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

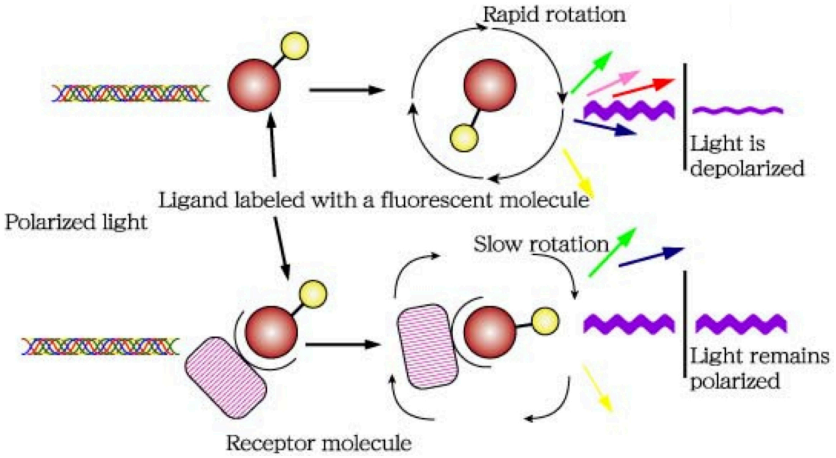
- Protein-protein
- Protein-small molecule
- Protein-DNA
- DNA-small-molecule
- ...

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

I. Methods for characterizing biomolecular interactions

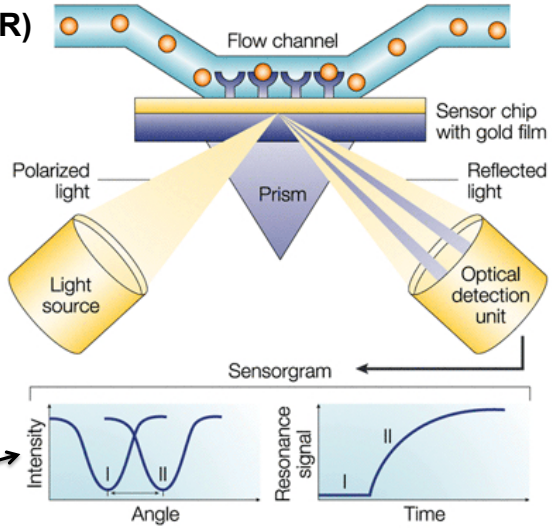
1. Spectroscopic methods

- 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"

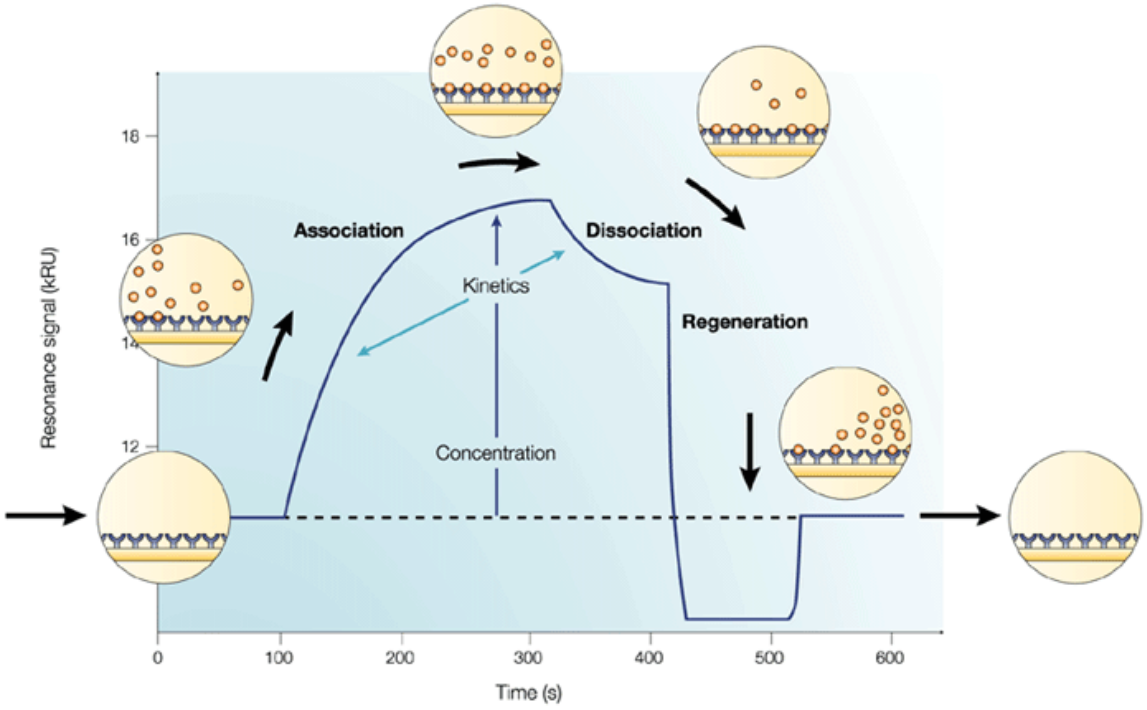
- 1st molecule is Immobilized on surface

- 2nd molecule passes over sensor surface

- The binding of the 2nd molecule to the 1st 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



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3. Isothermal Titration Calorimetry (ITC)

