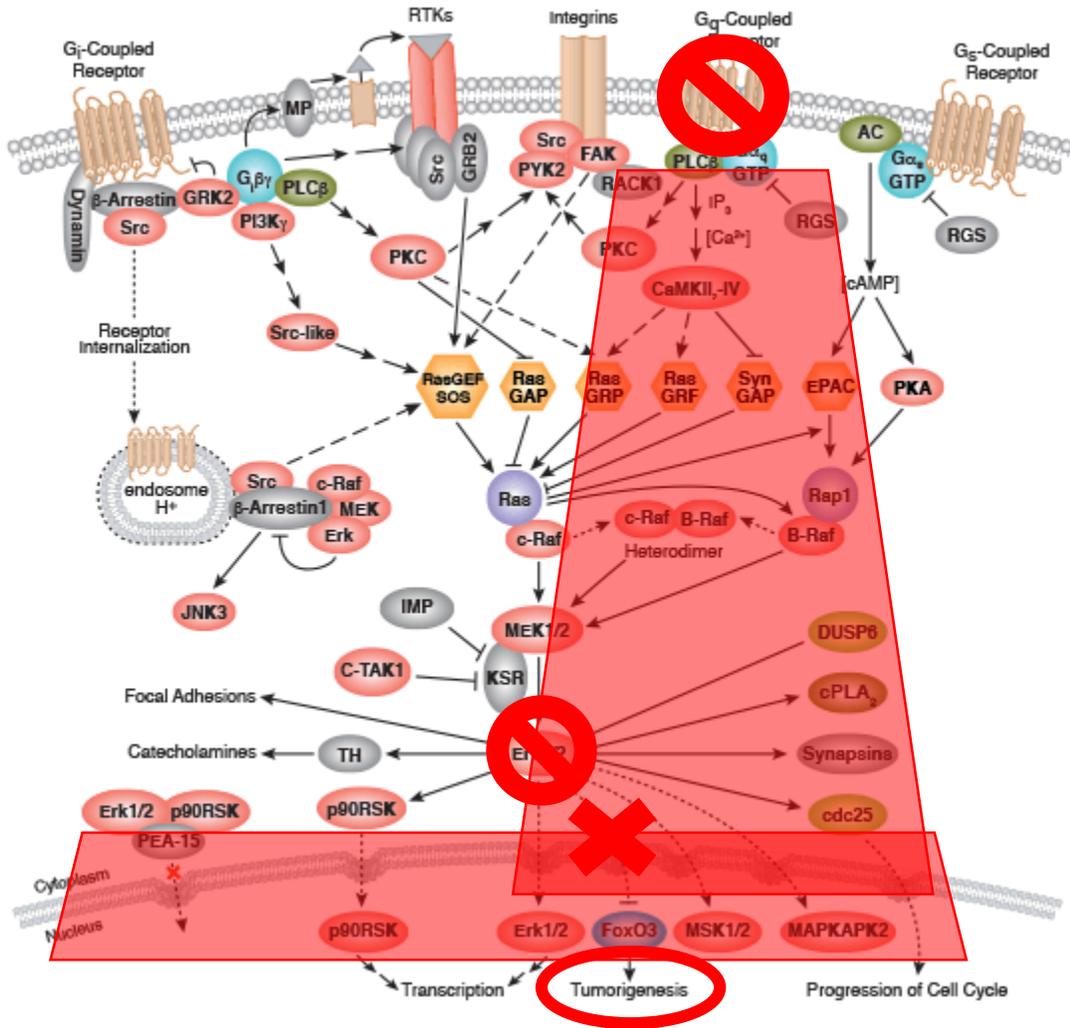


Peptide Design

Rosetta Workshop

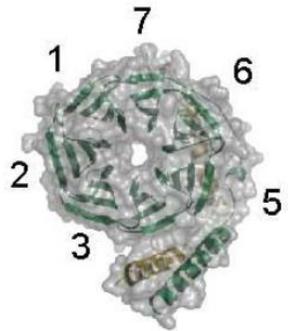
10 May 2018

Protein-Protein Interactions Regulate Majority of Cellular Processes

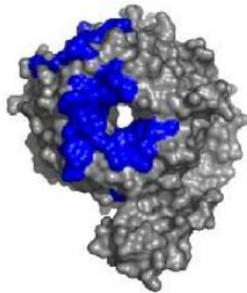


- Found significantly in signaling and regulatory networks
- Inhibiting a single protein may prove disastrous for the entire network

