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Introduction: the biology of the water molecule

P. Nicholls

Department of Biological Sciences, University of Essex, Central Campus, Wivenhoe Park, Colchester, Essex CO4 3SQ, (United Kingdom), Fax + 44 1206 872592

> "And where the ship's huge shadow lay, the charméd water burnt alway, a still and awful red"—Coleridge, 1798, the Ancient Mariner. "And fish for fancies as they pass, within the watery glass"—Blake, 1783, song from Poetical Sketches. "Sirrah, you giant, what says the doctor to my water?"—Shakespeare, 1598, Henry IV Pt. 2.

Water as threat, water as promise and water as diagnostic medium – the substance that forms an interface between science, medicine and literature also lies between the statistical world of physical chemistry, in which it is an essentially continuous medium, and the atomic world of crystal structure and molecular mechanism, where each molecule has its own name and place. What is water? How should a molecule of water be represented? How do such molecules interact with other water molecules and with biological macromolecules?

According to Cavendish [1], 'we must suppose that water consists of inflammable air united to dephlogisticated air.' In an alternative chemical language, Cavendish's contemporary Lavoisier [2, 3] remarks (through his translator) that 'we have ascertained with as much certainty as possible in physical or chemical subjects, that water is not a simple elementary substance but is composed of two elements, oxygen and hydrogen'. Figure 1 shows a Cavendish-Lavoisier water molecule (pace John Dalton) 200 years later, in space-filling computer graphics format. An atom of oxygen is bound covalently to two atoms of hydrogen. Each H–O bond length is a little under 1.0 Å, and there is a slightly less than tetrahedral angle of 103° between the two O–H bonds. In addition to these covalent bonds the oxygen atom has two lone electron pairs (fig. 1, right hand side). The hydrogen atoms can donate hydrogen bonds, the lone pairs can accept bonds from other hydrogens and a given water molecule can therefore take part in up to four H bonds. Such bonds can be either with donor and acceptor groups on macromolecules (fig. 2A) or with other waters (a typical triplet is shown in fig. 2B). Water molecules close to the surface and in the crevices of a macromolecule can form extensive H bonded networks.

Such H bonds are typically 2.7 Å long (oxygen to oxygen) for a distance of 1.7 Å (oxygen to remote hydrogen). Each water molecule can thus occupy a sphere of radius 1.35 Å and volume $= (4/3)\pi r^3$ or 9.2 $Å^3$. The resulting molar volume (1 mol $= 6.03*10^{23}$ molecules) is 5.55 ml. The molecular mass of water being 18.02 g, the predicted density exceeds 3.0. But according to Kamb even high-pressure ice forms do not usually reach such densities in the bulk phase [4]. Figure 3 shows that for the observed density of 1.00 at 20–30 °C the average (non bonded) oxygen-oxygen distance must be 3.85 Å, with each water molecule

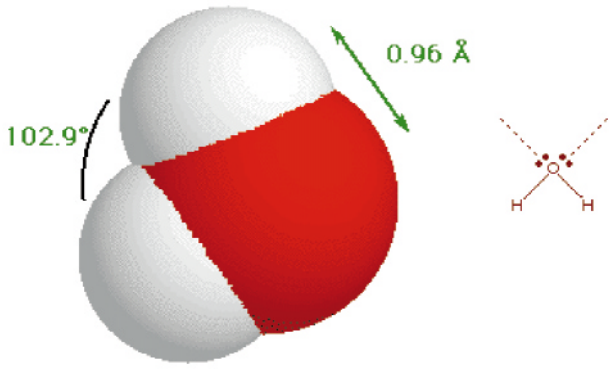

Figure 1. The water molecule (Lavoisier-Cavendish-Dalton). Left-hand side: Space-filling structure (SwissProt pdb viewer + Pov-Ray image). Right-hand side: Bonds and lone pairs (ChemDraw).

