17. Biomolecular Interaction

- Methods for characterizing biomolecular interactions
- Sequence-specific DNA binding ligands
- Molecular mechanisms of drug action and drug resistance
- *In silico* compound design and screening
- Chemical library: combinatorial approaches
- Phage library

- **Types of biomolecular interaction**
  - Protein-protein
  - Protein-small molecule
  - Protein-DNA
  - DNA-small-molecule
  - ...

  - Essential for life
  - Critical for understanding fundamental biology
  - Important for drug design

I. Methods for characterizing biomolecular interactions

1. Spectroscopic methods
   - Usually Require a “label”
   - Example: fluorescence polarization (fluorescence anisotropy)

   - Rapid rotation of free, labeled ligand → loss of polarization
   - Binding of ligand to macromolecule → slower tumbling; less loss of polarization
2. Surface Plasmon Resonance (SPR)

- Usually does not require a “label”

- 1\textsuperscript{st} molecule is Immobilized on surface

- 2\textsuperscript{nd} molecule passes over sensor surface

- The binding of the 2\textsuperscript{nd} molecule to the 1\textsuperscript{st} molecule changes resonance characteristics of the surface

- The SPR angle shifts when molecules bind to the surface and change the mass of the surface layer.

- This change in resonant angle can be monitored non-invasively in real time as a plot of resonance signal (proportional to mass change) versus time
3. Isothermal Titration Calorimetry (ITC)
- Usually does not require a “label”
- Heat evolved or absorbed when two molecules interact
- Titrate ligand into sample cell
- Measure power needed to maintain reference cell at same temperature as sample cell
- $K_d$, $\Delta G$, $\Delta H$, $\Delta S$
- A standard ITC trace consists of two panels:
  (a) Upper panel: shows the heat trace of the thermostat over the time of the experiment with the individual injections of ligand as peaks.
  (b) Lower panel: obtained by integrating the area of the peaks and plotting them against the molar ratio of ligand and protein

4. Mass Spectrometry
- Noncovalent complexes can be detected using mass spectrometry
- Usually does not require a “label”
5. Nuclear Magnetic Resonance (NMR)

- NMR has a strong history in study of biomolecular interactions, especially for drug discovery.
- Usually require a “label”
- Ligand binding is studied via chemical shift changes in $^{15}$N-HSQC spectra

A superposition of $^{15}$N-HSQC spectra of a protein in the presence and absence of a ligand

(HSQC: Heteronuclear single-quantum correlation spectroscopy)


6. Enzyme-linked immunosorbent assay (ELISA)

(a) Indirect ELISA

(b) Sandwich ELISA

(c) Competitive ELISA

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II. Identifying the compound binding site

- X-ray crystallography
- NMR
- Photoaffinity labeling

Light (hν) causes irreversible linkage between ligand and macromolecule

Commonly used photoaffinity probes

benzophenone

C–H insertion mechanism (insertion into C–H bonds within 3.1 Å of C=O oxygen)
aryl azide

NH$_2$ or OH nucleophilic additions

C/N/O–H insertions

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diazirine

C–H insertion
O–H insertion
N–H insertion

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III. Modulate biomolecular interactions

- Caged compounds

- Photorelease of active compound from inactive, protected ("caged") precursor

- Light ($h\nu$) enables both spatial and temporal control
An example from our own research

- Photo-control of protein interaction

SH2 domain complex with pTyr peptide

- Partially opened up the binding pocket

- Mutated away three chemically reactive side chains (a secondary alcohol, a thiol, and a primary amine)
### SH2-TM-R35ONBK $K_d$ (µM)

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#### O-Nitrobenzyl photochemistry

![Diagram of O-Nitrobenzyl photochemistry](image)
- Benzoin photochemistry

![Benzoin photochemistry diagram]

- O-Cinnamoyl photochemistry

![O-Cinnamoyl photochemistry diagram]

- An example in protein-protein interaction

![Electron microscopic projection of an artificially flattened cellulosome]

Electron microscopic projection of an artificially flattened cellulosome.


How do we control the assembly of this protein complex?
- Controlled Assembly of Cellulosome

- Cellulosome assembly: based on cohesin - dockerin interaction.
- Nonhomogeneous catalyst: \(6.6 \times 10^{16}\) variants within a single species.
- Synergistic action among cellulosomal enzymes.

**Construction of Cohesin & Dockerin Libraries**

- Cohesin (green) and dockerin (yellow) mutants are generated by the randomization of key interacting residues in each domain.
- Cohesin library (Asn37, Asp39, Tyr74, Arg77, and Glu131): \(3.4 \times 10^7\) (1.5 x \(10^8\)).
- Dockerin library (Ser45 and Thr46): \(10^3\) (2 x \(10^3\)).
Selection Scheme

A. Negative selection
[Diagram showing the selection process involving cohesin and dockerin with λcI operator, RNAP, and toxic to the host (λpirF) in the presence of 5-FOA.]

B. Positive selection
[Diagram showing the selection process involving cohesin and dockerin with λcI operator, RNAP, and essential to the host (λhisB) in the presence of 3-AT.]

C. Selection scheme
[Flowchart showing the process of selecting cohesin and dockerin mutants with negative selection involving wt dockerin and wt cohesin with interact with wt dockerin and interact with wt cohesin.]

Abbreviation: λcI, bacteriophage λ repressor protein; RNAP, α-subunit of RNA polymerase; P_{lacZ}, the lac promoter; 3-AT, 3-amino-1,2,4-triazole; 5-FOA, 5-fluoroorotic acid.

To visualize mutations that affect protein-protein interactions

PyMOL | pymol.org
UCSF Chimera