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Short Communication

Solid-State Structures and Liquid Crystals as Ordering Matrices for Proteins

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The prefix nano- has become the mot juste when it comes to describe efforts to design and/or apply atomic/molecular scale devices, i.e., nanoscale objects.[1] Thus we have e.g. nanochemistry, nanophysics, and the application of the latter: nanotechnology. In general, the objective is to circumvent Avogadrian statistics, either in terms of reducing the event density of chemical interactions, or to ensure that quantum effects are explicitly expressed.

The existence of zeolites,[2] archetypal solid-state nanostructures, has challenged chemists to find ways to synthesize similar, larger-pore materials extending beyond the ca. 7.5 Å aperture limit. Until recently, the efforts have been met with limited success, resulting in an approximate doubling of the available aperture sizes.[3–5] The eventual breakthrough,[6–9] the outcome of a successful mergence of solid-state chemistry and surface chemistry, has resulted in a range of solids, designated MCM-41, with pore sizes of ca. 15–100 Å. Presently the properties of the large-pore materials are under close scrutiny.

It is easy to envisage a large number of chemical applications of these porous solids in many different fields of chemistry. However, it is the aim of this paper to highlight a possible *nanotechnological* application where the porous solids are used as ordering matrices for proteins.

X-Ray crystallography is the single most useful technique to elucidate the secondary and tertiary structures of proteins, given that suitable protein crystals are at hand. Unfortunately, the crystallization of proteins is far from straightforward, being rather unpredictable and sluggish. Hence, in attempts to by-pass the difficulties, a number of crystallization methods, some of which include automated sequences involving crystallization robots, have been designed.[10] Nonetheless, the difficulty of preparing acceptable crystals still remains one of the major drawbacks of protein crystallography.

Confining atoms/molecules to rigid crystal lattices renders them suitable for analysis by diffraction techniques. Obviously, any confinement imposing order to a set of, e.g., molecules can act as a diffraction vehicle, generating a diffraction pattern pertinent to the order imposed. Most of the MCM-41 materials have the appearance of a hexagonal array of tubes (Fig. 1), where the walls of the tubes seem to be amorphous (at present the precise atomic order of the walls is not clearly understood, at any rate the walls do not contribute to the diffraction density). Provided that the dimensions of the tubes (*vide supra*) and the particular protein, see Table 1, are appropriately chosen it should be possible to produce 'inclusion compounds' of the kind depicted in Fig. 2, simply by contacting a protein solution with the solid silicate. It is evident from the dimensions presented in Table 1 that protein sizes in general and silicate apertures are remarkably well matched. The pseudo-crystalline arrangement of the protein molecules would result from a trade-off between protein–silicate wall interactions and protein–protein interactions, where the highly ordered tubes would be expected to provide the sequencing influence. Ideally, the protein arrangement in each tube would be exactly replicated in the other tubes, Fig. 2(a). This situation would arise if (i) protein–silicate interactions were uniform along the tubes, (ii) protein–protein interactions were preferentially directed, and, (iii) the presence of a protein molecule in one tube could be communicated across the walls to juxtaposed tubes. The first two conditions seem reasonable enough whereas the plausibility of the third is difficult to estimate. A tentatively more realistic expectation would be an arrangement as in Fig. 2(b) where the inter-protein organization is identical

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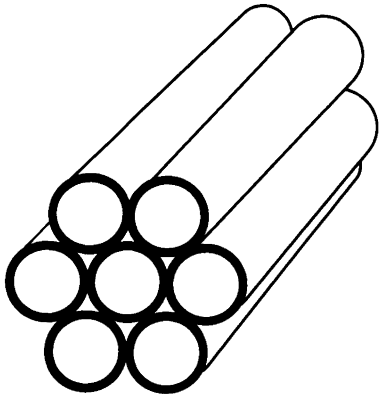

Fig. 1. MCM-41.

in each tube but the entire protein arrays are displaced with respect to the adjacent tubes. Fig. 2(b) illustrates perhaps the simplest distortion from the perfect idealized order of Fig. 2(a): a simple non-concerted sliding of the protein columns along the *c*-axis.

Apart from the possible complications regarding order vs. disorder, of relevance to the diffraction *per se*, some more chemically oriented limitations related to the unidimensional nature of the MCM-41 materials should be taken into account. It is well known that unidimensional systems in general suffer from restricted diffusion and are prone, e.g., to clogging. This phenomenon was, in part, found when silicate tubes with ca. 36 Å diameter were in contact with a 0.3 mg ml^{-1}, 0.9% saline solution of basic trypsin inhibitor (bovine pancreas). Assuming that the degree of aggregation is negligible and allowing for the hydration shell (0.86 g water per gram of protein[11]) the protein should fit nicely, see Table 1. The protein uptake, as monitored by means of absorbance measurements at 280 nm, was indeed very slow; after several days the decrease in protein concentration was approximately 10%. Hence it could be expected that a method intended to maximize scattering intensity and order, i.e., long contact time, high protein concentration, and, elevated temperature at the instance of inclusion, would presumably damage the protein.

Table 1. Dimensions of selected proteins, adapted from Ref. 11.