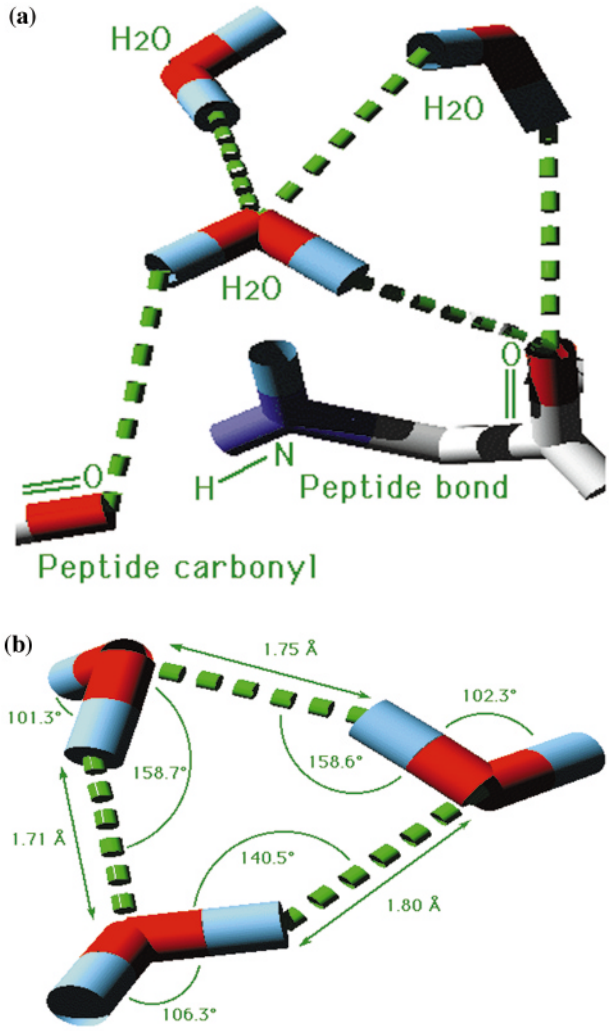

Figure 2. Hydrogen-bonded waters. (*A*) Donor and acceptor H bonds at a protein surface. (*B*) Linkage of water molecules by H bonds: a circular triplet of hydrogen-bonded waters – angles and distances. Data extracted from the pdb file for hydrated *Molpadia* (sea cucumber) myoglobin (1HLB).

occupying a sphere of radius 1.9 Å. How is this structure to be described in detail? Robinson and Cho [5] use a model in which liquid water is a mixture of ordinary ice like clusters (Ih type, 37% total, density = 0.81 @ 30 °C) and dense ice clusters (II-type, 63% total, density = 1.14 @ 30 °C). Figure 3 and the associated calculations show that water at a macromolecular surface or within a macromolecule can easily have a density substantially greater than that of bulk water (cf. Gerstein and Lynden-Bell, [6]), possibly as high as 2.0 and certainly as high as 1.15, the density of ice II.

Not only macromolecules contain trapped and surface waters. Even simple solutes such as monosaccharides and disaccharides bind water in solution. The Earl of Berkeley and Mr. Hartley [7] studied the osmotic pressure exerted by increasing aqueous concentrations of sucrose, as illustrated in figure 4. Haldane senior [8] interpreted the deviations from van't Hoff behaviour – the existence of a substantial second coefficient in the virial equation – in terms of the binding of water to sucrose. These waters of hydration do not behave like bulk water; they are not osmotically active. Figure 4 summarises the results, plotting the data in the form Π/C vs. C, where Π is the osmotic pressure and C is molal concentration. The least deviation from ideal behaviour below 1.0 M is obtained if it is assumed that an average six water molecules are sequestered by each sucrose molecule from the bulk phase. Rather less hydration may occur at high molalities [8]. At a lower temperature (5° C) the presence of six or even seven bound waters has been determined by dielectric relaxation methods (cf. Franks [9]).

The 'bulk' behaviour determined by osmotic pressure measurements can thus provide information about local molecular interactions between solute and water. What Haldane's idea offers us here is a structural interpretation of the abstract 'second virial coefficient'. It bridges the gap between a purely thermodynamic description of watery behaviour and the detailed descriptions of bound waters now reified for us in X-ray crystal structures (cf. Nicholls et al. [10]).

