

or deactivating specific endosomal-lysosomal enzymes that control interaction between TLR9 and CpG DNA.

Cathepsin K could also be important for the transit of TLR9 to the endosomal-lysosomal compartment. TLR9 signaling is sensitive to its position within the endosomal-lysosomal network (bacterial CpG-A and CpG-B oligodeoxynucleotides signal from early versus late compartments, respectively, affecting dendritic cell activation). Toll-like receptors move from the endoplasmic reticulum to the endosomal-lysosomal network by a yet undiscovered trafficking signal and/or chaperone carrier. Chaperoning could be analogous to that needed for major histocompatibility complex class II molecules (which present antigens at the cell surface to T cells) to pass from the endoplasmic reticulum to the lysosome

before antigen loading and passage to the cell surface. During this transport, cathepsins L, F, S, and V are important for the chaperoning role of a protein called the invariant chain. By analogy, cathepsin S has been proposed to control the function of CD1d, a glycoprotein that presents lipid antigens to T cells. Cathepsin S may influence the transport of CD1d by cleaving key proteins in the endosomal-lysosomal network.

One important question is the degree to which the immune effect of cathepsin K is limited to TLR9. Is the apparent role of cathepsin K in regulating the autoimmune diseases examined by Asagiri *et al.* consistent with a simple TLR9 inhibition mechanism? TLR9 agonists may contribute to both autoimmune disease models examined in the study and TLR9-deficient mice are partially resistant to

experimental autoimmune encephalomyelitis (4, 5). Nevertheless, it remains to be determined whether there is any pathogenic role for cathepsin K in human autoimmune or inflammatory diseases. The protease activity of cathepsin K is thought to be relatively nonspecific, including strong collagenase, elastase, and gelatinase activities, and how this translates into such an apparently specific effect on TLR9 activation remains to be elucidated.

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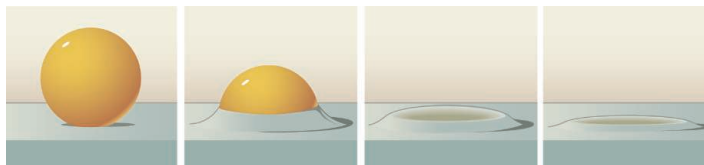
MATERIALS SCIENCE

Glass Surfaces Not So Glassy

J. R. Dutcher and M. D. Ediger

In many materials, atoms or molecules on surfaces can be more mobile than particles in the interior. For example, the diffusion of an atom along a single crystal surface is typically much faster than diffusion inside the crystal. The control of layer-by-layer growth of crystalline materials for semiconductor lasers and other devices is possible because of a sophisticated understanding of this process. Many technologies also rely on noncrystalline materials such as glass (a solid without the regular packing of a crystal). Is this same type of mobility present on the surface of a glass? On page 600, Fakhraai and Forrest (1) report elegant experiments establishing that a polymer glass surface can be many orders of magnitude more mobile than the interior. But their results cannot be explained by atoms or molecules skittering across a surface. Rather, they find that a liquid-like layer, at least several nanometers thick, exists at the surface of a polymer glass.

Fakhraai and Forrest measure surface mobility by preparing polystyrene films with well-defined nanoindentations on the surface



Watching glass relax. Gold particles are deposited, allowed to partially embed in the glass surface, and then gently removed. Fakhraai and Forrest used atomic force microscopy to image the filling of the nanoindentations over time at various annealing temperatures.

(see the figure). Gold spheres (with a nearly uniform 20-nm diameter) are deposited on a flat polystyrene surface at room temperature, well below the glass transition temperature T_g where polystyrene becomes a solid. Annealing the polymer films above T_g allows the gold spheres to sink a few nanometers into the surface. At room temperature, mercury dissolves away the gold spheres, leaving behind hemispherical nanoindentations about 5 nm deep. Surfaces prepared in this manner are then annealed below T_g for various periods of time, and atomic force microscopy is used to image the filling of the holes.

The most striking observation by Fakhraai and Forrest is that filling of the nanoindentations on the polystyrene glass surface is essentially complete on relatively short time scales. At 20 K below T_g the process takes a few minutes, whereas at 100 K below T_g the holes fill in a few weeks. These times are much faster (by a factor of $\sim 10^9$ at $T = T_g - 100$ K) than

Nanoscale indentations on a polymer glass relax rapidly, indicating the presence of a highly mobile liquid surface layer.

comparable relaxation processes in bulk polystyrene. Additionally, the surface relaxation process has a much weaker temperature dependence than bulk relaxation, and the two processes appear to merge slightly above T_g .

Owing to the wide application of polymer glasses, researchers have been interested in understanding the dynamics of these surfaces (2, 3). Prior studies measured the relaxation of a free surface upon which a pattern has been templated (4, 5). However, the present work uniquely combines a reproducible deformation with a simple geometry, a penetration depth of only a few nanometers (necessary for probing only the surface), and a gentle method for removing the template from the sample. Although previous studies have provided evidence for enhanced mobility at the surface of polymer glasses (6), this is the first quantitative characterization of the surface relaxation time over a wide temperature range. The merging of the surface and bulk relaxations near T_g explains why previous surface-sensitive experiments performed close to T_g show nearly bulk dynamics.

