Dynamical Heterogeneity in Supported Lipid Bilayers

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Abstract

Planar-supported phospholipid bilayers are responsive surfaces that reconstruct when macromolecules adsorb. This review outlines the phenomenon of lipid diffusion "slaved" to or significantly controlled by that of macromolecular adsorbates. To elucidate such systems, we discuss the value of spatially resolved experiments at the few-molecule level, lipid diffusion compared in outer and inner leaflets of the supported bilayer, and a simple method to minimize defects by the strategy of "electrostatic stitching."

Keywords: *adsorption, biological, diffusion, surface chemistry.*

Consider the following armchair experiment. Suppose that you survey the audience in a movie theater. About half of the audience wears eyeglasses (two eyes per person, usually); about half of the audience wears none. On average, the number of eyes bearing eyeglasses is one per person. But this calculation is too simple-minded—the naïve average masks a bimodal distribution that carries physical significance.

So what? As this review will show, similar ideas apply to the case of phospholipids, where lipid mobilities have nontrivial distributions that depend strongly on macromolecular adsorbates. Lipid mobility in supported phospholipid bilayers is so fundamental to their function that it is interesting and relevant to understand the distribution of mobility within them, not just for studying biological questions but also in applied problems such as their use as biosensors and nanoreactors. Of central importance is understanding what controls the lateral mobility of the individual molecules that comprise these fluid yet two-dimensional systems.

"Slaved" Diffusion and the Value of Spatially Resolved Experiments

A vast body of research shows that the presence of molecules such as cholesterol embedded within the lipid membrane produces heterogeneities; for example, lipid "rafts" and resulting nanodomains are well documented.[1–4] However, the simple process of allowing macromolecules to adsorb also produces dynamical heterogeneity, even when the bilayers are composed of one single type of phospholipid. It has been known for some time that lipid diffusion depends on the chemical composition and phase state of the bilayer,[5–7] but those studies dealt with naked bilayers (no adsorption). It has also been known for some time that mixtures of phospholipids partition spatially after interacting with an adsorbate,[8] but those studies did not address the mobility of these lipids. Other diffusion-related studies, such as binding-induced mobility,[9–11] anomalous subdiffusion,[12] and the influence of obstacles in the diffusing plane,[13–15] have also been widely considered, but few of them involved macromolecule adsorption.

The usual methods to study mobility in phospholipid bilayers employ area-averaged methods, such as FRAP (fluorescence recovery after photobleaching), but an area-averaged method suffers from the same potential liability as our naïve eyeglass calculation. Methods exist to measure local mobility. Fluorescence correlation spectroscopy (FCS) enables one to measure the mobility of fluorescent molecules within the diffraction-limited focus of a laser beam, a diameter of ~0.35 μm.[16,17] In experiments of this kind, it is convenient to design the experimental system so that on average one sole fluorescent molecule resides within the area sampled. Then the fluctuations of emitted fluorescence, when fluorescent molecules diffuse into and out of this planar area, reflect their translational diffusion, and the method carries spatial resolution.

Spatially resolved measurements of lipid diffusion were made[17] after macromolecules were allowed to adsorb to supported phospholipid bilayers at incomplete surface coverage, as summarized in Figure 1. To avoid the complexity of having permanent electric charge, a system was chosen in which the lipids carried a zwitterionic head group, a dipolar head group that carried no net electric charge. Depending on where the laser was focused, the rate of fluorescence fluctuation switched between two values; it varied from spot to spot on the bilayer, slow or fast, but not in between. Studies in the physical sciences rarely encounter this bimodal distribution. More typically, a heterogeneous distribution is evenly distributed around the mean, but it was not so here. In Figure 1, the intensity–intensity autocorrelation function computed from the observed fluorescence fluctuations is plotted against time lag after the cationic polymer, quaternized poly-4-vinylpyridine (QPVP), was allowed to adsorb to partial surface coverage. The physical meaning of the autocorrelation function is to quantify the time for Fickian diffusion through the spot illuminated by the focused laser beam; then, the translational diffusion coefficient D scales as the square of its linear dimension, divided by the time at which the autocorrelation function decayed to a given value. Quantitative elaboration of this idea, standard in using the FCS method, also takes into account the Gaussian shape of the spot illuminated by the laser beam.[18] Analysis showed that the translational diffusion coefficient was described by a bimodal distribution, taking either the faster value or the slower value, depending on where the laser beam was focused in space.[17] This finding was robust; the bimodal distribution of diffusion coefficients held over a wide range of surface coverage, so long as the surface coverage was less than ~50% of saturated adsorption (Figure 2).

