Lincoln College Biochemistry Tutorials

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Michaelmas Term, Tutorial 1

Title: Biophysical Methods I – Hydrodynamics (Lincoln)
This week's work will cover the global physical properties of biological molecules, the way that they interact with their environment and how these properties can be used to both isolate and characterise biomolecules. Concentrate on the classical techniques for studying hydrodynamic properties, techniques based on electromagnetic radiation will be dealt with later.

You should cover (make notes on) the following areas:
1. Bulk properties of biomolecules :
What is meant by hydrodynamics ? Have some idea of the size, shape and surface properties (hydrophobicity/hydrophilicity, charge, etc.) of proteins, nucleic acids, carbohydrates, lipids, etc., the amount of variation in these and how they effect the way that biomolecules interact with their surroundings.
2. Interaction between biomolecules and solvent:
Solubilities in different solvents, solvent extraction techniques. Hydration. Partial specific volumes. Relationship of diffusion and viscosity to molecular size and shape. Role of diffusion in reaction kinetics. Methods of measuring diffusion and viscosity.
3. Interaction between biomolecules and solvent under external applied forces :
Centrifugal forces (sedimentation and ultra-centrifugation).
Electric field forces
electrophoresis (paper, gel, SDS, capillary zone, proteomics).
4. Interaction between biomolecules and a matrix (chromatography) :-
Bulk interactions - gel filtration, diffusion in gels; Specific interactions - based on charges (anion/cation exchange columns), [specific ligand interactions (affinity columns) although strictly speaking these are not hydrodynamic properties].
5. The role of hydrodynamics in biological systems :-
Think about the way biomolecules interact with their surroundings (solvent, other molecules, lipids, membranes, etc). Role of diffusion and viscosity in biological systems. Control of diffusion in non-aqueous phases (e.g. membranes).

References
Standard general texts:

V.A. Bloomfield, (1977) Ann. Rev. Phys. Chem., **28**, p.233-259 – "Hydrodynamics in biological chemistry" – *a bit more advanced*.
J.G. de la Torre and V.A. Bloomfield, (1981) Quart. Rev. Biophys., **14**, p.81-139 – "Hydrodynamic properties of complex, rigid, biological macromolecules: theory and application" – *much more complex, rigorous and mathematical approach.*
T.E. Creighton, (1983) "Proteins: structure and molecular principles", pub. Freeman – *p.268-269, comparison of protein dimensions from different techniques*.

M. Dunn, (1998) Chemistry in Britain, **34**, p.54-58 – “Proteome analysis”.
H. Neurath and R.L. Hill, (1975) “The Proteins, Vol. 1”, pub. Academic Press.
D. Rickwood, (1984) “Centrifugation”, pub. IRL press.

Problems

All submitted material to be attached as one bundle from each separate student, to be clearly marked with the title of the tutorial, the date, the name of the student, and to clearly display “FAO Dr. Mark Leake, Clarendon Lab” on the first page. To be handed in to either the receptionist, or placed in the “L” pigeon-hole, of the Clarendon Laboratory, Dept of Physics by *12 noon* the day before the tutorial.

1). Discuss, with examples, the ways in which hydrodynamic studies can be used to obtain information about macromolecular assemblies.

2). What factors determine the resolution of protein chromatographic systems and how is high resolution achieved by HPLC technology? Discuss the advantages and disadvantages of other methods (including non-chromatographic systems) for separating protein mixtures.