

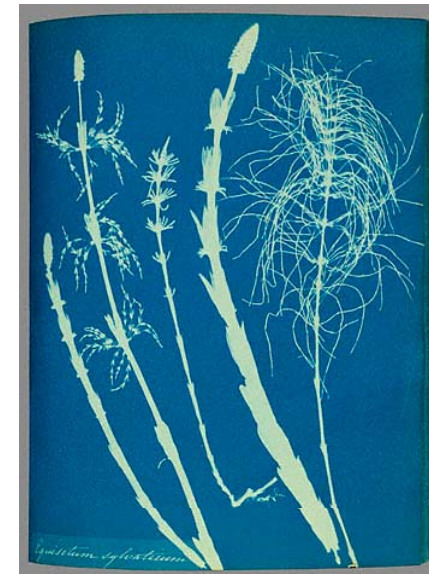
Angela K. Carrillo Alocén

Friday April 23th 2010

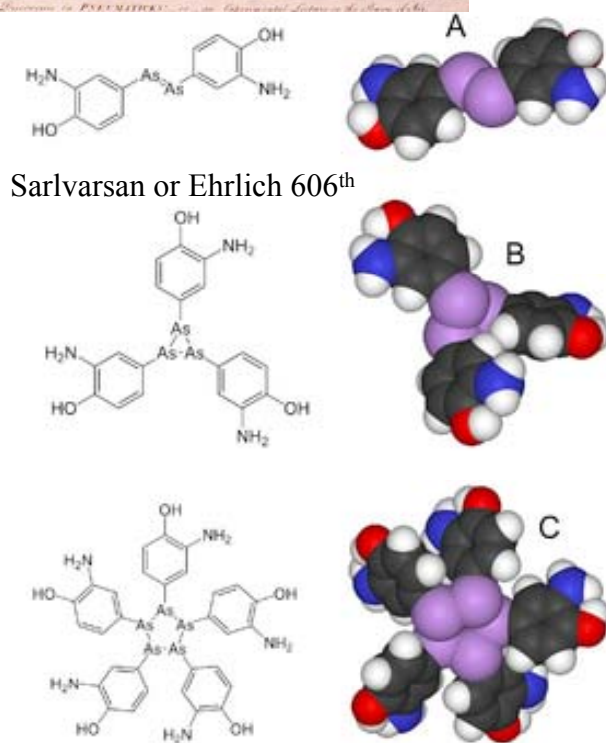
The origins of Chemical Biology



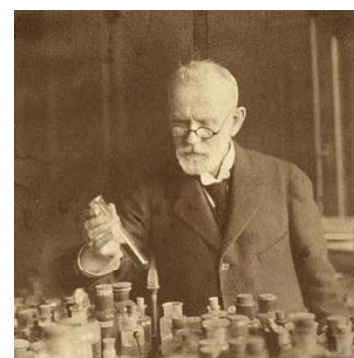
Sir Humphry Davy (1778-1829) presents his newly isolated airs to the Royal Institution.



A cyanotype from *British and Foreign Flowering Plants and Ferns* by Anna Atkins (1799-1871).



Sarlvarsan or Ehrlich 606th



Paul Ehrlich (1854-1915). Winner of the Nobel Prize in 1908.

Chemical Biology : Definitions

Nature Chemical Biology defines chemical biology as...

...both the use of chemistry to advance a molecular understanding of biology and the harnessing of biology to advance chemistry

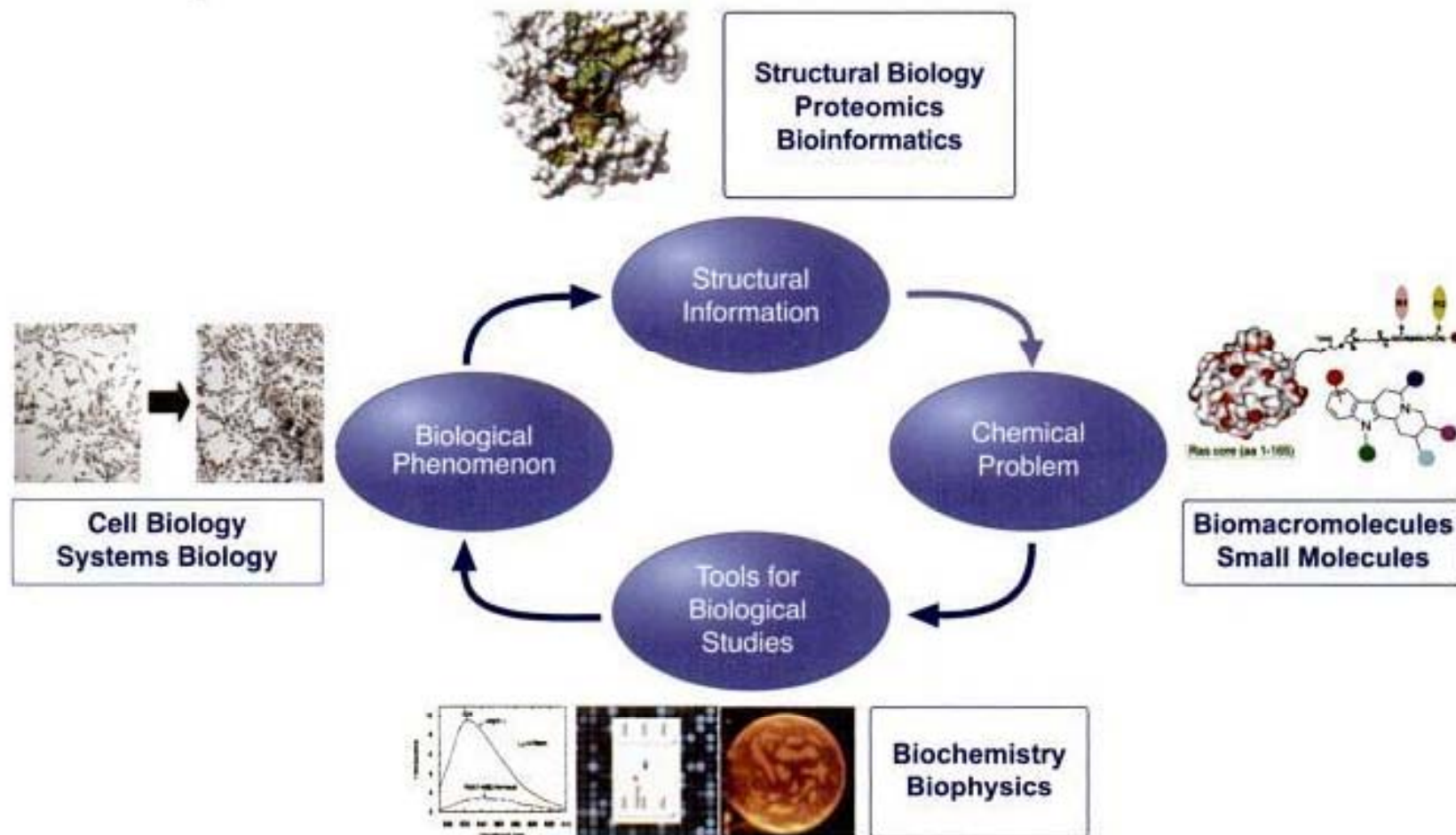
.- Anonymous. Nat. Chem. Biol. 2005, 1, 3,

Chemical Biology may also be defined as....

...the application of chemistry methods and techniques to the study of biological phenomena, that is chemical biology research seeks new insights into biology by means of an approach originating from an enabling chemistry tool box...

.- Waldmann and Janning, Chemical Biology. learning through case studies, 2009

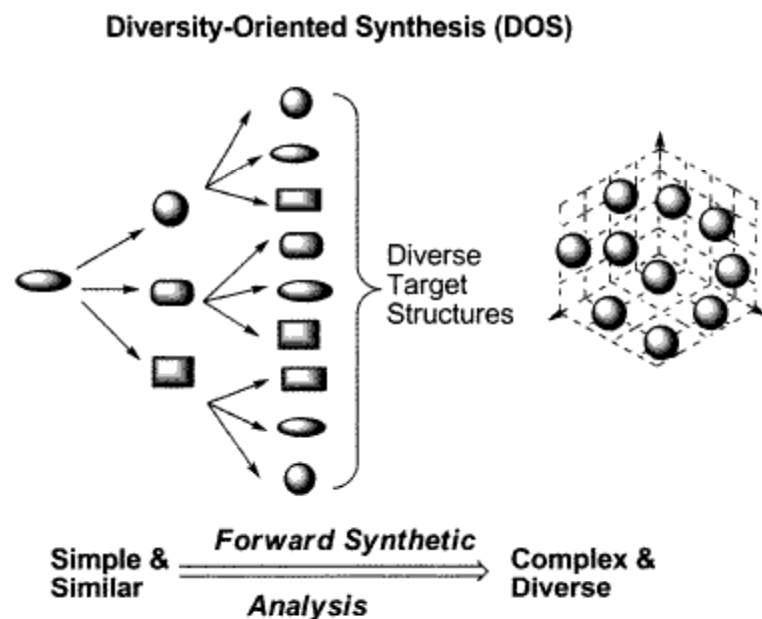
Chemical Biology Research



1. Waldmann, H; Janning, P; *Chemical Biology: Learning through Case Studies*, Wiley-VCH 2009, Weinheim, Germany.

Compound Collection Synthesis for Chemical Biology

Diversity-Oriented Synthesis (DOS)