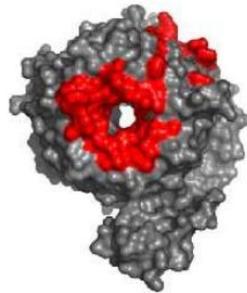
Challenges to Inhibiting Protein-Protein Interactions



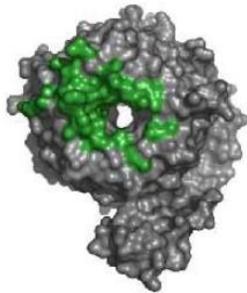
Gβ₁γ₁



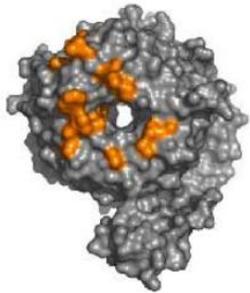
Gα_{t1}



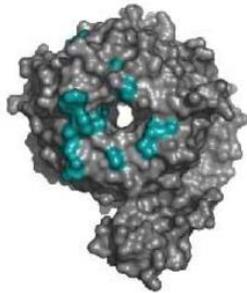
Phosducin



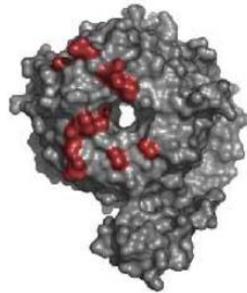
GRK2



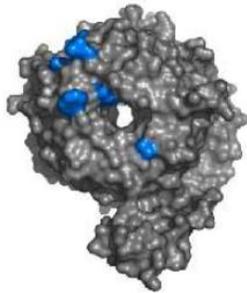
Adenylyl
Cyclase II



PLCβ₂



Ca²⁺-
Channels



GIRK
Channel

- Interactions are often flat surfaces as opposed to pockets
 - Small molecule drugs often need pockets
 - Small molecules are by definition small and may not cover entire binding surface
 - Peptides can bind along surface and with increased specificity than small molecules

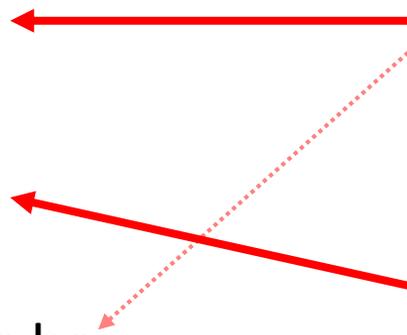
Using Peptides to Inhibit Protein-Protein Interactions

- Derived peptides must bind target with:

- Comparable affinities
- Similar binding mode
- (Would cyclized peptides be suitable?)

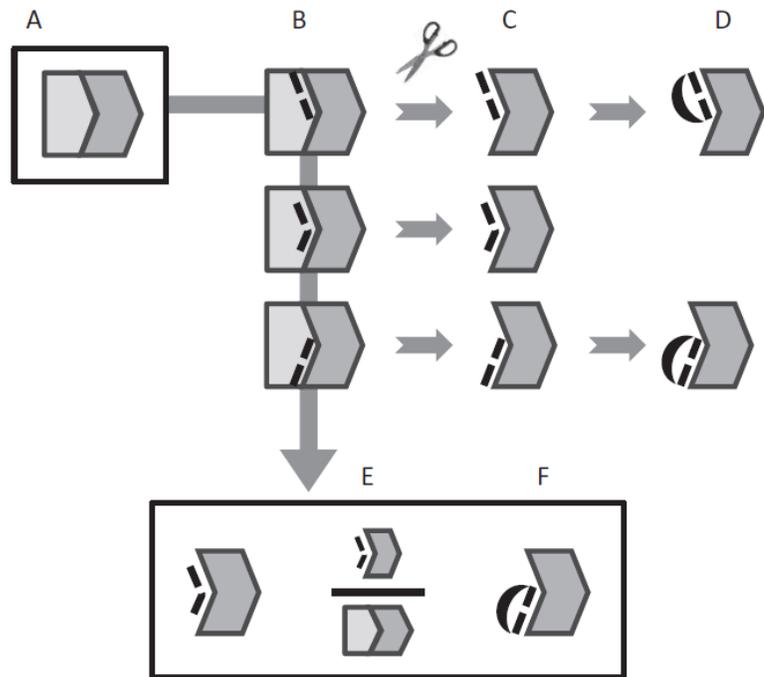
- Protocol for Peptide Design

- PeptiDerive
 - Peptides derived from protein-protein interaction that contribute majority of binding energy
- FlexPepDock
 - Are peptides binding in lowest energy state without presence of rest of protein?

