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Membrane Protein Dynamics and Functional Implications in Mammalian Cells

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Abstract

The organization of the plasma membrane is both highly complex and highly dynamic. One manifestation of this dynamic complexity is the lateral mobility of proteins within the plane of the membrane, which is often an important determinant of intermolecular protein-binding interactions, downstream signal transduction, and local membrane mechanics. The mode of membrane protein mobility can range from random Brownian motion to immobility and from confined or restricted motion to actively directed motion. Several methods can be used to distinguish among the various modes of protein mobility, including fluorescence recovery after photobleaching, single-particle tracking, fluorescence correlation spectroscopy, and variations of these techniques. Here, we present both a brief overview of these methods and examples of their use to elucidate the dynamics of membrane proteins in mammalian cells—first in erythrocytes, then in erythroblasts and other cells in the hematopoietic lineage, and finally in non-hematopoietic cells. This multisystem analysis shows that the cytoskeleton frequently governs modes of membrane protein motion by stably anchoring the proteins through direct-binding interactions, by restricting protein diffusion through steric interactions, or by facilitating directed protein motion. Together, these studies have begun to delineate mechanisms by which membrane protein dynamics influence signaling sequelae and membrane mechanical properties, which, in turn, govern cell function.

1. INTRODUCTION

The composition and organization of the plasma membrane are both highly complex and ever-changing. The modes by which proteins move in the plane of the membrane provide insights into the molecular interactions between these proteins and neighboring membrane proteins, membrane lipids, the underlying cytoskeleton, and counter-receptors on cells or other structures in the extracellular environment. Over the past several decades, investigators have developed increasingly sophisticated methods for probing the dynamics of membrane proteins, and, in so doing, have helped to define structure–function relationships among membrane receptors, counter-receptors, and structural proteins.

Membrane-associated proteins comprise a large subset of all proteins synthesized by mammalian cells. Understanding how these proteins move and interact with one another in their native environment is essential to understanding their cellular function. For example, regardless of their intrinsic affinity for binding to each other, two proteins expressed on the plasma membrane may have little chance for interaction if their positions are fixed at random locations, unless the protein concentration is very high. With slow random diffusion of both proteins, the opportunity for interaction increases. With directed movement of one or both proteins toward a membrane landmark, or with confinement or corralling of the proteins in specific sites (such as focal adhesions or membrane microdomains), the opportunities for interaction increase further. It is in this context that the diffusion modes and kinetics of membrane proteins direct their function.

2. THE FLUID MOSAIC MODEL AND BEYOND

The fluid mosaic model was a critical insight that has guided all subsequent refinements of our understanding of membrane protein dynamics. Developed by Singer and Nicolson in 1972 in the face of limited knowledge of the complexity of the plasma membrane's composition and organization, this model attempted to characterize biological membranes in a unifying manner (Singer & Nicolson, 1972). One main tenet of the model was that biological membranes consist of a phospholipid bilayer in which globular proteins are embedded. The nonpolar portions of membrane proteins were thought to be sequestered from contact with the aqueous extracellular and cytoplasmic environments, whereas the polar portions of the proteins were thought to be relatively exposed to the extracellular or cytoplasmic environment in order to minimize the free energy of the membrane. These concepts may seem obvious to cell biologists now, but, at the time, the fluid mosaic model was at odds with other ideas, such as the possibility that membrane proteins are in some way tethered to the membrane and are extended into the extracellular environment without any consideration for thermodynamic stability. The fluid mosaic model further predicted that proteins embedded in a lipid bilayer would be free to undergo translational diffusion at rates determined by the viscosity of the lipid bilayer. Despite this ability to undergo lateral diffusion, the proteins would maintain their membrane-embedded status; that is, their "degree of intercalation" with the membrane would not change (Singer & Nicolson, 1972). These predictions about mobility came with an important caveat—the lateral diffusion of a membrane protein would occur freely *unless* the protein interacts specifically with other proteins or lipids.

We now know that such interactions are the rule rather than the exception. In fact, interactions of membrane proteins with other membrane proteins, membrane lipids, and intracellular and extracellular structures are so common that measured diffusion rates of membrane proteins rarely approach the rate or pattern of free Brownian diffusion. Below is a useful categorization of the major classes of nonrandom interactions involving membrane proteins:

- A. *Interactions with membrane lipids.* The lipid bilayer surrounding any given membrane protein is typically inhomogeneous. This is evident on the molecular scale, in that adjacent phospholipid molecules have different molecular structures

such that the interface of the protein with the lipid bilayer is not radially symmetric. The inhomogeneity of the lipid bilayer is also manifested on the nanometer and micrometer scale, in that the plasma membrane contains areas that are enriched for certain lipids such as cholesterol and glycosphingolipids (Simons & Ikonen, 1997). These cholesterol-enriched membrane microdomains (CEMMs; sometimes called lipid rafts) are present in most cell types, and many membrane proteins have demonstrated a clear preference for localizing to these microdomains (Brown & London, 1998; Galbiati, Razani, & Lisanti, 2001). Indeed, compartmentalization of such proteins to CEMMs allows the proteins to serve as organizing centers for signal transduction at the membrane (Lingwood & Simons, 2010; Patel, Murray, & Insel, 2008). Observations of the lateral diffusion of membrane proteins that are preferentially localized to CEMMs reflect this relative confinement (see “*Confinement*” below).

- B. *Steric interactions and complex formation with other membrane proteins and with intracellular proteins.*** Any interaction with another protein may affect the lateral diffusion of a membrane protein. Relatively immobile protein obstacles that are either within the membrane or just below or above the membrane can present a corralling effect to confine the diffusion of membrane proteins. Binding interactions with other membrane proteins and with cytoskeletal or adaptor proteins can also constrain membrane protein diffusion. The affinity of the binding interaction, the degree of immobilization of the binding partner, and the number of proteins interacting with the membrane protein combine to determine the magnitude of the restriction on lateral diffusion (Frick, Schmidt, & Nichols, 2007). For example, a membrane protein anchored to the underlying cytoskeleton through a large number of other proteins may exhibit more restricted diffusion than a membrane protein that forms a heterodimer with another membrane protein but has no other significant protein–protein interactions.
- C. *Interactions with extracellular structures.*** Many membrane proteins are receptors or ligands for receptors on other cells or for extracellular matrix (ECM) proteins. These interactions can affect membrane protein diffusion through two mechanisms: first, the physical restriction of lateral diffusion through the receptor–counter-receptor binding interaction; and second, the binding-induced conformational change in the membrane protein that may alter downstream signaling and protein associations to further modulate diffusion.
- D. *Self-organization, self-assembly, and hierarchical ordering of membrane proteins.*** There is a growing understanding that all cellular proteins—including proteins found at the plasma membrane—are highly organized and dynamic. In order to function properly, membrane proteins must bind to and release from other proteins, form and dissolve protein complexes, and traffic to and from intracellular and membrane-localized sites. These dynamic interactions result in membrane protein mobility that is not consistent with free diffusion (Kusumi, Suzuki, Kasai, Ritchie, & Fujiwara, 2011; Watkins, Miller, Majewski, & Kuhl, 2011). In one extreme form, directed motion along a submembranous scaffold can be observed as a protein travels from a site of insertion in the membrane to a distant site of action

(Serge, Fourgeaud, Hemar, & Choquet, 2003). Small-scale directed motion can also be observed as proteins cluster at specific sites to form supramolecular complexes (Sander, Arora, & Smith, 2012; Wehrle-Haller, 2007).

Given the prevalence of these complex interactions involving proteins in the plasma membranes of mammalian cells, there is surprisingly little room for free diffusion of membrane proteins such as that predicted by the fluid mosaic model. Free diffusion may occur within some membrane sub-domains or with some mild restriction for certain proteins, but it is the exception rather than the rule. Moreover, the existence of interactions involving lipid rafts, protein complexes, extracellular structures, and self-organization all suggest that the distribution of membrane proteins is non-random across the plasma membrane.

Over the past 30–40 years, the dynamics of many different proteins in mammalian plasma membranes have been described and several distinct models for the regulation of these dynamics have been advanced. In general, these models have been developed in concert with the elucidation of the complex membrane protein interactions described above. The major current models for the regulation of membrane protein dynamics include (Fig. 3.1):

- A. *Random diffusion.* This mode of motion is based on a model of independent particles (such as proteins) that are randomly distributed in a homogenous, two-dimensional fluid (such as the plasma membrane). In such a model, the particles diffuse freely (also called Brownian motion) and the rate of diffusion is characterized by the particle's diffusion coefficient. The distance traveled by the particle as a function of time is expressed as its mean square displacement (MSD), which is related to the diffusion coefficient. In two dimensions, $MSD = 4Dt^\alpha$, where $\alpha = 1$ for Brownian motion (Mirchev & Golan, 2001). Therefore, for random diffusion, the diffusion coefficient $D = MSD/4t$. As an example, if a membrane protein randomly diffuses in two dimensions and travels an average of $0.2 \mu\text{m}$ in 1 s, then $D = (2 \times 10^{-5} \text{ cm})^2 / (4 \times 1 \text{ s}) = 1 \times 10^{-10} \text{ cm}^2/\text{s}$.
- B. *Confinement.* This mode of motion occurs when a membrane protein is preferentially localized to specific subdomains of the membrane (such as lipid rafts), or when a membrane protein encounters obstacles in its lateral motion (such as relatively immobile protein complexes or cytoskeleton-anchored membrane proteins) so that it is effectively corralled. In both scenarios, the probability that the membrane protein will move away from the local area is less than the probability that it will stay within the local area. In many cases, the lateral mobility of the protein within the area of confinement is consistent with random diffusion, and the escape of the protein from the local area results in its relocation to a new area elsewhere on the membrane, where random diffusion resumes. If the predominant behavior is confined diffusion with rare relocation events, this is known as *hop diffusion*. If the immobile obstacles or preferred membrane environments are sparse, then the confinement is transient and the larger-scale movements predominate. In either case, the lateral mobility (characterized by the diffusion coefficient D) differs during periods of confinement and nonconfinement. For this reason, it is useful to characterize the lateral mobility of membrane proteins both on small time and distance scales (D_μ , on the ms and nm scale) and on large time and

distance scales (D_M , on the s and μm scale). When D_μ exceeds D_M —a common situation for many proteins in mammalian plasma membranes—short time-scale diffusion is not constrained, but obstacles or areas of confinement become apparent over longer periods. When D_M and D_μ are equal (and not extremely low), confinement is not present.