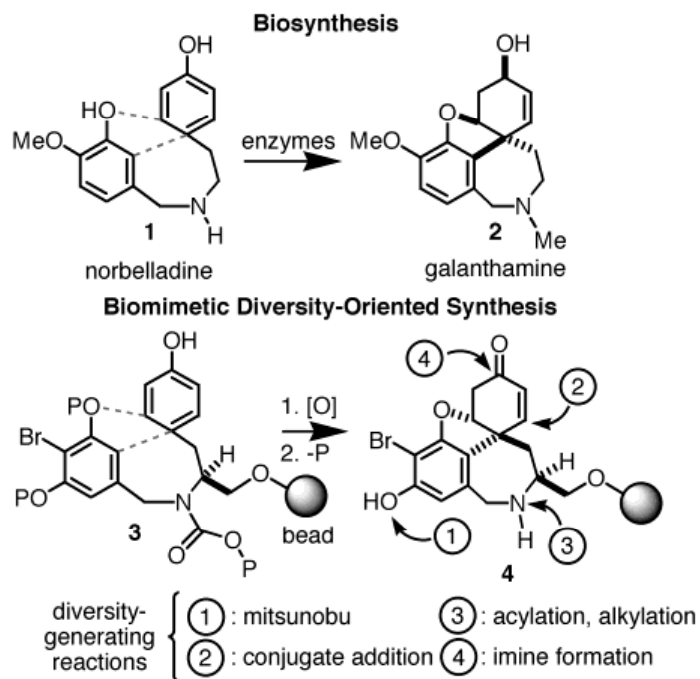


- Not directed toward a single biological target. DOS libraries used to identify ligands for a variety of targets. “Forward chemical genetics”
- Construct structurally complex and diverse architectures in a high-throughput manner.
- Access to natural product (NP)-like skeletons.
- Establishment of three-dimensional structural complexity by stereo- and enantio-selective reactions on solid phase

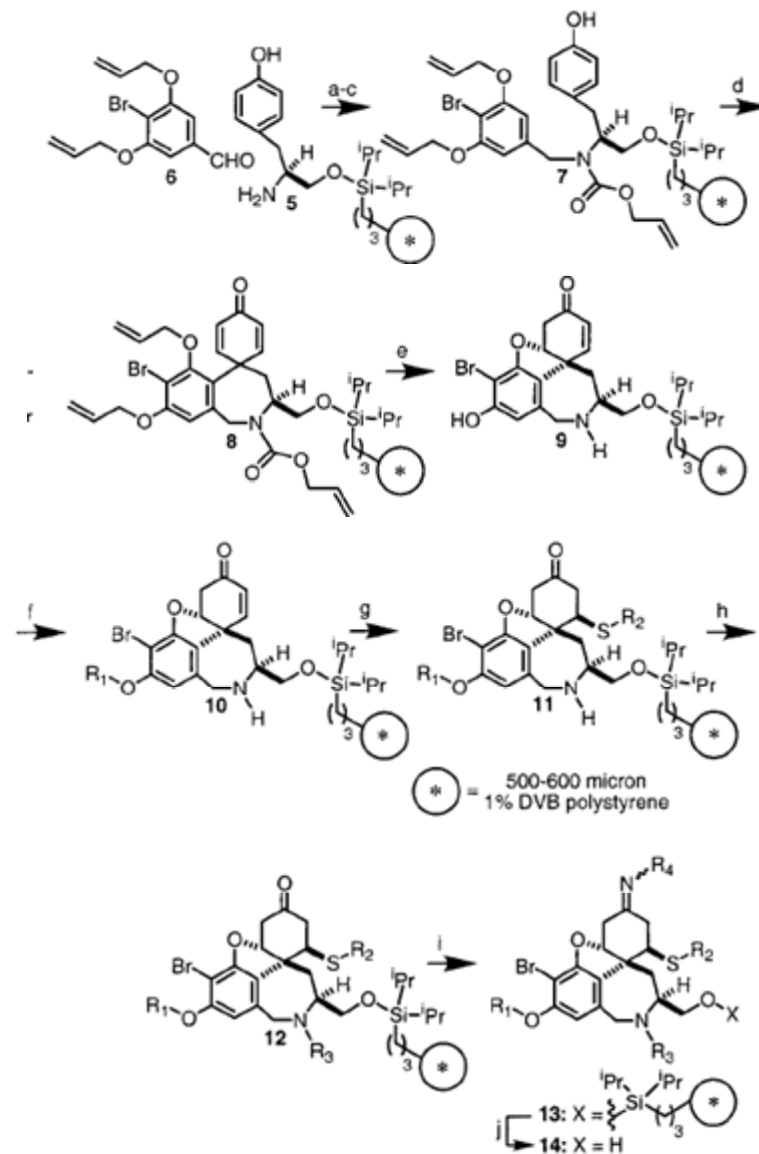
1. Waldmann, H; Janning, P; *Chemical Biology: Learning through Case Studies*, Wiley-VCH 2009, Weinheim, Germany.
2. Tan, D; *Nat. Chem. Biol.*, 2005, 1, 74-84

Compound Collection Synthesis for Chemical Biology

Diversity-Oriented Synthesis (DOS)

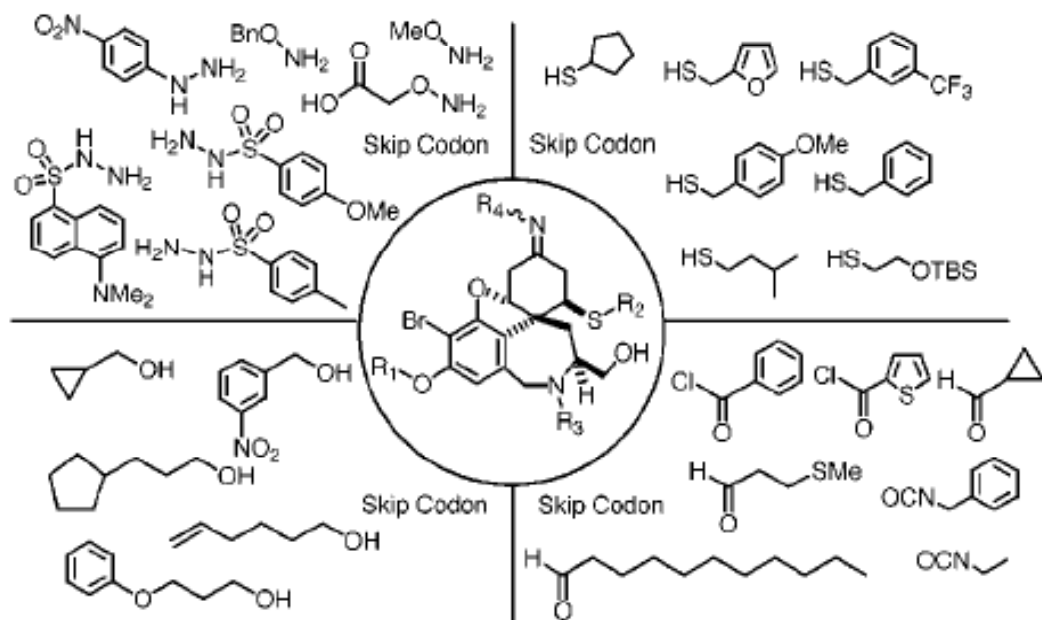


Galanthamine is a acetylcholinesterase inhibitor

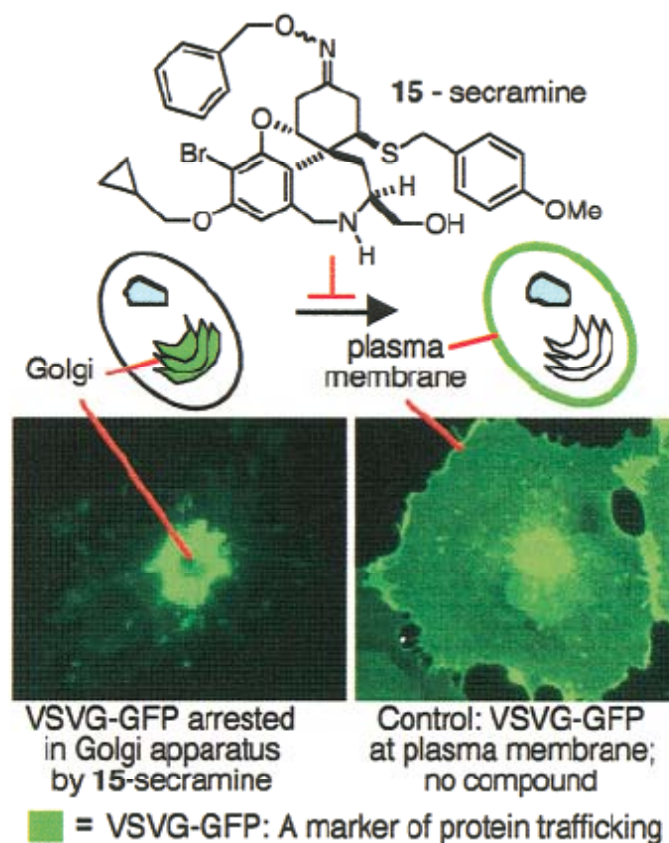


Compound Collection Synthesis for Chemical Biology

Diversity-Oriented Synthesis (DOS)