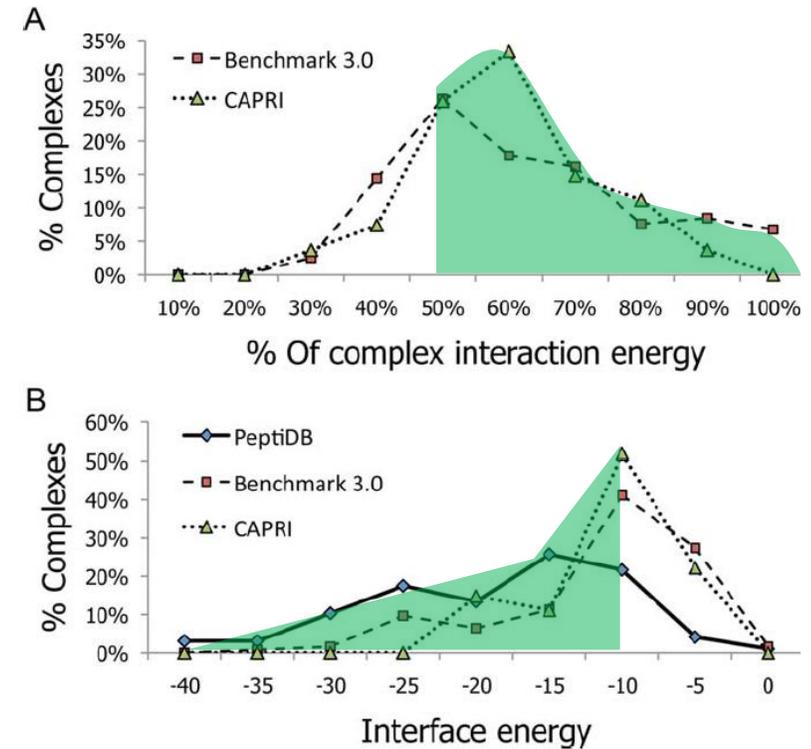


PeptiDerive Identifies Peptides from Complex that Make Up the Majority of Binding Energy

PeptiDerive Algorithm



Energetic Analysis



Is Peptide Cyclization Possible?

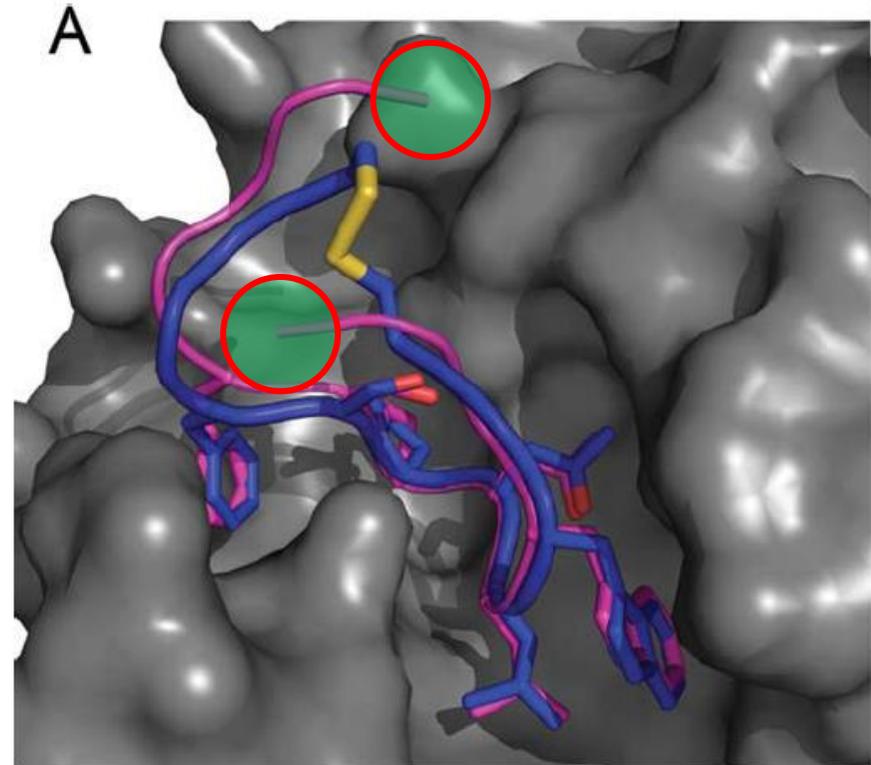
Cyclization:

- Increases conformational stability
- Reduces biological degradation

Derived Peptide



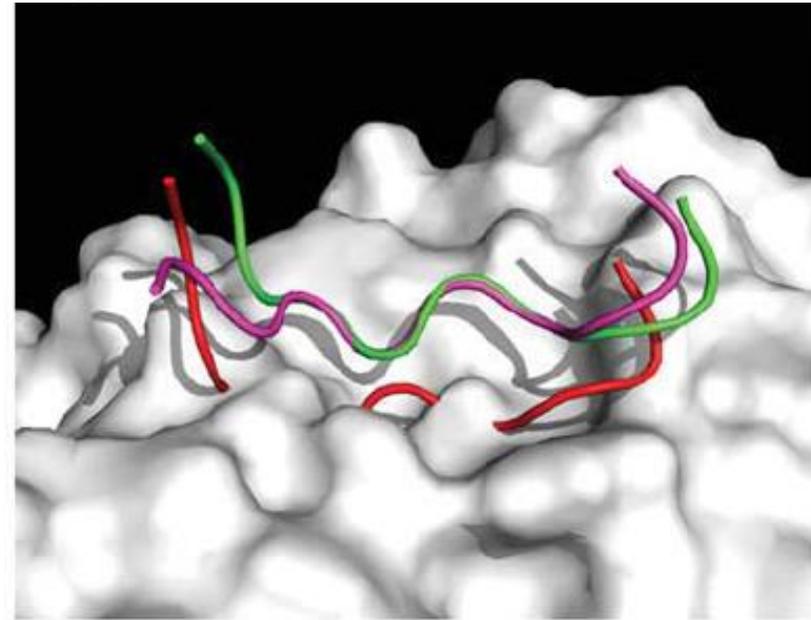
Cyclized Peptide



Calculated in PeptiDerive

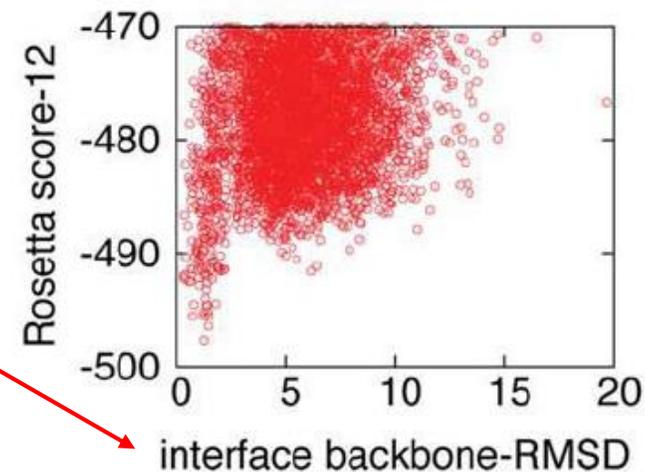
Does Derived Peptide Bind in Same Orientation?

- Redocking of Peptide (in absence of original protein) allows for energetic sampling of binding surface to ensure correct binding manner



Native
Derived
Redocked

RMSD of peptide interface residues to peptide in its native protein context



Peptide Design Tutorial

1. Submit Protein-Protein Complex to PeptiDerive Server on Rosie

- <http://rosie.rosettacommons.org/peptiderive>
- Necessary input: PDB of protein-protein complex
- Output: Protein-peptide complex

Identify peptides that can compete

2. Redock Output Peptide-Protein Complex with FlexPepDock

- <http://flexpepdock.furmanlab.cs.huji.ac.il/>
- Necessary input: PDB of protein-peptide complex (output from PeptiDerive)

Check if the peptide will bind in the same manner without larger protein

PeptiDerive Server

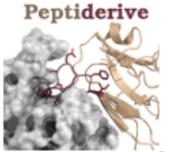
<http://rosie.rosettacommons.org/peptiderive>

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Rosetta Peptiderive Protocol



[\[Submit Peptiderive task\]](#)



[\[Peptiderive Queue\]](#)



[\[Peptiderive Server Documentation\]](#)

Please cite the following article when referring to results from our ROSIE server:

1. Yuval Sedan, Orly Marcu, Sergey Lyskov, Ora Schueler-Furman **Peptiderive server: derive peptide inhibitors from protein-protein interactions** *Nucleic Acids Research* 2016; doi: 10.1093/nar/gkw385 [online](#)
2. London N, Raveh B, Movshovitz-Attias D, Schueler-Furman O. (2010). **Can self-inhibitory peptides be derived from the interfaces of globular protein-protein interactions?** *Proteins*, **78**:3140-49. [PMID: [20607702](#)]
3. Lyskov S, Chou FC, Conchúir SÓ, Der BS, Drew K, Kuroda D, Xu J, Weitzner BD, Renfrew PD, Sripakdeevong P, Borgo B, Havranek JJ, Kuhlman B, Kortemme T, Bonneau R, Gray JJ, Das R., **"Serverification of Molecular Modeling Applications: The Rosetta Online Server That Includes Everyone"** *PLoS One*. 2013 May 22;8(5):e63906. doi: 10.1371/journal.pone.0063906. Print 2013. [Link](#)