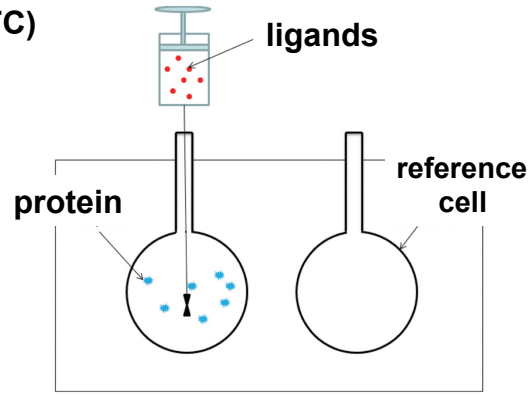
- Usually does not require a “label”

- 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

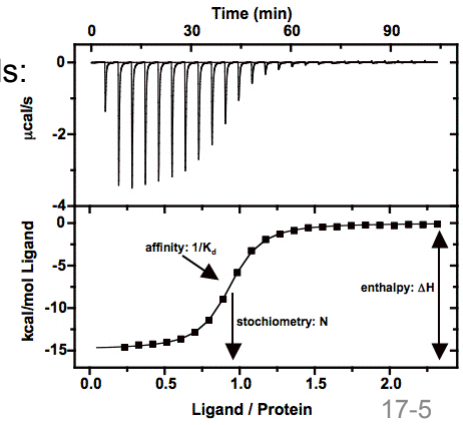
- K_d, ΔG, ΔH, ΔS



- 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.

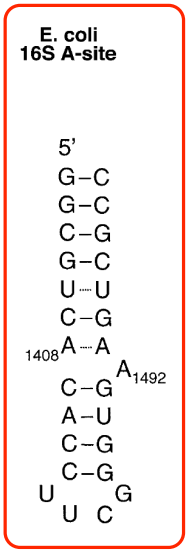
(b) Lower panel: obtained by integrating the area of the peaks and plotting them against the molar ratio of ligand and protein

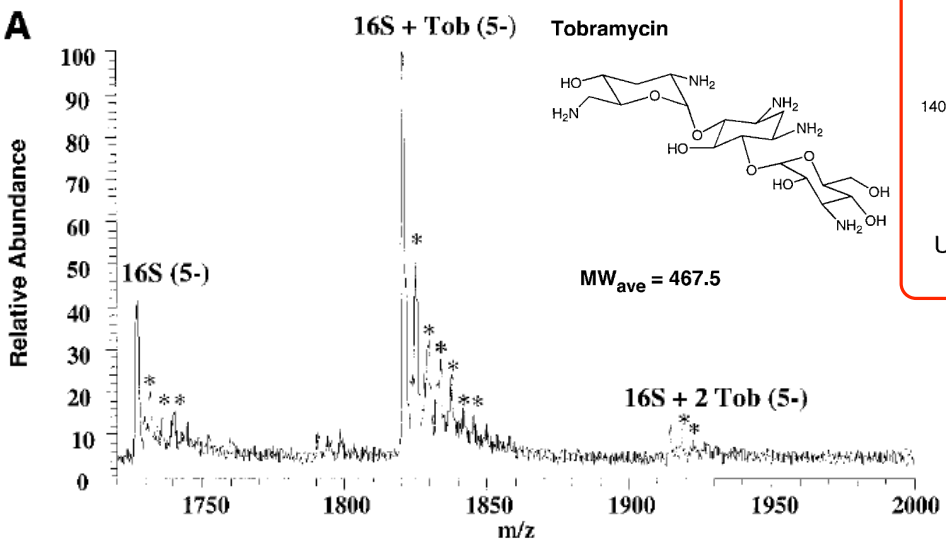


4. Mass Spectrometry

- Noncovalent complexes can be detected using mass spectrometry

- Usually does not require a “label”





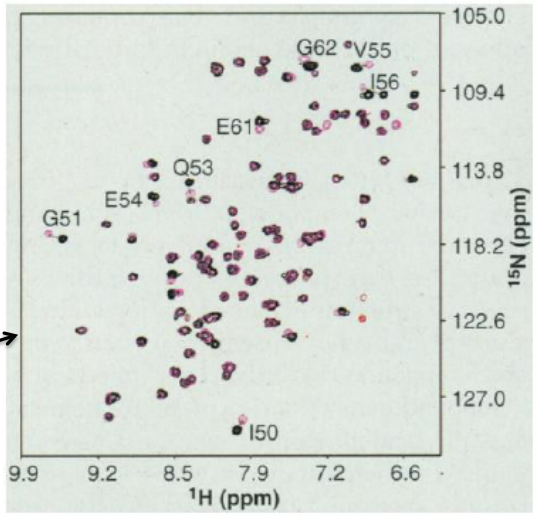
5. Nuclear Magnetic Resonance (NMR)

- NMR has a strong history in study of biomolecular interactions, especially for drug discovery.

