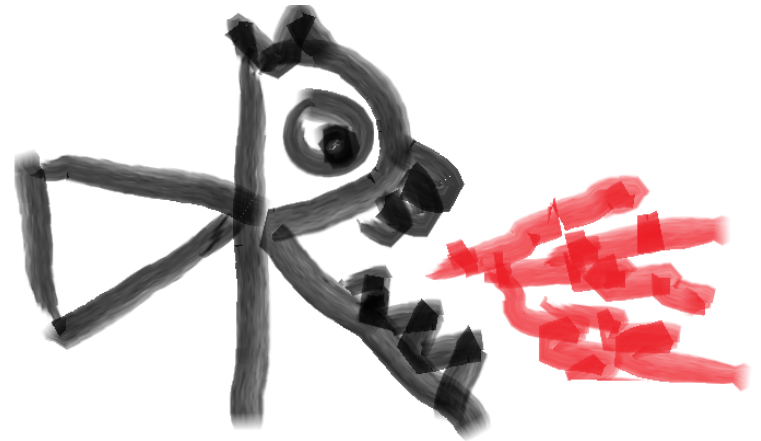
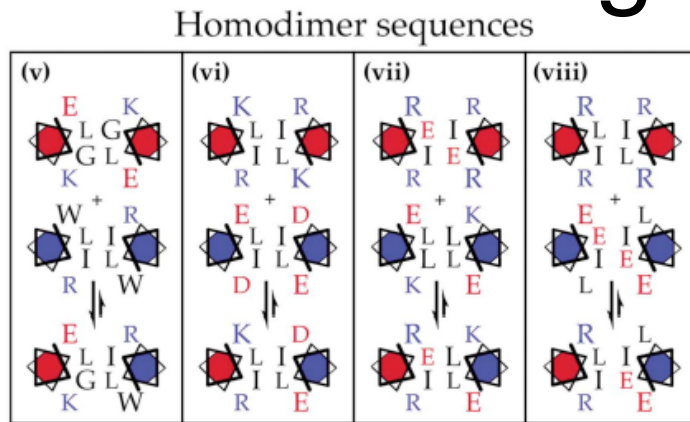




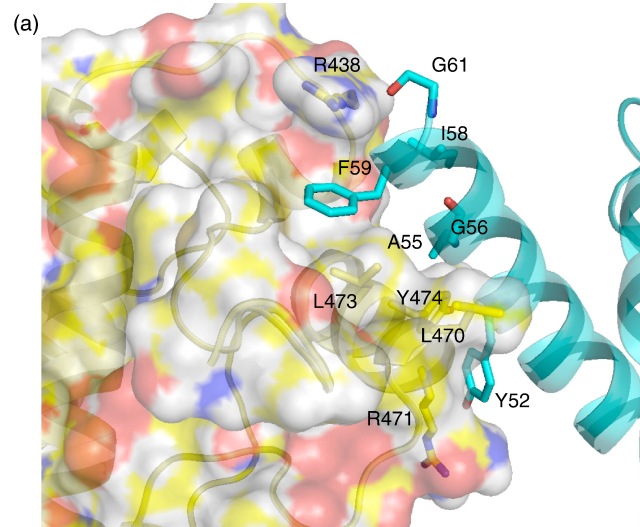
Accurate design of a symmetric homodimer using β -strand assembly

Ben Stranges
Kuhlman Lab
RosettaCon 2011

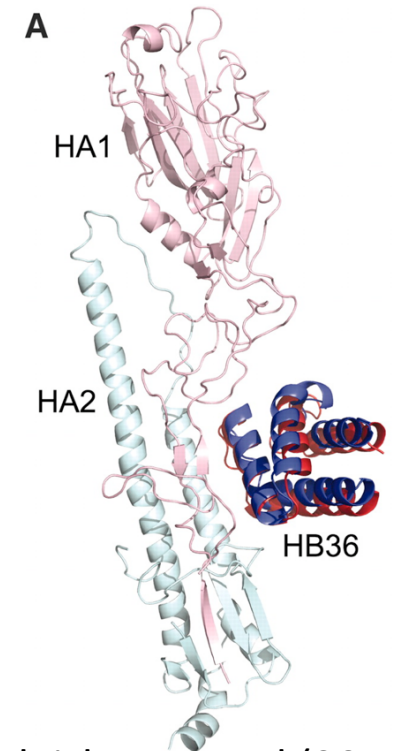
Examples of success in computational interface design