Submitting A Job

1. Public Description
2. **Input PDB (NECESSARY)**
 - Either supply own file or fetch from PDB
3. Specify receptor and partner
 - If not specified, output will contain peptides derivatives of both partners
4. Specify peptide lengths
 - Default is 10

Welcome to ROSIE
Rosetta Online Server that Includes Everyone

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Submit a new Peptiderive job

- 1 Job short description (visible in queue):
- 2 Input PDB file No file chosen or enter a PDB ID to be fetched from RCSB:
- 3 Restrict receptor role to chains
(comma separated list of chain IDs: all chains if empty):
- 3 Restrict partner role to chains
(comma separated list of chain IDs: all chains if empty):
- 4 Peptide lengths to derive
(comma separated list of amino acid counts):

Job Description (for your own records):

You have not logged in! If you already have a ROSIE account, please [login](#) to submit your job. Alternatively, you also can submit a job as Guest:

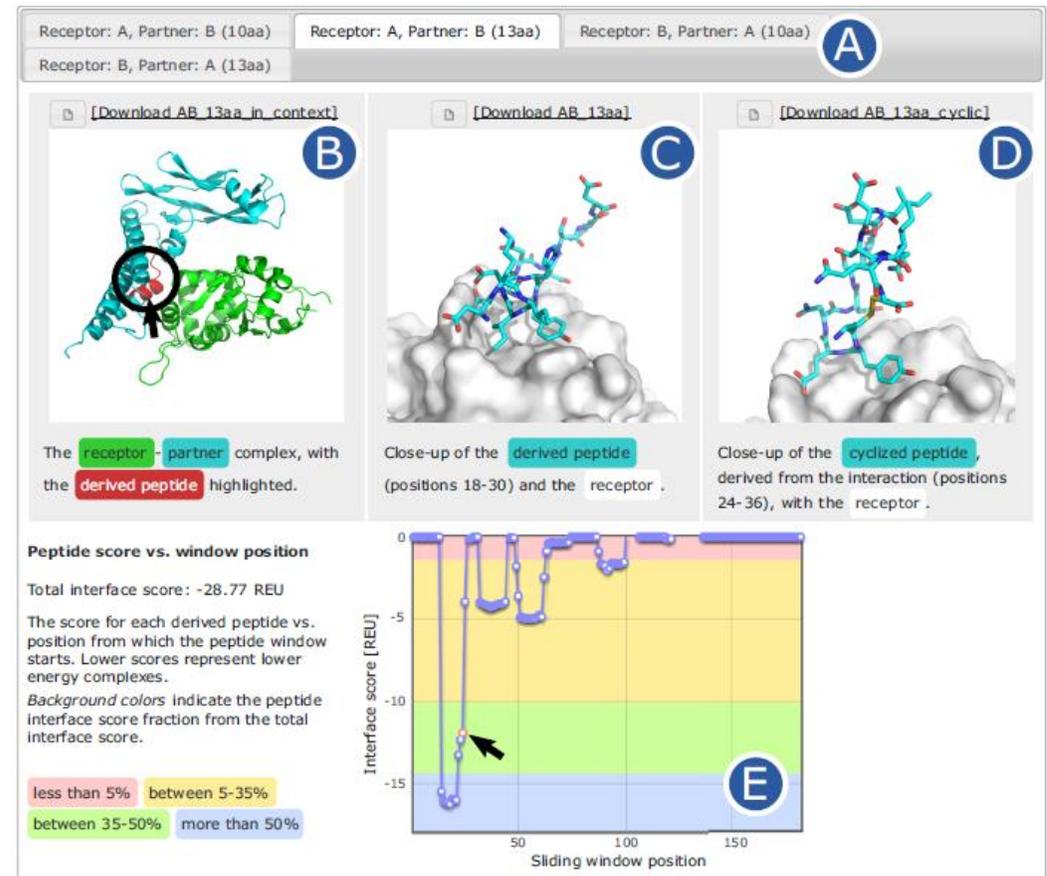
Keep my job-data public (Note that Public Jobs have higher priority and longer life time!)