The influence of polymer molar mass was also investigated. Always, a fast and a slow mode were observed, depending on where on the bilayer the focused laser

beam was directed. The higher the molar mass of the adsorbate, the slower the diffusion. In Figure 3, the diffusion coefficient D inferred from the slow mode is plotted on log–log scales against the degree of polymerization of the adsorbed polymer N; this chain length varies by nearly an order of magnitude, and the comparison is made at fixed surface coverage, 20% of saturated adsorption. The plot shows clearly an empirical power law relation, $D \propto N^{-1}$.

Why? Reflection and control experiments[17] led to the conclusion that the most plausible interpretation of the coexistence of fast and slow diffusion in the same system was that macromolecular adsorption created nanodomains of lipid whose mobility was determined by the occluded area of adsorbed polymer. The multivalency of these nanodomains (i.e., the multiple potential adsorption sites to which lipids can bind when exposed to an adsorbate whose occluded area is large) localized lipids because the tendency to adsorb at any individual spot is amplified by the large number of potential binding sites. The data in Figure 3 show that as $N \rightarrow 150$, D extrapolates to that characteristic of the naked lipid, implying that the slow mode disappeared below a critical adsorbed size, the projected area of ~80 head groups.

For lipids trapped within these nanodomains, these arguments suggest that collective diffusion as a unit replaced the independent diffusion of individual lipid molecules. The translational mobility of a particle embedded in biological membranes has been considered theoretically.[19] These experiments show that lipid mobility is itself affected. Adsorption of macromolecular objects of variable size modifies the mobility of lipids underneath the adsorbed object. This leads to dynamical heterogeneity, even though chemically the lipid comprises only one chemical species. The dependence on molar mass of the adsorbed macromolecule displays the same phenomenology as the diffusion of that same adsorbed macromolecule;[20,21] diffusion of the lipid appears to be slaved to the adsorbate.

The present work shows that the lipid mobility is spatially dependent, even in simple systems that only contain one lipid type. This bimodal distribution highlights the need for spatially resolved measurement techniques that can capture these variations and suggests that earlier work using average-area approaches misses this important attribute of the system. The present work also complements a growing number of theoretical studies that predict that local bending rigidity and the local spontaneous radius of curvature of

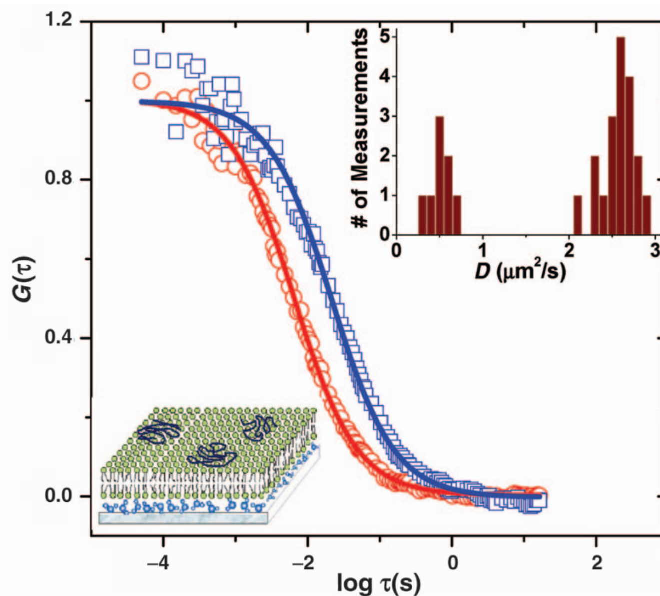

Figure 1. Fluorescence autocorrelation function G(τ) plotted as a function of logarithmic time lag τ for a DLPC (1,2-dilauroyl-sn-glycero-3-phosphocholine) supported lipid bilayer carrying adsorbed quaternized poly-4-vinylpyridine (QPVP) at the fractional surface coverage of 20% (see the schematic in the lower-left corner). The QPVP was 100% quaternized (charged by reaction with ethyl bromide) with a weight-average molar mass of 81,500 g mol^{-1}. Note that fast and slow diffusion modes coexist depending on where the interrogatory laser spot was focused. The mean diffusion coefficient D of the slow mode is 0.50 μm^2 s^{-1} with a standard deviation of 0.12 (open blue squares), while the mean D of the fast mode is 2.62 μm^2 s^{-1} with a standard deviation of 0.18 (open red circles). Inset, upper right: histogram of diffusion coefficients obtained from ~30 different measurements on a number of samples. Adapted from Reference 17.

