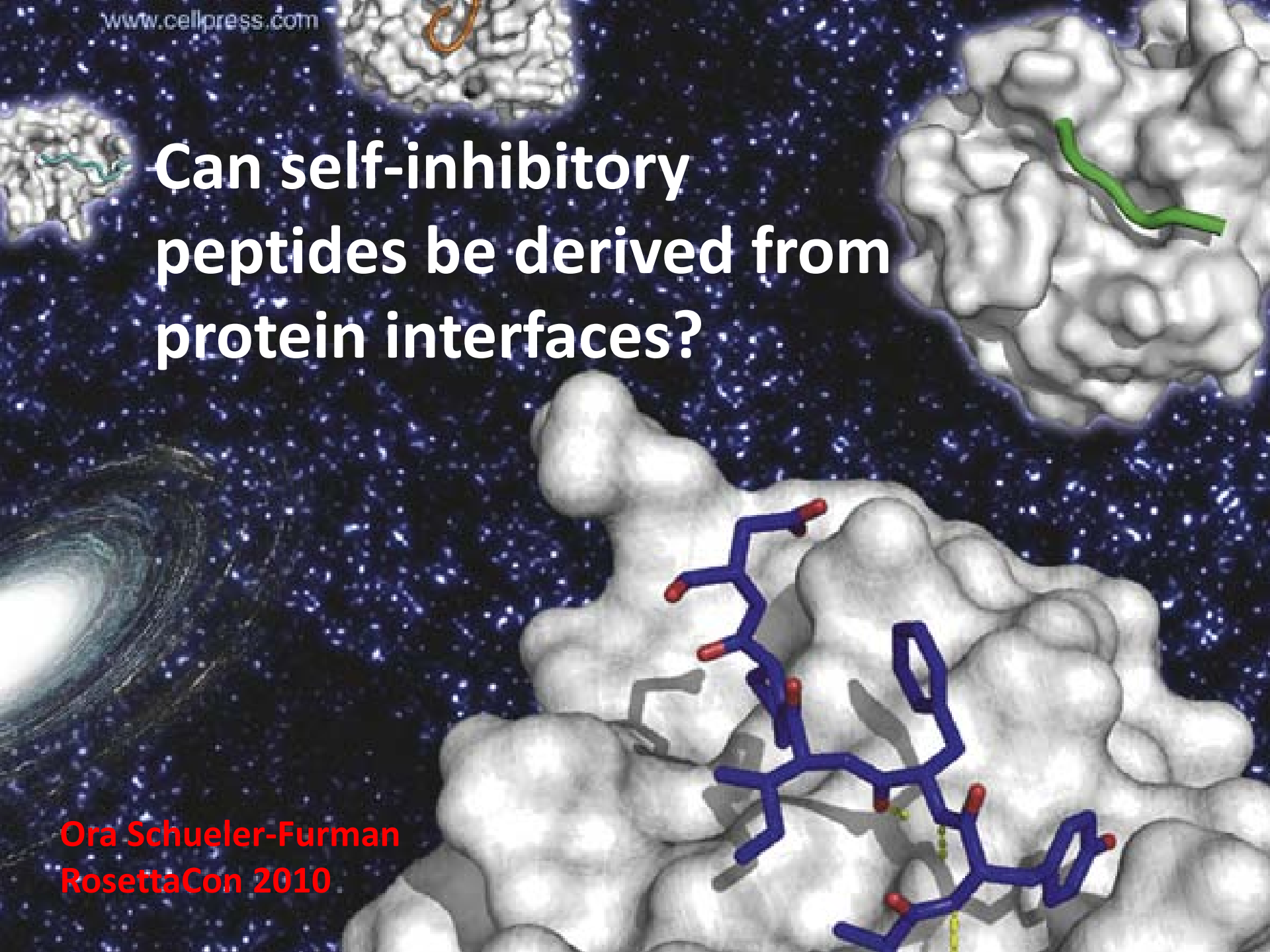


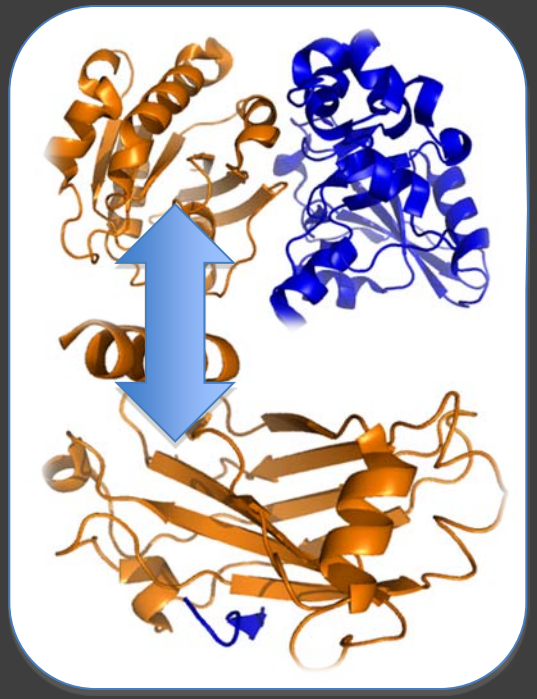
Can self-inhibitory peptides be derived from protein interfaces?

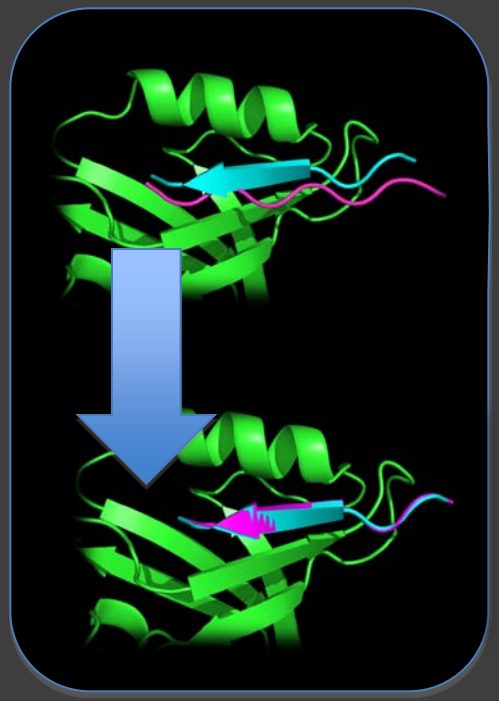
Ora Schueler-Furman
RosettaCon 2010

Outline of today's talk

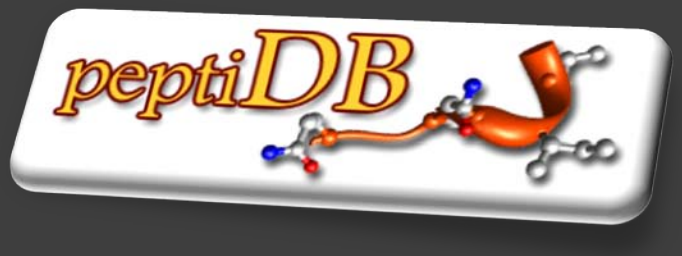
1. Short introduction on peptide-protein interactions and their modeling
2. Peptides in protein-protein interfaces
3. Inhibition of protein interactions by peptides : the antitoxin-toxin interaction
4. Outlook

1. Short introduction on peptide-protein interactions and their modeling



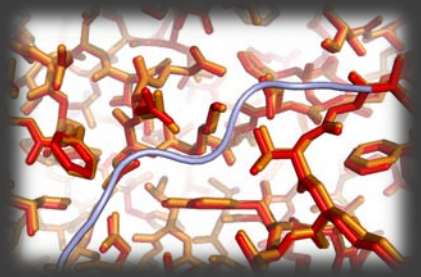


Features of peptide-protein interfaces



- Database of peptide-protein complexes
  (n=100; 87 free protein structures)

➢ Compare features to protein-protein complex structures



➢ Peptides optimize enthalpy and configurational entropy of binding:

- No conformational change in partner
- Optimized polar and VDW interactions
- Anchor hotspots in pockets

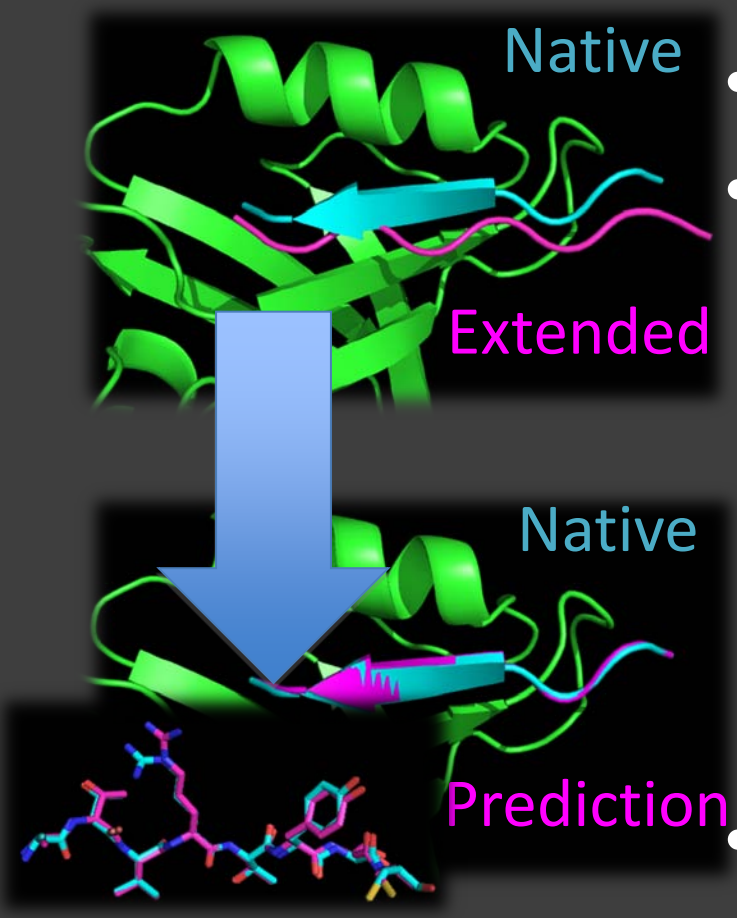
Structure prediction of peptide-protein complexes

