Designing Metal-Templated *de novo* Protein Complexes

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RosettaCon, August 4th 2010

ROSETTA
DESIGN GROUP

More Parlor Tricks with Metals

Interact dammit.

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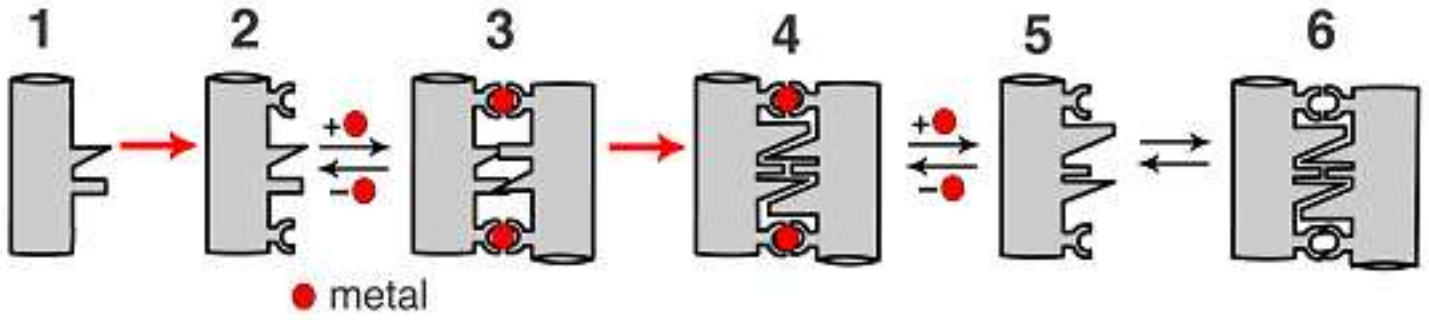
What is metal-templating?



1. Starting material: 'arbitrary' non-associating protein/proteins, '*de novo* complex design'

2. Introduce metal ligands: His, Asp, Glu, Cys, *etc.*

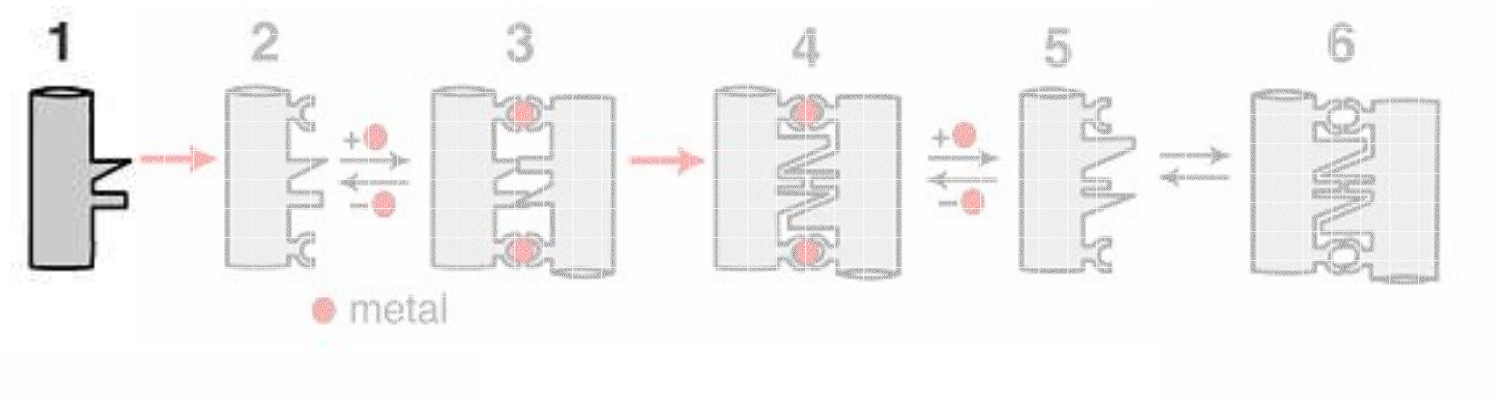
3. Characterize metal complex

4-5. Computationally redesign interface to create metal promoted complex

5-6. Metal 1-templated, metal-free *de novo* complex

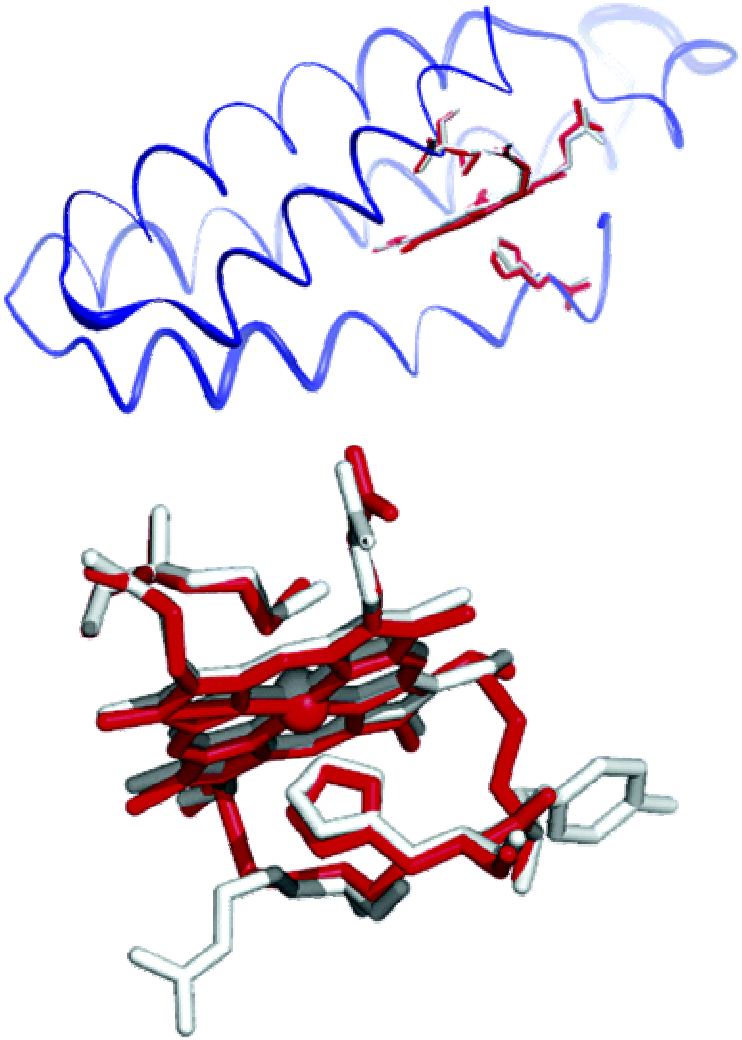
Proc. Natl. Acad. Sci. USA *107, 1827* (2010)

1. Starting material: ‘arbitrary’ non-associating protein/proteins



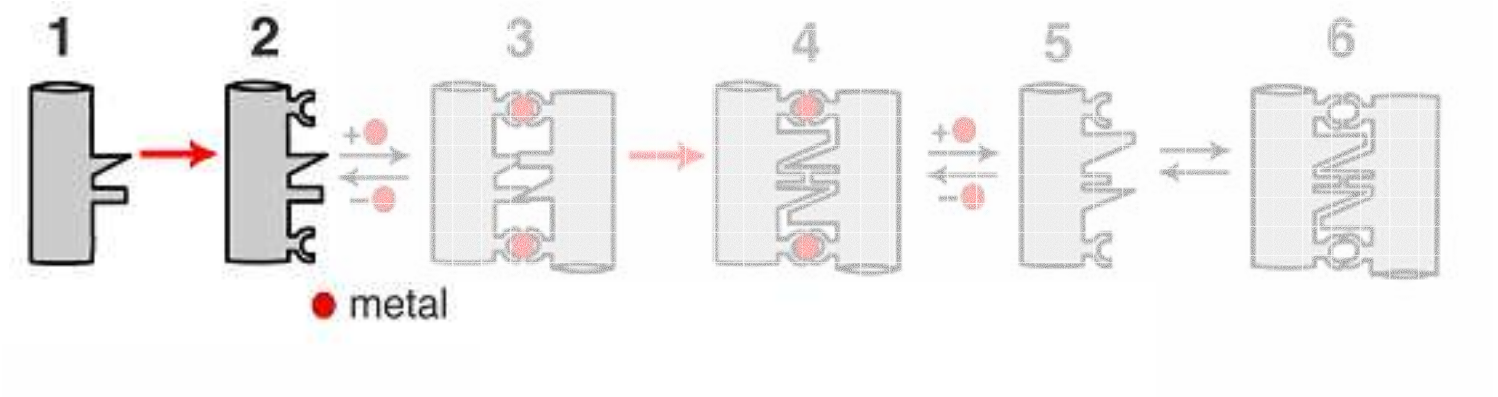
Starting Material: Engineered cytochrome b_{562}

- not exactly ‘arbitrary’
- 4-helix bundle (106 aa)
- Fe-protoporphyrin IX heme group
    - b-type axial ligation to Fe
- engineered c-type thioester linkages to heme group
- **highly stable** engineered fold (solution to flexbb problem, use Nobuteins?):
    - $[\ GuHCl\ ]_{1/2}$ ~ 4.2 M
    - ΔG fold: -42 kJ mol^{-1}
- **monomeric at millimolar concentrations**
- **roughly cylindrical shape**
- **experimentally tractable**



