Subject categories: s10, s16 [Materials chemistry and Polymer chemistry]
MOLECULARLY IMPRINTED POLYMERS

Smart hydrogel crystal gardens

Growing good-quality single crystals of proteins for high resolution X-ray diffraction relies on the use of a diverse range of materials as nucleating agents. Smart hydrogels, in the form of molecularly imprinted polymers, may provide a general solution.

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Proteomics and genomic studies are set to provide a much deeper understanding of the structure and function of living organisms at the molecular level by gaining insight into the role of newly discovered proteins in the cell. As more and more proteins are identified, it becomes a pressing matter to determine their 3-dimensional structures — particularly through the use of high-resolution X-ray diffraction studies. Proteins are, however, notoriously difficult to crystallise, especially in high enough quality to obtain good X-ray diffraction patterns.

Crystallographers know that the best method to produce good crystals is to grow them under metastable conditions — conditions under which nucleation will not spontaneously occur. The trick, therefore, is to find a substance that acts as a nucleation centre, kicking-off crystal growth which can then proceed at a controlled rate. The ideal candidate would be a small crystal of the protein in question, but for most proteins this represents the classic chicken-and-egg situation! Instead, a variety of materials — including objects such as hair, minerals, charged surfaces, thin films and porous solids — are used in the hope that they will provide the vital nucleation step. Now, writing in the *Proceedings of the National Academy of Sciences USA*, Naomi Chayen and colleagues have shown[1] that help may be at hand in the shape of molecularly imprinted polymers (MIPs).

MIPs are cross-linked materials formed in the presence of a molecular template. Interactions between the template and the functional groups of the monomers present in the polymerization mixture ensure that affinity sites — complementary in size and functionality to the templating agent — are formed (Fig. 1a) in the imprinted material and these remain after

the template molecules are removed[2]. As templates, proteins represent something of a special case due to their size, complexity and incompatibility with organic solvents and many different approaches to their imprinting have been tried[3], including the use of hydrogels[4]. These materials are significantly swollen with water and are less-heavily cross-linked than typical MIPs that are generally prepared with small molecule templates. Chayen and co-workers prepared hydrogel MIPs imprinted with seven proteins: lysozyme, trypsin, catalase, haemoglobin, intracellular xylanase IXT6-R217W, alpha crustacyanin and human migration inhibitory factor (MIF). Control materials (non-imprinted polymers — NIPS) were prepared in the same way, but in the absence of protein.

MIPs, NIPs and controls (no polymer) were then tested for their ability to induce crystallisation of a set of proteins. With the exception of catalase (which is know to be a special case) crystals of each protein formed in the presence of their respective MIP, while the NIP and control experiments remained clear. Catalase crystallises by a different mechanism to the other proteins studied; first precipitating, with crystals forming out of the precipitate. Catalase MIPs inhibited catalase crystallisation, while promoting crystallisation of other proteins. Crystals formed more rapidly and with better quality on their respective MIPs than in experiments without them. Furthermore, many of the MIPs were able to aid in the crystallisation of other proteins that had not been used as templates. This finding led to the application of MIPs to a series of screening experiments where their ability to nucleate crystallisation of four different proteins was compared to other materials such as horse hair, zeolites, human hair and bioglass powder (bioglass is a low silica biocompatible glass).
Of the protein targets used in the index screen, three were particularly challenging targets: both alpha crustacyanin and intracellular xylanase IXT6-R217W had not previously produced crystals of suitable quality for X-ray analysis and the third, MIF, would benefit from crystals able to produce higher-resolution structures. Trypsin, which crystallises relatively easily, was included as a comparison. The results showed that in 8-10% of the screening trials MIPs were effective at producing hits, whereas the non-specific nucleants had not produced any hits (except in the case of trypsin) after 4 weeks. These hits would have been missed if MIPs had not been included in the screen.

The successful formation of protein crystals induced by complementary MIPs suggests that there is a somewhat rational link between the templating and subsequent crystallization. To examine the interaction between the templating protein and the MIPs further, Chayen and

colleagues used an atomic force microscope (AFM) to probe one of the systems at the molecular level. By modifying an AFM tip with haemoglobin, it was shown that there are specific surface sites on the corresponding MIP that bind strongly to this target protein. The technique, previously demonstrated[5] by El Kirkat and co-workers, measures the force necessary to retract the tip of the AFM after allowing the attached molecule to interact with the gel surface. Pulling forces of 13.51 and 18.90 nN were measured on a control polylysine surface and the NIP, respectively, while a significantly higher force of 23.08 nN was required to pull the tip from the complementary MIP surface.