- C. *Restriction*. This mode of motion can be considered an extreme form of confinement. It occurs when a membrane protein is bound to a significantly less mobile element (such as a large protein complex that is anchored to the cytoskeleton) such that its diffusion is far less than what would be predicted by Brownian motion, even on small time and distance scales (see “*Confinement*” above). In the extreme example, fixation of cells results in the complete immobilization of membrane proteins (Umenishi, Verbavatz, & Verkman, 2000). When D_M and D_μ are both extremely low, restriction is present.
- D. *Directed Motion*. Active transport of a membrane protein results in its directed motion in the plane of the membrane. In such a situation, the calculated diffusion coefficient may be less informative than the velocity of the transport. The equation $\text{MSD} = 4Dt^\alpha$ may also be used to characterize directed motion since the parameter α describes the time dependence of D and indicates the mode of motion. As noted above, in Brownian diffusion $\alpha = 1$ and the equation reduces to $\text{MSD} = 4Dt$. In directed motion, $\alpha > 1$.

3. TECHNIQUES FOR MEASURING LATERAL MOBILITY OF MEMBRANE PROTEINS

Several different methods are used to measure membrane protein dynamics. The primary techniques currently in use include:

- A. *Fluorescence Recovery After Photobleaching (FRAP)* [also known as Fluorescence Photobleaching Recovery (FPR)]. In this technique (Fig. 3.2A), membrane proteins of interest are fluorescently labeled and a small area of the plasma membrane is rapidly photobleached by an intense laser (Axelrod, Koppel, Schlessinger, Elson, & Webb, 1976). Over time, fluorescently labeled proteins diffuse laterally from outside the bleached region into the bleached region. The rate of lateral diffusion can be quantified using the equation $D = r^2 / 4\tau_{1/2}$ (a modified version of $D = \text{MSD}/4t$), where r is the radius of the bleached spot and $\tau_{1/2}$ is the time for recovery of half the maximal postbleach fluorescence (Axelrod et al., 1976; Kang, Day, Kenworthy, & DiBenedetto, 2012; Lippincott-Schwartz, Snapp, & Kenworthy, 2001). The fractional mobility f is defined as the ratio of the maximal postbleach fluorescence intensity to the prebleach intensity. A value of $f < 1$ suggests that a fraction of the labeled membrane proteins is relatively immobile. One advantage of FRAP is its ability to characterize the average dynamics of the labeled membrane proteins, since the observed fluorescence signal represents the sum of the signals from many copies of the protein. At the same time, it is difficult to define the heterogeneity of dynamics of individual membrane proteins in a population using this technique.

- B. *Single-Particle Tracking (SPT)*.** In SPT (Fig. 3.2B), video microscopy is used to track the motion of labeled proteins within the plasma membrane (Qian, Sheetz, & Elson, 1991). SPT is often performed by binding 20–1000 nm-diameter gold, latex, or fluorescent beads to the protein of interest, and recording the position of the bead in x- and y-coordinates at high frame rates. Larger beads (~1000nm) can be tracked using light microscopy. Smaller (~20 nm) particles include colloidal gold, which can be tracked using differential interference contrast microscopy, and fluorescent nanoparticles or quantum dots, which can be tracked using high-speed fluorescence microscopy (fluorescent SPT). In all cases, high spatial resolution and reasonable time resolution are achieved by using short exposure times (Gonda, Watanabe, Ohuchi, & Higuchi, 2010). The primary advantage of SPT and its variations is, as the name indicates, the visualization of individual membrane proteins. Trajectories and MSD are recorded for each tracked particle, and, therefore, the heterogeneity of motion among a population of protein molecules can be better appreciated (Saxton, 1997; Saxton & Jacobson, 1997). SPT also provides information about the mode of motion of individual protein molecules, including confinement, restriction, and directed motion. As described above, this information can help elucidate the biological structures responsible for the nonrandom motion, such as membrane microdomains, cytoskeletal anchorage, hierarchical organization, and active transport. Fluorescent SPT can also be used to detect clustering of tracked particles, which is visualized as an increase in fluorescence intensity (Jaqaman et al., 2008). Fluorescence speckle microscopy (FSM) is used to analyze macromolecular assemblies. FSM has been used primarily to study the dynamics of cytoskeletal and cytoskeleton-binding proteins (Mendoza, Besson, & Danuser, 2012). In this technique, a small fraction of a protein of interest is labeled, often by fusion with a fluorescent protein. Because the protein concentration is heterogeneous in two and three dimensions and because the label is not saturating, the protein labeling appears speckled. In the context of membrane protein dynamics, speckles can represent transient clustering of the protein of interest. The motion, appearance, and disappearance of such speckles can be tracked and used to quantitatively describe local protein concentrations and dynamics. Although used primarily as a technique to study the dynamics of cytoskeletal proteins, FSM could also serve as a complement to SPT in the study of membrane proteins, particularly adhesion molecules (Hu, Ji, Applegate, Danuser, & Waterman-Storer, 2007; Mendoza et al., 2012).
- C. *Fluorescence Correlation Spectroscopy (FCS)*.** FCS is a third technique to measure membrane protein diffusion. In this method, membrane proteins of interest are fluorescently labeled and a small area of the plasma membrane is illuminated using a confocal microscope at the excitation wavelength of the fluorophore (Bacia, Kim, & Schwille, 2006; Haustein & Schwille, 2007). As the labeled proteins diffuse into the area of illumination, fluorescence emission is detected. The longer the protein remains in the illuminated area, the longer is the recorded burst of fluorescence emission. Analysis of the multiple transits of the labeled protein molecules yields a characteristic transit time, and this transit time, together with the size of the

illumination spot, allows for calculation of the diffusion coefficient (Haustein & Schwille, 2007). The transit time can also be expressed as a function of the size of the illumination spot; the larger the spot is, the longer is the transit time. In cases where there is active partitioning of proteins among different membrane subdomains, such as confinements conferred by lipid rafts, the transit time is less dependent on the size of the illumination spot (Bacia, Scherfeld, Kahya, & Schwille, 2004; Billaudeau et al., 2013; Kahya, Scherfeld, Bacia, Poolman, & Schwille, 2003). For example, if a small area of illumination happens to contain one or more lipid rafts in their entirety, then a raft-dependent protein that enters the area would likely be confined preferentially to those rafts, and shrinking the illumination area further would not change the transit time significantly if the rafts were still within the illumination area. Advantages of FCS are its ability to quantify particle density in the area of illumination and its use of low-intensity laser illumination (unlike FRAP).

All of the above techniques can be combined with other experimental modalities to measure protein–protein interactions (Lippincott-Schwartz et al., 2001). One such method is fluorescence resonance energy transfer (FRET). For example, FRET can be combined with fluorescent SPT to determine whether two fluorescently tagged proteins are clustering together or are otherwise associated at the plasma membrane (Rolfe et al., 2011). Similar analysis is also enabled using a combination of FCS and FRET (Haustein & Schwille, 2007). Protein diffusion before and after such protein–protein association can be measured and compared. FRAP, fluorescent SPT, and FCS can also be combined with total internal reflection fluorescence (TIRF) microscopy to gain better resolution of the plasma membrane and to measure the effect of protein–ligand interactions on membrane protein lateral mobility (Nechyporuk-Zloy, Dieterich, Oberleithner, Stock, & Schwab, 2008).

4. MEMBRANE PROTEIN DYNAMICS IN MAMMALIAN CELLS

Much of the early work on membrane protein dynamics was carried out on erythrocytes. Since that work, studies have expanded to include the dynamics of membrane proteins on all major hematopoietic lineages and many other nucleated mammalian cells. In this section, we review some of the studies on major membrane proteins in these various cell types.

4.1. Red cell membrane protein dynamics

The human red blood cell (RBC) membrane was an early model of choice for studying membrane protein dynamics. The reasons for this choice included: relative ease of obtaining the cells for study; relative uniformity of the cells; relatively abundant knowledge about the content, organization, and characterization of RBC membrane proteins and lipids (Bennett, 1985); and the availability of RBCs with abnormal membrane proteins that were known to be involved in several RBC diseases (Iolascon, Perrotta, & Stewart, 2003; Palek, 1987). Like the plasma membrane of other mammalian cells, the human RBC membrane consists of a lipid bilayer with embedded proteins. In the case of the RBC membrane, the lipid bilayer is biochemically and biophysically coupled to an underlying membrane skeleton. The major proteins in the membrane skeleton are spectrin tetramers and short actin filaments that, together, form a triangular lattice arrangement. The linking protein ankyrin has high-

affinity binding sites for both spectrin and band 3 (see Section 4.1.1 below), and thereby serves as an important connection between the membrane skeleton and the lipid bilayer (Bennett, 1985). Another important connection is provided by adducin, which links the spectrin–actin junctional complex to band 3 (Anong et al., 2009). In this section, we review the dynamics of some of the major RBC membrane proteins.

4.1.1 Band 3—Each human RBC contains ~1 million copies of band 3 protein (Steck, 1974). This protein has two important roles in RBC structure and function: (1) to serve as the major membrane anchor for the protein–protein interactions that couple the lipid bilayer to the underlying membrane skeleton and (2) to maintain anion exchange (HCO_3^- and Cl^-) between the interior of the cell and its extracellular environment. Molecular defects in band 3 are responsible for a fraction of cases of hereditary spherocytosis (Corbett, Agre, Palek, & Golan, 1994) and for all cases of Southeast Asian ovalocytosis (Liu et al., 1990; Mirchev, Lam, & Golan, 2011). Early studies of band 3 lateral mobility utilized FRAP on RBC ghost membranes (Golan & Veatch, 1980). Band 3 was found to be immobilized under conditions of low temperature (21 °C) and moderate ionic strength, whereas increasing temperature (toward 37 °C) and reducing ionic strength first increased the fractional mobility of band 3 and then significantly increased the diffusion coefficient (Golan & Veatch, 1980). Subsequent experiments suggested that this increase in mobility was not attributable to proteolysis of any major membrane protein. Rather, the progressive severing of the connections between the spectrin-based membrane skeleton and the overlying lipid bilayer, mediated by the release of ankyrin (and possibly adducin) from its binding site on spectrin, was responsible for the large changes in the fractional mobility and diffusion coefficient of band 3 (Cho, Eber, Liu, Lux, & Golan, 1998; Tsuji & Ohnishi, 1986). Indeed, RBCs from patients with ankyrin-deficient hereditary spherocytosis had markedly increased band 3 diffusion (Cho et al., 1998). Given that the ankyrin link to spectrin is critical for limiting band 3 diffusion, it followed that disruption of the spectrin-based membrane skeleton would also lead to higher diffusion coefficients for band 3. As expected, the diffusion coefficient of band 3 was eightfold higher on RBCs from patients with spectrin-deficient hereditary spherocytosis ($12 \times 10^{-11} \text{ cm}^2/\text{s}$) than on control RBCs ($1.6 \times 10^{-11} \text{ cm}^2/\text{s}$), without a change in band 3 fractional mobility (Corbett, Agre, et al., 1994).

