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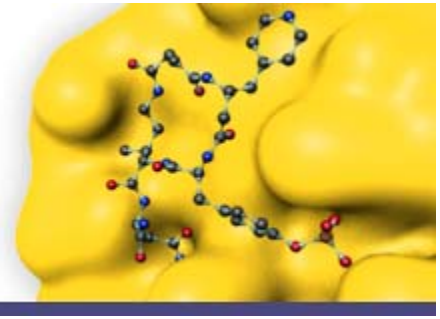
Moving in New Circles – Exploiting Macrocycles for Drug Discovery

SCI Fine Chemical Group

March 23rd 2011

The rise of the macrocycle



Nat Rev Drug Discov 2008, 7(7), 608-624

REVIEWS

The exploration of macrocycles for drug discovery — an underexploited structural class

Edward M. Driggers, Stephen P. Hale, Jinbo Lee and Nicholas K. Terrett

Abstract | Macrocyclic natural products have evolved to fulfil numerous biochemi
functions, and their profound pharmacological properties have led to their develop
as drugs. A macrocycle provides diverse functionality and stereochemical compl
conformationally pre-organized ring structure. This can result in high affinity and
for protein targets, while preserving sufficient bioavailability to reach intracellu
Despite these valuable characteristics, and the proven success of more than 1
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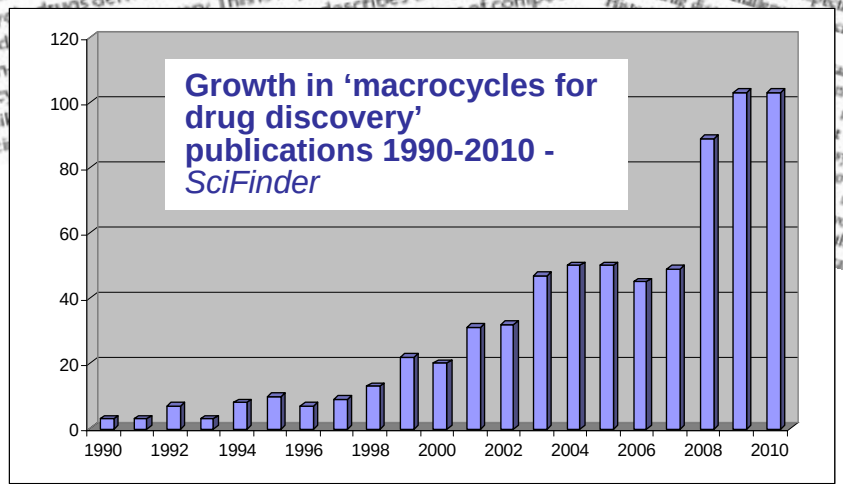
drugs derived from natural products, this structural class has b
This is in part due to concerns about synth
describes the growing body o
of compoun



Growth in 'macrocycles for drug discovery' publications 1990-2010 - *SciFinder*

Journal of Medicinal Chemistry

J Med Chem 2011

Macrocycles Are Great Cycles: Applications, Opportunities, and Challenges of Synthetic Macrocycles in Drug Discovery

PERSPECTIVE

pubs.acs.org/jmc

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1. INTRODUCTION

Macrocycles occupy a unique segment of chemical space. In the past decade, their chemical diversity expanded significantly, supported by advances in bioinformatics and synthetic methodology. As a consequence, this structural type has now been successfully tested on most biological target classes. The goal of this article is to put into perspective the current applications, opportunities, and challenges associated with synthetic macrocycles in drug

candidates have originated
natural products, provided
rapamycin, vancomycin,
reviews are dedicated
synthetic and medicinal
covered here.[1−3] From
medicinal chemistry
volved direct use as a
the natural product
ent advances in the

molecular weights tend to be on the higher end (often in the 500−900 g·mol^{-1} range), their numbers of H-bond donors and acceptors, as well as their polar surface area (PSA), tend to be on the far side of the accepted druglike spectrum.[18] For an equal number of heavy atoms, macrocycles inherently possess a lower-number of rotatable bonds than their acyclic analogues. a beneficial feature for oral bioavailability (in the following "acyclic" will be used in the sense of "nonmacrocyclic").[18] As a result, macrocycles are mere conformationally restricted than their acyclic analogues, which potentially can impart higher target binding and selectivity and improved oral bioavailability (in this assessment, endocyclic bonds are considered to be nonrotatable which is only an approximation; see ref 18). For a systematic chemoinformatic analysis of biologically active macrocycles the reader is referred to the recent review of Brandt et al.[19] Topologically, macrocycles have the unique ability to span larger surface areas while remaining conformationally restricted compared to acyclic molecules of equivalent molecular weight. This characteristic makes them especially suited for targets displaying

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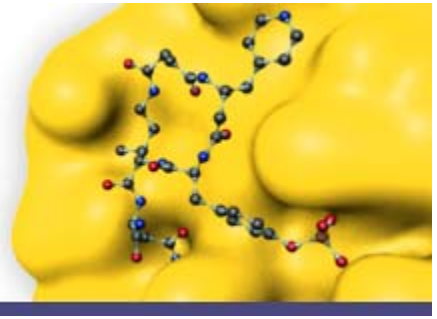
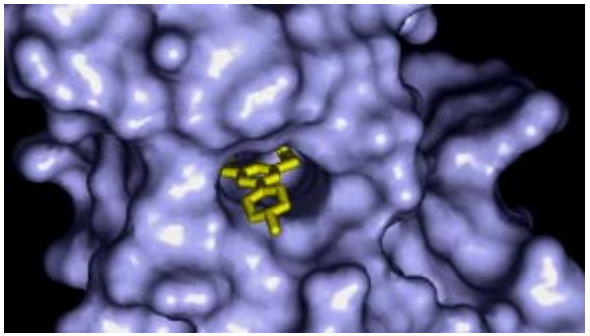
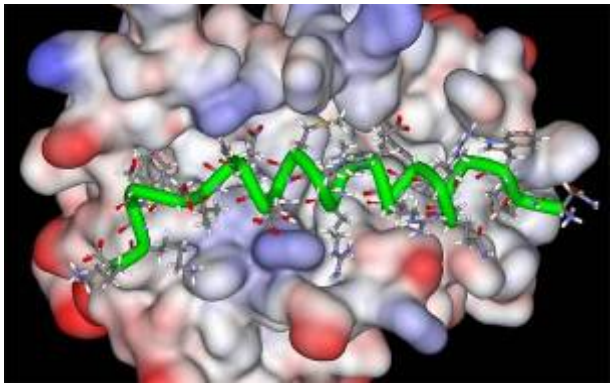
Macrocycles ideal to address extended-binding site targets



Abl Kinase with inhibitor bound



BCL-xL/Bad complex



Compact Binding Motifs:

- Discrete, concave binding site
- Known small-molecule starting points
- *Limited opportunities for drug discovery*

Extended Binding Motifs:

- Large interface, multiple interactions
- Evolved to selectively recognize protein/peptidic substrates
- Not readily addressed by small (Ro5) molecules
- *~80% of disease targets possess extended binding regions*

Protein Complexes, Proteases, Phosphatases:
Huge medical need and market opportunity

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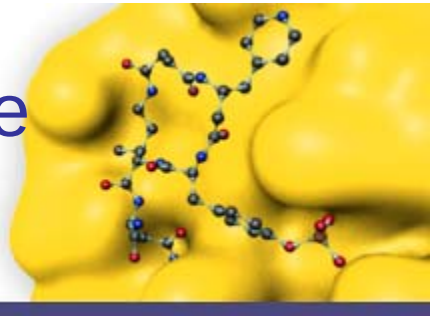
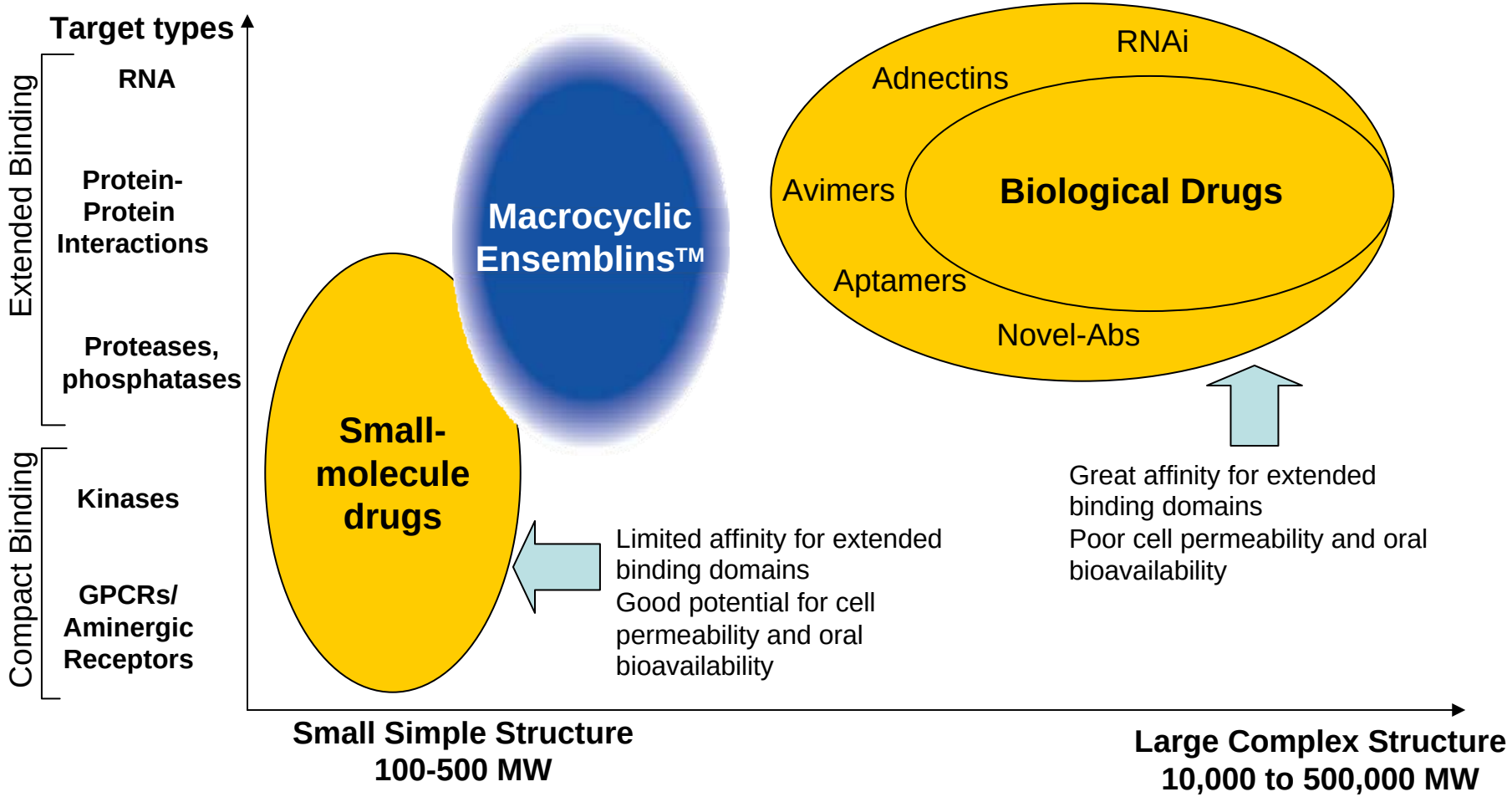
Ensemblins: exploiting a new chemical space
"small molecule biologics"





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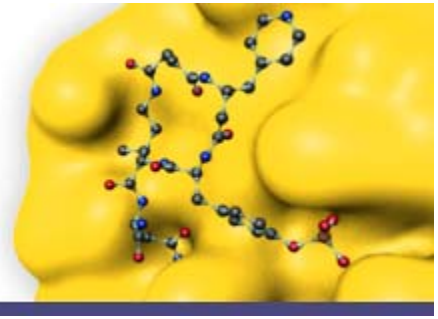
Modern approach taps nature's solution for extended binding sites

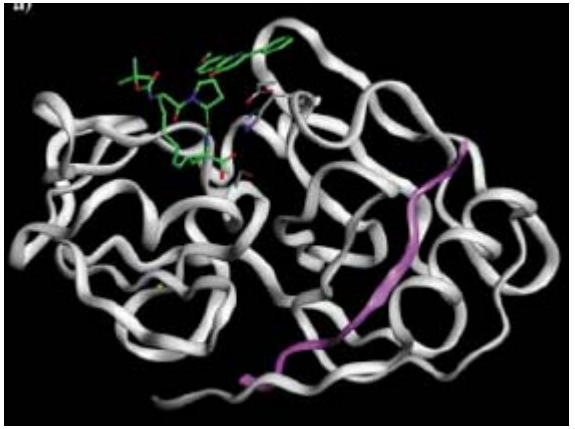


Macrocycles: Well-precedented source of drug molecules

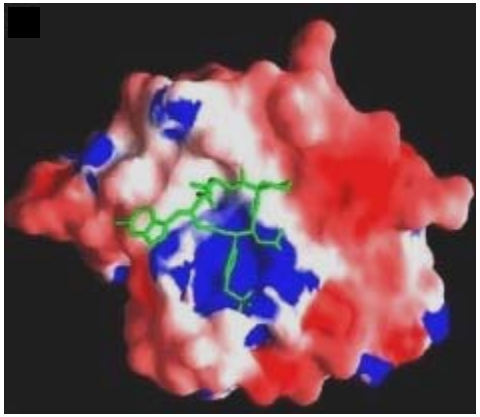
- Many important drugs
  - Antibiotics, immune modulators, anticancer
  - Most often from natural sources
- Often target extended binding surfaces