✓Localize search on largest pocket(s)

✓No need to model conformational changes in protein

London, Movshovitz-Attias, Schueler-Furman. Structure (2010)

FlexPepDock – modeling of peptide-protein complex structures



- General and accurate
- Successful cross docking
- Effective sampling range for flexible-peptides refinement :
  - 5Å for near-native conformations (<2Å bbRMSD)
  - 3Å for high-resolution conformations (<1Å bbRMSD)

  Often, high-quality models are sampled from much larger deviations (>10Å)
- Good sampling & selection of high-resolution tetramers (<1Å all atom RMSD)

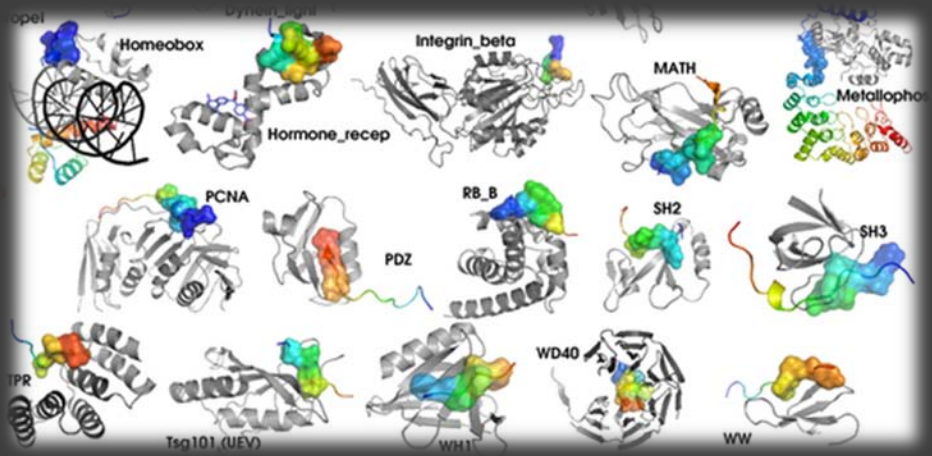
Raveh, London*, Schueler-Furman. Proteins (2010)*

Outline of today's talk

1. Short introduction on peptide-protein interactions and their modeling
2. Peptides in protein-protein interfaces
3. Inhibition of protein interactions by peptides: the antitoxin-toxin interaction
4. Outlook

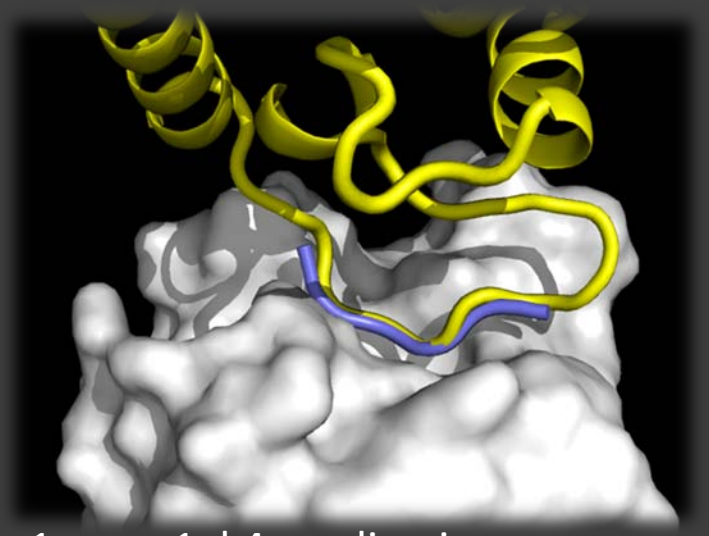
Peptide-mediated PPI's

1. Linear motifs within unstructured regions



2. Continuous stretch at PPI interface. Contributes most of binding energy. Similar structure as free peptide

3. Free peptide



1awr – 1ak4: proline isomerase – HIV capsid protein (HAGPIA peptide)

Many PPI's are mediated by a single peptide

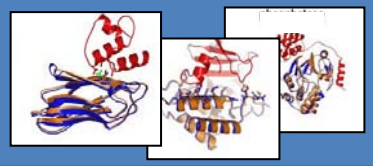
PeptiDeriver

Given a complex :

- minimize the structure
- for each protein partner
- extract each decamer peptide at interface
- calculate binding energy ΔΔG *(Rosetta Interface score12)*

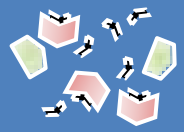
- **Select peptide with best binding energy**

dataset of protein-protein complexes



detect high-affinity segments at the interface

high–affinity segments → self-inhibitory peptides?



Computational Validation : Flexible Peptide Docking (using FlexPepDock)

Optional: redesign peptide for increased affinity

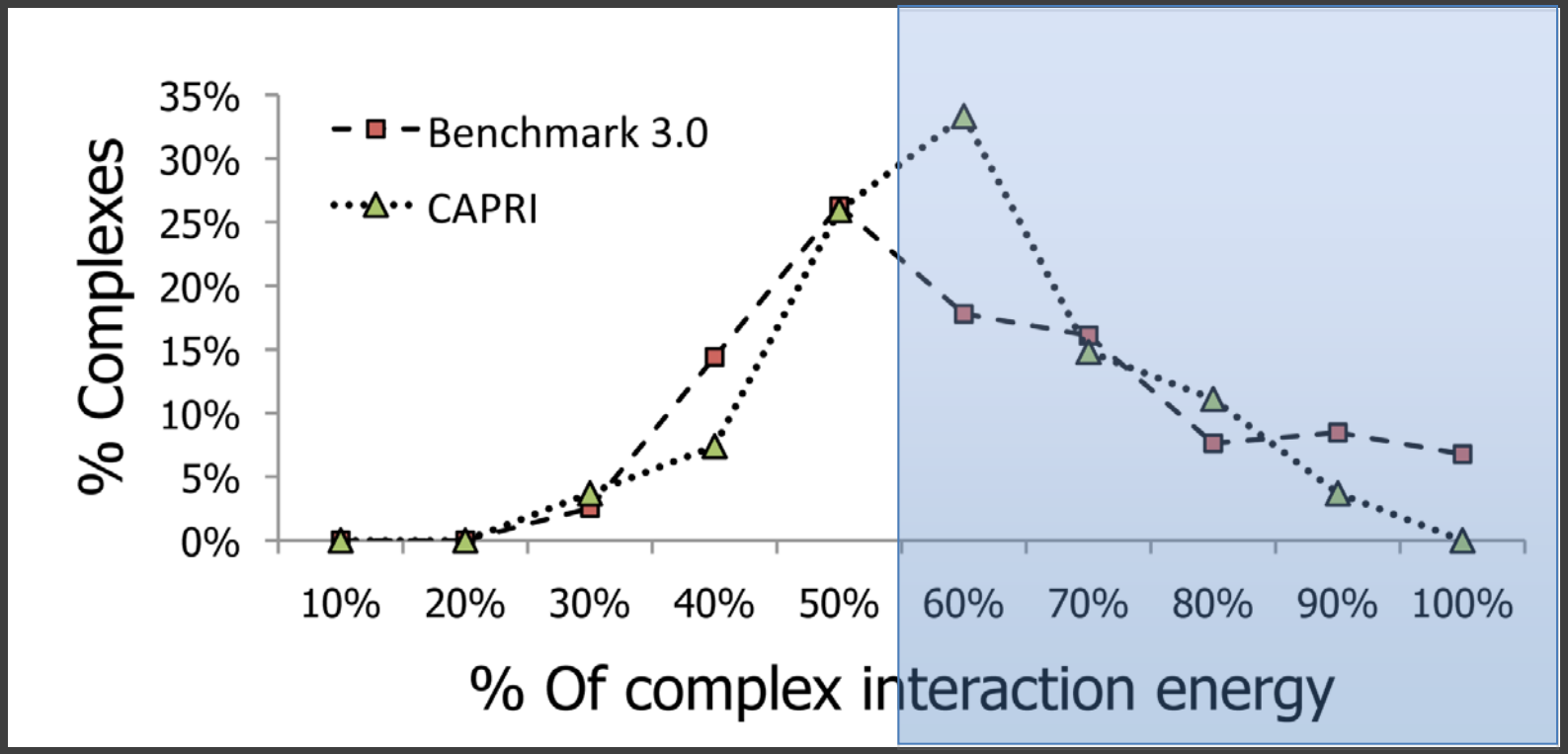
London, Raveh*, Movshovitz-Attias, Schueler-Furman. Proteins (2010)*