Band 3 mobility has also been measured using SPT. The first SPT analyses of band 3 suggested that one third of the protein was relatively tightly confined, likely tethered to spectrin, whereas the remainder demonstrated confinement on a larger time and distance scale with episodic hops between the zones of confinement (Tomishige, Sako, & Kusumi, 1998). These findings, together with measurements of band 3 mobility under conditions of membrane skeletal disruption, led to the hypothesis that corralling of the cytoplasmic domain of band 3 by the underlying membrane skeleton was the mechanism most likely responsible for band 3 confinement and hopping (Tomishige et al., 1998). In another SPT study on normal RBCs, about half of the band 3 molecules displayed confinement and the other half were not confined (Mirchev et al., 2011). Interestingly, RBCs with the Southeast Asian ovalocytosis (SAO) band 3 mutation demonstrated even tighter areas of band 3 confinement. The SAO mutation causes band 3 to form linear oligomers in the plane of the RBC membrane, and this tighter confinement likely reflected a greater degree of mobility

constraint on the larger protein complex. A third set of SPT studies also confirmed that a fraction of band 3 is quite confined—this is likely to represent the molecules that are associated with spectrin through ankyrin or adducin linkages—whereas another fraction is corralled in larger confinement zones (Kodippili et al., 2012).

4.1.2 Glycophorins—Glycophorins are the major sialoglycoproteins that span the membrane of the RBC. The predominant RBC glycophorin is glycophorin A (0.5 million copies per RBC). Several early studies measured the lateral mobility of nonspecifically labeled RBC membrane glycoproteins (including glycophorin A) in RBCs and RBC ghosts (Schindler, Koppel, & Sheetz, 1980) and the lateral mobility of purified glycophorin in synthetic lipid bilayers (Kapitza, Ruppel, Galla, & Sackmann, 1984). Our laboratory used fluorescein thiosemicarbazide to label RBC glycophorins specifically and FRAP to measure glycophorin lateral mobility in intact RBCs. The measured diffusion coefficient for the glycophorins was $2\text{--}5 \times 10^{-11} \text{ cm}^2/\text{s}$, similar to that of band 3 (Corbett, Cho, & Golan, 1994). The lateral mobility of both glycophorin and band 3 decreased as a function of cell density in RBCs from patients with sickle cell disease, likely due to the irreversible damage that characterizes the membrane of dense sickle RBCs (Corbett & Golan, 1993). These and other data led to the hypothesis that a fraction of band 3 and glycophorin molecules are physically linked in the RBC membrane. Consistent with this hypothesis, FRAP studies showed that extracellular engagement of glycophorin A induced lateral immobilization both of this protein and of band 3, probably through a mechanism involving rigidification of the underlying membrane skeleton (Knowles, Chasis, Evans, & Mohandas, 1994).

4.1.3 Complement components—Decay-accelerating factor (DAF) is a membrane protein that inhibits activation of the C3 complement component and thereby protects RBCs from complement-mediated pore formation and eventual cell lysis (Brodbeck, Mold, Atkinson, & Medof, 2000; Michaels, Abramovitz, Hammer, & Mayer, 1976). As a glycosylphosphatidylinositol (GPI)-anchored protein, DAF is well suited for this function, since the lateral mobility of GPI-linked proteins is generally high in mammalian plasma membranes. High lateral mobility allows DAF to “patrol” the RBC membrane and inactivate small amounts of newly deposited complement proteins wherever they are located on the cell. Using SPT, our laboratory found that the majority (~80%) of DAF molecules exhibited Brownian lateral motion with a diffusion coefficient of $41 \times 10^{-11} \text{ cm}^2/\text{s}$ in untreated (noncomplement activated) cells (Karnchanaphanurach et al., 2009).

In contrast, cells treated with abundant amounts of complement showed relative immobilization of DAF at sites of complement deposition. Under these conditions, 70% of DAF molecules exhibited a diffusion coefficient of $6 \times 10^{-11} \text{ cm}^2/\text{s}$. Moreover, this immobilization of DAF was accompanied by immobilization of complement component C3b and of the major RBC membrane proteins glycophorin A (which is also the RBC receptor for C3b) and band 3. Mass spectrometry analysis showed that, in such complement-treated cells, band 3, glycophorin A, and C3b formed a macromolecular complex that was immobilized via links to the underlying spectrin–ankyrin membrane skeleton. Finally, as measured using laser optical tweezers, the mechanical properties of the complement-treated RBCs were affected in the form of increased stiffness and membrane viscosity. Together,

these data suggested that complement activation caused a membrane skeleton-linked DAF-C3b-glycophorin A-band 3 complex to form on the RBC surface, and that formation of this complex increased RBC stiffness and membrane viscosity (Karnchanaphanurach et al., 2009). These changes in membrane stiffness may facilitate removal of senescent RBCs from the circulation.

4.1.4 Aquaporins—Approximately 50,000 tetramers of the aquaporin water transport protein (AQP1) are expressed on the human RBC membrane (Smith & Agre, 1991). FRAP-based measurements showed that fluorescently labeled AQP1 had a relatively high mobile fraction (66%) and a relatively low lateral diffusion coefficient (3.1×10^{-11} cm²/s) in the membrane of intact RBCs. The fractional mobility of AQP1 was not significantly altered by immobilization of band 3 or glycophorin A, indicating that AQP1 is not tightly associated with either of these membrane proteins. Interestingly, stretching the membrane (and thereby dilating the underlying membrane skeleton) caused a 20-fold increase in the lateral diffusion coefficient of AQP1. Membrane deformation did not have the same effect on the lateral mobility of band 3 and glycophorin A, suggesting that aquaporin dynamics may be uniquely responsive to cell deformation. These findings also suggested that, unlike other RBC membrane proteins, AQP1 is not tightly associated with the underlying membrane skeleton; instead, the spectrin-based lattice may serve to corral the aquaporin molecules in confinement zones that are susceptible to enlargement and/or rupture upon stretching of the membrane (Cho et al., 1999).

4.1.5 Toward a model of functional organization—Measurements of the lateral mobility of erythrocyte membrane proteins have provided insights into the organization of the RBC membrane and the functional implications of RBC membrane structure. For example, the lateral mobility of two of the most abundant RBC membrane proteins, band 3 and glycophorin A, is markedly confined in normal RBC membranes. Both of these proteins are intimately associated with the underlying membrane skeleton, the former through ankyrin and adducin linkages and the latter through interaction with band 3. These direct-binding interactions are responsible for the restricted (immobile) fraction of band 3 and glycophorin A molecules observed in FRAP and SPT experiments. The band 3 and glycophorin A molecules that are not directly bound to the membrane skeleton show confined, not free, lateral mobility, due to the corralling effect of the spectrin-based membrane skeleton. Disruption of the membrane skeleton in diseases such as hereditary spherocytosis leads to both release of band 3 and glycophorin A from these mobility constraints and to increased fragility of the RBCs. In other diseases such as sickle cell anemia, band 3 and glycophorin A are immobilized in the RBC membrane due to oxidative crosslinking reactions catalyzed by membrane-associated aggregates of hemoglobin S, and the immobilization (clustering) of band 3 contributes to the premature removal of these cells.

Lateral mobility measurements on less abundant membrane proteins have also been informative. For example, the complement inhibitory protein DAF is highly mobile on the RBC membrane until it encounters its target (deposited complement components), at which point it becomes immobile and associates with a number of other RBC membrane proteins

to alter cell stiffness. AQP1, in contrast, is relatively confined at baseline and becomes increasingly mobile with deformation of the spectrin-based membrane skeleton. The functional consequence of this change in AQP1 mobility remains an open question. The high mobility of AQP1 under membrane deformation may allow for rapid redistribution of this important homeostatic channel, and this redistribution may be prevented in stiff, senescent RBCs that are rapidly cleared from the circulation (Yip et al., 1983).

4.2. Membrane protein dynamics in other hematopoietic cells

The erythrocyte provided an early model for studying membrane protein dynamics, and the field then turned to other hematopoietic cells. The larger number and variety of membrane proteins on these cells, many of which are receptors or counter-receptors, have yielded new insights into the functional consequences of membrane protein mobility regulation. In this section, we review the dynamics of membrane proteins in erythroblasts, lymphocytes, and other cells derived from the bone marrow.

4.2.1 Erythroblasts—Erythroblasts undergo massive cytoskeletal changes during their maturation into erythrocytes, eventually losing much of the actin cytoskeleton and assembling a spectrin-based membrane skeleton (Liu, Guo, Mohandas, Chasis, & An, 2010). The signals and mechanisms that regulate these changes are critical for erythroid maturation. Along with these cytoskeletal changes, receptors that are important for maintaining adhesive interactions between the erythroblast and its microenvironment become downregulated and disappear at the end of erythroid maturation. Integrins, for example, mediate cellular adhesion between the erythroblast and the ECM and neighboring cells (Mohandas & Chasis, 2010). Integrins signal bidirectionally across the plasma membrane, physically linking the extracellular environment to intracellular signaling molecules and to the cytoskeleton (Giancotti & Ruoslahti, 1999). Understanding the dynamics of critical erythroblast membrane proteins, such as integrins, could provide insights into the relative importance for cellular function of different membrane proteins at each stage of erythroblast maturation, and could allow for identification of structural and/or signaling complexes at the membrane at each stage of development.