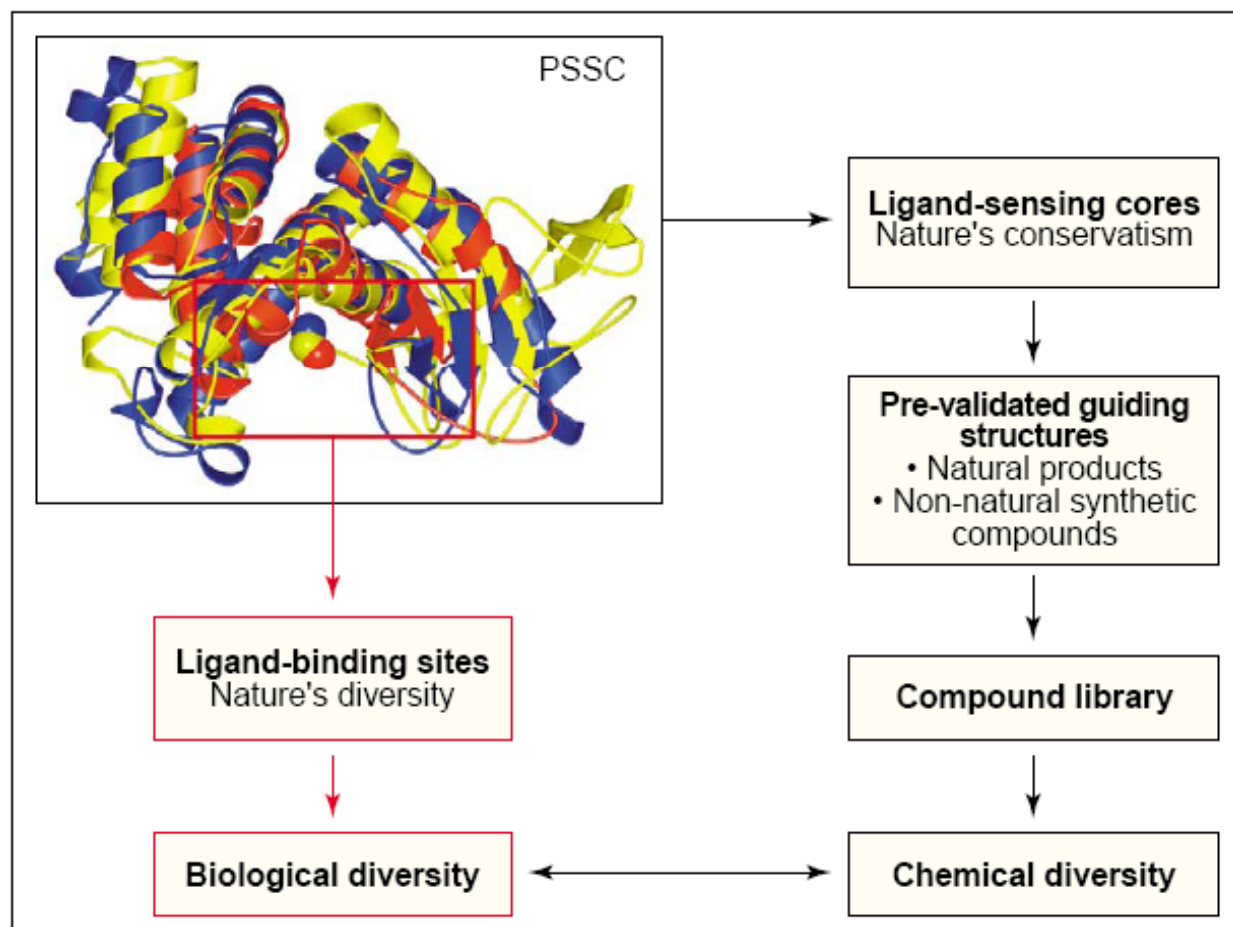
2527 out 2946 (86%) potential compounds were prepared



Discovery of sercramine, a galanthamine-based molecule that perturbs protein trafficking.

Compound Collection Synthesis for Chemical Biology

Biology-Oriented Synthesis (BIOS)



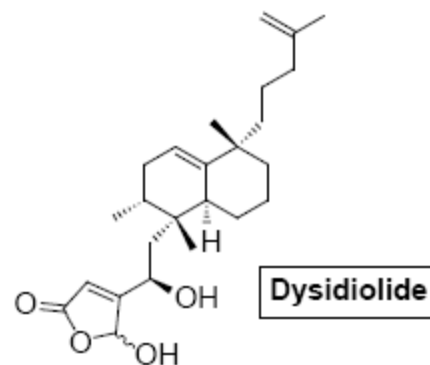
- Correlates the evolutionary relationship between NP and the protein world.
- Spatial structure is more conserved than AA sequence.
- Discovery of ligands for proteins of a protein structure similarity cluster (PSSC). PSSC determined by Bioinformatic techniques.
- Relevant Scaffolds of NP space determined by Cheminformatic techniques

Compound Collection Synthesis for Chemical Biology

Biology-Oriented Synthesis (BIOS)



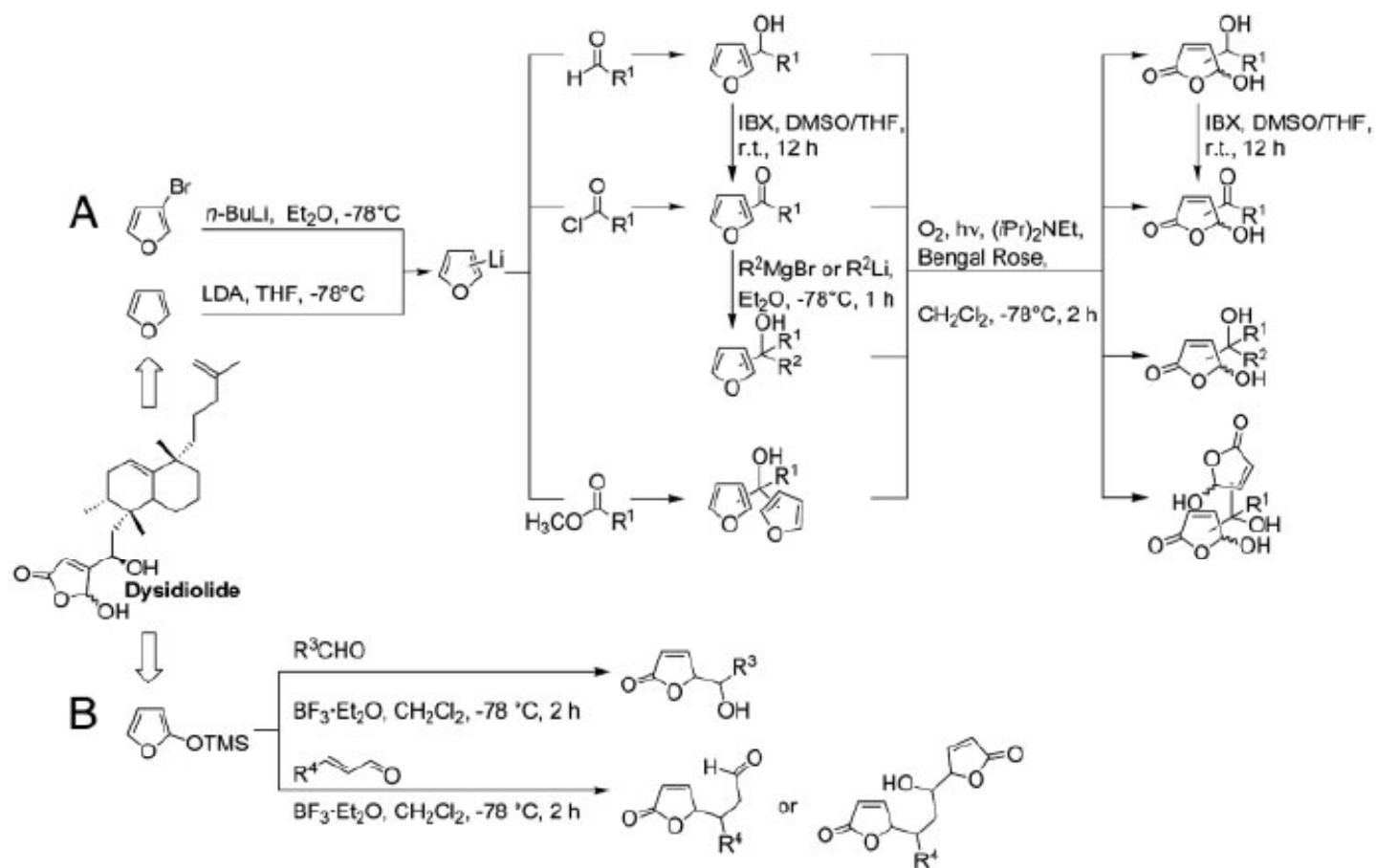
Top view of the catalytic sites of Cdc25A (red), 11βHSD1 (green) and Ache (blue).