Many PPI's are mediated by a single peptide

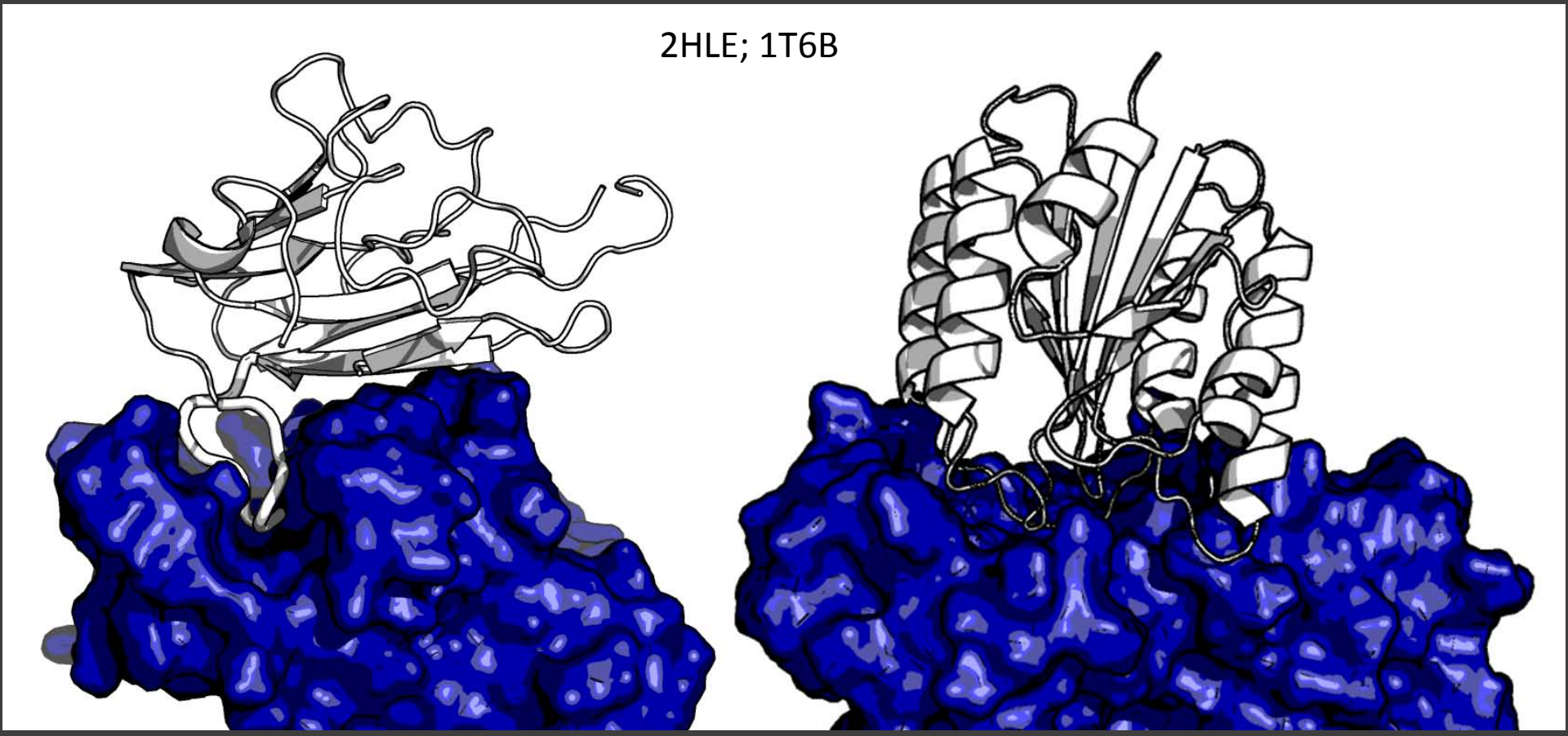
Datasets:

- Benchmark 3.0 (N=124)
- CAPRI (N=27)

57% (Bench 3.0)
63% (Capri)



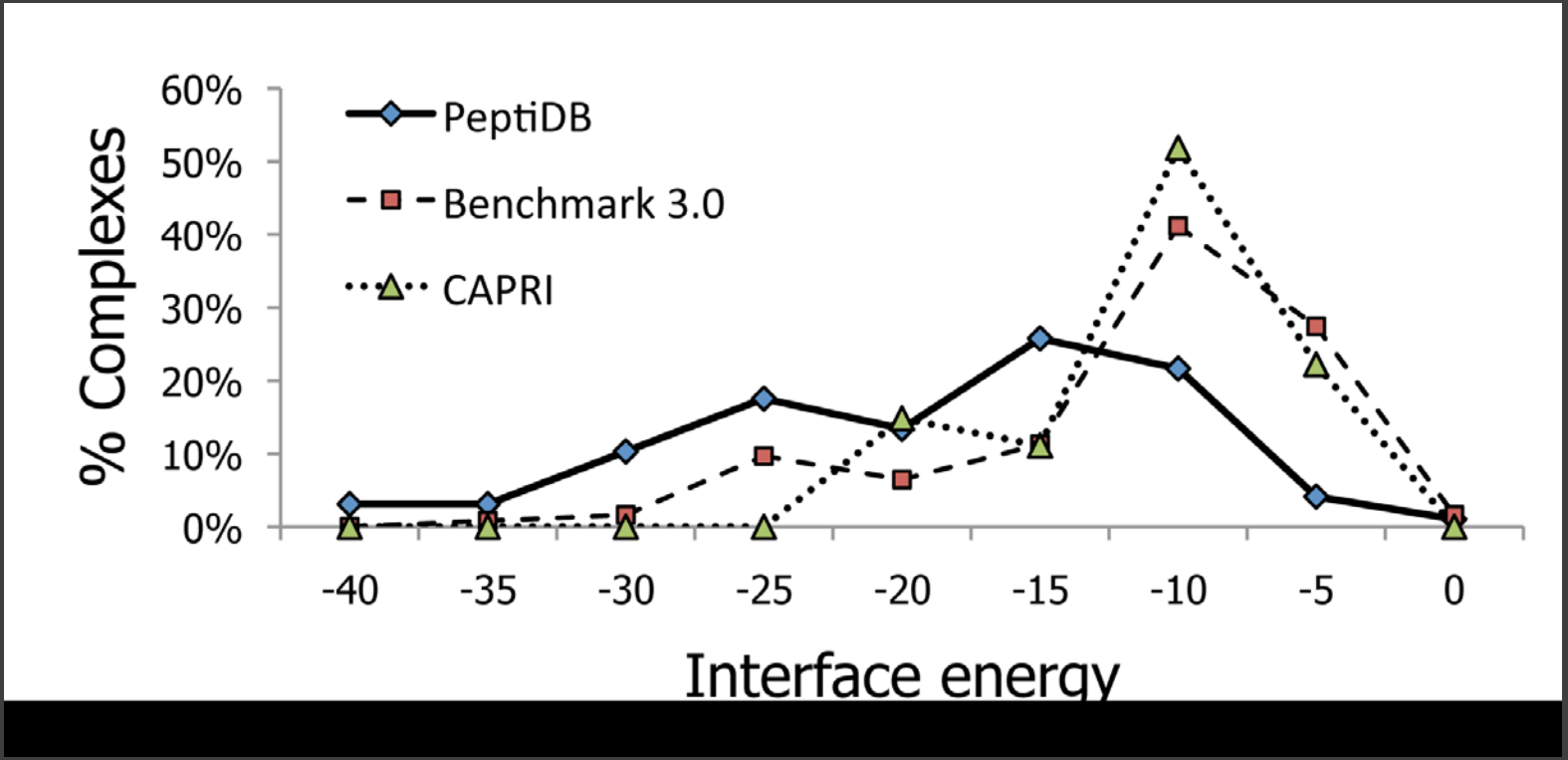
Examples



65% / -19.5 REU

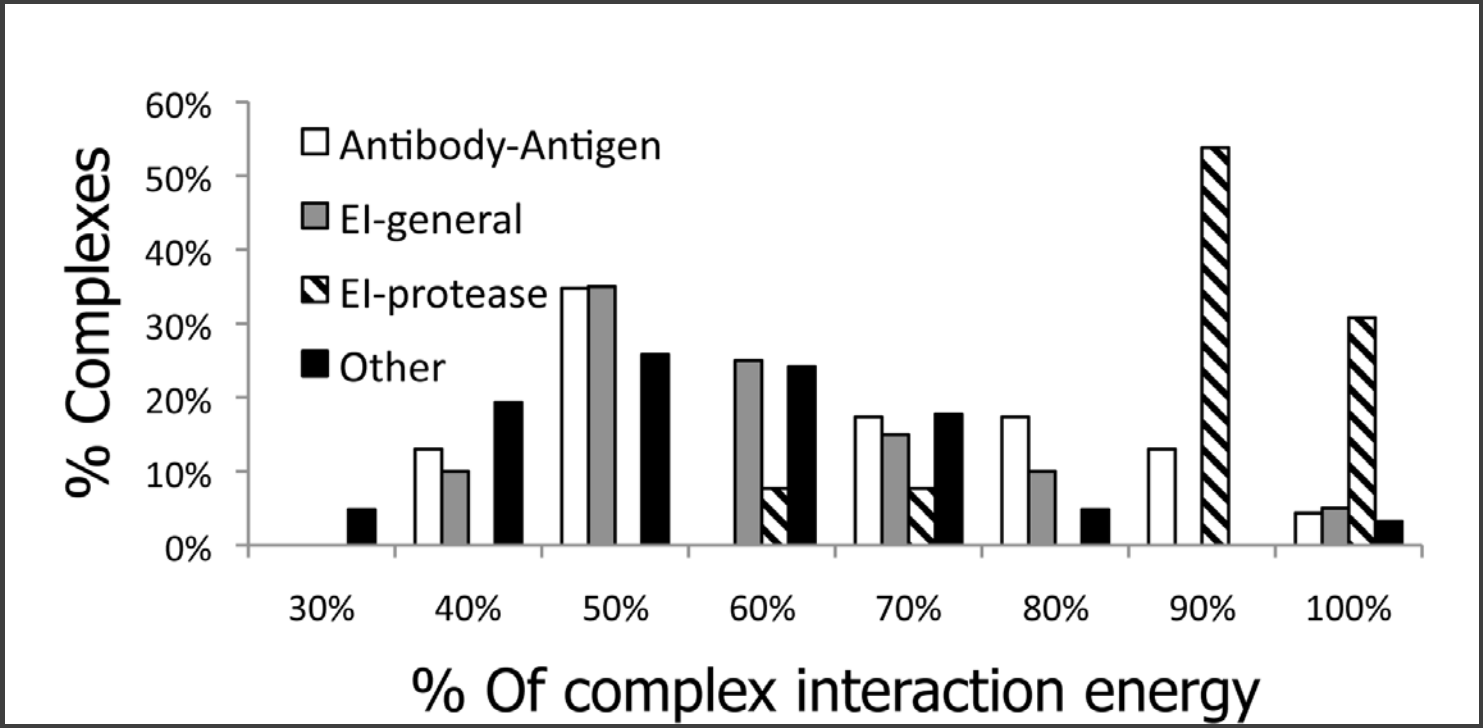
43% / -8.5 REU

Can these derived peptides bind ?