Ensemble's Platform enables access to this rich source of drug molecules

Macrocyclic HCV protease inhibitor



Macrocyclic Grb2 SH2 domain binder



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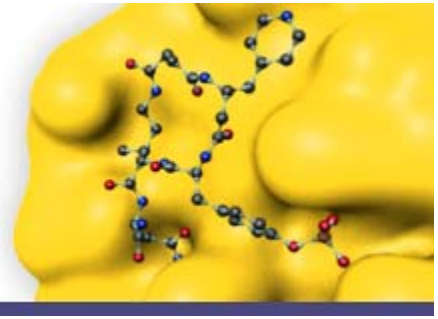
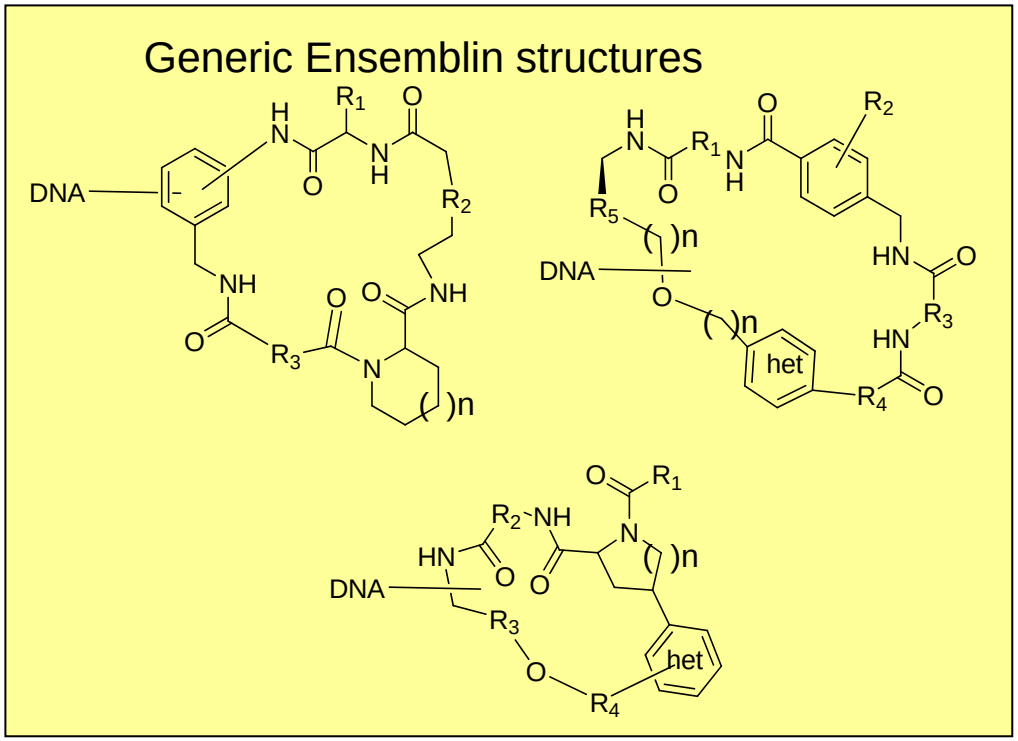
Novel macrocyclic therapeutics with unique design elements for addressing PPIs



- Goal: orally bioavailable synthetic macrocycles
- Cyclic structure for unique combination of chemical and biological properties
- Drug utility well-precedented among macrocyclic natural products
- Highly modifiable for affinity, specificity, delivery and drug-like qualities
- Generated though highly cost-effective and rapid processes



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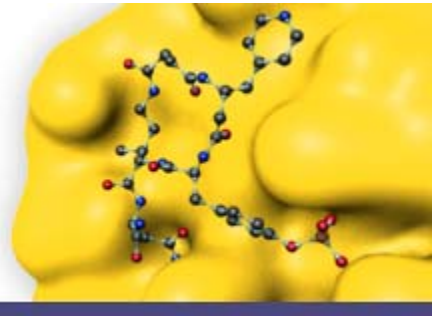
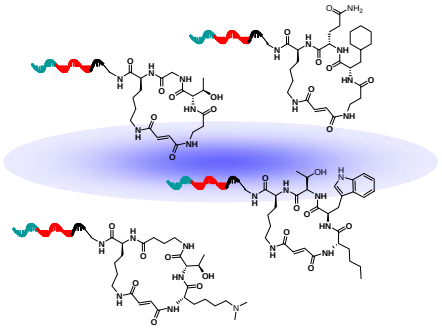
Ensemble employs two integrated chemistry platforms for drug discovery

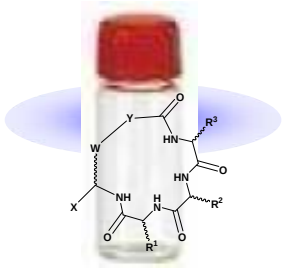


DNA-Tagged Libraries



- >1,600,000 macrocyclic Ensemblins available for affinity-based selection assays
  - Prepared by proprietary DNA-programmed chemistry (DPC™)
  - Many distinct structural architectures
  - 5th generation libraries in 2011
    - Protein motif mimics
    - 2.6 million Ensemblins in Q1 2011

Discrete Compounds



- Macrocycle synthesis “know-how”
  - >2000 prepared in multiple structural classes
  - Solid-phase and solution-phase synthesis (mg-g scale)
  - Single and combinatorial synthesis methodologies
  - Scalable
    - Up to 20 g produced in-house

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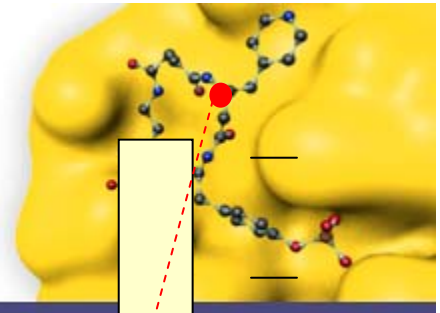
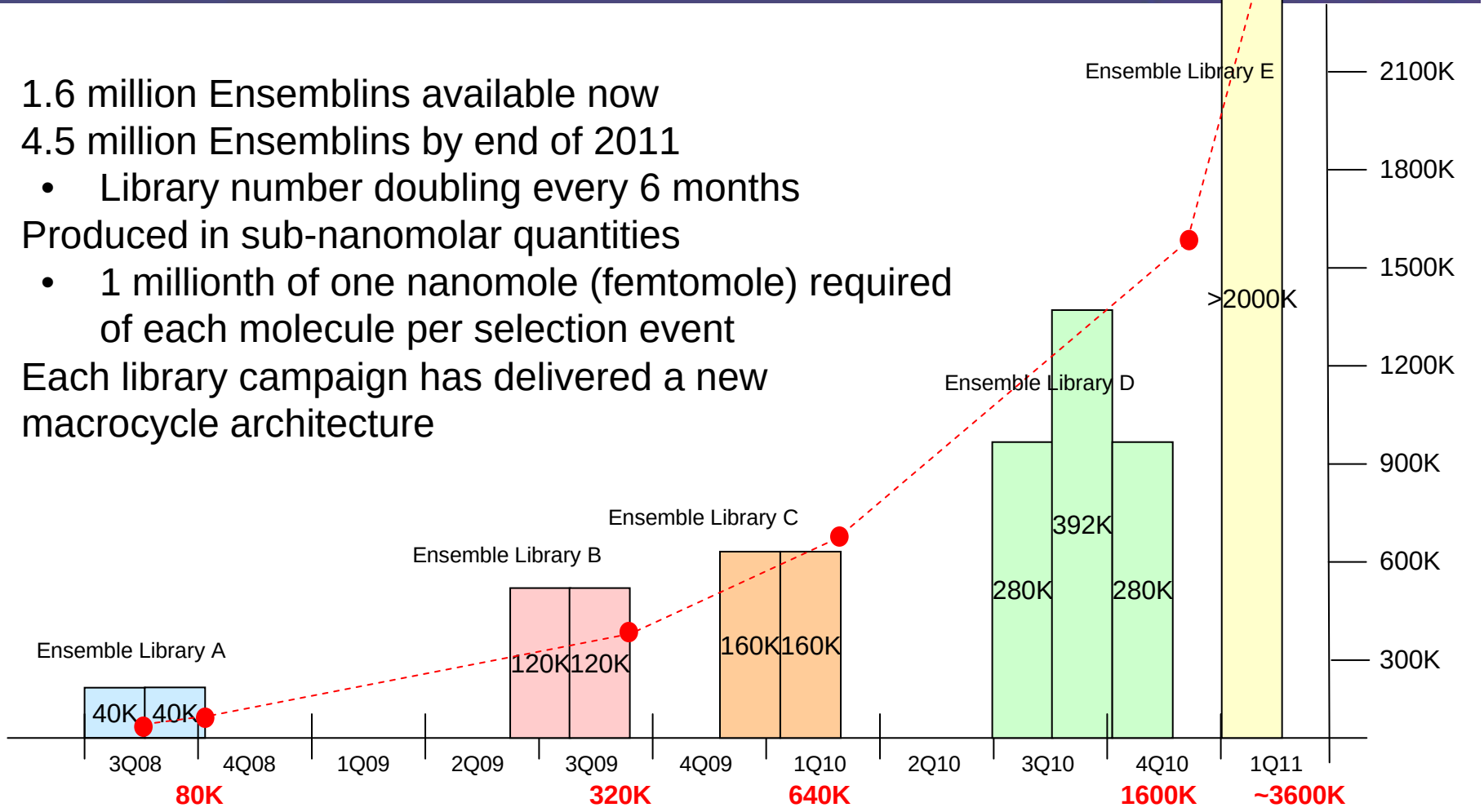
Ensemble Library Synthesis 2008-11



- 1.6 million Ensemblins available now
- 4.5 million Ensemblins by end of 2011
  - Library number doubling every 6 months
- Produced in sub-nanomolar quantities
  - 1 millionth of one nanomole (femtomole) required of each molecule per selection event
- Each library campaign has delivered a new macrocycle architecture



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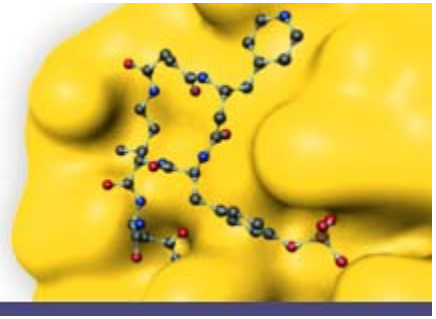
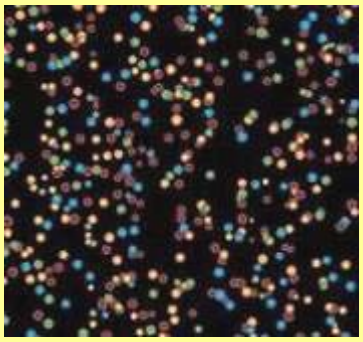
Replacing conventional HTS with fmole synthesis and selection



Million-member libraries in small volumes

Simple, low-tech selection assay set-up

PCR of DNA tags



DNA Sequencing of libraries and hits

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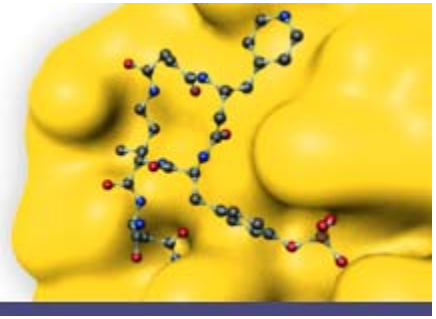
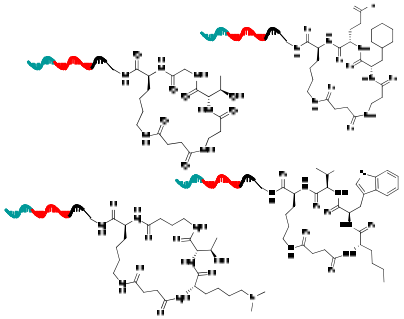
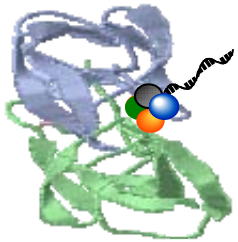
Integration of Ensemblin platforms for a powerful discovery engine