- Natural inhibitor of protein phosphatase Cdc25A which is essential for cell proliferation.
- It contains a dehydrodecaline part and a hydroxybutenolide part.
- Core structure: hydroxybutenolide. Critical motif for phosphatase inhibition.

Compound Collection Synthesis for Chemical Biology

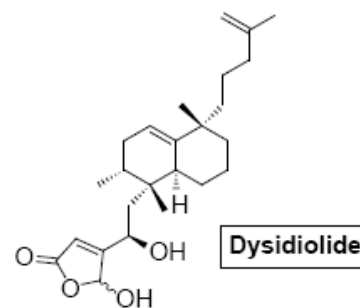
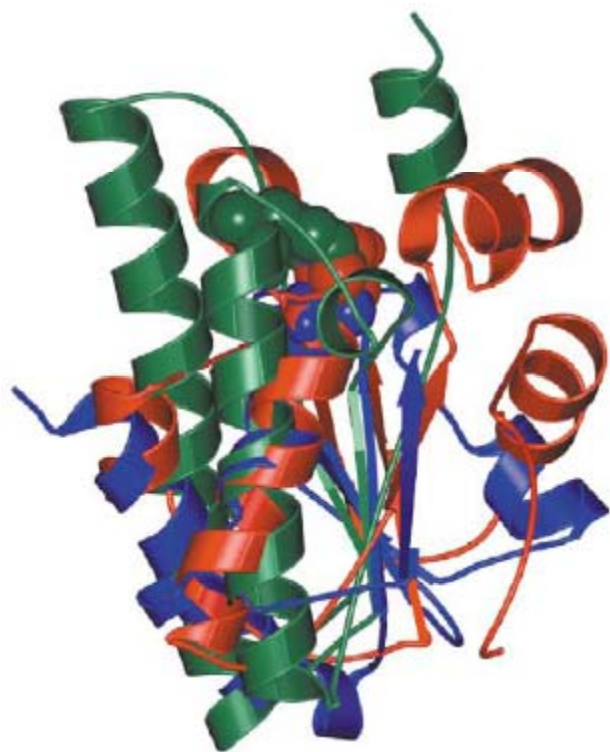
Biology-Oriented Synthesis (BIOS)



1. Waldmann et al, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, 101, 16721-16726.

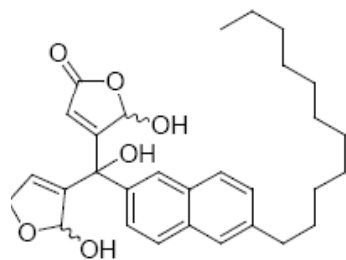
Compound Collection Synthesis for Chemical Biology

Biology-Oriented Synthesis (BIOS)

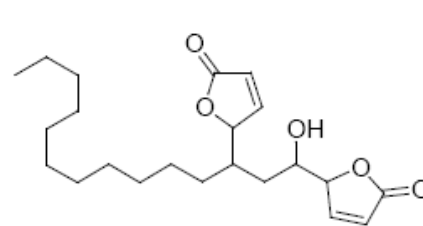


Cdc25A: 9.4 μM

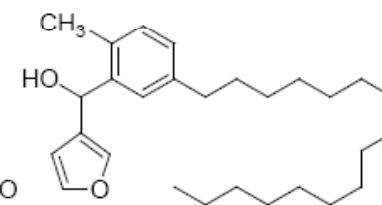
Library of analogs



Cdc25A: 0.35 μM
AChE: >20 μM
11 β HSD1: 14 μM
11 β HSD2: 2.4 μM



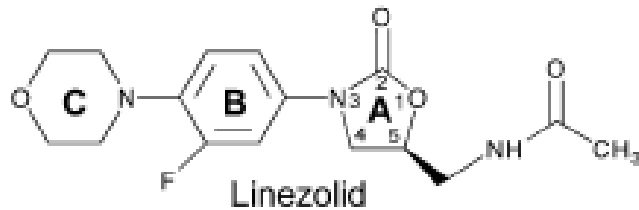
Cdc25A: 45 μM
AChE: >20 μM
11 β HSD1: 10 μM
11 β HSD2: 95 μM



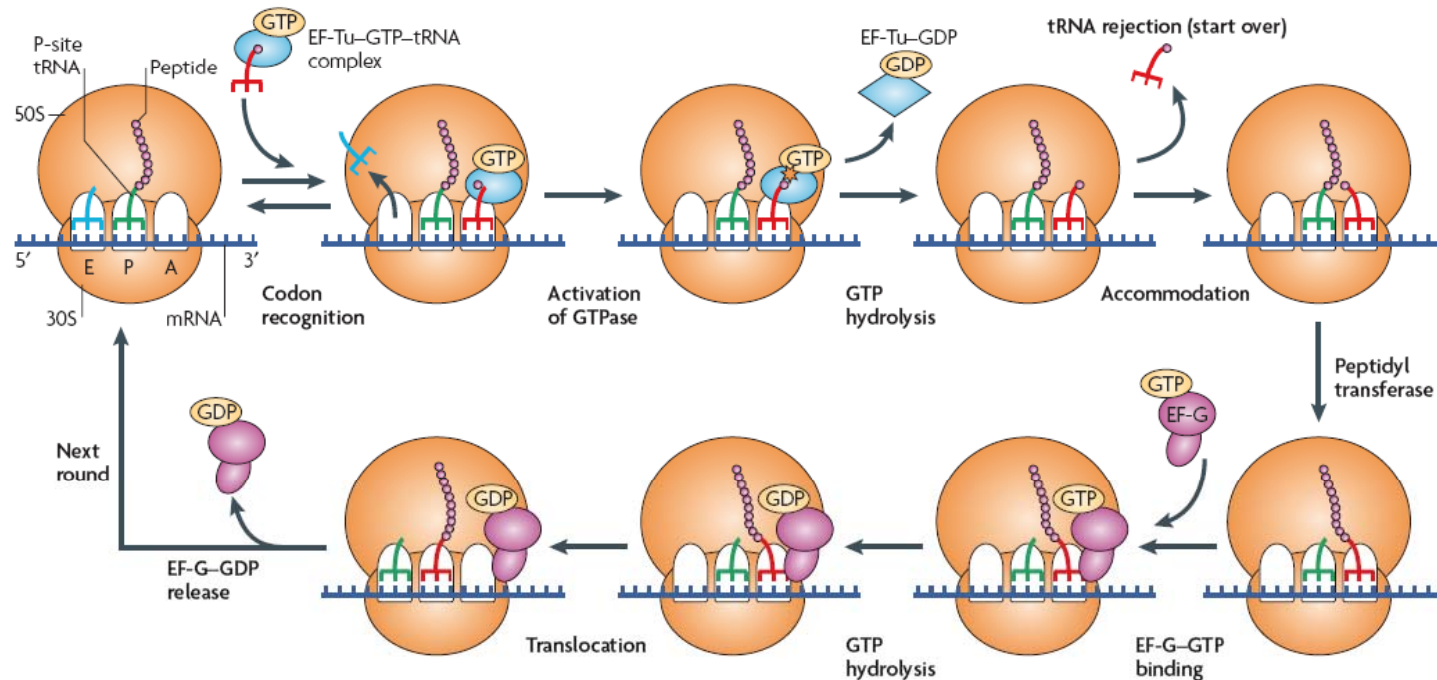
Cdc25A: 1.8 μM
AChE: >20 μM
11 β HSD1: 19 μM
11 β HSD2: 6.7 μM

Photoaffinity Labeling

Identification of the Binding Site of the Antibiotic Linezolid



- Inhibits bacterial growth by binding to the ribosome and interfering with protein biosynthesis.
- Peptidyl-transferase-center (PTC) the main site of oxazolidinone action.
- Mutation does not allow exact location of the inhibition, nucleotide exchange may confer resistance allosterically.
- Exact binding location through covalent interaction.