Protein molecules, much larger than sucrose molecules, also interact with water in ways that can be

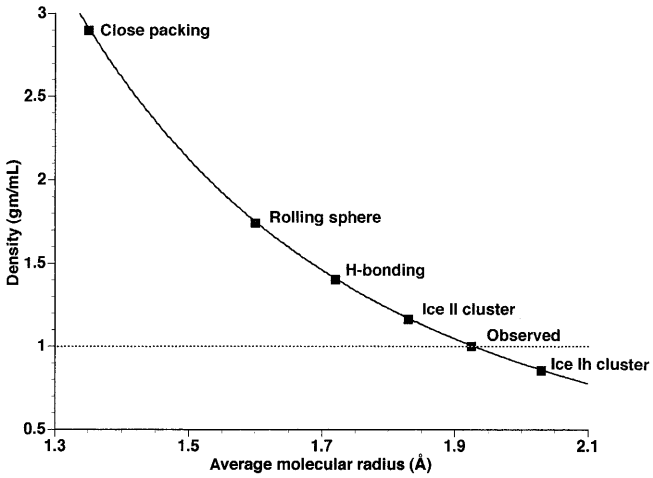

Figure 3. The relationship between molecular size and density. Bulk water density is plotted against average molecular radius. Close packing size: see text. Rolling sphere size: cf. Kuhn et al. [24]. Hydrogen-bonding size:see text. Ice Ih: cf. Robinson and Cho [5] and Kamb [4]. Ice II: cf. Robinson and Cho [5] and Kamb [4].

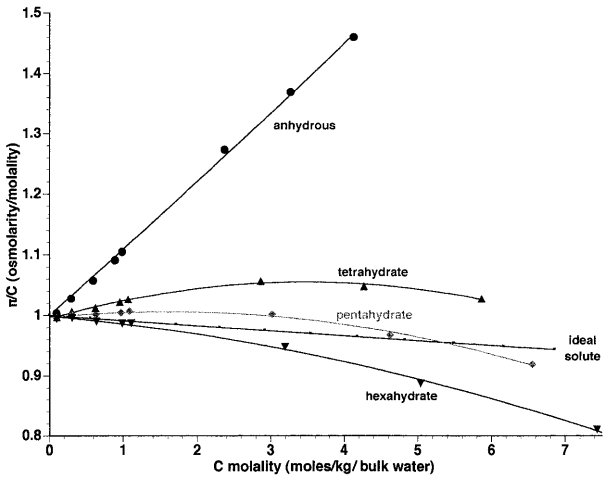

Figure 4. Water binding to sucrose: the osmotic pressure of sucrose solutions. Π/C (osmotic pressure/molality) is plotted against molality. Molality is in moles of solute (moles of sucrose or sucrose-water complex per kilogram of bulk water). Data of Berkeley and Hartley [7] are plotted for the cases of anhydrous sucrose as solute (all water bulk water) and progressively hydrated sucroses (bulk water = total water minus bound water); the ideal solute plot was obtained assuming an ideal second virial coefficient of 0.009 (cf. Nicholls et al. [10]).

be described both globally and specifically. The following papers in this multi-author review show how water molecules bound to macromolecules can be treated either as individuals (cf. Belton's description [11] of the use of nuclear magnetic resonance (NMR) analysis; and Pocker's analysis [12] of carbonic anhydrase activity and structure) or as thermodynamic populations (cf. Rand, Parsegian and Rau's discussion [13] of osmotic stress, and Symons' use [14] of the information provided by infrared (IR) spectroscopy).

Water in protein hydration networks – both shell and interior – is essential to maintaining protein structure as well as moulding the active site of enzymes. Figure 5A illustrates the roles played by structural waters in an exemplary enzyme protein, lysozyme. Such waters stabilise part of what is otherwise an α-helical region in the native protein [15]. Figure 5B shows the corresponding pattern of waters in substrate-free lysozyme at the latter's active site, awaiting replacement by the substrate to be cleaved. Analogous events for carbonic anhydrase are described by Pocker [12].

