Transplantation of a complex binding site using computational design and *in vitro* evolution

Bill Schief
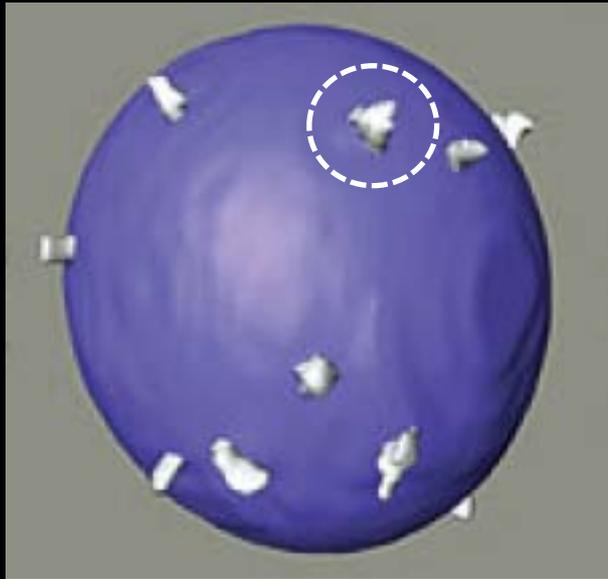
Department of Biochemistry – UW

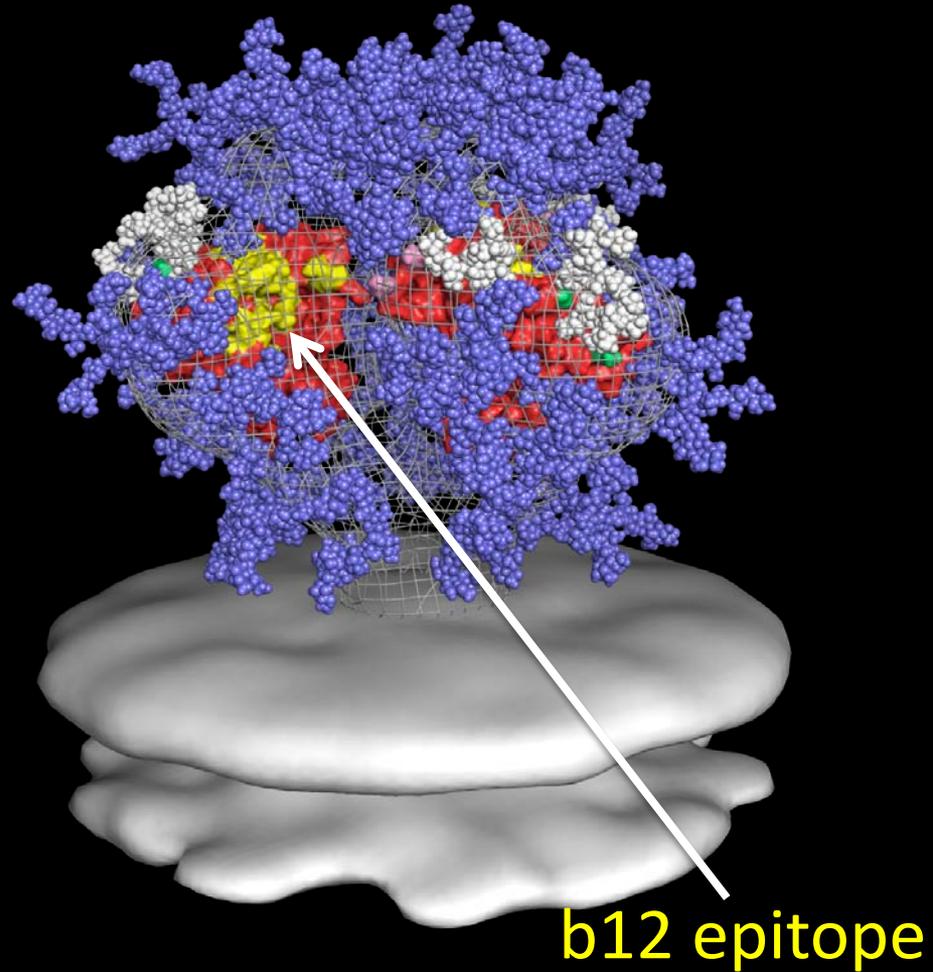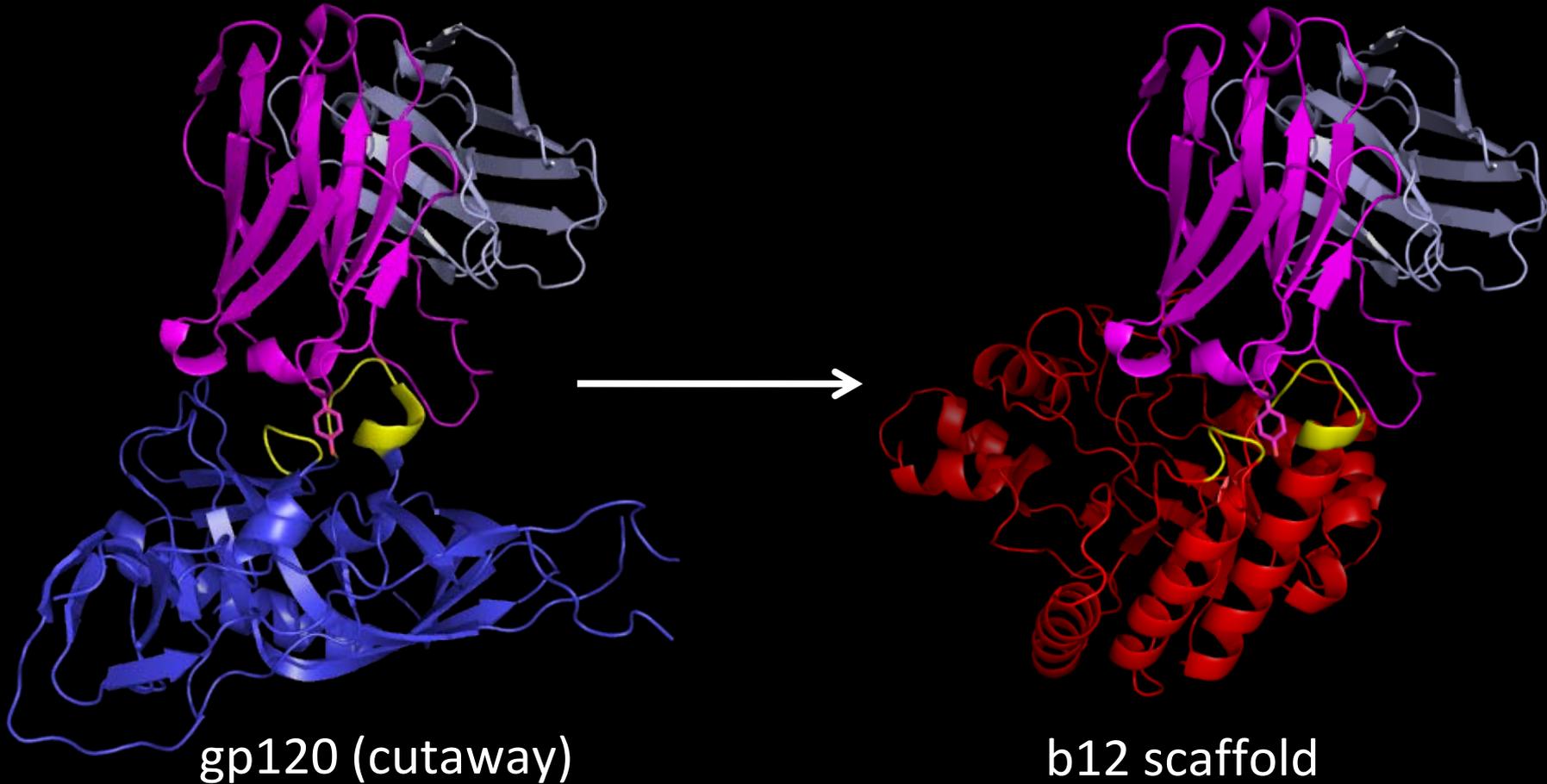RosettaCON Aug 4, 2010