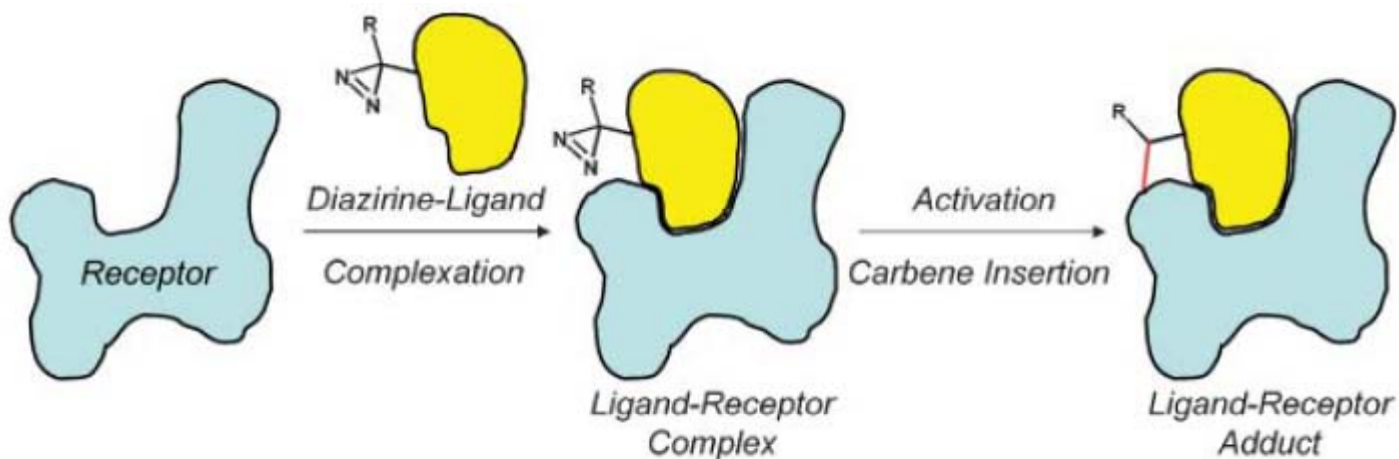
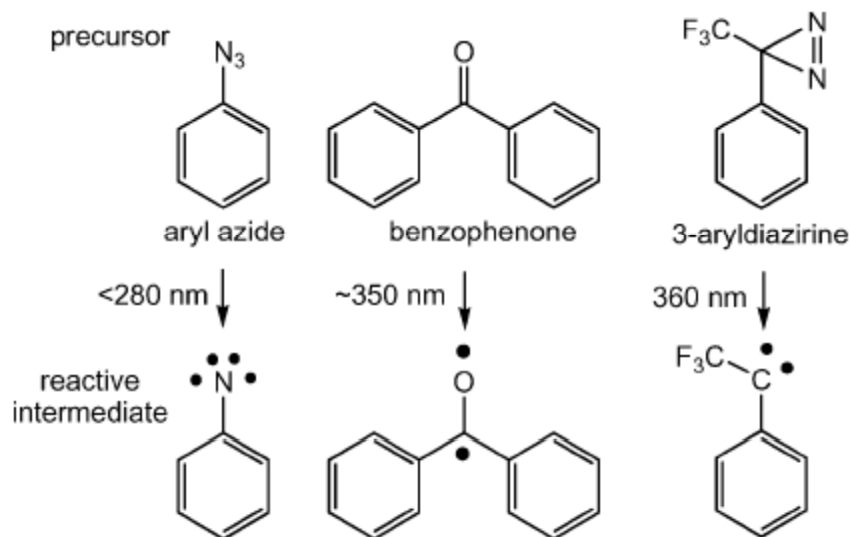
Photoaffinity Labeling

Identification of the Binding Site of the Antibiotic Linezolid

Photoaffinity labeling allows identification of initially reversible bound interaction partners by forming covalent bonds between them after activation of a photoreactive group with light.

Photoaffinity labels should be:

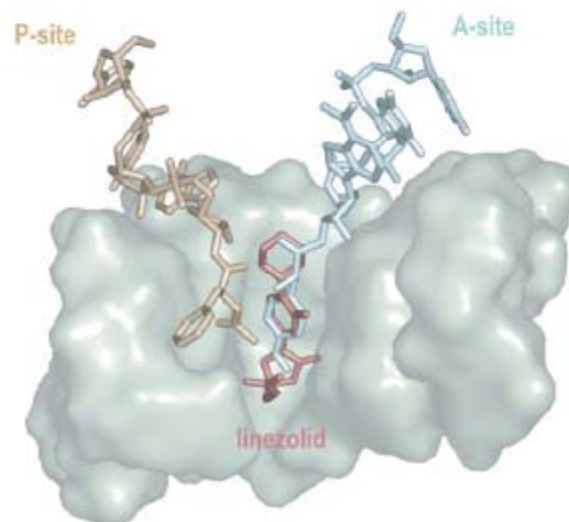
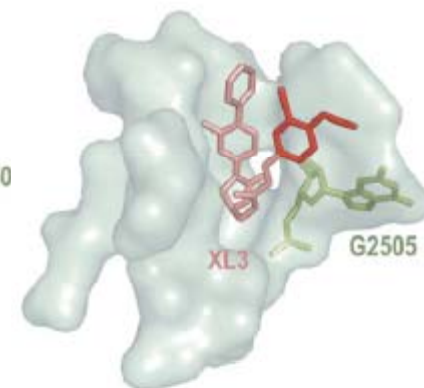
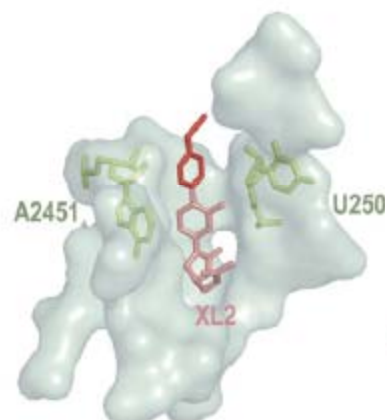
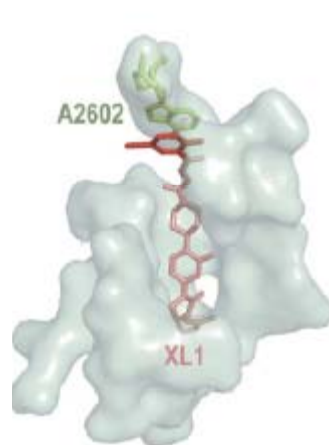
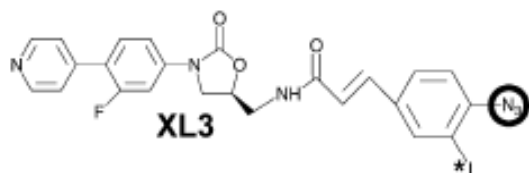
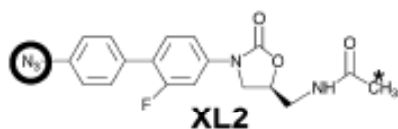
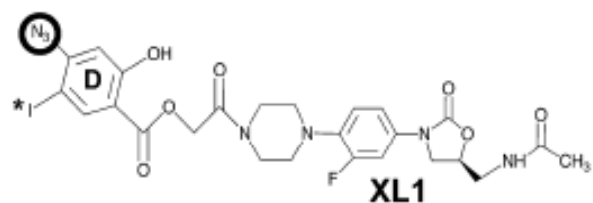
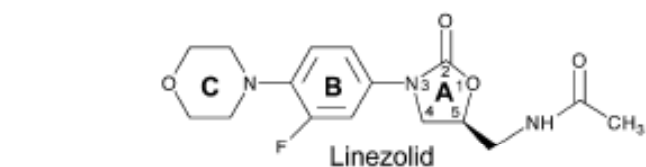
- Stable to daylight
- A photochemically excited state is generated. Life-time shorter than dissociation of the ligand-receptor complex.
- Should not react with the solvent
- Activation energy > 300 nm



1. Waldmann, H; Janning, P; Chemical Biology: Learning through Case Studies, Wiley-VCH 2009, Weinheim, Germany.
2. Hatanaka *et al*, *Eur. J. Org. Chem.* 2008, 2513–2523.

