## Posters on Folding, Dynamics and Solvation of Macromolecules

### 085

STUDIES ON THE DISSOCIATION OF αβ DIMERS OF HEMOGLOBIN A.
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Adult human hemoglobin tetramers (HbA = $\alpha_2\beta_2$) dissociate into, and exist in equilibrium with, small amounts of αβ dimers. To determine if αβ dimers dissociate into α and β monomers, isolated $^3$H-α chains (~0.1 mg/ml) were incubated with either 0.1, 1, or 10 mg/ml of unlabeled CO-HbA in 0.01 M $PO_4(K^+)$ pH 7.0 at 25°C for several days. Samples were withdrawn at various times, and the free α monomers and HbA tetramers were separated by electrophoresis on cellulose acetate or chromatography on DEAE-cellulose. Radioactivity analysis showed a slow, steady transfer of $^3$H-α chains into HbA (e.g., ~55% of $^3$H-α chain cpm were incorporated into HbA, 10 mg/ml, after 72 h). The initial rate of this transfer increased directly with the calculated concentration of αβ dimer. Additional evidence (e.g., separation of the globin chains on a CM-cellulose column in 8 M urea or Sephadex chromatography in 0.10 M NaCl) showed that the $^3$H-cpm in HbA were in intact α chains that were an integral part of, and not adsorbed to, the tetramer. In a complementary experiment, unlabeled α chains (~6 mg/ml) were incubated with 12 mg/ml of $^3$H-HbA (α/β cpm ratio = 0.28), and 5% of the $^3$H-HbA cpm were transferred into free α chains after 240 h. These results show there was an exchange of α chains between free monomers and those in HbA subunits and were used to calculate a rate constant k for the dissociation of αβ dimers into α and β monomers of ~7 x $10^{-3}$ $h^{-1}$.

### 086

EFFECTIVE INTER-RESIDUE CONTACT ENERGIES FROM PROTEIN CRYSTAL STRUCTURES.
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Contact energies for proteins in solution are estimated from the numbers of residue-residue contacts observed in crystal structures on the basis of the quasi-chemical approximation with an approximate treatment of the effects of chain connectivity. A protein is regarded as a close-packed mixture of unconnected residues and effective solvent molecules whose size is the average size of a residue. A basic assumption is that the average characteristics of residue-residue contacts formed in a large number of protein crystal structures reflect actual differences of interactions among residues, as if contacts among residues and solvent molecules in each protein were in quasi-chemical equilibrium. The number of effective solvent molecules for each protein is chosen to yield the number of residue-residue contacts equal to its expected value for the hypothetical case of hard sphere repulsions. A residue is represented by the center of its side chain atom positions, and contacting residues are defined to be close pairs within a distance of 6.5A; nearest neighbor pairs along a chain are excluded. Coordination numbers, for each type of residue and solvent, are estimated and used to evaluate the numbers of residue-solvent and solvent-solvent contacts. Estimated contact energies have reasonable residue-type dependences; e.g., non-polar-in and polar-out are seen as well as the segregation between these residue groups. There is a linear relationship between the average contact energies for non-polar residues and their hydrophobicities reported by Nozaki and Tanford.

### 087

ON THE ELECTRICAL INTERACTION IN BIOFLUIDS
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The effect of the ions on the forces between charged groups of macromolecules is considered. It is pointed out that the main action of the ions is due to the charge screening. This screening can be included in the Coulomb's law as an effective dielectric permittivity and will depend on the salinity of the medium in such a way that departures from the physiological concentration could disturb the force balance. The screening effectivity can be roughly quantizied by a time and a length. The time being a measured of how fast the rearragement of the ions take place, while the length gives an idea of the size of the ion cloud around the charges. In this work the former is estimated through the Maxwell's relaxation time and the latter using the Mean Spherical Approximation (MSA). The computed screening length for a number of salts allows to order the ions in a series that resembles the well known Hoffmeister series.

### 088

ESR STUDIES ON IRRADIATED SINGLE CRYSTALS OF OXYMYOGLOBIN
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X-Irradiation of single crystals (type A) of oxymyoglobin at 77 K results in electron addition to the $Fe\text{-}O_2$ unit. Besides the nitrosyl, the superoxide derivative that is formed is the only species whose paramagnetism does not stem from the metal ion.

An ESR crystallographic study was pursued with the dioxygen ligand enriched to 30% in $^{17}O$ and with $^{57}Fe$-substituted myoglobin. There are two spectroscopically distinct $FeO_2^-$ species. For the more abundant species, P I, the g-tensor (2.23, 2.13, 1.97), the $^{17}O$ Hf-tensor (-27, 16, 6 G) and the $^{57}Fe$ Hf-tensor (16, 6, 5 G) were determined showing most of the unpaired spin density in the iron $d_{z^2}$ and $d_{yz}$ orbitals with an oxygen $\pi$-orbital population of approximately .3 mainly in the "in plane" orbital. The resulting Fe-ligand geometry was found to be strongly tilted with a bond angle of 118°, the O-O direction pointing towards the distal histidine.

Upon annealing the samples above 180 K two new paramagnetic centers were irreversibly formed, possibly as a result of protonation of the ligand. Both secondary species and the other primary center showed no $^{17}O$ Hf-splitting suggesting a shift of spin density to the iron. Possible interactions between the ligand and the protein moiety are discussed.

### 089

MUCONATE LACTONISING ENZYME AT 6·5 Å RESOLUTION
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We have obtained crystals of Muconate Lactonising Enzyme from Pseudomonas putida which diffract to better than 2·4 Å resolution. The enzyme has a molecular weight of 40 kD, and contains one Manganese atom per monomer. The cell dimensions are: 139·3 by 139·3 by 84·1 Å in the space group I4, giving a $V_m$ of 2·5 $Å^3$ per Dalton, assuming two monomers in the asymmetric unit. The two monomers are related by a non-crystallographic two-fold axis, which is perpendicular to the crystallographic four-fold axis, and is inclined at an angle of 24 degrees to the x-axis.