Despite this promise, the field of erythroblast membrane protein dynamics is still in its infancy. Band 3 dynamics have recently been described in erythroblasts (Kodippili et al., 2012). Using SPT, a decrease in the lateral mobility of band 3 was found to occur with progressive stages of erythroblast development (Kodippili et al., 2012). The gradual immobilization of band 3 was thought to be due to the integration of the protein in a cooperative membrane assembly process such that, by the end of terminal erythroid maturation, band 3 was in its proper place and fully functional. A potential model is as follows. Band 3 is present on the membrane in early-stage erythroblasts, but the protein is not cytoskeletally anchored in these cells. Indeed, the spectrin and ankyrin required for membrane skeletal attachment are not yet present in these early cells. With erythroblast maturation, as the requisite membrane skeletal elements accumulate, band 3 becomes progressively anchored. This model may also apply to other erythroblast membrane proteins that increase in expression during erythroid maturation, that is, such proteins may become more tightly anchored and/or confined as they become more functional. It remains to be

determined whether the lateral mobility of downregulated membrane proteins, such as integrins, the transferrin receptor, and the erythropoietin receptor, also changes with erythroid maturation. This is an area of active investigation.

4.2.2 Lymphocytes—The interactions of lymphocytes with other cells are highly regulated. For example, T cells interact with endothelial cells and with antigen-presenting cells (APCs) in order to home to sites of inflammation and to modulate inflammatory responses. Lymphocyte function-associated antigen 1 (LFA-1, also called CD11a/CD18 and $\alpha_L\beta_2$) is an integrin heterodimer expressed on T cells that interacts with ICAM-1 on endothelial cells and APCs. The affinity of LFA-1 for its counter-receptors and its extent of clustering on the membrane influence both T cell adhesion and activation (Kim, Carman, & Springer, 2003; van Kooyk & Figdor, 2000). The topological alignment of LFA-1 and ICAM-1 is necessary for optimal receptor–counter-receptor binding and clustering to take place (Dustin, 1998). Therefore, the lateral mobility of LFA-1 has great functional consequence for determining its ability to engage ICAM-1 effectively.

Substantial work has been done to characterize the lateral mobility of lymphocyte adhesion and activation receptors. An early study showed that T-cell activation with PMA caused a 10-fold increase in the diffusion coefficient of LFA-1, from 2.3×10^{-11} cm²/s in native cells to 2.9×10^{-10} cm²/s after 10 min of PMA treatment. Cytochalasin D also increased the rate of LFA-1 diffusion in resting T cells, suggesting that the mobility of nonactivated LFA-1 was constrained by the underlying actin cytoskeleton (Kucik, Dustin, Miller, & Brown, 1996). In another study, removing the portion of LFA-1 that is known to interact with the actin cytoskeleton did not completely release LFA-1 from its mobility constraints. Instead, it appeared that disruption of the entire actin network was required to substantially increase LFA-1 diffusion. These findings suggested that tight binding of LFA-1 to the cytoskeleton was not the only determinant of confined mobility, and that LFA-1 confinement was, at least in part, due to corraling of the integrin by the underlying actin network (Peters et al., 1999). More recently, the observation that treatment with cytochalasin D increases the lateral mobility of LFA-1 was replicated in neutrophils (Gaborski, Clark, Waugh, & McGrath, 2008). An important study showed that the increased lateral mobility of LFA-1 induced by disruption of the actin cytoskeleton was capable of driving integrin clustering and cell adhesion, and suggested that such clustering is especially important in adhesion strengthening after integrin binding to multivalent ligands (Kim, Carman, Yang, Salas, & Springer, 2004).

Our group has used FRAP and SPT to measure the lateral mobility of active and inactive conformations of LFA-1 in the plasma membrane of the T cell. Using a conformation-nonspecific antibody to label LFA-1, FRAP studies showed that exogenous activation of T cells with PMA significantly increased the average lateral diffusion coefficient of this receptor, and SPT measurements indicated that activation with PMA caused a higher fraction of LFA-1 molecules to become laterally mobile. Interestingly, however, using an antibody that was specific for the active, high-affinity conformation of LFA-1, we found that activation of T cells with PMA increased the *immobile* fraction of LFA-1 molecules that were in the active (open) conformation (Cairo, Mirchev, & Golan, 2006). Conversely, PMA increased the *mobile* fraction of LFA-1 molecules that were in the inactive (closed)

conformation. These results were consistent with a model in which T cell activation mobilizes non-ligated, closed-conformation LFA-1 in order to maximize the opportunity for receptor-counter-receptor interactions, whereas the same stimulus acts to anchor the ligated, open-conformation LFA-1 in order to reinforce the receptor-counter-receptor interaction and strengthen T-cell adhesion. FRAP studies were used by other investigators to demonstrate immobilization of high-affinity LFA-1 in focal zones on the membranes of rapidly migrating T cells, and talin was shown to be the critical adaptor protein mediating the interaction of activated LFA-1 with the underlying actin cytoskeleton (Smith et al., 2005).

The T cell receptor (TCR) is the centerpiece of the immunological synapse that forms between a T cell and an APC (Grakoui et al., 1999). Investigators used SPT to show that T-cell activation by an agonist signal from an APC caused relatively fast directed movement of unligated TCR molecules to the interface between the two cells, allowing these TCR molecules to participate in the growing synapse (Moss, Irvine, Davis, & Krummel, 2002). The directed movement of the TCR to the growing synapse was in stark contrast to the baseline motion of the TCR in nonactivated T cells, which was confined (diffusion coefficient $\sim 1 \times 10^{-10} \text{ cm}^2/\text{s}$) but not directed (Sloan-Lancaster et al., 1998). The mechanism underlying this switch from confined motion to directed motion was unclear, but interaction of the TCR with the cortical actin cytoskeleton was likely to be responsible since the cortical actin cytoskeleton is required for immunological synapse formation between T cell and APC (Valitutti, Dessing, Aktories, Gallati, & Lanzavecchia, 1995; Varma, Campi, Yokosuka, Saito, & Dustin, 2006; Wulfig & Davis, 1998). Calcium may also be involved in TCR mobility regulation, as T-cell activation by ionomycin (which increases intracellular calcium) reduced TCR mobility in an actin-dependent manner (Dushek et al., 2008). Future measurements of membrane protein dynamics will likely contribute to elucidating the mechanism by which the cytoskeleton guides directed motion of the TCR.

The diffusion properties of other participants in the immunological synapse have also been characterized. In resting T cells, the adhesion molecule CD2 had a relatively high lateral diffusion coefficient ($7.9 \times 10^{-10} \text{ cm}^2/\text{s}$) and fractional mobility (75%). Binding of CD2 to its counter-receptor CD58 did not alter either the diffusion coefficient or the fractional mobility of CD2 (Zhu, Dustin, Cairo, & Golan, 2007). In combination with T-cell activation, however, binding of CD2 to CD58 decreased the fractional mobility of CD2. These results were consistent with the role of the CD2-CD58 interaction at the immunological synapse, since high lateral mobility would facilitate rapid diffusion of CD2 to the nascent synapse, whereas, upon cell activation, immobilization of bound CD2-CD58 complexes at the mature synapse would strengthen adhesion (Zhu, Dustin, Cairo, Thatte, & Golan, 2006). Measurements of CD2 fractional mobility were combined with measurements of the number of CD2 molecules per cell, the surface area of the cell, the size of the contact area, and the bound and free counter-receptor (CD58) densities in the contact area to calculate the two-dimensional affinity of binding ($2D K_d$) between CD2 and CD58. The $2D K_d$ was shown to represent a quantitative measure of the mechanisms that regulate cell-cell adhesion (Zhu et al., 2007).

CD45 is a membrane protein expressed on T cells and certain other hematopoietic cells. The cytoplasmic domain of CD45 has phosphatase activity that is critical to T-cell activation. FRAP measurements showed that CD45 had a lateral diffusion coefficient of 4×10^{-10} cm²/s in T-cell plasma membranes (Goldman et al., 1992). SPT measurements showed that CD45 mobility decreased upon cell activation, and this decrease in mobility was attenuated by disruption of the actin cytoskeleton. Peptide fragments of β 1 spectrin were used to show that CD45 utilized spectrin to maintain cytoskeletal linkages with actin and with ankyrin (Cairo et al., 2010). These spectrin–ankyrin and spectrin–actin links stabilized CD45 in the membrane. SPT and time-resolved TIRF have been used to measure the lateral mobility of several other T-cell membrane proteins that are important for immunological synapse formation, including ZAP70, SLP76, CD3, and CD4 (Hsu et al., 2012; Mascalchi, Lamort, Salome, & Dumas, 2012).

Investigators have measured the lateral mobility of major histocompatibility complex (MHC) class I and class II molecules on APCs with the goal of understanding how such mobility could affect the interaction of APCs with T cells (Bierer, Herrmann, Brown, Burakoff, & Golan, 1987; Wilson, Morrison, Smith, Fernandez, & Cherry, 1996). Fluorescent SPT was used to track the motion of transfected HLA-DR molecules on fibroblasts. Multiple modes of motion were observed, including random diffusion, confined diffusion, and directed motion (Wilson et al., 1996). Reported lateral diffusion coefficients of MHC class II molecules have varied widely, from 1×10^{-13} cm²/s to 32×10^{-10} cm²/s, depending on cell type and experimental conditions (Umemura et al., 2008; Wilson et al., 1996; Yang, Kohler, Davis, & Burroughs, 2010). MHC class II molecules in which the transmembrane and cytoplasmic domains were replaced by GPI linkers exhibited random diffusion with higher diffusion coefficients. This finding, coupled with experiments using latrunculin to perturb the underlying actin cytoskeleton, suggested that the cytoplasmic domain of MHC class II molecules interacted sterically with the actin-based membrane skeleton. In this model, membrane skeletal proteins do not bind the MHC class II molecules directly but rather serve as boundaries to confine and compartmentalize MHC class II diffusion. The MHC class II molecules periodically hop to adjacent regions of confinement, facilitated by transient perturbation of the membrane or transient disruption of the underlying actin lattice (Umemura et al., 2008; Vrljic, Nishimura, Brasselet, Moerner, & McConnell, 2002).