Photoaffinity Labeling

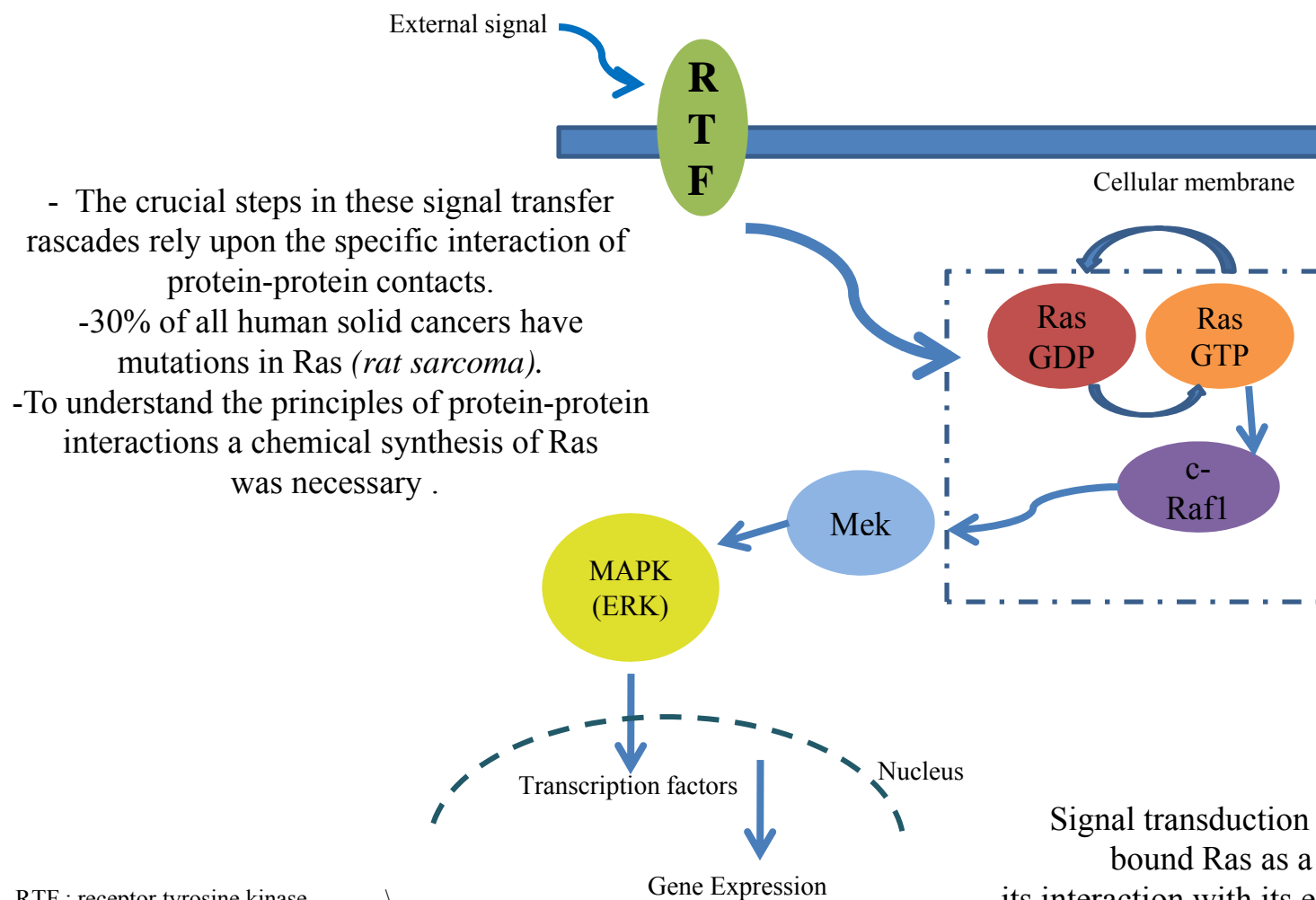
Identification of the Binding Site of the Antibiotic Linezolid



- All inhibited growth of *Staphylococcus aureus*
- Oxazolidinones prevent binding or proper placement of aminoacyl-tRNA in the peptidyl transferase active site.

Native Chemical Ligation (NCL)

NCL as a Tool for Chemical Protein Synthesis



RTF : receptor tyrosine kinase
GEF : guanine nucleotide exchange

Signal transduction cascade. Rol of membrane-bound Ras as a molecular switch and its interaction with its effector protein c-Raf1 (via the Ras-binding domain (RBD)).

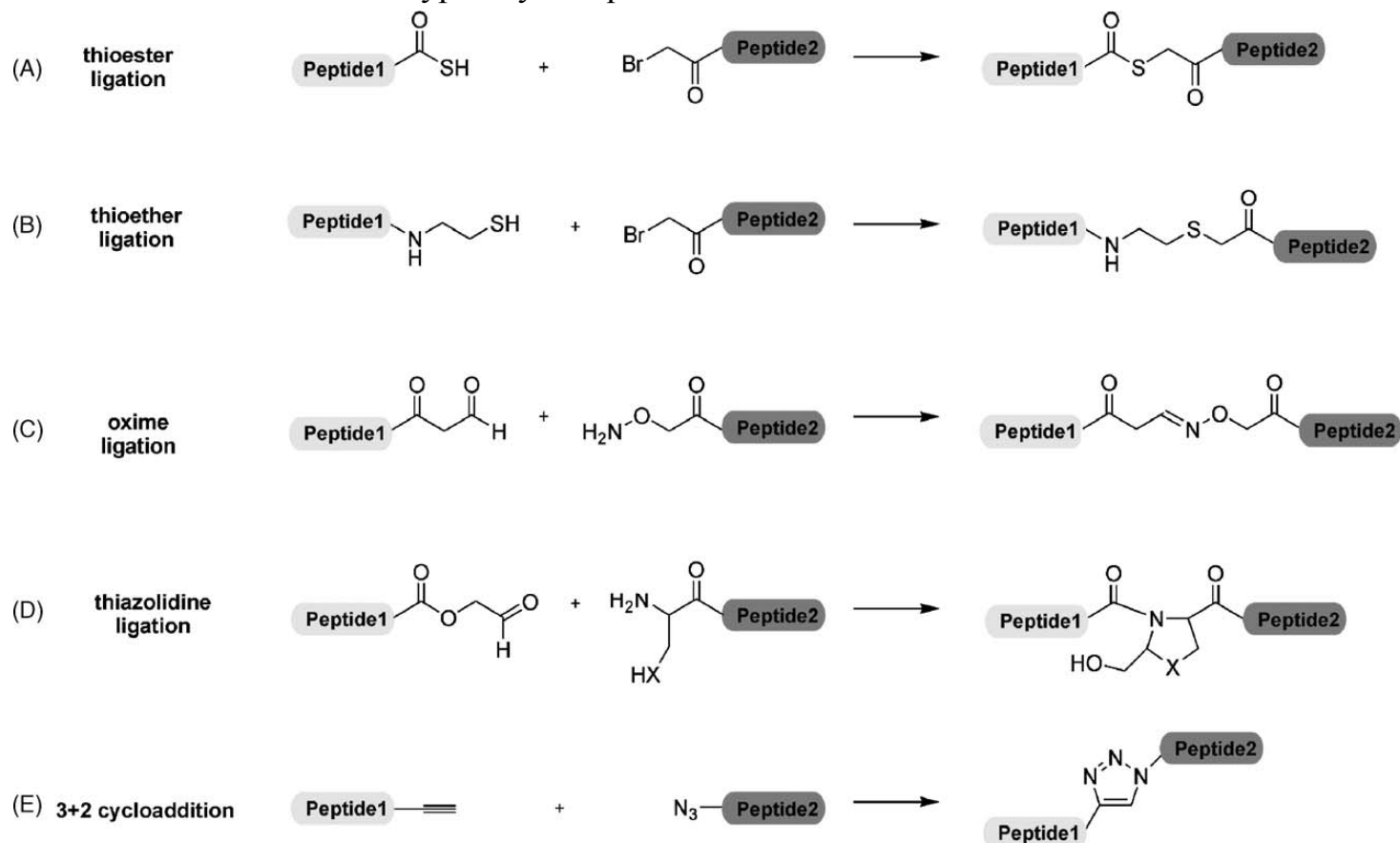
Native Chemical Ligation (NCL)

NCL as a Tool for Chemical Protein Synthesis

Native chemical ligation (NCL): a useful tool for the assembly of tailor-made small to medium-sized protein and protein domains from synthetic peptides.