HIV Overview

HIV

Zhu et al, Nature, 2006

b12 epitope

-Cell entry is mediated by the CD4-gp120 interaction
-b12 antibody neutralizes HIV at the CD4 binding site

Transplantation of the b12 discontinuous epitope

gp120 (cutaway)

b12 scaffold

b12-gp120 structure: Zhou et al. Nature 2007

-gp120 is an ineffective immunogen
-Challenging test for protein design

Epitope transfer to scaffolds (Rosetta Multigraft)
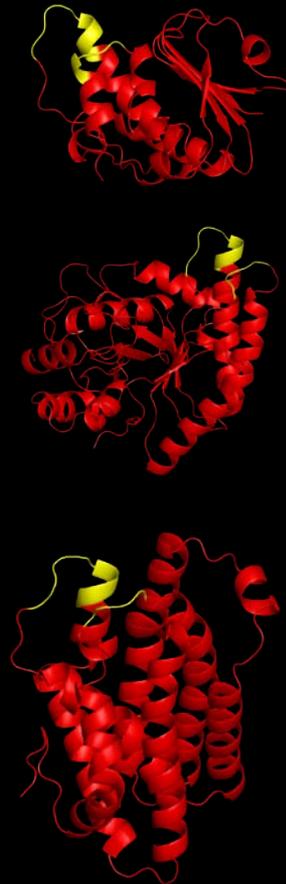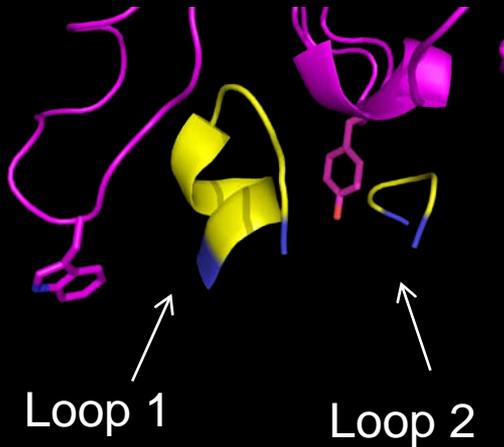
b12-bound epitope structure

b12 mAb

gp120

Epitope transfer to scaffolds (Rosetta Multigraft)

b12-bound
epitope structure

Scaffold Search
(based on structural
features)

Loop 1

Loop 2

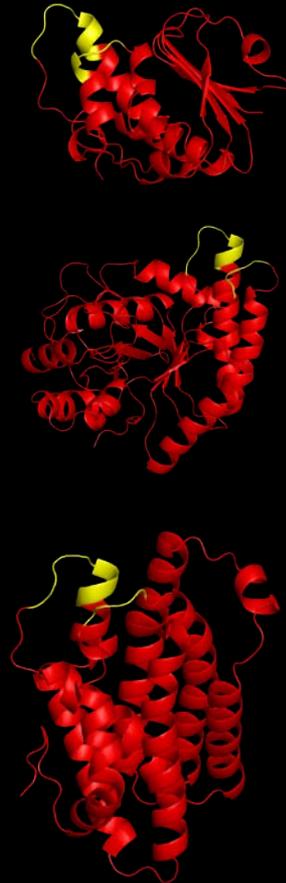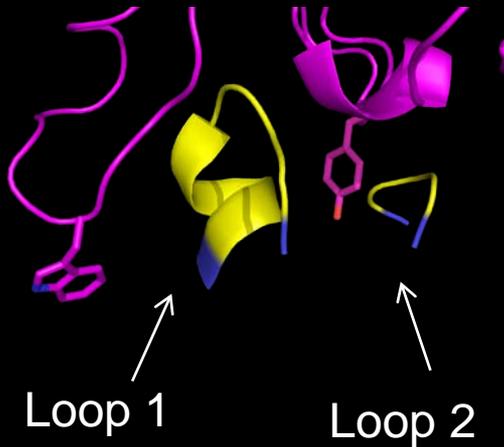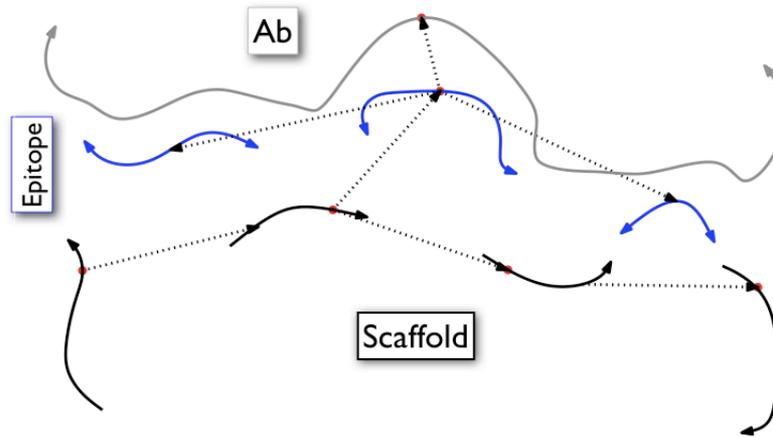# Epitope transfer to scaffolds (Rosetta Multigraft)

| b12-bound epitope structure	Scaffold Search (based on structural features)	Epitope grafting (backbone reconnecting, structural stabilization)

