

## Water-protein interactions

In a recent paper, Madhusudhan and Vijayan<sup>1</sup> reported that it was possible to delineate the flexible and rigid regions of the lysozyme molecule as a function of hydration. This rather interesting study, consistent with earlier reports of a similar kind<sup>2-5</sup>, brings to fore the need of some generalizations to explain the underlying mechanisms. The need becomes more acute since we (Sitaramam and Mathai<sup>6</sup>) have reported recently a similar situation with membrane proteins on the basis of altogether different kinds of experiments. The common problem is the variable contact between the solvent and the proteins (water in the case of lysozyme and the lipid in the case of membrane proteins) and structural perturbations associated with the proteins.

The experimental situations are apparently different. In the case of lysozyme the water of hydration of the crystal in a capillary was controlled by altering the salt content of a salt solution, which was only in 'vapour-phase equilibrium' with the crystal and not in direct contact, such that hydration alone was changed. In the case of membrane proteins, we could show that the lipid domain 'moves' on (i.e. interpenetrates into) the polypeptide chains, exposing and burying charged groups, which was detected through binding of the fluorescent probe 8-anilino-naphthalene-1-sulfonic acid (ANS). In the case of lipid, the motive force was definitely identified as an exergonic reaction such as respiration, in that the variable lipid/protein contact was observed only during respiration and was abolished when respiration ceased in submitochondrial particles<sup>6</sup>.

These two experimental situations may be quite comparable and not as diverse as they may appear to be. Since conformation of a polymer pertains to a state of equilibrium, what is the motive force for structural perturbations in the lysozyme molecule? Since water of hydration alone is controlled experimentally, a simple way to look at the problem is to assume that the dielectric profile intrinsic to the molecule, which exhibits spatial heterogeneity, would couple hydration to structural perturbations. Since water is associated largely with the charged groups of the protein, which are mostly on the surface, a

solution for the relevant molecular dynamics would relate charge density and the geometry (e.g. radius) *vis-a-vis* the dielectric.

If we consider the simplest case of the electrostatic energy of a homogeneous charged surface of a sphere,  $E = Q^2/2Dr$ , where  $Q$  are the total charges on the surface,  $D$  the dielectric, and  $r$  the radius of the sphere, the dielectric and the charge density would contribute to defining the free energy of the system and hence the configuration (geometry). What was important to recognize in our membrane studies was the fact that the effective charge density (of the exposed/unscreened charges) needs to be defined not only in terms of the ionic strength but also in terms of the local pH milieu. Since water ionizes very little, small changes in pH are energetically equivalent to relatively large shifts in ionic strength. Thus ionic strength as well as local pH become important extrinsic variables, which influence the intrinsic variables, viz. the dielectric profile and the charged groups, to define the energy profile, i.e. the configuration of the proteins and fluctuations thereof. Variation in local pH at the membrane surface, particularly in relation to migration of surface protons, has attracted considerable interest in recent years from an energetics point of view.

How justified are we in extrapolating water-protein interactions to lipid-protein interactions on the pretext that these represent primarily solvent-polymer interactions? The homology, if any, is not restricted to this class of observations alone. Catalysis by proteins is generally considered to be associated with structural fluctuations to account for enthalpy-entropy compensation as expected of processes at equilibrium<sup>7</sup>. However, determined efforts have yielded < 5% (notional) changes in volume/activity as an effect of hydrostatic pressure on globular proteins<sup>8</sup>. On the other hand, we could demonstrate that membrane proteins exhibit 200-300% changes in activity with (osmotic) stretch/compression of the bilayer, instantiating the appropriateness of membrane proteins for such structure-activity relationships<sup>9</sup>. Solvent-polymer interactions will be of major importance in understanding the molecular dynamics associated with catalysis<sup>10</sup>. The full impact of the crystallographic detail will also become more evident if these

studies increasingly focus on the mechanistic basis of structural fluctuations.

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## Fullerenes from flames

Research on fullerenes has become the rage of the day after the discovery of a method of preparing them in macroscopic quantities by the contact-arc vaporization of graphite. More recently, Howard *et al.* (*Nature*, 1991, **352**, 139) have found C<sub>60</sub> and C<sub>70</sub> in varying proportions in laminar flames of benzene and oxygen. These authors stabilized the flame in a partial helium atmosphere employing a specially designed low-pressure chamber and found the fullerenes preferentially in non-sooty flames. We have been examining the nature of condensible compounds and soot in benzene-air flames for some time and have found fullerenes C<sub>60</sub> and C<sub>70</sub> in sooty flames from a simple laboratory spirit lamp using benzene as the fuel. We consider this to be of some general interest.