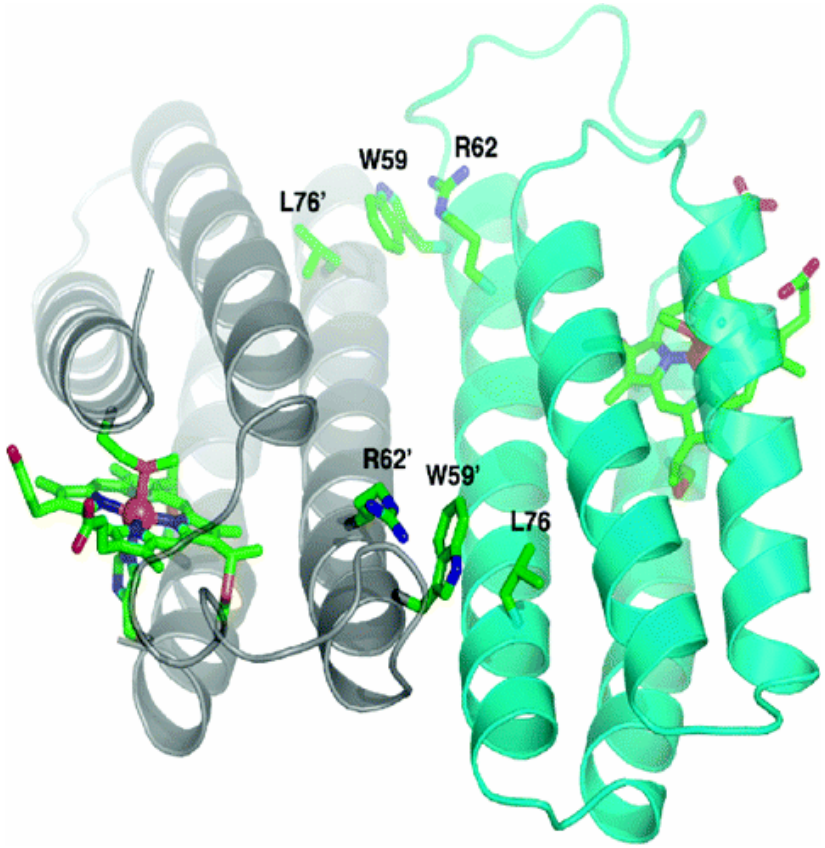
Faraone-Mennella J, et al. Biochemistry. 2006
Barker PD, et al. Biochemistry. 1995

2. Introduce metal ligands: His, Asp, Glu, Cys, *etc.*



Introducing Metal-ligands

- K59W for fluorescence studies results in anti-parallel crystal contacts at helix 3
- Q: Zinc mediated dimer with *i*, *i* + *4* di-His motifs at N- & C-termini of helix 3?
    - 59,63 and 73,77 (Asp at 60,64)



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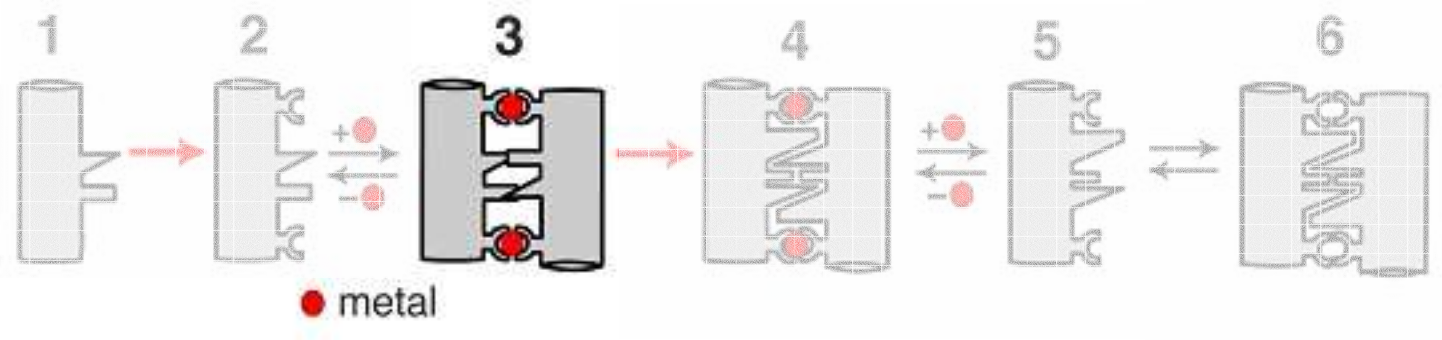
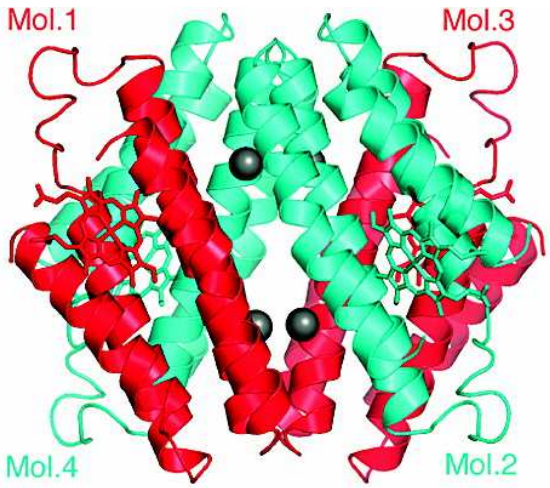
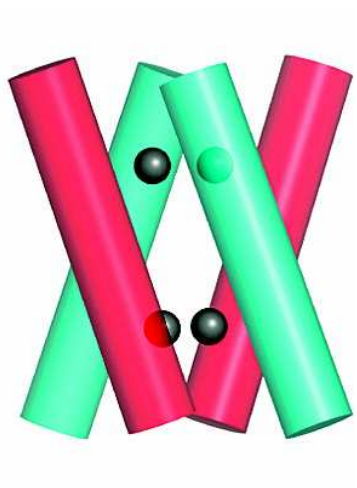
3. Characterize metal complex



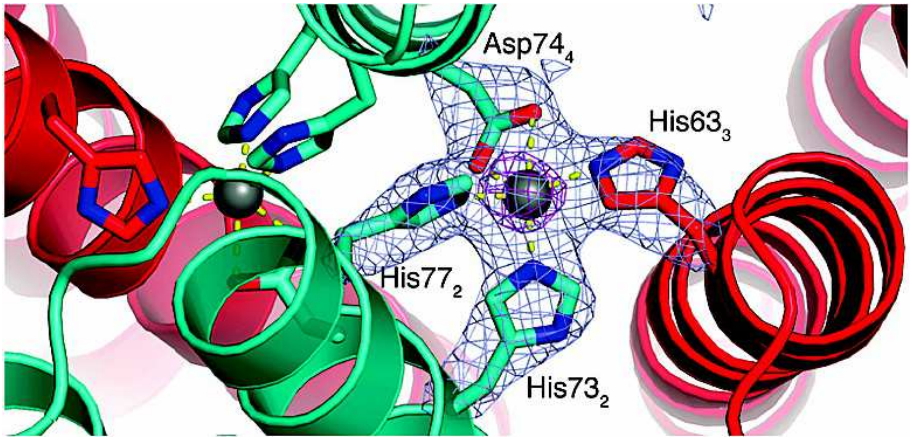
- **AUC**
- **crystallography**
- **computational interface analysis**