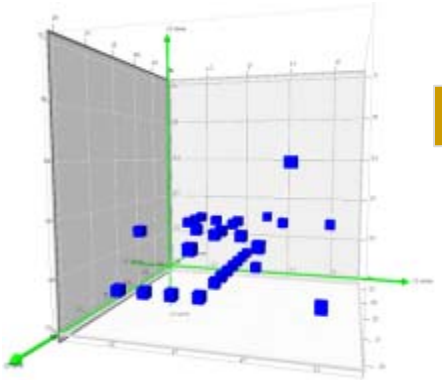
DNA-Programmed Chemical Synthesis



Diverse Libraries



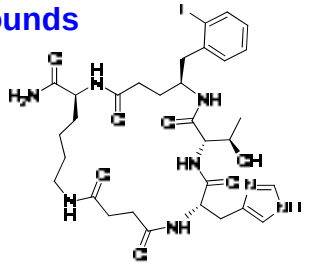
Affinity-Based Selections



Comprehensive Data Set

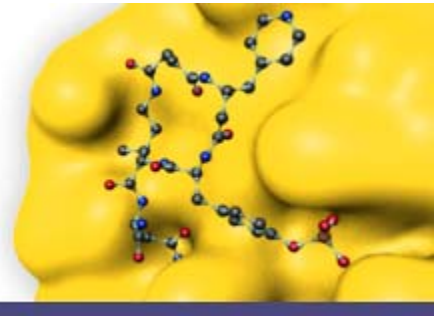
"Instant SAR"

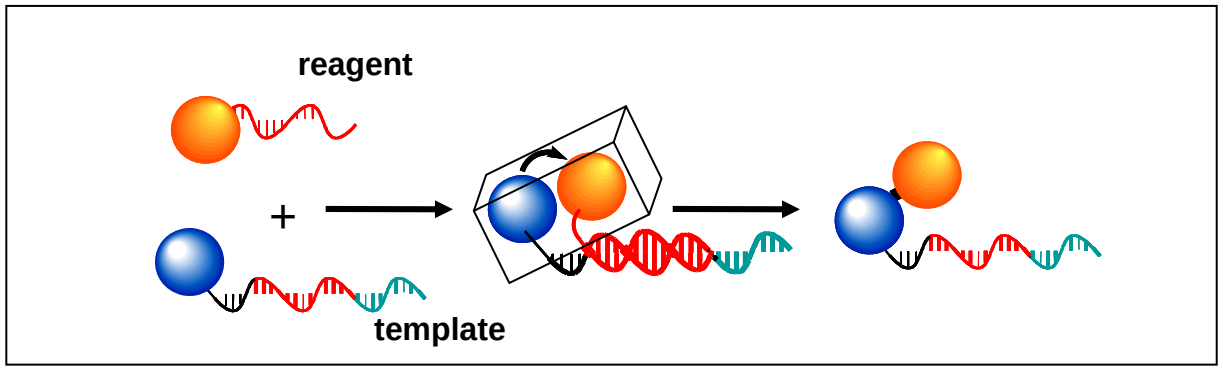
Discrete compounds



Biochemical, biophysical, cell, and in vivo evaluation → development candidates

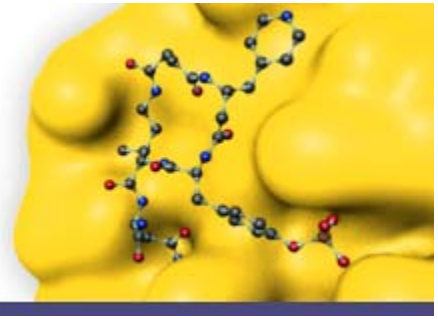
DNA-programmed chemistry (DPC)

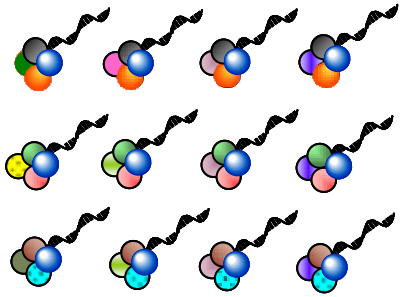




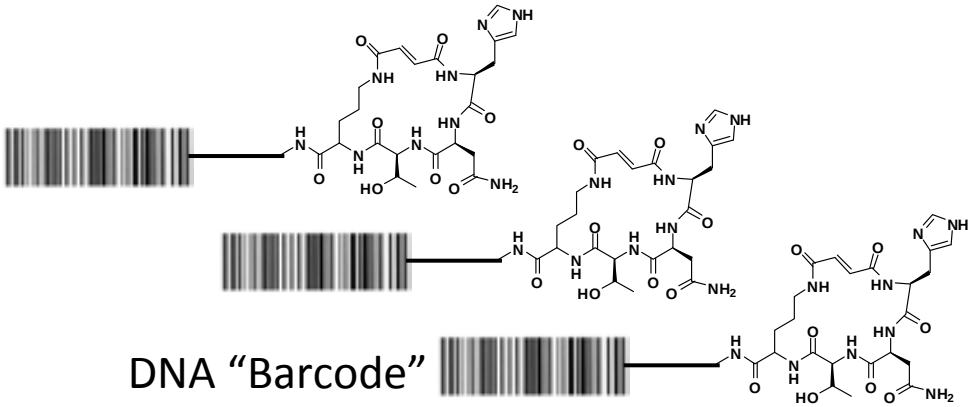
- DNA hybridization directs chemical reactions in “nanoreactor”
  - enthalpic benefit of DNA hybridization > entropic loss of chemical reaction
  - increases effective reactant molarity, promotes reaction specificity
- Reactions occur under mild aqueous conditions
- Purification and analysis at every step

DNA-encoded compounds:
'Screenable' Ensemblin mixtures





A mixture/library of 40-200K compounds is created in one reaction vessel. Each compound is coded for by a unique DNA sequence, allowing the behavior of each individual within a mixture to be tracked.



DNA "Barcode"

"Small Molecule" Chemical Diversity

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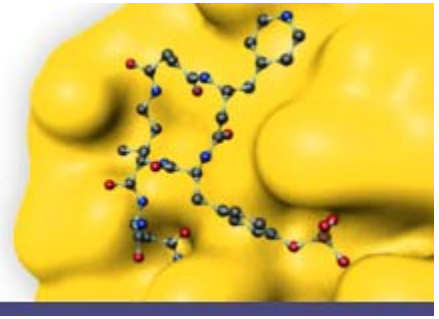
Affinity-based screening of DNA-encoded compounds



Coded Diversity Collection

Affinity-Based Screening

Recovery of Active Compounds

Signal Amplification and Compound ID

- <1 fmole/compound/screen

- Target Protein

- PCR amplification
- Next Gen DNA sequencing
- >20,000,000 per run
- Decoding/analysis

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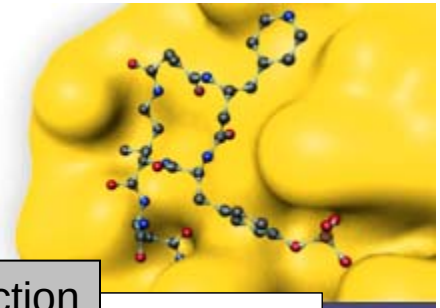
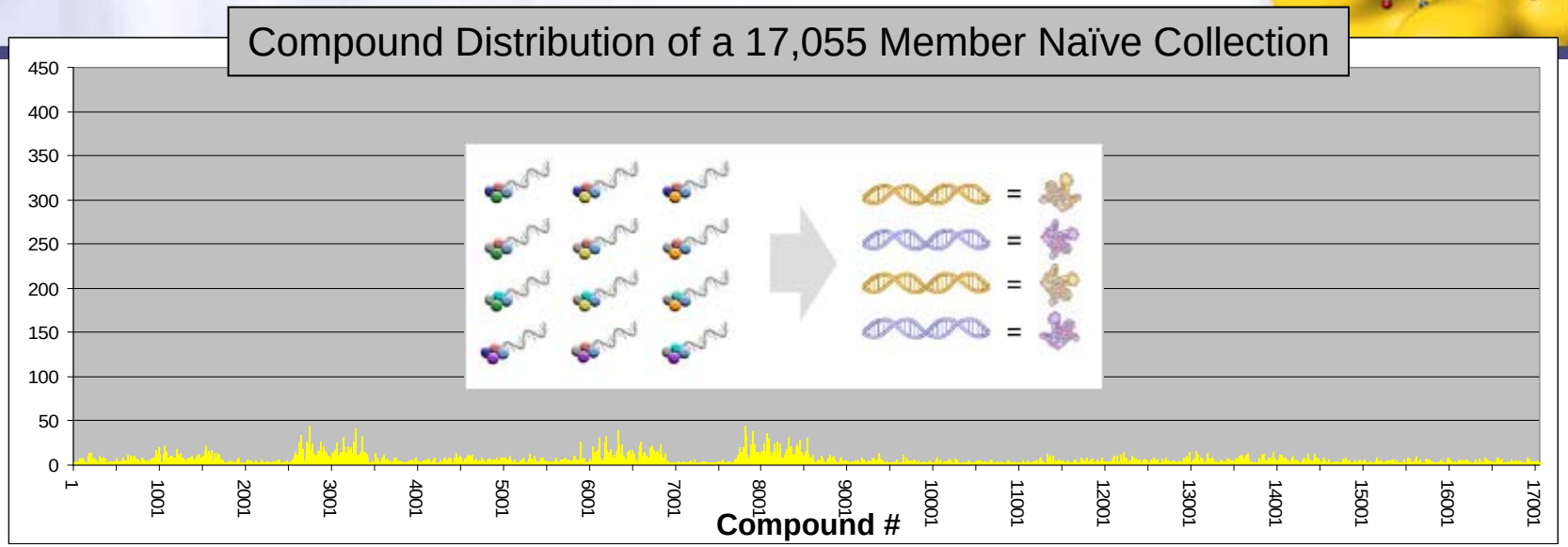
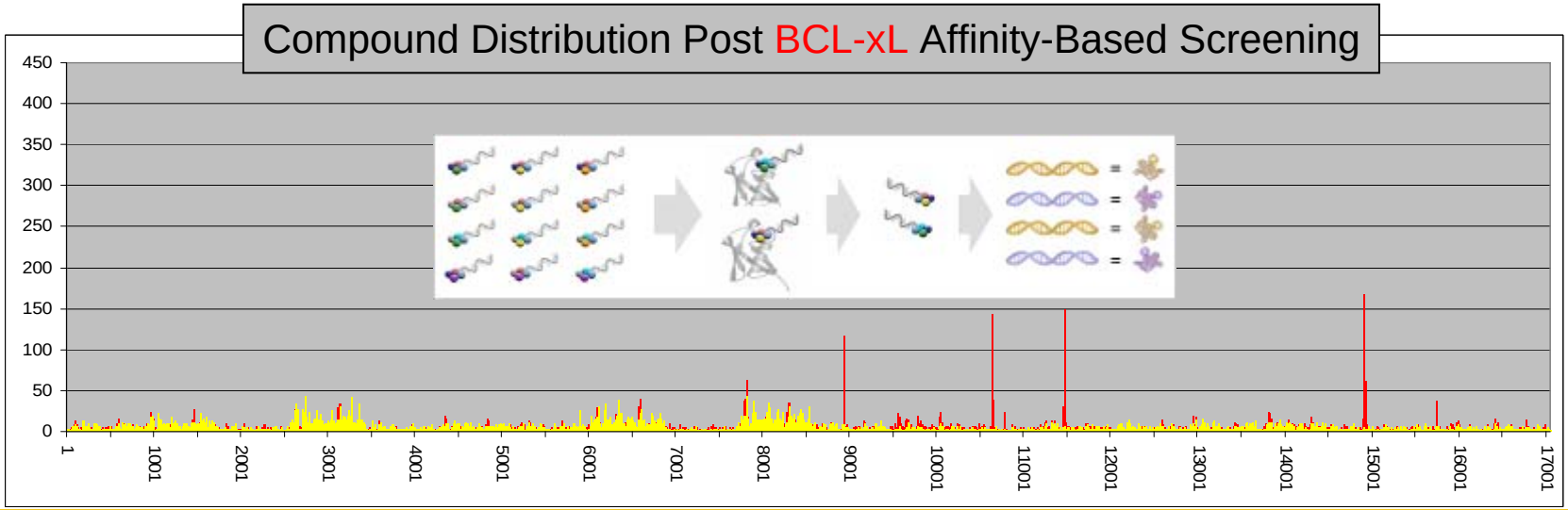
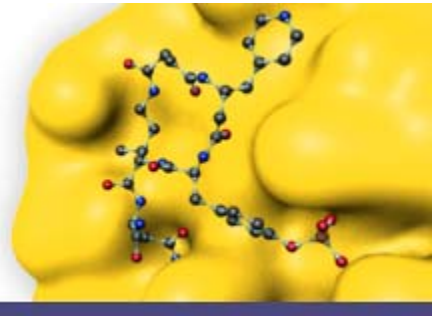
BCL-xL affinity selection: raw data