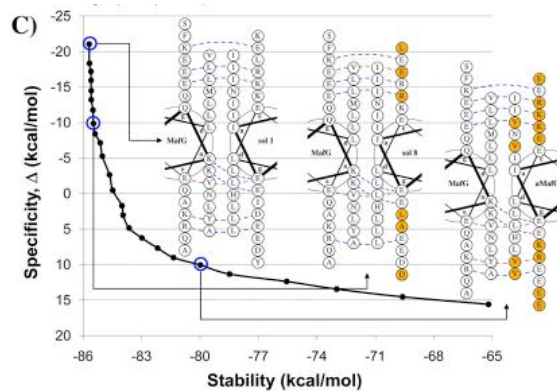
Havranek & Harbury (2002)



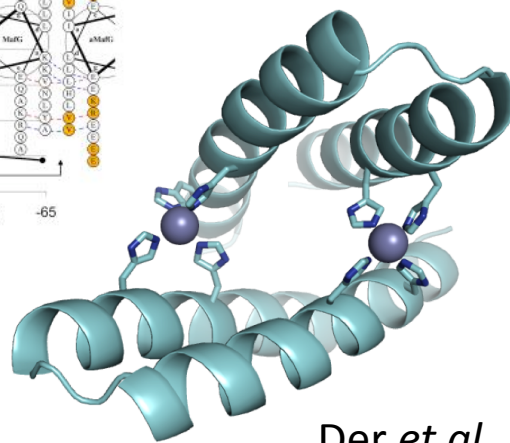
Jha *et al* (2010)



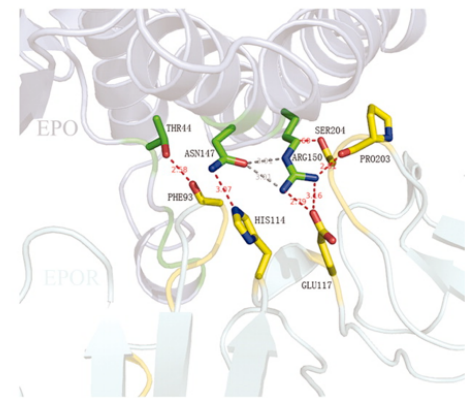
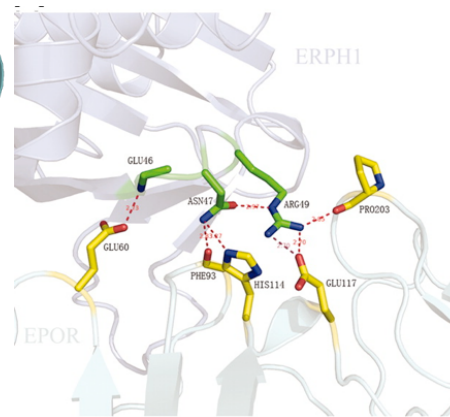
Fleishman *et al* (2011)



Grigoryan, Reinke
& Keating (2009)