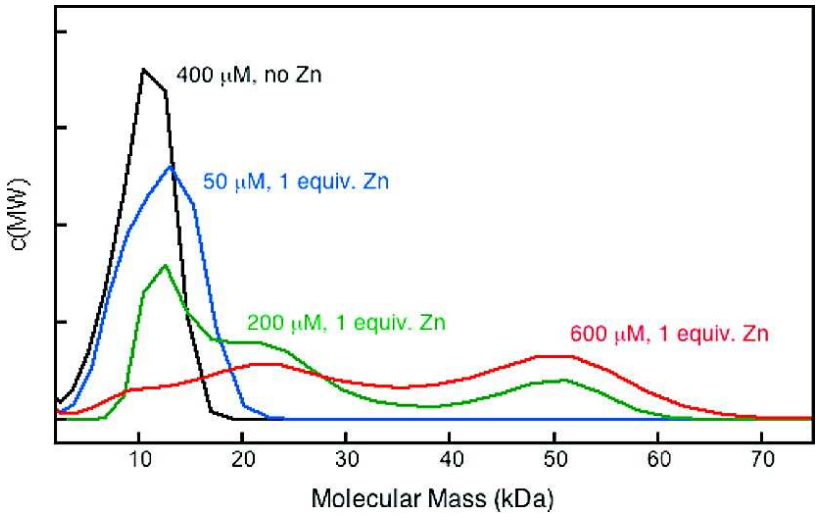
Zinc Complex





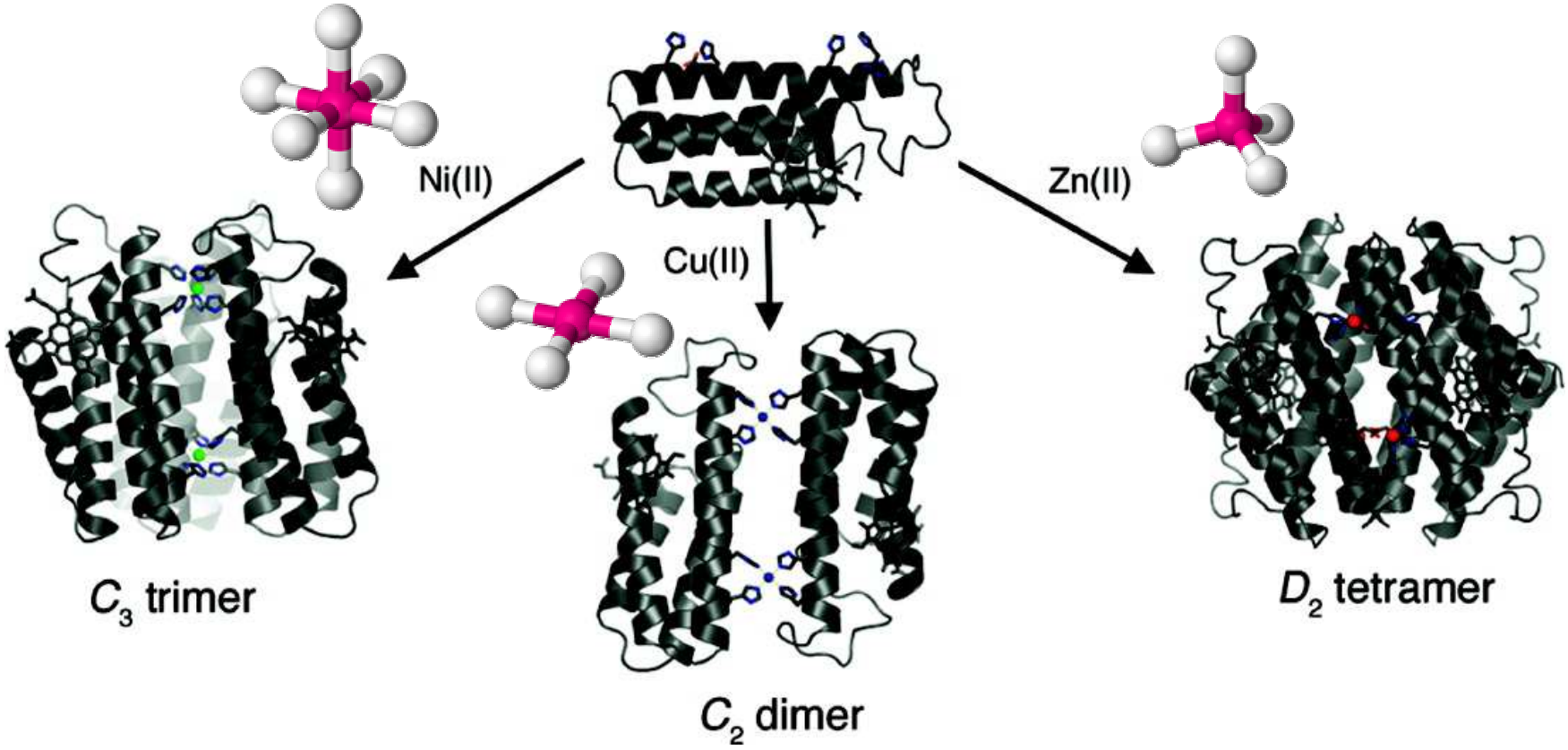
- AUC revealed a weak stoichiometric Zn-dependent tetrameric complex
- “Crisscrossed” tetrameric crystal structure





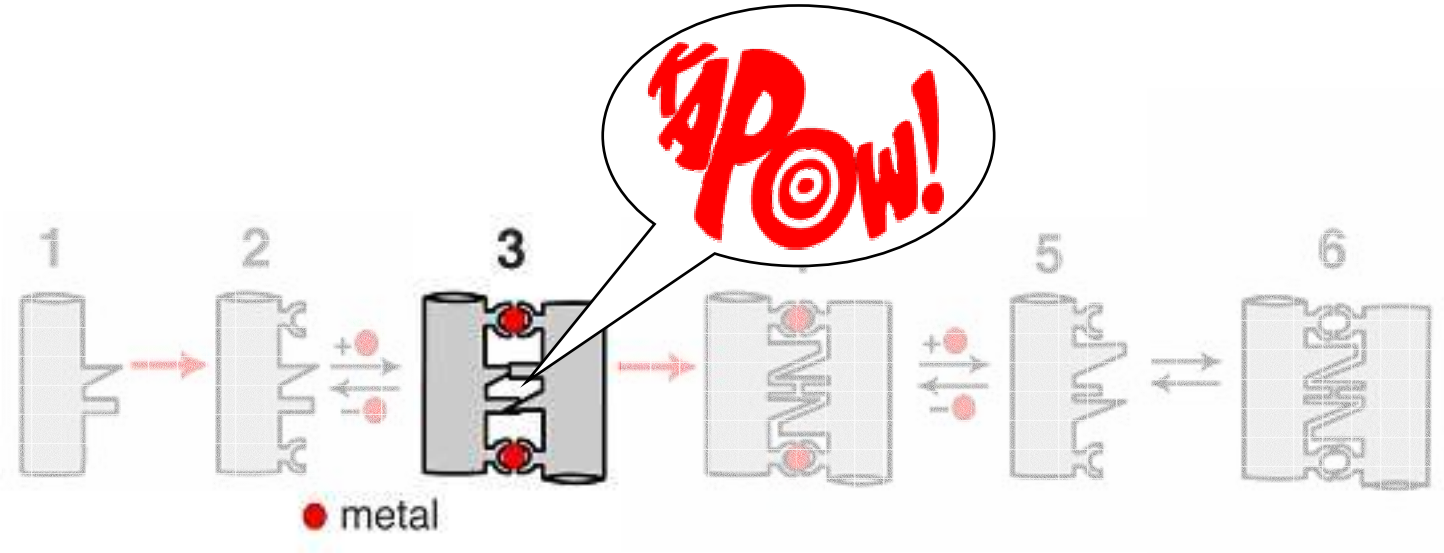
Salgado, EN et al. *J. Am. Chem. Soc.* 2007

Additional Metal Complexes



Published in: Eric N. Salgado; Richard A. Lewis; Susanne Mossin; Arnold L. Rheingold; F. Akif Tezcan; *Inorg. Chem.* **2009,** 48, 2726-2728.
DOI: 10.1021/ic9001237

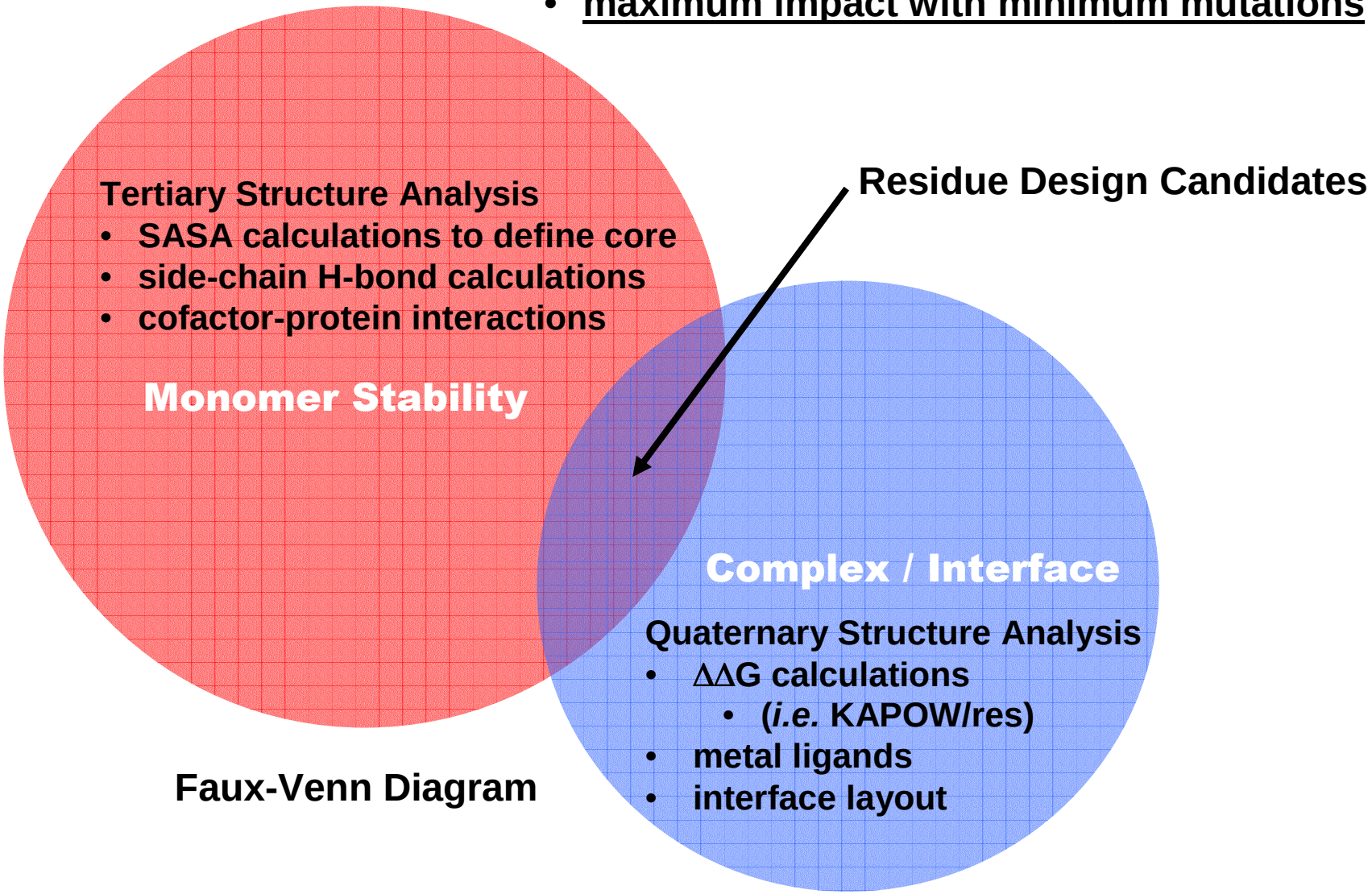
3. Characterize metal complex



- AUC
- crystallography
- **computational interface analysis**

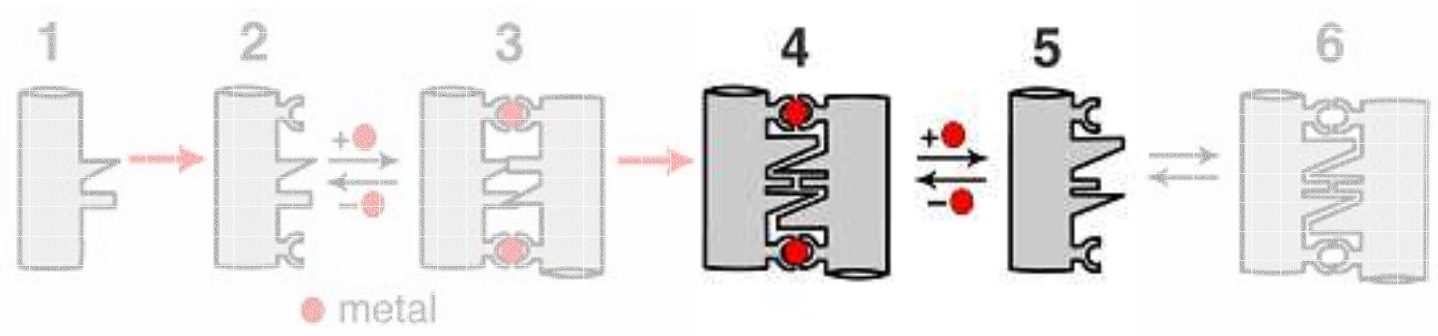
Design Considerations

- **maximum impact with minimum mutations**



Faux-Venn Diagram

4-5. Computationally redesign interface to create metal promoted complex



Design: Under the hood

MaxiRosetta/Rosetta++/OldRosetta/Rosetta2/Dinosetta BRANCH command line:

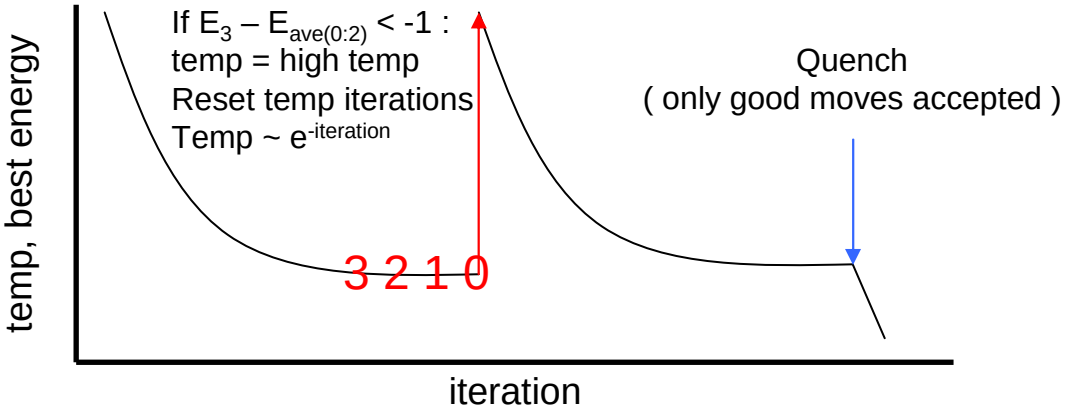
-parallel_design simple -soft_rep_design -ex1 –ex2aro_only -extrachi_cutoff 1 -try_both_his_tautomers -no_his_his_pairE

What is this “simple parallel_design” (besides out-of-date)????

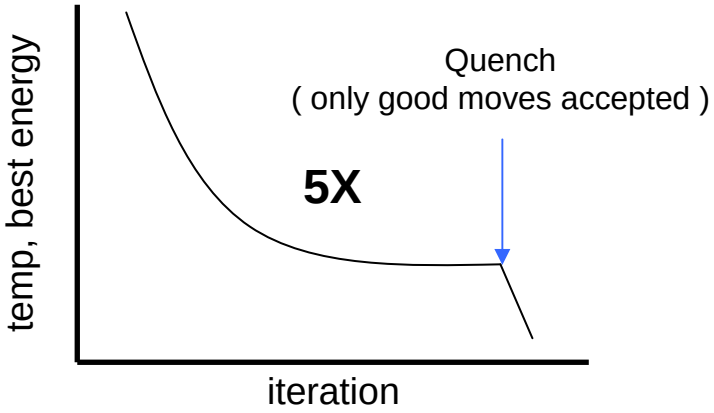
https://svn.rosettacommons.org/source/branches/RosettaX/

Ambroggio & Kuhlman 2006 multi-state algorithm + changes in rotamer packing:

OLD (Really old, but maybe not?)



NEW (actually old)

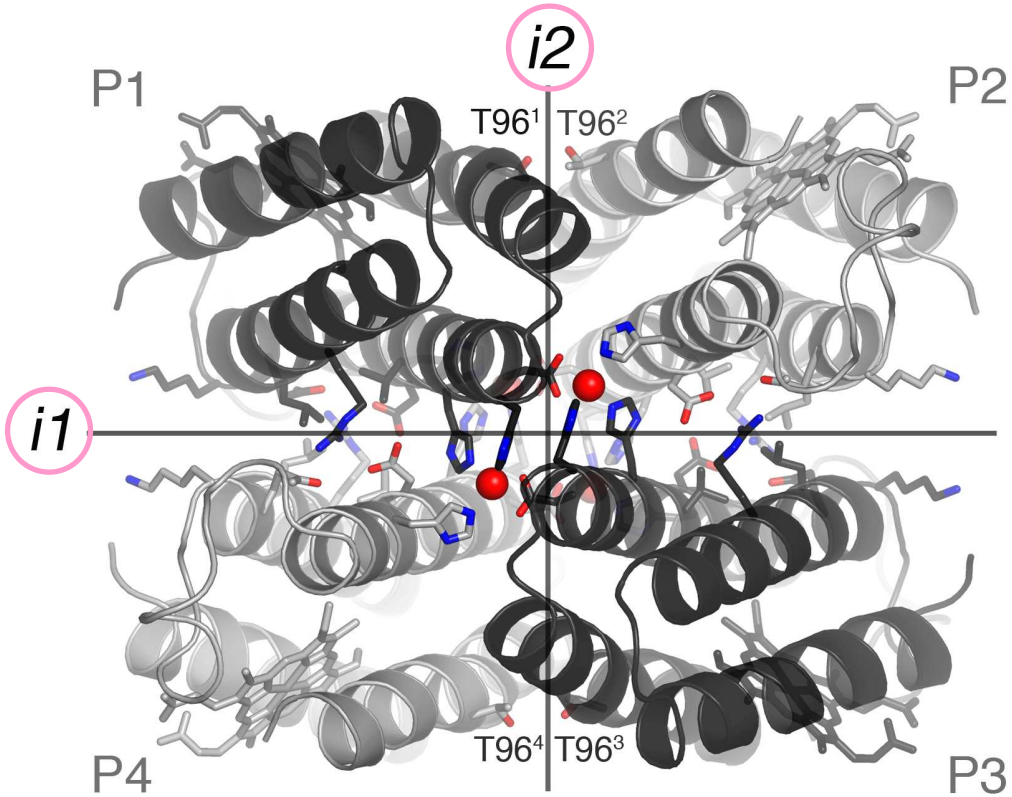


OLD Rotamer selection:
- select random rotamer
- if quench, randomly try every rotamer

NEW Rotamer selection:
- randomly try every rotamer
- *if accepting a rotamer with ΔE < 0, add another copy to rotamer list*

