

# Electrical Manipulation of Glycan-Phosphatidyl Inositol-Tethered Proteins in Planar Supported Bilayers

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**ABSTRACT** Electric fields have been used to manipulate and concentrate glycan-phosphatidyl inositol (GPI)-tethered proteins in planar supported bilayers. Naturally GPI-linked CD48, along with engineered forms of I-E<sup>k</sup> and B7-2, in which their transmembrane domains have been genetically replaced with the GPI linkage, were studied. The proteins were labeled with fluorescently tagged antibodies, allowing the electric field-induced behavior to be followed by epifluorescence microscopy. All three protein complexes were observed to migrate toward the cathode with the B7-2 and CD48, each tethered to the membrane by a single GPI linker, moving significantly faster than the I-E<sup>k</sup>, which has two GPI linkers. Patterns scratched into the membrane function as barriers to lateral diffusion and were used to isolate the proteins into highly concentrated corrals. All field-induced concentration profiles were completely reversible, indicating that the supported bilayer provides a stable, fluid environment in which GPI-tethered proteins can be manipulated. The ability to electrically control the spatial distribution of membrane-tethered proteins provides new opportunities for the study of biological membranes and the development of membrane-based devices.

## INTRODUCTION

Unilamellar phospholipid vesicles spontaneously fuse with an appropriate hydrophilic surface to form a continuous supported bilayer (Brian and McConnell, 1984; Sackmann, 1996). The vesicle fusion process is quite general, accommodating a variety of substrates and lipid compositions as well as the incorporation of proteins (Kalb et al., 1992; Rädler et al., 1995; Salafsky et al., 1996; Watts et al., 1984). The supported membrane is separated from the solid support by a 10–20-Å film of water (Bayerl and Bloom, 1990; Johnson et al., 1991) and retains many of the properties of free membranes, including lateral fluidity. This fluidity is truly long-range, with mobile components of both leaflets of the bilayer freely diffusing over the entire surface of the support. Molecules confined to a supported bilayer are thus uniquely sensitive to electrical manipulation. Microelectrophoresis in supported bilayers has been demonstrated (Stelzle et al., 1992) and, more recently, combined with patterns of lateral diffusion barriers to generate steady-state concentration gradients of charged lipid probes in the supported membrane (Groves and Boxer, 1995). It was shown that these steady-state concentration profiles can be quantitatively described by a competition between electric field-induced drift and diffusion, thus providing a simple and predictable way of electrically creating spatial variations in the composition of a bilayer membrane.

Planar supported membranes were originally developed for studies of cell-cell recognition in the immune system, where they have proven to be highly useful (McConnell et al., 1986; Watts and McConnell, 1987). It was shown that

purified major histocompatibility complex (MHC) protein incorporated into a supported membrane can replace the antigen-presenting cell in the presentation of a preprocessed antigen to a helper T-cell. However, MHC is immobile in the supported bilayer, despite the persistent fluidity of the surrounding lipid. This has also been observed for the photosynthetic reaction center, which is fully functional, yet immobilized, in the supported membrane (Salafsky et al., 1996). It is likely that this immobilization, which appears to be a general feature of transmembrane proteins in supported bilayers, results from direct interactions between the protein and the solid support (Kühner et al., 1994). Proteins tethered to the membrane by glycan-phosphatidyl inositol (GPI) linkages do not present this problem and are highly mobile (Chan et al., 1991; Fein et al., 1993). There are numerous naturally occurring GPI-linked proteins, including hydrolytic enzymes, parasite coat proteins, lymphoid antigens, small molecule receptors, as well as a variety of cell adhesion molecules (Cross, 1990; Englund, 1993). In addition, many more proteins can be genetically engineered to have a GPI linkage (Caras et al., 1987; Whitehorn et al., 1995). Incorporation of a GPI attachment signal into a gene will cause the protein to be posttranslationally modified by the cell, resulting in a GPI linkage at the signal position. This type of alteration generally does not affect the molecular recognition properties of proteins such as the ones described here (Lin et al., 1990; McHugh et al., 1995; Wettstein et al., 1991).

In this work the electric field-induced motion of GPI-tethered proteins in supported bilayers is examined. The I-E<sup>k</sup> and B7-2 studied here have been genetically modified to replace their transmembrane domains with the GPI linkage; CD48 is naturally GPI-linked. All three proteins are mobile in supported bilayers and can easily be corralled into highly concentrated regions or spread into shallow concentration gradients through the use of electric fields and dif-

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fusion barriers. Nearly close-packed densities can be achieved while preserving the fluidity and structure of the supported membrane. Measurement of drift velocities and steady-state concentration profiles of the proteins are described and compared with those of the lipid probe. All of the field-induced concentration patterns were completely reversible. The results demonstrate that the supported bilayer provides a suitable environment for the electrical manipulation of GPI-tethered proteins.

## MATERIALS AND METHODS

### Proteins

Expression systems for CD48 (Kato et al., 1992; Wong et al., 1990) and B7-2 (Azuma et al., 1993; Freeman et al., 1993; Hathcock et al., 1993) were established. Although this is not strictly necessary for CD48, it facilitates isolation of the protein in larger amounts. Mouse CD48 was amplified by polymerase chain reaction (PCR) from a mouse lymph node cDNA using the oligonucleotides 5'-AGTCATCTAGATTCTTAAG-TATTATGTGCTTC-3' and 5'-AGTCAGAATTCGTTCTTGTCAGGT-TAACAGG-3'. The PCR product was sequenced and cloned into the polylinker of the eukaryotic expression vector, pBJ1Neo (Lin et al., 1990), using the restriction endonucleases *EcoRI* and *XbaI*. Similarly, the extracellular domain of B7-2 (amino acids 1–235) was PCR amplified from a mouse lymph node cDNA using oligonucleotides 5'-ACTGAGAAATTC-CGGAAGCACCCACGATGG-3' and ACTGAGCGGCCGCTTGAGT-GAAATTGAGAGGTTTGG-3'. The PCR product was sequenced and cloned into a pBJ1Neo derivative (Whitehorn et al., 1995) that contains a modified polylinker and the human placental alkaline phosphatase (HPAP) GPI linkage signal (Fig. 1), using restriction endonucleases *EcoRI* and *NotI*. A similarly constructed GPI-linked version of I-E<sup>k</sup> has already been described (Wettstein et al., 1991). Chinese hamster ovary (CHO) cells were transfected by electroporation at 0.23 kV/960  $\mu$ F. Transfectants were selected by fluorescence-activated cell sorting for high surface expression.