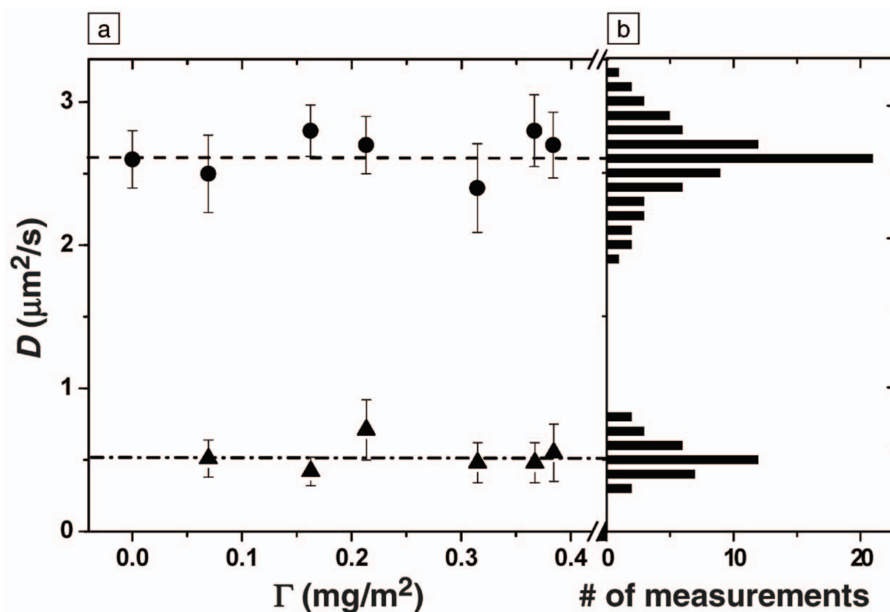

Figure 2. Surface coverage dependence when surface coverage is <50% of saturated adsorption. (a) Diffusion coefficients of the fast (solid circles) and slow (solid triangles) modes of lipid motion, plotted against surface coverage Γ for the same system as in Figure 1. Error bars show standard deviation. (b) Histograms of fast (top) and slow (bottom) diffusion coefficients obtained from ~100 different measurements on a number of samples. Adapted from Reference 17.

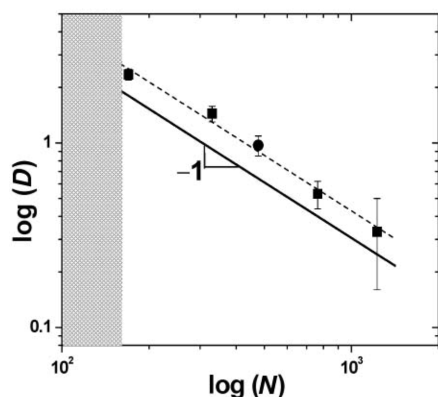

Figure 3. Slow-mode diffusion coefficient plotted against degree of polymerization of the adsorbed polymer on log–log scales at the fractional surface coverage of 20%; the reference solid line has slope −1. The fully quaternized poly-4-vinylpyridine (QPVP) samples were prepared from parent PVP samples with molar masses $M_w = 18,100$, 34,200, 81,500, and 130,000 g mol^{-1} (ratio of weight-average to number-average molar mass $M_w/M_n = 1.11$, 1.23, 1.18, and 1.24, respectively) (solid squares). The adsorbed poly(methacrylic acid) (PMA) had $M_w = 40,000$ g mol^{-1} ($M_w/M_n = 1.05$) (solid circle). These data extrapolate as $N \rightarrow 150$ to a diffusion coefficient D characteristic of the naked lipid (shaded area), implying that the slow mode disappears below a critical adsorbate size because the polymer size is not big enough to slave lipids. This occurs at a projected area of ~80 lipid head groups. Adapted from Reference 17.

the membranes is modified by anchored or adsorbed polymers,[22–28] which may change the diffusion of underneath lipids.