- best of 5
- if little change in lowest energy (< 0.01) after 3rd trial, quit

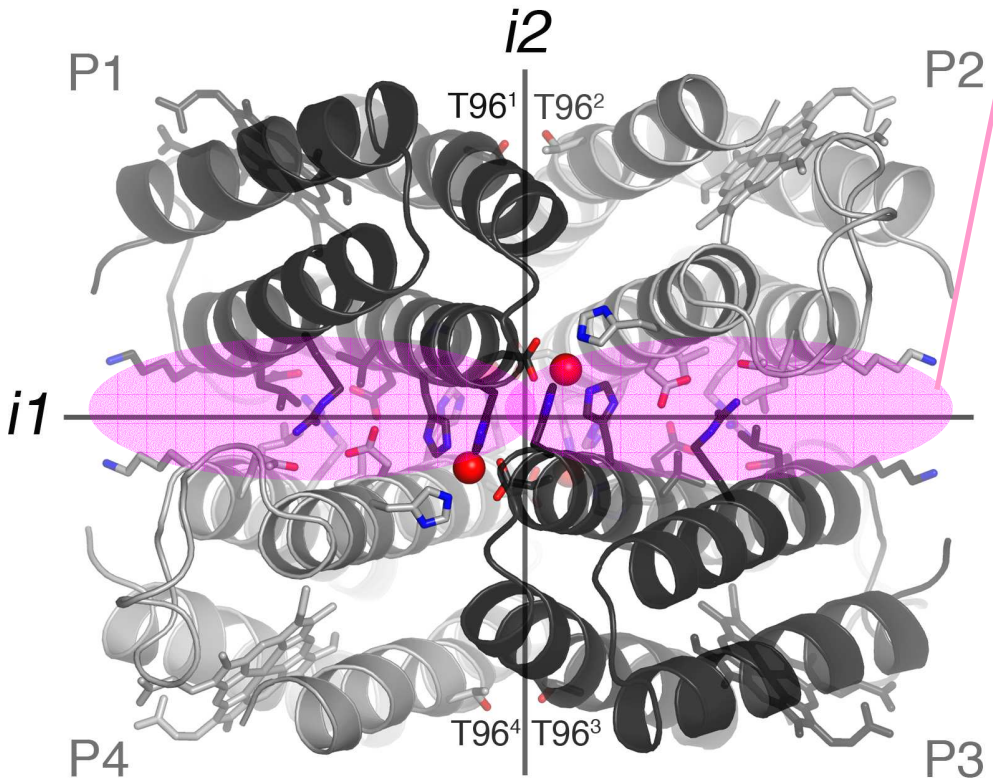
Zinc Complex Interfaces



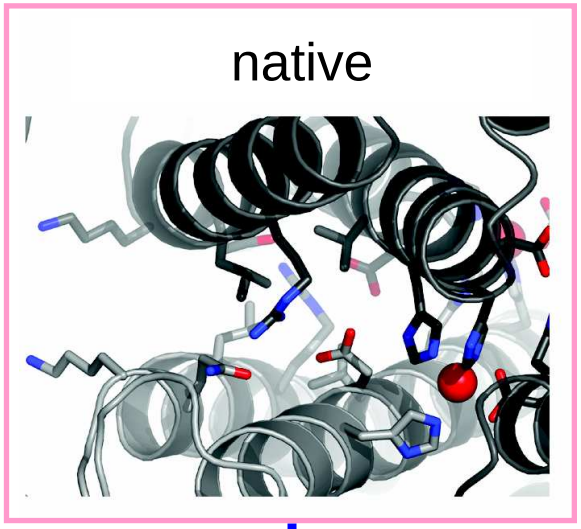
Zn_4:MBPC-1_4

- **2 ‘independent’ interfaces: *i1, i2***

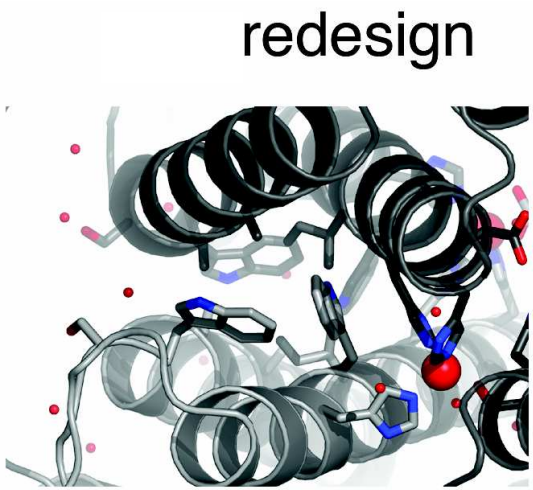
i1 Redesign



Zn_4:MBPC-1_4

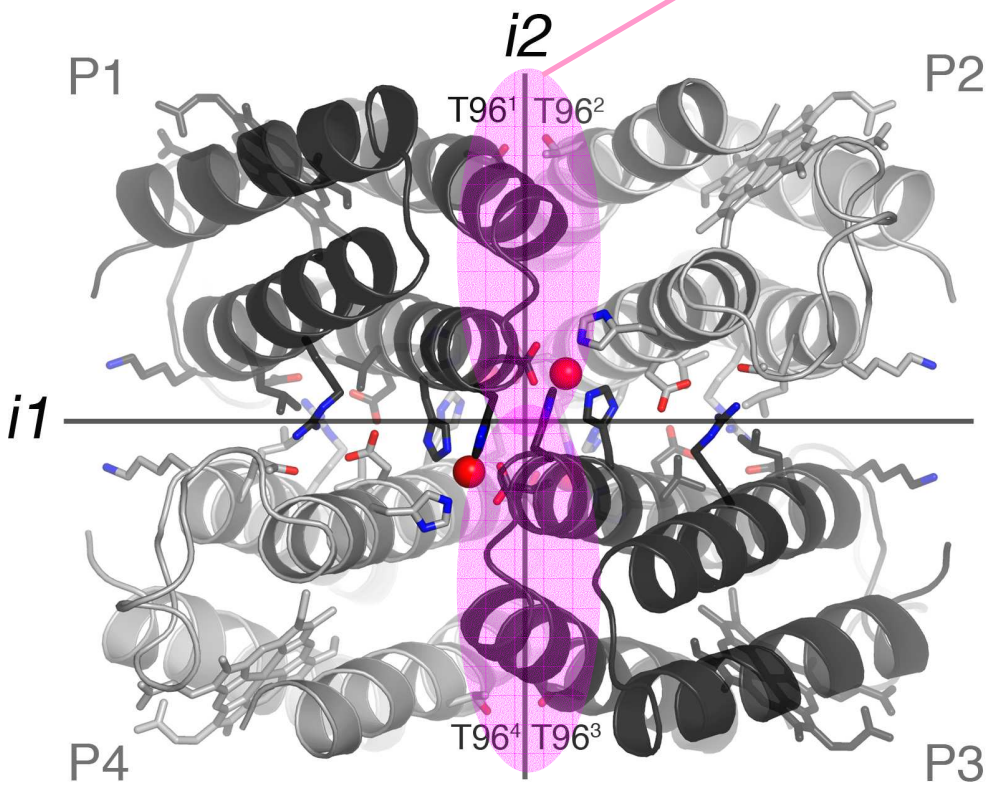


R34A, L38A, Q41W,
K42S, D66W, V69I

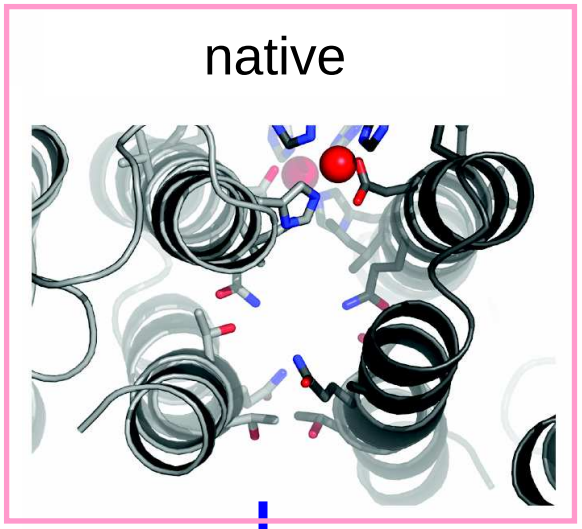


RIDC-1

i2 Redesign

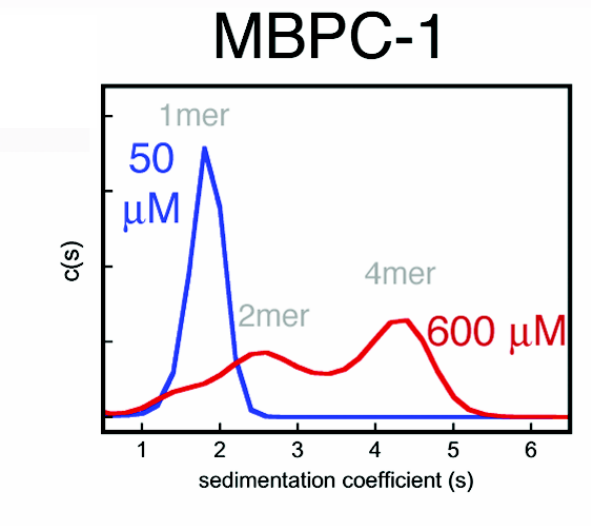


Zn_4:MBPC-1_4



RIDC-2

AUC with Equimolar Zn (II)



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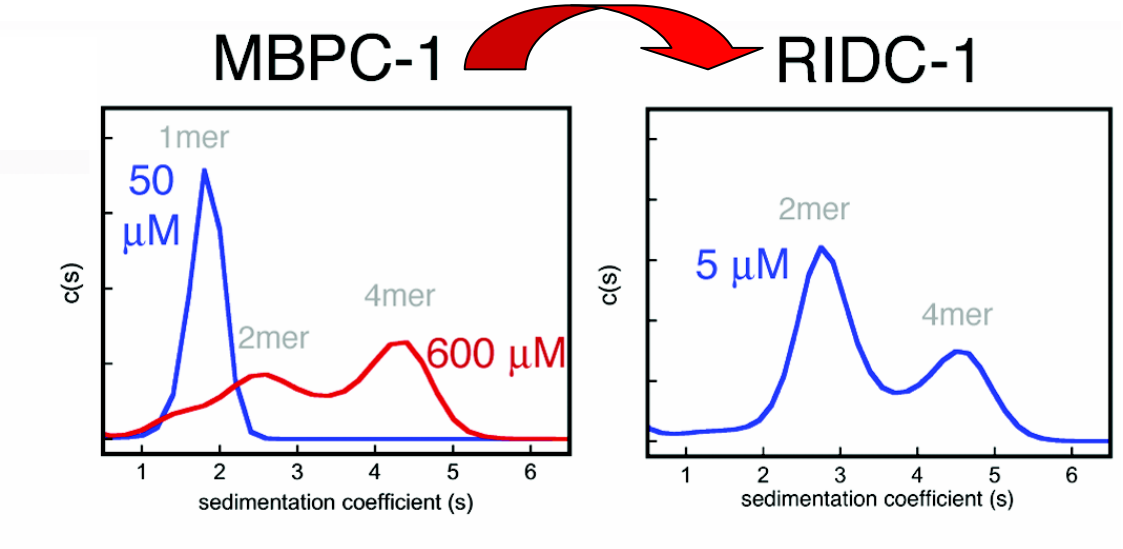

