NIRT: Protein Crystals as Materials

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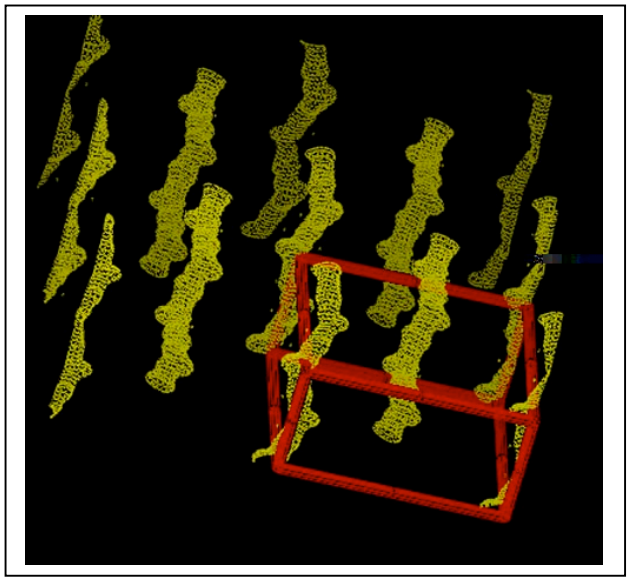
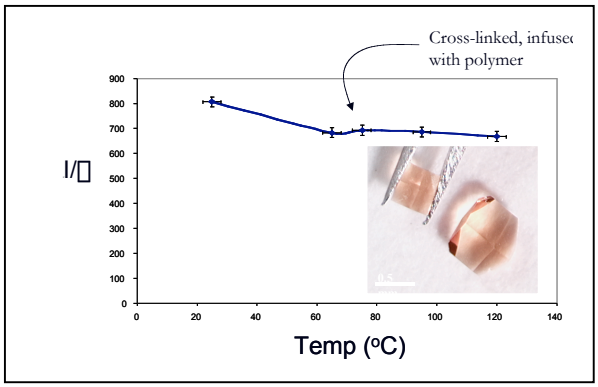
Patterning the nanometer composition of monolithic solids can be a powerful tool for materials design. For example, block co-polymers can form tough and pliable plastics, a result of the alternating nanoscopic regions of rubbery and glassy materials of which they are comprised. When nanoscopic patterns contain air as one component, the resulting structures are micro- or mesoporous. These can find great value in separation, catalytic, and electrochemical technologies. If compositional patterns are ordered over long length scales, then the resulting materials can exhibit strong optical diffraction, which is useful in numerous photonic applications. Existing chemical strategies for generating three dimensional organization use sacrificial phases (e.g., copolymer or surfactant templates) as scaffolds for the deposition of more functional materials. These templates provide only a limited number of different patterns. In addition, they present a homogeneous scaffolding surface so that the coordinated placement of different materials is difficult. The aim of this project to develop a previously unexploited class of materials, namely protein and virus crystals, as functional materials for designing nanoscopic three dimensional structures.

We have demonstrated that protein crystals offer a unique system for nanoscopic materials synthesis. Protein crystals are relatively easy to fabricate once a structure has been reported, and the protein databank has detailed maps for over 1000 different biomolecules. Straightforward methods for growth of large (greater than 1 mm) single crystals with typical repeat units of 15 – 50 Å are standard in structural biology. Structural analysis which focuses on the water regions of most protein crystals reveals that they often possess an open architecture. Typically, biomolecular crystals consist of 40-70% water in the form of ordered channels with nanometer length scale and complex but regular symmetries. The picture to the left shows the porous structure in a model lysozyme system. The pores are more than 25 Å in diameter. Virus particles, in contrast, exhibit more conventional packing (e.g., body-centered cubic), with interconnected voids of 40-50 Å diameter.



