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New and Notable

New Dimensions in Two Dimensions

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Reversibility in the binding of soluble molecules to biological surfaces appears to be both common and physiologically functional. Some examples are spectrin and myosin S-1 binding to phospholipids on the cytofacial surface of the plasma membrane, blood coagulation factor binding to foreign tissue, and agonist and hormone-specific binding to receptors and nonspecific binding to the surrounding membrane. In most studies, such binding is modeled by an overly simple reaction scheme $A + B \rightleftarrows C$, where A is the bulk concentration, B is the concentration of free binding sites on the surface, and C is concentration of bound surface sites. This reaction is most commonly characterized by the single equilibrium dissociation constant K_d ($=AB/C$), and in somewhat greater but still oversimplified detail by the forward and backward reaction rates k_1 and k_2, and by the surface diffusion coefficient by which species C might skim the surface. A combination of total internal reflection with fluorescence recovery after photobleaching (TIR-FRAP or TIR-FPR) can conveniently be used to measure these kinetic surface-binding parameters of fluorescent-labeled molecules.

Even in the simple scheme, k_1 and k_2 separately contain interesting information not found in their ratio k_2/k_1 ($=K_d$). Rate k_1 is proportional to the probability of binding per collision of the bulk-dissolved adsorbate with the surface. Rate k_2 is the reciprocal of the mean residence time on the surface, given that a successful adsorption has occurred. A stronger equilibrium binding can result from either a larger k_1 or a smaller k_2, but only a direct measurement of at least one of the kinetic rates can determine which change is the cause of the stronger binding. The separate kinetic rates also affect the efficacy by which surface diffusion might enhance reaction rates of nonspecifically adsorbed agonists at specific membrane targets (Adam and Delbruck, 1968; Berg and Purcell, 1977; Axelrod and Wang, 1994; and others). The paper by Huang et al. in the October issue of *Biophysical Journal* (pp. 1754–1766) uses a modification of TIR-FPR (called TIR-FPPR for *f*luorescence *p*attern *p*hotobleaching *r*ecovery, employing a stripe interference pattern for the initial bleached imprint) to measure surface diffusion coefficients for a biologically important adsorbate (prothrombin fragment 1) that is so weakly bound that it remains associated with the phospholipid surface for less than 1 s. That difficult measurement is the main point of the paper.

There is a complication that may turn out to be as interesting as the main point. Almost all systems upon which the adsorption kinetics have been measured by TIR-FPR relax with a nonexponential fluorescence recovery, indicating that the binding event is not just a simple single on/off event. Nonexponential fluorescence recovery can arise from numerous mechanisms. Formally, the simplest can be written as (1) a "parallel" scheme whereby two independent types of binding sites coexist, each with its own K_d and kinetic rate constants and maximum number of available sites. This scheme naturally gives rise to discussions about the discrete "high affinity" site and the "low affinity" site, discussions which are ubiquitous in much of the biochemical binding literature. Another is (2) a "series" scheme in which all the binding events progress through the same series of intermediate steps. Obviously, even more complex schemes can be imagined, with more types of sites, more intermediate steps, combinations of the two, and even loops in the pathways.

Apart from this phenomenological description, actual binding might be physically pictured as the adsorbate falling by Brownian motion into a multidimensional free energy well near the target surface, with the dimensions describing not just distance from the surface or lateral position along the surface, but also orientation and macromolecular conformation factors. The multidimensional well may have more than a single minimum, with the adsorbate undergoing random Brownian motion from one energy minimum to the next, and also spending considerable time between the positions of the minima. The various minima might correspond to discrete binding states, but if the shape of the well is complex, any discrete state phenomenological model is only an approximation. The results in the Huang et al. paper are sufficiently noisy to preclude resolving between even the simplest of these alternatives, but they can resolve two phenomenological sets of kinetic rates while simultaneously detecting surface diffusion. In combination with an earlier theoretical paper from the same group (Hsieh and Thompson, 1994), the Huang et al. paper is a step up in sophistication and provides a framework on how to measure surface diffusion in the presence of multiple desorption rates and potentially how to distinguish between different surface binding mechanisms.

Surface diffusion itself is also interesting and complex. It is not just bulk diffusion occurring near a surface. What TIR-FPR reports as surface diffusion is essentially the (possibly slight) preference for a Brownian molecule to move in a parallel rather than isotropic fashion (within the available

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This New and Notable paper addresses "Translational Diffusion of Bovine Prothrombin Fragment 1 Weakly Bound to Supported Planar Membranes: Measurements by Total Internal Reflection with Fluorescence Pattern Photobleaching Recovery," by Zhengping Huang, Kenneth H. Pearce, and Nancy L. Thompson and appears in *Biophysical Journal* October, 1994 (Vol. 67, pp. 1754–1766).

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half-space) proximal to a surface. Surface diffusion can exist if an adsorbate is trapped by a free energy well that is steeply attractive in the normal direction to the surface but is more open in the lateral direction. The complexity in surface diffusion arises for several reasons. (1) It is of interest to know how far a reversible adsorbate might diffuse while it is adsorbed. The answer is not so clear when the average adsorption time arises from a multiplicity of parallel or series states. (2) Surface diffusion of a molecule may be a skimming or gliding on a surface, limited only by bulk solvent viscosity and the bumpiness of the lateral surface potential field, or it may involve actual penetration into the mono- or bilayer of the surface, limited by the viscosity of the surface molecular layers. (3) If reversible adsorption is strong, the effective concentration of solute at a surface may be much higher than it is in the bulk, leading to molecular crowding effects.

For prothrombin binding at phospholipids, Huang et al. have no information on the first point above, but they have interesting results on the second and third points. They find that prothrombin surface diffusion is limited by the physical state of the lipids: gel state membranes adsorb prothrombin about as well as liquid state membranes, but they virtually abolish surface diffusion. Either prothrombin becomes partly intercolated into the gel membrane and becomes laterally stuck, or it associates with a particular set of lipids and does not move around unless those lipids move too. Huang et al. also find a crowding effect among the reversibly adsorbed prothrombin: 70% saturation of the surface with protein reduces the surface diffusion by about 60% relative to the value at 20% saturation. Such crowding effects could significantly alter the rates of two-dimensional chemistry in some systems.

Given the kind of techniques and analysis presented in Huang et al., coupled with an actual assay for the chemical products of a surface reaction, it should be possible in the future to confirm or refute experimentally the role of surface diffusion in enhancing reaction rates of reversible adsorbates at membranes. But even if surface diffusion is essentially nonexistent or if reactions with specific targets in the membrane can only occur by approach from the bulk, reversible nonspecific adsorption to a surface might increase reaction rates with specific surface targets in some cases simply by increasing the local bulk concentration of reactive groups near the surface. This is possible, for example, if one end of an elongated molecule adsorbs nonspecifically while the other end containing a reactive group for a specific membrane target diffuses around at the end of its tether.

Aside from the useful data analysis theory and the novel results on prothrombin adsorption to membranes, the Huang et al. paper contains a rather complete reference list of recent papers involving TIR-FPR theory, optics, and applications based around supported phospholipid bilayers. These techniques are now also becoming usable for studying the dynamics of reversible adsorption to biological membranes (Hellen and Axelrod, 1991; Fulbright and Axelrod, 1993; Stout and Axelrod, 1994).

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