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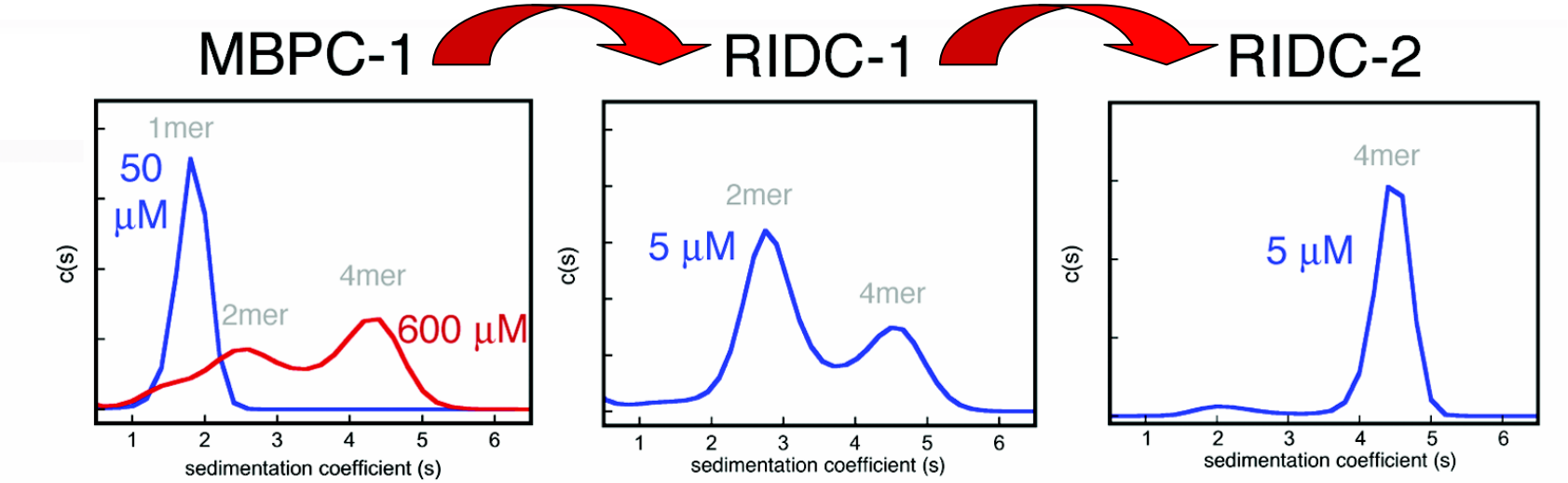

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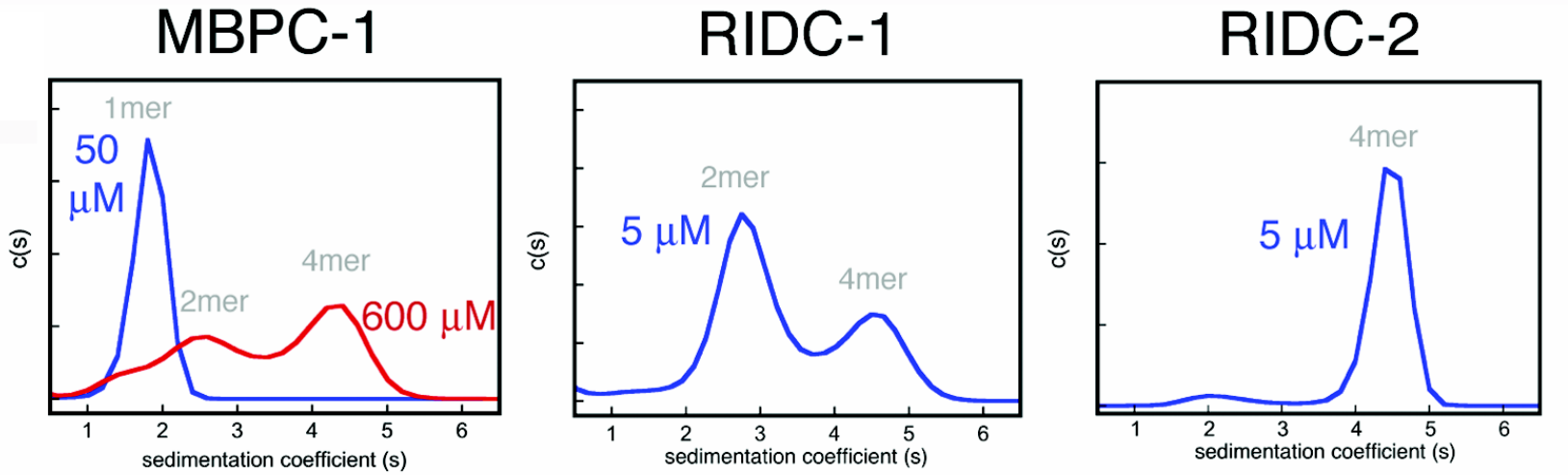
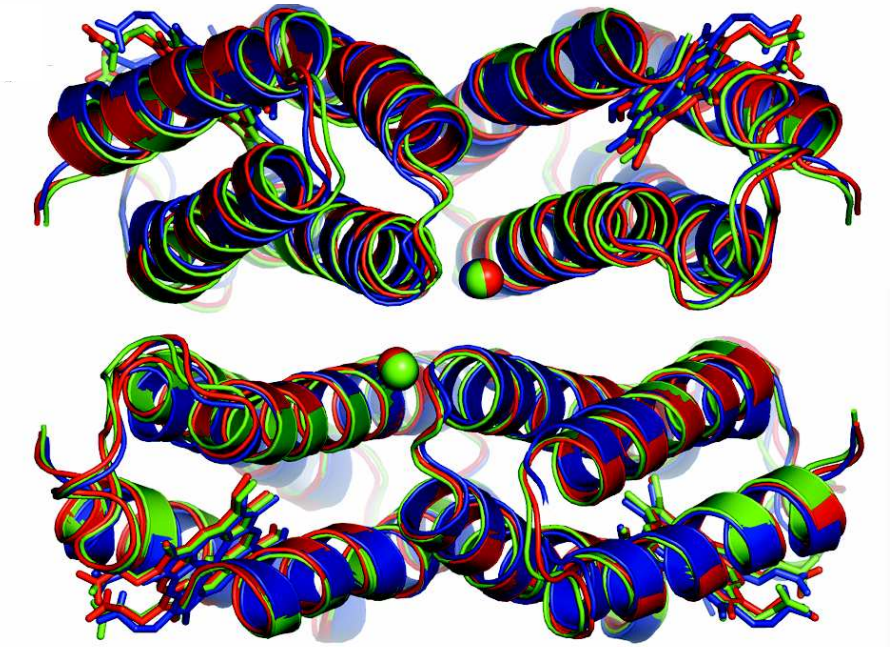
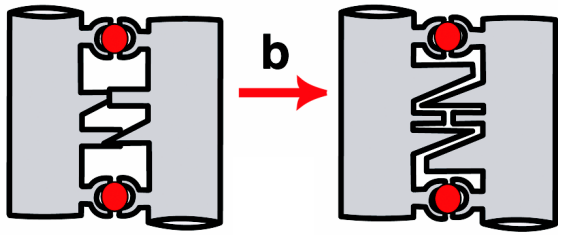
"Template-and-Stabilize" strategy works







green- Zn_4:MBPC-1_4
blue- Zn_4:RIDC-1_4
red- Zn_4:RIDC-2_4

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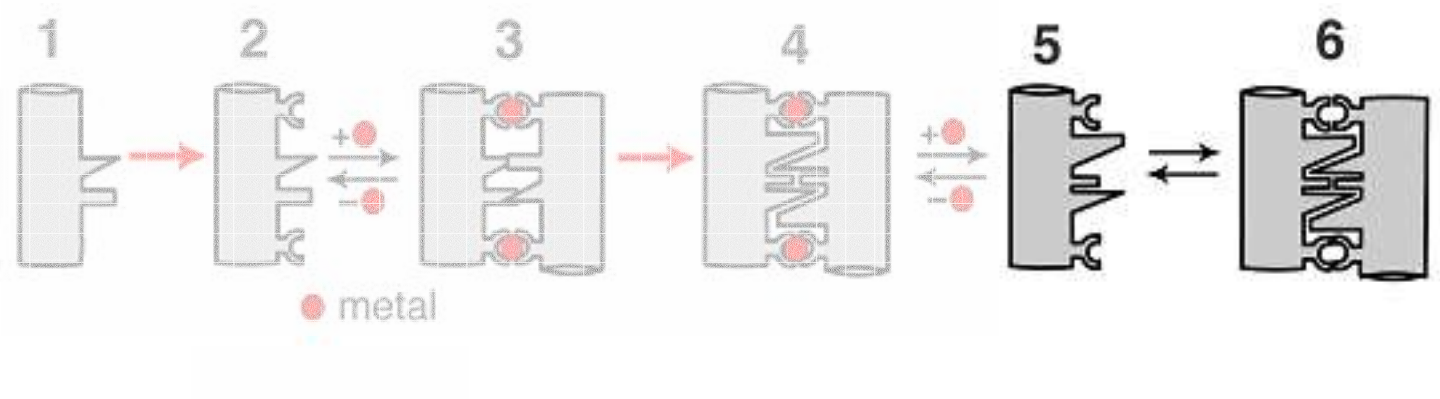
6. Metal-templated, metal-free *de novo* complex



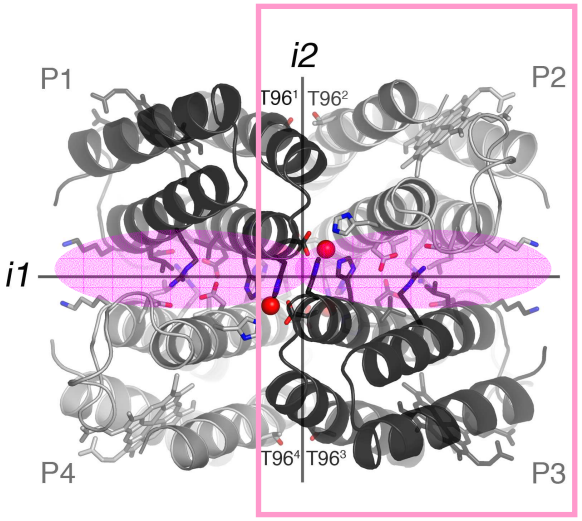
Is the redesign predicted to adopt the metal-templated structure?