The work of Fakhraai and Forrest highlights a critical difference between mobility at crystal surfaces and at glass surfaces. Because the polymer segments in these experiments are connected to each other in long chains that extend

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20 nm or more into the glass, the nanoindentations cannot be filled by polymer segments skittering across the surface; each segment at the surface is chemically tethered to other segments that are below the surface. Instead, mobility at the surface of a glass can be viewed as the motion of a thin liquid-like layer that responds to surface tension, filling the nanoindentations to minimize surface area. These experiments and others indicate that the range of enhanced surface mobility is at least several nanometers (and perhaps more than 10 nm) (6); for comparison, the polymer segments are roughly 1 nm. A liquid-like layer of several nanometers is reasonable given that molecular motions near T_g are highly cooperative. That is, the packing is so tight that one polymer segment can move only when a large number of neighbors collectively

adjust their positions (7). The size of this group of segments has been estimated to be several nanometers (8), and it makes sense that the presence of a surface would perturb motions over a length scale at least this large (9). Whether this viewpoint can yield a predictive theory of glass surface mobility is unknown.

The surface mobility of polymer glasses is relevant for understanding adhesion, friction, and instabilities in thin polymer films and nanostructures used in advanced lithography (10). If strategies can be devised to decrease surface mobility, photoresists could be used to transfer patterns on even smaller length scales. Surface mobility is also likely to be important in a wide range of nonpolymeric glasses (11, 12). Given the enormous magnitude of this effect in polystyrene, the surface

mobility of glasses likely has important consequences that have not yet been considered.

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MOLECULAR BIOLOGY

The Art of Assembly

Francis Szoka

Small RNAs were chosen as the “Breakthrough of the Year” molecule for 2002 (1). One of these, small interfering RNA (siRNA), which is 20 to 23 nucleotides in length, can base pair with a target messenger RNA (mRNA) sequence and direct its degradation, thus blocking production of the encoded protein. Although siRNAs have surpassed expectations when used in experiments to alter gene expression, the challenge of turning them into effective drugs has been the lack of an efficient in vivo delivery system. On page 627 in this issue (2), Peer *et al.* describe a strategy that offers cautious optimism for siRNA becoming

a therapeutic reality to treat human disease in the coming decade.

Although small by nucleic acid standards, siRNAs are large compared to most drugs. A 22-nucleotide siRNA has a molecular weight of about 15,000, about 50 times that of a typical drug. Because they also have a strong negative charge, siRNAs cannot readily cross biological membranes and enter cells. Furthermore, siRNAs are metabolized in the blood, requiring a chemical modification or a specific formulation solution to prevent their degradation during delivery.

Despite these limitations, delivering siRNA by attaching it to a lipid modestly reduced gene expression in mouse hepatocytes (3). Substantial improvement in siRNA delivery in nonhuman primates was achieved by packaging the siRNA in a cationic lipo-

Advances in delivering small interfering RNAs to specific tissues may bring these nucleotides closer to reality as therapeutic agents.

some that was coated with polyethylene glycol polymer chains (4). At a single dose of 2.5 mg of siRNA per kilogram of body weight, this approach reduced expression in the liver of the cholesterol-carrying protein apolipoprotein B by more than 90%, and also decreased serum cholesterol (4). Both effects persisted for more than 2 weeks. By comparison, a 2-week supply of an oral cholesterol-reducing drug is about 2 mg per kilogram of body weight.

Most in vivo siRNA delivery studies have been directed to tumor tissue or to the liver, so the study by Peer *et al.* targeting leukocytes is an exciting new development. Promising delivery approaches described by this group and others have a unifying theme: attaching a targeting ligand—either a carbohydrate (5), small molecule (6), antibody (2), or the mole-

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Performance of siRNA carriers with proven intravenous delivery capabilities

Species tested	Carriers of siRNA	Ligand attached to carrier	Target of siRNA	Effects	Adverse side effects
Primate (4)	Liposome–polyethylene glycol complex	None	Apolipoprotein B (liver)	Decreases serum cholesterol	Transient increase in serum transglutaminases
Mouse (5)	Polymer	Carbohydrate	Apolipoprotein B (liver)	Decreases serum cholesterol	Transient increase in serum transglutaminases
Primate (7)	Polymer	Transferrin	Ribonucleotide reductase (tumor)	Inhibits M2 subunit of ribonucleotide reductase	Immune response to transferrin; increase in inflammatory cytokines
Mouse (6)	Liposome–protamine complex	Anisamide	Epidermal growth factor receptor (tumor)	Blocks epidermal growth factor activity and tumor proliferation	Transient increase in serum transglutaminases
Mouse (2)	Liposome–hyaluronan–protamine complex	Antibody to β_1 integrin	Cyclin D1 (leukocytes)	Reduces leukocyte proliferation and inflammation	None reported