHBs provide a versatile platform for examining many aspects of membrane protein function beyond the scanning and detection of membrane proteins.

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Supporting Information Available: Movie showing DHB formation, successive DHB scans on αHL, current−voltage characterization of Kcv, single-channel recording of gramicidin C, and DHB gel scanning of porin and porin-free *E. coli* extracts. This material is available free of charge via the Internet at http://pubs.acs.org.

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