



# The Rapid Creation and Screening of Peptidic-Macrocylic Libraries against Protein-Protein Interactions

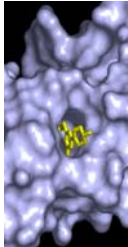
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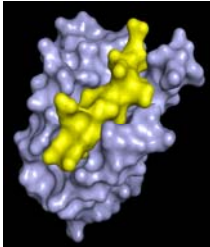
Conformation-restricted peptidic macrocycles can present functionally diverse chemical groups over a relatively large and distributed surface. This class of molecules is well suited to bind to the extended binding surfaces typical of protein-protein interactions that define key therapeutically-relevant pathways. DNA-programmed-chemistry is applied to generate libraries totaling more than 750,000 members. The libraries are applied to modified in vitro selection methodologies to screen for families of compounds and epitopes that selectively bind targets of interest. The platform has been used successfully for the discovery of compounds that interact with a number of targets such as the oncology target Bcl-xL. Screening and analysis of the libraries are under way against a number of targets relevant to the oncology, inflammation, and anti-viral therapeutic areas.

## Protein-Protein Interactions— The Need for a New Drug Modality

Abl Kinase with Inhibitor Bound



Bcl-2 with BH3 peptide



### Defined binding site

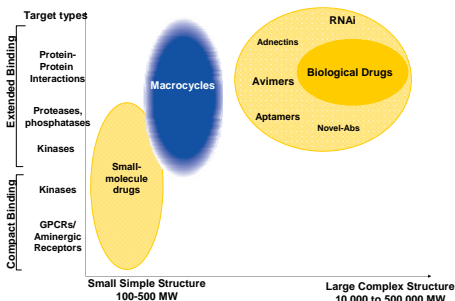
- Discrete, well defined, concave
- Natural ligand is small molecule
- Small molecule hits are abundant

### Protein-Protein Interactions

- Interfaces dispersed, complex
- Diversity of multiple specific interactions
- Small molecule hits are rare

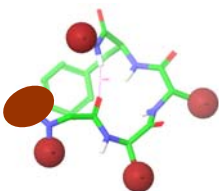
## Macrocycles:

### An underexploited class to modulate PPIs

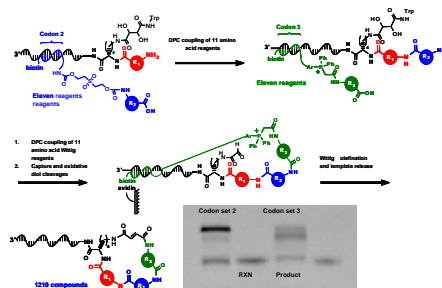


### Macrocycles present stabilized protein epitope mimetics

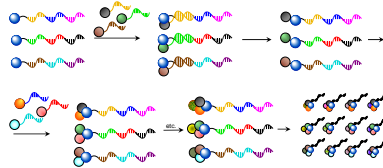
- Conformational analysis of key Ensemblins show they can readily adopt turn conformations
- Cyclic structure confers structural pre-organization
  - Reduced entropic loss on binding
- Designed with pharmaceutical properties
  - Metabolic stability over linear peptides
  - Observed oral availability
  - Intramolecular H-bonds provide conformers with amphipathic properties (e.g. Cyclosporine)



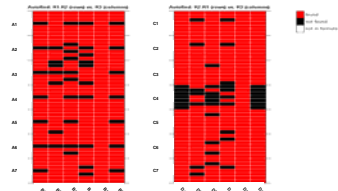
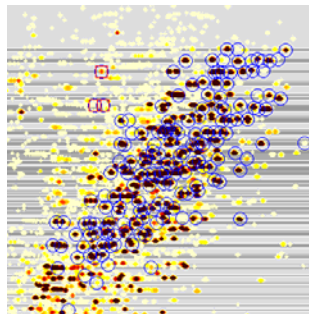
## DNA templated chemistry enables large numbers macrocycles



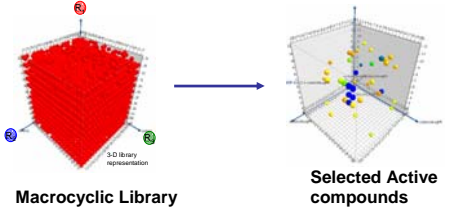
## Ensemble's DNA Programmed Chemistry enables unique reactivity for library generation



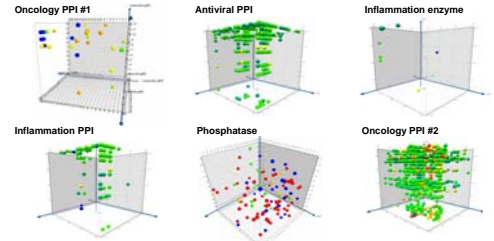
## LC-MS Analysis of DPC Products



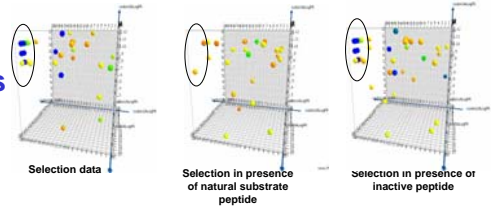
## Ensemble's Affinity Selections result in rapid SAR



- Compounds in a library are displayed as points in 3D space. The identity of each molecule defines its spatial positioning. The position along each of the three coordinates is a function of chemical building blocks employed.

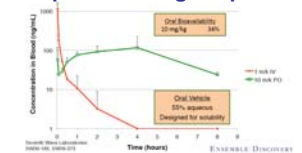


- Unique Ensemblins have been selected against targets within important therapeutic areas
- The above selections employ differing libraries all typically employing ~40K members



- Competitive selections in the presence of a known binder provide a mechanism for the confirmation of identified hits and binding with the target at a known position.

## Ensemblins: An attractive physicochemical and pharmacological profile



- Hit to Lead Compounds**
  - Solubility → Low  $\mu\text{M}$  to mM
  - High metabolic stability → >1hr in human liver microsomes
  - Log D values → Log D -0.2 to 4.0
  - Plasma protein → UP to 100 nm/sec
  - PK studies in rat → Rapid tissue distribution
  - Minimal metabolism
  - Plasma levels maintained >8 hrs

ENSEMBLE THERAPEUTICS