The Rapid Creation and Selection of Macrocyclic Libraries for the Modulation of Protein-Protein Interactions

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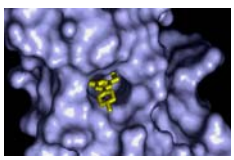
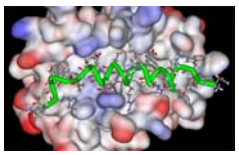
Conformation-restricted peptidic macrocycles can present functionally diverse chemical groups over a relatively large and distributed surface. This class of molecules is well suited to bind to the extended binding surfaces typical of protein-protein interactions that define key therapeutically-relevant pathways. DNA-programmed-chemistry is applied to generate libraries totaling more than 500,000 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 currently ongoing 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



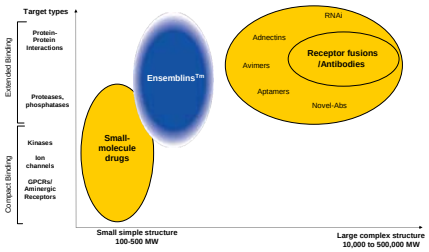
Bcl-2 with BH3 peptide



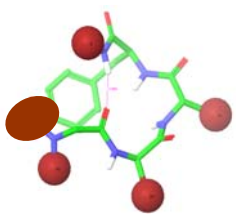
Defined binding site
- Discrete, well defined, concave
- Natural ligand is small molecule
- Small molecule hits are abundant

Protein-Protein Interactions
- Interfaces disperse, complex
- Diversity leads to multiple specific interactions
- Small molecule hits are rare

Macrocycles: An underexploited space between biologicals and small molecules

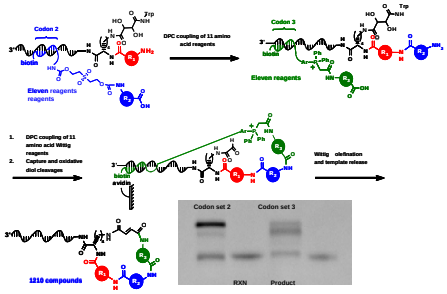


Macrocycles present stabilized protein epitope mimetics

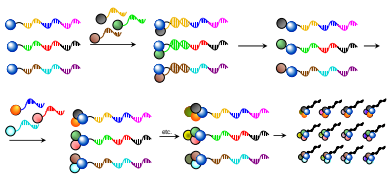


- Conformational analysis of key Ensemblins show they can readily adopt turn conformations
- Cyclic structure confers structural pre-organization
  - Reduced entropic loss on binding
- Designed with pharmaceutical properties
  - Metabolic stability over linear peptides
  - Observed oral availability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

DNA-templated chemistry enables synthesis of large number libraries

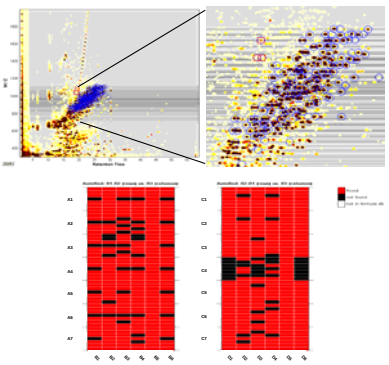


Ensemble's DNA Programmed Chemistry enables unique reactivity for library generation

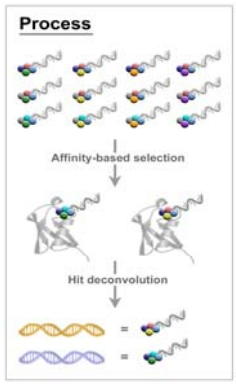


Ensemble's DPC Advantage
- DNA Programmed Chemistry enables rapid generation of diverse libraries directed by the selectivity of DNA hybridization
- Purification is coupled to synthetic process
- Ring closure is challenging via traditional chemistry; controlled by DPC
- 500,000 compounds in house with 1.5 million in total by end of 2010

Analysis of DPC intermediates



- DPC-reactions are analyzed via LC-MS of digested DNA-macrocycle intermediates
- Generation of 'Hit Maps' identifies presence of individual species



Selection against therapeutically relevant targets

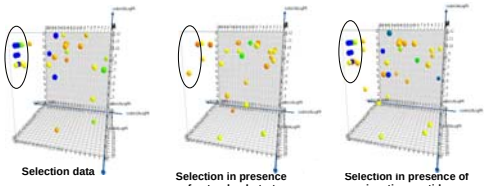
- Library mixture of > Thousands
  [D] + [T] ⇌ [DT]
- Target Driven Hit ID

D = Drug T = Target

Iterative Parallel Enrichment
- All compounds screened simultaneously
- Affinity based hit discovery
- DNA is amplified for sequencing to decode functional molecules

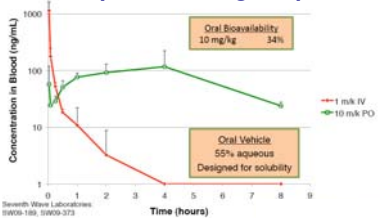
Ensemble's Affinity Selections result in rapid SAR

- 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.



- The above selections employ differing libraries all typically employing ~40K members
- Compounds are structurally related
- 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 µM 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

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