Epitope-targeted peptide inhibitors of Myc-Max Dimerization

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Motivation and Objectives

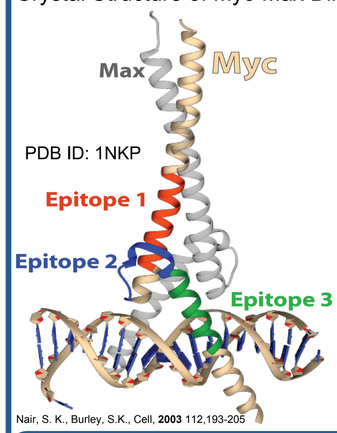
Motivation: Myc dimerizes with Max to promote the transcription of genes associated with cellular proliferation, differentiation, and survival. Deregulation of Myc expression initiates and maintains approximately 30% of human cancers, making Myc an excellent target in oncology.

Objective: Develop potent inhibitors of Myc-Max dimerization that are suitable as an *in vivo* therapy.

Challenge: Myc-Max dimerization is difficult to inhibit due to the relatively large interface over which Myc-Max interactions occur. As a result, Myc has been considered "undruggable."

Introduction

Crystal Structure of Myc-Max Dimer



- Myc-Max dimers recognize DNA and promote transcription
- Deregulation of Myc initiates and maintains nearly 30% of human cancers
- Large inter-protein interface makes Myc-Max dimerization difficult to inhibit

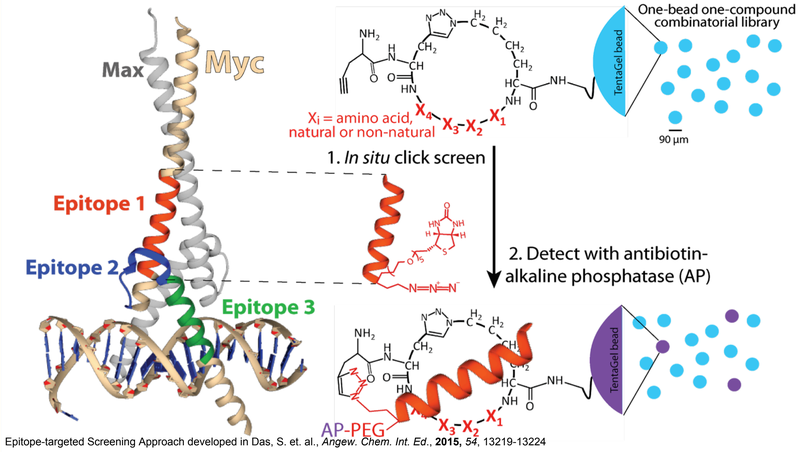
Nair, S. K., Burley, S.K., Cell, **2003** 112,193-205

Hypothesis

We hypothesize that epitope-targeted peptide ligands that adsorb at the Myc-Max dimer interface will disrupt the interprotien interactions and prevent dimerization.

Gabay, M., et. al., *Cold Spring Harbor Perspectives in Medicine*, **2014**, *4*

Approach: Epitope-targeted Capture Agents

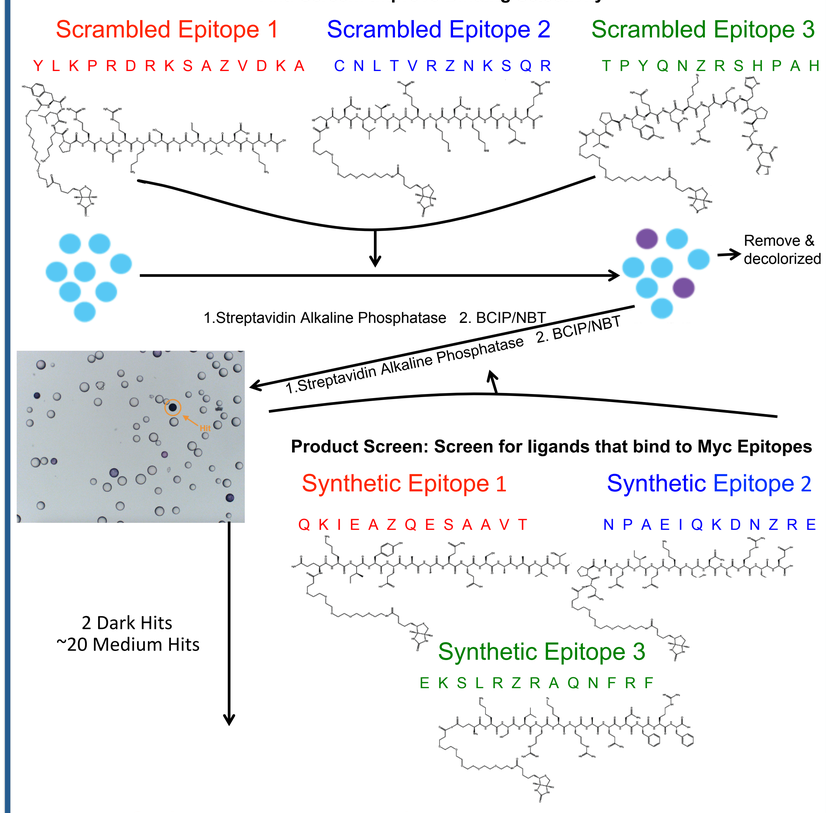


Epitope-targeted Screening Approach developed in Das, S. et. al., *Angew. Chem. Int. Ed.*, **2015**, *54*, 13219-13224

