TETHERING: Fragment-Based Drug Discovery

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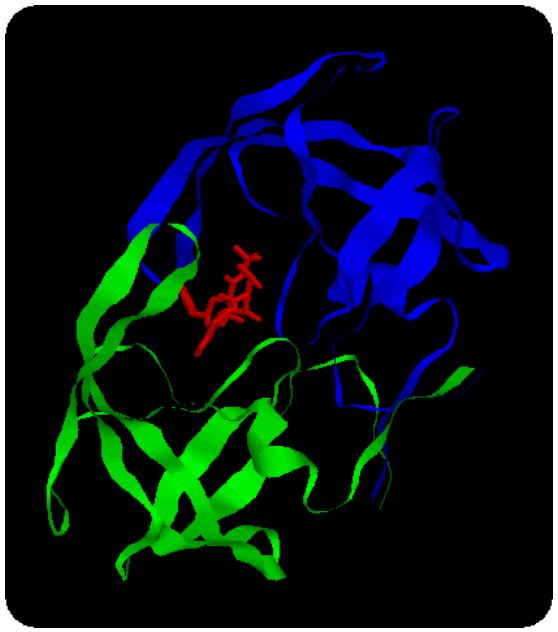
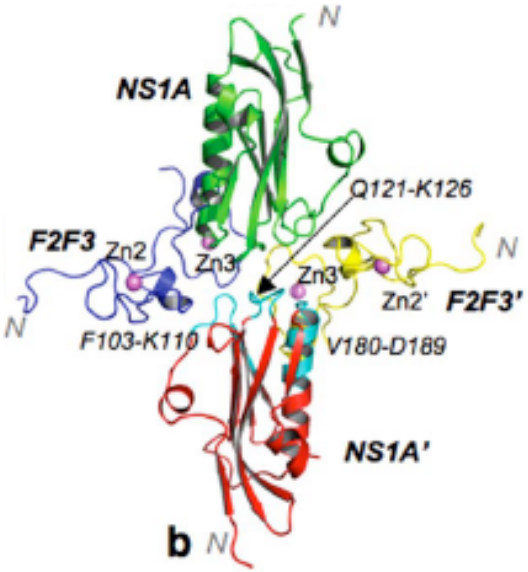
Explosion of New Drug Targets



Enzymes



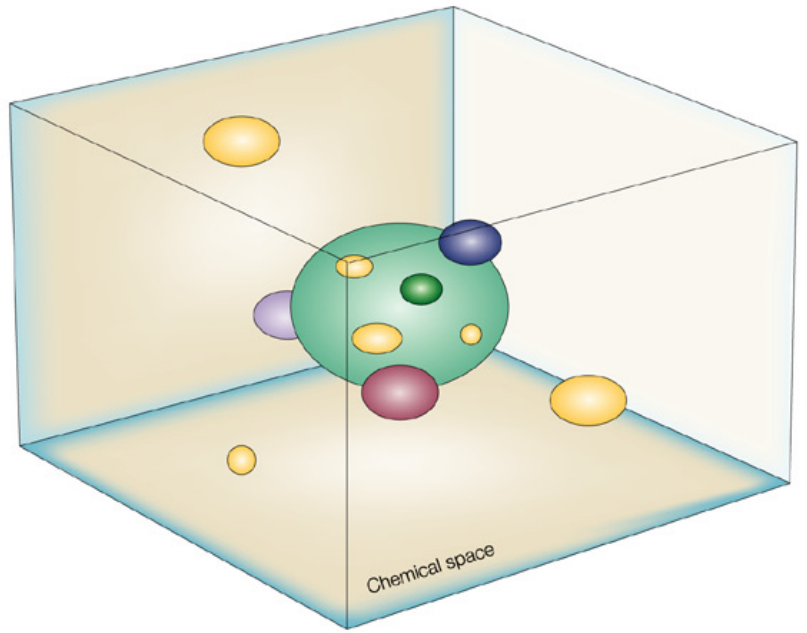
Protein to Protein Interactions

G-protein Coupled Receptors

The Problem

Chemical Space is Vast

- Imagine all sets of structures that could be made from a mixture of just 30 non-hydrogen atoms
- It is Multidimensional
- One estimate puts the possibilities at greater than 10^{60}