If you decide to keep your job private and submit it as Guest, your ROSIE Job will be allocated an obscure URL (**anybody with a link will be able to access the results**). For better security please consider to [Create a ROSIE Account](#) and use it to submit private jobs.

[optional] Notify me when my job completes by sending a mail to:

Output from PeptiDerive Server

- A. Output Files
- B. Visualization of **Derived Peptide** in Context of Complex
- C. Visualization of **Derived Peptide**
- D. (Visualization of **Cyclized Peptide**)
- E. Energy of Peptide Binding Versus Sliding Window



FlexPepDock Server

<http://flexpepdock.furmanlab.cs.huji.ac.il/>

NECESSARY INPUT:
PDB of Protein-Peptide
Complex

Generates 200 models

flex pep dock High resolution modeling of peptide-protein interactions

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Rosetta

FlexPepDock is a high-resolution peptide-protein docking (refinement) protocol for the modeling of peptide-protein complexes, implemented in the [Rosetta framework](#).

For more details see the "[Overview](#)" and "[Usage & FAQ](#)" sections.
Recent publicly shared jobs: [ClusPro model 006.15 used as input](#)

Input PDB file: No file chosen

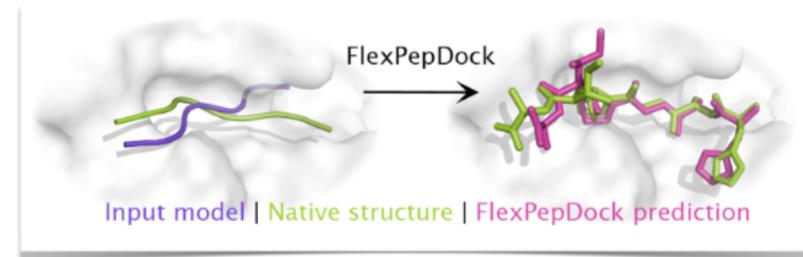
or

Your e-mail:

Optional but
recommended

[Advanced Options](#)

(click to toggle)



FlexPepDock Server Output

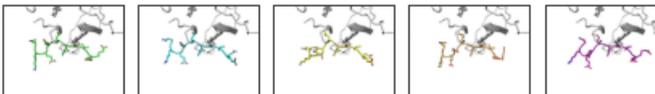
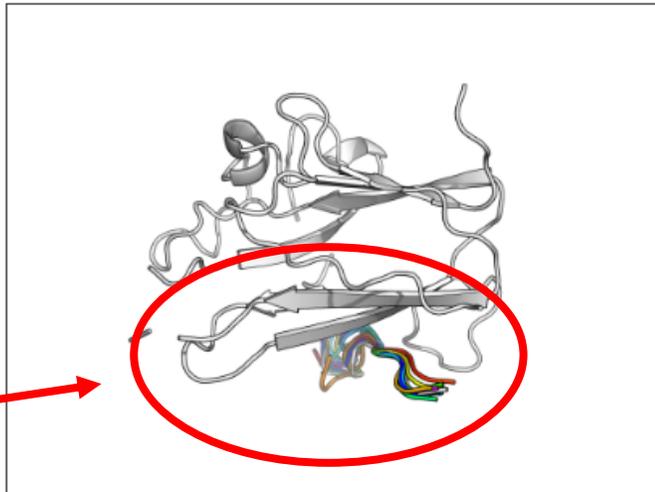


For download:

- Top 10 model pdbs and scores

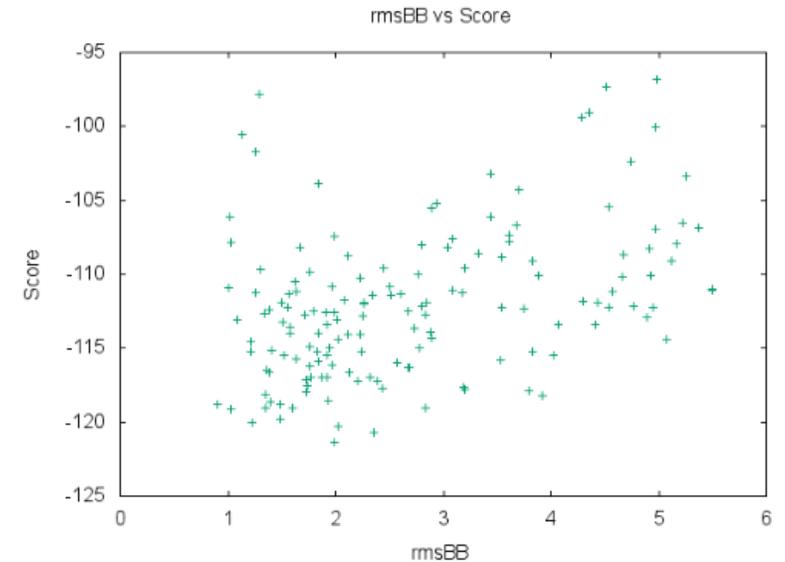
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Rosetta