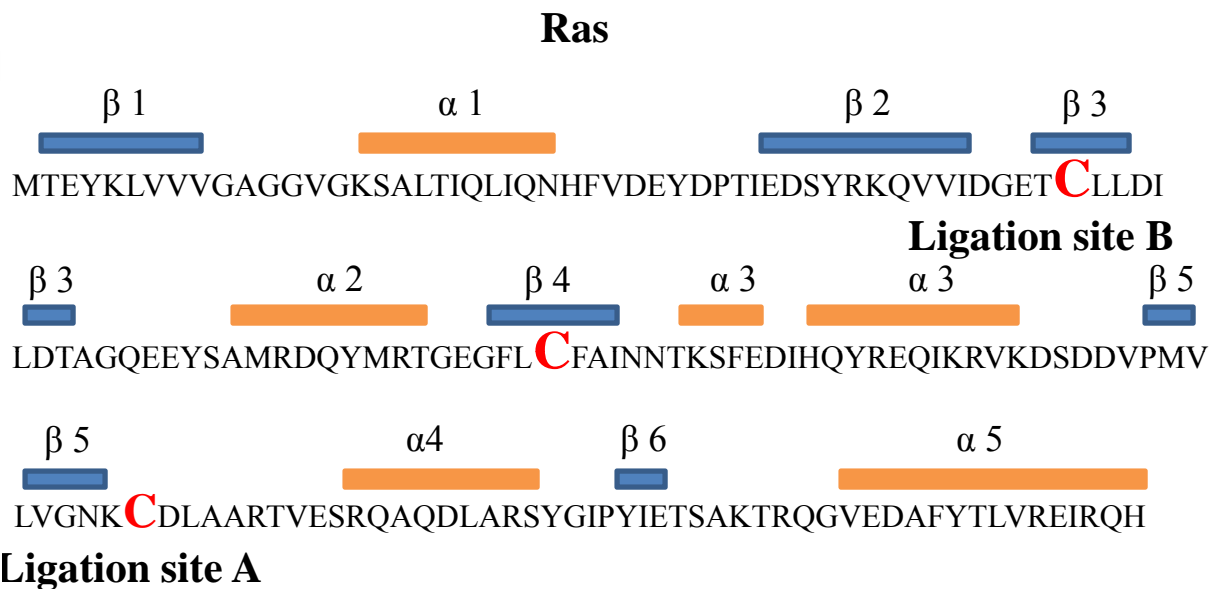
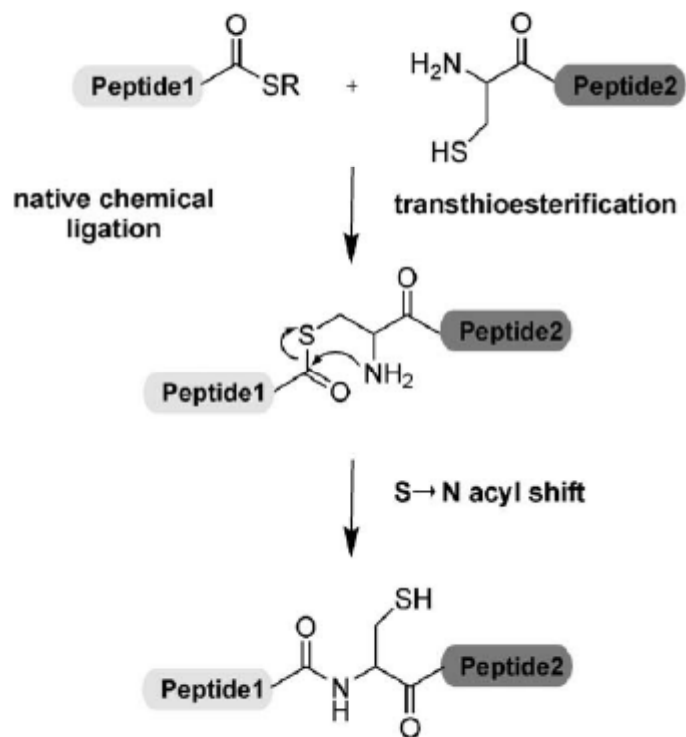
Sequences of 50 to 100 AA residues can be prepared through SPPS depending on sequence.

Chemoselective reactions: Used to modify (un)protected peptides or to selectively link two polymers, typically in aqueous environment.



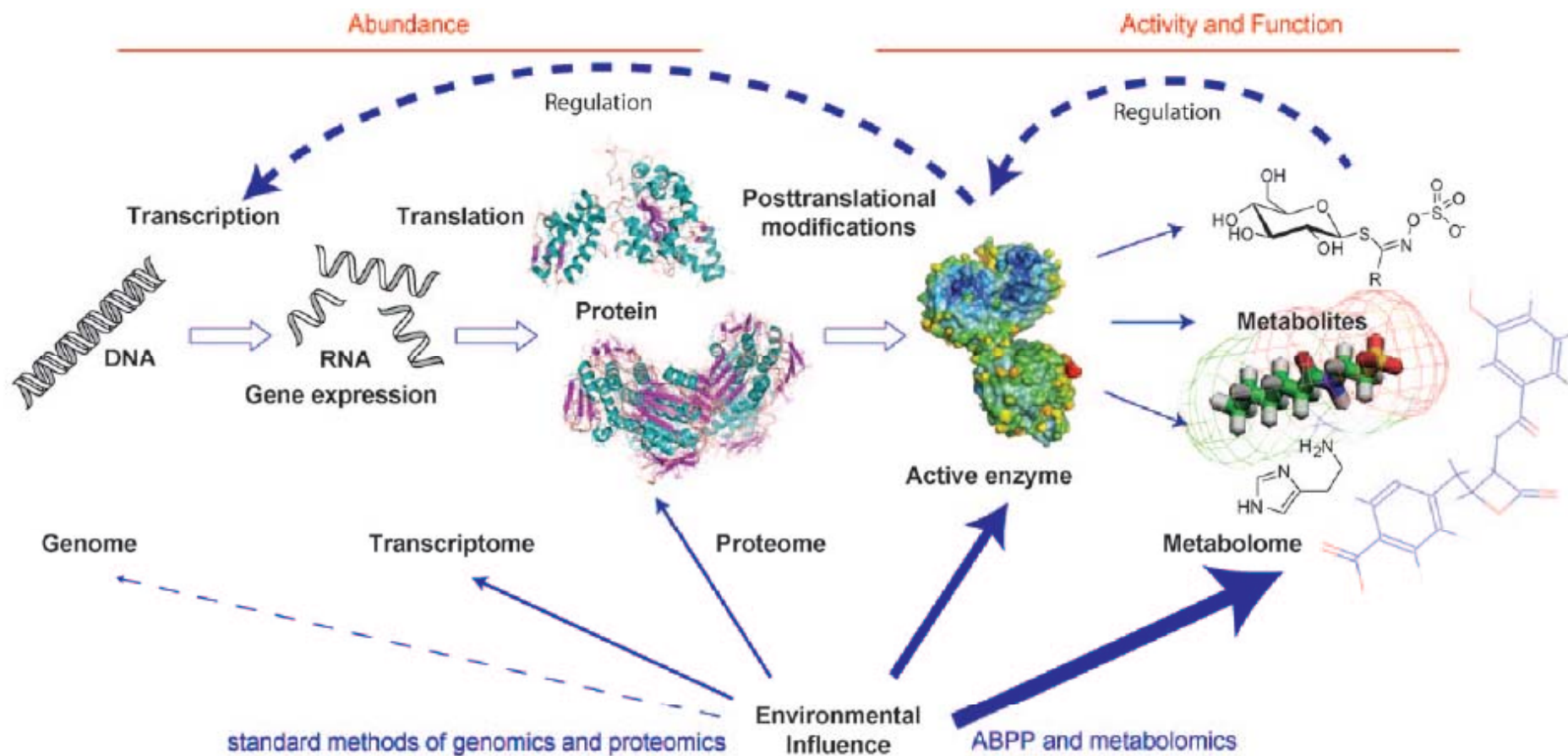
Native Chemical Ligation (NCL)

NCL as a Tool for Chemical Protein Synthesis



- Transthioesterification was introduced by Kent and co-workers in 1994.
- Limitation: requirement of a cysteine residue at the ligation site
- CD spectra of synthetic Ras adopted correct secondary structure.
- Kinetic parameters obtained from interactions of synthetic and recombinant Ras with RBD were identical.

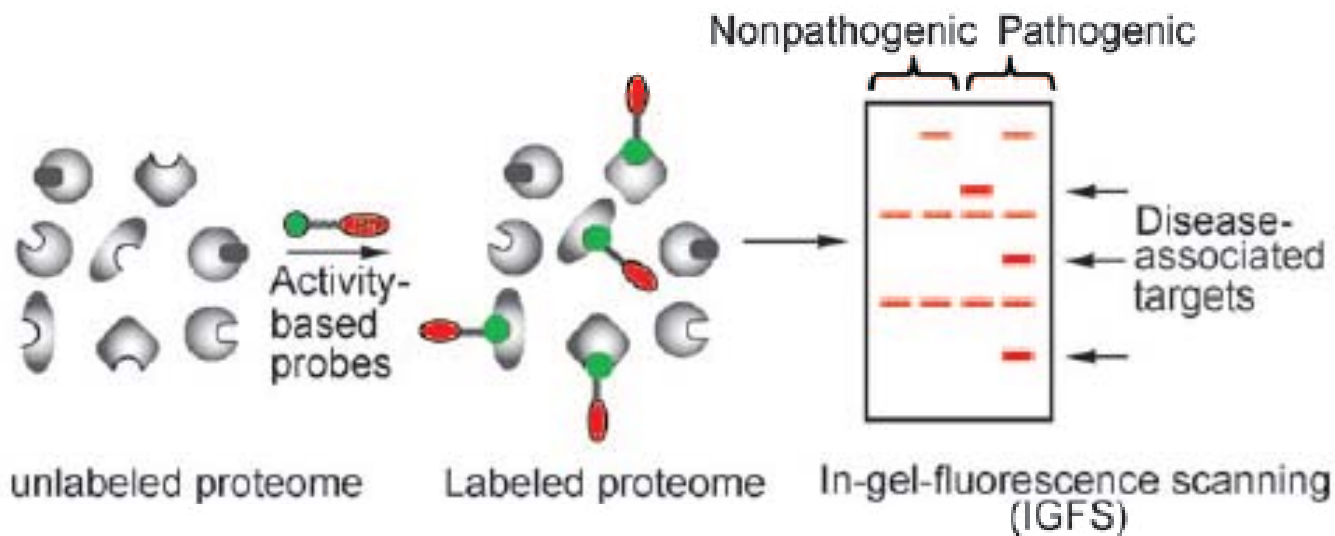
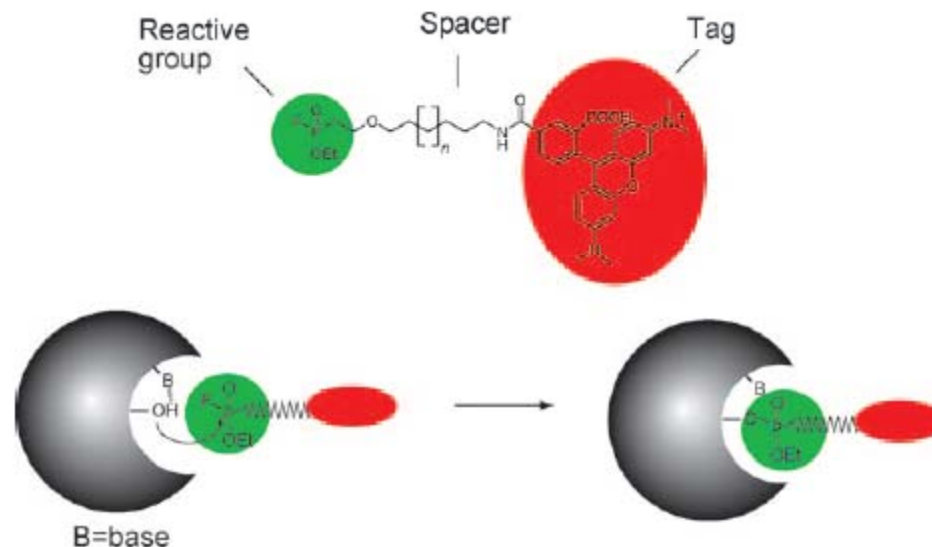
Activity-Based Protein Profiling (ABPP)