GPI-linked proteins were purified from the membrane of transfected CHO cells by detergent extraction (Schild et al., 1994). Briefly, almost confluent CHO cells were washed free of medium with phosphate-buffered saline (PBS) containing a cocktail of proteinase and phosphatase inhibitors. The cells were lysed on ice in the same buffer containing 0.5% NP-40. Nuclei and cell debris were spun out, and the supernatant was loaded on an antibody affinity column. The following antibodies were used: 14.4.4, anti-I-E<sup>k</sup> (Ozato et al., 1980), GL1, anti-mouse B7-2 (Hathcock et al., 1993), and HM48-1, anti-mouse CD48 (Kato et al., 1992). The detergent was exchanged to 1% octylglucoside (OG) on the column, and the proteins were eluted by base (pH 11.5) containing 1% OG. After elution, either the proteins were stored in neutralized elution buffer or the buffer was exchanged with 1% OG in PBS.

CGGTATCGAT AAGCTTGATA TCGAATTCCT GCAGCCCGGG GGATCCACTA

GTCTAGAGC GGCCGCCTGC CTGGAGCCCT ACACCGCCTG GCACCTGGCG  
C L E P Y T A C D L A

CCCCCGCCG GCACCAACGA CGCCGCGCAC CCGGGGCGGT CCGTGGTCCC  
P P A G T T D A A H P G R S V V P

CGCGTTGCTT CCTCTGCTGG CCGGACCCCT GCTGCTGCTG GAGACGGCCA  
A L L P L L A G T L L L L E T A T

CTGCTCCCTG AGATT  
A P \*

FIGURE 1 Sequence of the polylinker and the HPAP GPI linkage signal in the modified version of pBJ1Neo. The sites used for cloning the extracellular domain of B7-2 are underlined, and the protein sequence of the GPI linkage signal is given in one-letter code.

### Proteoliposomes

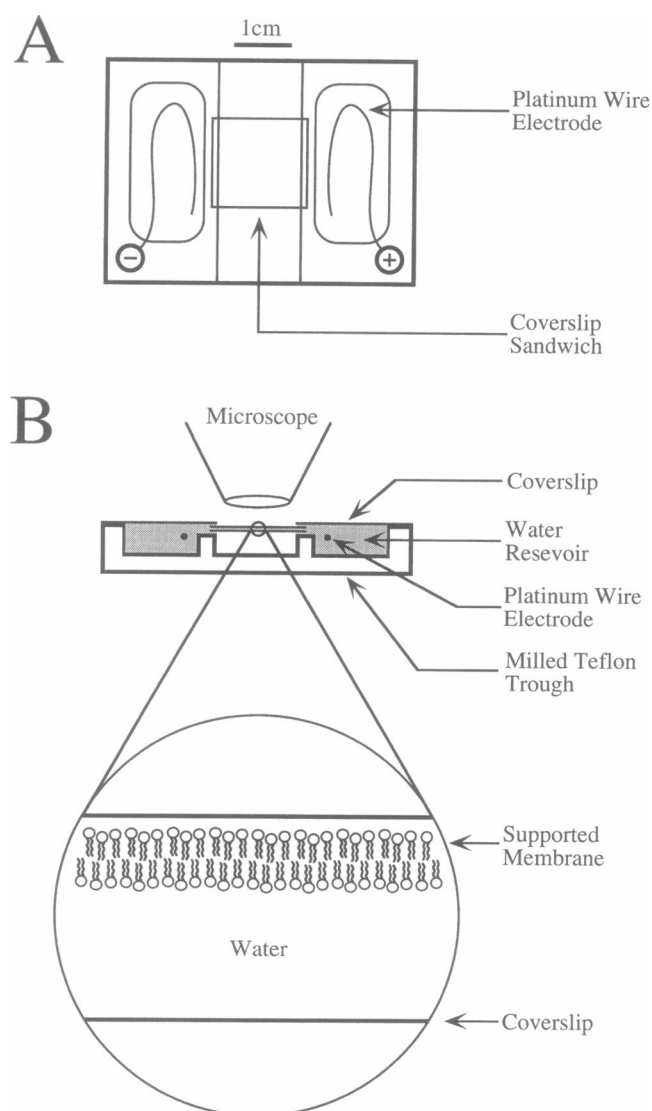
Proteoliposomes were prepared by the detergent dialysis method with preformed small unilamellar vesicles (SUVs). The SUVs were made roughly according to the Barenholz procedure (Barenholz et al., 1977). A lipid solution in chloroform was evaporated onto the walls of a round-bottomed flask, which was then evacuated overnight. Lipids were resuspended in 25 mM Tris, pH 8.0, 50 mM NaCl (TN25/50) by vortexing moderately for several minutes. The lipid concentration at this point was around 6 mg/ml. The lipid dispersion was then probe sonicated to clarity on ice under a steady flow of argon to minimize oxidation of the unsaturated lipids. The SUVs were separated from other lipid structures by ultracentrifugation for 5 h at 192,000 *g*. The supernatant contained the SUVs with typical yields of 50–75%. SUVs were frozen and stored at  $-80^{\circ}\text{C}$ . Before each proteoliposome preparation, the freshly thawed SUVs were centrifuged briefly at 20,000 *g* to remove fused lipid structures. The SUVs were composed of L- $\alpha$ -phosphatidylcholine from egg (egg-PC) (Avanti Polar Lipids, Alabaster, AL) with 1% by mole of the fluorescent probe, *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt (NBD-PE) (Molecular Probes, Eugene, OR).