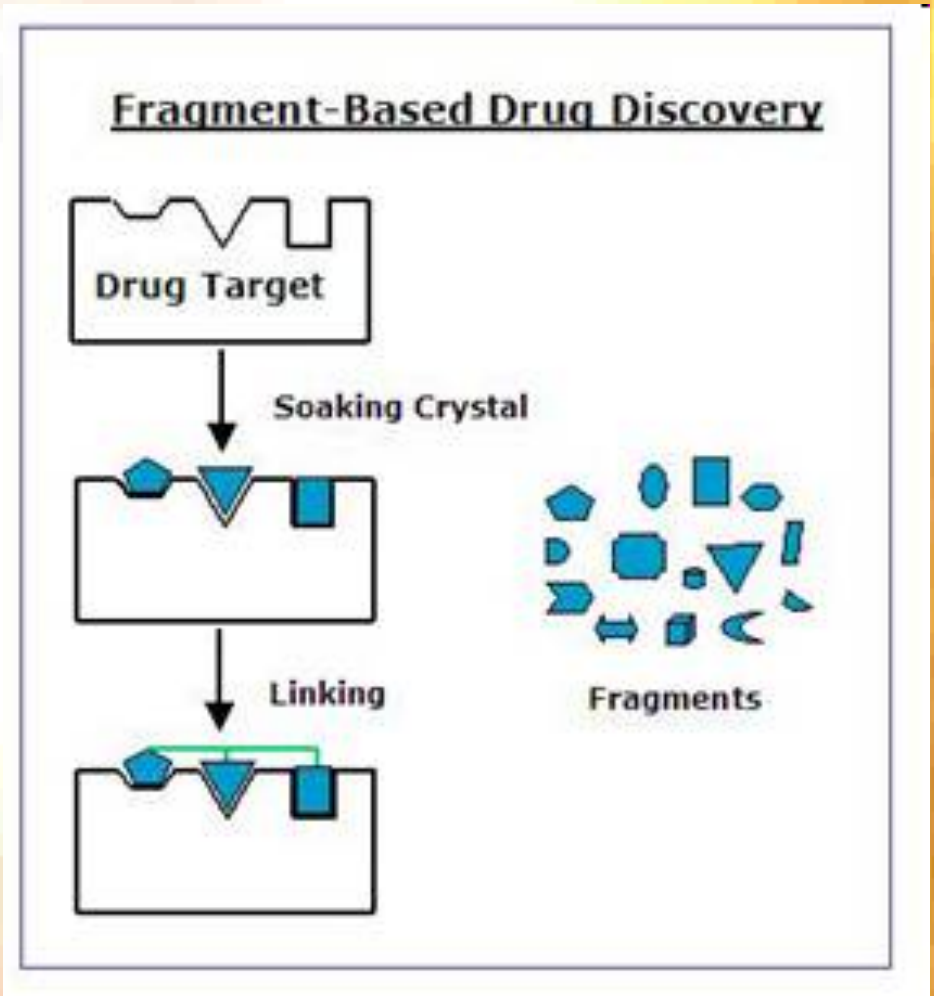


Solutions

- Use biochemical, enzymological, biophysical, and structural techniques to identify important binding interactions
- Screen smaller molecules and then either build from these or combine them.

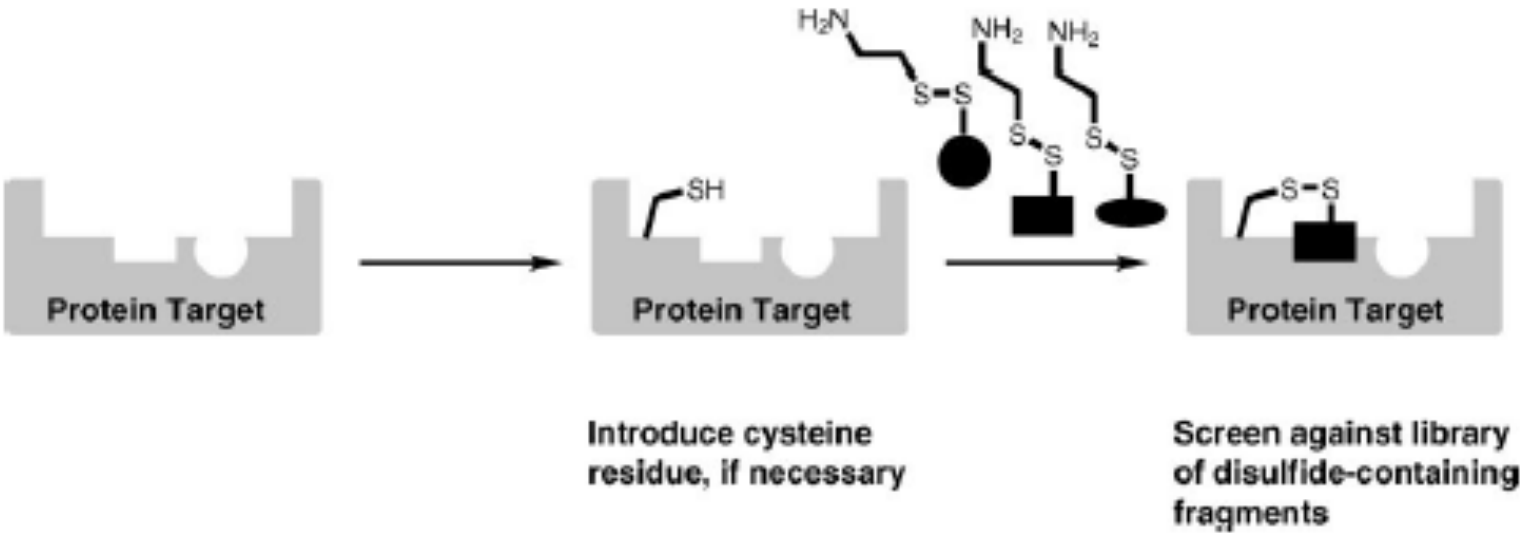
Fragment-Based Drug Discovery

- Link many small molecules with weak binding to form a molecule with strong affinity.
- Screen by positive selection rather than negative selection.
- Use techniques such as NMR, X-ray crystallography and Mass Spectrometry