Chayen and co-workers speculate (Fig. 1b) that the MIPs are so good at nucleating protein crystallisation because they aid in the separation of a protein-rich liquid phase under conditions that are far from those where phase separation normally occurs in the absence of MIPs. At the conditions (protein concentration and temperature) used in the screen this demixing, in the case of lysozyme, would normally require a high NaCl content (at least 7%), whereas in the presence of MIP it occurs at only 2.8%. This phase could be seen to form in some experiments and its presence was associated with the subsequent growth of crystals. The ability of some of the MIPs to crystallise non-template proteins was postulated to be related to size, because when the target protein was much larger that the template used for MIP production, no hits were seen.

The results reported in this study represent a new niche for MIPs which has promise to significantly accelerate the discovery of new protein crystal structures. The real value of the method lies in the ability (at least in these hands of these researchers) to make imprinted materials that are specific but not overly selective; specific in the sense that MIPs show the ability to nucleate, while non-imprinted gels (chemically identical except lacking the protein-shaped cavities) do not; non-selective in that other proteins, apparently of similar size to the template can be crystallised. This combination of features is why the smart hydrogels hold the promise of being a general solution to the problem of protein crystallisation. While the application is undoubtedly new, the use of MIPs to nucleate crystallisation is not. The first attempts used inorganic crystals as templates[6] and subsequent reports have shown the formation of crystals of small organic species on MIPs[7] and even crystals of another protein — lysozyme — have been observed to grow on MIPs imprinted with that template[8]. Nevertheless, this systematic report is promising and should stimulate more work in this blossoming field.

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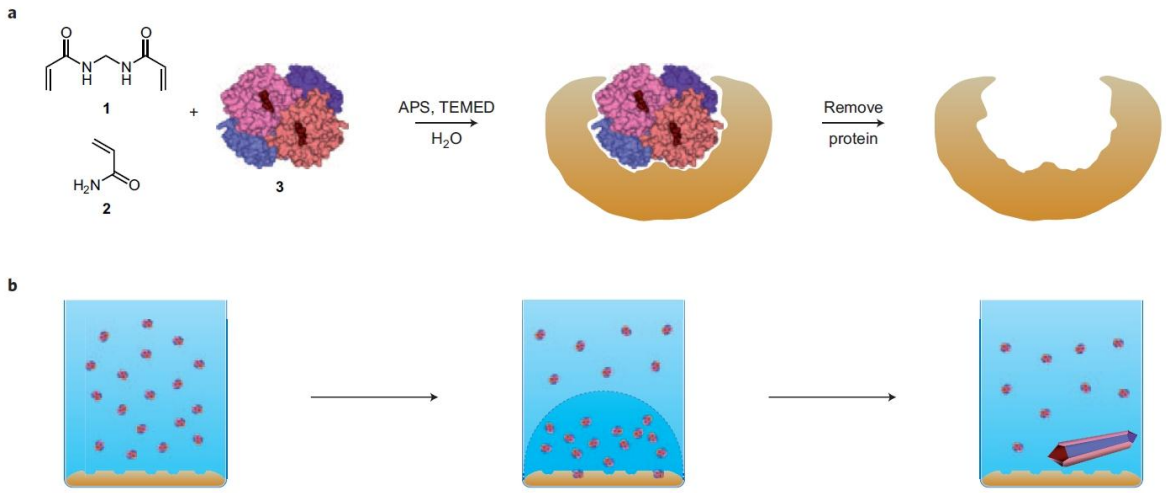

Figure caption

Molecularly imprinted polymers for protein crystallization. a, An imprinted hydrogel is formed when acylamide is polymerised with a cross linker (methylene-bis-acylamide) in the presence of a protein template. Polymerisation in water with ammonium persulphate (APS) and tetramethylethylenediamine (TEMED) gives rise to the hydrogel with embedded protein molecules. Removal of the template protein results in the formation of the imprints, cavities complementary in size and shape to the protein templates. **b,** Stages of the protein crystallisation process on a MIP. First of all, a piece of hydrogel with many protein cavities is brought into contact with a solution containing the target protein. A drop of protein-rich liquid then phase separates from the bulk solution at the surface of the hydrogel and following nucleation at the surface of the gel, a crystal begins to grow.