Cellular interactions through the regulation of proteins and metabolites. The essential Information regarding activity and function of biomolecules can not be determined with classical molecular biology.

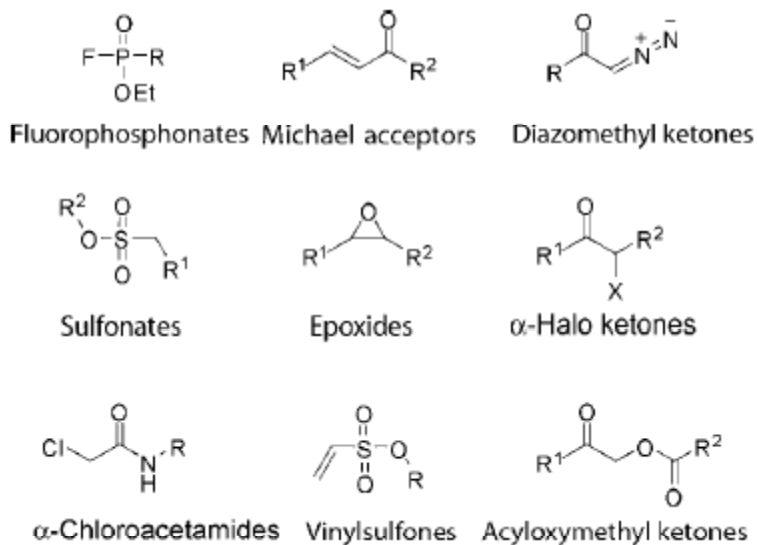
Activity-Based Protein Profiling (ABPP)

- ABPP makes possible to study the activity, function, and regulation of enzymes *in vitro* as well as *in vivo*.
- Small molecules that have high affinity for active sites of enzymes can be equipped for visualization (fluorescence dye) or enrichment (biotin).
- Enzymes not bound cannot be visualized.
- The pathogenesis-associated enzymes can be investigated more thoroughly and later serve as therapeutic targets.

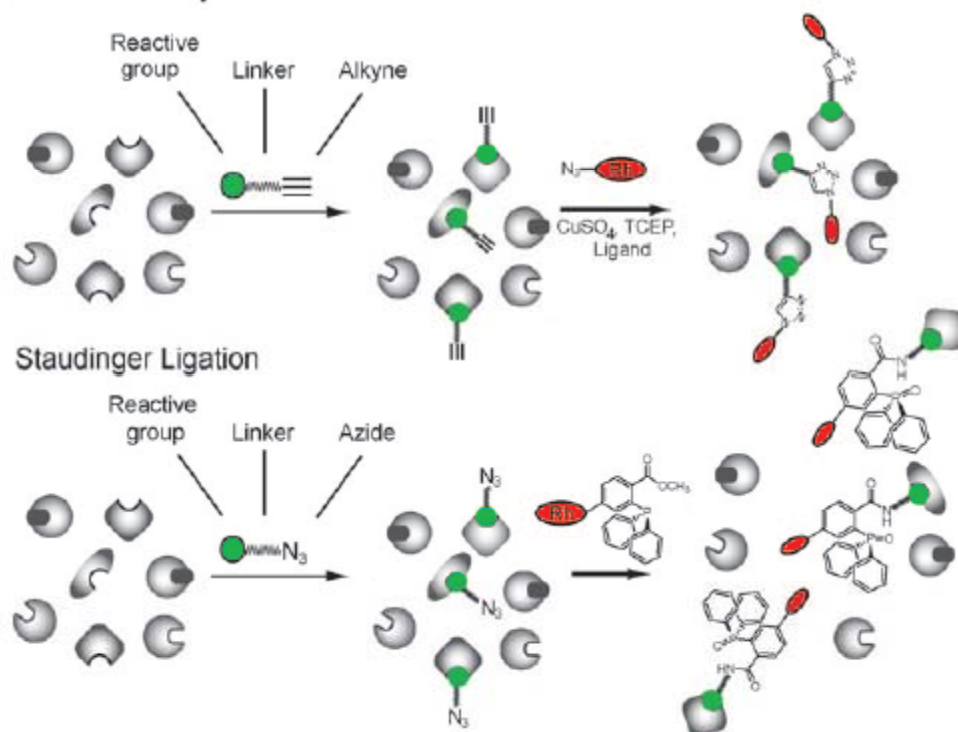


Activity-Based Protein Profiling (ABPP)

Reactive groups for ABPP



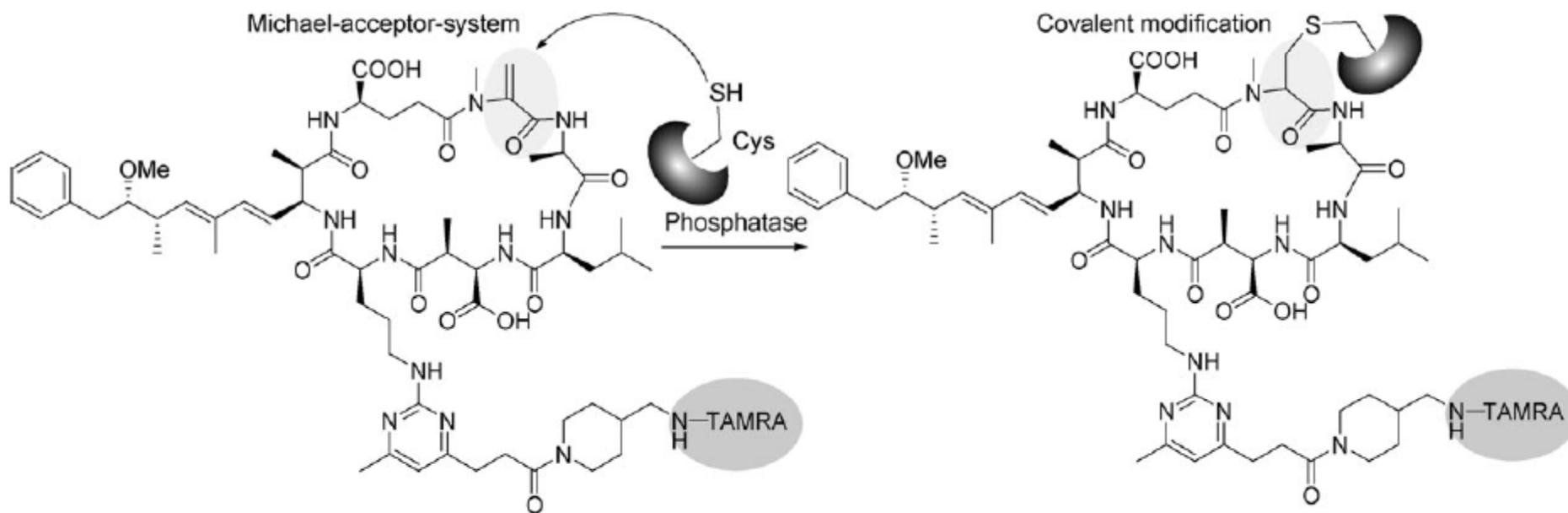
Click Chemistry



Orthogonal reactions can be conducted in aqueous media since they show slow reactivity towards other biomolecules such as DNA

Activity-Based Protein Profiling (ABPP)

ABPP probes based on natural products structures



- *Microcystins* are nonribosomally synthesized cyclic heptapeptides produced by cyanobacteria.
 - Inhibitors of the serine/threonine protein phosphatase families PP1 and PP2A.
 - They attack Michael-acceptor systems by a conserved cysteine in their active site.
 - Specific binding to Jurkat cell (immortalized T lymphocyte cell line) was found.

Fin