- Usually Require a “label”

- Ligand binding is studies via chemical shift changes in ^{15}N-HSQC spectra

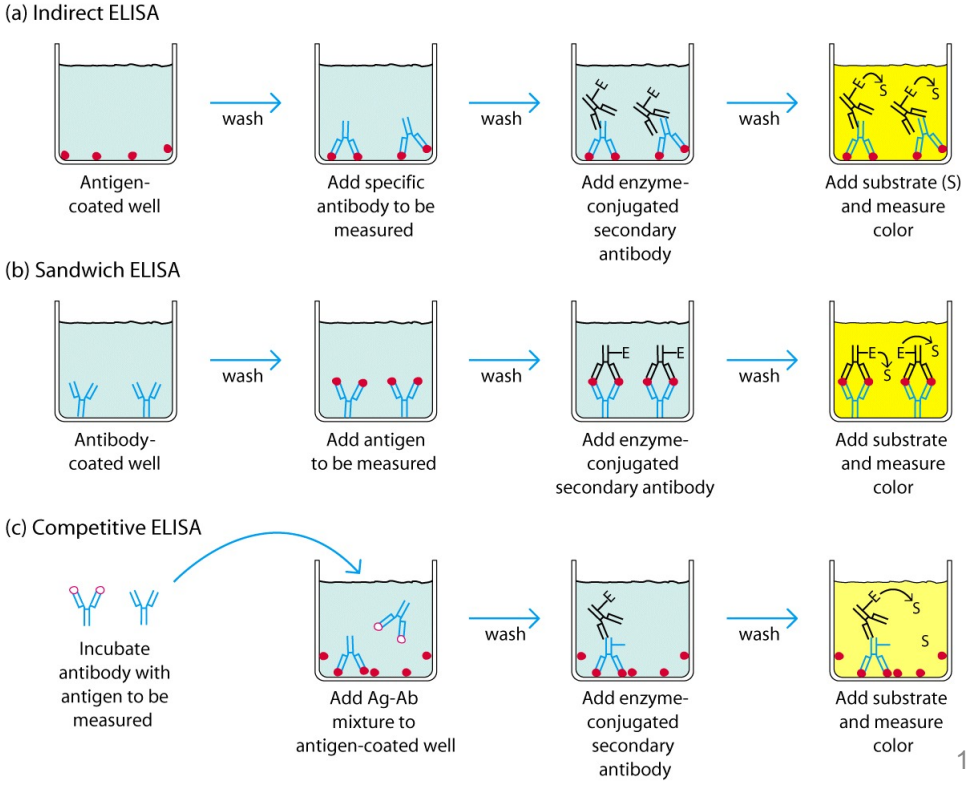
A superposition of ^{15}N-HSQC spectra of a protein in the presence and absence of a ligand



(**HSQC**: Heteronuclear single-quantum correlation spectroscopy)

Science **1996,** *274,* 1531.

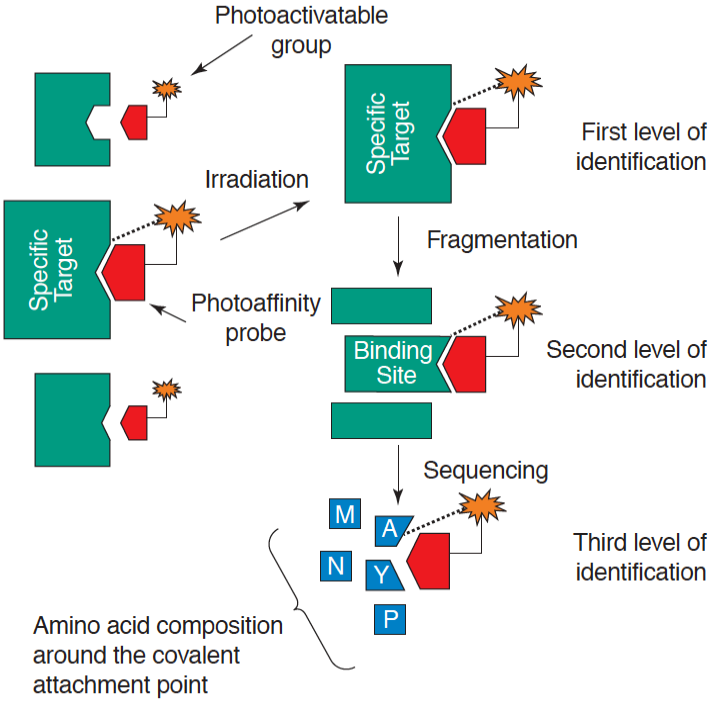
6. Enzyme-linked immunosorbent assay (ELISA)



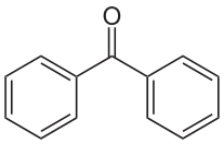
II. Identifying the compound binding site