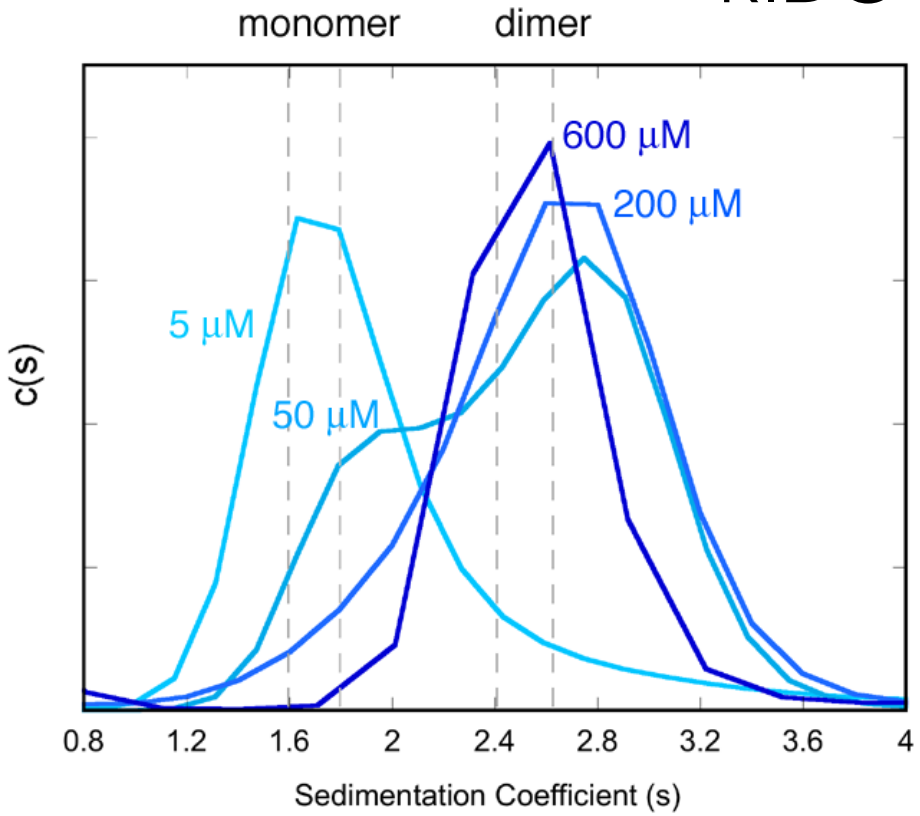
Pursued the answer in parallel with:

- computational predictions
  - Docking with Rosetta
- experimental characterization
  - AUC
  - Crystallography

Metal-free AUC

RIDC-1





Variant with the redesigned 1st interface (RIDC-1) forms a dimer with a K_d = 26 µM.

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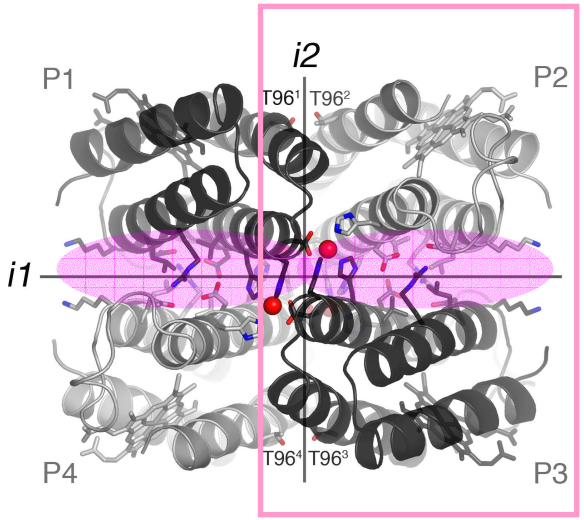
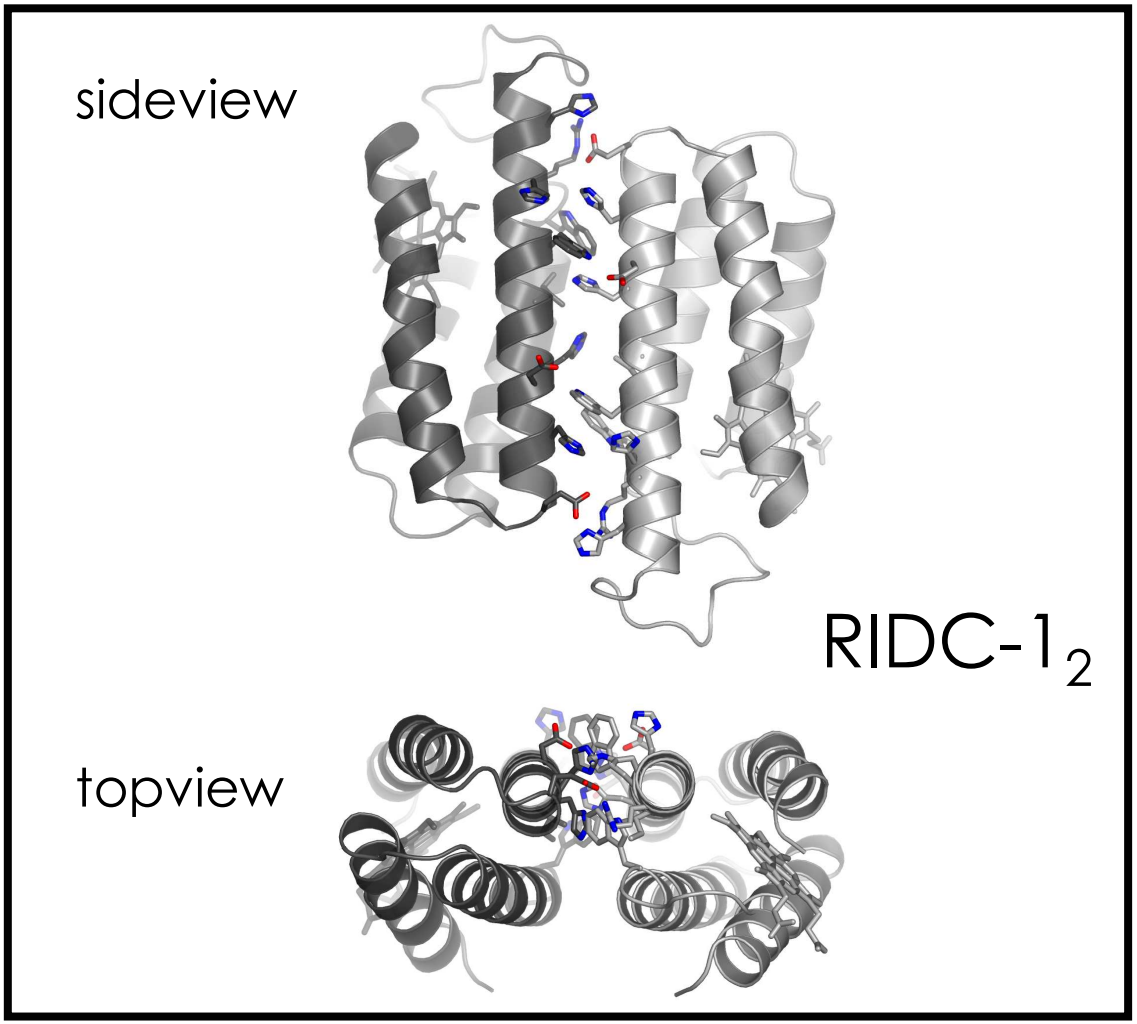
Metal-free Crystal Structure





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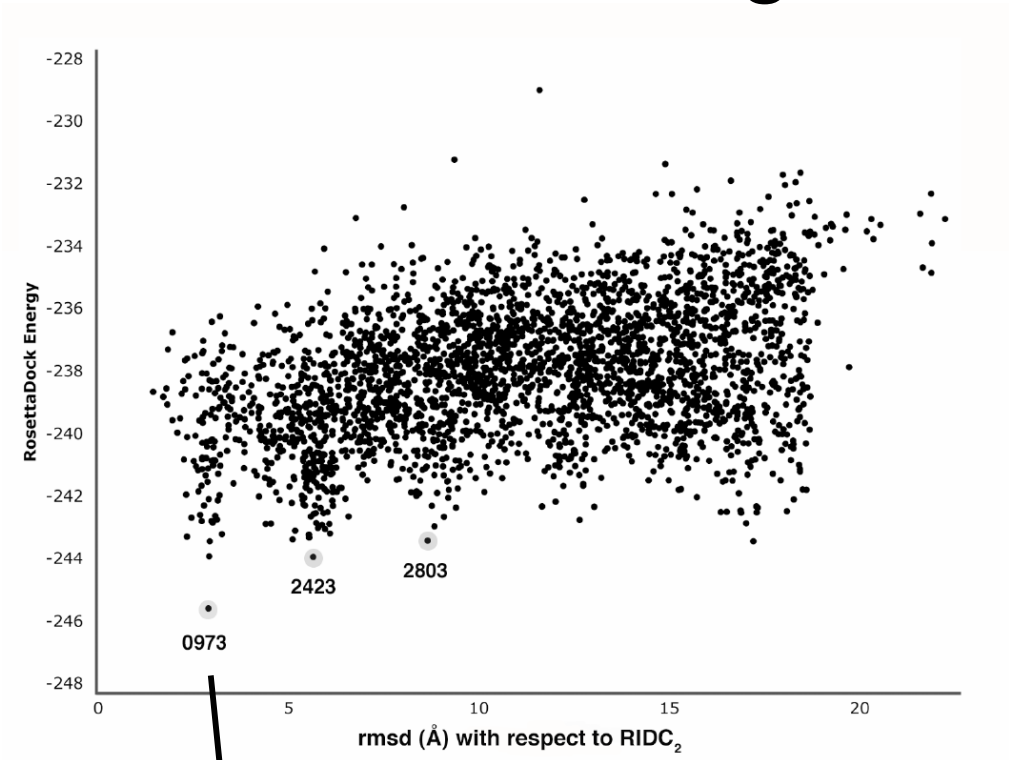
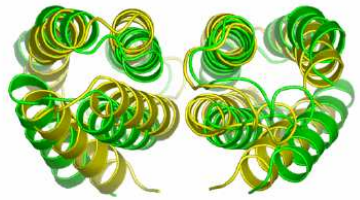
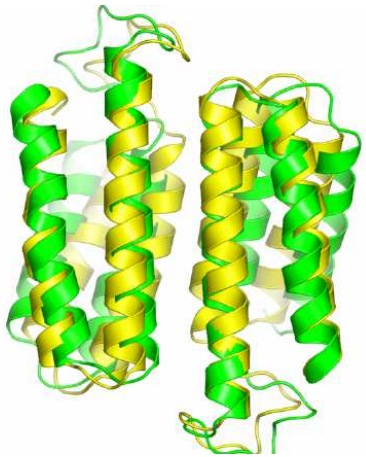
Blind Metal-free Docking Simulations