Solutions of the GPI-linked proteins, at concentrations around 100 nM, were mixed with SUVs, at a lipid concentration of 1 mM, in TN25/50; the total OG concentration did not exceed 0.15%. The detergent was removed by dialysis against three changes of 1 liter of TN25/50 at  $4^{\circ}\text{C}$ . After dialysis the lipid concentration was determined using the NBD-PE absorption at 465 nm and adjusted to 0.2 mg/ml.

### Supported bilayers

Planar supported bilayers were produced by spontaneous fusion of proteoliposomes or SUVs with clean glass coverslips (Corning, Corning, NY). The glass surfaces were prepared by rinsing with purified water (Millipore system, 10 MW-cm), drying under a nitrogen stream, and heating at  $400^{\circ}\text{C}$  for 5 h and were used within 8 h. The bilayer was allowed to self-assemble by placing a coverslip over an 80- $\mu$ l drop of vesicle suspension in a petri dish for several minutes. The dish was then carefully filled with PBS, taking care not to allow the concentrated vesicle dispersion to contact the top of the coverslip. The supported membrane was rinsed by shaking gently. The coverslip and membrane were then transferred under buffer to a two-well coverglass chamber (Nunc, Naperville, IL) for antibody staining. Nonspecific binding of the antibody was blocked by pretreatment with 10% calf serum in RPMI; any binding of proteins from the calf serum had no apparent effect on the quality or fluidity of the supported membrane. The membrane was then incubated with a 10  $\mu$ g/ml solution of the appropriate antibody in the same buffer for 1 h at room temperature. The antibodies used were the same as for the purification, all directly conjugated to the fluorescent protein phycoerythrin (PE) (Pharmingen, San Diego, CA). Unbound antibody was washed away with PBS.

Two different enzyme-linked immunosorbent assay (ELISA) protocols were used to quantitate the amount of protein incorporated into the supported bilayers. The membranes were deposited on 0.5-cm round coverslips (Bellco Glass, Vineland, NJ) in a 96-well plate. In the first protocol, the bilayers were incubated with nonconjugated antibodies, as in the staining protocol. The incubation with the primary antibody was followed by a similar incubation with an appropriate alkaline phosphatase-conjugated secondary antibody: anti-mouse IgG for I-E<sup>k</sup>, anti-hamster IgG for CD48, and anti-rat IgG for B7-2, all from Sigma (St. Louis, MO). The ELISA was developed with Sigma 104 alkaline phosphatase substrate. Values were compared to ones obtained by directly coating I-E<sup>k</sup> to the 96-well plate. Because it cannot be assumed that directly coated protein is recognized by antibody in the same way as membrane-tethered protein, a second ELISA protocol was used for quantification of I-E<sup>k</sup> in a comparison under identical conditions. The bilayer was redissolved in 1% OG, and the amount of I-E<sup>k</sup> was determined with a sandwich ELISA in the presence of detergent. 14.4.4 was covalently bound to MicroBIND-HZ (Dynatech, Chantilly, VA) following the instructions of the manufacturer. The solubilized membrane was bound to 14.4.4 in comparison to known amounts of