- X-ray crystallography
- NMR
- **Photoaffinity labeling**

Light (hν) causes irreversible linkage between ligand and macromolecule

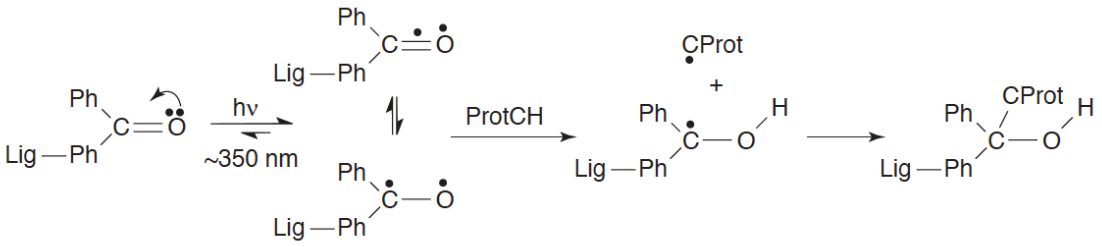


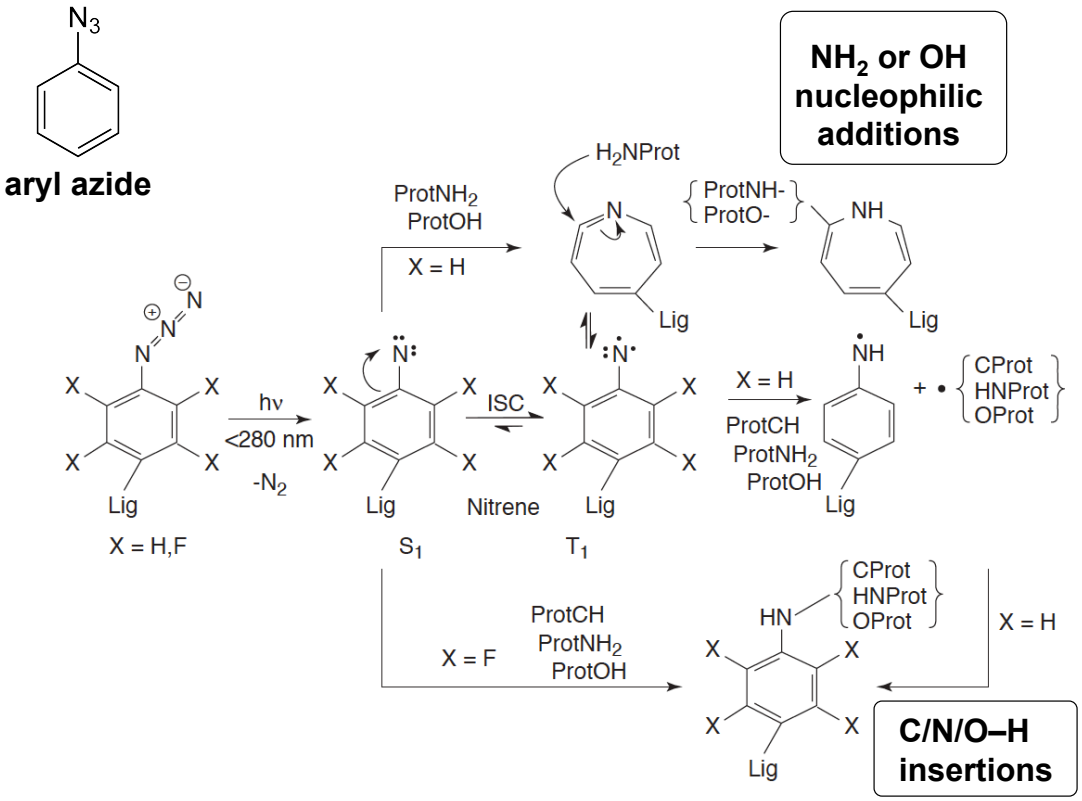
Commonly used photoaffinity probes

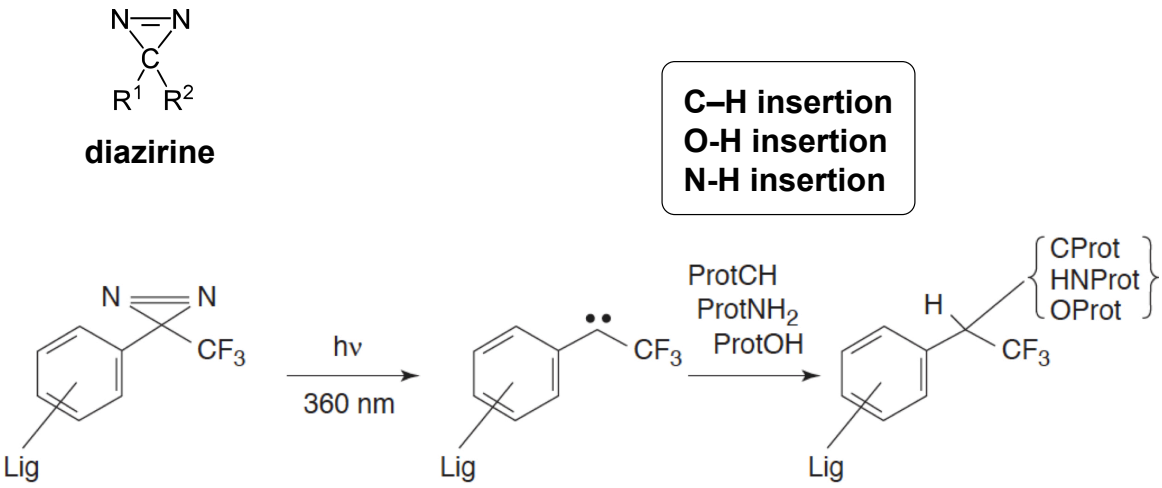


benzophenone

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

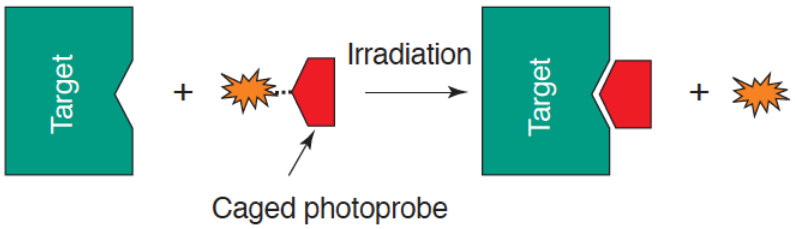



N3
aryl azide
NH2 or OH nucleophilic additions
ProtNH2
ProtOH
X = H
H2NProt
ProtNH-
ProtO-
NH
Lig
hν
<280 nm
-N2
X = H,F
ISC
Nitrene
S1
T1
X = H
ProtCH
ProtNH2
ProtOH
CProt
HNProt
OProt
X = F
HN
C/N/O–H insertions


diazirine
C–H insertion
O-H insertion
N-H insertion
CF3
hν
360 nm
ProtCH
ProtNH2
ProtOH
CProt
HNProt
OProt
H
Lig