Protein	Dimensions/10 nm
Basic trypsin inhibitor	29 × 19 × 19
Cytochrome C	25 × 25 × 37
Ribonuclease-A	38 × 28 × 22
Lysozyme	45 × 30 × 30
Myoglobin	44 × 44 × 25
Carbonic anhydrase B	47 × 41 × 41
Superoxide dismutase	72 × 40 × 38
Hemoglobin, oxy	70 × 55 × 55
Carboxypeptidase	50 × 42 × 38
Alcohol dehydrogenase	45 × 55 × 110
Adenylate kinase	40 × 40 × 30
Trypsin	50 × 40 × 40
Chymotrypsinogen A	50 × 40 × 40
Lactate dehydrogenase	74 × 74 × 84
Serum albumin	140 × 40 × 40

At least partly to circumvent these problems one could instead use a bicontinuous cubic structure where diffusional limitations are comparatively attenuated. Such materials could be made available by utilization of the same synthetic procedure as that used to produce the tubular silicates.[6] Currently only one cubic structure is accessible, Fig. 3(b), but drawing on the known characteristics of liquid–crystal structures,[12,13] related structures such as 3(a), (c) should be attainable. As in the case of tubular silicates, it is to be expected that the aperture size of the cubic structures would be tunable in order to accommodate proteins of different sizes. Also, in terms of pseudo-crystalline order, in the cubic cases the positioning of individual protein molecules should be less ambiguous, Fig. 3(a)–(c). Recalling the three criteria considered essential for the formation of a perfectly ordered set of protein molecules, it is reasonable to assume that in the cubic case they can all be met; the channel systems of the cubic solids are continuous, sustaining uninterrupted protein communication.

A number of other matrices may likewise be utilized to force the protein molecules into a crystalline array. In particular, the bicontinuous cubic phases consisting of surfactant alone (e.g., the MCM-41 template) may be sufficiently rigid to order the protein molecules into a lattice, the symmetry and topology of which reflect those of the surfactant assembly. The dimensions, symmetry, and topology of these cubic phases can be finely tuned by varying the water content and the surfactant molecular architecture to accommodate proteins of various dimensions.

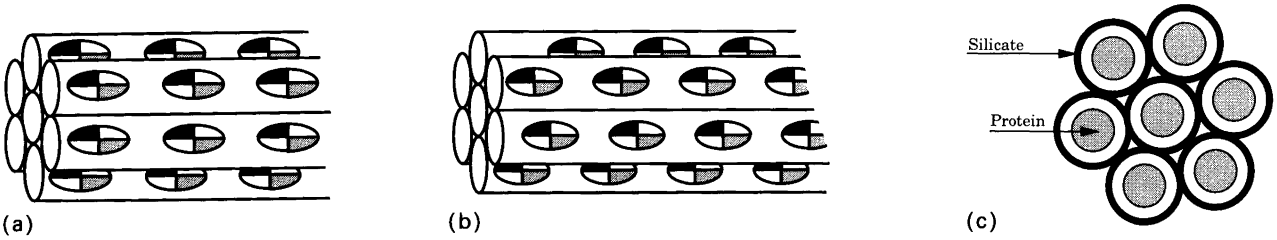

Fig. 2. Schematic representation of protein molecules inside tubular silicates: (a) idealized optimum packing; (b) sliding disorder; (c) view parallel to crystallographic *c*-axis.

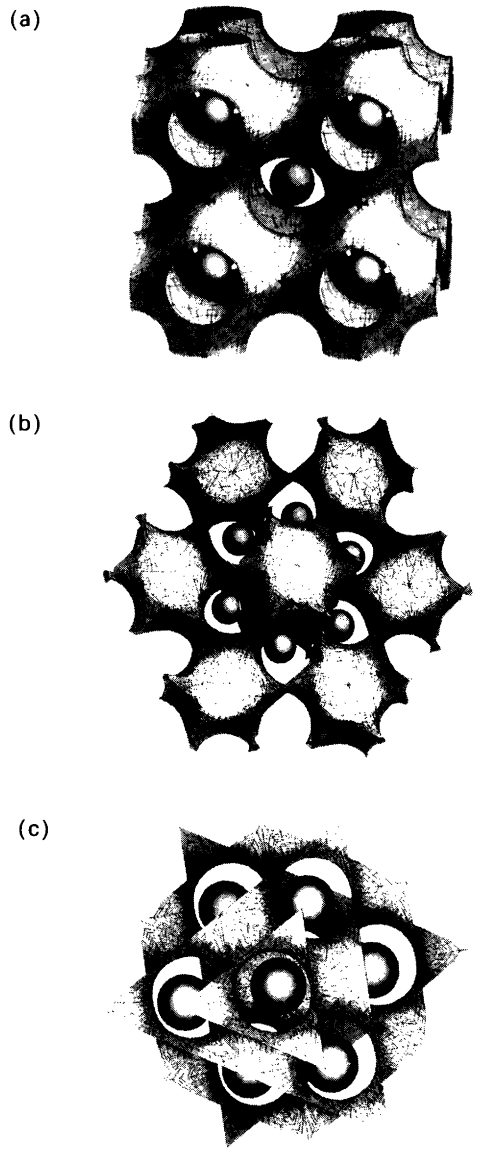

Fig. 3. Cubic structures found in liquid crystal systems. Spheres indicate tentative protein positions: (a) P-surface, space group *Im*3*m*; (b) gyroid, space group *Ia*3*d*; (c) D-surface, space group *Pn*3*m*.

In this respect it is worth noting that detergents (i.e., surfactants) are routinely used to crystallize membrane proteins. The action of these additives is at present poorly understood. In our opinion, they serve to promote crystallization via the intrinsic self-assembly properties of the protein–surfactant mixture, which results in a crystalline assembly. In this respect, it is not surprising that such additives are required for proteins that exist as monomers *in vivo*. The crystallization process requires a degree of self-association which is often not possible under physiological conditions (with the notable exception of structural proteins). In order to crystallize such proteins, the addition of surfactants is essential to constrain the molecules into a lattice, just as can be achieved by the range of micro- or meso-porous matrices proposed in this article.

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