Protein cross-linking chemistry is an essential step for using these crystals as materials. Biomolecular crystals are held together by non-covalent interactions. They are quite fragile, difficult to handle, and will shatter when dehydrated. For decades structural biologists have relied on glutaraldehyde to ‘fix’ crystals by cross-linking adjacent lysine residues. This covalent bond anchors the proteins and creates stable crystals, usually at the expense of some resolution in

the x-ray diffraction. We rely on extreme versions of this cross-linking in many of our examples, and sacrifice some amount of order in the crystal to obtain thermally and mechanically stable crystals. Remarkably, once sufficient cross-links are developed lysozyme crystals can be dehydrated and heated to temperatures approaching 100°C without any significant loss of diffraction resolution. In the figure above, the y-axis is a measure of the disorder present in the crystal. The inset is a photograph of two cross-linked crystals after heat treatments to 100 C.

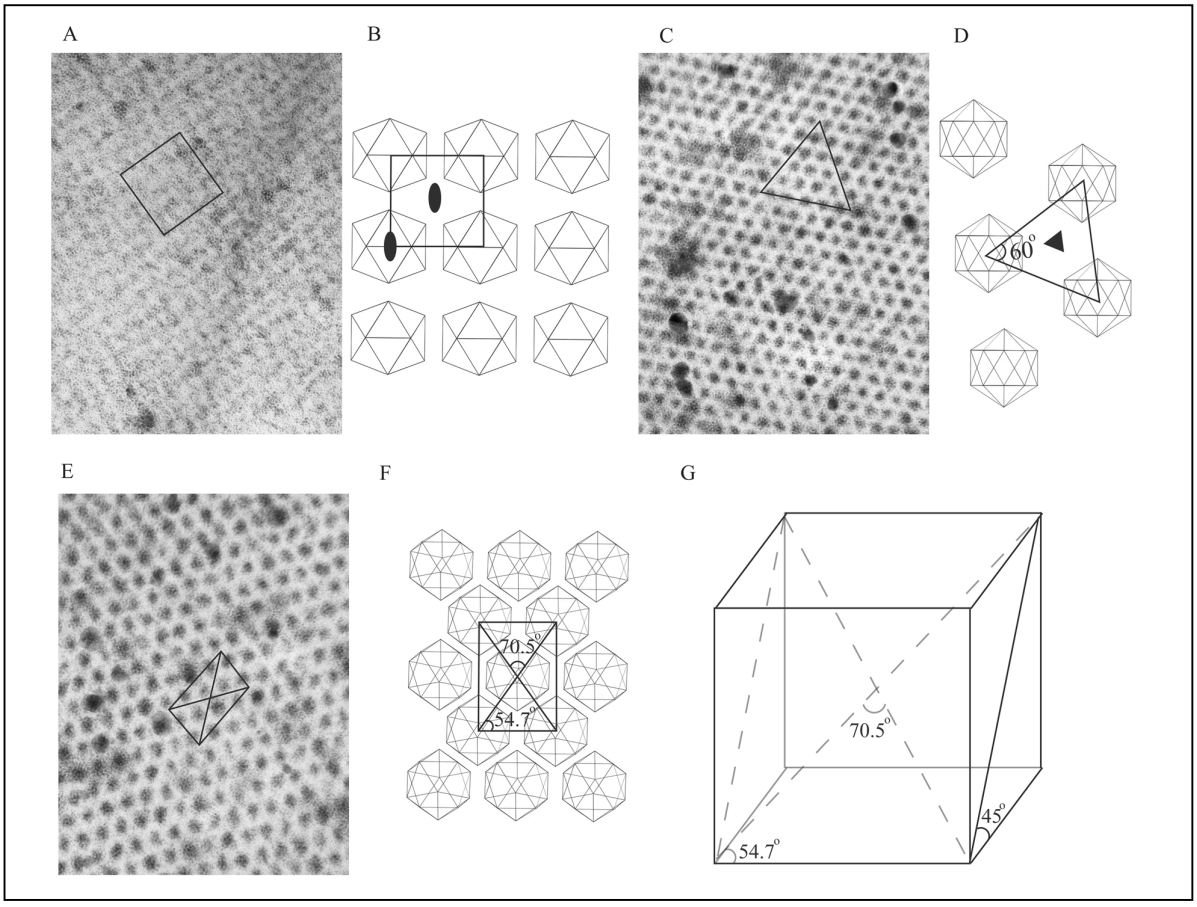


Once stabilized, the water within the crystals can be replaced with polymers or metals with only minimal disruption to the protein crystal structure. We demonstrate this fact explicitly in the case of lysozyme, in which epoxy monomer was infiltrated and then polymerized. Single-crystal x-ray diffraction studies of filled crystals found evidence of aligned epoxy chains within the pores at a significant filling fractions. These crystals still diffract x-rays with high resolution (to 3 Å) and are valuable as stabilized standards for beam alignment at synchrotron x-ray beamlines. Surprisingly, the polymerization reactions lead to significant reduction in unit cell sizes, with 10-12% shrinkage typical, without complete loss of diffraction.

Ideally, we aim to fill protein crystals with only minimal disruption to the underlying framework. However, it is generally true that disorder is increased in the x-ray diffraction pattern of the protein after filling reactions are completed. In order to evaluate whether this disorder is due to mechanical or thermal stresses, or simply to a smooth homogeneous disruption of the entire lattice we initiated a collaboration with Robert Thorne at Cornell University, to perform x-ray tomography. In these preliminary experiments, crystals are imaged and dark lines appear where the crystal structure is not perfect. This data illustrated how disorder is primarily introduced during the cross-linking stage; from its appearance one can speculate that mechanical stresses in the crystal lead to an increased in the mosaicity.