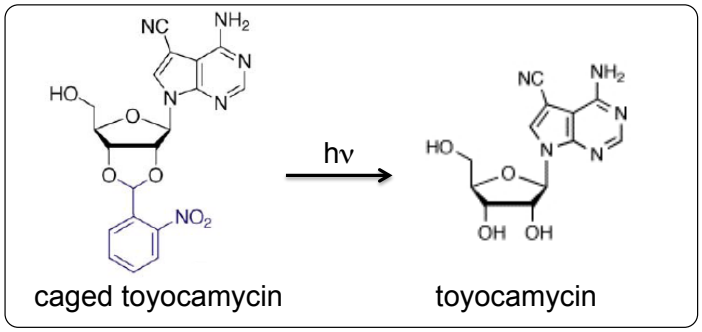
III. Modulate biomolecular interactions

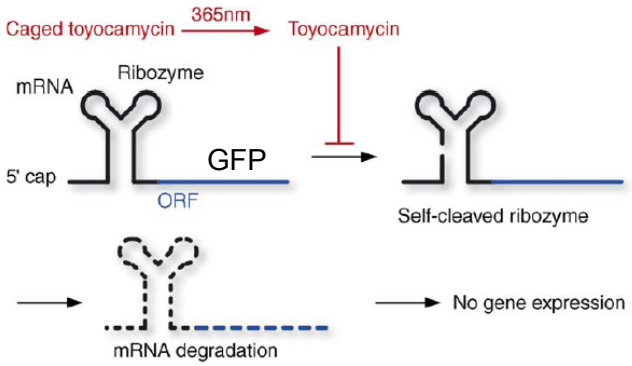
- Caged compounds

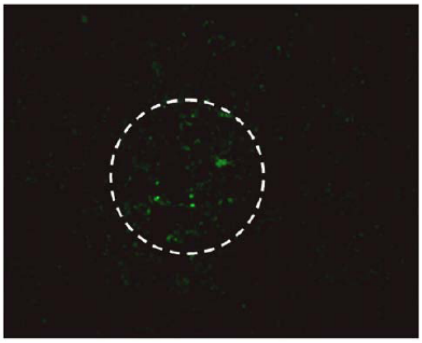


- Photorelease of active compound from inactive, protected ("caged") precursor

- Light (hν) enables both spatial and temporal control

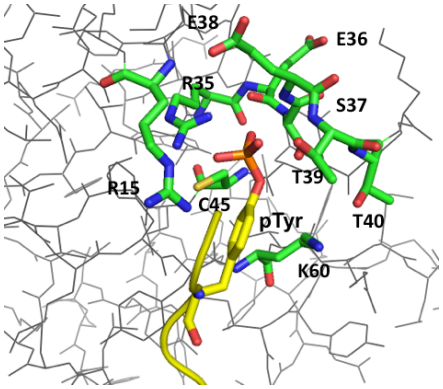


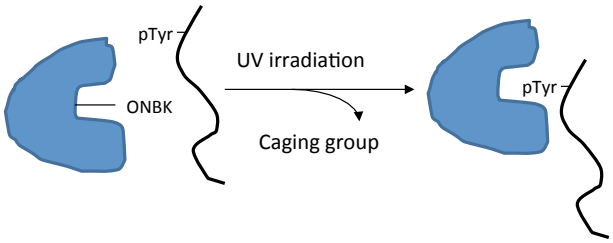


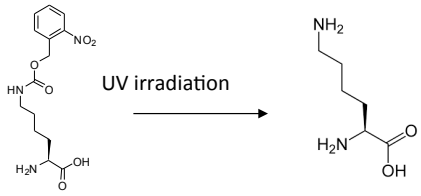


An example from our own research