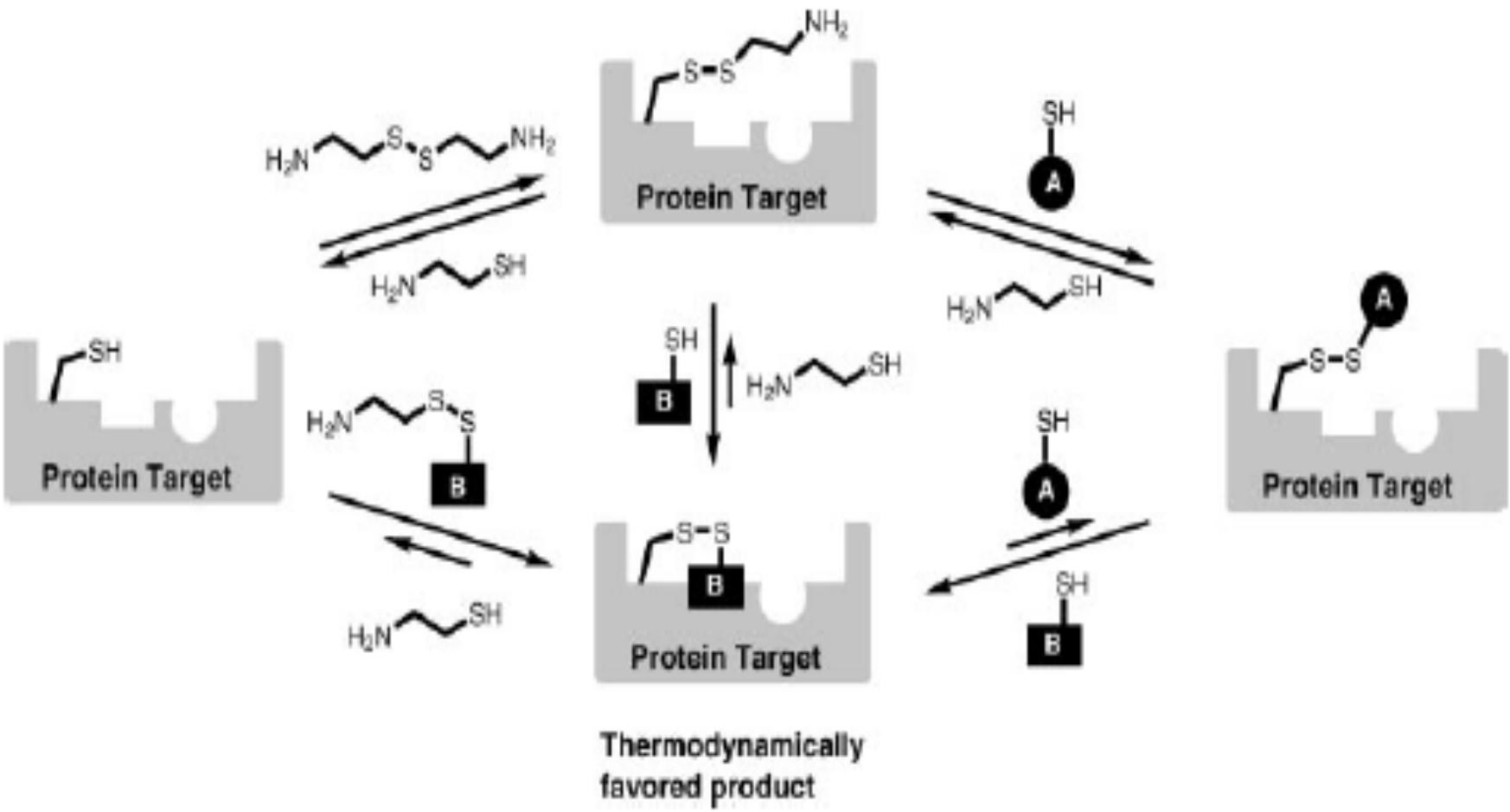


Tethering

1. Use of a cysteine near the targeted area which is either found naturally or introduced via site directed mutagenesis
2. Protein is reacted with a library of disulfide containing fragments in a partially reducing environment
3. Identify the dominate species using mass spectrometry



Tethering (continued)

