



these interactions. The basic principle relies on the resolution of protein-bound (single or multiple complexes) and unbound DNA molecules by migration in native conditions through a matrix (agarose or acrylamide) under the influence of an electric potential. Many factors, including mass, charge and shape of the molecules, as well as the migration conditions (composition and concentration of gel matrix, ionic strength, pH, etc) will affect on their resolution and relative migration distances. As any other technique, EMSA has its strengths and shortcomings. The method is most frequently used for qualitative purposes, but when ap-

plied correctly, it may also provide quantitative data. In this lecture advantages and shortcomings of the technique will be reviewed and it will be discussed how EMSA can be used to determine apparent equilibrium dissociation constants, binding specificity, the number of binding sites, the stoichiometry of the interaction, binding cooperativity, the effect of allosteric co-factors on the protein-DNA interaction, and intrinsic and protein induced DNA deformations.

L12**UNRAVELLING SEQUENCE-SPECIFIC PROTEIN-DNA INTERACTIONS****D. Charlier***Research group of Microbiology, Vrije Universiteit Brussel*

Proteins may bind DNA in a sequence-specific, structure-specific (or a combination of these) or random manner. Random binding is for instance involved in the compaction of pro- and eukaryotic genomes by nucleoid associated proteins. Examples of exquisite structure-specific recognition can be found in various processes such as the binding of the sliding clamp at the primer:template junction in DNA replication or branch migration and resolution of the Holliday (4-way) junction in DNA recombination by the RuvAB and RuvC proteins.

However, here the focus will be on sequence-specific binding. The recognition of a short specific target sequence (frequently not more than 15-20 base pairs) among the millions of bp of a cell's genome is a crucial aspect of the onset and the control of numerous cellular processes involving DNA transactions, such as origin recognition in bacterial DNA replication and promoter selection and transcription regulation.

Techniques that are used to study sequence-specific protein-DNA interactions can be divided in two major classes: protection studies and premodification binding interference studies. In protection studies the protein is first allowed to bind to the DNA, then the DNA sequence stretch protected by the protein against enzymatic or chem-

ical cleavage is revealed by gel electrophoresis of the reaction products in denaturing conditions. DNaseI, hydroxyl radical and 'in gel' copper-phenanthroline footprinting will be discussed. In the premodification binding interference approaches target DNA is first chemically modified, then it is evaluated how these modifications affect on the protein-DNA interaction. This involves protein binding to the heterogeneous mixture of sparingly modified DNA molecules and their separation on basis of the affinity for the protein in an EMSA, recovery of free and bound forms from the gel, cleavage of the phosphodiester bond at the modified positions and resolution of the reaction products by gel electrophoresis in denaturing conditions. Premodification binding interference studies allow the identification of phosphates, bases or base-specific groups that contribute significantly to complex formation within a zone of interaction with the protein. Modifications may be of different kinds and be backbone-specific (e.g. phosphate ethylation) or base-specific (e.g. groove-specific purine methylation, thymine oxidation, base removal, (missing contact probing) etc.). Various techniques will be discussed and analyzed, including advantages and shortcomings, and experimental data will be interpreted.

L13**LIGAND-BINDING THEORY AND PRACTICE****Jannette Carey***Department of chemistry, Princeton University, Frick Laboratory, 360
Princeton, NJ 08544, USA
jcarey@princeton.edu*

NMR in studies of ligand binding. The focus will be on applications of NMR to studies of molecular interactions rather than on NMR methods.



Lectures – Saturday, July 4

L14

Wei-Feng Xue

*School of Biosciences, The University of Kent,
Canterbury, Kent, UK
W.F.Xue@kent.ac.uk*

Seminar: Global computational analysis of students' own data

Exercises

E1

GEL FILTRATION

Jannette Carey

*Department of chemistry, Princeton University, Frick Laboratory, 360
Princeton, NJ 08544, USA
jcarey@princeton.edu*

The method of Hummel and Dreyer (1962 *Biochim. Biophys. Acta* 63, 532-534) will be used to monitor binding of small ligands to macromolecules. Students can examine their own systems provided that the small-molecule ligand is available in adequate quantity and solubility to be

included in the running buffer. We hope to make a standard test system available for those whose own systems do not suit.

E2

ELECTRONIC SPECTROSCOPY

Andrzej Górecki

*Department of Physical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, ul. Gronostajowa 7 30-387 Kraków, Poland
andrzej.gorecki@uj.edu.pl*

Electronic spectroscopy is a powerful tool to investigate structural parameters of molecules. Since the structure of proteins or ligands most often depends on their complex formation, it is possible to find spectroscopic parameters that identify apo and holo state of the proteins. In case of multiple binding sites electronic spectroscopy can also help to monitor fractional saturation of individual sites. The binding constants are the most significant parameters of the reversible binding of ligands to sites. In order to obtain an experimental measurement of that parameter it is necessary to measure the concentration of fractional saturation of the

protein as a function of ligand concentration. During the lab exercises the student's own systems will be used. Initially spectroscopic parameters that monitor complex formation will be searched. Suitability of the system as an experimental model for protein-ligand binding will be discussed and ligand binding curves will be determined. A linear form of the binding equation will be derived or nonlinear least-squares regression of the binding data will be performed. In case of lack of the suitable student's system, an illustrational experimental model for protein-ligand binding will be used.