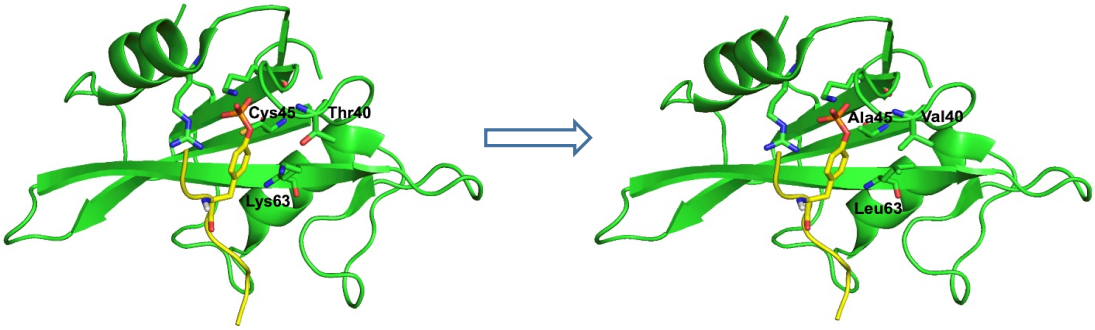
- Photo-control of protein interaction







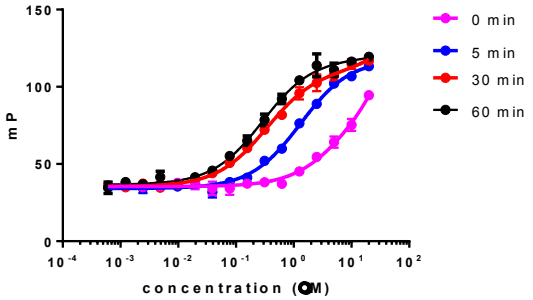
SH2 domain complex with pTyr peptide



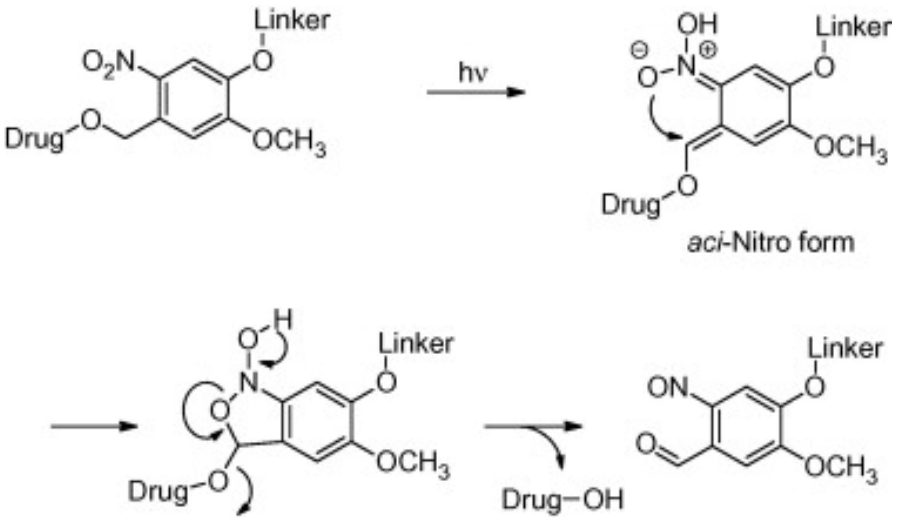
- Partially opened up the binding pocket

- Mutated away three chemically reactive side chains (a secondary alcohol, a thiol, and a primary amine)

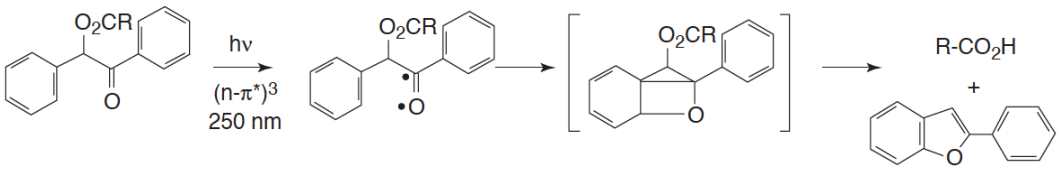
Time	0 min	15 min	30 min	60 min
SH2-TM-R35ONBK K_d (μM)	14.05±13.39	0.6546±0.0331	0.4544±0.0299	0.2947±0.0215