Loop 1

Loop 2

MultiGraft

- All-in-on continuous & discontinuous epitope grafting
- Full control over epitope insertion
  - Style of graft closure
  - Relative rigid body orientation of discontinuous loops (fixed or broken)
  - Flexibility/rigidity of epitope
  - Variable length linkers
  - Linker secondary structure & a.a. identity
  - Optimization of antibody vs. epitope-scaffold orientation
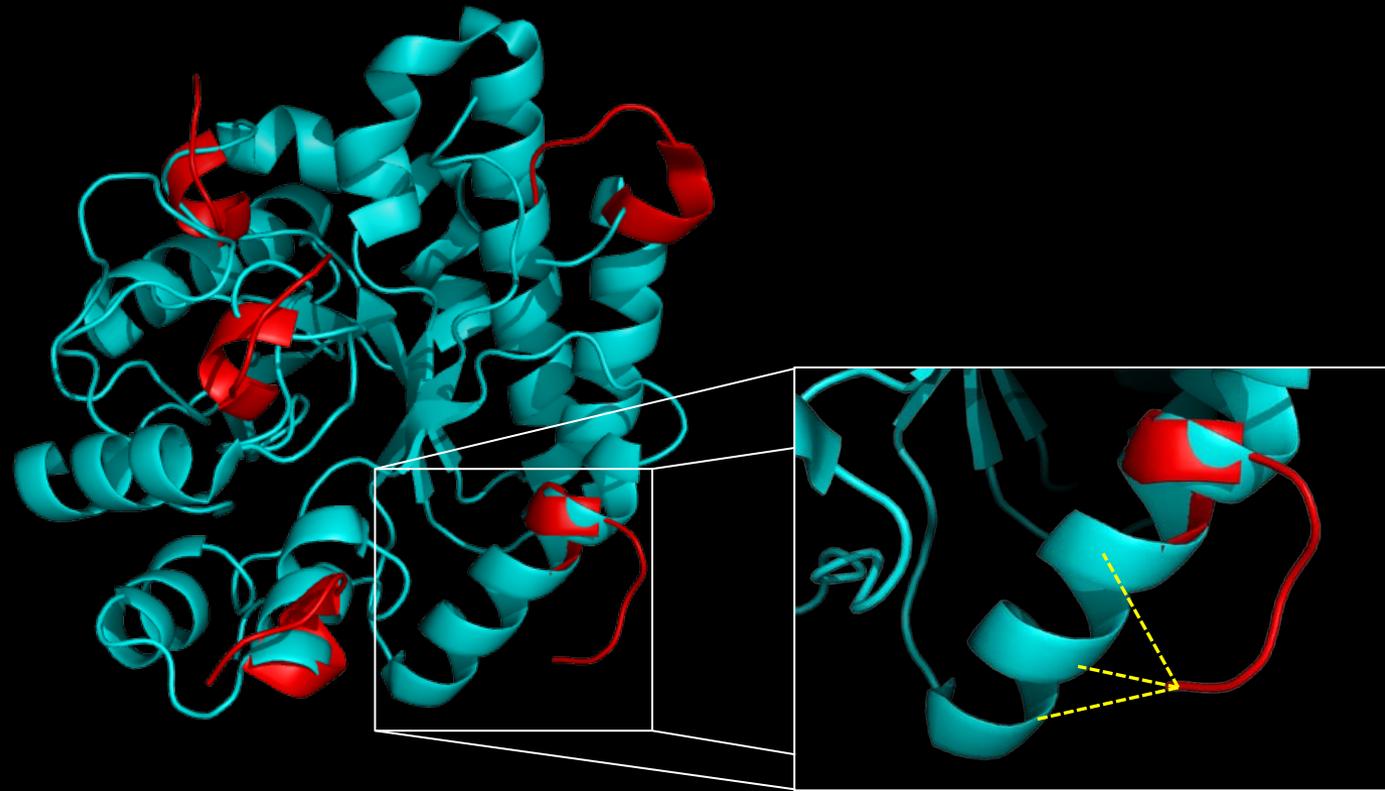
Scaffold Search
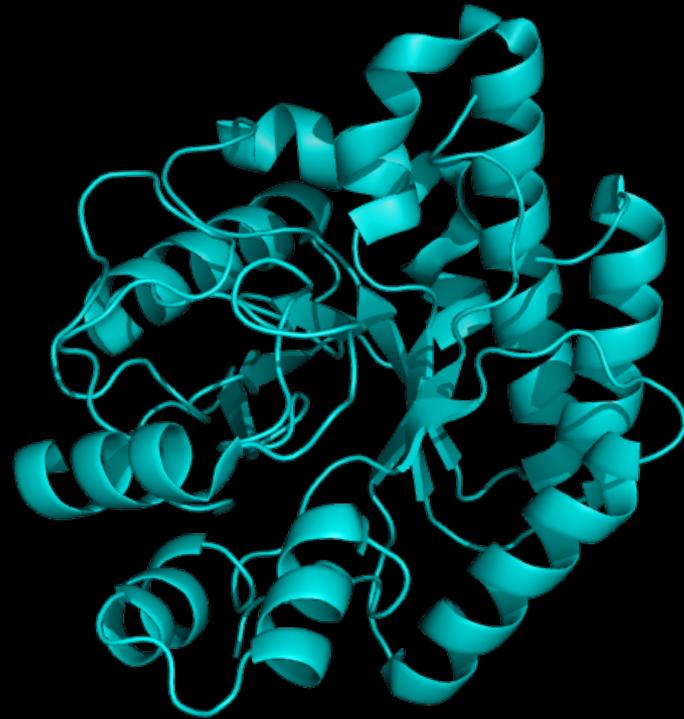
Putative Scaffold

Scaffold Search

Exhaustive Search for Loop 1 Match

Scaffold Search

Match Found for Loop 1

Scaffold Search

Recovery of the original complex epitope
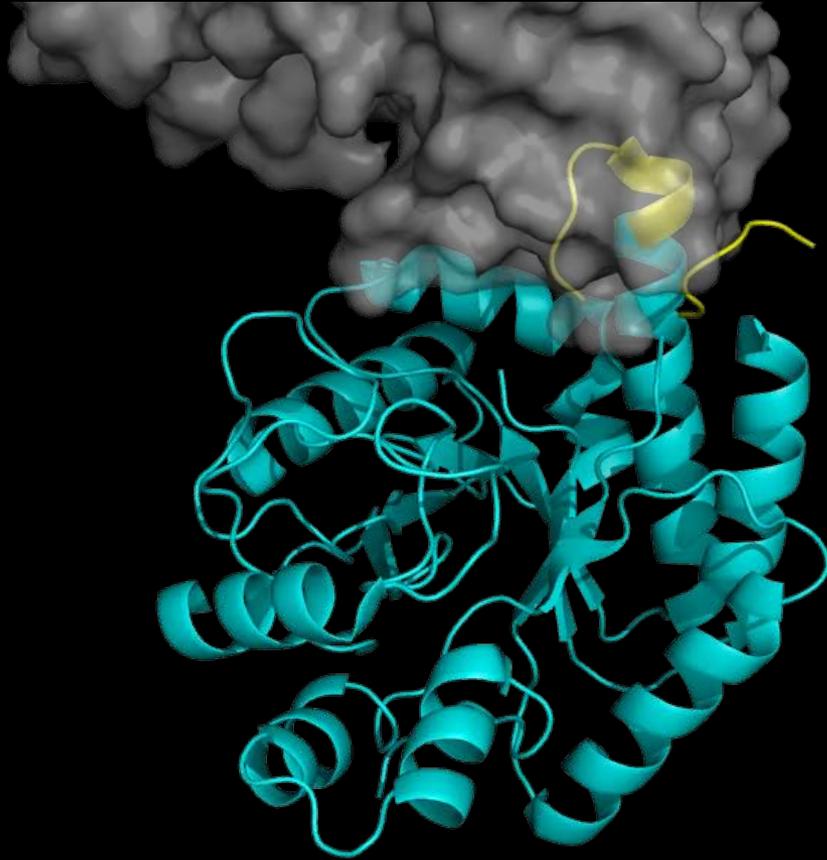-Check for Loop 2 matches