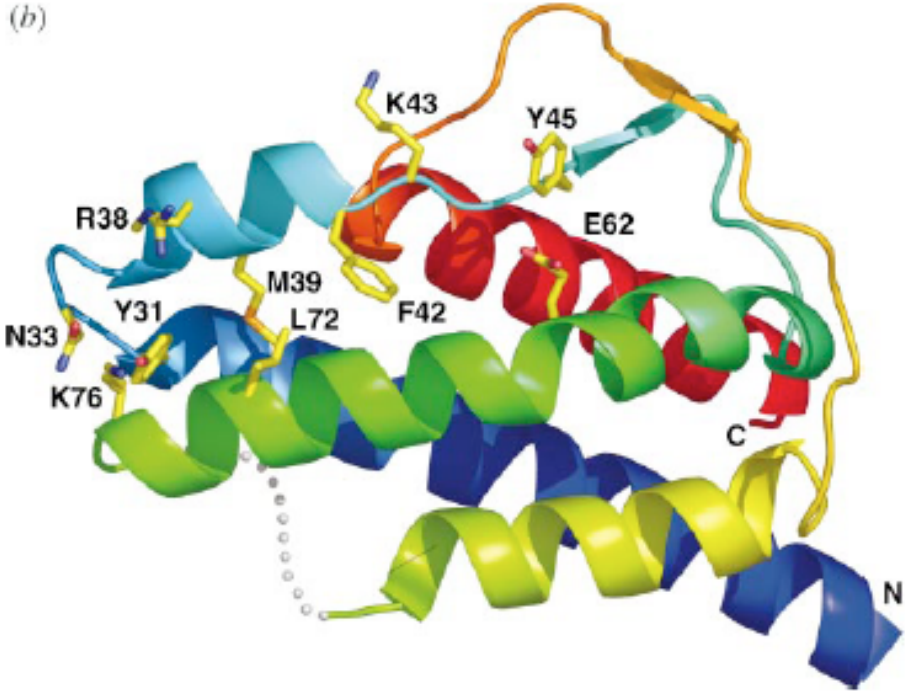


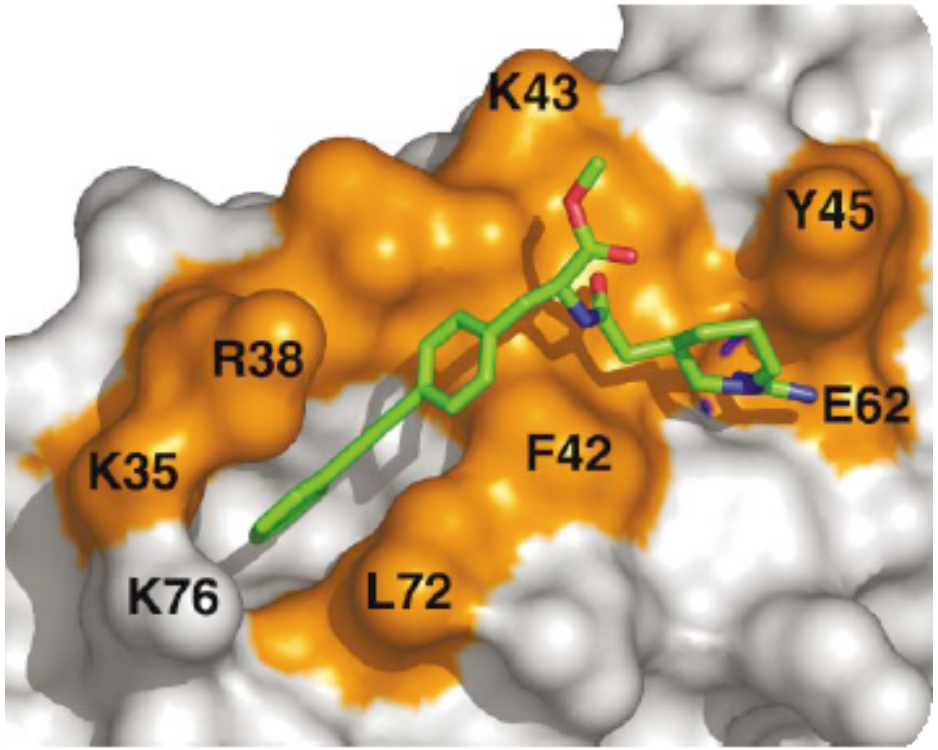
Advantages

- Screen at much lower concentrations
- Disulfide bonds can be formed and broken under mild conditions.
- Stringency can be altered

IL-2: Probing an Adaptive Binding Site

- cytokine interleukin-2 is an important protein involved in immune response
- Hoffman-La Roche found a molecule (compound 1) which bound to IL-2 with 3μM affinity.





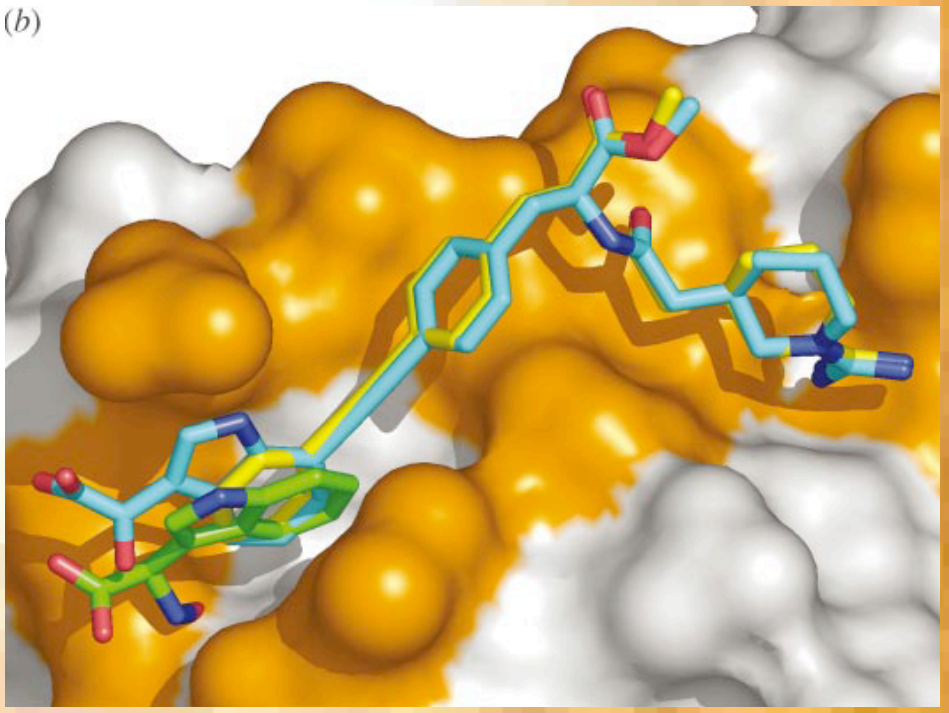
Results Summary

- Found a new target molecule (compound 2) that had a 500 μM kD
- Made a molecule that was a combination of compound 1 and 2 which had a 1.5 μM kD

Compound 1- yellow

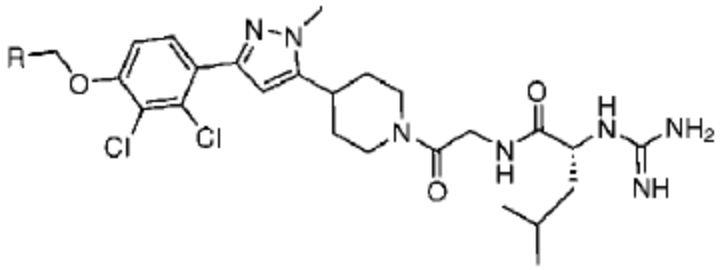
Compound 2- green

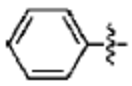
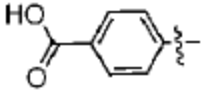
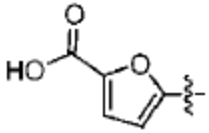
Compound 3- blue