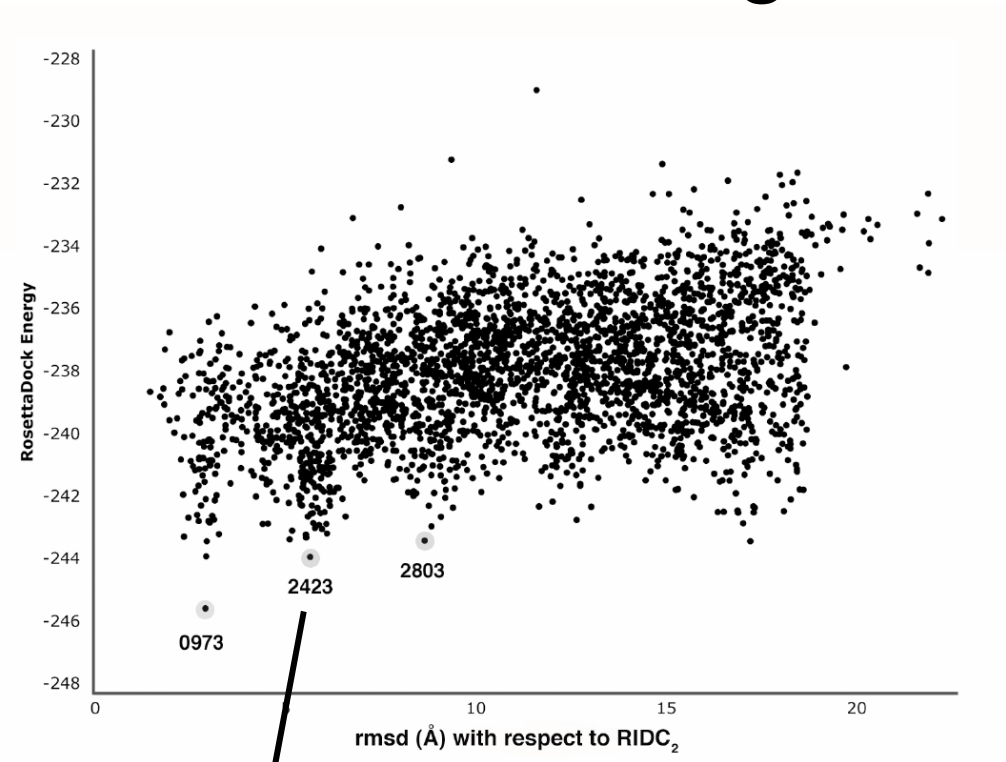


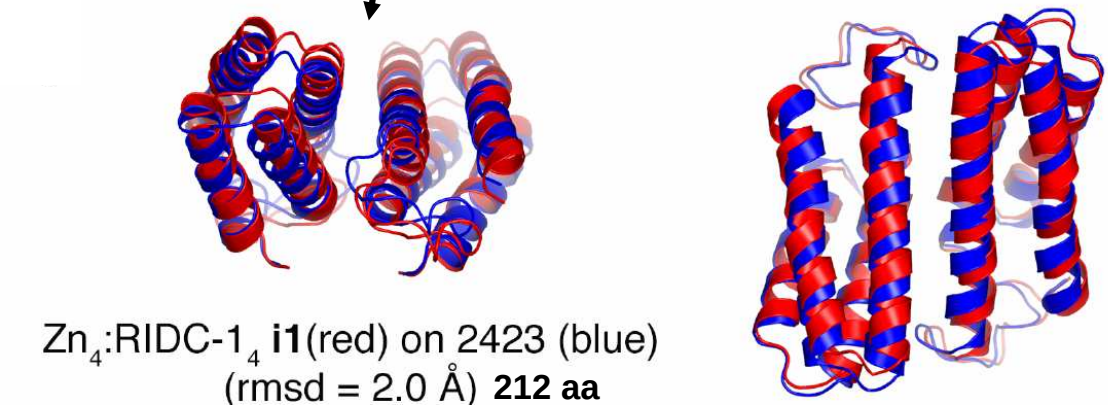




RIDC-1_2 (green) on 0973 (yellow)
(rmsd = 2.9 Å) **212 aa**

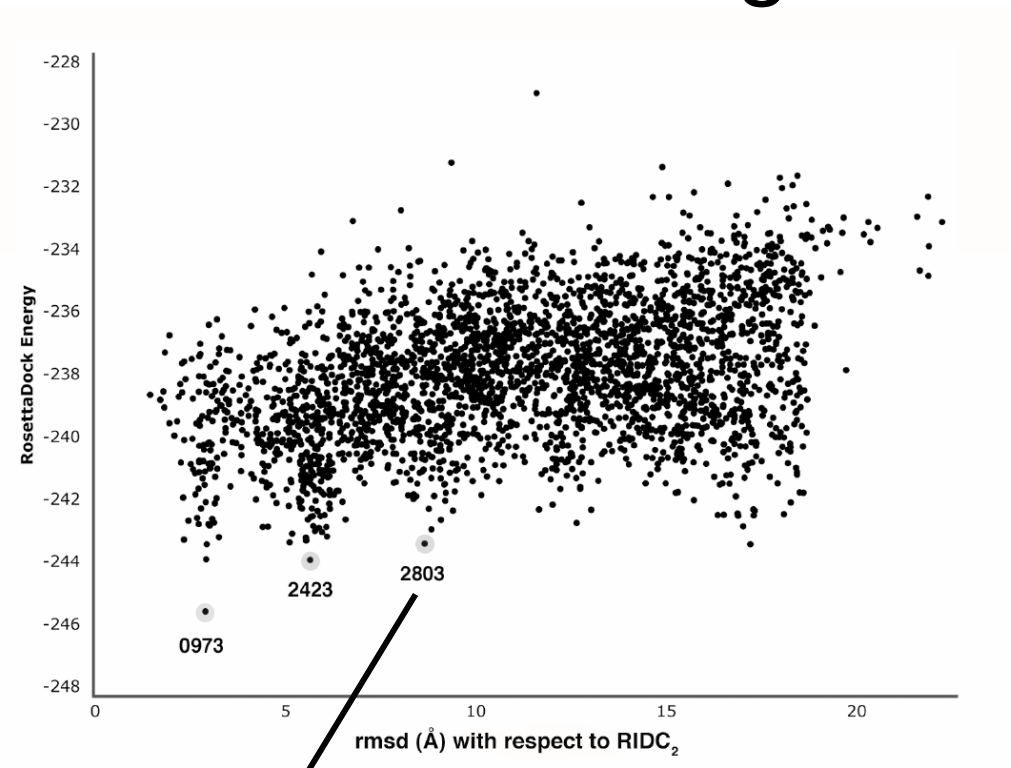
Blind Metal-free Docking Simulations

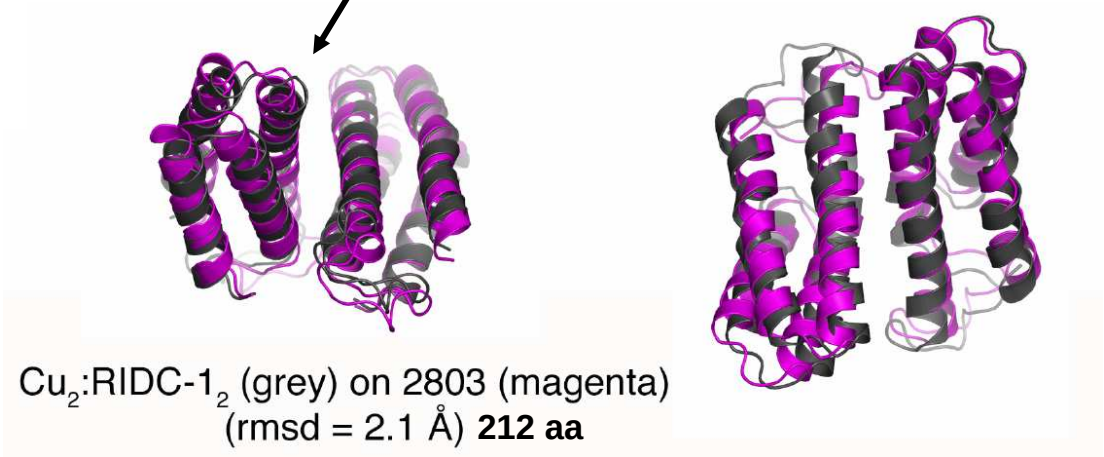




Zn_4:RIDC-1_4 **i1**(red) on 2423 (blue)
(rmsd = 2.0 Å) **212 aa**

Blind Metal-free Docking Simulations





Cu_2:RIDC-1_2 (grey) on 2803 (magenta)
(rmsd = 2.1 Å) **212 aa**

Acknowledgements

Eric N. Salgado
Jeffrey D. Brodin
F. Akif Tezcan
Brian Kuhlman

Thank You!