Finally, water chains provide channels within proteins – enzymes and carriers – that move protons and other ions. We and others have been interested for a number of years in the proton translocating capability of cytochrome *c* oxidase [16, 17]. Despite the availability of several X-ray crystal structures for this enzyme [18, 19] no unambiguous pathway for the pumped protons can be seen in the published structures [20, 21]. Yet it seems most likely that an aqueous pathway exists along which the protons can move [22]. Analogies are needed. One example of a possible proton-transferring sequence of waters is that leading to the heme iron of cytochrome *f*, illustrated two-dimensionally in figure 6A. Yet it is not at all clear why this cytochrome, with an essentially straightforward electrical electron transferring role, should need such a chain. The bacterial photosynthetic reaction center, such as that of *Rhodobacter* (fig. 6B), does not appear to pump protons. But it contains waters that may be involved in transmitting protons to the reduced quinones generated during the photochemical events [23].

Other examples are described below. Let the authors speak for themselves. But we should take care to remember the 400-year-old reply to Falstaff's question: 'He said, sir, the water itself was a good healthy water; but for the party that owed it, he might have more diseases than he knew for.'

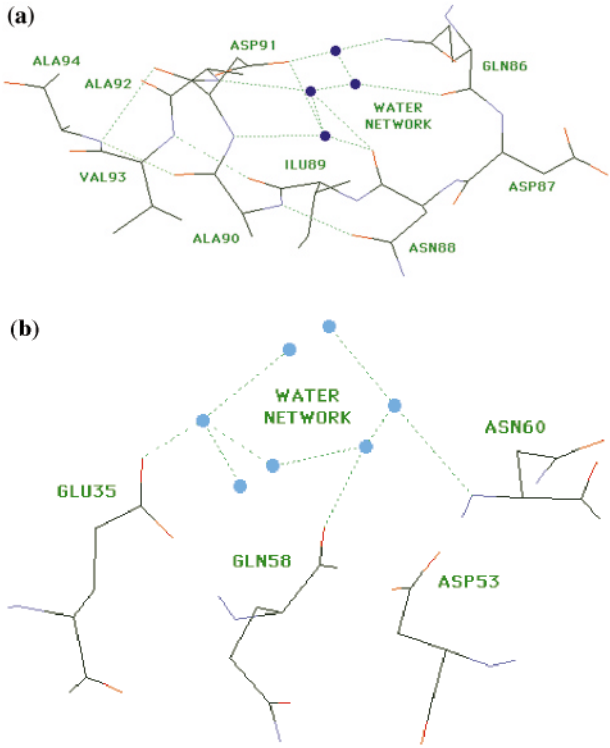

Figure 5. Water binding to a protein: the true catalyst? (*A*) Waters in lysozyme stabilise a piece of irregular α helix. (*B*) Waters in lysozyme stabilise the active site in absence of substrate. Structures from lysozyme pdb file 2HEB (Takano et al. [15]).