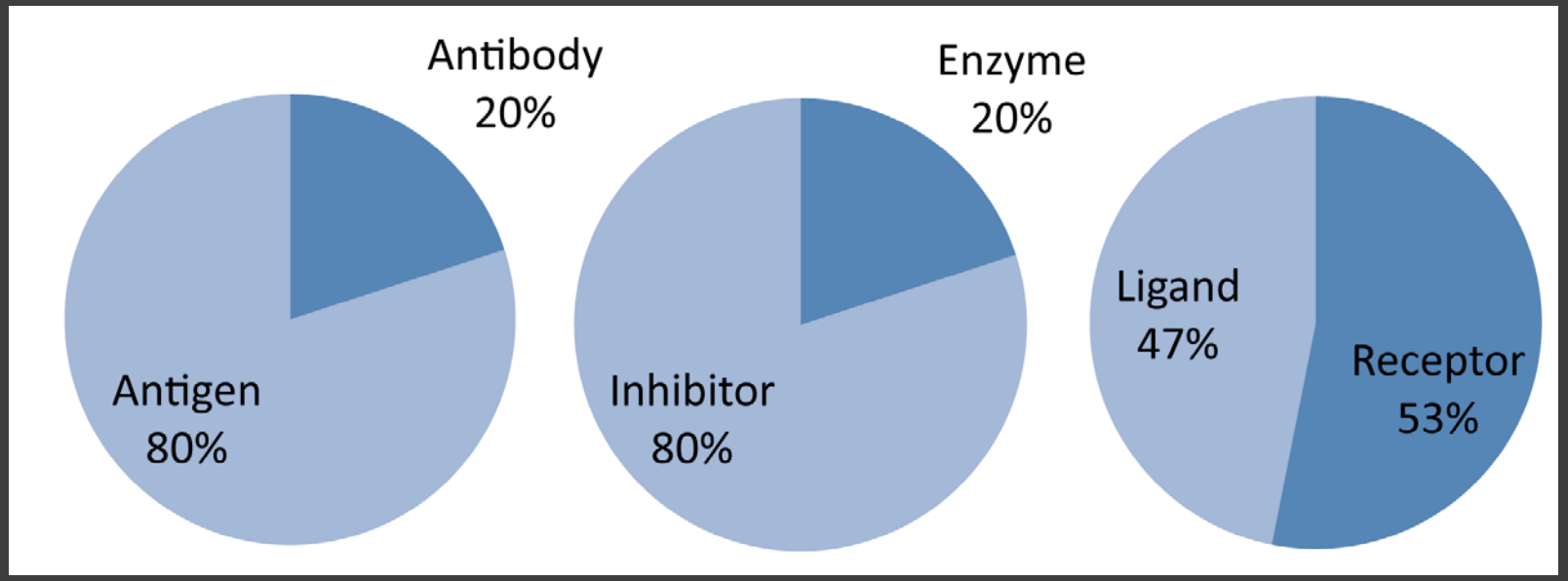
- Similar binding energy between solved structures of peptide-protein complexes and peptides derived from PPI interfaces

Asymmetry in peptide utilization



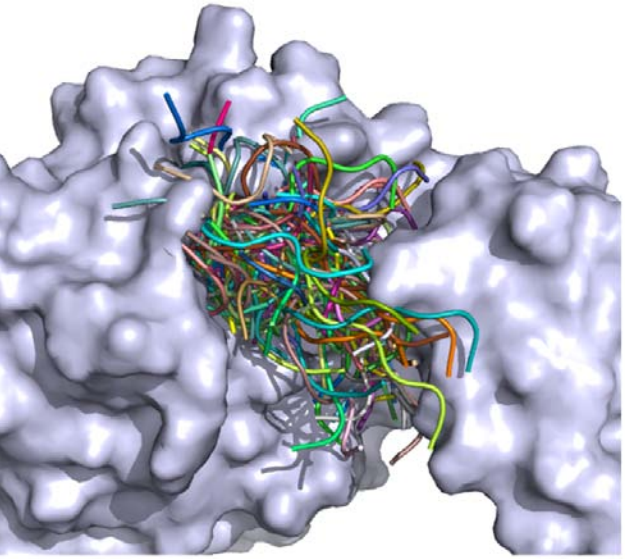
- Energy contribution of peptides is similar for different classes of interactions
- Proteases use peptides to achieve very tight binding

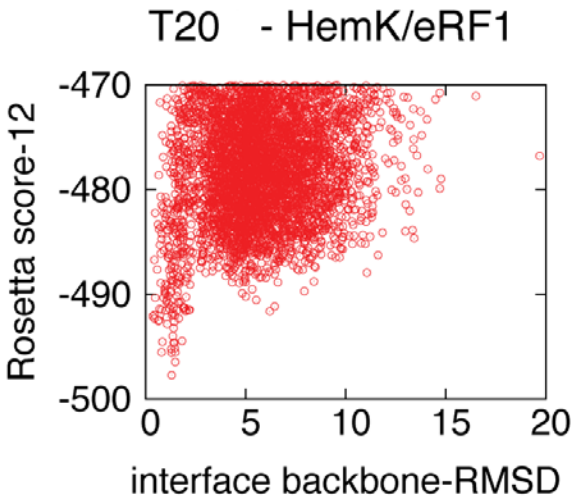
Asymmetry in peptide utilization

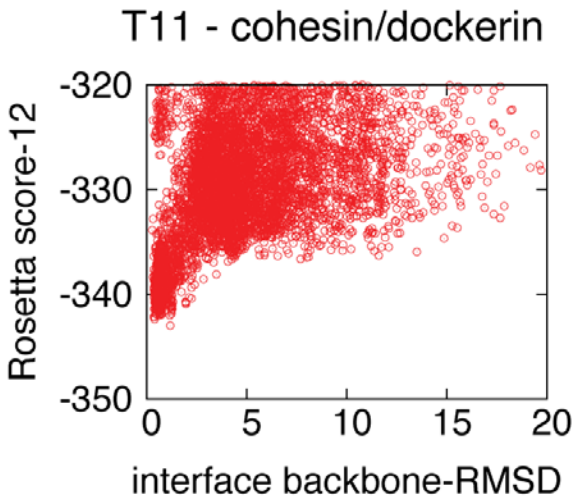


- Asymmetric inhibitory peptide distribution for Ab/Ag and E/I

Mapping the energy landscape of derived peptides (using FlexPepDock)