Scaffold Search

Loop 2 Match found
Backbone Replacement

Scaffold Search
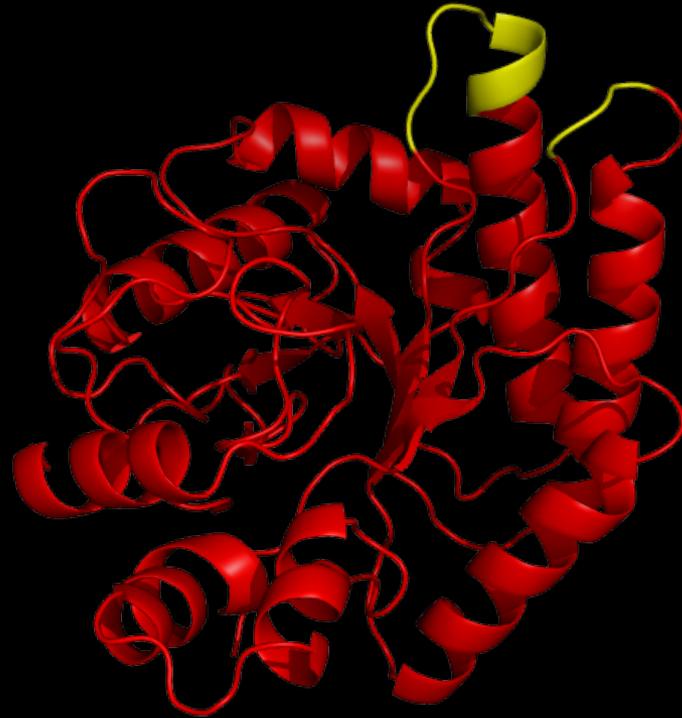

Clash Checking
Final Match Found

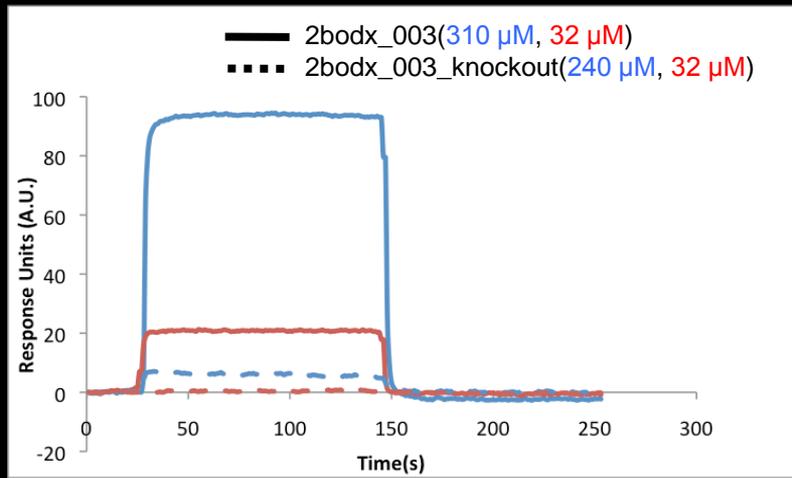# Loop Modeling & Design

Backbone Rebuilt and Designed to close gaps
Wang et al. JMB 2007; Kuhlman & Baker PNAS 2000

Computational Design

-12 different scaffolds expressed in *E. coli*
-2 showed good expression and solubility
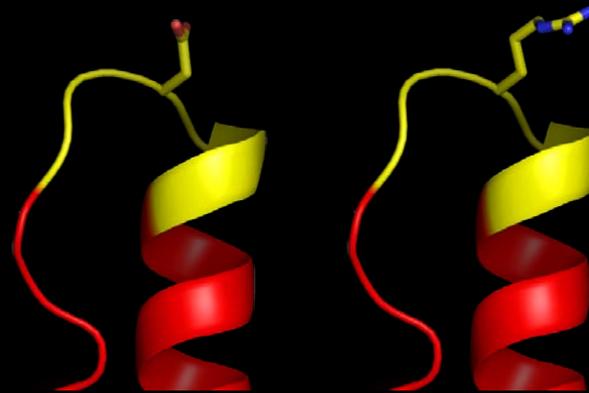

2bodx

Surface Plasmon Resonance

— 2bodx_003($310\ \mu M$, $32\ \mu M$)
···· 2bodx_003_knockout($240\ \mu M$, $32\ \mu M$)

WT   Knockout

Li et al. Nat Med 2007

- 2bodx showed detectable and specific binding
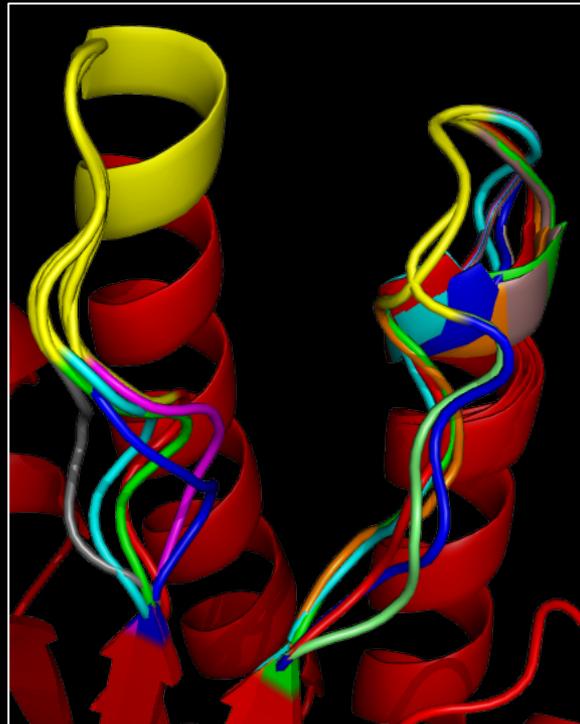
Directed Library Design

Conformational Ensembles

Designed Positions

Directed Library Design
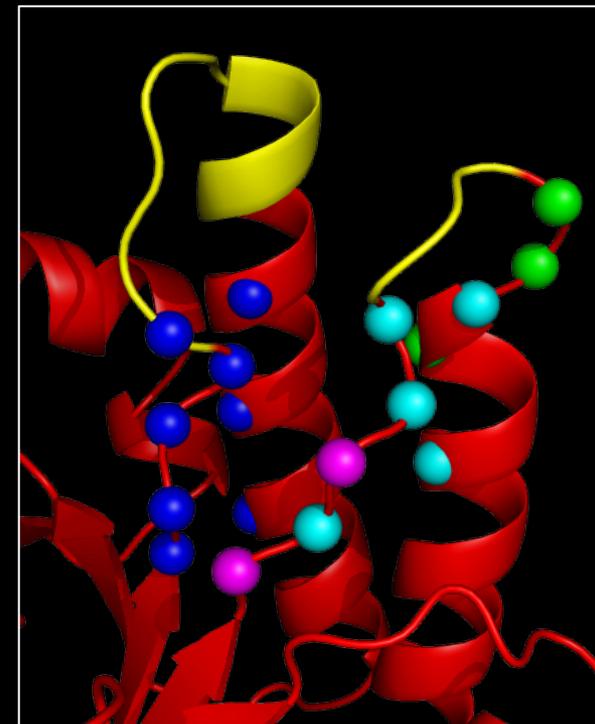
Conformational ensembles of epitope connecting segments