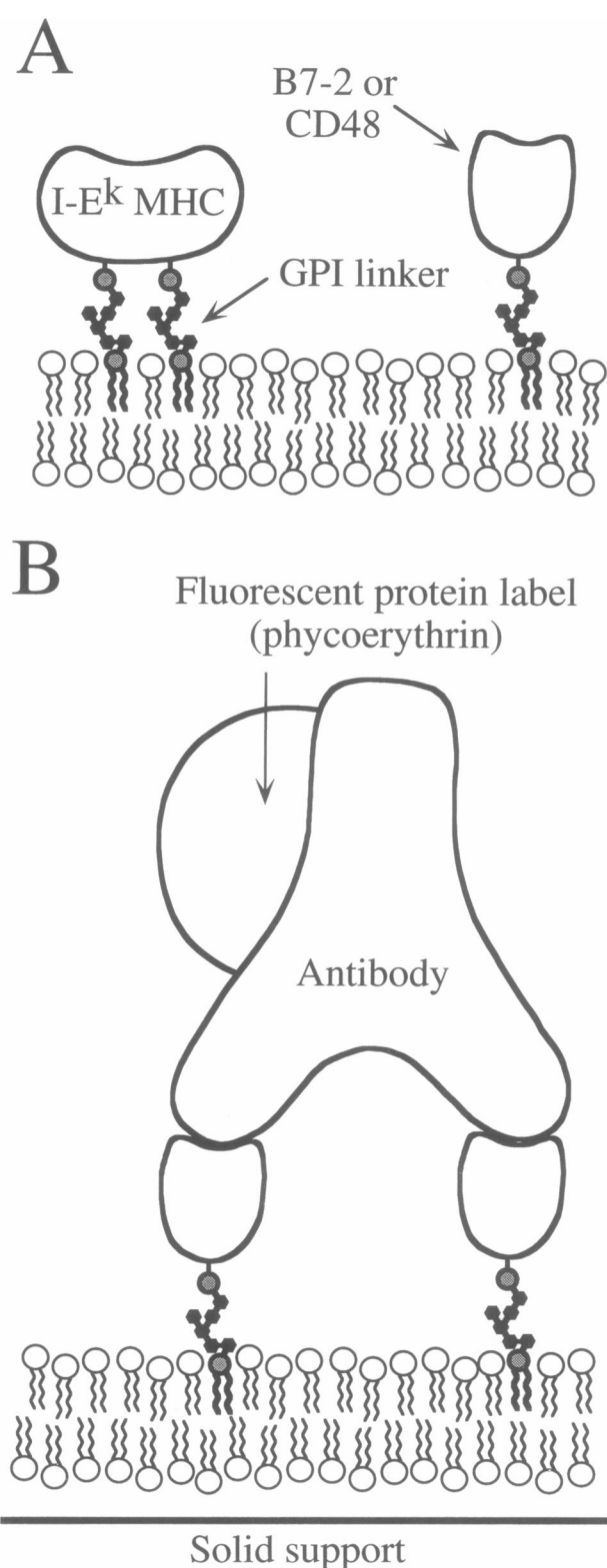


**FIGURE 2** (A) Top view of the bilayer electrophoresis cell. The coverslip sandwich forms a bridge between the two electrode-containing wells in the Teflon trough. (B) Side view of the same arrangement, showing the solution-filled wells and the electrical contact achieved through the coverslip sandwich. The inset depicts a cross-section of the coverslip sandwich, illustrating the location of the supported membrane (not drawn to scale). The water layer thickness in the sandwich is in the range of 10–50  $\mu\text{m}$ , which is much greater than the typical membrane thickness of 60  $\text{\AA}$ .

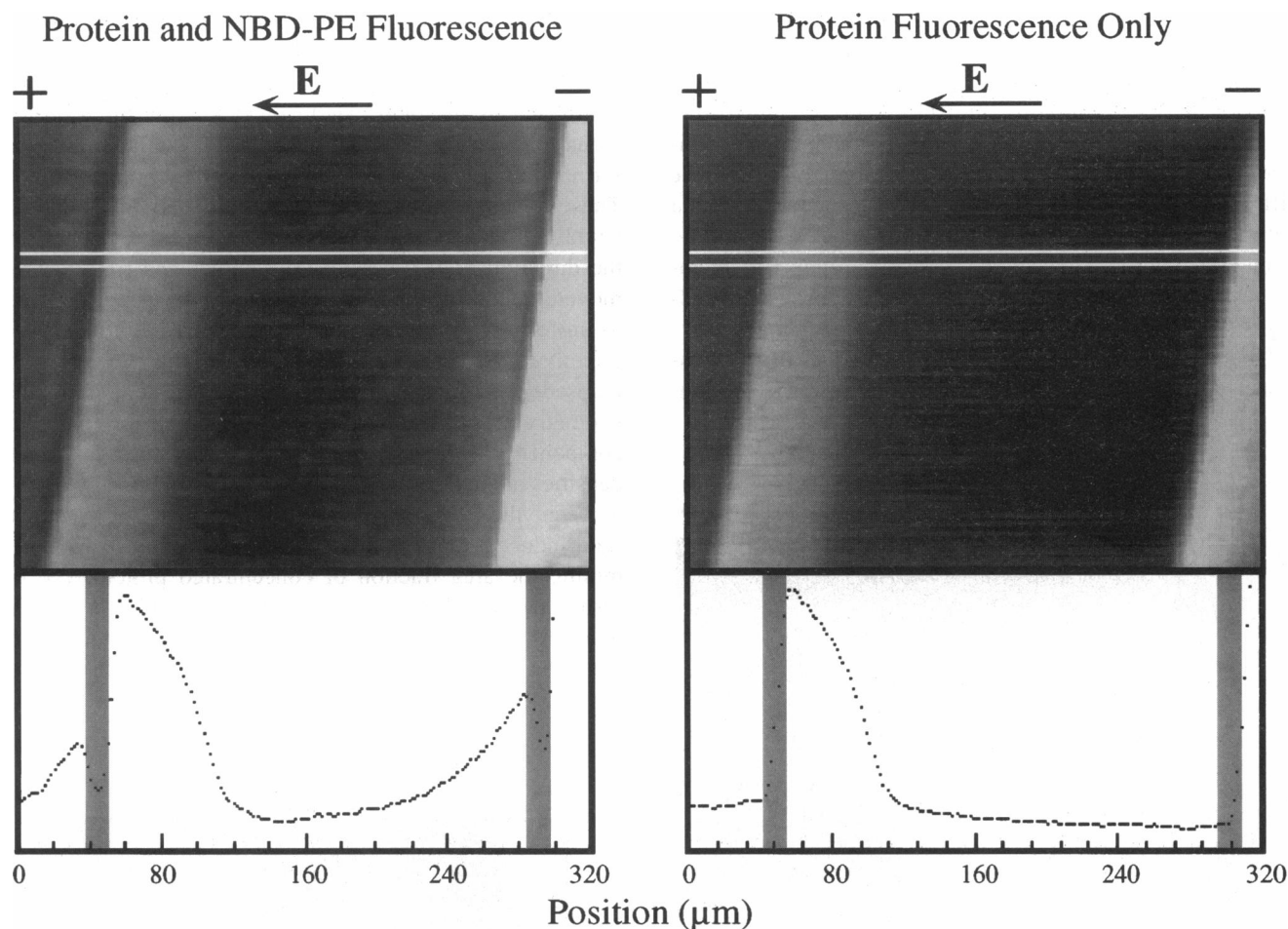
soluble I-E<sup>k</sup>. The sandwich ELISA was developed with a rabbit anti-I-E<sup>k</sup> serum (Reay and Davis, unpublished result). The two methods gave similar results: a 100 nM solution of the GPI-linked I-E<sup>k</sup> in the detergent dialysis produced a supported bilayer with a protein density in the range of  $10^{10}$  to  $10^{11} \text{ cm}^{-2}$ .