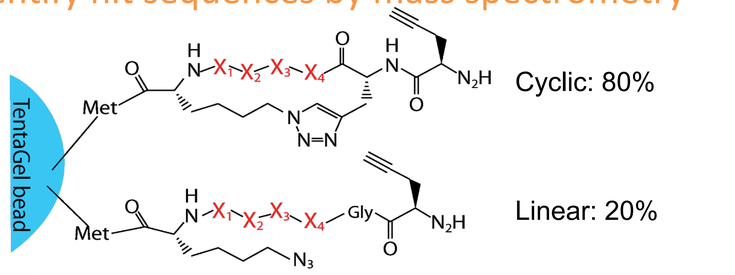
- Typically yields ligands with high affinity (K_D of 100 pM - 10 uM) to target epitope
- Rapid sub-month development time
- Judiciously target three epitopes on Myc dimer interface

In situ click screen to identify high-affinity ligands to the Myc dimer interface

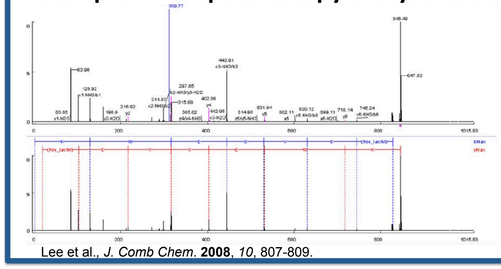
Anti Screen: Improve Binding Selectivity



Identify hit sequences by mass spectrometry



Example mass spectroscopy analysis to sequence a peptide



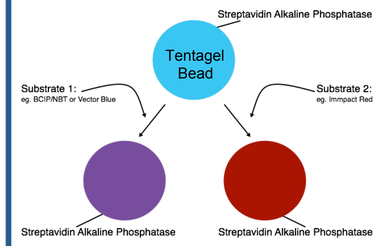
Lee et al., *J. Comb Chem.* **2008**, *10*, 807-809.

- Linear peptides can be sequenced by using Mass Spectroscopy
- Library encoded by 20% linear peptides
- Collaborate with Indi Molecular to sequence hits from our screen

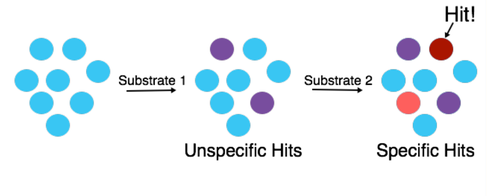
Faster Screening with Counterstaining Strategy

Motivation: Eliminate need to manually remove beads from anti-screen (1 day to 2 weeks)

Example Enzymatic Development Scheme

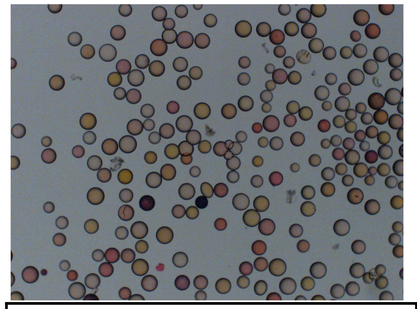


Counterstaining Approach

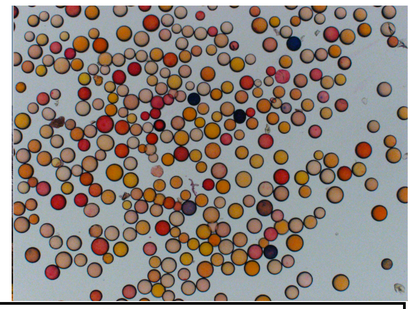


Optical Images of Enzymatically-Developed Beads

BCIP/NBT (purple) and ImmPact Red (red/orange)



Vector Blue (blue) and ImmPact Red (red/orange)



- Counterstain procedures yield different colored beads for anti-screen and product screens

- Yields different colored beads for anti-screen and product screens
- Some beads exhibit yellow color, which likely interferes with pre-clear
- Red and blue beads have unique fluorescence

Summary

Conclusion

- Discovered 2 strong and ~20 medium peptide binders to Myc-Max dimer interface
- Counterstaining is a promising approach to accelerate screening for epitope-targeted peptides

On-Going Work

- Optimize counterstaining procedures
- Identify Myc ligand hits, with Bert Lai at Indi Molecular
- Synthesize ligands and test their efficacies to inhibit Myc-Max dimerization

Acknowledgements

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