Adsorption-Induced Surface Reconstruction

The considerations raised in the previous section put into curious perspective a large amount of prior research. In the fields of polymer science and biomaterials, it is known that macromolecules adsorb spontaneously from solution because a small adsorption energy per segment adds up to a large net adsorption energy per molecule.[29,30] In the field of polymer science, this is traditionally considered to occur on surfaces having a “frozen,” unresponsive structure, and definitive treatises exist on this subject.[29,30] This does not describe the situation in the previous section.

In contrast, in the study of phospholipid membranes, drug delivery, and gene therapy, interactions with polymers are known phenomenologically to have the capacity to make membranes leaky—for example, for the outflow of drugs from vesicles[31] or the inflow of encapsulated DNA into cells.[32] In those cases, the membrane structure is clearly disrupted. Bacteriocidal action has even been demonstrated[33] by polymer disruption of phospholipid membranes. This said, it is evident that when phospholipid bilayers are used in sensor and materials applications, macromolecules must be able to adsorb heavily without disruption of the membrane structure.

It is fascinating that these different communities developed with little crosstalk. The adsorption and biology communities have focused on the extreme limits—the adsorption community focusing on the polymer side of the interface, and the biologically minded community focusing on the practical consequences, especially when membranes are disrupted.

In fact, recent studies of surface equilibration dynamics at supported phospholipid bilayers find patterns of dynamic physical behavior that differ remarkably from what is characteristic of adsorption onto frozen surfaces.[29,30,34–38] This has bearing not just on biological and biophysical problems[39] but also on industrial applications such as creating formulations for many cosmetics and pharmaceutical products.[40] The dynamics of adsorption and surface equilibration when the surface possesses reciprocal mobility is a largely unsolved problem and remains a challenge for the field of surface science[41] and the phospholipid bilayer community.

Lipid Diffusion Compared in Outer and Inner Leaflets of Supported Bilayers

It may seem paradoxical to observe single diffusion processes at each laser focus spot (e.g., Figure 1); one might have expected to find a distinction such that lipid diffusion would fall into two populations corresponding to the inner and outer leaflets (monolayers that comprise the bilayer) of the bilayer. But when arguments produce a paradox, from this one learns about limitations of the model on which the argument is based!

In pioneering work, Bayerl and coworkers studied phospholipid bilayers wrapped around spherical silica beads and concluded, using nuclear magnetic resonance (NMR), that lipid diffusion in the inner leaflet was slower by a factor of two than in the outer leaflet.[42] The relevance of this study to planar-supported bilayers was uncertain, however, first because bilayers that coat a colloidal-sized substrate necessarily possess much higher curvature; and second because these colloids are typically rougher than planar surfaces.

Using Langmuir–Blodgett methods to form supported bilayers one leaflet at a time, several groups used FRAP to discriminate between lipid diffusion in the outer and inner leaflets,[43,44] but the generality of the conclusion was unclear because analysis was based on the assumption that lipid “flip-flop” between leaflets was slower than the experimental time scale of hours. However, the validity of this assumption was called into question by recent sum frequency generation experiments which found lipid flip-flop to be considerably faster than this in the fluid state.[45,46]

To further test this question, it seemed worthwhile to revisit it on planar solid supports using few-molecule fluorescence methods. Iodide quenching of dyes in the outer leaflet was used to distinguish diffusion in the inner leaflet from that in the outer leaflet and to confirm the generality of the findings. The bilayers were prepared not only by vesicle fusion but also by Langmuir–Blodgett deposition. These studies concluded that regardless of whether the bilayers were supported on quartz or on a polymer cushion, translational diffusion in the outer and inner leaflets was the same within an experimental uncertainty of ±10% but with a small systematic tendency to be slower (by <5%) within the inner leaflet.[47] Theoretical arguments have interpreted such behavior to indicate that leaflet–leaflet coupling is stronger than leaflet–substrate coupling across an intervening thin water film or polymer cushion,[47,48] but the precise nature of this strong coupling remains to be elucidated.

