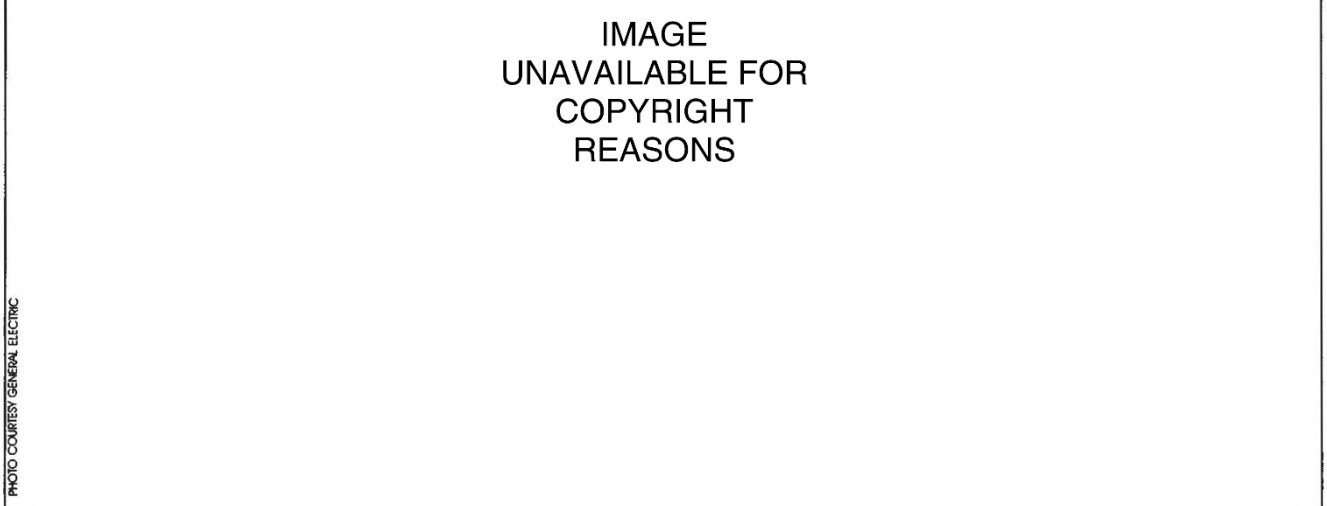
PROTEIN STRUCTURE

LOOKING AT TWO-DIMENSIONAL CONFIGURATIONS



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Transmission electron micrograph of two-dimensional IgG crystal growth. The stain-excluding regions of this ordered hexagonal array are clusters of the FAb portions of six antibody molecules. The slender threads connecting the clusters are the Fc portions of the molecules.

SCHENECTADY, N.Y.—Protein engineers could soon have a new technique to help them analyze crystal structures. The method, developed by General Electric staff biophysicist Egidijus E. Uzgiris and collaborators, allows visualization of the overall configuration of a protein sample rapidly—in a day or so. The trade-off is somewhat lower resolution than yielded by current techniques: whereas X-ray crystallography gives resolution down to one Angstrom, the new technique only resolves structures to 7–10 Angstroms. This is not precise enough to allow calculation of atomic coordinates, but it certainly gives information on molecular packing in crystals and may indicate how proteins interact with each other under physiological conditions.

The highly ordered protein arrays are "grown" on electron microscope grids. A grid is dipped into an aqueous solution upon which a monolayer of phospholipid floats. When the grid is removed through the air/liquid interface, it brings with it a layer of the phospholipid. Uzgiris has obtained phospholipid bilayers by dipping the grid twice. The grids are first UV-irradiated to render them hydrophilic; this allows uniform distribution of the phospholipid over nearly 100 percent of the grid's surface. After the phospholipid-coated grid is dried, it is placed face-down on the protein solution of interest. The bilayers are stable, and can endure the prolonged exposure needed to bind proteins from dilute solutions. The grids can be incubated on drops as small as ten microliters containing 0.5 micrograms of protein.

Uzgiris has used these phospholipid-coated grids to bind IgE and IgG_1 monoclonal antibodies, employing a phospholipid with the DNP (dinitrophenyl) hapten derivitized to the molecules' polar head groups. The antibodies are anti-DNP, so they bind to the DNP moieties on the coated grids. While the antibody solutions Uzgiris used were about 99 percent pure, non-binding contaminants would have no effect on the results.

When IgG_1 binds to the haptenated phospholipid, it forms two types of crystals, either linear strands or hexagonal arrays (see photo), depending on temperature, pH, and salt concentration. At room temperature the phospholipid is fluid, and lateral diffusion allows the bound antibody molecules to rearrange themselves in a highly ordered array. In the two-dimensional hexagonal lattice, the distance from the vertex of one cluster to that of the next is 150 Angstroms. Uzgiris says that each vertex is formed by a cluster of the FAb (antigen binding) portions of six antibody molecules. The slender threads that connect adjacent clusters are the Fc (constant) domains.

Since antibody molecules have a hinge to allow for flexibility during antigen binding, Uzgiris speculates the antibody molecules bend over slightly to form Fc:Fc contacts with other molecules of the lattice. In fact, there is some evidence to support this notion. When dithiothreitol (DTT, a compound that preferentially reduces the hinge's disulfide bonds) was added to the mixture, very little crystallization occurred.

The crystallization and packing of IgE is different from that of IgG_1. Uzgiris attributes this to IgE's extra Fc domain. This means that Fc contacts can't be made in the same way.

Phospholipids can be altered with moieties other than the DNP hapten. Uzgiris has used saccharide moieties to study bacterial toxins, for instance. Before this technique becomes widely adaptable, however, he stresses that it will require one more technological advance. As the technique now stands, it must be tailored to each new protein. By refining or omitting some of the chemistry, Uzgiris hopes to eliminate the problem of hooking on an appropriate ligand for each new protein. Once the technique is easy to adapt to individual needs, it could become an adjunct to traditional electron microscopy. The inventor sees the technique contributing to studies on protein folding and structure; it may also prove useful for monitoring pH-induced conformational changes.

—Jennifer Van Brunt