Apply tethering to Medicinal Chemistry

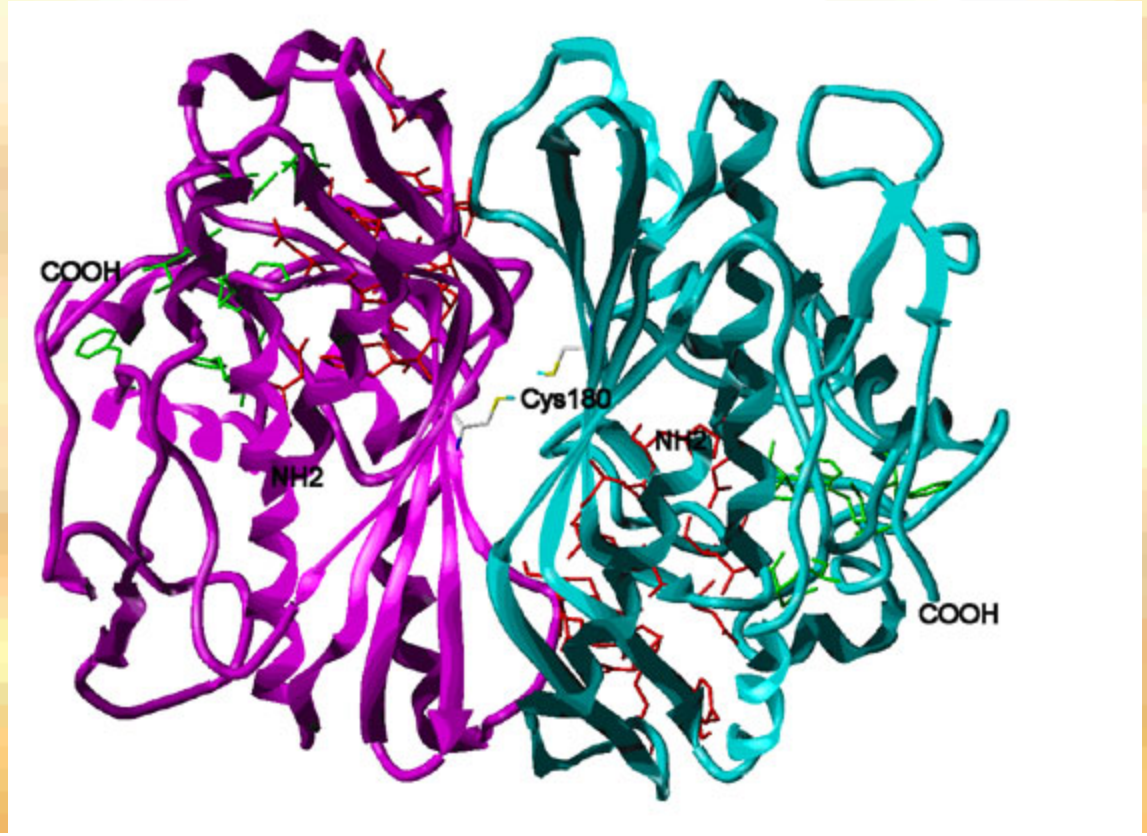
- Compound 4 identified using medicinal chemistry showed affinity in the 3 μM range
- Designed additional groups based on previous tethering experiments.

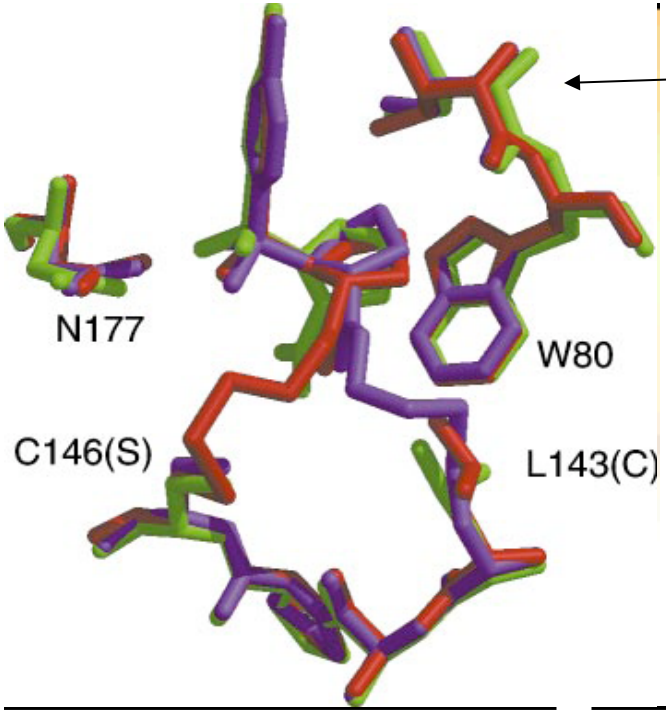


R			R			R		
	**5**	35 μM		**6**	0.20 μM		**7**	0.060 μM

Thymidylate Synthase: Discovering and Elaborating a Core Fragment

Tethering can be used not only to identify novel regions on a protein, but also to identify fragments that can bind in a known ligand site.



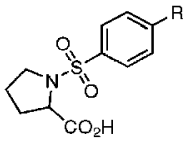


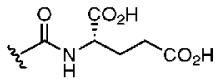
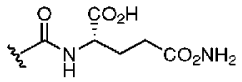
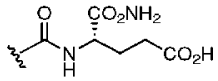
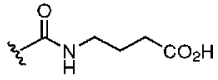
Key

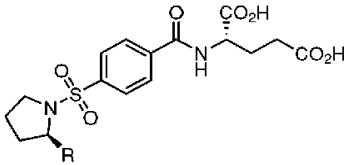
Green- *N*-tosyl-D-proline non-covalently bound to the enzyme

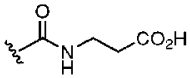
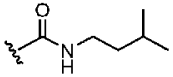
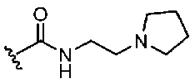
Purple- *N*-tosyl-D-proline covalently bound to L143C

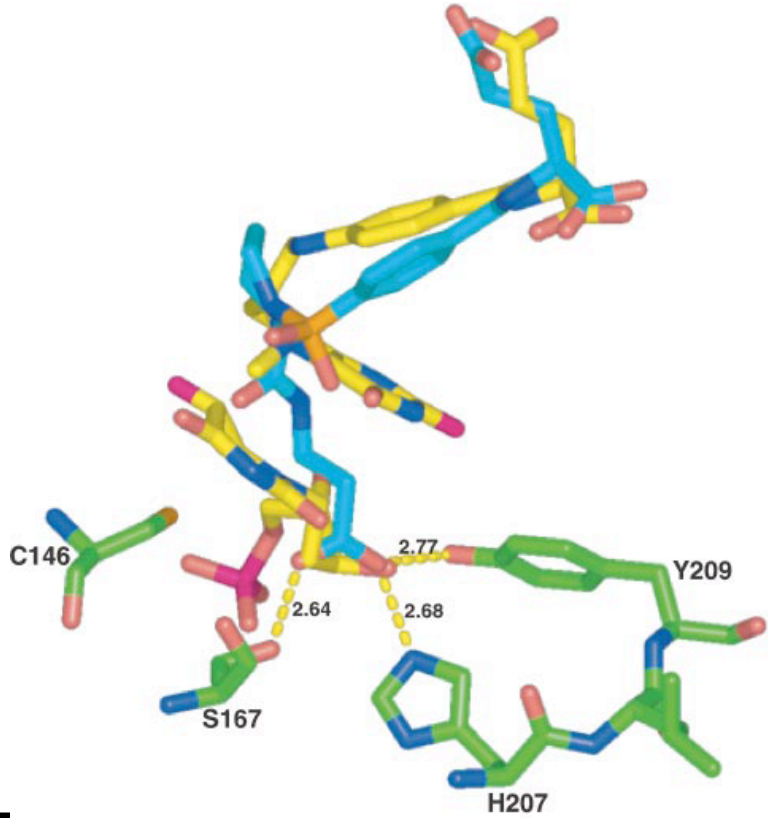
Red- *N*-tosyl-D-proline covalently bound to C146