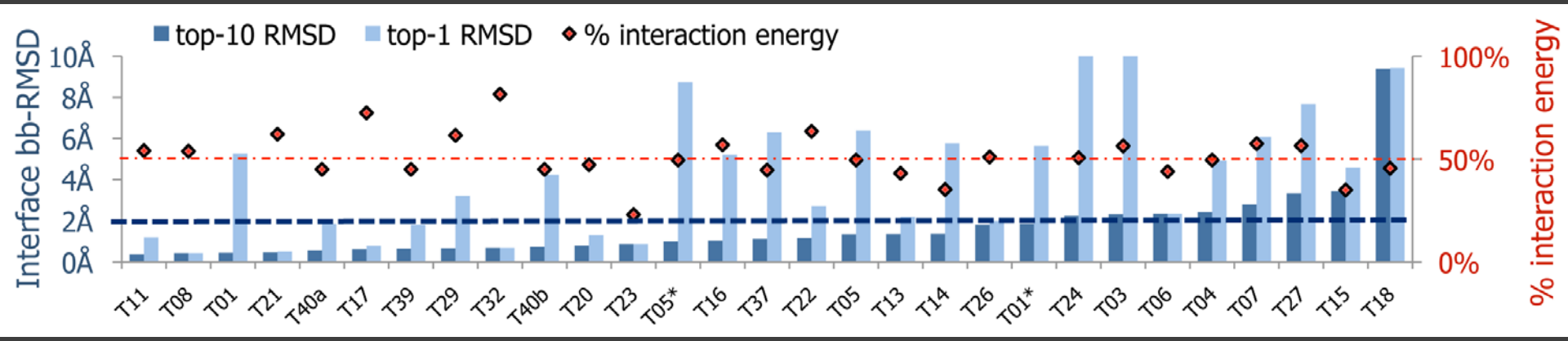






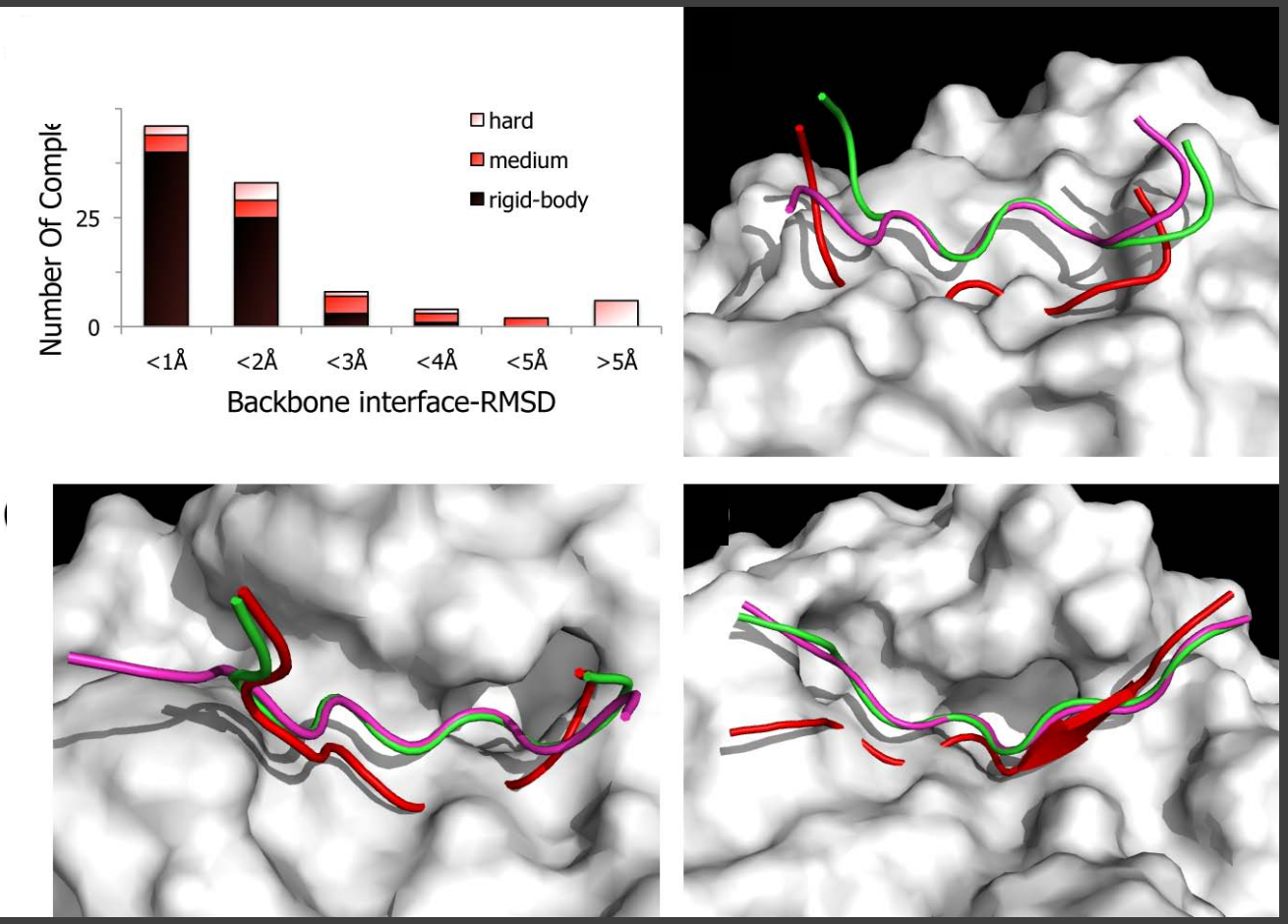
- Typical near-native binding funnels suggest similar binding mode for free peptides

Mapping the energy landscape of derived peptides



- Many peptides show similar structural preference to whole protein

Modeling of peptide segment starting from the free protein



- Most peptides are near their bound conformation
- For those that change – FlexPepDock is able to recover the 'bound' binding mode

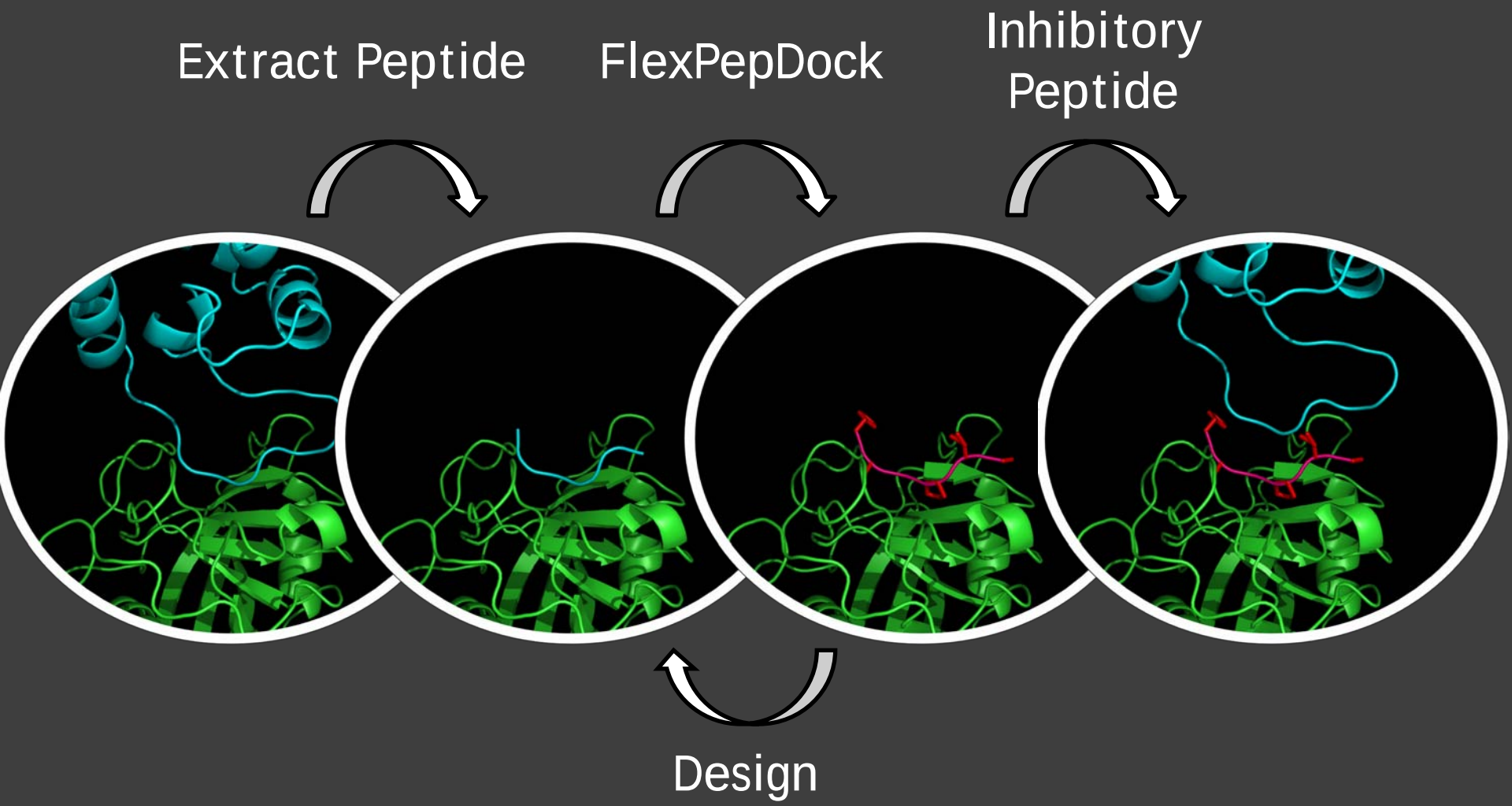
native bound peptide unbound peptide
top ranking FlexPepDock prediction

Peptides at PPIs - conclusions

- Protein-protein interfaces are often dominated by one contiguous peptide stretch (e.g. EI: protease - inhibitor)
- The local peptide sequence exhibits structural features needed for binding (FlexPepDock: Near native energy values; Binding funnels)
  - ➢ might bind independently
- Peptide-mediated interactions may occur within domains, in addition to unstructured regions

➢ Manipulate interactions with peptides

Towards PPI inhibitory peptides



Any system can use a peptide…

- HIV integrase – LEDGF *(Friedler – HUJI)*
- Analgesia *(Yin - Colorado U)*
- Super Antigen Inhibition *(Kampfer – HUJI)*
- β–TRCP Signaling *(Ben Neriah – HUJI)*
- AML *(Ben Yehuda – Hadassah Medical School)*

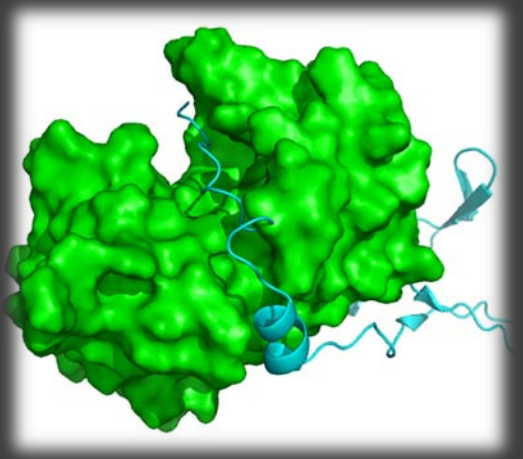
Outline of today's talk

1. Short introduction on peptide-protein interactions and their modeling
2. Analysis of peptides in protein-protein interfaces
3. Inhibition of protein interactions by free peptides: the antitoxin-toxin interaction
4. Outlook

