Chemical Biology
In-class discussion: "Tethering"

This discussion will focus on various "Tethering Methods" described by workers at Sunesis (for a review, see Erlanson *et al. Ann. Rev. Biophys. Biomol. Str.* **2004**).

Regarding Erlanson *et al. JACS* **2003.**

a. This paper describes the discovery of a phosphotyrosine mimetic as a potential protein tyrosine phosphatase (PTP) inhibitor using "breakaway tethering". What reaction do PTPs catalyze?
b. Summarize what advance this method offers over the more straightforward "tethering" method introduced on Tuesday.
c. How do these workers direct alkylation selectively to the distal cysteine thiol? How do they verify that their strategy was successful?
d. What is the point of Figure 2?
e. Describe the ways the autors validate that the "extender" provides an advantage in the inhibitor-discovery process.
f. One can imagine combining this method with phage display to identify conjugates of short peptides and small molecules that bound cooperatively to a protein surface. How might this work?

Regarding Erlanson *et al. Nat. Biotech.* **2003.**

a. Tethering is in many ways a variation of dynamic combinatorial chemistry. What is dynamic combinatorial chemistry?
b. In both papers this approach was used to discover inhibitors of enzymes that process peptides and/or proteins. Why do the authors claim that this method is ideally suited for the discovery of inhibitors of this type?
c. Describe the potential of this method to increase the size of the "druggable genome".
d. Explain how the aryloxymethyl ketone "warhead" works.
e. The tethering strategy (in all of its manifestations) offers a strategic advantage in the drug discovery process. What might this advantage be?
f. At the end of the day, this paper describes a reasonably (although not incredibly) selective inhibitor for caspase 3. How might you alter their approach to increase the chance that a selective inhibitor will be identified?

Chemical Biology
In-class discussion: "Liu vs. Harbury"

Consider the DNA Display method described by Halprin & Harbury (PLOS **2004**)

a. Explain how this method works.
b. Sometimes the machines that synthesize oligonucleotides make mistakes and delete a base during synthesis. How would this deletion be detected? How would it affect the routing scheme?
c. The conditions used to route the DNA in this scheme seem pretty harsh – 1.5 M NaCl! What is the likely role of the high salt concentration?
d. What are three advantages of DNA display over DNA-templated synthesis?
e. Are there any disadvantages?
f. What other uses might be imagined for this method?
g. Explain how this method could be used to prepare 10^{12} distinct small molecules from 30 pmol DNA starting material.
h. What improvements could you imagine that would further increase the diversity of the pool or the ease with which this method can be applied?

Chemical Biology
In-class discussion: Molecular arrays

I. Questions about Newman & Keating, *Science* **2003**, 300, 2097.

1. Why are these researchers interested in the pairing specificities of bZIP proteins?
2. Ordinarily, it would be extremely time-consuming to purify 49 different proteins. What clever trick did these researchers use to simplify the purification process?
3. What chemistry did these researchers use to immobilize the peptides on the glass slide? Does this method lead to homogeneity or heterogeneity within an individual spot? Can you think of a method that would lead to the other alternative (homogeneous or heterogeneous)?
4. How did the researchers insure that the bZIP peptides they prepared were presented on the slides as monomers?
5. What is meant by the statement "the interaction array showed very high symmetry" and why is this observation important?
6. What controls did these researchers perform to provide evidence that the interactions between the proteins were specific?
7. What experiments did they perform to demonstrate that the interactions involved formation of a coiled coil?
8. Name three experiments that build on the results described herein. What do you think these workers are doing now? What would you do if you were in the Keating Lab?

II. Questions about MacBeath et al., *JACS* **1999**, *121*, 7967.

1. Why are these researchers interested in presenting small molecules in arrays?
2. The method described in this paper is different than the "on-bead binding assay" described previously. What is the "on-bead binding assay" and what benefits do molecular arrays have over the "on-bead binding assay"?
3. What chemistry did these researchers use to immobilize the small molecules on the glass slide?
4. Does this method lead to homogeneity or heterogeneity within an individual spot? Can you think of a method that would lead to the other alternative (homogeneous or heterogeneous)?
5. How does this chemistry limit the types of molecules that can be displayed on the slide?
6. What controls did these researchers perform to provide evidence that the observed interactions were specific?

7. What are two limitations of this method as a way to assess small molecule-protein interactions? How do these limitations compare to ones in which the protein is immobilized?
8. Name three experiments that build on the results described herein. What do you think these workers are doing now?

Chemical Biology
In-class discussion: Chemical complementation

1. List three criteria that should be considered when designing a molecule that can recruit two receptors at once (a "chemical inducer of dimerization").
    a. modification must not decrease affinity or specificity
    b. modification molecule must be easy to synthesize
    c. modification should not alter cellular localization

2. Let's say you have synthesized a potential CID, and you are interested in determining if it binds its respective receptors with affinities that are comparable to the wild type molecule.
    a. How would you design and analyze this binding experiment?
    b. How would you determine if binding of the CID to receptor A was altered by the presence of a bound receptor B?
    c. What techniques would you use to monitor binding?

3. In Lin et al. 2000, it was observed that *lacZ* transcription was dependent on the concentration of Dex-Mtx when [Dex-Mtx] ranged between 0.01 and 10 μM.
    a. Should this correlation hold at concentrations much higher than 10 μM.
    b. These authors also observed that addition of a 10-fold excess of Ex did not decrease the extent of laxZ transcription (while a 10-fold excess of Mtx did). What do you think about the author's explanation of the seemingly failed control experiment?
    c. Why is Dex-Mtx a better CID than Dex-FK506?

4. The authors of Baker et al 2002 describe an interesting strategic choice. They chose to insert a bond to be *cleaved* between Mtx and Dex, and select for molecules that decrease lacZ transcription, rather than inserting a bond to be *synthesized* between Dex and Mtx, and selecting for increased lacZ transcription.
    a. Was this choice wise? What are two advantages and disadvantages of each strategy?
    b. Can this method be extended easily to identify enzymes that do not make or break a bond between the activation and DNA-binding domains?