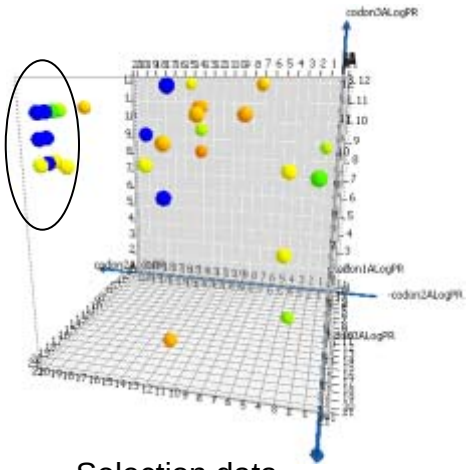




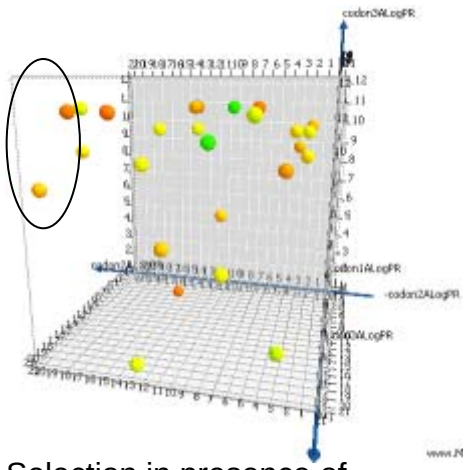


Macrocycle discovery vs BCL-xL protein complex

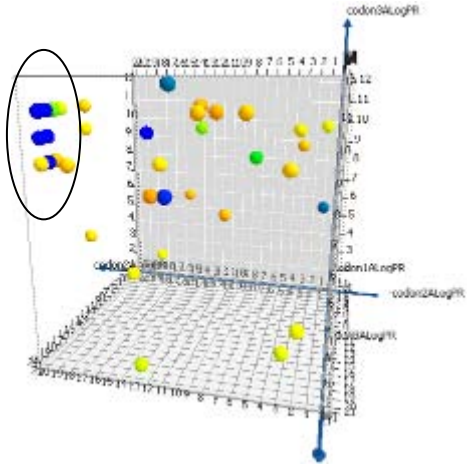




Selection data

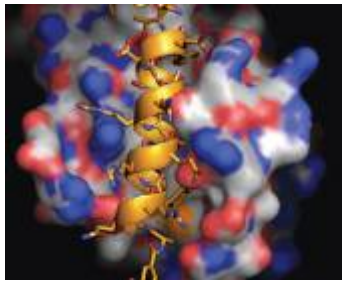


Selection in presence of natural substrate peptide



Selection in presence of inactive peptide

Each sphere represents a unique Ensemblin structure; color coded by degree of enrichment (Blue = highest enrichment)



- Significant enrichment of macrocycles that bind to BCL-XL
- Compound binding blocked by natural substrate peptide
- Binding affinity improved by design, synthesis of follow-on libraries

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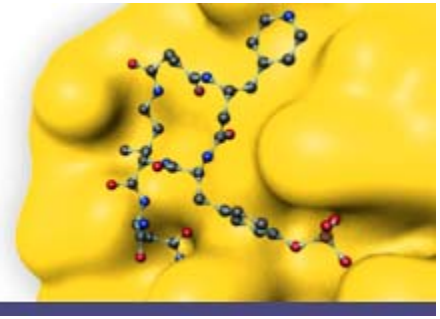
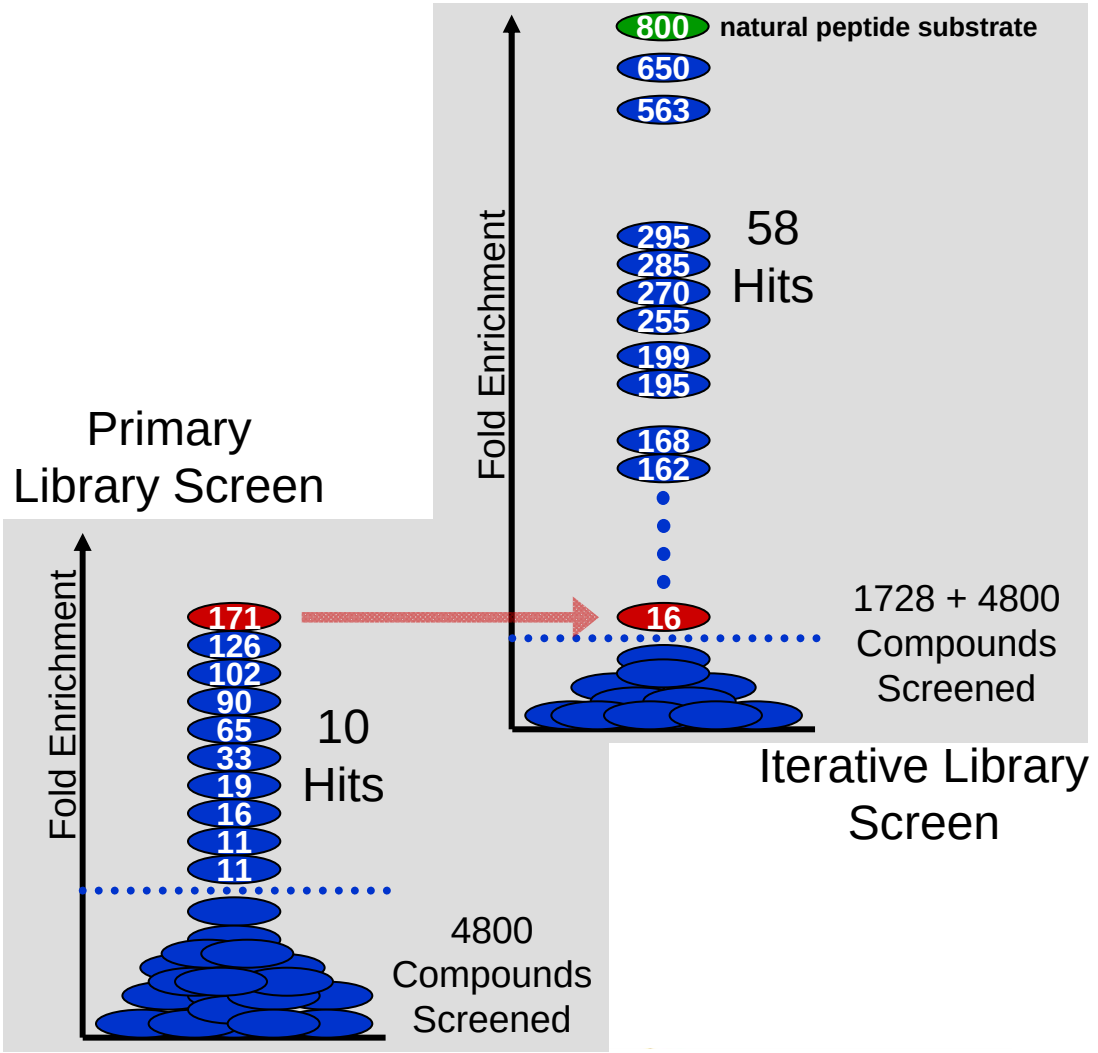
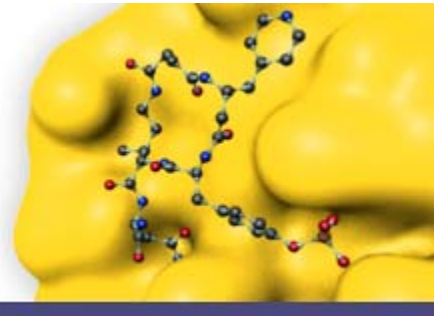
BCL-xL inhibitor iterative library follow-up



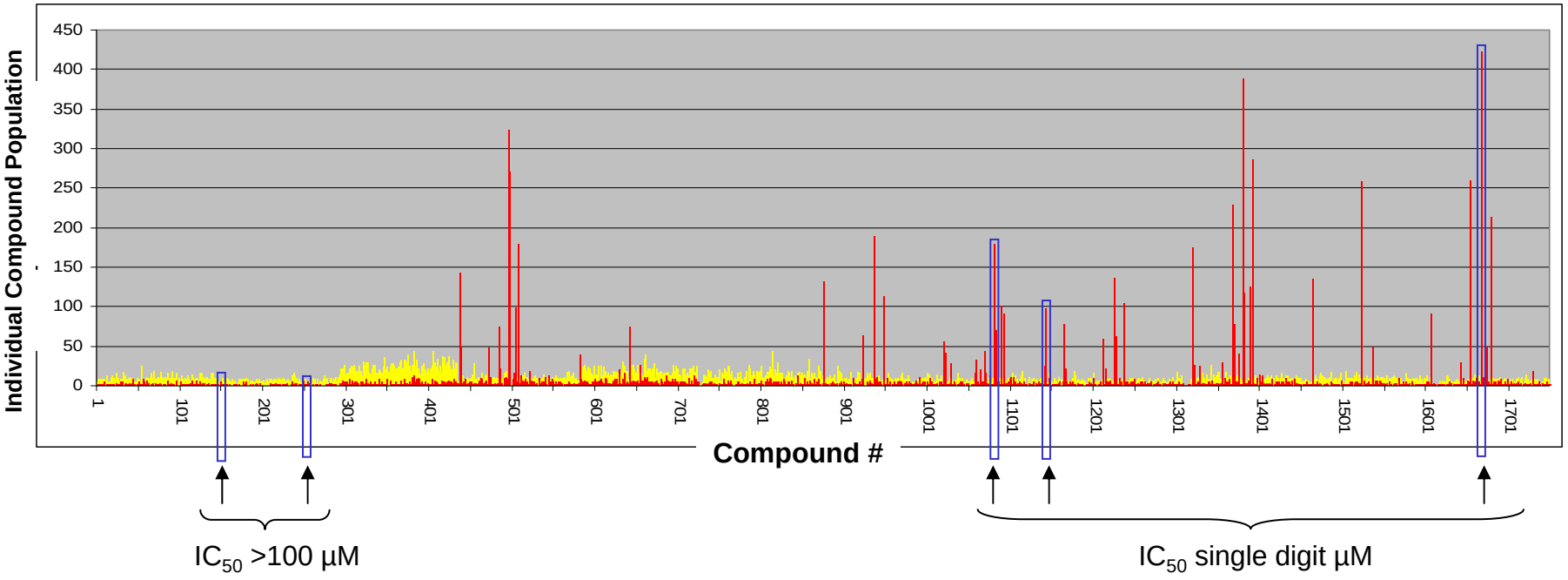
- Library of 1,728 compounds made based on best actives
- Affinity selection of iterative library
- Considerable enrichment of numerous compounds observed
- Selection in presence of natural substrate (Ki = 200 nM) indicated best compounds have similar enrichment



Analysis of “off-DNA” discrete compounds: Macrocycle SAR



- Compounds are selected for synthesis and assay based on DNA enrichment and SAR patterns
- Activity determined through Biacore or FP assay, with FITC labeled BAK peptide
- Clear correlation between enrichment in affinity selection and biochemical IC50 of discrete



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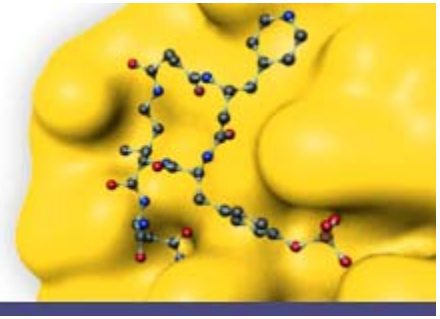
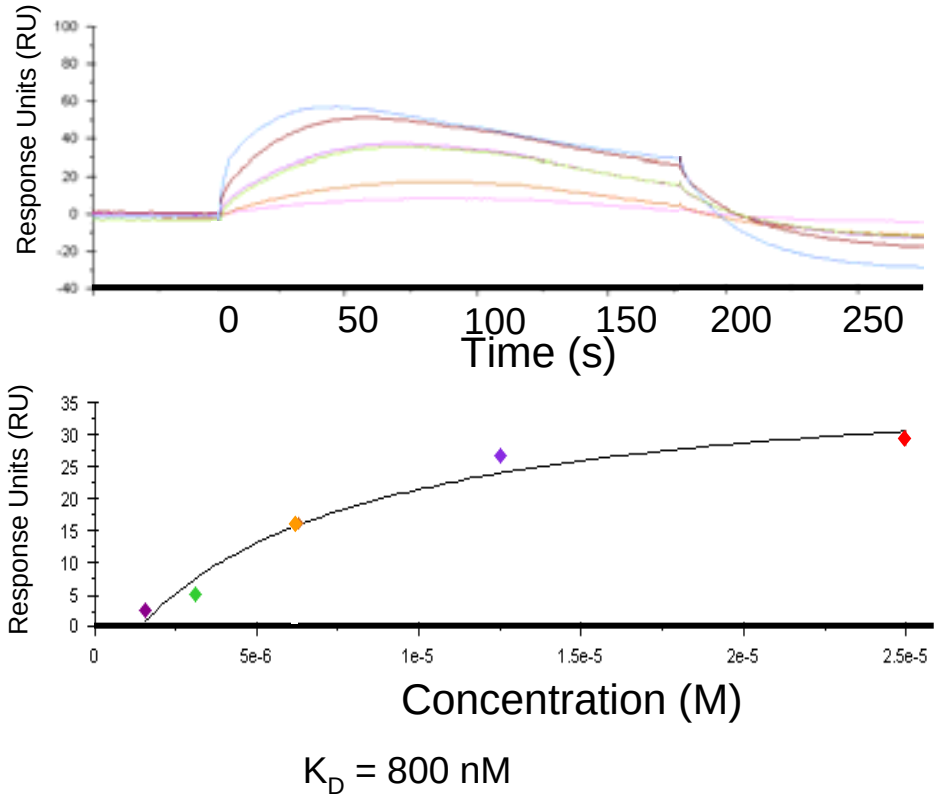
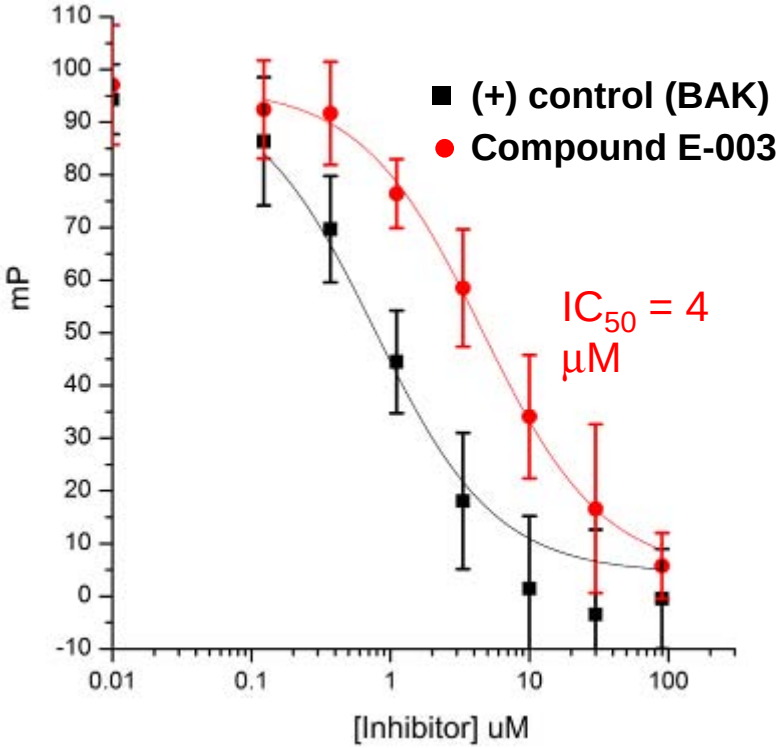
Ensemblin binding to BCL-xL confirmed by Biacore/SPR and fluorescence polarization