Results for job #9326 (receptorB_partnerA_10aa_best_linear_linear_peptide_complex.pdb):



Check for large
movement of
peptides here

Score vs RMSD of all 200 poses



FlexPepDock Server Output

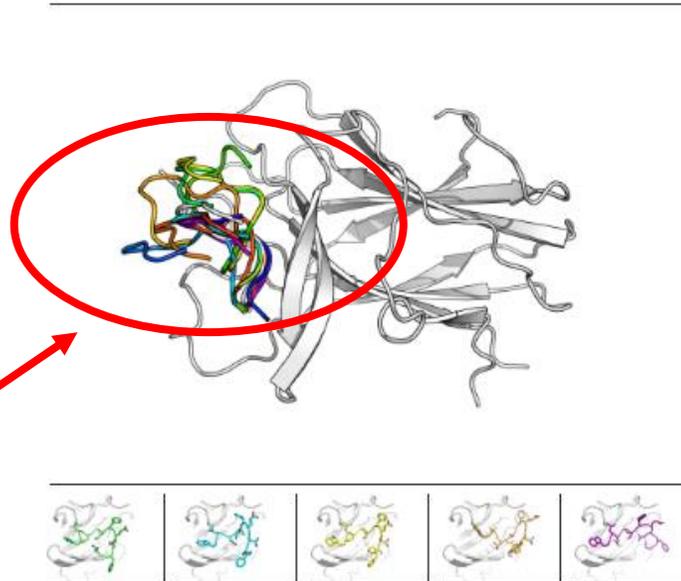
For download:

- Top 10 model pdbs and scores



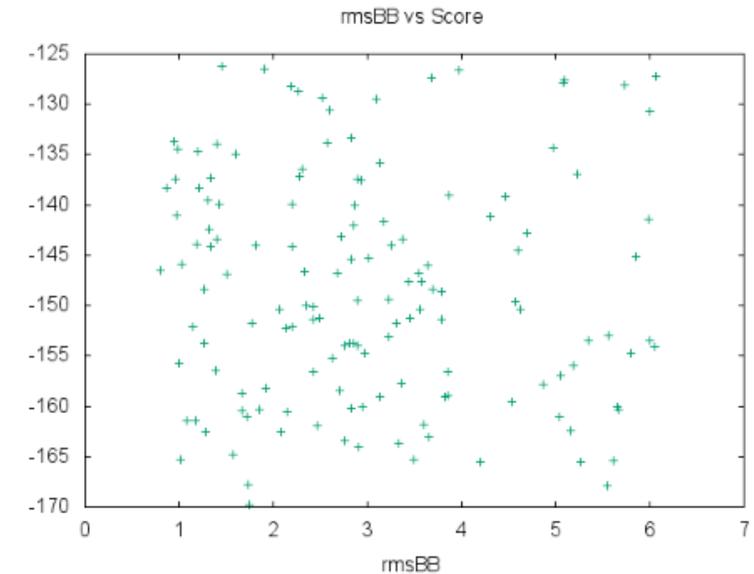
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Results for job #9327 (receptorA_partnerB_10aa_best_linear_linear_peptide_complex.pdb):