Removing Heterogeneity by “Electrostatic Stitching”

The discussion so far concerned the “fluid” phase of supported lipid bilayers, but also interesting is the “gel” phase that occurs when the mobile “liquid-crystalline” ordering crystallizes as the temperature is lowered. Studies of the materials science of the fluid-to-gel phase transition show a high density of surface defects in the gel phase, presumably because the area per head group of zwitterionic phospholipids shrinks by ~20% upon crystallization.[49–52]

A strategy of doping the bilayer with a small amount of cationic lipid shows promise in eliminating the heterogeneities caused by this shrinkage. The idea is simple: the charged lipid is expected to be well dispersed within the bilayer because of electrostatic repulsion, and its electrostatic interactions with zwitterionic lipids may act to stitch together the bilayer, imparting dimensional stability through the phase transition. Computer simulations first proposed that this effect is expected

from simple electrostatic considerations.[53] Recent experiments appeared to confirm this idea (Figure 4).[54] On the functional side, this suggests a simple method to minimize defects in gel-phase supported lipid bilayers, thereby extending their potential range of practical usefulness.

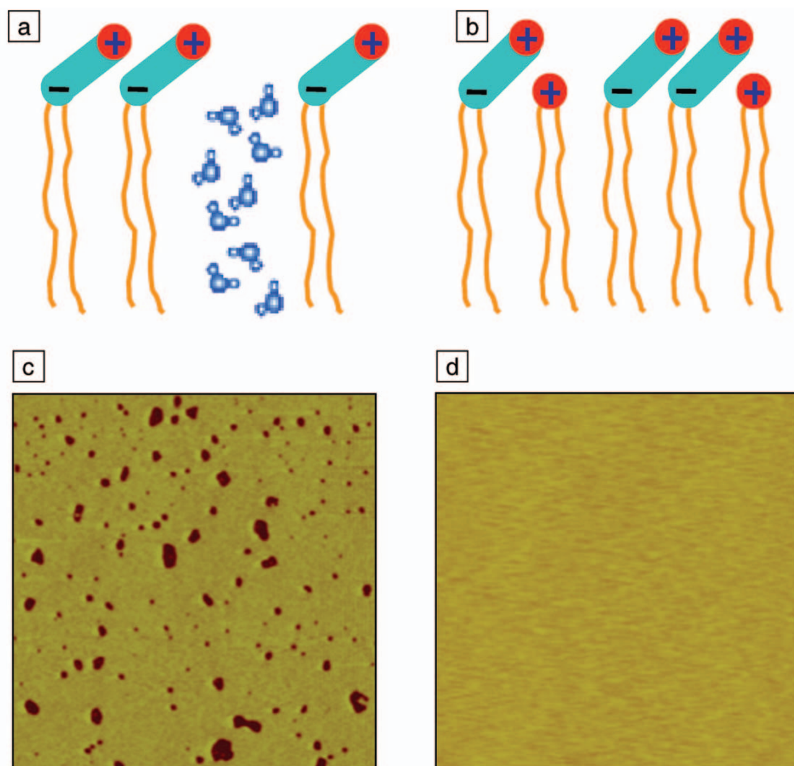

Figure 4. Distinction between gel-phase morphologies of single-component zwitterionic lipid bilayers (a) and (c) and two-component zwitterionic lipid bilayers mixed with up to 20% cationic lipid (b) and (d). (a) Schematic illustrating that in the single-component bilayers, mutual repulsion between p–n+ head-group dipoles in the head groups facilitates defect formation. (b) Schematic illustrating that electrostatic interaction of zwitterionic lipid with cationic lipid head groups favors a compact structure. (c) Representative magnetic acoustic code (MAC)-mode atomic force microscopy (AFM) images of single-component zwitterionic, DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine) bilayers. (d) Representative MAC-mode AFM images of bilayers containing 10% cationic lipid DMTAP (1,2-dimyristoyl-3-trimethylammonium-propane). In (c) and (d), the temperature is 15°C and the image area is 5 μm $\times$ 5 μm. Adapted from Reference 54.

Outlook

Studies of planar-supported phospholipid bilayers offer crossroads between areas of materials science that, historically, evolved with too little cross-fertilization. Among the many areas of opportunity for future advances this review has called attention to are (1) the instructive distinction between single-molecule behavior and the ensemble average, especially when the ensemble average does not add up to a unimodal distribution; (2) the practical importance not only of adsorption but also of reciprocal surface reconstruction when macromolecules adsorb; and (3) the potential of using simple electrostatic arguments as a guide to engineer the fundamental issue of defect abundance when bilayers undergo a common phase transition.

