Lincoln College Biochemistry Tutorials

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Michaelmas Term, Tutorial 2

Title: Biophysical Methods 2 – Scattering and Microscopy (Lincoln)

This week's work will concentrate on methods of determining shape and size using techniques based on electromagnetic radiation rather than bulk measurement (week 1).
You should cover (make notes on) the following areas:
1. Microscopy:
Experimental factors; types of radiation that can be used (light, electron), experimental details (keep it very brief), difference between standard and scanning microscopy, nature of sample needed (depends on technique used), resolution (scale on which things can be studied), contrast (under-focussing to improve contrast), filtering.
Sample preparation; embedding, staining, shadowing, freeze-fracture, imaging in ice, immunolabelling, artifacts due to sample preparation method.
Specific techniques; light microscopy, fluorescence microscopy, transmission electron microscopy, scanning electron microscopy, scanning tunnelling microscopy, atomic force microscopy.
Image reconstruction to obtain 3D models.
2. Scattering:
Types of radiation that can be scattered (X-rays, UV, IR, neutrons, etc.). What causes scattering, intensity of scattering from different species. Elastic, quasi-elastic and non-elastic scattering. Relationship between scattering, particle size, shape and wavelength (Bragg and Guinier equations).
Fibre and solution scattering. Refractive index and birefringence. Raman spectrscopy. Determination of size, shape, radius of gyration, diffusion, etc. from elastic and quasi-elastic scattering studies. Variable contrast (changing scattering power of solvent, particular for neutrons).
Optical tweezers and their applications.

References

Standard general texts + lecture notes + OLIS + Medline + ….
K. Gast, D. Ziraer, A-M. Ladhoff, J. Schreiber, R. Koelsch, K. Kretschner and J. Lasch,
(1982) Biochim. Biophys. Acta, **686**, p.99-106 – "Auto-oxidation-induced fusion of lipid vesicles" – *quasi-elastic scattering and electron microscopy.*
Microscopy:
M. Adrian, J. Dubochet, J. Lepault, A.W. McDowall, (1984) Nature, **308**, p.32-36 "Cryo-electron microscopy of viruses" – *improvements on standard freezing techniques.*
B. Austen and M. Manca, (2000) Chemistry in Britain, **36**(1), p.28-31 – "Proteins on the brain" – *examples of electron microscopy and atomic force microscopy.*

R.A. Crowther, (1989) Nature, **339**, p.426-427 – "Probing biological structure" – *short News and Views on scanning tunneling microscopy and atomic force microscopy.*
R.A. Crowther and A. Klug, (1975) Ann. Rev. Biochem., **44**, p.161-182 – "Structural analysis of macromolecular assemblies by image reconstruction from electron micrographs".
U. Dammer, O. Popescu, P. Wagner, D. Anselmetti, H.J. Guntherodt and G.N. Misevic (1995) Science, **267**, p.1173-1175 – "Binding strength between cell adhesion proteoglycans measured by atomic force microscopy".
H.W. Fisher and R.C. Williams, (1979) Ann. Rev. Biochem., **48**, p.649-679 – "Electron microscopic visualization of nucleic acids and of their complexes with proteins".
T.E. Fisher, A.F. Oberhauser, M. Carrion-Vazquez, P.E. Marszalek and J.M. Fernandez (1999) TIBS, **24**(10), p.379-384 – "The study of protein mechanics with the atomic force microscope" – mostly about mechanics of folding.
O.H. Giffith and G.B. Birrell, (1985) TIBS, **10**, p.-336-339 – "Photoelectron microscopy".
P.K. Hansma, V.B. Ellings, O. Marti and C.E. Bracker, (1988) Science, **242**, p.209-216 – "Scanning Tunneling Microscopy and Atomic Force Microscopy: Application to Biology and Technology".
Henderson and Unwin, (1975) Nature, **257**, p.28-32 – "Three-dimensional model of purple membrane obtained by electron microscopy" – *the classic paper.*
E.D. Korn, (1966) Science, **153**, p.1491-1498 – "Structure of biological membranes".
C.A. Mannella, M. Marko and K. Buttle, (1997) TIBS, 22, p.37-38 – "Reconsidering mitochondrial structure : new views of an old organelle" – *example of electron microscopic tomography.*
S. Mann, (1987) Chemistry in Britain, **23**, p.137-140 – "Biomineralisation of iron oxides" – *some bioinorganic applications of electron microscopy.*
O.L Miller, (1973) Sci. Am., **228(4)**, p.34-42 – "The visualisation of genes in action".
D. Shotton, (1980) Nature, **283**, p.12-14 – "Quick-freezing - the new frontier in freezefracture" – *new freezing technique.*
D. Shotton and N. White, (1989) TIBS, **14**, p.435-439 – "Confocal scanning microscopy: three-dimensional biological imaging".
U. Skogland and B. Daneholt, (1986) TIBS, **11**, p.499-503 – "Electron microscope
tomography".
G. Stoffler and M. Stoffler-Meilicke, (1984) Ann. Rev. Biophys. Bioeng., **13**, p.303-330 – "Immunoelectron microscopy of ribosomes".
H.K. Wickramasingle, (1989) Sci. Am., **261(4)**, p.74-81 – "Scanning-probe microscopy".
Scattering :-
S.M. Block, (1992) Nature, **360**, p.493-5 – "Making light work with optical tweezers".