### Membrane electrophoresis

For the electrophoretic studies, the supported membrane in PBS was diluted to 1 mM total ionic strength. Barriers to lateral diffusion were created by scratching patterns into the membrane-coated surface with a pair of tweezers. This was then assembled, under buffer, into a sandwich with another coverslip. The electrophoresis cell consisted of two 0.01" diameter platinum wire electrodes in solution-filled wells of a Teflon trough (Fig. 2).



**FIGURE 3** (A) Schematic drawings of I-E<sup>k</sup> MHC, B7-2, and CD48 tethered to a membrane with GPI linkers. The I-E<sup>k</sup> is a dimeric protein with two GPI linkages, whereas the monomeric B7-2 and CD48 each have one linkage. (B) Schematic of the fluorescently tagged antibody complexed with two GPI-tethered proteins in a supported bilayer. The exact stoichiometry of the antibody-receptor complex is not known. The sizes are roughly to scale, illustrating the extension of the protein complex away from the membrane surface.



**FIGURE 4** Steady-state concentration profiles of GPI-tethered B7-2 protein complex and NBD-PE lipid in a confined region of the supported membrane at a field strength of 15 V/cm. The confined region was created by scratching the membrane-coated surface with a pair of tweezers. The scratch boundaries are roughly vertical, slanting slightly to the right. The left image was taken through filters passing fluorescence from both the protein and the NBD-PE. The right image is of the same region just seconds later, viewed through filters that passed only the protein fluorescence. The fluorescence profile appearing on the anode side of the bounded region (visible in the left image only) is from the NBD-PE lipid; the protein concentration profile has built up toward the cathode. Beneath each region is a trace of the fluorescence intensity enclosed in the region between the two horizontal white lines. The vertical gray bars in these lower panels mark the positions of the scratched boundaries. It is apparent from these traces that the NBD-PE and the GPI-tethered B7-2 protein complex have different characteristic concentration profiles.

The coverslip sandwich was arranged to form a bridge between the two electrode wells. The electrical connection was achieved through the solution in the coverslip sandwich. All glass was rinsed before use to remove any residual salt deposits. Fields up to 60 V/cm were applied with a standard power supply. Currents were monitored with a Keithley picoammeter (Cleveland, OH) and were typically around 3  $\mu$ A for a single 18-mm square coverslip sandwich at 15 V/cm. This corresponds to a total power dissipation of  $9 \times 10^{-5}$  W, which should produce a negligible amount of Joule heating. Membranes were observed in a temperature-controlled room (21°C) with an epifluorescence microscope (Zeiss, Oberkochen, Germany) through a 10 $\times$  objective. Images were monitored with a low-light video camera (Cohu, San Diego, CA) and recorded with an S-VHS VCR (JVC, Elmwood Park, NJ). The camera's gamma factor ( $I_{\text{out}} = I^{\gamma}$ ) was set at  $\gamma = 1$ , providing linear imaging of the fluorescence intensity.

## RESULTS AND DISCUSSION

### Supported membranes

Three different GPI-linked proteins have been incorporated into planar supported bilayers: CD48, B7-2, and I-E<sup>k</sup>. CD48

is a naturally GPI-linked, monomeric lymphocyte receptor. B7-2 is a monomeric receptor on professional antigen presenting cells. A GPI-linked form of B7-2 was genetically engineered by replacing its transmembrane domain with the GPI attachment signal from HPAP. I-E<sup>k</sup> is a heterodimeric mouse MHC class II protein with one transmembrane domain per monomer, both of which have been replaced with the GPI attachment signal. After formation of the supported bilayer, the membrane-tethered proteins were fluorescently labeled with PE-tagged antibodies (Fig. 3). The antibody does not appear to dissociate from the membrane over the time-scale of these experiments (several hours). It is expected that virtually all receptor proteins are bound to an antibody, given the vast excess of antibody used during the staining procedure. However, the exact stoichiometry of the antibody-receptor complex is not known. The relative concentrations of antibody and membrane-tethered receptors along with the observation of an apparently slow dissocia-

tion rate suggest that there is a substantial fraction of doubly bound antibody. This issue was not pursued in depth for the general study presented here. The egg-PC membrane was doped with 1% by mole of the fluorescently tagged lipid NBD-PE. Observation of the supported membranes by epifluorescence microscopy reveals that they are uniform to the diffraction-limited resolution of the microscope and that both the protein and the lipid are fluid with essentially no immobile fraction. Control studies with protein-free membranes indicate that there is no detectable nonspecific binding of the PE-labeled antibodies.

Under the influence of an electric field, all three membrane-tethered protein complexes drifted toward the cath-

ode, whereas the negatively charged lipid probe NBD-PE drifted toward the anode. The major component of these membranes, egg-PC, is neutral and thus is expected to be unaffected by the field. At steady state, concentration gradients of both the NBD-PE and the GPI-tethered proteins formed in confined regions of the supported bilayer (Fig. 4). These concentration profiles were completely reversible and could be switched back and forth repeatedly by changing the direction of the electric field. When the field was removed, lateral diffusion caused the gradients to relax back to uniformity. Patterns scratched into the membrane were effective guides to lateral motion, allowing proteins to be focused into highly concentrated corrals. The triangular geometry was particularly useful for concentrating dilute components such as the I-E<sup>k</sup> (Fig. 5). Average protein densities in the supported bilayers were estimated by ELISA to be in the range of  $10^{10}$  to  $10^{11}$  cm<sup>-2</sup>. The degree of field-induced concentration could be ascertained by determining the area fraction of concentrated protein. For the results described here, the proteins were typically concentrated 5- to 50-fold, corresponding to nearly close-packed densities. It proved possible to create regions of supported