Compound	R	K_i (μM)
8		1100
9		35
10		61
11		378
12		246



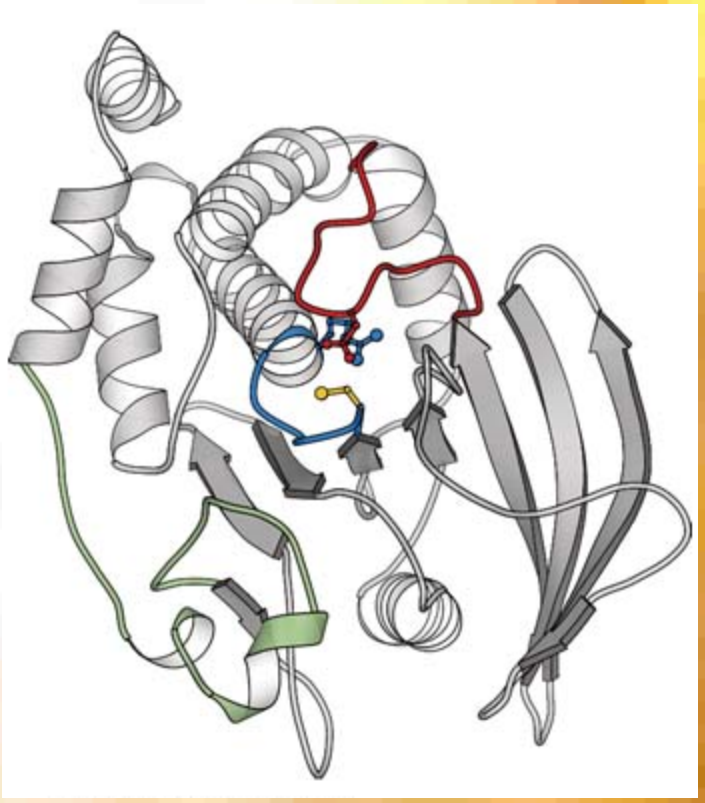
Compound	R	K_i (μM)
13		37
14		noncompetitive
15		0.33
16		12
17		> 100, only partially competitive

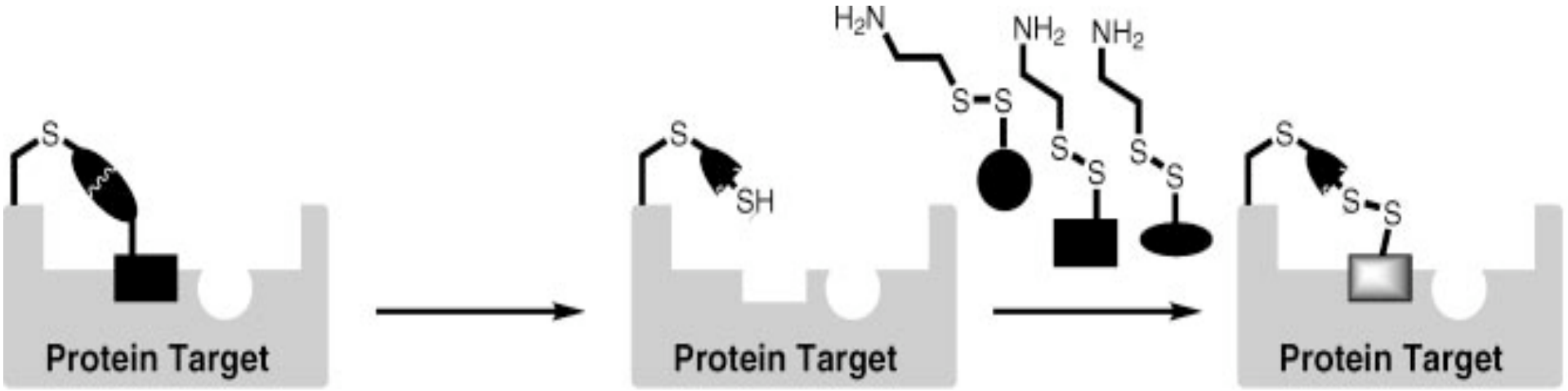


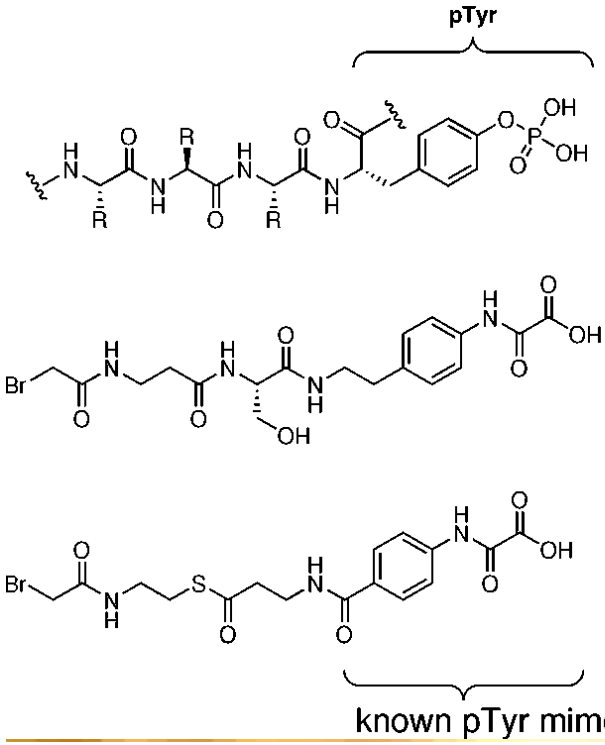
Target enzyme with small, easily disrupted active sites.