Design & Filter

⇩

Sequence Profiles

⇩

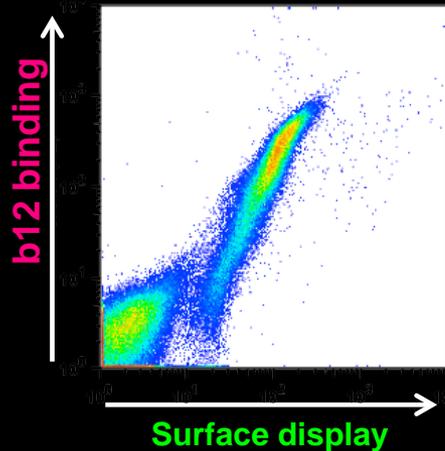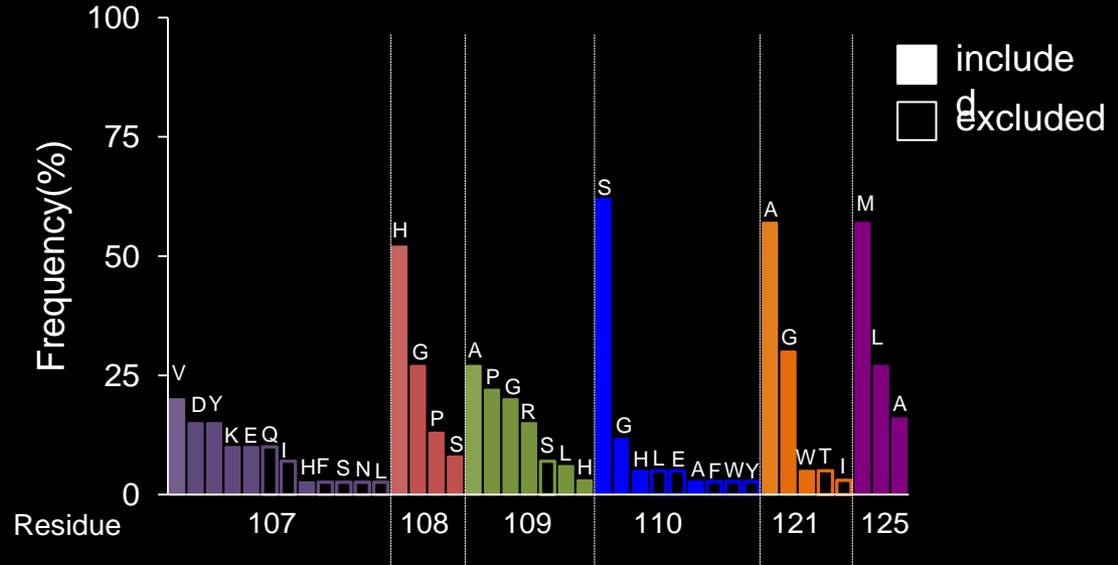Library Design
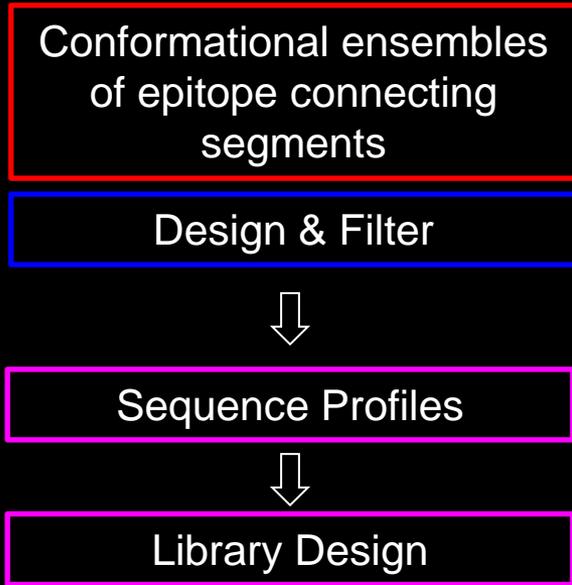
Rosetta preferences for Loop 1 residues

Yeast Display

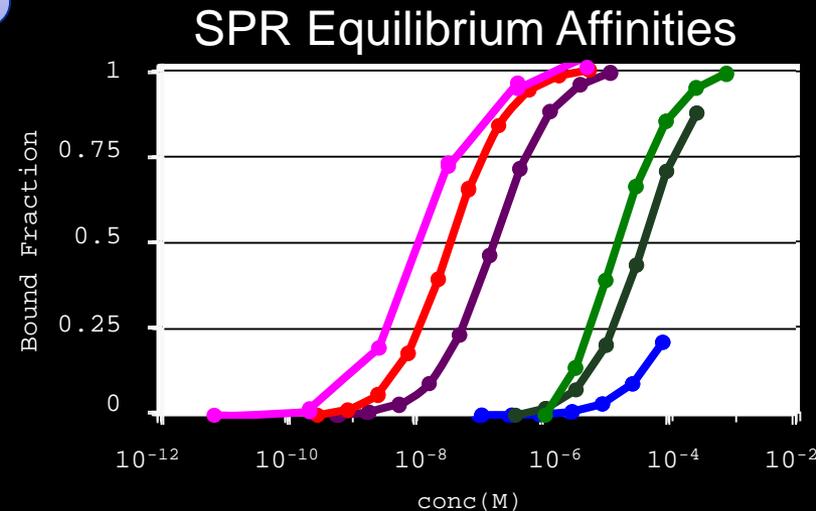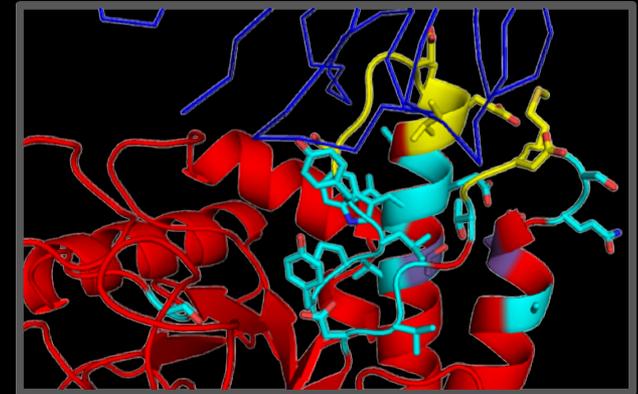Library Sizes:
   All amino-acids – 10^{21}
   Rosetta – 10^{17}
   Manual Inspection – 10^{12}