Ensemblin binding to BCL-xL confirmed by Biacore/SPR direct binding analysis



Ensemblin exhibits functional competition of BH3 binding to BCL-xL in FP assay

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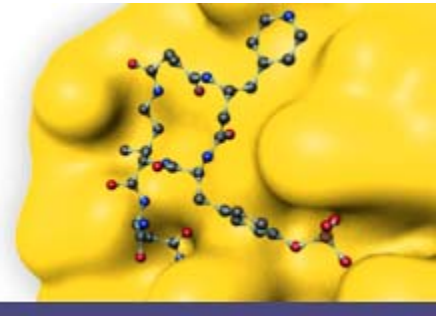
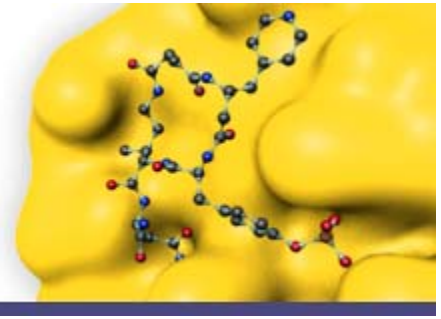
Productivity in action



Identification and optimization of macrocycles against two important disease targets

Case Study 1	Intracellular dual-binding target
Case Study 2	Extracellular cytokine target

Macrocycle Case Study 1



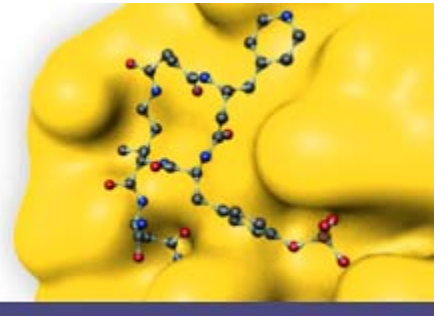
DPC enables discovery of macrocyclic candidates with nM IC50 against Partner's intracellular PPI target

Background:

- Intracellular protein-protein interaction target
- Candidate profile requires affinity for two related binding sites – i.e. inhibitor with dual activity
- Limited SAR in the literature
- No pre-existing molecule with this profile known

Case Study 1

Aggressive criteria set by partner
Success leads to project transfer to partner

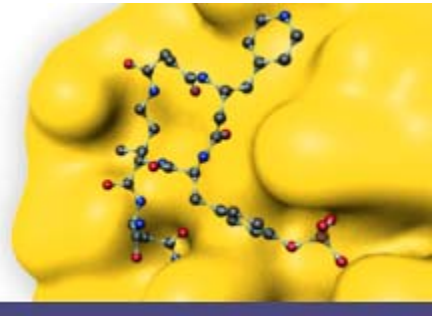


Requirements for successful project for transfer to Partner:

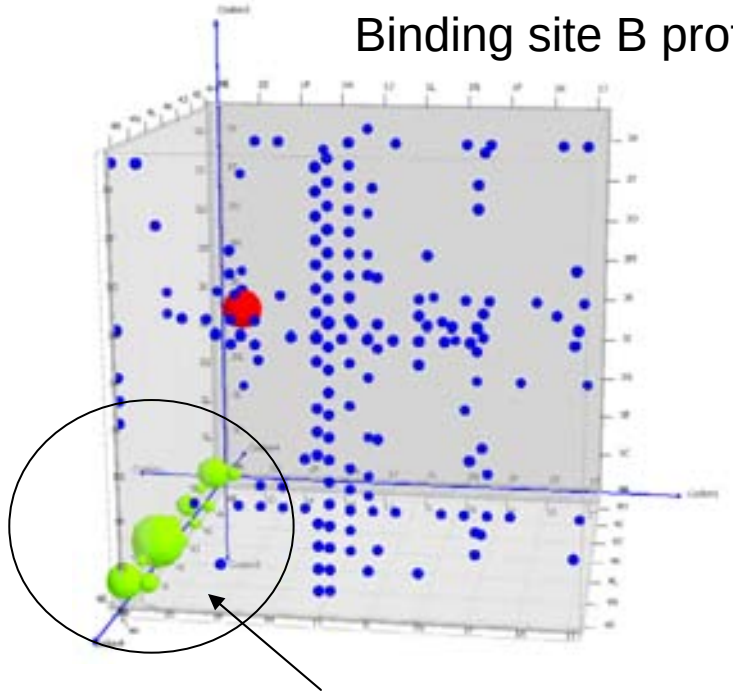
- Low nM IC_{50} against both binding sites in same molecule
- Sub-micromolar cell assay activity
- No manufacturing limitations
- Acceptable ADME and PK properties
- Acceptable off-target, HERG, Cyp panel profile

Case Study 1

Screening the DPC libraries against the two target proteins



- 320K Ensemblin Library Screened
- Active leads identified for Binding Site B protein
  - no hits for Binding Site A
- Hits synthesized as discretes molecules (no DNA – mg scale):
- Confirms low micromolar affinity for Site B, no binding to Site A
- A focused library prepared to expand on observed SAR



Case Study 1

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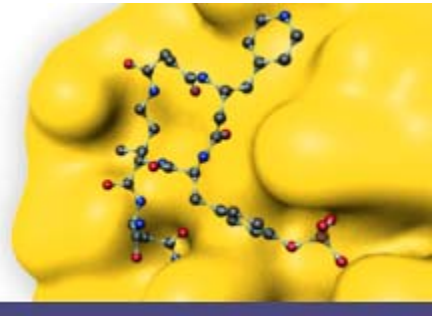
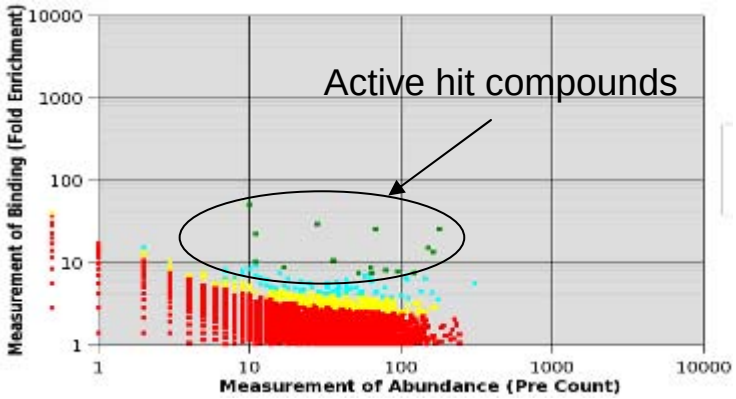
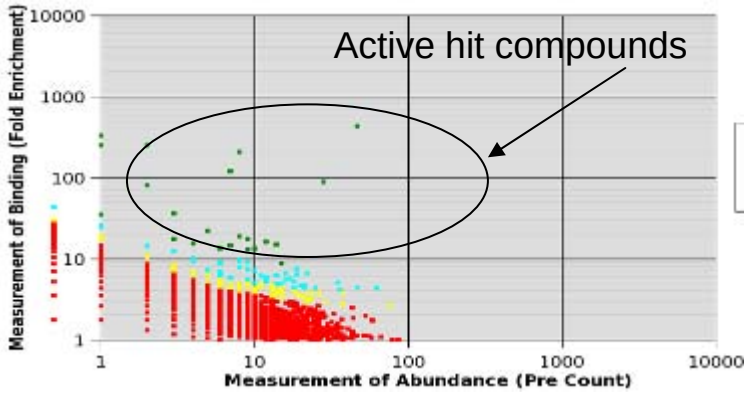
Iterative focused libraries produced hits with affinity for both sites



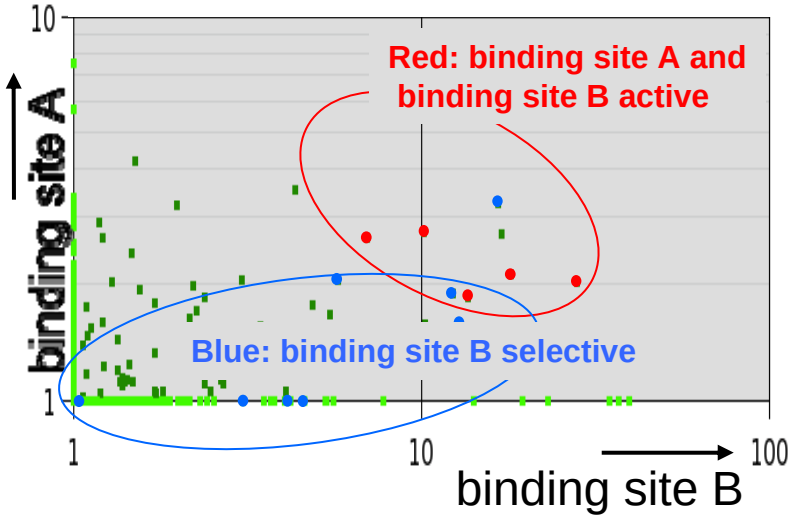
Binding site A protein



Binding site B protein

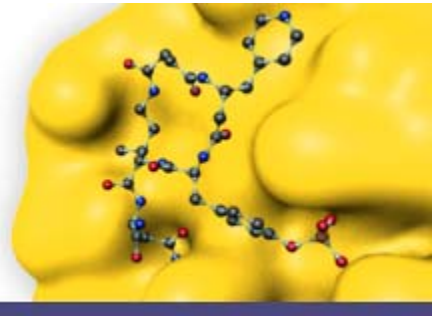


- Focused secondary macrocycle library
  - 1800 compounds
- Hits identified for both Binding Site A and B proteins
- Correlation analysis showed multiple hits with dual affinity
- Compounds synthesized as discrete molecules confirmed as dual actives with low µM affinity for both sites



Case Study 1

Medicinal chemistry campaign by Ensemble rapidly improved dual binding potency



- Initial leads were single digit micromolar
- Two series were pursued
  - Best compounds in Series 1: 100-200 nM for both sites
  - Best compounds in Series 2: 1-50 nM for both sites
- Current best compounds:
  - Balanced affinity ~10 nM for both sites
- On-mechanism activity confirmed in multiple biophysical and functional assays
- Several X-ray co-crystal structures generated with our macrocycles bound to target proteins