Looking to the future, beyond the scope of this review, we call attention to the need to develop methods to investigate phospholipid vesicle structures, sometimes called liposomes,[55,56] which would possess the portability, long lifetime, and dimensional stability that underlie much of the attraction of planar-supported phospholipid bilayers. When it becomes possible to use liposomes of submicrometer size as engineering materials (as planar-supported bilayers can already be used), versatility in applying this engaging biomaterial in new functional applications will be expanded greatly.

Acknowledgment

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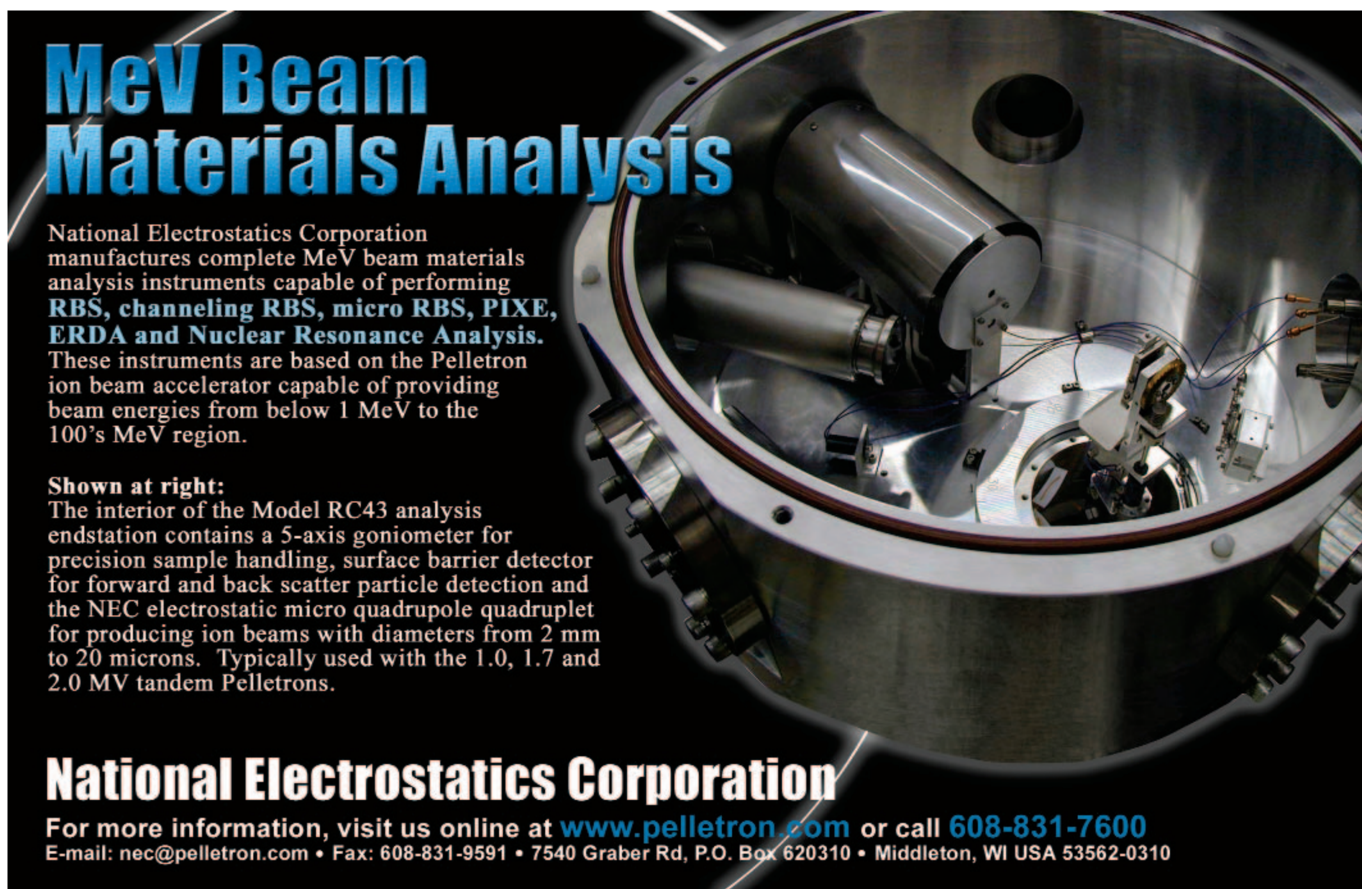

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