The technique employed by us involved quenching part of the sooting end

of the flame by argon coming through a glass nozzle (1 mm dia) at a stagnation pressure of 1.5 atm and collecting the soot on a cooled copper surface positioned opposite the nozzle. We could collect ~5 g of the soot after 3 h of operation, expending 250 ml of benzene. The soot was washed with ether, and the taluene extract of the residue dried and analysed by mass spectrometry. The mass spectrum showed the 720 and the 840 mass peaks (as well as the  $m/2$  peaks). The proportion of  $C_{60}$  was higher and the total yield of fullerenes was ~0.1% with respect to the soot. The yield can be improved by quenching a larger area of the flame. The observation of fullerenes in such simple benzene flames is not only of significance in understanding the origin of fullerenes as well as the formation of soot, but also suggests the possible occurrence of these species in the environment.

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## Non-reflective surface for insect eye

In compound eyes of insects, the function of the 'dioptric apparatus', which is a modified cuticle, seems to be extensive and varied. The eye cuticle plays an essential role as an interference filter, in reflecting specific wavelengths from the trachea behind the receptive cells, and also in reducing reflection at the eye surface<sup>1-3</sup>. Although the lens is a refracting structure, small reflections are produced in it in the region where there is a sharp transition in refractive index. It has been calculated from the Fresnel formula that the intensity of reflection from the ocular refractive surface is 4% of the incident beam. Owing to this reflection from the refractive surface image quality is degraded, because of glares and ghosts, and intensity of the image is also lost.

Similar reflections are produced in the surfaces of optical lens, camera and microscope. To reduce this effect, good-quality lenses are coated with thin layers designed to reduce the reflection<sup>4</sup>.

It is interesting that corneal cuticles of some insect species, e.g. moths, have some mechanisms to make the surface non-reflective. The reduced reflection observed in the moth cornea was attributed to the presence of an array of conical cuticular protuberances, termed corneal nipple<sup>2</sup>. These protuberances, a characteristic feature of the nocturnal moth, were also detected in some diurnal lepidopterans that contain reflecting tapeta in their eyes. Corneal nipples are classified according to their height as Group I (50 nm or less), Group II (between 50 nm and 200 nm) and Group III (200 nm or greater) protuberances. Although the lower-height Group I protuberances have been detected in many insect species of different orders, reports of occurrence of Group II and Group III protuberances in corneal cuticle of insects other than moths are scanty<sup>5,6</sup>. Here I report the occurrence of group II protuberances in the corneal surface of a diurnal fly, *Lonchae* sp.

The eyes were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer for 4 h at 4°C, post-fixed in osmium tetroxide, and dehydrated through increasing concentration of acetone. Dehydrated samples were critical-point dried using acetone as intermediate fluid and CO<sub>2</sub> as transitional fluid. The dry samples were secured horizontally to brass stubs by electroconductive paint, and coated with gold in a fine-coat ion sputter. Observations were made with a JEOL JSM 35 CF scanning electron microscope operated at 15 kV. Tilt control was fixed at 0° for setting the specimen stage in a horizontal position.

The corneal surface of *Lonchaea* (Figure) is covered with nipples with a height of 160 nm and a periodicity (centre-to-centre distance) of about 200 nm. However some variations were observed in height and periodicity of the nipple in certain parts of the facet.



The corneal nipple, which covers the cornea of refractive index  $n_3 = 1.5$ , can be treated as a thin film  $n_2$  of thickness  $d$ . The refractive index of the nipple increases smoothly from  $n_1$  of air at the tip to that of cornea at the base. It is known that if  $n_2$  is a gradual transition between  $n_1$  and  $n_3$  with a thickness  $d = \lambda_0/2$ , a broad-band anti-reflection effect is achieved<sup>4</sup>. Since corneal nipples with a height of more than 50 nm fulfil the criteria for thin-film anti-reflection coatings, it is certain that the protuberances detected in the present case also perform a similar function.

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## Solar plasma physics

Though belated, I wish to draw attention to the discussion on solar plasma physics at the International Astronomical Union symposium on 'Basic plasma processes on the Sun' held during 1-5 December 1989 in Bangalore. The symposium was hosted by the Indian Institute of Astrophysics. Our knowledge of plasma physics comes mainly from laboratory studies, though, more recently, space missions to solar-system bodies and extremely high-quality observations of the Sun have triggered tremendous theoretical activity at a rather advanced level. The study of plasma processes in and on the Sun acquires special significance in view of the fact that most plasma processes like magnetic recon-