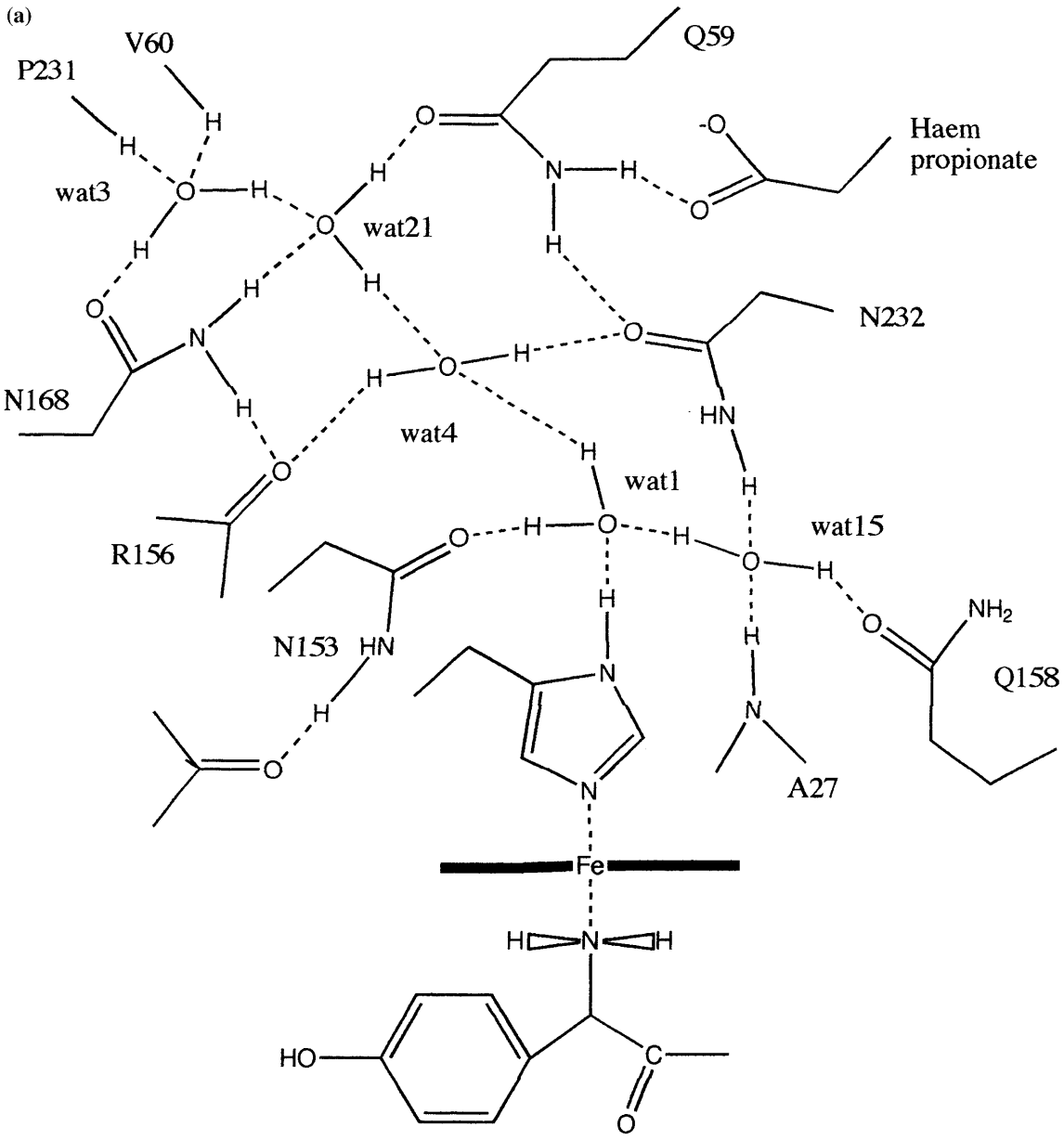

Figure 6. Water binding in proton channels of membrane proteins. (*A*) A chain of five waters in cytochrome *f*. Structure redrawn from protein data bank information and published Kinemage file (Martinez et al. [25]). (*B*) Rhodobacter photosynthetic complex: a chain of waters. Structure redrawn from protein data bank file 1AIJ (Stowell et al. [23]).