Loop 1
10^5

Loop 2
10^6

Selection of a b12 high affinity binder

Computational design
$K_D > 300\mu M$

Random mutagenesis
$K_D \approx 40\mu M$
2 mutations

loop2 directed library
$K_D = 13\mu M$
8 mutations

loop1 directed library
$K_D = 145nM$
15 mutations

Combined mutations
$K_D = 35nM$
17 mutations

3rd directed library
$K_D = 10nM$
19 mutations

SPR Equilibrium Affinities
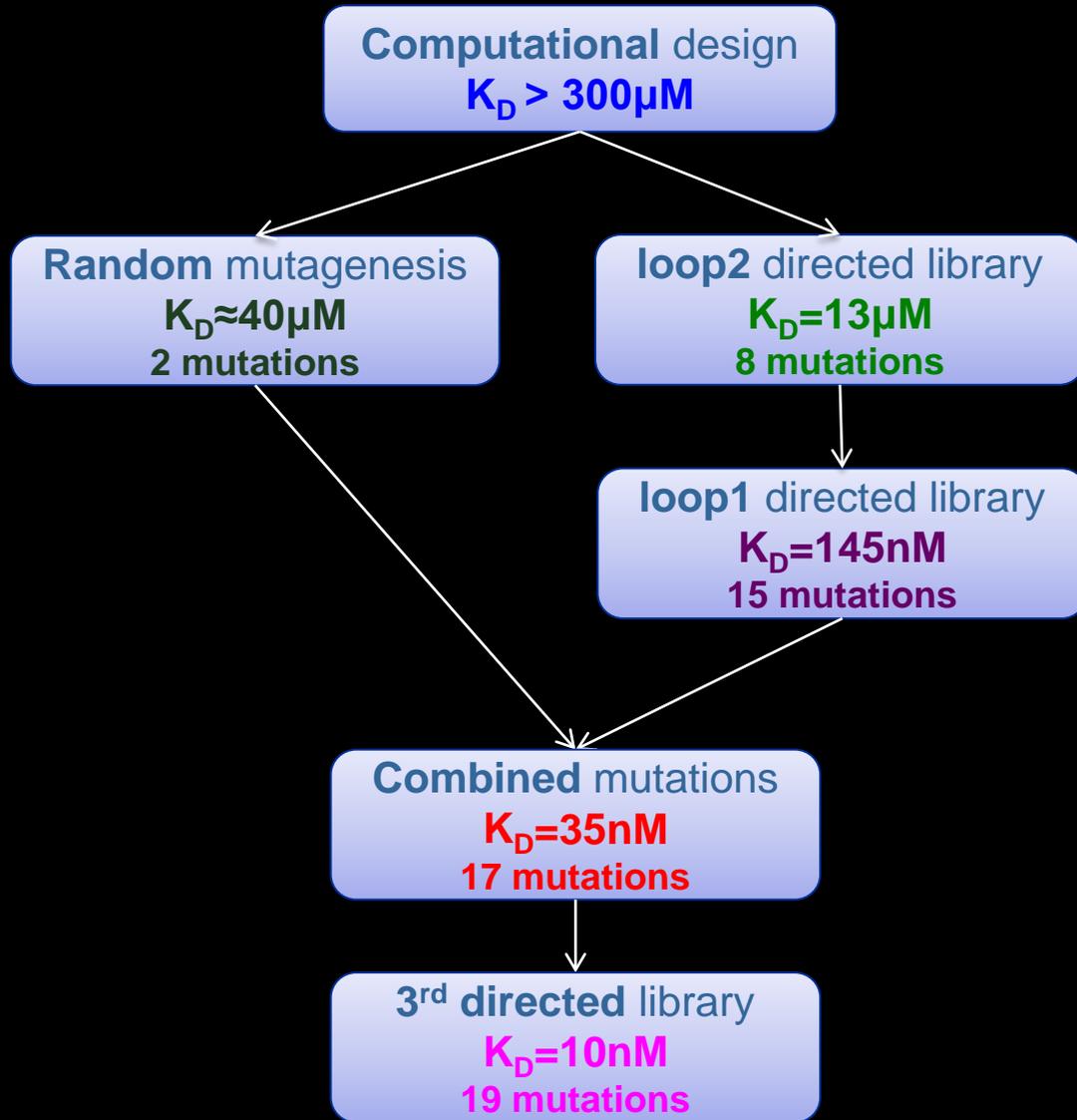
b12 binding specificity

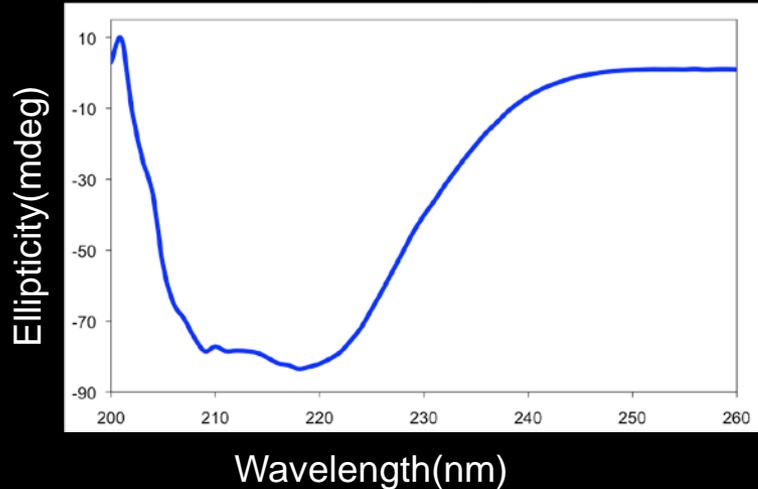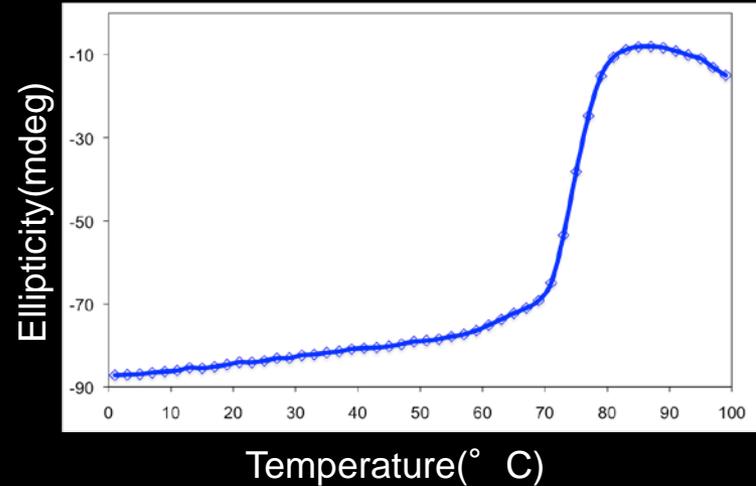## Surface Plasmon Resonance