Membrane protein dynamics have been less well studied in hematopoietic cells of the myeloid lineage (Flannagan, Harrison, Yip, Jaqaman, & Grinstein, 2010). Such studies are at least partially complicated by high rates of membrane turnover associated with endocytosis and phagocytosis. One outstanding study used fluorescent SPT to measure the dynamics of CD36, the receptor for oxidized LDL, on macrophage membranes. This work showed that cortical F-actin, in cooperation with microtubules, organized to form linear troughs that confined CD36 lateral mobility and thereby promoted its clustering and priming for endocytosis of oxidized LDL (Jaqaman et al., 2011).

4.2.3 Common themes of functional organization in hematopoietic cells—

Extending from lateral mobility measurements in erythrocytes, which focused primarily on the structural organization and architecture of the membrane, studies of membrane protein

dynamics in other hematopoietic cells have helped to explain the mechanisms by which membrane protein lateral mobility affect cell–cell interactions. Characterization of the membrane protein dynamics of receptor–counter-receptor interactions involving lymphocytes has been especially illuminating. The functional consequence of changes in protein mobility in response to APC contact, and the dependence of these changes on underlying cytoskeletal elements, have become key tenets of immune system physiology. As initially shown in erythrocytes, the role of the underlying cytoskeleton in regulating membrane protein dynamics is now a common theme and has recently been reviewed in detail (Jaqaman & Grinstein, 2012). The potential for future studies to move beyond the standard three-dimensional K_d , as measured in solution chemistry, to the more physiologically relevant two-dimensional K_d , derived in part from measurements of membrane protein dynamics, has important physiologic and pharmacologic implications.

4.3. Membrane protein dynamics in non-hematopoietic cells

Measurements of membrane protein lateral diffusion have been performed in a wide array of non-hematopoietic cell types to address substantial questions about membrane physiology and cell function. The current discussion cannot cover every cell type that has been studied; instead, we illustrate the range by discussing one representative cell type derived from each of the three germ layers. In the neuron, the lateral diffusion and active transport of neurotransmitter receptors govern the cell's response to synaptic activity. In the endothelium, the availability and lateral mobility of counter-receptors for interacting leukocytes and of receptors for ECM proteins govern how the endothelial cells interact with their environment to direct inflammatory processes and vascular remodeling. In the epithelial parenchyma of most major organs, such as the alveolar epithelium, changes in receptor lateral mobility can determine cell-specific physiologic responses to drugs and natural ligands.

4.3.1 Neurons—SPT has been a useful tool for measuring the dynamics of neurotransmitter receptors as they move into and out of synapses (Alcor, Gouzer, & Triller, 2009). The lateral motion of these receptors is in addition to, and distinguished from, receptor turnover mediated by endocytosis and exocytosis. In many of the experiments on neurons, the beads used for SPT were brought into controlled contact with the cultured neuron of interest using optical tweezers. An early study of neurotransmitter receptor diffusion found that the 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid (AMPA) receptor in rat hippocampal neurons switched rapidly from fast, relatively unconstrained diffusion to relative immobilization, and that the fraction of time spent immobilized at postsynaptic terminals increased as the neurons matured. Increases in intracellular calcium also promoted AMPA receptor immobilization (Borgdorff & Choquet, 2002). A similar rapid switching phenomenon was observed for the neuronal glycine receptor (Meier, Vannier, Serge, Triller, & Choquet, 2001). Switching between modes of lateral mobility required the scaffolding protein gephyrin and was regulated by connections to the actin and microtubule cytoskeletons (Calamai et al., 2009; Charrier, Ehrensperger, Dahan, Levi, & Triller, 2006; Meier et al., 2001). Gephyrin was also implicated in controlling the motion of the GABA receptor during inhibitory clustering activity, but not during excitatory declustering (Mukherjee et al., 2011; Niwa et al., 2012). Using both SPT

and FRAP, investigators found that ECM proteins acted in part as restrictive barriers to the lateral diffusion of AMPA receptors, and the interaction between the ECM proteins and the receptors may have modulated synaptic plasticity (Frischknecht et al., 2009).

Regulation of membrane protein lateral mobility appears to be important in axonal growth. For example, the ECM receptor $\beta 1$ integrin exhibited restricted diffusion in axonal membranes in the absence of nerve growth factor (NGF), and treatment with NGF induced both threefold faster receptor diffusion (the apparent diffusion coefficient increased from $4.4 \times 10^{-10} \text{ cm}^2/\text{s}$ to $14.5 \times 10^{-10} \text{ cm}^2/\text{s}$) as well as rapid directed receptor motion toward the end of the growth cone (at a typical rate of $37 \text{ }\mu\text{m}/\text{min}$, with brief periods of sustained forward excursions at rates of $75\text{--}150 \text{ }\mu\text{m}/\text{min}$). The directed motion of $\beta 1$ integrin required an intact actin cytoskeleton and active actin–myosin coupling, since treatment with cytochalasin D or butanedione monoxime (a myosin ATPase inhibitor) blocked this response (Grabham, Foley, Umeojiako, & Goldberg, 2000). A similar pattern of response to NGF was observed with GABA receptors; in this case, the directed motion was dependent on microtubules (Bouzigues, Morel, Triller, & Dahan, 2007).

Recent work has probed the lateral mobility of ion channels in neuronal membranes. Sodium channels at the axonal initial segment, which are critical for action potential initiation, were restricted in their lateral diffusion by ankyrin G. Overexpression of ankyrin reduced the fractional mobility of the channels from 95% to 65% and decreased the lateral diffusion coefficient by 50% (Brachet et al., 2010). FRAP studies on cells cotransfected with neurofascin, a neuronal membrane protein, and fluorescently tagged ankyrin G have helped to define the domains of ankyrin G that are responsible for immobilizing the neurofascin–ankyrin G complex in the membrane. Neurofascin immobilization is important for the organization of sodium channels in the axonal initial segment. The lateral diffusion coefficient of neurofascin decreased by approximately 10-fold, and its fractional mobility decreased from 70% to 10%, upon formation of the neurofascin–ankyrin G complex (Zhang & Bennett, 1998). Ankyrin G appears to have a critical role in regulating the lateral mobility of both sodium channels and neurofascin in neuronal membranes, likely due to its function as a linking protein that mediates interactions between membrane proteins and the underlying cytoskeleton (Bennett & Baines, 2001; Galiano et al., 2012).

4.3.2 Endothelial cells—Given the myriad of cell–cell interactions involving the endothelium, it is likely that the dynamics of proteins in the endothelial cell membrane are highly regulated. Somewhat surprisingly, however, these cells have not been extensively studied in this context. For example, while the lateral mobility of LFA-1 on circulating lymphocytes has been probed in detail, the mobility of LFA-1's counter-receptor ICAM-1 on endothelial cells has only recently received attention. FRAP was used to measure the lateral mobility of GFP–ICAM-1 fusion proteins in the plasma membrane of human umbilical vein endothelial cells (HUVEC), and the lateral diffusion coefficient was $2.9 \times 10^{-10} \text{ cm}^2/\text{s}$. A variant of ICAM-1 that lacked the cytoplasmic tail had a diffusion coefficient three- to fourfold higher than this value. Crosslinking of ICAM-1 (which mimics ICAM-1 engagement) increased the density of actin stress fibers in the cells in a cortactin-dependent manner while concomitantly restricting the lateral diffusion of the wild-type ICAM-1 but not the ICAM-1 variant lacking the cytoplasmic tail. Thus, the lateral mobility

of ICAM-1 was dynamically linked to the actin cytoskeleton (Yang et al., 2006). A second FRAP study investigated the regulation of ICAM-1 clustering at the apical (luminal) surface of endothelial cells upon leukocyte engagement. Such clustering was associated with a decrease in ICAM-1 fractional mobility, and the relative immobilization of the clustered molecules was reversed by disruption of the actin cytoskeleton (van Buul et al., 2010).

FRAP has been used to measure the lateral mobility of integrins in the plasma membrane of endothelial cells in the context of focal adhesions. Here, disruption of the actin cytoskeleton with cytochalasin D or latrunculin B *decreased* the fractional mobility of the integrins (Tsuruta et al., 2002). This result suggested a model in which integrins require the actin cytoskeleton in order to translocate within focal adhesion sites, where the endothelial cell engages with the underlying ECM. Integrin translocation likely occurs with the cooperation of other focal adhesion complex proteins; more work is needed to dissect the interactions that determine integrin trafficking in this context (Lele, Thodeti, Pendse, & Ingber, 2008). FRAP has also been used to measure the lateral mobility of GFP-tagged VE-cadherin in endothelial cell–cell junctions. Inhibition of the activity of tyrosine phosphatase, specifically SHP2, significantly decreased the fractional mobility and diffusion coefficient of VE-cadherin. This result suggested that SHP2-containing pathways may be important for promoting increased diffusion of VE-cadherin, and thereby controlling the recovery of cell–cell junctions in response to inflammation (Timmerman et al., 2012).

4.3.3 Epithelial cells—Few studies have been conducted to date on membrane protein dynamics in epithelial cells. One interesting set of observations involved the dynamics of the G protein-coupled β -adrenergic receptor in the plasma membrane of alveolar epithelial cells and model cell lines. FCS experiments showed that epinephrine binding caused β_2 receptors to exhibit very rapid lateral diffusion for several minutes (diffusion coefficient, $288 \times 10^{-10} \text{ cm}^2/\text{s}$), after which the diffusion rate slowed to more typical values ($10 \times 10^{-10} \text{ cm}^2/\text{s}$). This behavior could correspond to ligand binding and free diffusion of the ligand-bound receptor within CEMMs, followed by migration of the ligand-bound receptor out of CEMMs in preparation for receptor internalization (Hegener et al., 2004). FRAP experiments showed that the lateral diffusion coefficient of unligated, GFP-tagged β_2 receptor was $40 \times 10^{-10} \text{ cm}^2/\text{s}$ in the plasma membrane of a transfected cell line (Barak et al., 1997). Initial SPT studies in a model cell line showed that ligated β_2 receptors demonstrated a heterogeneous mobility profile. Global stimulation of β_2 receptors by terbutaline markedly immobilized the labeled receptor through regulatory mechanisms downstream of the cAMP pathway (Sieben, Kaminski, Kubitscheck, & Haberlein, 2011). Future SPT studies in which CEMMs and cAMP pathways are disrupted may further elucidate the mechanisms underlying these agonist-induced changes in membrane protein dynamics.