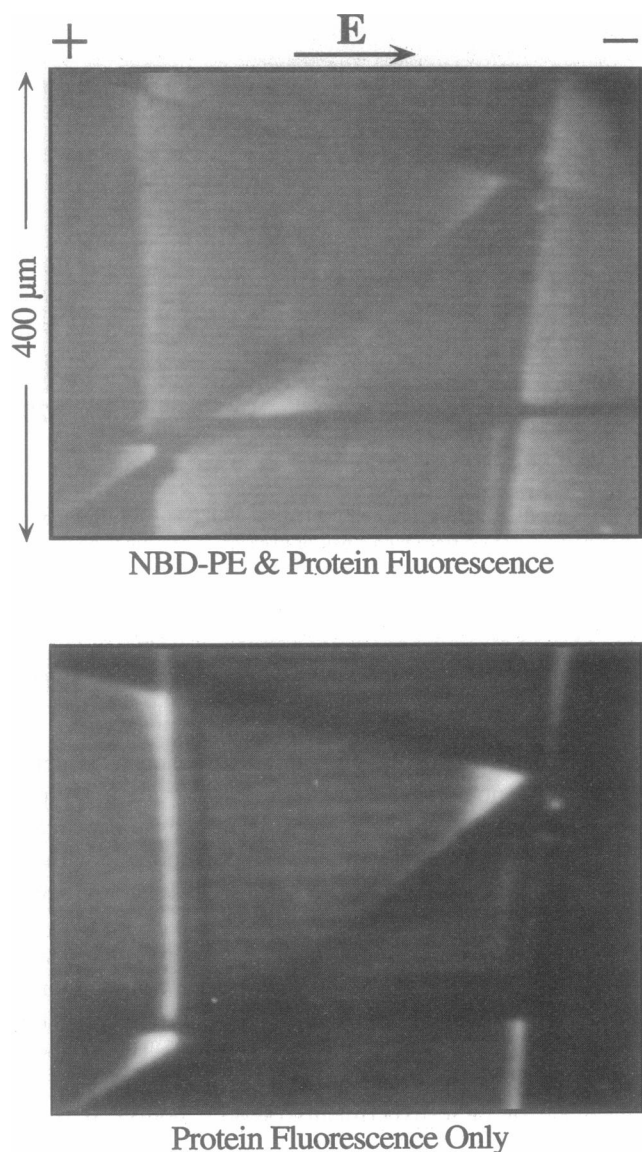


FIGURE 5 Steady-state concentration profiles of I-E<sup>k</sup> and NBD-PE in bounded triangular regions of the supported membrane. This type of pattern is effective for generating highly concentrated domains of dilute components. The top image shows fluorescence from both the protein and the NBD-PE; the bottom shows protein fluorescence only.

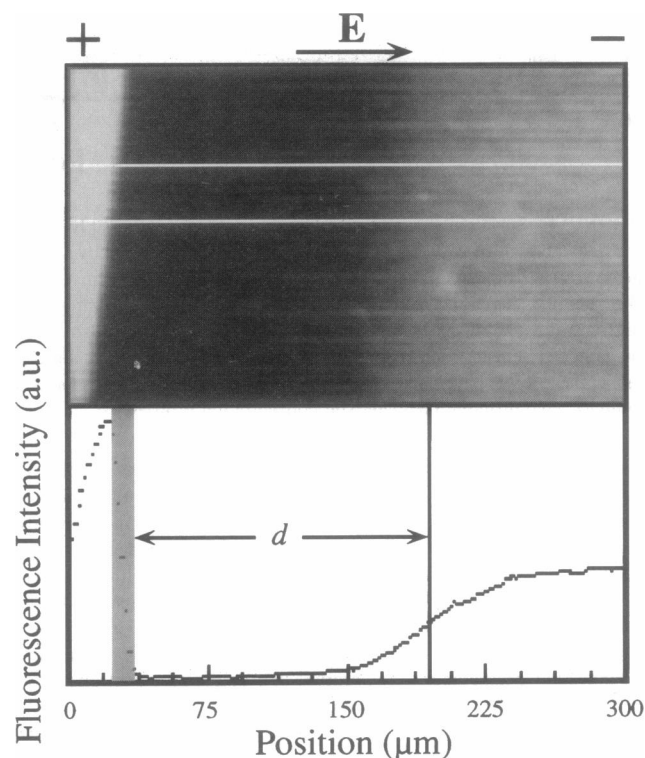


FIGURE 6 Video micrograph of the drifting image of a boundary during approach to steady state at a field strength of 15 V/cm. Below is a fluorescence intensity trace across the region between the two horizontal white lines with the scratch diffusion boundary marked with a gray bar. An image of the boundary has drifted to the right a distance  $d$ , as marked with a vertical line. Although the image shows significant diffusive spread, this distance can be determined accurately by locating the midpoint of the intensity profile. This image is from a CD48-containing membrane; B7-2 drift velocities were also measured in this way.

bilayer with protein concentrations several times the level at which the vesicle suspensions became unstable.