b12_43(1 uM)
b12_43_knockout(1uM)
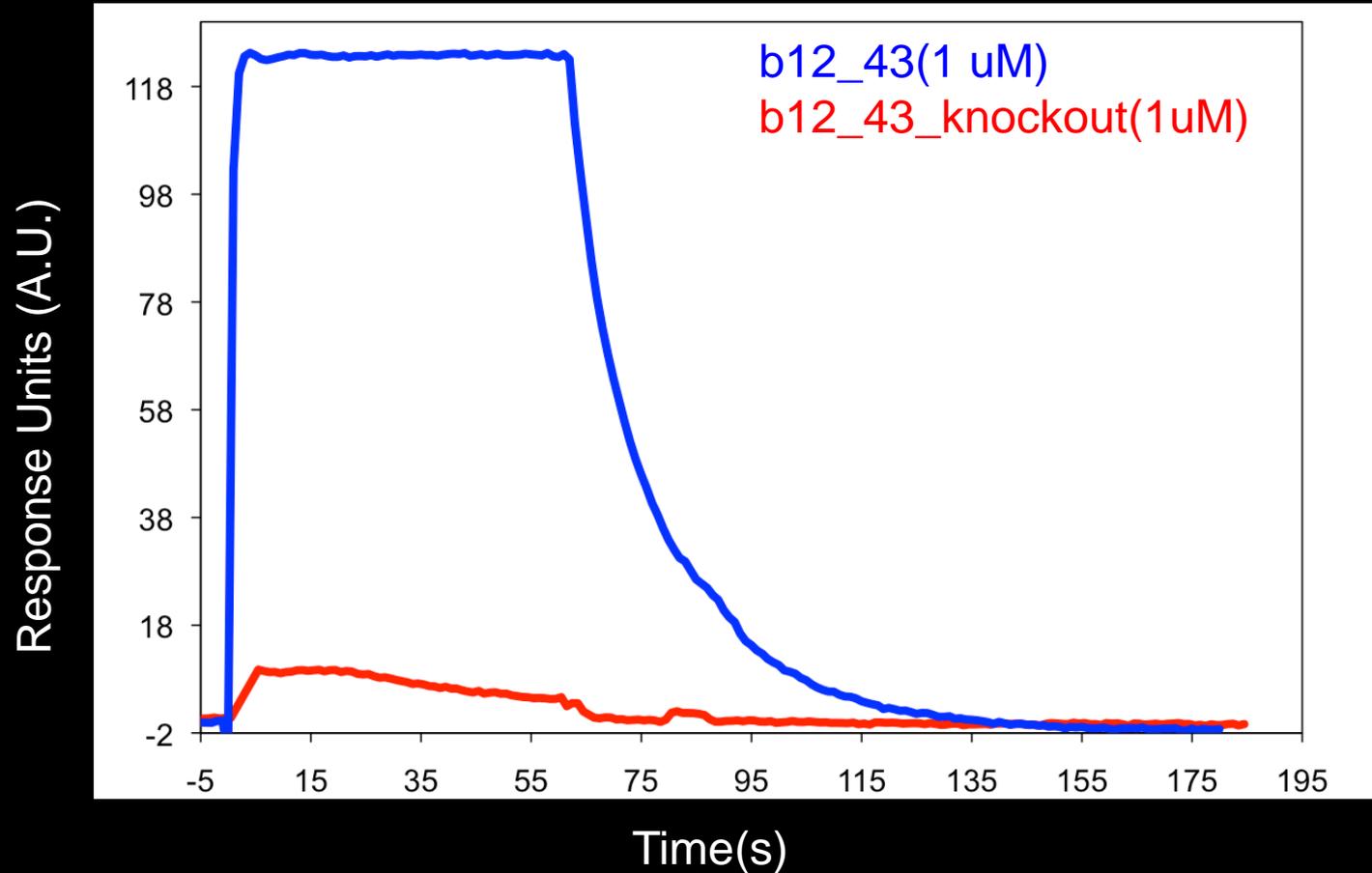
-b12_43 binding ablated by known knockout mutation of b12-gp120 interaction
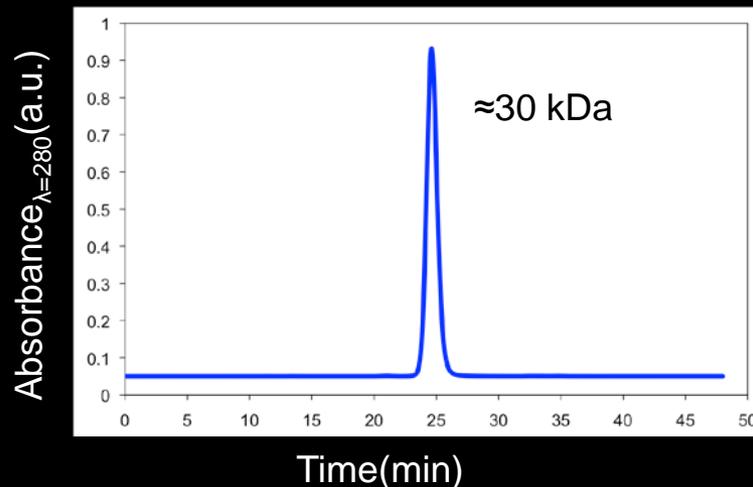
b12_43 solution properties