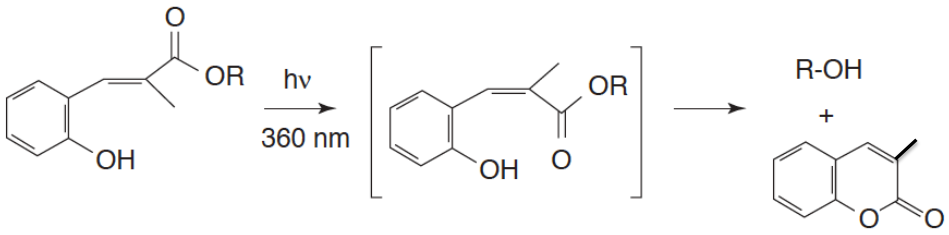
O-Nitrobenzyl photochemistry



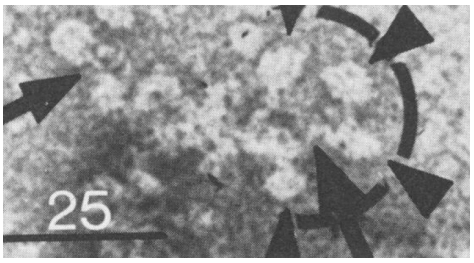
- Benzoin photochemistry



- *O*-Cinnamoyl photochemistry

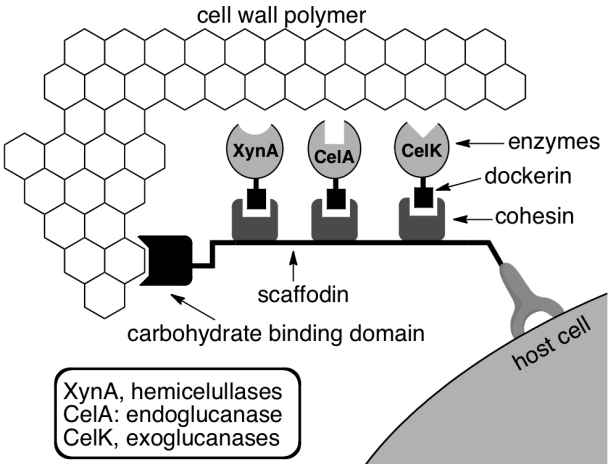


- An example in protein-protein interaction



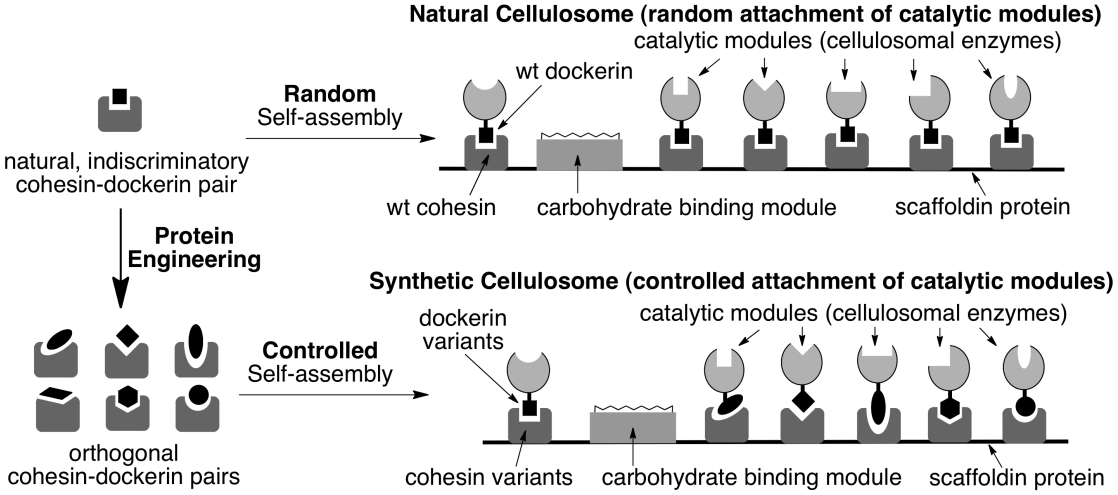
Electron microscopic projection of an artificially flattened cellulosome.

Mayer, Coughlan, Mori, Ljungdahl, *Appl. Environ. Microbiol.* **1987**, *53*, 2785.



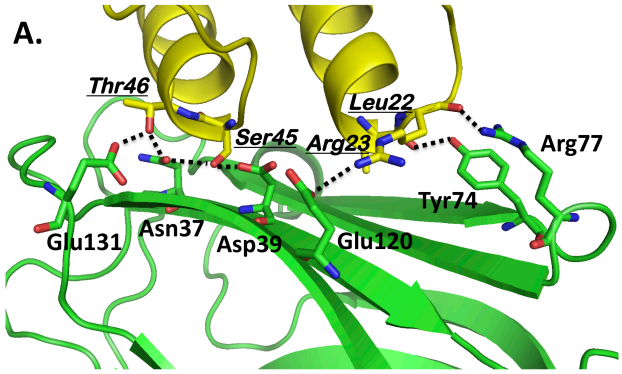
How do we control the assembly of this protein complex?

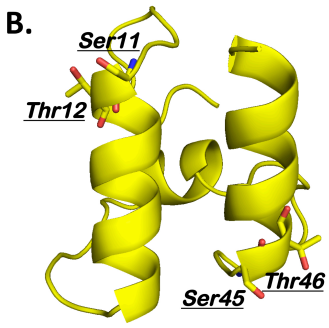
- Controlled Assembly of Cellulosome



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

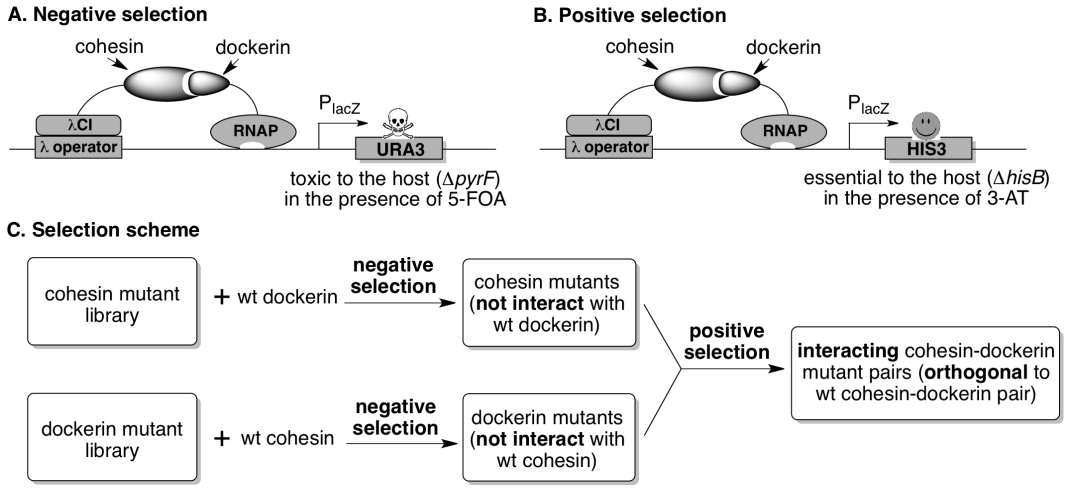
Construction of Cohesin & Dockerin Libraries





- 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 x 10^7 (1.5 x 10^8).
- Dockerin library (Ser45 and Thr46): 10^3 (2 x 10^3).