Case Study 1

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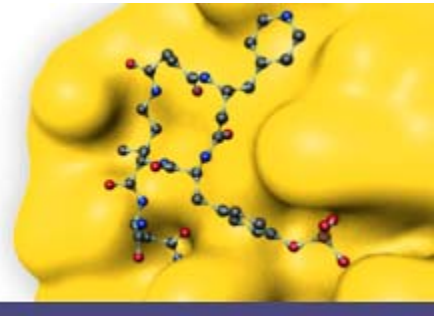
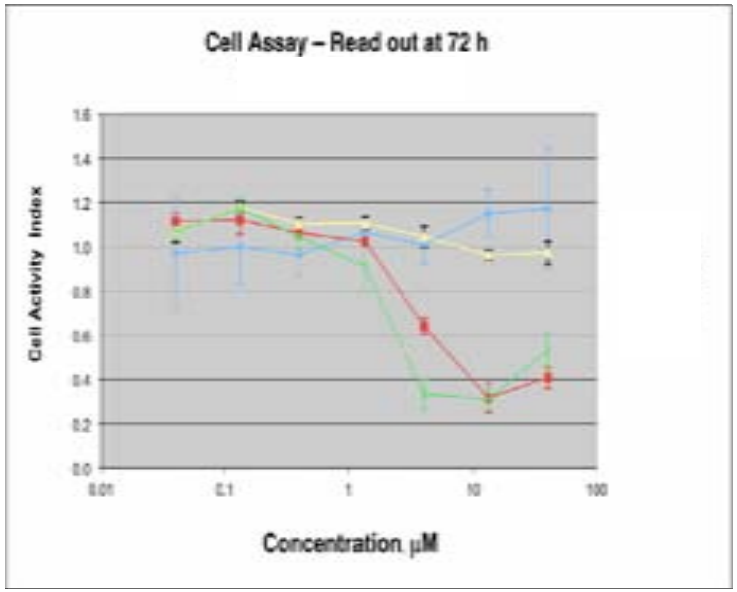
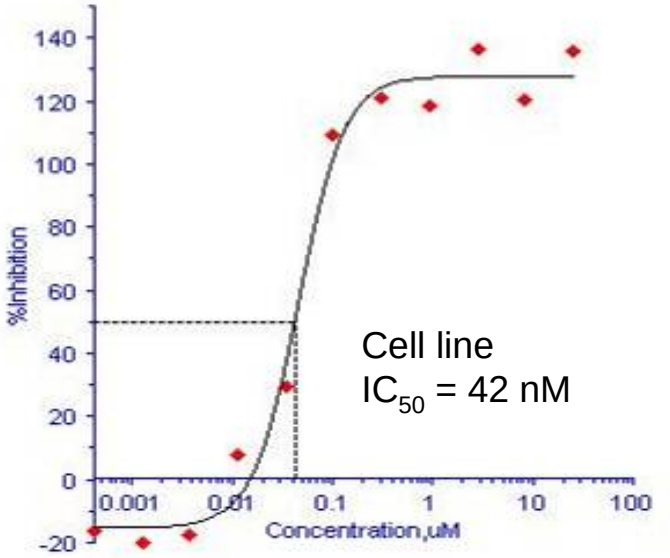
Activity in whole cell assay, initially poor, but quickly improved to low nM IC_{50}





Initial whole cell assay results showed relatively weak activity despite good binding to target protein – only low micromolar cell potency, at best



A rational series of analogs designed for improved permeability led to dramatically increased cell potency: best compounds are now ~10 nM IC50

Case Study 1

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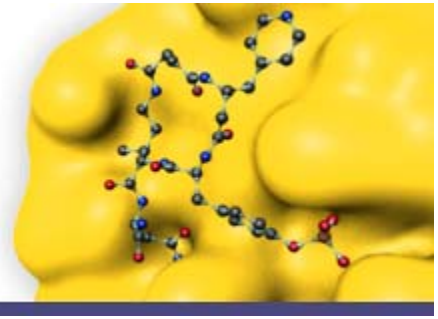
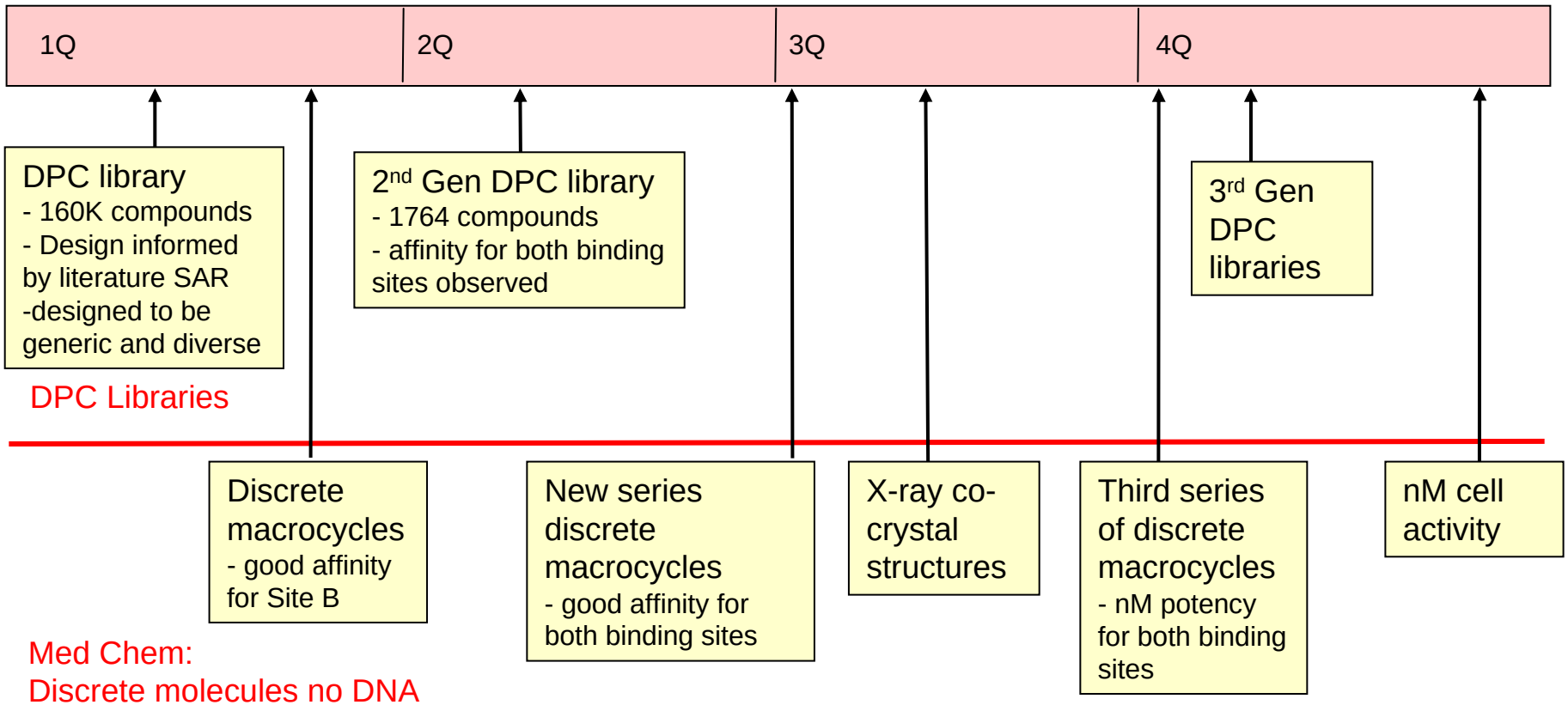
Rapid process gives hits, SAR, crystal structures and nM cell activity





Case Study 1

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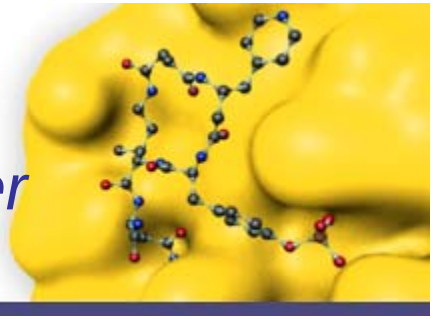
Aggressive criteria set by partner
Met by Ensemble's Team -- project ready for transfer



Requirements for successful project for transfer to Partner:

- ✓ Low nM IC_{50} against both binding sites in same molecule
  - ✓ MET: Single digit to double digit nM affinity and IC_{50}
- ✓ Sub-micromolar cell assay activity
  - ✓ MET: Single digit nM IC_{50}
- ✓ No constraints on scale-up and analog synthesis
  - ✓ MET: rapid synthetic methods developed
- ✓ Acceptable off-target, HERG, cyp panel profile
  - ✓ MET: e.g. HERG >80 µM
- ✓ Acceptable ADME and PK properties
  - ✓ MET: good exposure to drug in rodent model

Case Study 1

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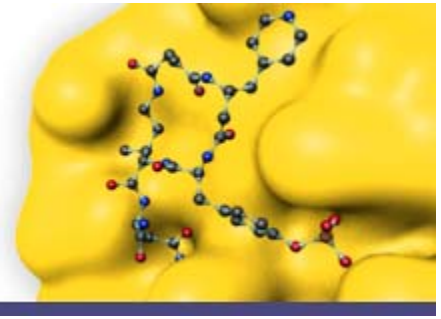
Ensemblin™ Case Study 2



Ensemble's macrocycle platform used to discover small molecule inhibitors with nM Kd against partner's PPI target

Background:

- Extracellular cytokine protein-protein interaction target
- Candidate profile requires affinity for solution phase cytokine
- Mechanism clinically validated with mAb product
- No small molecule ligand or SAR known for this target

Case Study 2

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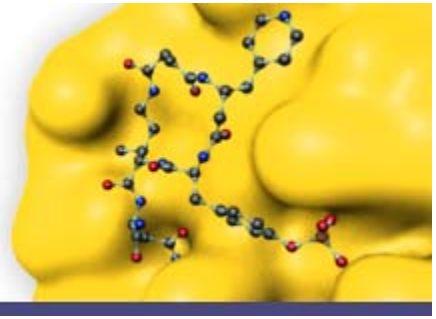
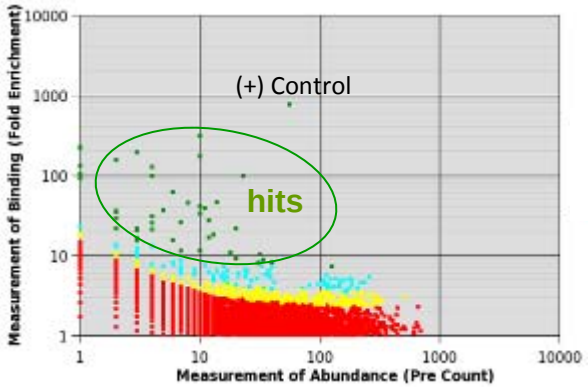
Selection assay with 500K+ macrocycles

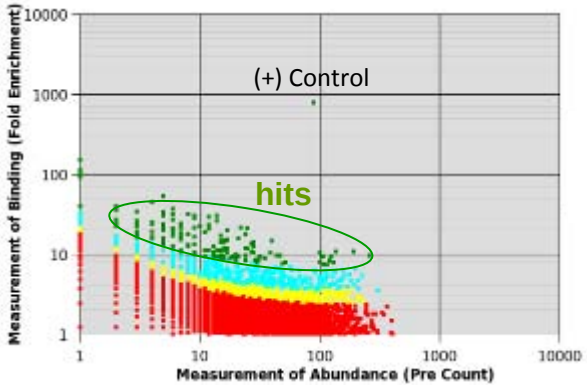
Results reveal structurally distinct chemotypes
With good affinity

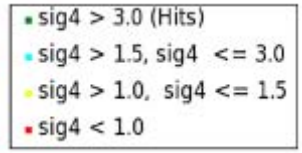


ELB-2

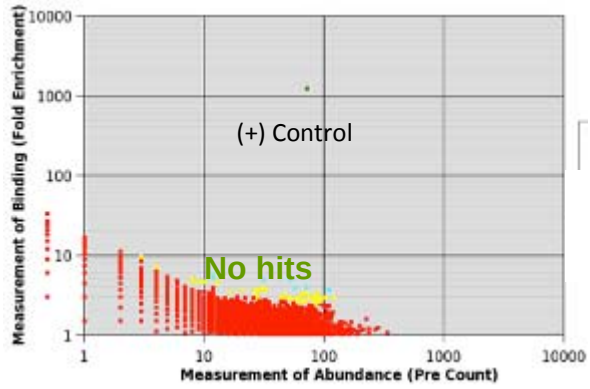


ELB-5





ELD-3



- Compelling screening result
- Large number of highly enriched compounds in certain pools but none in many others – indicates specific binding interaction
- Compounds with up to 300-fold enrichment observed – indicates strong binding interaction
- Extensive and detailed SAR observed to guide medicinal chemistry

Case Study 2

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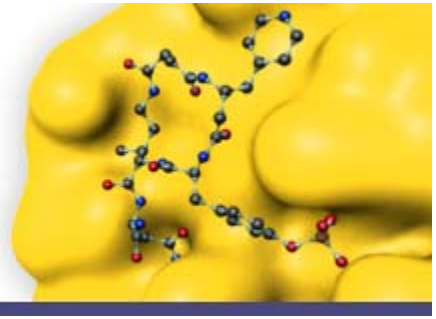
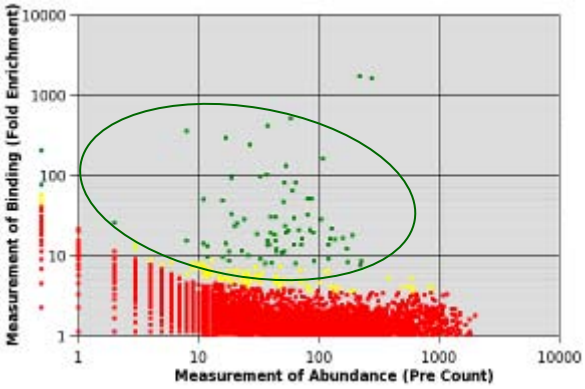
Selection repeated in "competition mode"
Hits competed by known hi-affinity peptide