Far-UV CD spectrum
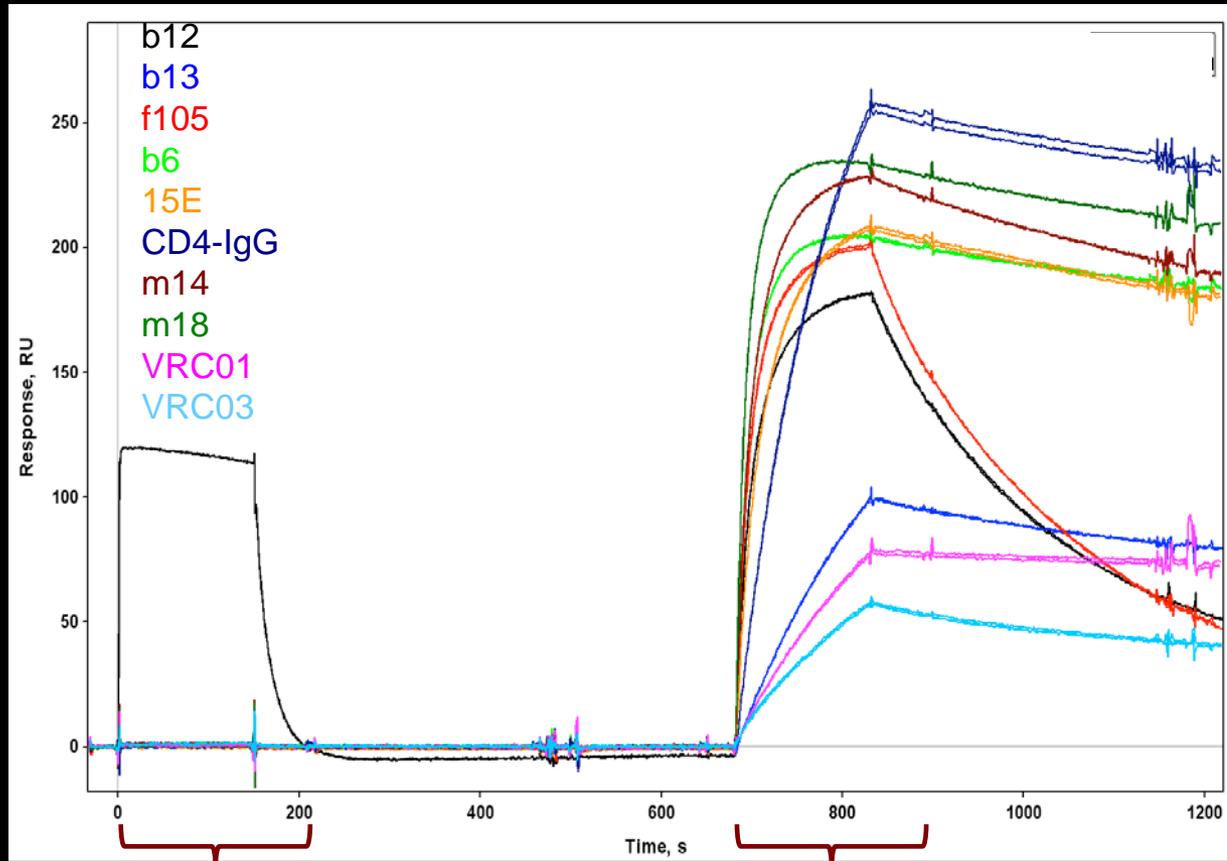
Thermal Denaturation

Size Exclusion-Light Scattering

≈30 kDa

-b12_43 unfolds cooperatively with a Tm of 75 °C and is a monomer in solution

Highly specific b12 binder

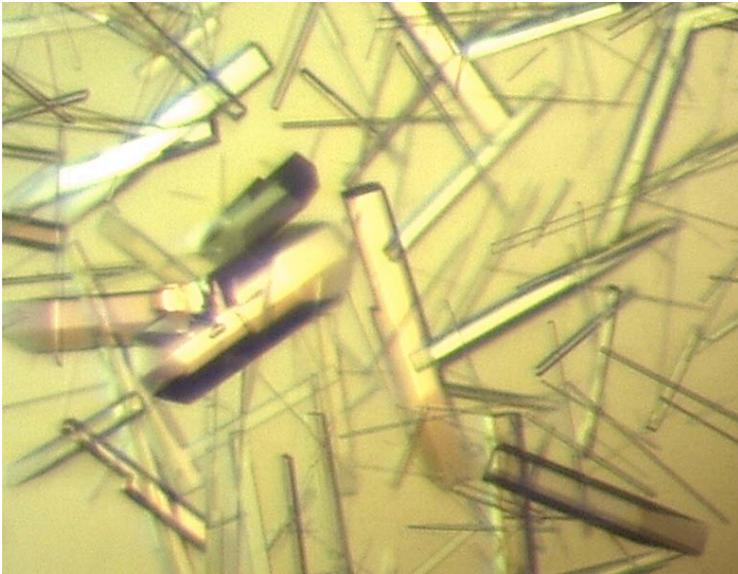Surface Plasmon Resonance

b12 scaffold (1.2uM)   gp120 (96nM)

-Scaffold only binds to b12 and not several antibodies that target the cd4-bs on gp120
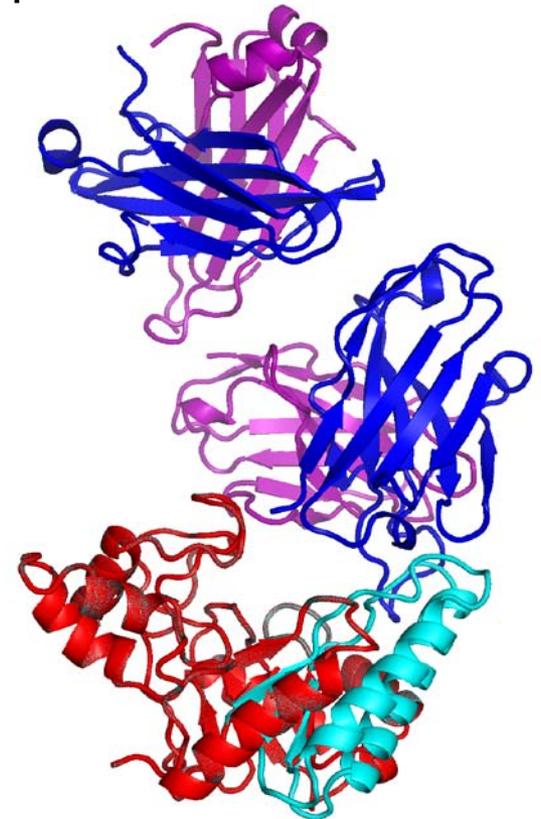
2bodx43:b12 Fab Crystals

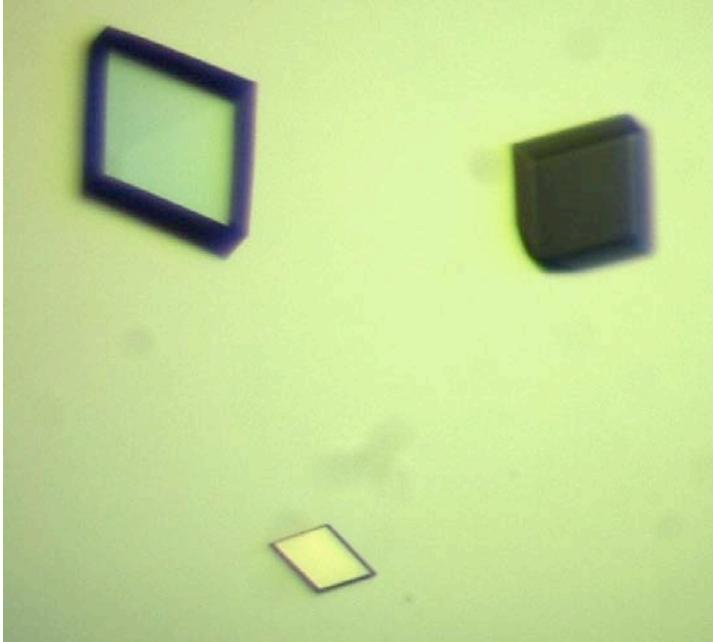- 2.07 A data
- MR phasing works
- Currently refining

B/R/P: search model
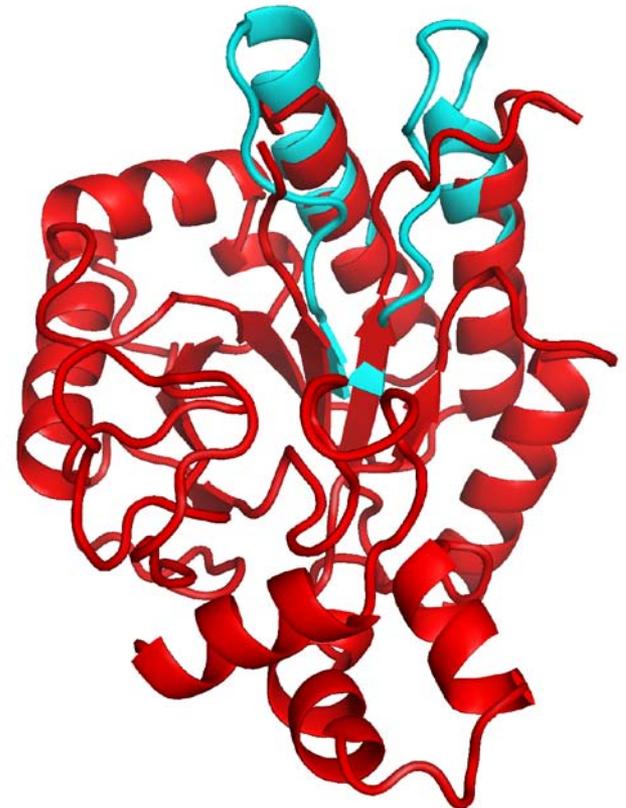
Cyan: input model

2bodx45 Unliganded Crystals

- 1.52 A data
- MR phasing works
- Currently refining

Red: Current Refinement

Cyan: input model

Conclusions & Future Work

- Development of a general computational procedure for transplantation of complex binding sites

- Computation-guided libraries achieved 30,000 fold improvement in affinity over the initial computational design

- Best scaffold binds to b12 with the same affinity as gp120

- Scaffold binds with high specificity to b12 and not to other cd4 binding site antibodies

- Crystallographic characterization of both bound and unbound scaffold

- Immunization experiments to investigate the capability to re-elicit b12 like antibodies

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(most of) Schief Lab on Goat Peak, Aug 2009