4.3.4 Common themes of functional organization in non-hematopoietic cells—

In the future, we expect that the dynamics of a much broader array of membrane proteins will be investigated in many different non-hematopoietic cell types. The techniques of FRAP, SPT, FCS, variations thereof, and complementary methods such as FRET are widely applicable. Much like the systems discussed here—that is, neurotransmitter receptor trafficking in neurons, cell–cell and cell–ECM interactions in endothelium, and β_2 -

adrenergic responses in lung—the elucidation of cell physiology and pathophysiology will be advanced in other mammalian and model systems through studies of membrane protein dynamics. In these systems, as in the non-hematopoietic cells described above, we expect that a common theme will be the role of interactions between membrane proteins and the underlying cytoskeleton in regulating protein lateral mobility and cell function.

5. MEMBRANE DIFFUSION, PHYSIOLOGY, AND PHARMACOLOGIC IMPLICATIONS

Studies of membrane protein dynamics provide insight into the real-time behavior of receptors, counter-receptors, and structural proteins in living mammalian cells. When coupled with interventions such as ligand binding, crosslinking, or disruption of downstream signaling pathways or cytoskeletal linkages, a rich picture can emerge of the mechanisms by which membrane protein dynamics influence signaling sequelae and membrane mechanical properties. Ultimately, the regulation of membrane protein lateral mobility translates into the local availability of these proteins for interactions with the intracellular and extracellular environment, which includes not only other cells and ECM components but also pharmacologic agents that are designed to modulate some aspect of the protein's activity or binding capacity. Studies of membrane protein dynamics are, therefore, one important component of a complete depiction of the role of membrane proteins in cell function.

REFERENCES

- Alcor D, Gouzer G, Triller A. Single-particle tracking methods for the study of membrane receptors dynamics. *The European Journal of Neuroscience*. 2009; 30:987–997. [PubMed: 19735284]
- Anong WA, Franco T, Chu H, Weis TL, Devlin EE, Bodine DM, et al. Adducin forms a bridge between the erythrocyte membrane and its cytoskeleton and regulates membrane cohesion. *Blood*. 2009; 114:1904–1912. [PubMed: 19567882]
- Axelrod D, Koppel DE, Schlessinger J, Elson E, Webb WW. Mobility measurement by analysis of fluorescence photobleaching recovery kinetics. *Biophysical Journal*. 1976; 16:1055–1069. [PubMed: 786399]
- Bacia K, Kim SA, Schwille P. Fluorescence cross-correlation spectroscopy in living cells. *Nature Methods*. 2006; 3:83–89. [PubMed: 16432516]
- Bacia K, Scherfeld D, Kahya N, Schwille P. Fluorescence correlation spectroscopy relates rafts in model and native membranes. *Biophysical Journal*. 2004; 87:1034–1043. [PubMed: 15298908]
- Barak LS, Ferguson SS, Zhang J, Martenson C, Meyer T, Caron MG. Internal trafficking and surface mobility of a functionally intact beta2-adrenergic receptor-green fluorescent protein conjugate. *Molecular Pharmacology*. 1997; 51:177–184. [PubMed: 9203621]
- Bennett V. The membrane skeleton of human erythrocytes and its implications for more complex cells. *Annual Review of Biochemistry*. 1985; 54:273–304.
- Bennett V, Baines AJ. Spectrin and ankyrin-based pathways: Metazoan inventions for integrating cells into tissues. *Physiological Reviews*. 2001; 81:1353–1392. [PubMed: 11427698]
- Bierer BE, Herrmann SH, Brown CS, Burakoff SJ, Golan DE. Lateral mobility of class I histocompatibility antigens in B lymphoblastoid cell membranes: Modulation by cross-linking and effect of cell density. *The Journal of Cell Biology*. 1987; 105:1147–1152. [PubMed: 3115995]
- Billaudeau C, Mailfert S, Trombik T, Bertaux N, Rouger V, Hamon Y, et al. Probing the plasma membrane organization in living cells by spot variation fluorescence correlation spectroscopy. *Methods in Enzymology*. 2013; 519:277–302. [PubMed: 23280115]
- Borgdorff AJ, Choquet D. Regulation of AMPA receptor lateral movements. *Nature*. 2002; 411:649–653. [PubMed: 12050666]

- Bouzigues C, Morel M, Triller A, Dahan M. Asymmetric redistribution of GABA receptors during GABA gradient sensing by nerve growth cones analyzed by single quantum dot imaging. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:11251–11256. [PubMed: 17592112]
- Brachet A, Leterrier C, Irondelle M, Fache MP, Racine V, Sibarita JB, et al. Ankyrin G restricts ion channel diffusion at the axonal initial segment before the establishment of the diffusion barrier. *The Journal of Cell Biology*. 2010; 191:383–395. [PubMed: 20956383]
- Brodbeck WG, Mold C, Atkinson JP, Medof ME. Cooperation between decay-accelerating factor and membrane cofactor protein in protecting cells from autologous complement attack. *The Journal of Immunology*. 2000; 165:3999–4006. [PubMed: 11034410]
- Brown DA, London E. Functions of lipid rafts in biological membranes. *Annual Review of Cell and Developmental Biology*. 1998; 14:111–136.
- Cairo CW, Das R, Albohy A, Baca QJ, Pradhan D, Morrow JS, et al. Dynamic regulation of CD45 lateral mobility by the spectrin-ankyrin cytoskeleton of T cells. *The Journal of Biological Chemistry*. 2010; 285:11392–11401. [PubMed: 20164196]
- Cairo CW, Mirchev R, Golan DE. Cytoskeletal regulation couples LFA-1 conformational changes to receptor lateral mobility and clustering. *Immunity*. 2006; 25:297–308. [PubMed: 16901728]
- Calamai M, Specht CG, Heller J, Alcor D, Machado P, Vannier C, et al. Gephyrin oligomerization controls GlyR mobility and synaptic clustering. *The Journal of Neuroscience*. 2009; 29:7639–7648. [PubMed: 19535575]
- Charrier C, Ehrensperger MV, Dahan M, Levi S, Triller A. Cytoskeleton regulation of glycine receptor number at synapses and diffusion in the plasma membrane. *The Journal of Neuroscience*. 2006; 26:8502–8511. [PubMed: 16914675]
- Cho MR, Eber SW, Liu SC, Lux SE, Golan DE. Regulation of band 3 rotational mobility by ankyrin in intact human red cells. *Biochemistry*. 1998; 31:17828–17835. [PubMed: 9922149]
- Cho MR, Knowles DW, Smith BL, Moulds JJ, Agre P, Mohandas N, et al. Membrane dynamics of the water transport protein aquaporin-1 in intact human red cells. *Biophysical Journal*. 1999; 16:1136–1144. [PubMed: 9916045]
- Corbett JD, Agre P, Palek J, Golan DE. Differential control of band 3 lateral and rotational mobility in intact red cells. *The Journal of Clinical Investigation*. 1994; 94:683–688. [PubMed: 8040322]
- Corbett JD, Cho MR, Golan DE. Deoxygenation affects fluorescence photobleaching recovery measurements of red cell membrane protein lateral mobility. *Biophysical Journal*. 1994; 66:25–30. [PubMed: 8130343]
- Corbett JD, Golan DE. Band 3 and glycophorin are progressively aggregated in density-fractionated sickle and normal red blood cells. Evidence from rotational and lateral mobility studies. *The Journal of Clinical Investigation*. 1993; 91:208–217. [PubMed: 8423219]
- Dushek O, Mueller S, Soubies S, Depoil D, Caramalho I, Coombs D, et al. Effects of intracellular calcium and actin cytoskeleton on TCR mobility measured by fluorescence recovery. *PLoS One*. 2008; 3:e3913. [PubMed: 19079546]
- Dustin ML. Making a little affinity go a long way: A topological view of LFA-1 regulation. *Cell Adhesion and Communication*. 1998; 6:255–262. [PubMed: 9823476]
- Flannagan RS, Harrison RE, Yip CM, Jaqaman K, Grinstein S. Dynamic macrophage “probing” is required for the efficient capture of phagocytic targets. *The Journal of Cell Biology*. 2010; 191:1205–1218. [PubMed: 21135140]
- Frick M, Schmidt K, Nichols BJ. Modulation of lateral diffusion in the plasma membrane by protein density. *Current Biology*. 2007; 17:462–467. [PubMed: 17331726]
- Frischknecht R, Heine M, Perrais D, Seidenbecher CI, Choquet D, Gundelfinger ED. Brain extracellular matrix affects AMPA receptor lateral mobility and short-term synaptic plasticity. *Nature Neuroscience*. 2009; 12:897–904.
- Gaborski TR, Clark A Jr, Waugh RE, McGrath JL. Membrane mobility of beta2 integrins and rolling associated adhesion molecules in resting neutrophils. *Biophysical Journal*. 2008; 95:4934–4947. [PubMed: 18689449]
- Galbiati F, Razani B, Lisanti MP. Emerging themes in lipid rafts and caveolae. *Cell*. 2001; 106:403–411. [PubMed: 11525727]