In addition to the polymeric systems, we developed metal infiltration schemes for biological crystals. For this work, we adapted the electroless plating of platinum and gold on colloidal templates to the problem of protein templating. Here we first treat stabilized crystals with palladium complexes, such as palladium acetate; it has been established that Pd will preferentially bind at histidine residues on proteins. Once bound, the metal center serves as a catalyst for the reduction of a platinum salt; this forms a nanocrystal nucleated near histidine residues. We have shown that this chemistry can fill a variety of protein crystals (thaumitin, lysozyme, myoglobin) and virus crystals (CPMV) with ordered structures of nanoscale metals. Loading densities as high as 70% volume can be achieved. X-ray diffraction and electron microscopy allow for visualization of these complex ordered three dimensional architectures. The figure on the next page shows several perspectives of a metallized CPMV crystal; our collaborators at Scripps, Dr. Tianwei Lin and Dr. Jack Johnson, matched the observed electron microscopy images with known structural characteristis of the native virus crystals. Each dot in figure c is 15 nm across.

We have recently begun to explore applications of these solid-filled proteins in several areas, including catalysis and photonics. First, we have shown that platinum nanocrystals loaded into protein crystals are able to catalyze the hydrogenation of alkenes much as they do in the unsupported phase. We are most interested here in developing enantiomeric selectivity in the catalysis process. The pores of the protein walls are not smooth and straight; in a complex



cavity, the transition state of one enantiomer could be stabilized, in analogy with existing enantiomeric separation schemes.

Photonic applications are naturally suggested given the long-range order present in these materials. Since the repeat distances of protein crystals are 1 to 50 nanometers, applications in the soft x-ray region are suggested. The metallic subphase has a strong scattering efficiency which may be the basis for developing new types of soft x-ray mirrors and monochromators. The polymer encapsulated crystals will find use as stable standards for x-ray beamlines. We also observe that the metallic templated crystals exhibit unusually strong reflective properties at visible wavelengths. This is not easily understood from a simple consideration of diffraction from ordered dielectrics. This may be a manifestation of metallodielectric photonic effects, for which ordering on the length scale of the optical wavelength is not necessarily required. In this case, they could provide unusual photonic properties distinct from a typical photonic band gap material. We are completing calculations of the interaction of light with nanoscale metal meshes to better understand the origin of the angle-dependent reflectivity in these structures.