Using two heavy-atom derivatives, $Sm(Ac)_3$ and $K_2Pt(NO_2)_4$, we have calculated a map at 6·5 Å resolution. Symmetry-averaging and molecular replacement have improved the map; the final figure of merit is above 0·8. This map shows that the enzyme is a tightly-packed octomer with D4 symmetry, with more intra-octomer than inter-octomer contacts. This contradicts earlier solution measurements (1), which indicated that the oligomeric molecular weight was 240 to 255 kD, consistent with the enzyme being a hexamer. We are currently collecting data to high resolution and will use molecular replacement to improve that map.

(1) G. Avigard *et al*, J. Mol. Biol., 89, 651-662 (1974)

### 090

**The impact of hydration patterns observed in oligonucleotide crystals on our view of the stabilisation of different DNA conformations.**

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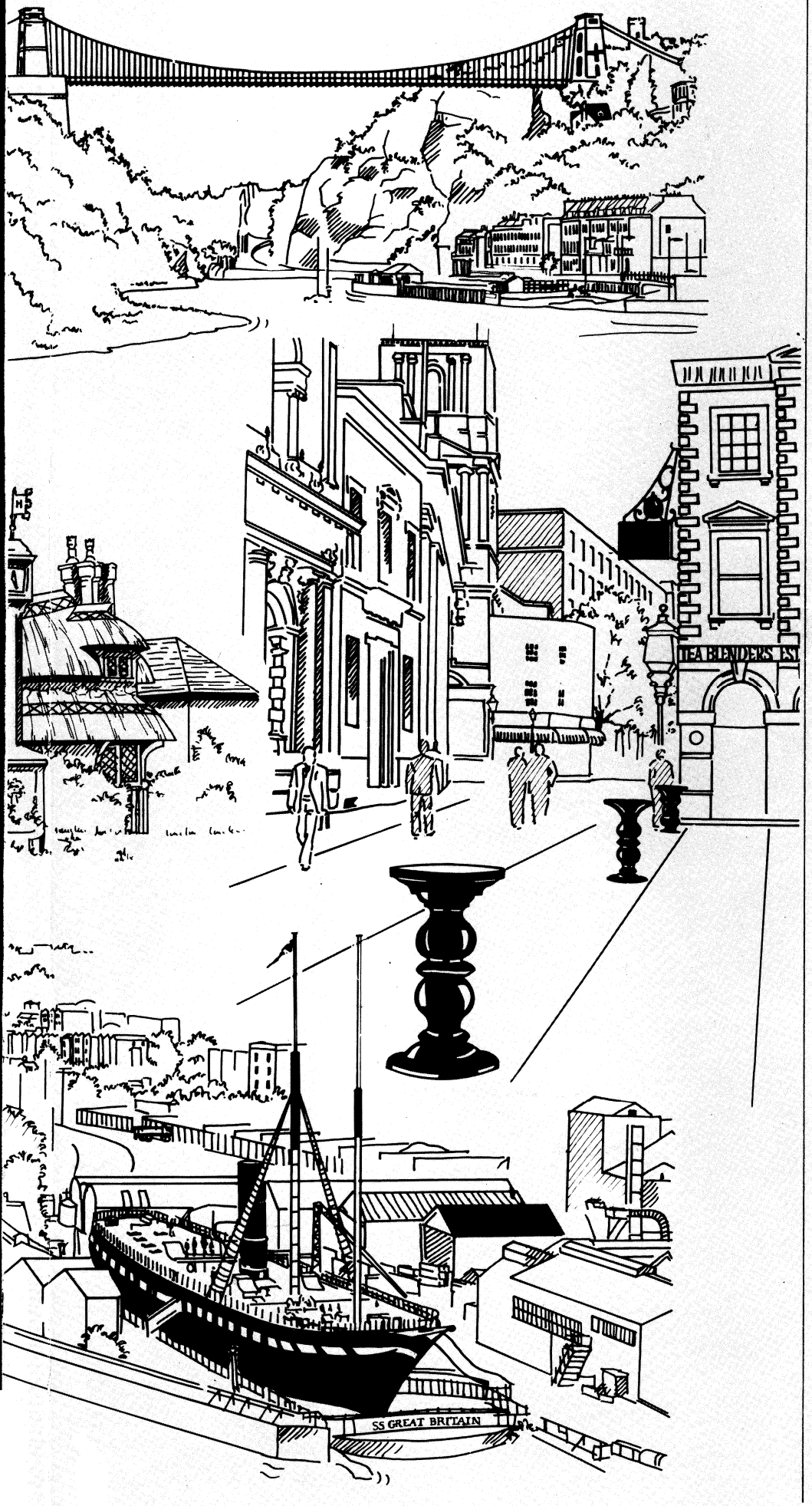
Oligonucleotides generally crystallise with only 40-60% DNA per cell volume, the remainder of the cell being taken up by water and counter-ions. At a resolution of around 2A it is possible to identify, with some confidence, a substantial number of water molecules particularly in the first hydration shell, and thus study the water structure around the DNA double helix.

The hydration pattern observed in two isomorphous A-type octamers with different base sequence [d(GGTATACC) and d(GGGGCCCC)] will be described. The results will be compared with the hydration of the B-dodecamer d(CGCGAATTCGCG) [1] and used to re-examine our views on the role of water in B to A transitions and conformational stabilisation.

[1] H. Drew and R.E. Dickerson. J. Mol. Biol (1981) 151, 535-556

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