- Chose to work with Protein tyrosine phosphatases, and in this case **PTP-1B**

- Needed a different approach from the one used for Thymidylate Synthase







Generic peptide substrate

Prototype Extender **18**

Breakaway Extender **19**

Screened against a library of 15,000 fragments.

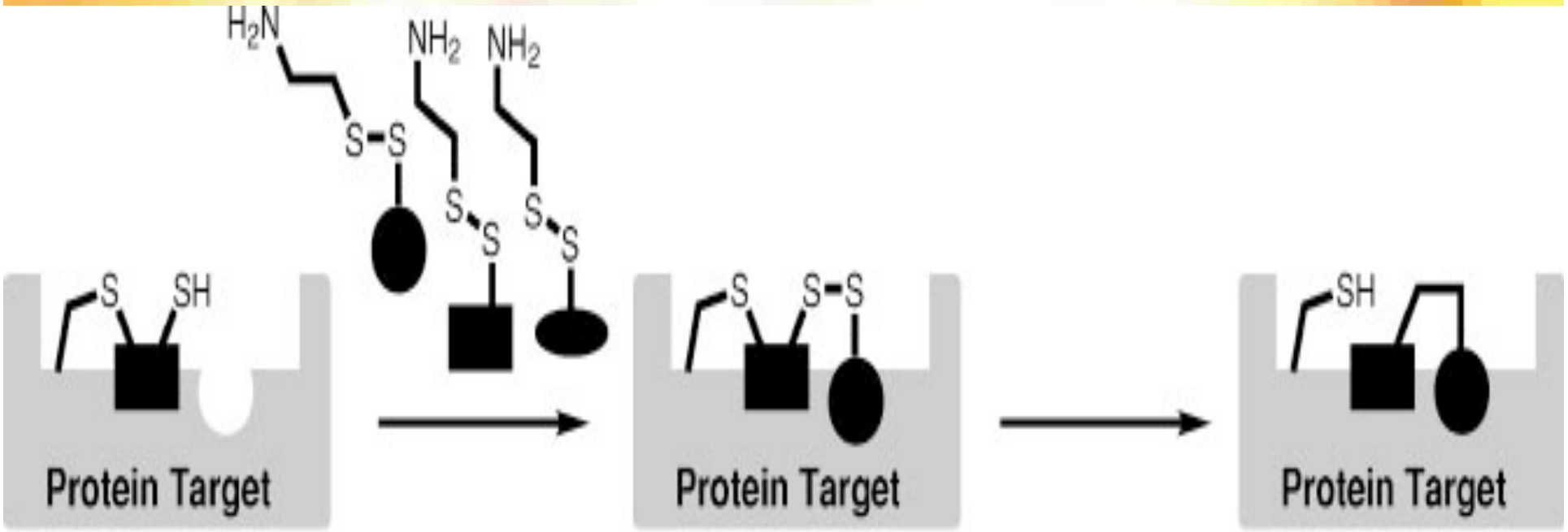
Most hits were negatively charged

Best hit had affinity of about 4.1mM

Seems very weak, but it is very similar to the natural substrates affinity of about 4.9mM

This hit is a good starting point to work from.

Assemble Fragments through Tethering



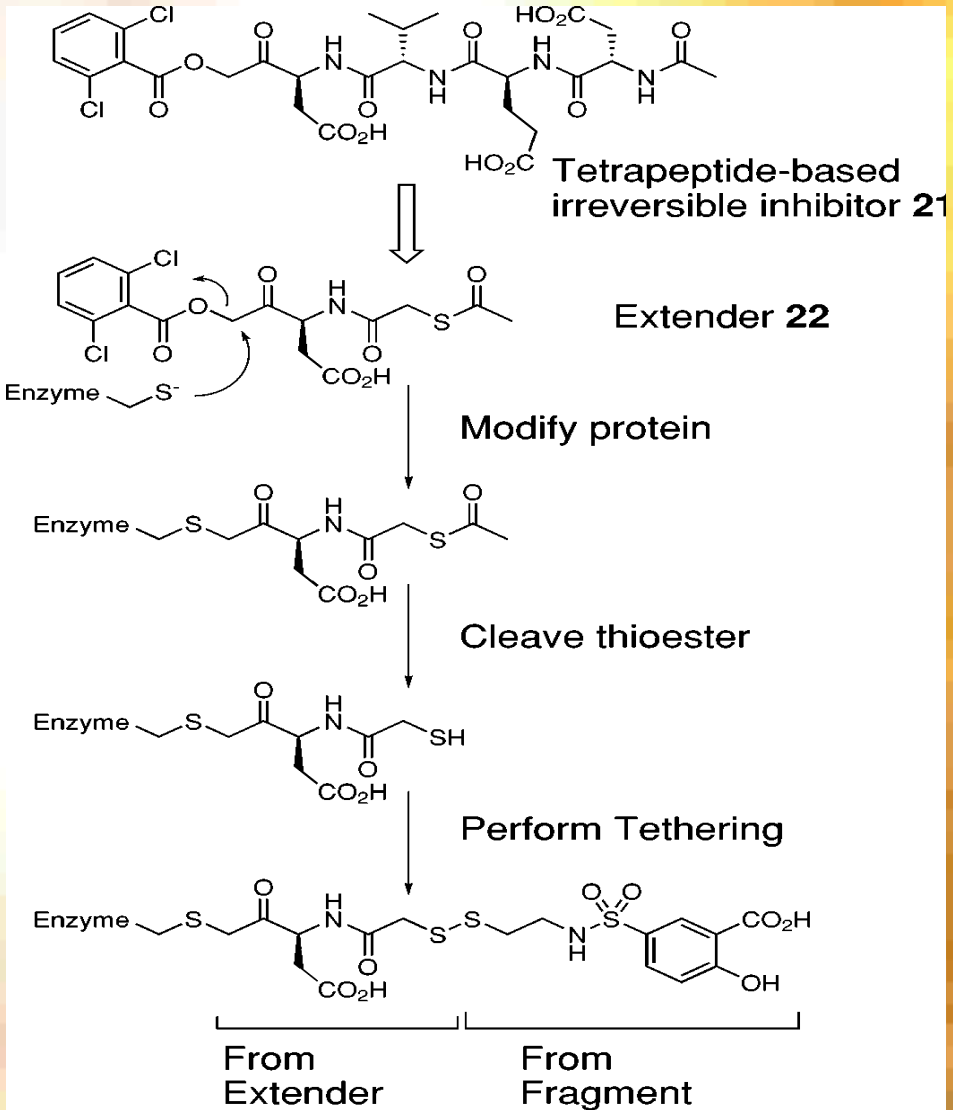
Introduce Extender, unmask thiol if necessary