### Drift velocities

The drift velocities of B7-2 and CD48 complexes were measured directly by tracking the drifting image of a boundary, as depicted in Fig. 6. Upon application of the field, all charged membrane components begin to drift with a constant average drift velocity; thus an image of the boundary will drift along with this velocity (Groves and Boxer, 1995). The image will also spread because of diffusion, but image analysis can be effectively used to locate the midpoint of this profile, allowing the image to be tracked for several minutes over more than 100  $\mu\text{m}$ . Measurements taken at various times and positions in the otherwise homogeneous membrane are shown in Fig. 7. The drift velocities of both complexes are uniform and constant at  $0.91 \pm 0.05 \mu\text{m/s}$  for B7-2 and  $0.57 \pm 0.03 \mu\text{m/s}$  for CD48 at an electric field strength of 15 V/cm. It was qualitatively apparent, based on the time required to reach steady state, that I-E<sup>k</sup> drifted substantially more slowly than either B7-2 or CD48. A plausible explanation of the reduced mobility of the I-E<sup>k</sup> complex is that its double GPI linkage provides more drag in the viscous membrane; however, charge differences cannot be ruled out. Direct measurement of the drift velocity of the I-E<sup>k</sup> protein complex could not be performed because it was not possible to clearly resolve the image of the boundary. The diffusive spread of a slowly drifting image can make it difficult to track.

The direction of electric field-induced drift does not necessarily indicate that the protein complex carries a net positive charge. For these experiments there generally was a bulk electroosmotic flow of solution toward the cathode on the order of 100  $\mu\text{m/s}$ , as estimated by the motion of

stray vesicles. Frictional coupling to this flow contributes to the drift velocity of membrane components. It has been shown that electroosmotic contributions reduce the drift velocity of negatively charged probe lipids by roughly 40% under similar experimental conditions (Groves and Boxer, 1995; Stelzle et al., 1992). Unlike the lipids, the protein complex protrudes far out of the membrane plane and is thus deeply immersed in the bulk electroosmotic flow. McLaughlin and Poo (1981) have analyzed the role of electroosmosis on the field-induced motion of molecules on the surfaces of living cells. Their analysis predicts that it is the relative difference between the zeta potentials of the protein and membrane that determines the direction of motion. If the protein is less negative than the membrane, it will move toward the cathode. Thus electroosmotic effects can dominate the motion of membrane-associated proteins.

Related experiments also provide evidence suggesting that the drift velocities of the protein complexes are sensitive to the local composition of the membrane. This sensitivity becomes apparent if the field is reversed after a concentration gradient of the protein has formed. The entire concentrated domain of protein drifts away from the boundary as a unit for a short time before substantial diffusive spreading occurs. Tracking the position of these drifting domains gave variable results. The apparent drift velocity was not constant and was always slower, by as much as 60%, than the measurements presented above for relatively uniform membranes. This discrepancy may arise from heterogeneities in surface charge density, which will, in turn, cause local variation in the electroosmotic flow. This variation can be observed directly through the trajectories of stray vesicles. Other effects such as cluster formation and percolation of oppositely drifting components may also affect the motion of protein in these concentrated domains (Scalettar and Abney, 1991).

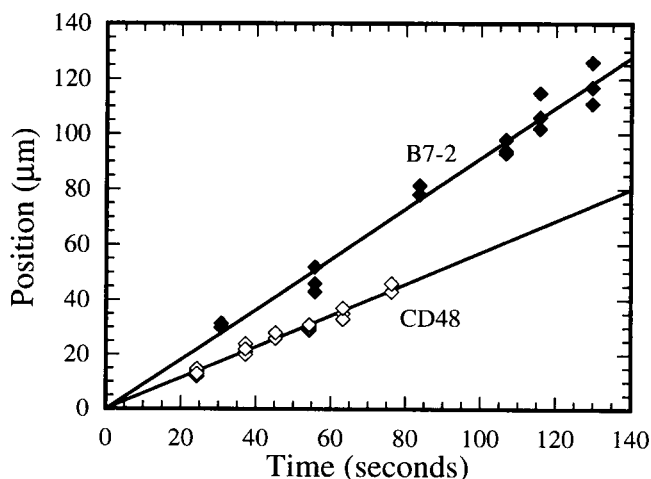


FIGURE 7 Plots of image position against drift time for the B7-2 and CD48 protein complexes. At a field strength of 15 V/cm, the drift velocity was found to be  $0.91 \pm 0.05 \mu\text{m/s}$  for B7-2 and  $0.57 \pm 0.03 \mu\text{m/s}$  for CD48.

### Steady-state concentration gradients

The NBD-PE and protein built up concentration gradients in opposite directions and with different characteristic profiles (Fig. 4). Fluorescence intensity traces across these concentration profiles are plotted in Fig. 8 on a semilog scale, from which it is evident that the NBD-PE profile is single exponential, whereas the protein profile clearly shows two distinct regions, labeled I and II. Only region I appeared in the most dilute samples.

The single exponential profile observed for NBD-PE agrees with a description of this behavior as competition between field-induced drift and diffusion (Groves and Boxer, 1995). This model, which ignores all intermolecular interactions, predicts steady-state concentration profiles of the form

$$C(\mathbf{r}) = C_0 e^{\mathbf{V} \cdot \mathbf{r} / D}$$

in confined regions of the membrane;  $\mathbf{V}$  is the drift velocity,  $D$  is the diffusion coefficient, and  $\mathbf{r}$  is the position vector.

The drift velocity of the NBD-PE at 15 V/cm was estimated to be  $0.13 \pm 0.03 \mu\text{m/s}$  from this relationship by combining exponential fits of the steady-state concentration profile (giving  $V/D$ ) with known values of the diffusion coefficient of NBD-PE in supported bilayers ( $4.4 \pm 0.5 \mu\text{m}^2/\text{s}$ ) (Stelzle et al., 1992). This value of the drift velocity is comparable to that obtained by direct measurement of a drifting boundary for Texas Red dihexadecanoyl-phosphoethanolamine (DHPE) under essentially identical conditions (Groves and Boxer, 1995). It is also interesting to note, however, that the single exponential character of the NBD-PE profile continues all the way to the scratch boundary, in contrast to the nonexponential roll-off, which was observed near boundaries in profiles of the Texas Red DHPE lipid probe.