- Galiano MR, Jha S, Ho TS, Zhang C, Ogawa Y, Chang KJ, et al. A distal axonal cytoskeleton forms an intra-axonal boundary that controls axon initial segment assembly. *Cell*. 2012; 149:1125–1139. [PubMed: 22632975]
- Giancotti FG, Ruoslahti E. Integrin signaling. *Science*. 1999; 285:1028–1032. [PubMed: 10446041]
- Golan DE, Veatch W. Lateral mobility of band 3 in the human erythrocyte membrane studied by fluorescence photobleaching recovery: Evidence for control by cytoskeletal interactions. *Proceedings of the National Academy of Sciences of the United States of America*. 1980; 77:2537–2541. [PubMed: 6930650]
- Goldman SJ, Uniyal S, Ferguson LM, Golan DE, Burakoff SJ, Kiener PA. Differential activation of phosphotyrosine protein phosphatase activity in a murine T cell hybridoma by monoclonal antibodies to CD45. *The Journal of Biological Chemistry*. 1992; 267:6197–6204. [PubMed: 1532579]
- Gonda K, Watanabe TM, Ohuchi N, Higuchi H. In vivo nano-imaging of membrane dynamics in metastatic tumor cells using quantum dots. *The Journal of Biological Chemistry*. 2010; 285:2750–2757. [PubMed: 19917603]
- Grabham PW, Foley M, Umeojiako A, Goldberg DJ. Nerve growth factor stimulates coupling of beta1 integrin to distinct transport mechanisms in the filopodia of growth cones. *Journal of Cell Science*. 2000; 113(Pt 17):3003–3012. [PubMed: 10934039]
- Grakoui A, Bromley SK, Sumen C, Davis MM, Shaw AS, Allen PM, et al. The immunological synapse: A molecular machine controlling T cell activation. *Science*. 1999; 285:221–227. [PubMed: 10398592]
- Haustein E, Schwille P. Fluorescence correlation spectroscopy: Novel variations of an established technique. *Annual Review of Biophysics and Biomolecular Structure*. 2007; 36:151–169.
- Hegener O, Prenner L, Runkel F, Baader SL, Kappler J, Haberlein H. Dynamics of beta2-adrenergic receptor-ligand complexes on living cells. *Biochemistry*. 2004; 43:6190–6199. [PubMed: 15147203]
- Hsu CJ, Hsieh WT, Waldman A, Clarke F, Huseby ES, Burkhardt JK, et al. Ligand mobility modulates immunological synapse formation and T cell activation. *PLoS One*. 2012; 7:e32398. [PubMed: 22384241]
- Hu K, Ji L, Applegate KT, Danuser G, Waterman-Storer CM. Differential transmission of actin motion within focal adhesions. *Science*. 2007; 315:111–115. [PubMed: 17204653]
- Iolascon A, Perrotta S, Stewart GW. Red blood cell membrane defects. *Reviews in Clinical and Experimental Hematology*. 2003; 7:22–56. [PubMed: 14692233]
- Jaqaman K, Grinstein S. Regulation from within: The cytoskeleton in transmembrane signaling. *Trends in Cell Biology*. 2012; 22:515–526. [PubMed: 22917551]
- Jaqaman K, Kuwata H, Touret N, Collins R, Trimble WS, Danuser G, et al. Cytoskeletal control of CD36 diffusion promotes its receptor and signaling function. *Cell*. 2011; 146:593–606. [PubMed: 21854984]
- Jaqaman K, Loerke D, Mettlen M, Kuwata H, Grinstein S, Schmid SL, et al. Robust single-particle tracking in live-cell time-lapse sequences. *Nature Methods*. 2008; 5:695–702. [PubMed: 18641657]
- Kahya N, Scherfeld D, Bacia K, Poolman B, Schwille P. Probing lipid mobility of raft-exhibiting model membranes by fluorescence correlation spectroscopy. *The Journal of Biological Chemistry*. 2003; 278:28109–28115. [PubMed: 12736276]
- Kang M, Day CA, Kenworthy AK, DiBenedetto E. Simplified equation to extract diffusion coefficients from confocal FRAP data. *Traffic*. 2012; 13:1589–1600. [PubMed: 22984916]
- Kapitza HG, Ruppel DA, Galla HJ, Sackmann E. Lateral diffusion of lipids and glycophorin in solid phosphatidylcholine bilayers. The role of structural defects. *Biophysical Journal*. 1984; 45:577–587. [PubMed: 6713070]
- Karnchanaphanurach P, Mirchev R, Ghiran I, Asara JM, Papahadjopoulos-Sternberg B, Nicholson-Weller A, et al. C3b deposition on human erythrocytes induces the formation of a membrane skeleton-linked protein complex. *The Journal of Clinical Investigation*. 2009; 119:788–801. [PubMed: 19258706]

- Kim M, Carman CV, Springer TA. Bidirectional transmembrane signaling by cytoplasmic domain separation in integrins. *Science*. 2003; 301:1720–1725. [PubMed: 14500982]
- Kim M, Carman CV, Yang W, Salas A, Springer TA. The primacy of affinity over clustering in regulation of adhesiveness of the integrin $\{\alpha\}L\{\beta\}_2$. *The Journal of Cell Biology*. 2004; 167:1241–1253. [PubMed: 15611342]
- Knowles DW, Chasis JA, Evans EA, Mohandas N. Cooperative action between band 3 and glycophorin A in human erythrocytes: Immobilization of band 3 induced by antibodies to glycophorin A. *Biophysical Journal*. 1994; 66:1726–1732. [PubMed: 8061221]
- Kodippili GC, Spector J, Hale J, Giger K, Hughes MR, McNagny KM, et al. Analysis of the mobilities of band 3 populations associated with ankyrin protein and junctional complexes in intact murine erythrocytes. *The Journal of Biological Chemistry*. 2012; 287:4129–4138. [PubMed: 22147703]
- Kucik DF, Dustin ML, Miller JM, Brown EJ. Adhesion-activating phorbol ester increases the mobility of leukocyte integrin LFA-1 in cultured lymphocytes. *The Journal of Clinical Investigation*. 1996; 97:2139–2144. [PubMed: 8621804]
- Kusumi A, Suzuki KG, Kasai RS, Ritchie K, Fujiwara TK. Hierarchical mesoscale domain organization of the plasma membrane. *Trends in Biochemical Sciences*. 2011; 36:604–615. [PubMed: 21917465]
- Lele TP, Thodeti CK, Pendse J, Ingber DE. Investigating complexity of protein–protein interactions in focal adhesions. *Biochemical and Biophysical Research Communications*. 2008; 369:929–934. [PubMed: 18331831]
- Lingwood D, Simons K. Lipid rafts as a membrane-organizing principle. *Science*. 2010; 327:46–50. [PubMed: 20044567]
- Lippincott-Schwartz J, Snapp E, Kenworthy A. Studying protein dynamics in living cells. *Nature Reviews Molecular Cell Biology*. 2001; 2:444–456.
- Liu J, Guo X, Mohandas N, Chasis JA, An X. Membrane remodeling during reticulocyte maturation. *Blood*. 2010; 115:2021–2027. [PubMed: 20038785]
- Liu SC, Zhai S, Palek J, Golan DE, Amato D, Hassan K, et al. Molecular defect of the band 3 protein in southeast Asian ovalocytosis. *The New England Journal of Medicine*. 1990; 323:1530–1538. [PubMed: 2146504]
- Mascalchi P, Lamort AS, Salome L, Dumas F. Single particle tracking reveals two distinct environments for CD4 receptors at the surface of living T lymphocytes. *Biochemical and Biophysical Research Communications*. 2012; 417:409–413. [PubMed: 22166195]
- Meier J, Vannier C, Serge A, Triller A, Choquet D. Fast and reversible trapping of surface glycine receptors by gephyrin. *Nature Neuroscience*. 2001; 4:253–260.
- Mendoza MC, Besson S, Danuser G. Quantitative fluorescent speckle microscopy (QFSM) to measure actin dynamics. *Current Protocols in Cytometry*. 2012; 62:2.18.1–2.18.26.
- Michaels DW, Abramovitz AS, Hammer CH, Mayer MM. Increased ion permeability of planar lipid bilayer membranes after treatment with the C5b-9 cytolytic attack mechanism of complement. *Proceedings of the National Academy of Sciences of the United States of America*. 1976; 73:2852–2856. [PubMed: 1066698]
- Mirchev R, Golan DE. Single-particle tracking and laser optical tweezers studies of the dynamics of individual protein molecules in membranes of intact human and mouse red cells. *Blood Cells, Molecules & Diseases*. 2001; 27:143–147.
- Mirchev R, Lam A, Golan DE. Membrane compartmentalization in Southeast Asian ovalocytosis red blood cells. *British Journal of Haematology*. 2011; 155:111–121. [PubMed: 21793815]
- Mohandas N, Chasis JA. The erythroid niche: Molecular processes occurring within erythroblastic islands. *Transfusion Clinique et Biologique*. 2010; 17:110–111. [PubMed: 20655267]
- Moss WC, Irvine DJ, Davis MM, Krummel MF. Quantifying signaling-induced reorientation of T cell receptors during immunological synapse formation. *Proceedings of the National Academy of Sciences of the United States of America*. 2002; 99:15024–15029. [PubMed: 12415110]
- Mukherjee J, Kretschmannova K, Gouzer G, Maric HM, Ramsden S, Tretter V, et al. The residence time of GABA(A)Rs at inhibitory synapses is determined by direct binding of the receptor $\alpha 1$ subunit to gephyrin. *The Journal of Neuroscience*. 2011; 31:14677–14687. [PubMed: 21994384]