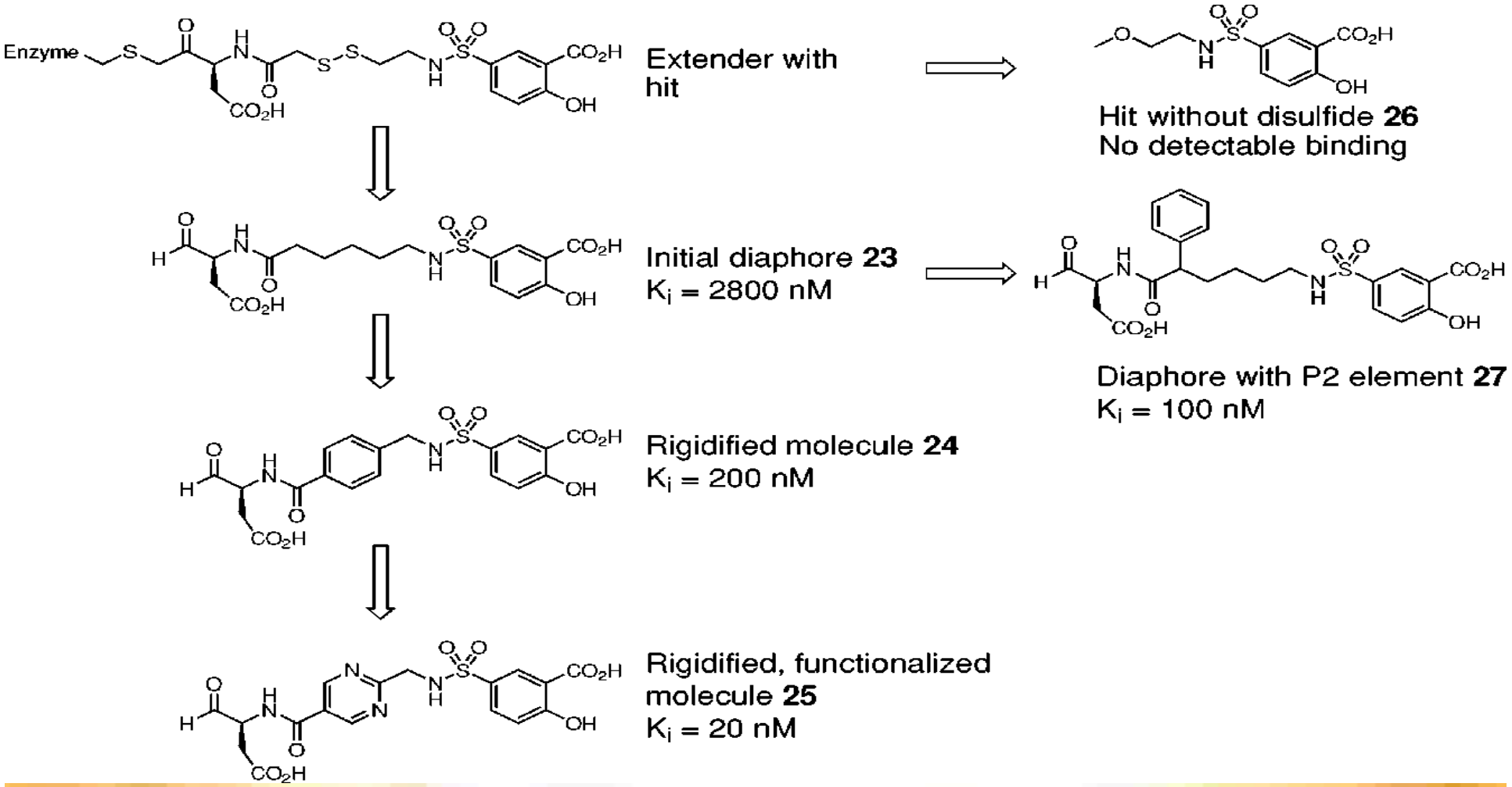
Screen against library of disulfide-containing fragments

Replace disulfide with alternate linker

Discovery of Nonpeptidic Caspase Inhibitors

- Great for this procedure because the active site contains a catalytic cysteine
- Irreversible inhibiters have already been designed





Since they know the distance between the extender molecule and the hit because they are connected by a disulfide bond, they can mimic the length in whichever linker they decide to synthesize.

This hit would have most definitely been missed through a High Throughput screen, and might very well have been missed using NMR because adding salicylic acid at even high millimolar concentrations by itself demonstrated no measurable inhibition of the enzyme.

Looking Forward

- Since fragments based drug discovery relies on weak interactions it is important that they are selected for at one to one ratios with their target molecule.
- The disulfide bond does not compromise the structure as shown by X-ray Crystallography
- Has many advantages over functional based screens eg. Can target kinases in active and inactive form

Citations

- Erlanson, Daniel. "TETHERING: Fragment-Based Drug Discovery." Biophys. Biomol. Struct 33 (2004): 199-223.
- Das, K.; Ma, L-C.; … Krug, R.M.; Montelione, G.T. Proc. Natl. Acad. Sci. U.S.A. 2008, 105: 13092 - 13097. Structural basis for suppression by influenza A virus of a host antiviral response.
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- http://projects.villa-bosch.de/mcm/pictures/img/img3_4_full