E3

FLUORESCENCE

Catherine Royer

*Biological Sciences and Chemistry, Constellation Professor - Bio-computation and Bio-informatics,
Rensselaer Polytechnic Institute 110 Eighth St. BT3244B*

Fluorescence, due to its sensitivity and versatility, is widely used as a means to observe and quantify interactions between biomolecules. There are several possible fluorescence observables and multiple fluorophores, hence providing innumerable strategies for designing the experiment which is most appropriate for the biomolecular sys-

tem at hand. I will briefly define the observables and their properties: intensity, color, anisotropy, lifetime, fluctuations and FRET. The advantages and disadvantages of a number of popular fluorophores will be examined. Then via a few examples, I will discuss how to design and carry out a successful fluorescence-based binding assay.

E4

PRACTICAL EXPERIMENTS USING ISOTHERMAL TITRATION CALORIMETRY (ITC)

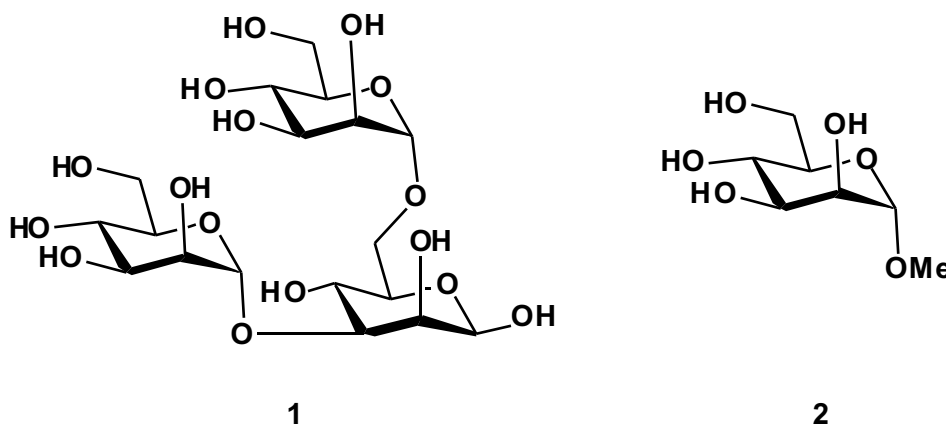
Bruce Turnbull

*School of Chemistry and Astbury Centre for Structural Molecular Biology, University of Leeds,
Leeds LS2 9JT, UK*

A practical illustration of the isothermal titration calorimetry (ITC) technique will involve a combination of experiments performed on the participants' own systems of interest and experiments using the plant lectin concanavalin A (con A) which binds to mannosyl oligosaccharide ligands. Whereas the branched trisaccharide **1** binds with a K_d in the micromolar range, binding of the monosaccharide **2** has a millimolar K_d .¹ These examples will be used to show how the shape of the isotherm changes with affinity and concentration.^{2,3} Other experiments that could be performed include competition ITC experiments for tackling higher and lower affinity systems⁴ and the use of ITC to determine detergent critical micelle concentrations. There will also be an opportunity to use curve fitting software to process raw data including more complex datasets for systems involving multiple binding sites with different affinities.

Useful references:

1. D. K. Mandal, N. Kishore and C. F. Brewer, "Thermodynamics of lectin-carbohydrate interactions. Titration microcalorimetry measurements of the binding of N-linked carbohydrates and ovalbumin to concanavalin A", *Biochemistry* 1994, **33**, 1149-1156.
2. W. B. Turnbull, "Divided we fall? Studying low affinity fragments of ligands by ITC", *G. E. Life Sciences Application Note*. 2011, <http://bit.ly/ITC-Turnbull>.
3. W. B. Turnbull and A. H. Daranas, "On the value of c : can low affinity systems be studied by isothermal titration calorimetry?", *J. Am. Chem. Soc.* 2003, **125**, 14859-14866.
4. W. B. Turnbull, B. L. Precious and S. W. Homans, "Dissecting the cholera toxin-ganglioside GM1 interaction by isothermal titration calorimetry", *J. Am. Chem. Soc.* 2004, **126**, 1047-1054.





E5

PRACTICAL EXPERIMENTS USING ISOTHERMAL SURFACE PLASMON RESONANCE (SPR)

Wei-Feng Xue

*School of Biosciences, The University of Kent,
Canterbury, Kent, UK
W.F.Xue@kent.ac.uk*

This laboratory session will show the basic operations of surface plasmon resonance (SPR) instruments, as well as the design and workflow of SPR experiments aimed to measure the association rate, the dissociation rate and the affinity of protein-ligand interactions. Principles of ligand-binding kinetics analysis using SPR will be demon-

strated on a BiacoreX instrument. Experiments will be carried out using a standard protein interaction system. Opportunity will also be available for the analysis of students' own ligand-binding systems.