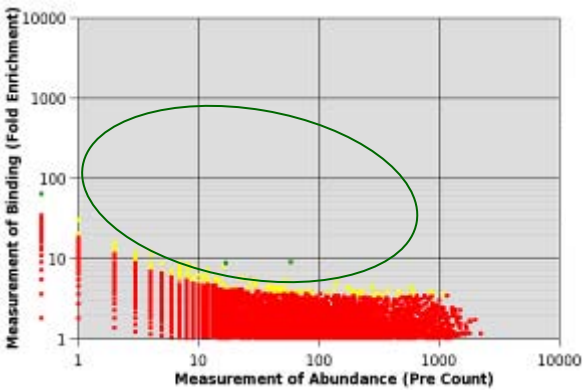


Target Selection of key library



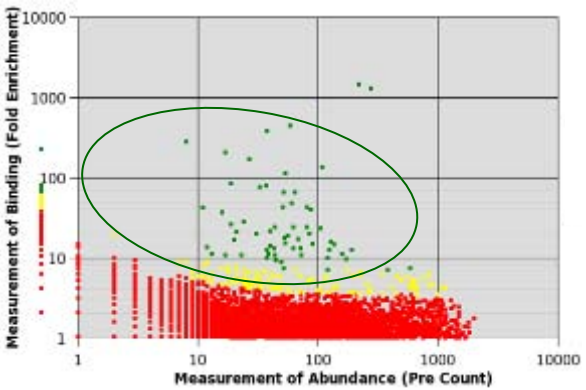
✓Hits identified as having affinity for the target

Target Selection repeated in the presence of "on mechanism" high affinity peptide



✓Hits are effectively competed by target-specific peptide—hits are "on mechanism"

Target Selection repeat in the presence of non-binding "random" peptide



✓Hits not affected by inactive peptide—hits confirmed as having affinity for the target

Case Study 2

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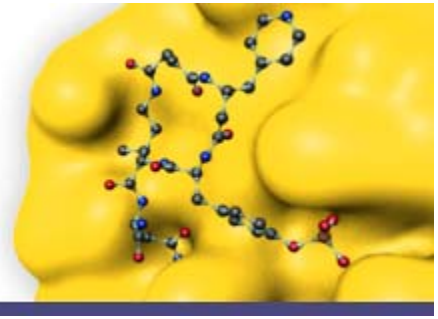
Compelling selection SAR confirmed by discrete macrocycles

Road map for medicinal chemistry campaign



Ordered by enrichment	Clear SAR	Mainly one component	Clear SAR	Linker fixed in subpool	Correlates with enrichment

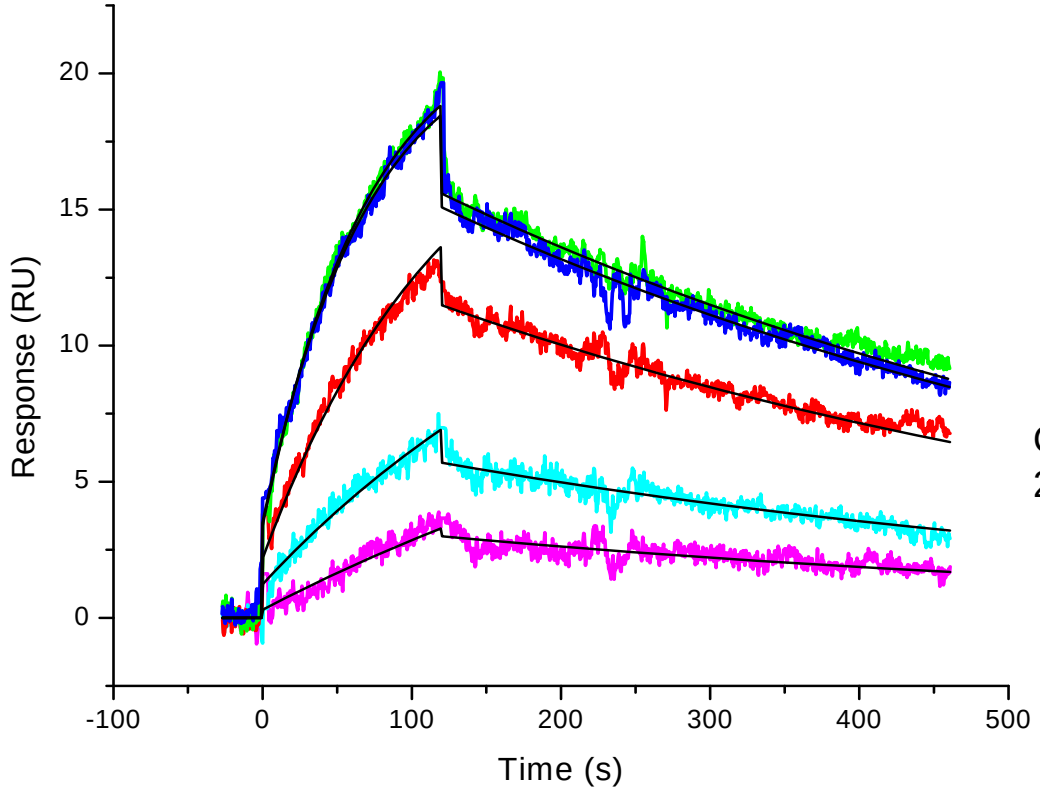
Most enriched compounds from selection of the ELB2 library

Code	FEsigma4	R1	R2	R3	Linker	Discrete: Target Affinity
1R2R3C4M	108.9	A	J	R	W	Kd = 543 nM
1R2R3K4M	59.9	A	J	S	W	
1R2R3O4M	39.0	A	J	T	W	
1K2R3C4M	34.5	B	J	R	W	Kd = 4.5 µM
1M2R3C4M	29.7	C	J	R	W	Kd = 1.1 µM
1M2R3K4M	26.8	C	J	S	W	
1M2R3O4M	26.3	C	J	T	W	
1R2T3C4M	19.0	A	K	R	W	
1S2R3C4M	17.2	D	J	R	W	Kd = 2.6 µM
1S2R3K4M	14.8	D	J	S	W	
1S2R3O4M	14.3	D	J	T	W	
1K2R3O4M	14.0	B	J	T	W	
1K2R3K4M	11.4	B	J	S	W	
1Q2R3K4M	10.3	E	J	S	W	
1Q2R3C4M	9.8	E	J	R	W	
1Q2R3O4M	8.2	E	J	T	W	
1P2R3K4M	8.1	D	J	S	W	
1R2T3K4M	7.9	A	K	S	W	

Case Study 2

SPR employed to confirm discrete Ensemblins bind to protein target

Representative Ensemblin binding to biotinylated target protein on a streptavidin SPR chip



K_{on} = 6.4E+02 (1/M*s)
K_{off} = 1.69E-03 (1/s)
Kd = 2.6 µM

Concentrations tested:
20, 10, 3.3, 1.1 µM

Case Study 2

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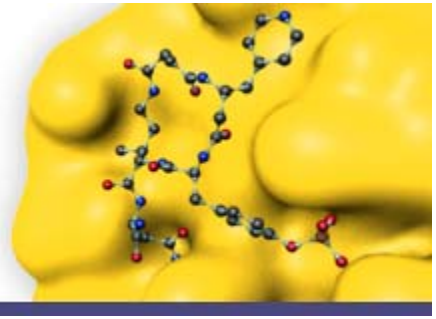
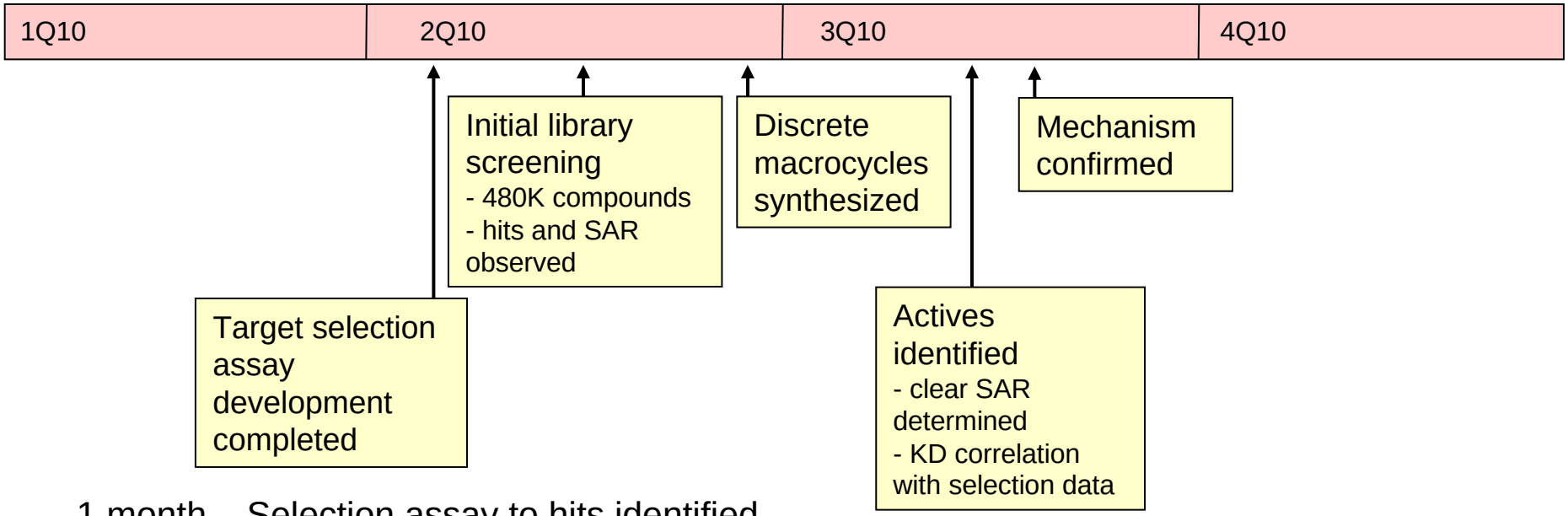
Ensemblin platform rapidly discovers first small molecule inhibitors of cytokine target





1 month – Selection assay to hits identified
1 month – Synthesis of discrete Ensemblin hits
1.5 months – Binding and inhibition data with SAR correlating with selection results
0.5 months – on-target mechanism confirmed

Case Study 2

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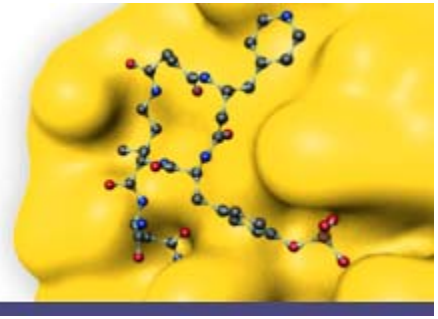
Macrocycles that inhibit cytokine/cytokine receptor interaction



Program Achievements:

- ✓ DPC hits identified that bind to cytokine
  - – Actives from at least two different macrocycle series
  - – Bind to site that is competed with known active peptide
- ✓ Discrete chemistry (non-DPC) produced good affinity leads
  - – 15 macrocycles with Kd values between 0.5 and 1.5 µM against cytokine
- ✓ Activity confirmed in biophysical competition assays (SPR and NMR)
  - – Binding affinity of discrete Ensemblins correlate well with selection enrichments
- ➢ *First known small molecule inhibitors of this cytokine target within 4 months of initiating selection assay*

Next steps: iterative libraries in screening, x-ray co-crystals in progress

Case Study 2

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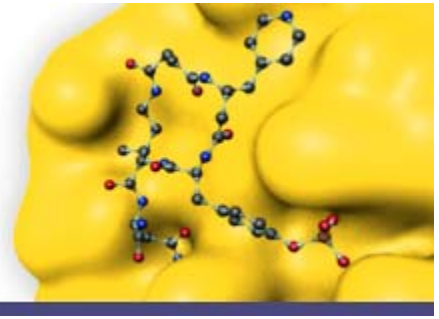
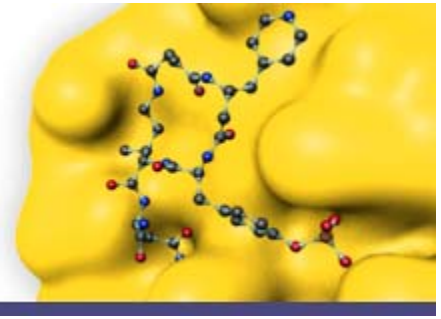


Ensemblins – Demonstrating Diversity and Druggability

Making macrocycles drug-like



- Ensemblins have MW > 500 and yet can be drug-like with oral bioavailability
- Natural product macrocycles provide a compelling precedent
  - At least 8 macrocyclic natural products have resulted in orally bioavailable drugs (see next slide)
    - erythromycin alone has led to three oral drugs (erythromycin, azithromycin and clarithromycin)
  - Rule of 5 requirements are violated by these compounds and yet they remain drug-like
- This provides compelling evidence for macrocycles existing within a non-Lipinski drug-like space
  - macrocycle drugs are not constrained by “rule of 5” limitations

