The goal of any branch of molecular biology, or human physiology more broadly, is an understanding of the relationship between structure and function. Both terms can have a broad and a narrow definition. In drug discovery, since most of our targets are proteins, the goal is relating protein structure to protein function. The simplest function is ligand binding, and ligands can be proteins or small molecules. Fortunately, we have at our disposal a number of high resolution biophysical tools which provide exquisite sensitivity in this analysis. Applications include basic characterization of proteins used in high throughput compound screening and a more high resolution analysis that might be used in new chemotype selection. It is important that these technologies be used in combination, first to establish the basic steps of the interaction and then to use that model to do a complete kinetic and thermodynamic analysis. When such an analysis is accomplished, one can have confidence in the results and in their application to subsequent steps in the drug discovery process.

Horse heart myoglobin will be dissolved in Tris Buffer and diluted to 5 to 50 uM. Absorbance spectra will be collected in the visible range (650-500 nm) and Soret (460-400 nm). The sample will then be titrated with stepwise additions of sodium fluoride from a 0.5 M solution and the absorbance spectra will be collected after each addition. The fractional ligand saturation will be calculated from the absorbance spectra and plotted as a function of fluoride and the experimental data will be fitted to a hyperbola using a non linear least squares minimization routine.
FLUORESCENCE
Andrzej Gorecki

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Fluorescence 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 the apo and holo state of the proteins. In case of multiple binding sites electronic spectroscopy can also help to monitor the 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 fluorescence 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. Nonlinear least-squares regression of the binding data will be performed. In case of no suitable student’s system, an illustrational experimental model for protein-ligand binding will be used.

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 Kd in the micromolar range, binding of the monosaccharide 2 has a millimolar Kd. These examples will be used to show how the shape of the isotherm changes with affinity and concentration. Other experiments that could be performed include competition ITC experiments for tackling higher and lower affinity systems 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:
SURFACE PLASMON RESONANCE (SPR)

Wei-Feng Xue

School of Biosciences, The University of Kent, Canterbury, Kent, UK

This laboratory practical session will demonstrate 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 demonstrated 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 protein-ligand binding systems.

BIOMOLECULAR INTERACTION ANALYTICS USING MICROSCALE THERMOPHORESIS

David Witte

NanoTemper Technologies GmbH Munchen Germany

Microscale Thermophoresis (MST) detects changes in the hydration shell, charge or size of molecules by measuring changes of the mobility of molecules in microscopic temperature gradients. It allows fast detection of a wide range of biomolecular interactions from ion or fragment binding up to interactions of large complexes (e.g. liposomes and ribosomes). The main key feature of MST is the possibility to work under close-to-native conditions: Measurements can be performed label-free, immobilization-free and in any buffer or complex bioliquid (e.g. serum and cell lysate). This approach combines versatility with a low sample consumption.

During the practical course the participants will have the opportunity to use their own samples for MST measurements either employing intrinsic tryptophan fluorescence or fluorescence originating from a dye.