Selection Scheme

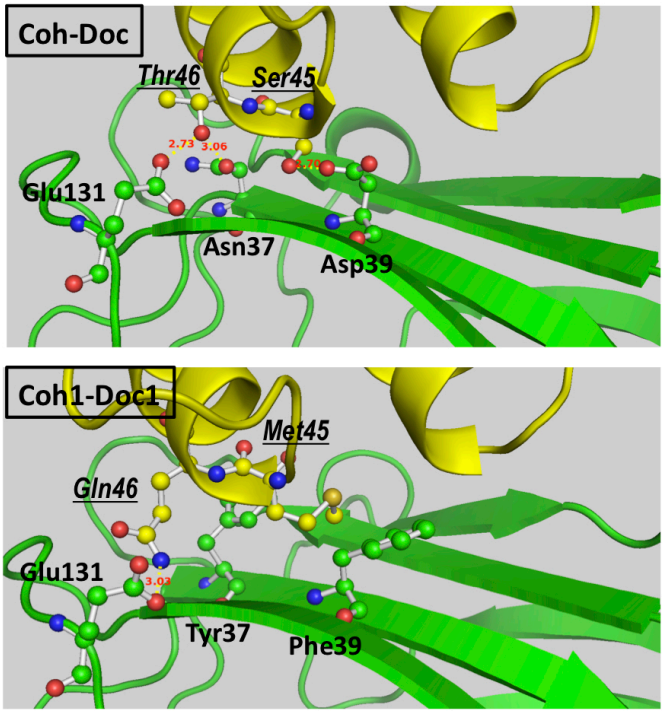


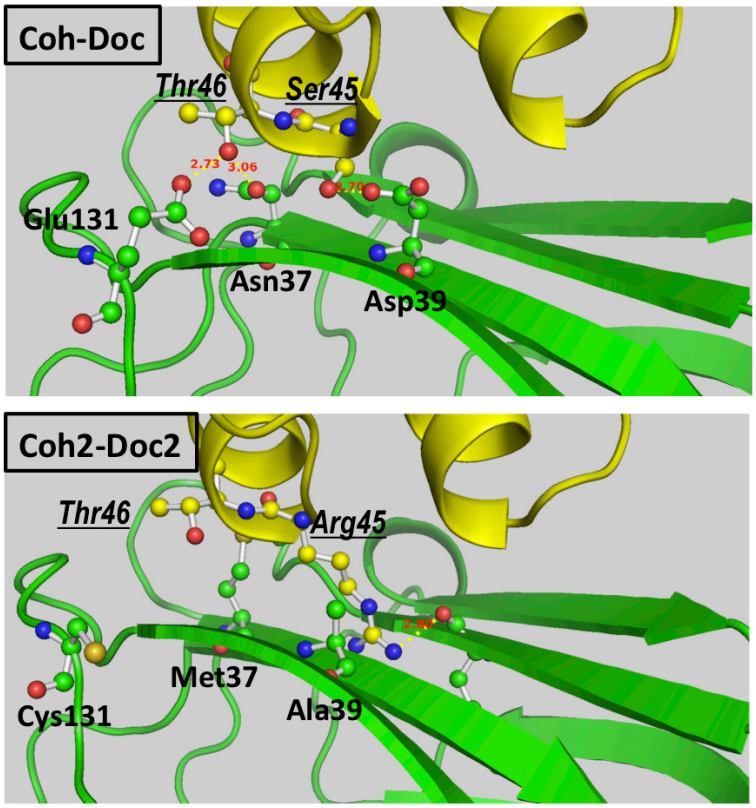
Abbreviation: λcI, bacteriophage λ repressor protein; RNAP, α-subunit of RNA polymerase; P_{lacZ}, the lac promoter; 3-AT, 3-amino-1,2,4-triazole; 5-FOA, 5-fluoroorotic acid.

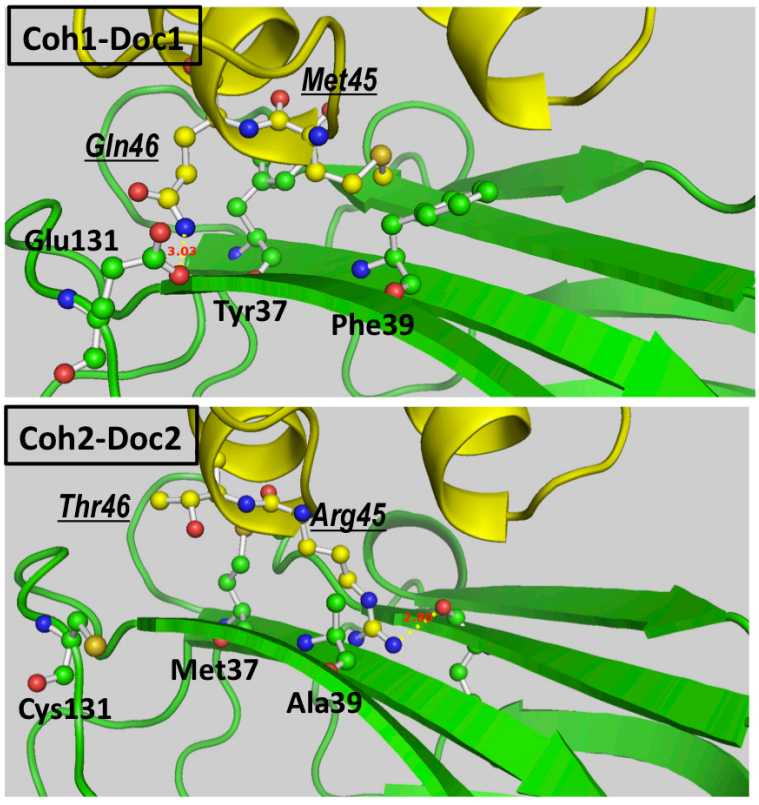
To visualize mutations that affect protein-protein interactions

PyMOL | pymol.org

UCSF Chimera




Coh-Doc
Thr46
Ser45
2.73
3.06
Glu131
Asn37
Asp39
Coh2-Doc2
Thr46
Arg45
Met37
Cys131
Ala39


Coh1-Doc1
Met45
Gln46
Glu131
3.02
Tyr37
Phe39
Coh2-Doc2
Thr46
Arg45
Met37
Cys131
Ala39