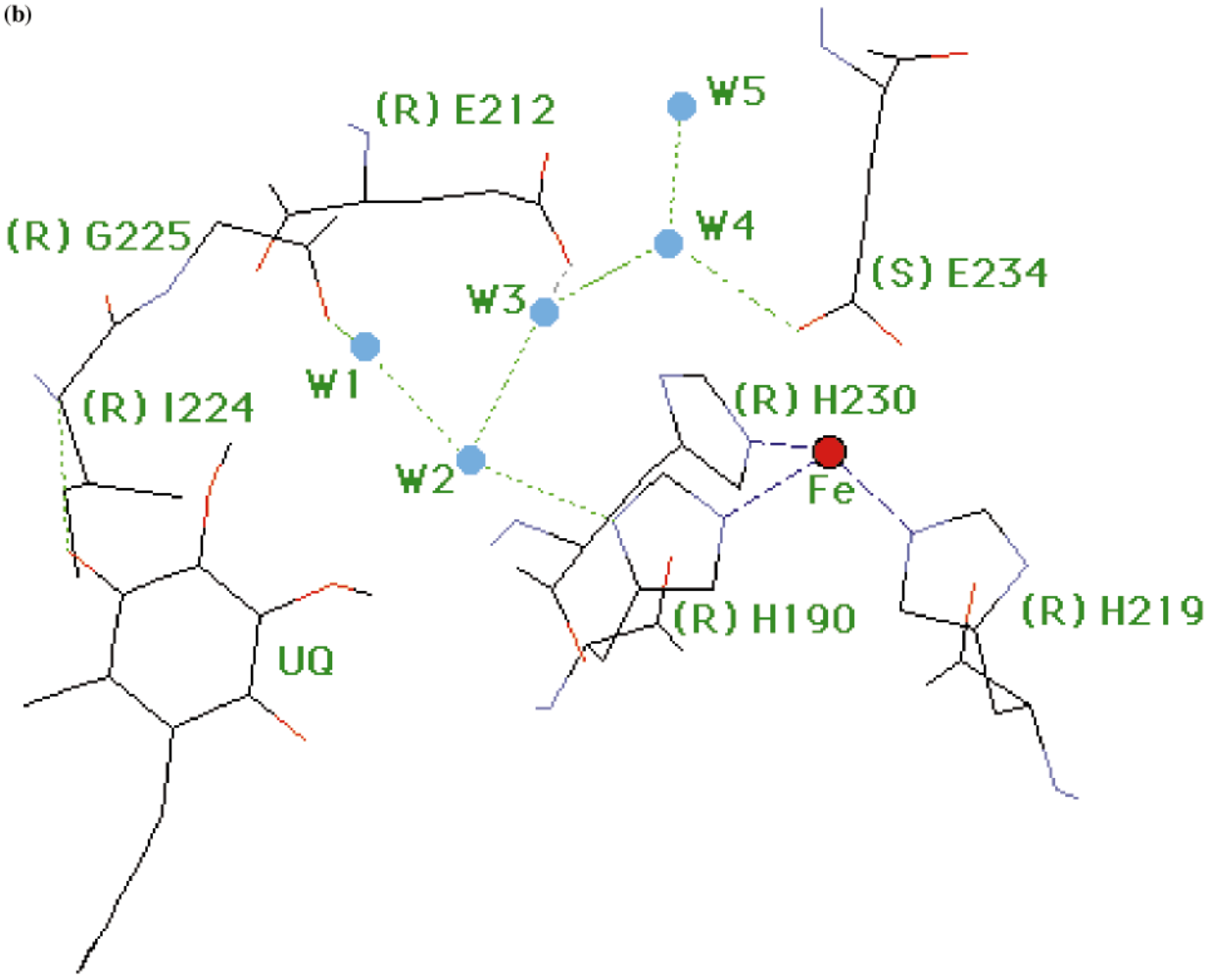

Fig. 6. (see legend, previous page)