- Nechyporuk-Zloy V, Dieterich P, Oberleithner H, Stock C, Schwab A. Dynamics of single potassium channel proteins in the plasma membrane of migrating cells. *American Journal of Physiology Cell Physiology*. 2008; 294:C1096–C1102. [PubMed: 18287336]
- Niwa F, Bannai H, Arizono M, Fukatsu K, Triller A, Mikoshiba K. Gephyrin-independent GABA(A)R mobility and clustering during plasticity. *PLoS One*. 2012; 7:e36148. [PubMed: 22563445]
- Palek J. Hereditary elliptocytosis, spherocytosis and related disorders: Consequences of a deficiency or a mutation of membrane skeletal proteins. *Blood Reviews*. 1987; 1:147–168. [PubMed: 3332099]
- Patel HH, Murray F, Insel PA. Caveolae as organizers of pharmacologically relevant signal transduction molecules. *Annual Review of Pharmacology and Toxicology*. 2008; 48:359–391.
- Peters IM, van Kooyk Y, van Vliet SJ, de Grooth BG, Figdor CG, Greve J. 3D single-particle tracking and optical trap measurements on adhesion proteins. *Cytometry*. 1999; 36:189–194. [PubMed: 10404967]
- Qian H, Sheetz MP, Elson EL. Single particle tracking. Analysis of diffusion and flow in two-dimensional systems. *Biophysical Journal*. 1991; 60:910–921. [PubMed: 1742458]
- Rolfe DJ, McLachlan CI, Hirsch M, Needham SR, Tynan CJ, Webb SE, et al. Automated multidimensional single molecule fluorescence microscopy feature detection and tracking. *European Biophysics Journal*. 2011; 40:1167–1186. [PubMed: 21928120]
- Sander S, Arora N, Smith EA. Elucidating the role of select cytoplasmic proteins in altering diffusion of integrin receptors. *Analytical and Bioanalytical Chemistry*. 2012; 403:2327–2337. [PubMed: 22234402]
- Saxton MJ. Single-particle tracking: The distribution of diffusion coefficients. *Biophysical Journal*. 1997; 72:1744–1753. [PubMed: 9083678]
- Saxton MJ, Jacobson K. Single-particle tracking: Applications to membrane dynamics. *Annual Review of Biophysics and Biomolecular Structure*. 1997; 26:373–399.
- Schindler M, Koppel DE, Sheetz MP. Modulation of membrane protein lateral mobility by polyphosphates and polyamines. *Proceedings of the National Academy of Sciences of the United States of America*. 1980; 77:1457–1461. [PubMed: 6929496]
- Serge A, Fargeaud L, Hemar A, Choquet D. Active surface transport of metabotropic glutamate receptors through binding to microtubules and actin flow. *Journal of Cell Science*. 2003; 116:5015–5022. [PubMed: 14625395]
- Sieben A, Kaminski T, Kubitscheck U, Haberlein H. Terbutaline causes immobilization of single beta2-adrenergic receptor-ligand complexes in the plasma membrane of living A549 cells as revealed by single-molecule microscopy. *Journal of Biomedical Optics*. 2011; 16:026013. [PubMed: 21361697]
- Simons K, Ikonen E. Functional rafts in cell membranes. *Nature*. 1997; 387:569–572. [PubMed: 9177342]
- Singer SJ, Nicolson GL. The fluid mosaic model of the structure of cell membranes. *Science*. 1972; 175:720–731. [PubMed: 4333397]
- Sloan-Lancaster J, Presley J, Ellenberg J, Yamazaki T, Lippincott-Schwartz J, Samelson LE. ZAP-70 association with T cell receptor zeta (TCRzeta): Fluorescence imaging of dynamic changes upon cellular stimulation. *The Journal of Cell Biology*. 1998; 143:613–624. [PubMed: 9813084]
- Smith BL, Agre P. Erythrocyte Mr 28,000 transmembrane protein exists as a multisubunit oligomer similar to channel proteins. *The Journal of Biological Chemistry*. 1991; 266:6407–6415. [PubMed: 2007592]
- Smith A, Carrasco YR, Stanley P, Kieffer N, Batista FD, Hogg N. A talin-dependent LFA-1 focal zone is formed by rapidly migrating T lymphocytes. *The Journal of Cell Biology*. 2005; 170:141–151. [PubMed: 15983060]
- Steck TL. The organization of proteins in the human red blood cell membrane. A review. *The Journal of Cell Biology*. 1974; 62:1–19. [PubMed: 4600883]
- Timmerman I, Hoogenboezem M, Bennett AM, Geerts D, Hordijk PL, van Buul JD. The tyrosine phosphatase SHP2 regulates recovery of endothelial adherens junctions through control of beta-catenin phosphorylation. *Molecular Biology of the Cell*. 2012; 23:4212–4225. [PubMed: 22956765]

- Tomishige M, Sako Y, Kusumi A. Regulation mechanism of the lateral diffusion of band 3 in erythrocyte membranes by the membrane skeleton. *The Journal of Cell Biology*. 1998; 142:989–1000. [PubMed: 9722611]
- Tsuji A, Ohnishi S. Restriction of the lateral motion of band 3 in the erythrocyte membrane by the cytoskeletal network: Dependence on spectrin association state. *Biochemistry*. 1986; 25:6133–6139. [PubMed: 3790510]
- Tsuruta D, Gonzales M, Hopkinson SB, Otey C, Khuon S, Goldman RD, et al. Microfilament-dependent movement of the beta3 integrin subunit within focal contacts of endothelial cells. *The FASEB Journal*. 2002; 16:866–868.
- Umemura YM, Vrljic M, Nishimura SY, Fujiwara TK, Suzuki KG, Kusumi A. Both MHC class II and its GPI-anchored form undergo hop diffusion as observed by single-molecule tracking. *Biophysical Journal*. 2008; 95:435–450. [PubMed: 18339737]
- Umenishi F, Verbavatz JM, Verkman AS. cAMP regulated membrane diffusion of a green fluorescent protein-aquaporin 2 chimera. *Biophysical Journal*. 2000; 78:1024–1035. [PubMed: 10653816]
- Valitutti S, Dessing M, Aktories K, Gallati H, Lanzavecchia A. Sustained signaling leading to T cell activation results from prolonged T cell receptor occupancy. Role of T cell actin cytoskeleton. *The Journal of Experimental Medicine*. 1995; 181:577–584. [PubMed: 7836913]
- van Buul JD, van Rijssel J, van Alphen FP, Hoogenboezem M, Tol S, Hoebe KA, et al. Inside-out regulation of ICAM-1 dynamics in TNF-alpha-activated endothelium. *PLoS One*. 2010; 5:e11336. [PubMed: 20596527]
- van Kooyk Y, Figdor CG. Avidity regulation of integrins: The driving force in leukocyte adhesion. *Current Opinion in Cell Biology*. 2000; 12:542–547. [PubMed: 10978887]
- Varma R, Campi G, Yokosuka T, Saito T, Dustin ML. T cell receptor-proximal signals are sustained in peripheral microclusters and terminated in the central supramolecular activation cluster. *Immunity*. 2006; 25:117–127. [PubMed: 16860761]
- Vrljic M, Nishimura SY, Brasselet S, Moerner WE, McConnell HM. Translational diffusion of individual class II MHC membrane proteins in cells. *Biophysical Journal*. 2002; 83:2681–2692. [PubMed: 12414700]
- Watkins EB, Miller CE, Majewski J, Kuhl TL. Membrane texture induced by specific protein binding and receptor clustering: Active roles for lipids in cellular function. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108:6975–6980. [PubMed: 21474780]
- Wehrle-HaUer B. Analysis of integrin dynamics by fluorescence recovery after photobleaching. *Methods in Molecular Biology*. 2007; 370:173–202. [PubMed: 17416995]
- Wilson KM, Morrison IE, Smith PR, Fernandez N, Cherry RJ. Single particle tracking of cell-surface HLA-DR molecules using R-phycoerythrin labeled monoclonal antibodies and fluorescence digital imaging. *Journal of Cell Science*. 1996; 109(Pt 8):2101–2109. [PubMed: 8856506]
- Wulfeing C, Davis MM. A receptor/cytoskeletal movement triggered by costimulation during T cell activation. *Science*. 1998; 282:2266–2269. [PubMed: 9856952]
- Yang J, Kohler K, Davis DM, Burroughs NJ. An improved strip FRAP method for estimating diffusion coefficients: Correcting for the degree of photobleaching. *Journal of Microscopy*. 2010; 238:240–253. [PubMed: 20579262]
- Yang L, Kowalski JR, Yacono P, Bajmoczy M, Shaw SK, Froio RM, et al. Endothelial cell cortactin coordinates intercellular adhesion molecule-1 clustering and actin cytoskeleton remodeling during polymorphonuclear leukocyte adhesion and transmigration. *The Journal of Immunology*. 2006; 111:6440–6449. [PubMed: 17056576]
- Yip R, Mohandas N, Clark MR, Jain S, Shohet SB, Dallman PR. Red cell membrane stiffness in iron deficiency. *Blood*. 1983; 62:99–106. [PubMed: 6860798]
- Zhang X, Bennett V. Restriction of 480/270-kD ankyrin G to axon proximal segments requires multiple ankyrin G-specific domains. *The Journal of Cell Biology*. 1998; 142:1571–1581. [PubMed: 9744885]
- Zhu DM, Dustin ML, Cairo CW, Golan DE. Analysis of two-dimensional dissociation constant of laterally mobile cell adhesion molecules. *Biophysical Journal*. 2007; 92:1022–1034. [PubMed: 17085486]

Zhu DM, Dustin ML, Cairo CW, Thatte HS, Golan DE. Mechanisms of cellular avidity regulation in CD2-CD58-mediated T cell adhesion. *ACS Chemical Biology*. 2006; 1:649–658. [PubMed: 17168569]

Figure 3.1. A general framework for the major modes of membrane protein mobility

Random diffusion (A) is most closely approximated by the Brownian motion of a protein embedded in a homogeneous lipid bilayer. Protein mobility is relatively free from encumbrances due to interactions with extracellular ligands or receptors, other membrane proteins, cytoskeletal elements, or cytoplasmic proteins. *Confinement* (B) occurs when a protein diffuses preferentially within specific domains in the membrane. These can be cholesterol-enriched membrane microdomains (CEMMs) that stabilize the protein or contain docking partners for the protein, patches bounded by less mobile membrane-embedded molecules that corral the diffusing protein, or areas overlying a meshwork of cytoskeletal or other structural proteins that corral or otherwise interact sterically with the cytoplasmic domain of the protein. Proteins experiencing *restriction* (C) have diffusion coefficients substantially lower than those predicted by Brownian motion. Such proteins may be restrained through direct or indirect binding to relatively immobile structures such as extracellular binding partners or macromolecular complexes anchored to the cytoskeleton. *Directed motion* (D) is characterized by either channeled movement or active transport of the protein and is typically guided by linearly polarized cytoskeletal elements or driven by subjacent motor mechanisms.

Figure 3.2. Fluorescence recovery after photobleaching (FRAP) and single-particle tracking (SPT) measurements are the basis for the majority of studies of membrane protein dynamics In *FRAP* (A), a small area of membrane containing a fluorescently labeled protein is photobleached using a high-intensity laser. As labeled proteins from the surrounding (unbleached) area migrate laterally into the bleached area, fluorescence recovers in the bleached area. The half-time for maximal fluorescence recovery is used to calculate the lateral diffusion coefficient of the protein. The ratio of the maximal fluorescence recovery to the prebleach fluorescence corresponds to the fractional mobility of the protein. In *SPT*(B), the membrane protein is tagged at very low density with a micro-particle, nanoparticle, or fluorescent molecule, and this tag is tracked using video microscopy, often at high frame

rates. The mean square displacement (MSD) from the origin is plotted versus time. The lateral diffusion coefficient is calculated based on the MSD versus time relationship. Certain modes of motion have characteristic MSD versus time relationships (e.g., directed motion, random diffusion, restricted diffusion). Because each tracked particle is monitored individually, SPT can be used to elucidate molecular heterogeneity among lateral diffusion coefficients and modes of motion.