

Why not train for and live in an interdisciplinary world?

Molecular Biology / Protein Biochemistry / Structure-Dynamics-Themodynamics / Simulations

"**Scientific progress is driven by ideas, data and technology**" is an obvious statement and that these three aspects of research are intimately related is clear also.

While the complexity of technology and knowledge is constantly increasing, an interdisciplinary approach is often essential in order to obtain a deep understanding of a system. In the study of the molecular biophysics of signaling molecules we endeavor to have the latest and best technology available, either in the laboratory or its extended surroundings. In addition, we have the expertise to use such equipment and interpret the data. Often it is also fun to think of novel, if not entirely new experiments. Our aim is not to use instruments for their own sake, but to answer questions for our specific system of study, i.e. a problem driven approach. We are part of the Cleveland Center for Structural Biology (structuralbiology.case.edu) and use both experimental and computational tools.

Specific instrumentation and technology that is used in the Buck laboratory includes:

Molecular Biology:

Cloning of proteins out of cDNA libraries (sometimes we have to)

Subcloning of fragments into plasmid amplification and protein expression vectors (vector preparation, ligation)

Site directed mutagenesis

Preparation of competent cells

Equipment Touchgene PCR thermal cycler; DNA gels; Pharmacia Ultrospec3000 UV/Vis; Labline environmental shaker; Sovall pico microfuge gel documentation system; floor centrifuges; autoclave

Protein Biochemistry:

Purification of untagged and tagged protein (His6, ubiquitin, GST)

Strong and Weak Ion exchange, size exclusion and hydrophobic interaction chromatography, HPLC; Protein refolding from inclusion bodies, dialysis; Protein concentration using amicons (stirred, ultra); Protein labeled with fluorophores; Activity determination using photo-spectrometric assays.

SDS- and native PAGE, protein transblotting for sequencing

Equipment Pharmacia Akta Purifier & Columns; Pierce Dialysers; Cary 100 Bio UV/Vis Spectrometer; Biorad power supply and gel apparatus;

Refrigerated bench top centrifuge Juan CR4.22; Branson 450 Sonicator; Balances; pH meter; water baths; stir & hotplates

Labconco Freezone4.5 Freeze Dryer

Protein Biophysics:

Limited proteolysis; pull down binding assays;

Characterization of protein secondary and tertiary structure using circular dichroism (CD); measurement of protein stability using CD; intrinsic fluorescence and fluorescent probes; density scanning calorimetry (DSC) and NMR. Determination of binding affinity using gel filtration, fluorescence spectroscopy and isothermal scanning calorimetry (ITC). Protein size using light scattering and analytical ultracentrifugation. Protein conformational changes and stability using NMR in conjunction with relaxation and amide hydrogen exchange experiments. Mapping of protein-protein/protein-ligand interaction surfaces using site directed mutagenesis, binding measurements and NMR. Structure determination using NMR and X-ray crystallography (OK... we are very new at this, but have seen many crystals - salt that is!)

Equipment Aviv215 Circular Dichroism spectrometer; crystallization screening.

Fluorimeters; Mass Spectrometer

NMR: 300MHz, 500MHz, 3 x 600MHz Varian Avance, 600MHz Bruker ICE + cryoprobe; purchased 800 MHz Bruker with cryoprobe, 900 MHz Bruker;

X-ray: Raxis IV++ detectors

Hydrodynamics: Beckman-Coulter XL-1 analytical ultracentrifuge; light scattering; Thermodynamics: Microcal VP ITC and DSC microcalorimetry; Biacore

Computational:

Processing and analysis of NMR spectra; e.g. relaxation and exchange rates; Protein structure determination from NMR (and X-ray data). Equilibrium and activated molecular dynamics to examine conformational stability and change. Docking and free energy calculations.

Equipment 4 x Dell Windows XP (Pentium IV); 3 x Linux number crunchers

vars. SGI indigos and octanes - being phased out

CHARMM; X-PLOR/CNS; MOLMOL; DNASTAR;

Origin; Cary Biosuite; Mathematica; nmrcode; nmrview; modelfree

Legend: in lab.; in neighboring lab.; in Department; other Dept., CCSB

Coworkers are expected to become familiar with most of these techniques, and become experts at some... all over a period of time of course.

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For questions or comments, e-mail the [Webmaster](#)

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