H. Durchslag, G. Puchwein, O. Kratky, I. Schuster and K. Kirschner, (1969) FEBS Lett,
4, 75-78 – “X-ray small-angle scattering of yeast glyceraldehyde-3-phosphate dehydrogenase as a function of saturation with nicotinamide-adenine-dinucleotide” – *one of the first papers in the field*.
O. Kratky and I. Pilz, (1978) Quart. Rev. Biochem., **11**, p.39-70 – “A comparison of Xray small-angle scattering results to crystal structure analysis and other physical techniques in the field of biological macromolecules”.
R.C. Macdonald, T.A. Steitz and D.M. Engleman, (1979) Biochemistry, **18**, p.338-342 – “Yeast hexonkinase in solution exhibts a large conformational change upon binding glucose or glucose 6-phosphate”.
A.D. Mehta, M. Rief, J.A. Spudich, D.A. Smith and R.M. Simmons, (1999) Science, **283**, p.1689-95 – “Single-molecule biomechanics with optical methods”.
S.J. Perkins, (1988) Biochem. J., **254**, p.313-327 – “Structural studies of proteins by high-flux X-ray and neutron solution scattering” – *good review*.
O.B. Ptitsyn, M.Y. Pavlov, M.A. Sinev and A.A. Timchenko, (1986) in “Multidomain Proteins; proc. of the UNESCO workshop on structure and function of proteins” ed. L. Patthy and P. Friedrich, p.11-25 – “Study of domain displacements in proteins by diffuse X-ray scattering”.
T.G. Spiro, G. Smulevich and C. Su, (1990) Biochemistry, **29**, p.4497-4508 – “Probing protein structure and dynamics with resonance raman spectroscopy”.
Leake MC, Chandler JH, Wadhams GH, Fan, B, Berry RM & Armitage JA. Stoichiometry and turnover in single, functioning membrane protein complexes. Nature (2006) **443**, 355-8.

Problems

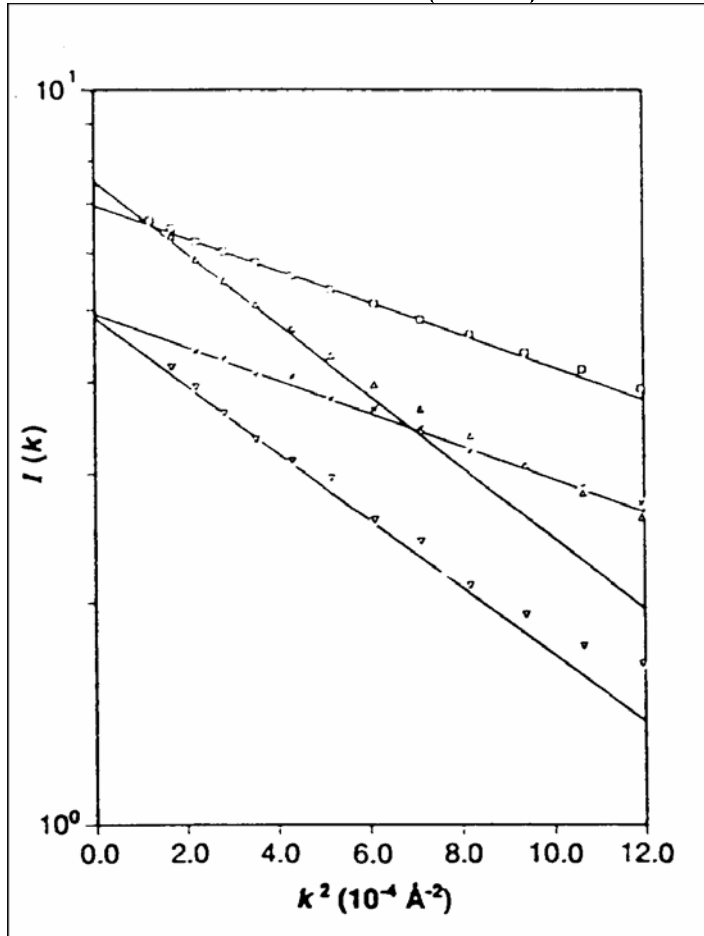
All submitted material to be attached as one bundle from each separate student, to be clearly marked with the title of the tutorial, the date, the name of the student, and to clearly display “FAO Dr. Mark Leake, Clarendon Lab” on the first page. To be handed in to either the receptionist, or placed in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

1). Discuss the contribution that microscopy has made to the study of biological membranes.

2). Briefly describe the principles of
a) atomic force microscopy
b) scanning tunneling microscopy
c) confocal microscopy
d) optical tweezers
Have any of these techniques made a significant contribution to our understanding of biological systems?

3). Plasminogen is a single chain polypeptide of 790 amino-acids located within six domains (five kringles and a serine protease). There is one strong lysine

binding site which can also be occupied by 6-AHA. A series of Guinier plots of low-angle neutron scattering from plasminogen in the presence and absence of 50mM 6-aminohexanoic acid (6-AHA) and in 1H2O or 2H2O are shown below.



Discuss the form of the data and the structural information that can be extracted.

Plasminogen in 2H2O

Plasminogen in 1H2O

Plasminogen + 6-AHA in 2H2O

Plasminogen + 6-AHA in 1H2O

What extra information could be obtained from the high-angle scattering data? In the presence of 6-AHA, the radius of gyration of plasminogen increases from 39 to 56 Å. Phosphoglycerate kinase (PGK) is a two-domain protein that binds ATP. The radius of gyration of PGK decreases from 23.9 to 23.3 Å on binding ATP. Comment on these values.