MazE-MazF toxin-antitoxin

- MazF toxin – stable
- MazE antitoxin – unstable

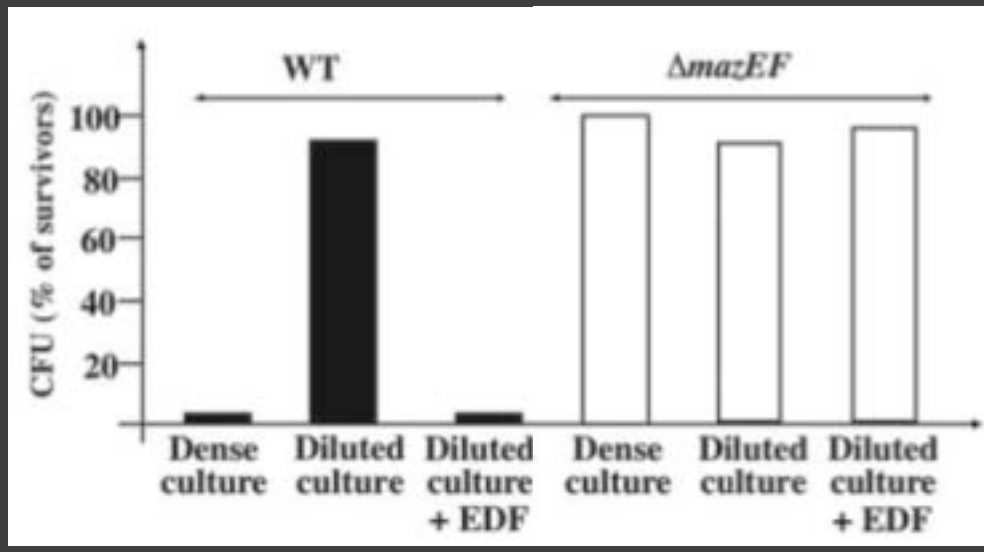
➢ MazE expression needed for survival



- EDF: extracellular death factor
  - leads to cell death in dense cultures
  - Peptide: **NNWNN**

Our assumption:

EDF expels MazE



Kolodkin-Gal et al., Science (2007)

Approach:
Thread EDF onto MazE to find the binding site

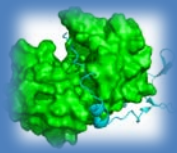
PeptiDefiner:

Given a complex:

- go over each possible pentamer peptide
- replace by NNWNN
- sample local energy landscape with FlexPepDock

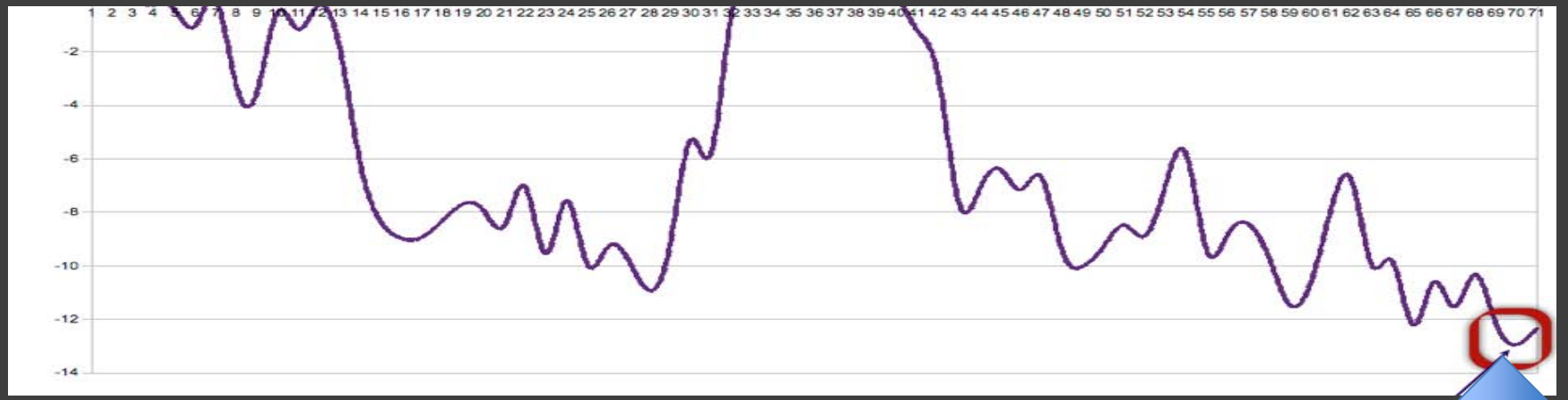
- Extract peptide pentamer with best binding energy

MazE-MazF

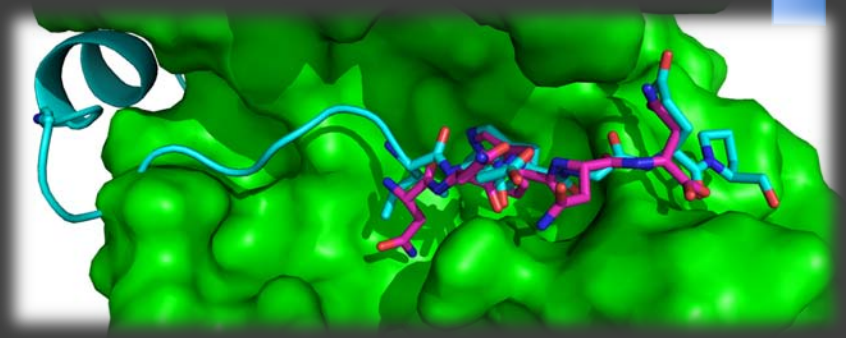


detect high-affinity segment at the interface (using FlexPepDock)