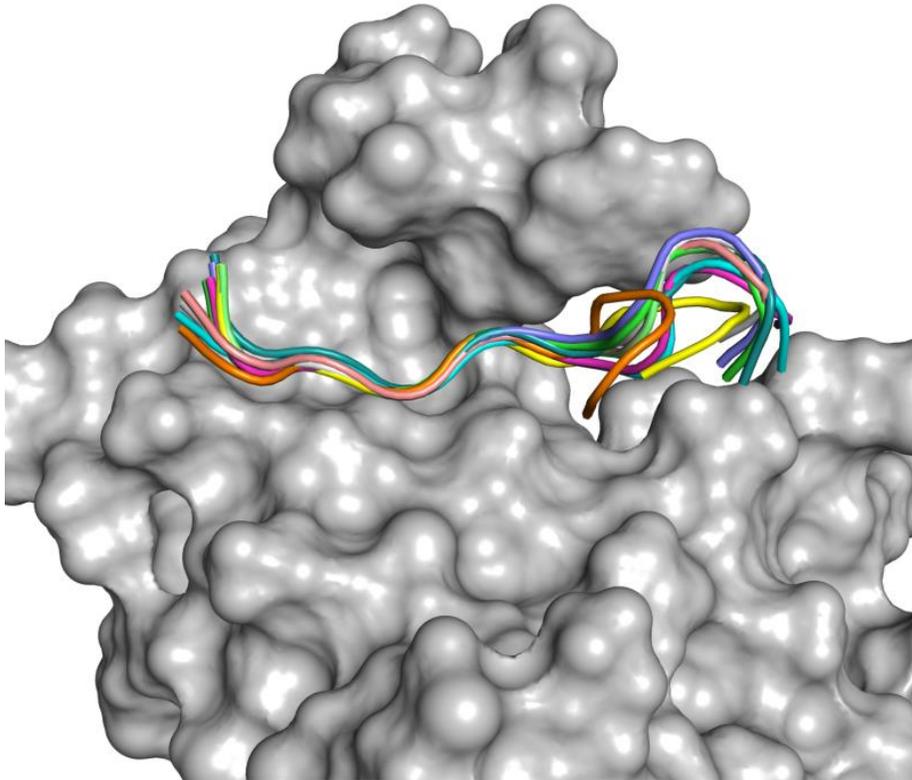
Check for large
movement of
peptides here

Score vs RMSD of all 200 poses

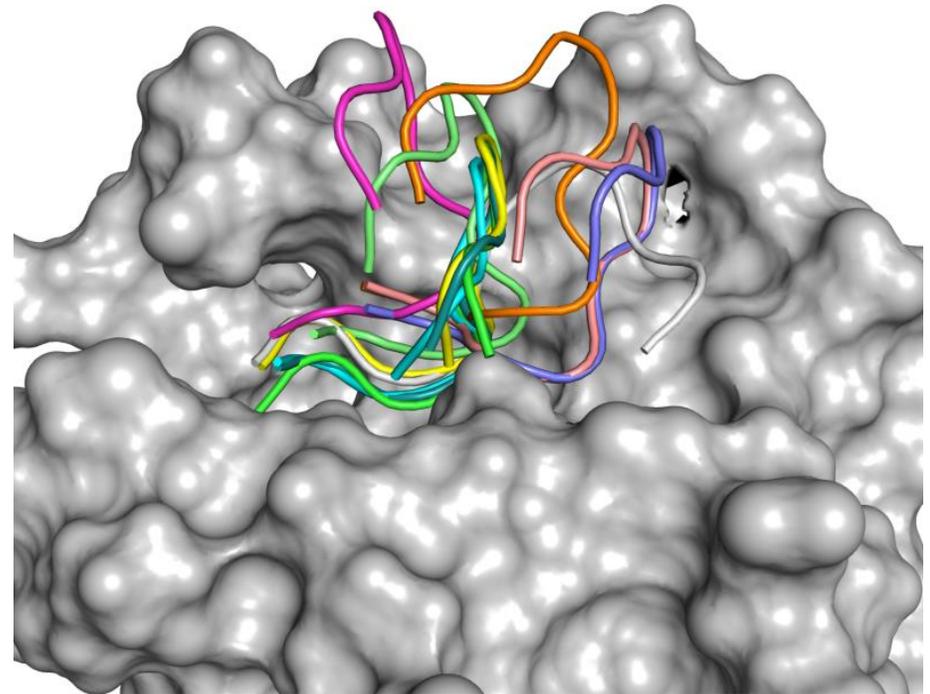


Compare Receptor Pairs

Receptor B



Receptor A



PeptiDerive Without Server

- Utilizes an XML with Rosetta_Scripts
- Needs an input file and an options file

```
{path_to_Rosetta}/main/source/bin/rosetta_scripts.linuxgccrelease \  
  -database {path_to_Rosetta}/main/database/ \  
  @ options.txt \  
  -in:file:s complex.pdb
```

FlexPepDock without Server

- Step 1: pre-pack your initial complex

```
{path_to_Rosetta}/main/source/bin/FlexPepDocking.linuxgccrelease \  
-database {path_to_Rosetta}/main/database \  
-in:file:s start.pdb \  
-flexpep_prepack -ex1 -ex2aro
```

- Step 2: Refine the pose (100-10000 decoys)

```
{path_to_Rosetta}/main/source/bin/FlexPepDocking.linuxgccrelease \  
-database {path_to_Rosetta}/main/database \  
-in:file:s start_0001.pdb -native native.pdb \  
-pep_refine -ex1 -ex2aro -use_input_sc -nstruct 10
```