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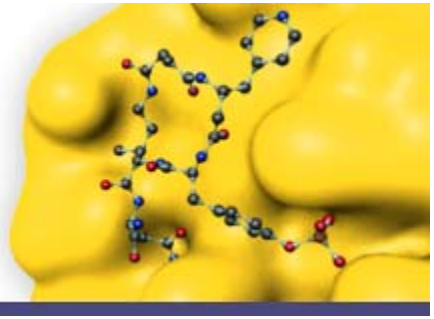
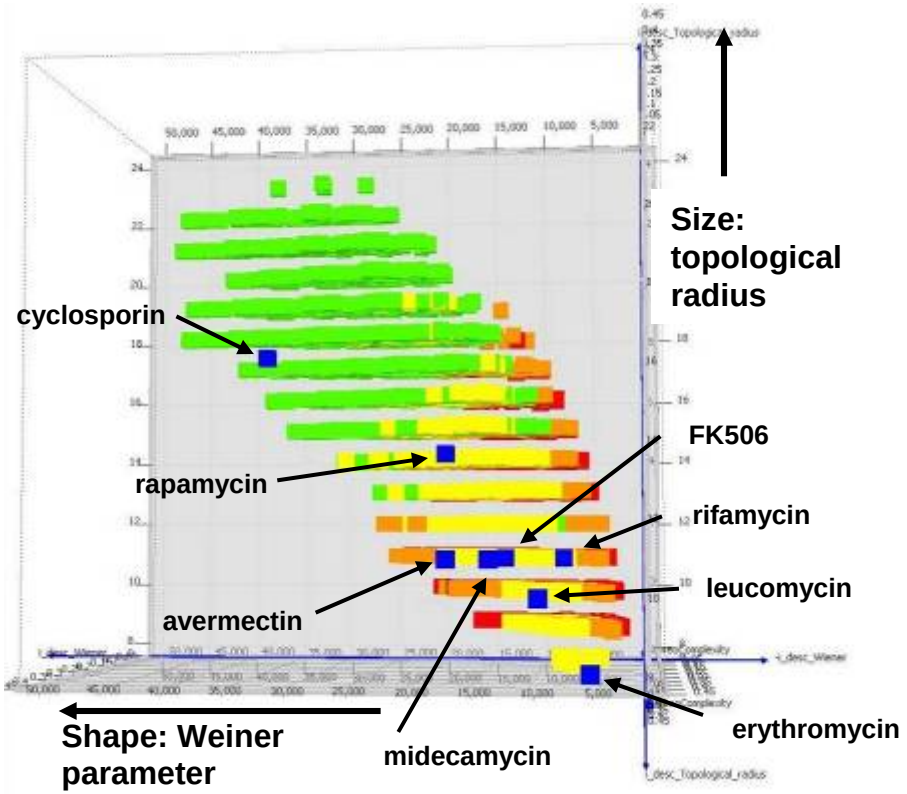
Ensemblins represent same space as orally bioavailable natural product macrocycles



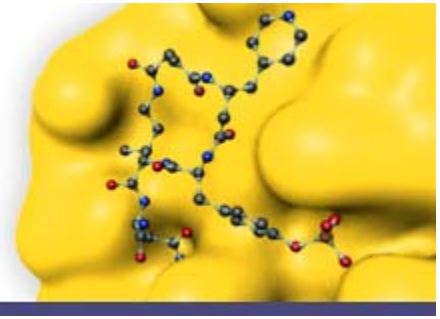
- Orally bioavailable natural product macrocycles have led to a number of marketed orally active products (oral bioavailability in green)

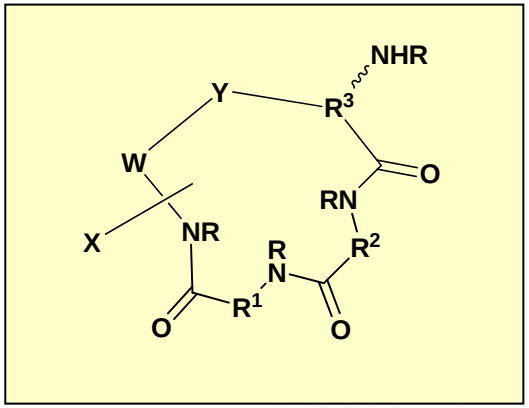
avermectin B1a	873	ivermectin	~100%
cyclosporin A	1203	ciclosporin	30%
erythromycin	734	erythromycin	25%
FK506	804	tacrolimus	20%
leucomycin	702	kitasamycin	
midecamycin	814	miocamycin	~100%
rapamycin	914	sirolimus	20%
rifamycin	698	rifampicin	90-5%

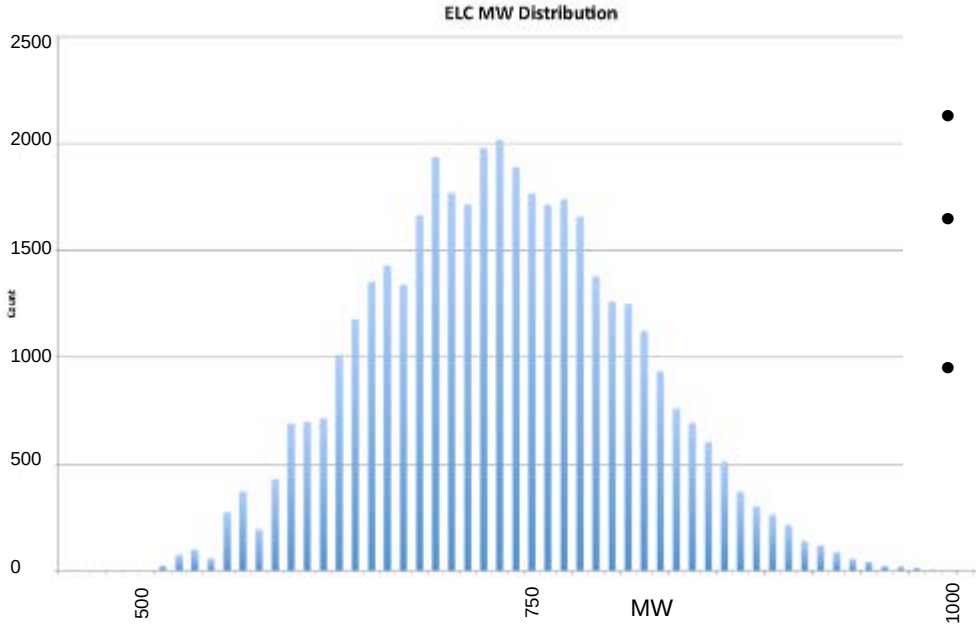
- Representative compounds distributed by size and shape
- Orally bioavailable natural product macrocycles in blue



Ensemblin Libraries: Diversity & Design







Macrocycles represent substantial opportunity for structural diversity

- Structural variation achieved through:
  - Macrocycle architecture and ring-closing chemistry
  - R-groups
  - N-alkylation of peptide bonds
  - Stereochemical variation
- Spacer groups enable molecular domains and incorporation of larger pharmacophores
- Linker-spacer chemistry developed to readily provide multiple types of chemical ligations with variable ring size and flexibility
- For the ELC library (1Q10)
  - Ring sizes range from 19-26; median=21
  - MW range 510-990; mean=720

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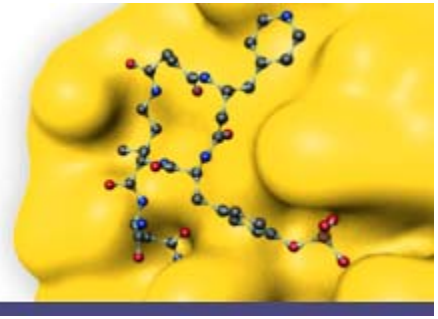
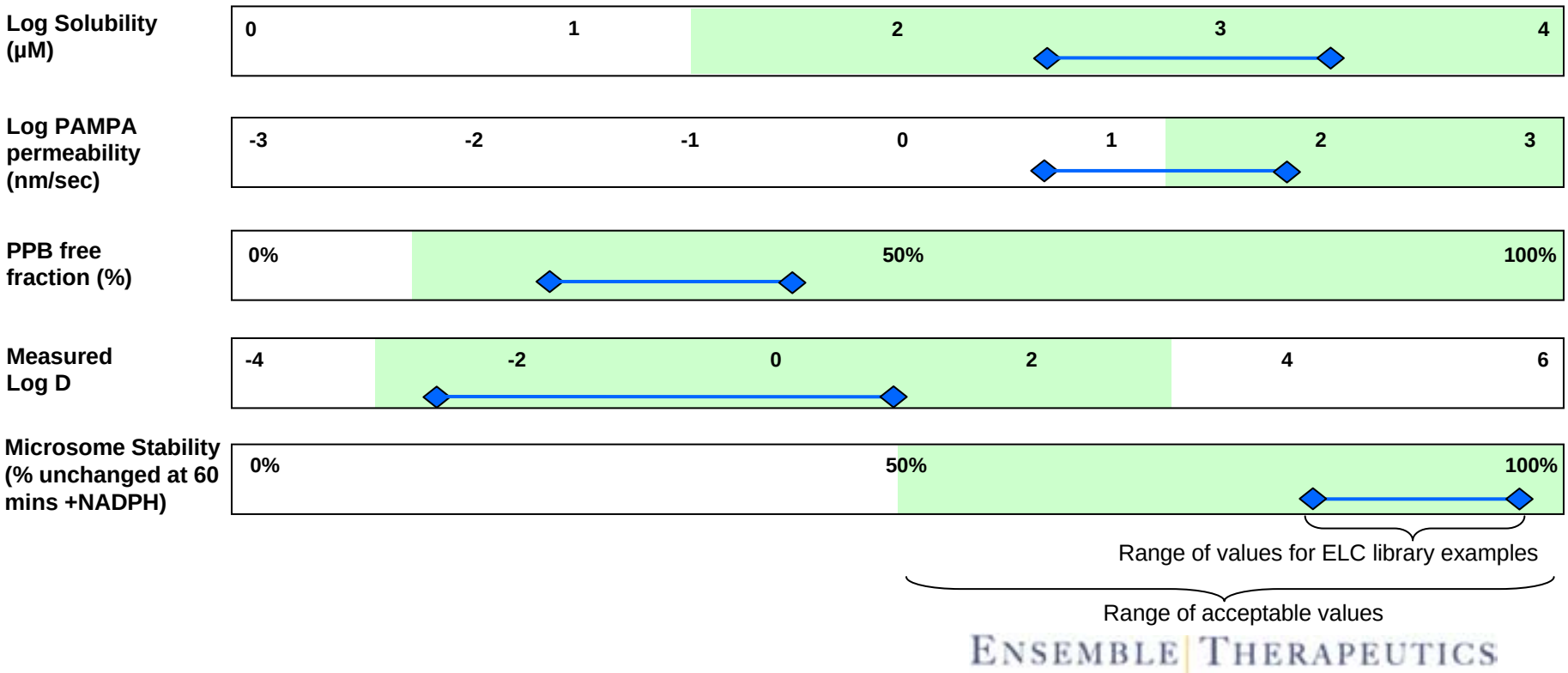
Ensemblins display a range of physical properties



- Macrocycles cover a range of physical and ADME properties
- We have built correlations between macrocycle structure and these properties and have incorporated them into new designs
- ELC library compounds designed to demonstrate good ADME properties



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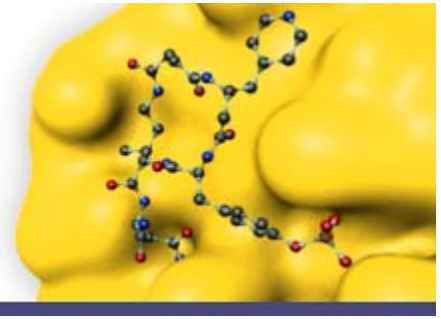
Summary

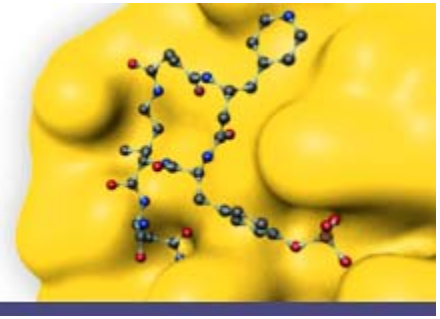


- Significant number of macrocyclic Ensemblins on DNA (>1.6 million) available for selection-based screening
  - Additional libraries in 2010 for total of 1.5 million Ensemblins
  - Largest macrocycle library in the industry

- Macrocycles represent considerable diversity and drug-like properties
  - Multiple novel macrocycle templates; protein function mimics
  - Physicochemical and other druggable properties incorporated
  - Oral bioavailability for numerous macrocycles

- Hits identified for multiple protein-peptide and protein-protein targets
  - Current collaborations with BMS and Pfizer
  - Significant success with partnered targets

Acknowledgements



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