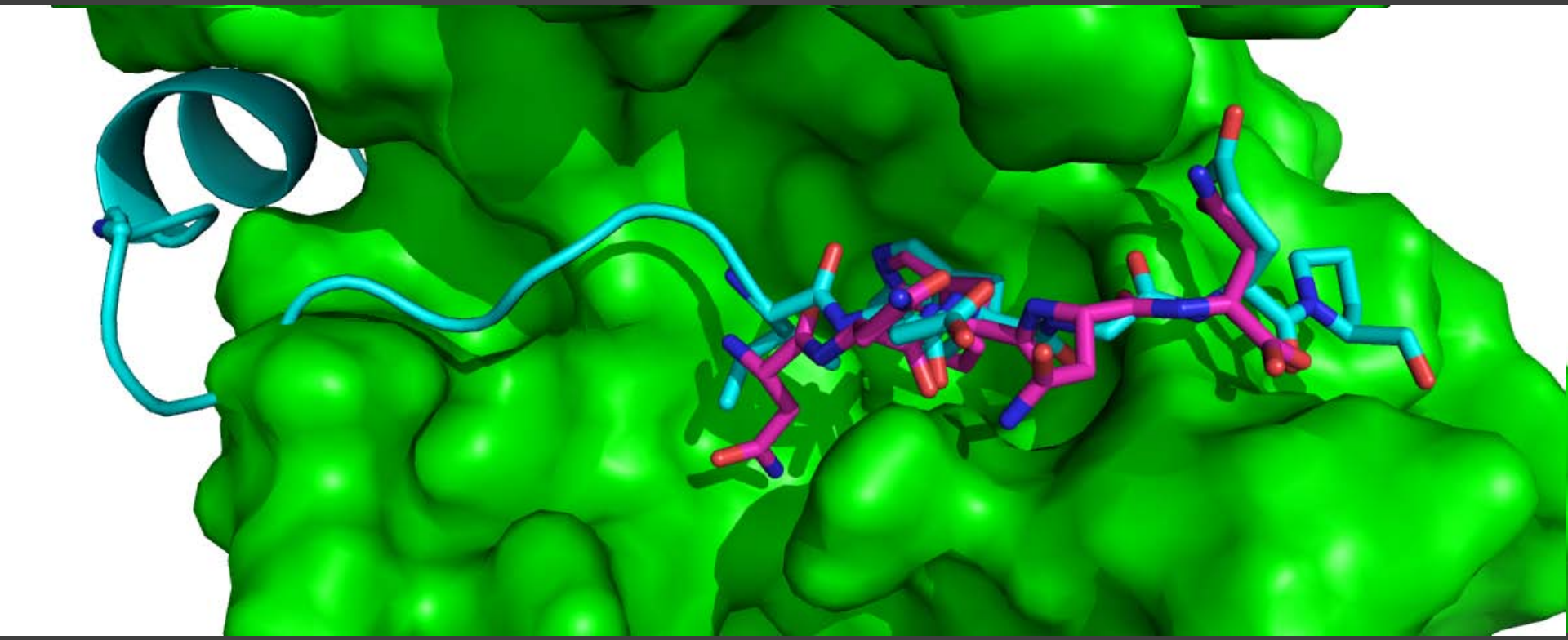
Identification of inhibitory site



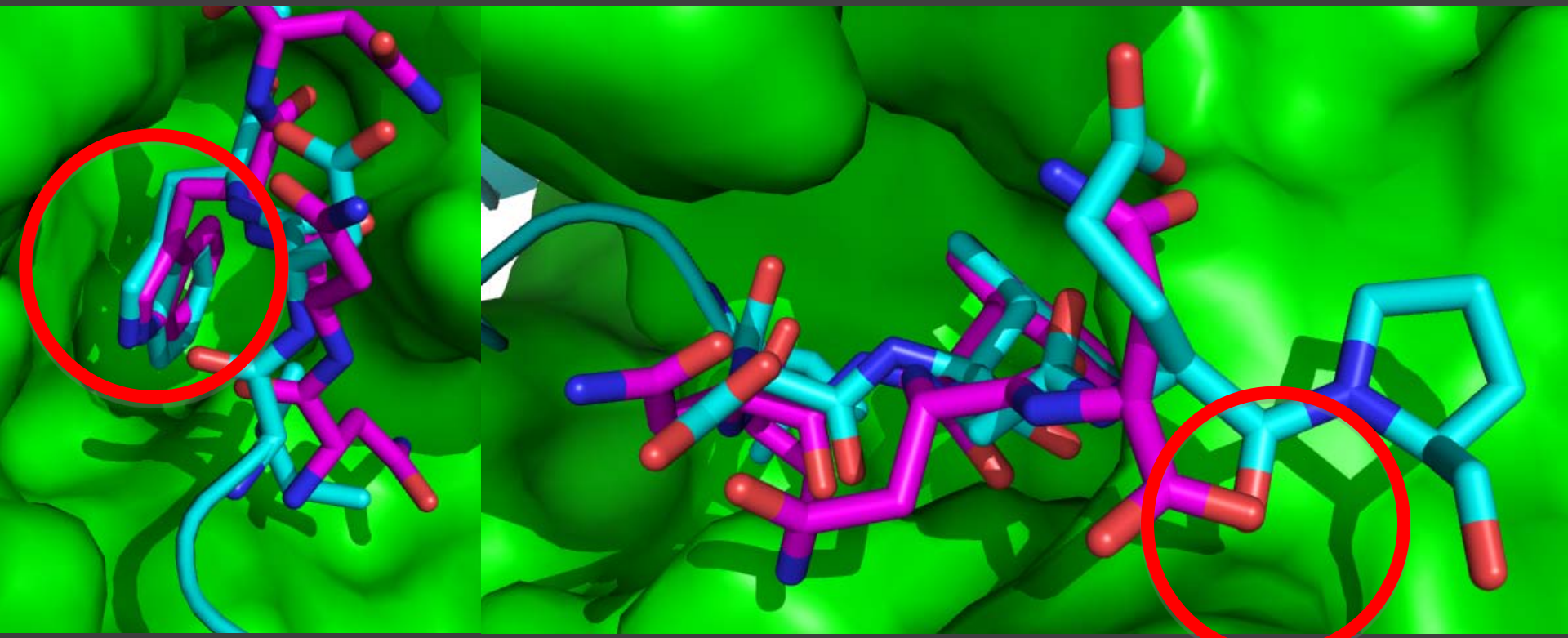
- Protocol identifies region of interest for binding of EDF to MazF
- Replace IDWGE
  with NNWNN (EDF)



Details of inhibitory site



W and Hydrogen bonds are conserved



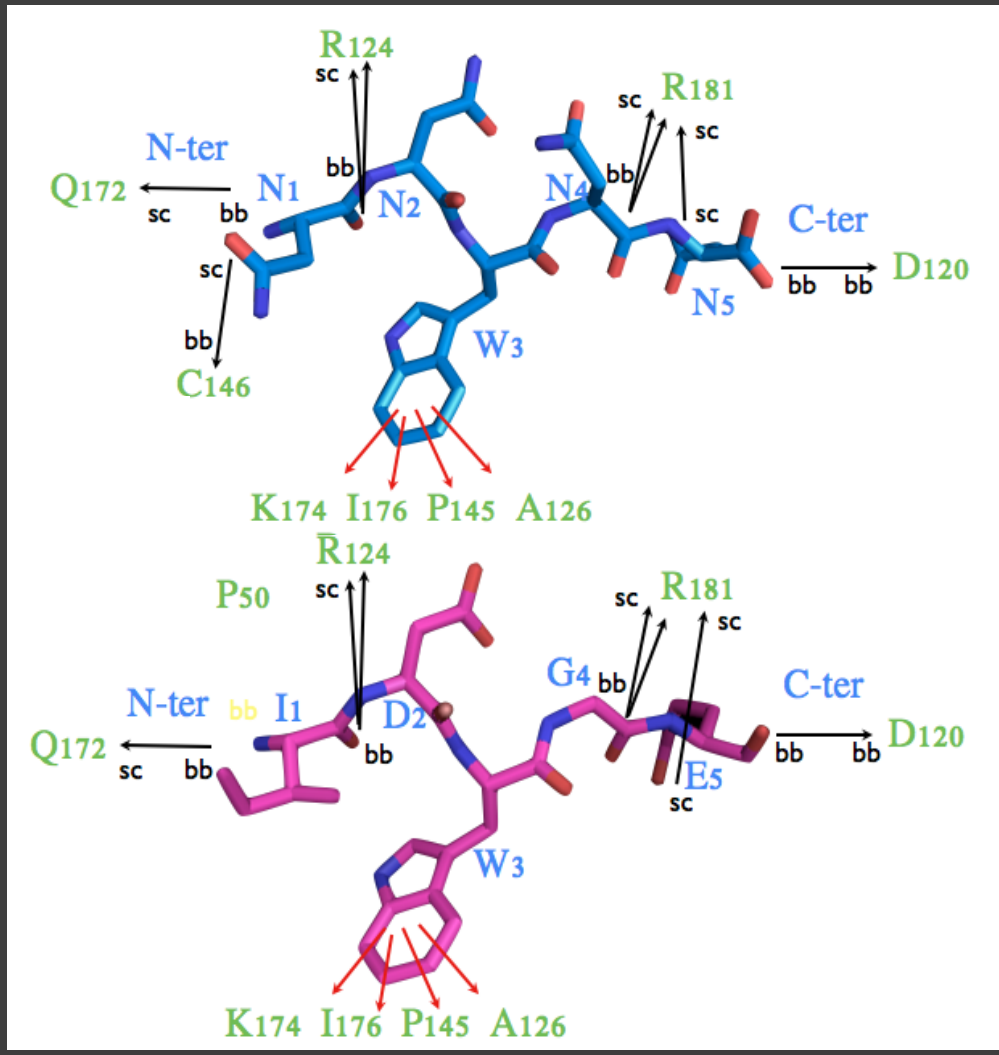
- Backbone rearrangements preserve critical interactions

Conserved binding pattern in EDF-MazF and MazE-MazF interactions

- Experimental testing of IDWGE for toxic activity is under way

Next :

- Redesign peptide
- Characterize effect of peptide on MazF function



Outlook

- *Peptides* that interact with proteins occur
  - In unstructured regions (e.g. PDZ, Sh3, *etc*)
  - In globular domains (e.g. HAGPIA)
  - As free particles (e.g. EDF)
- All can be modeled (and also redesigned) by our tools
- We can manipulate, manage and change peptide-mediated interactions

Thank you!

Rosetta Community

- Nir London
- Barak Raveh
- Michal Sperber
- Dana Movshovitz-Attias

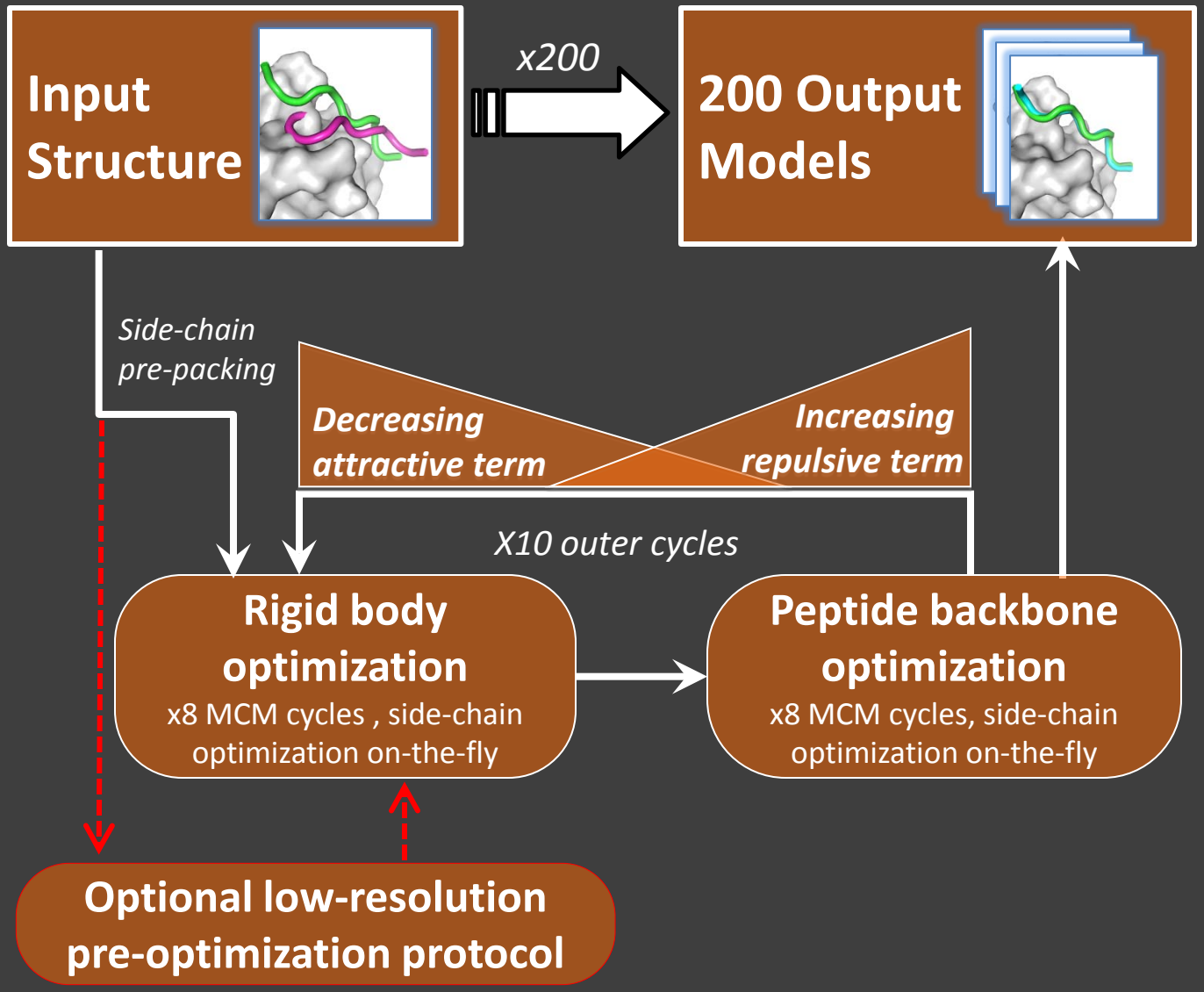
Funding:

Converging Technologies & Clore Scholarships

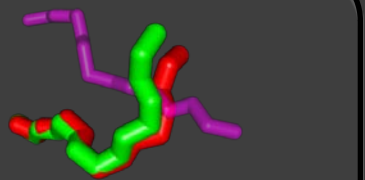
ISF, GIF Young investigator, NIH, BSF

Assaf Faragy
Lior Zimmerman
Dan Reshef
Eran Kuchuk

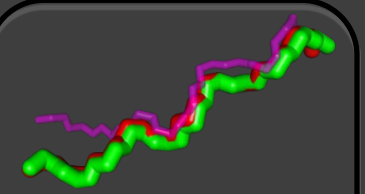
Outline of Rosetta *FlexPepDock*



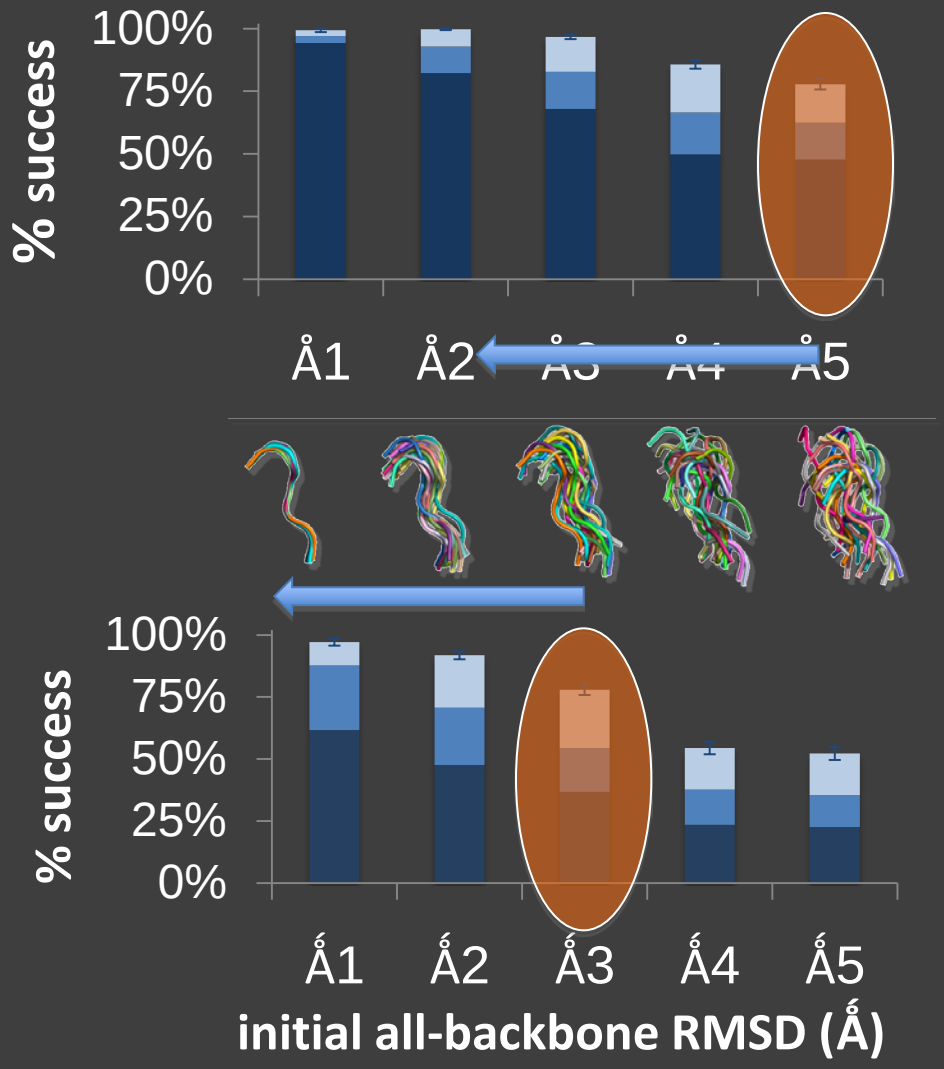
Quality of Models (Bound) and Sampling



1.5 Å model of *1ihj*



0.6 Å model of *1zuk*

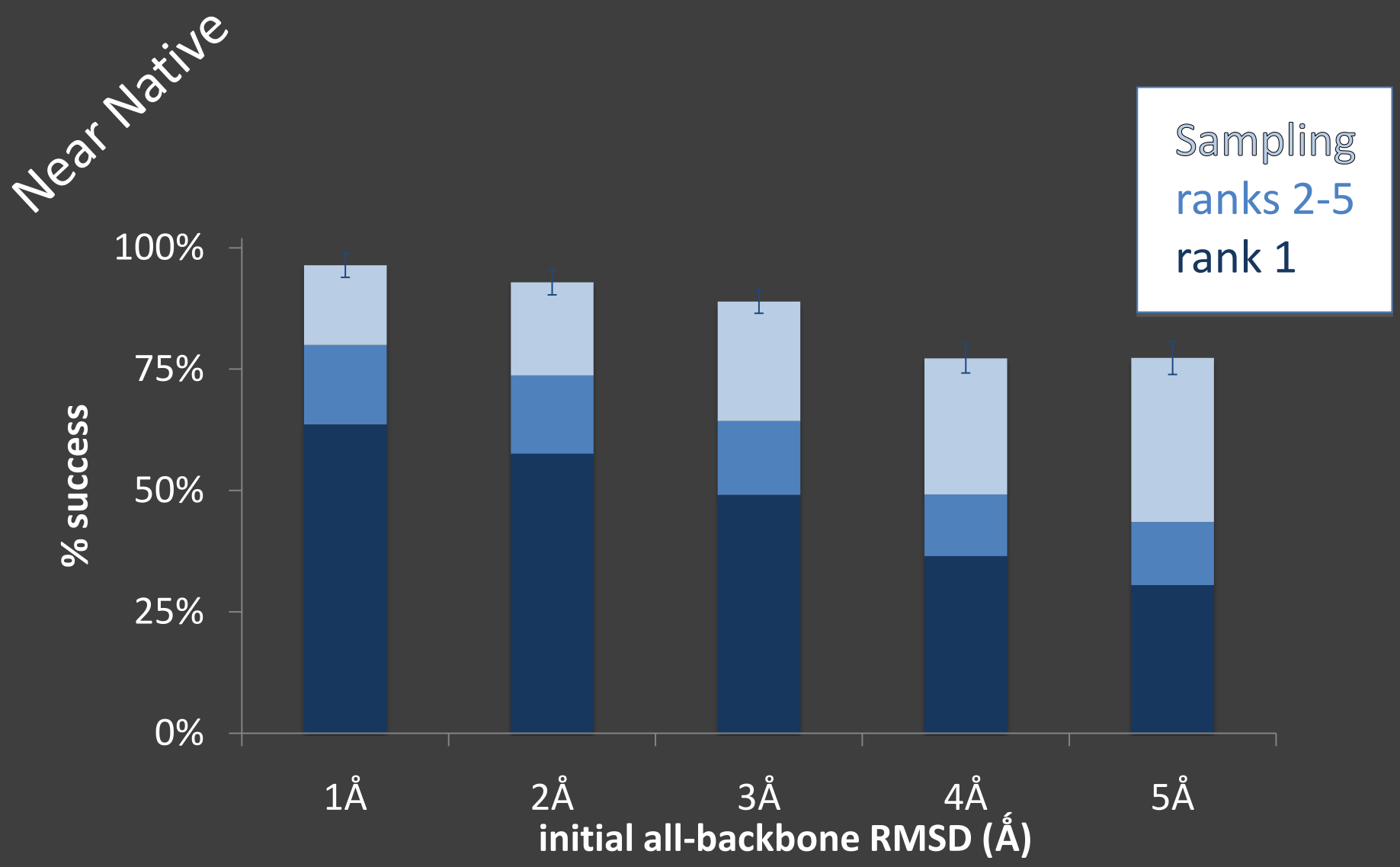


Near Native

Sub Angstrom

Sampling
ranks 2-5
rank 1

Quality of Models (Unbound) and Sampling



High-Resolution (<1Å **All-Atom** RMSD) Modeling of Tetramers onto **Bound** Receptor