The situation with the protein is clearly more complex, as at least two well-defined regions are observed. Applying this simple model to the more dilute region I of each concentration profile, exponents ( $V/D$ ) for B7-2 and CD48 were found to be similar, ranging from 0.09 to  $0.19 \mu\text{m}^{-1}$ , whereas the  $I-E^k$  profiles typically had exponents from 0.06 to  $0.10 \mu\text{m}^{-1}$ . The distinctly shallower  $I-E^k$  profile is consistent with the slower drift velocity observed for this protein. The experimental variation in these exponents is substantially greater than that observed for the NBD-PE, likely because of the composition dependence of the drift velocity, which is much more pronounced for the protein complexes. The local drift velocities in the most dilute concentration profiles (which generally had the highest  $V/D$  ratios) are expected to correspond most closely to the drift velocity measurements for uniform membranes described

above. Combining fit values of the exponential profiles in region I from these samples with drift velocity measurements such as those shown in Figs. 6 and 7 implies diffusion coefficients for the GPI-linked proteins equal to  $3\text{--}6 \mu\text{m}^2/\text{s}$ , which is comparable to the lipid.

Although it seems reasonable that the model of a simple competition between field-induced drift and diffusion can be applied to region I, it is less clear that this model applies to region II, where the protein is considerably more concentrated and intermolecular interactions may be significant. It is also possible that the two regions correspond to two states of the protein complex, for example, singly and doubly bound antibodies. Further work is necessary to make connections between molecular-scale interactions and electric field-induced concentration profiles in such highly concentrated regions. However, there is evidence suggesting that these profiles can indeed be informative. Preliminary studies of streptavidin tethered to supported bilayers with biotinylated lipid reveal substantially different behavior in the highly concentrated regions. In contrast to the nearly exponential profiles observed for the GPI-tethered proteins in region II, flat profiles are observed in these regions for streptavidin. Further evidence suggests that this flat profile corresponds to the formation of two-dimensional crystalline or polycrystalline domains. More detailed studies of this system are currently under way.

## CONCLUSION

The use of electric fields to manipulate GPI-tethered protein in supported bilayers has been explored. GPI-linked forms of CD48,  $I-E^k$ , and B7-2 were incorporated into supported bilayers, labeled with fluorescently tagged antibodies, and studied by epifluorescence microscopy. Under the influence of an electric field, all three protein complexes drifted toward the cathode, whereas the NBD-PE lipid probes drifted toward the anode. Steady-state concentration profiles of the NBD-PE were single exponential, in contrast to the protein profiles, which showed two distinctly different regions. Patterns scratched into the membrane were used to guide and trap both the protein and the NBD-PE lipid probe in highly concentrated corrals. Protein densities approaching close-packed were achieved. All of the field-induced concentration gradients were completely reversible and relaxed back to uniformity when the field was turned off. The GPI linkage tightly associates proteins with the supported membrane in a manner that preserves their fluidity and thus renders them susceptible to electrical manipulations. This linkage mechanism is also fairly general; the GPI attachment signal can be genetically incorporated into many different proteins.

Much of the motivation for this investigation comes from a parallel line of work in which we have integrated patterns of lateral diffusion barriers with microelectronics to construct devices capable of manipulating molecules on small length scales and in complex patterns in a supported bilayer.

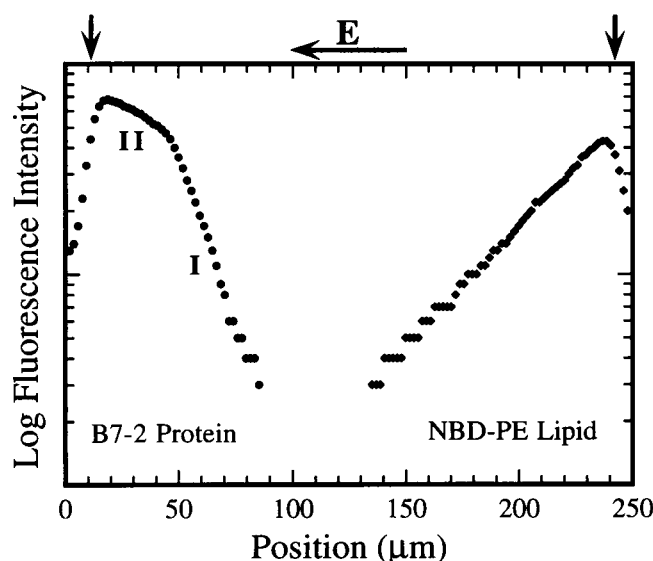


FIGURE 8 Semilog plot of the steady-state concentration profiles of NBD-PE ( $\blacklozenge$ ) and B7-2 protein ( $\bullet$ ) in a bounded region of membrane. The scratch boundaries are marked with vertical arrows; the field strength was 15 V/cm as indicated. The NBD-PE concentration gradient is single exponential, whereas the protein shows two distinct, although still essentially exponential regions (labeled I and II). Only region I is observed in the most dilute samples.



One mode of operation involves the assembly of a membrane on such a microfabricated surface and subsequent electrical separation into an array of membrane patches, each with a different and precisely controlled composition. The ability to manipulate GPI-tethered proteins allows for the application of this type of device to numerous biological assays, such as screening for biological response as a function of membrane composition or protein density. It may also prove possible to manipulate proteins anchored by transmembrane helices. Electrical control of membrane-tethered proteins offers new opportunities for studies of intermolecular interactions on the surface of a membrane. Preliminary investigation of streptavidin tethered to supported bilayers with biotinylated lipid indicates that such electrical manipulations can be used to control the crystallization process.

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