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1 Cavendish H. (1784, reprinted 1911) Experiments on Air. Phil. Trans. Roy. Soc. 74: 119–153 (Alembic Club reprint # 30)
2 Lavoisier A. (1789) Traité Élémentaire de Chimie, vol. 1, p.100, Paris, chez Cuchet.
3 Lavoisier A. (1806) Elements of Chemistry, vol. 1 p.117 5th. English edn, trans. Robt. Kerr, Evert Duyckinck and James & Thomas Ronalds, New York
4 Kamb B. (1968) Ice polymorphism and the structure of water. *In*: Structural Chemistry and Molecular Biology, Rich A. and Davidson N. (eds), pp. 507–542. W. H. Freeman, San Francisco
5 Robinson G. W. and Cho C. H. (1999) Role of hydration water in protein unfolding. Biophys. J. **77:** 3311–3318
6 Gerstein M. and Lynden-Bell R. M. (1993) What is the natural boundary of a protein in solution? J. Mol. Biol. **230:** 641–650
7 Berkeley, Earl of, & Hartley, E. G. J. (1916) Further determinations of direct osmotic pressures. Proc. Roy. Soc. A 92, 477–492.
8 Haldane, J. S. (1928) Gases and Liquids: A Contribution to Molecular Physics. Oliver and Boyd, Edinburgh
9 Franks F. (1977) Solvation and conformational effects in aqueous solutions of biopolymer analogues. Phil. Trans. Roy. Soc. Lond. B **278:** 33–57
10 Nicholls P., Sternin Y., Loewen J., Jennings T. and Tattrie B. (1996) Hydration as a control mechanism in membrane proteins: the case of cytochrome c oxidase. Faraday Discuss. **103:** 313–323
11 Belton, P. (2000) Cell Mol. Life Sci., this issue.
12 Pocker, Y. (2000) Cell and Mol. Life Sci., this issue.
13 Rand, R. P., Parsegian, V. A. & Rau, D. C. (2000) Cell and Mol. Life Sci., this issue.
14 Symons, M. C. R. (2000) Cell Mol. Life Sci., this issue.

15 Takano K., Funahashi J., Yamagata Y., Fujii S. and Yutani K. (1997) Contribution of water molecules in the interior of a protein to the conformational stability. J. Mol. Biol. **274:** 132–140
16 Verkhovsky M., Jasaitis A., Verkhovskaya M. L., Morgan J. E. and Wikstrom M. (1999) Proton translocation by cytochrome c oxidase. Nature **400:** 480–483
17 Nicholls P., Cooper C. E. and Wrigglesworth J. M. (1990) Control of proteoliposomal cytochrome c oxidase: the overall reaction. Biochem. Cell Biol. **68:** 1128–1134
18 Iwata S., Ostermeier C., Ludwig B. and Michel H. (1995) Structure at 2.8 Å resolution of cytochrome c oxidase from *Paracoccus denitrificans*. Nature **376:** 660–669
19 Tsukihara T., Aoyama H., Yamashita E., Tomizaki T., Yamaguchi H., Shinzawa-Itoh K. et al. (1996) The whole structure of the 13-subunit oxidized cytochrome c oxidase at 2.8 Å. Science **272:** 1136–1144
20 Kannt A., Lancaster C. R. D. and Michel H. (1998) The coupling of electron transfer and proton translocation: electrostatic calculations on *Paracoccus denitrificans* Cytochrome c Oxidase. Biophys. J. **74:** 708–721
21 Yoshikawa S., Shinzawa-Itoh K., Nakashima R., Yaono R., Yamashita E., Inoue N. et al. (1998) Redox-coupled crystal structural changes in bovine heart cytochrome c oxidase. Science **280:** 1723–1729
22 Kornblatt J. A. and Kornblatt M. J. (1992) Cytochrome c oxidase: the presumptive channel holds at least four water molecules. Biochim. Biophys. Acta **1099:** 182–184
23 Stowell M. H., McPhillips T. M., Rees D. C., Soltis S. M., Abresch E. and Feher G. (1997) Light-induced structural changes in photosynthetic reaction center: implications for mechanism of electron-proton transfer. Science **276:** 812–816
24 Kuhn L. A., Siani M. A., Pique M. E, Fisher C. L., Getzoff E. D. and Tainer J. A. (1992) The interdependence of protein surface topography and bound water molecules as revealed by surface accessibility and fractal density measures. J. Mol. Biol. **228:** 13–22
25 Martinez S. E., Huang D., Ponomarev M., Cramer W A. and Smith J. L. (1996) The heme redox center of chloroplast cytochrome f is linked to a buried five-water chain. Protein Sci. **5:** 1081–1092