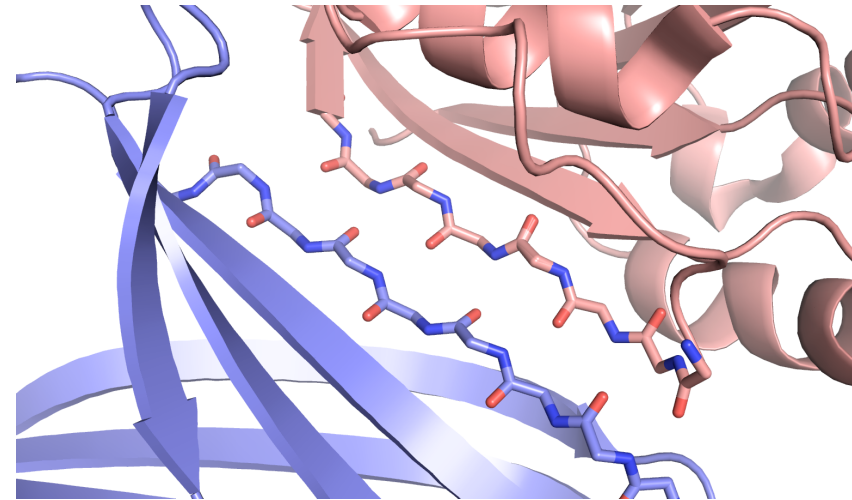
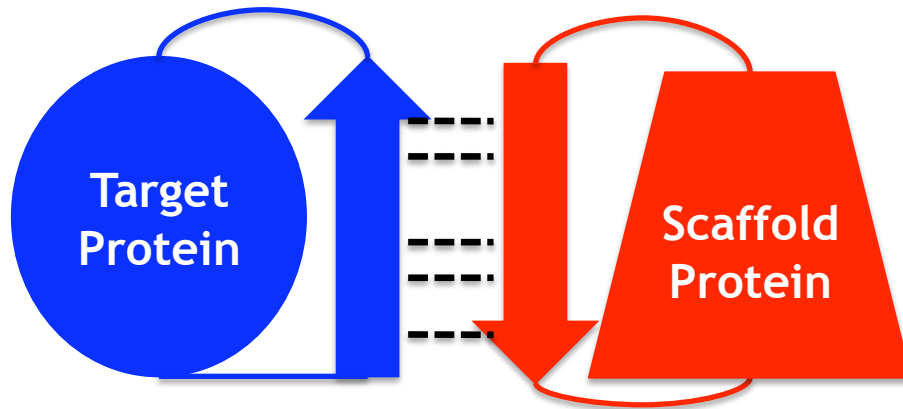


Der *et al*
in preparation



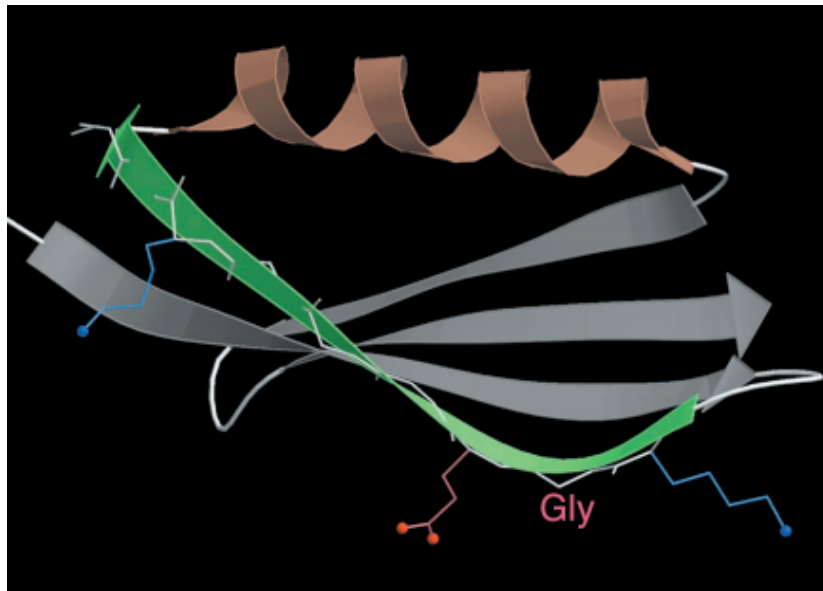
Liu *et al* (2007)

β -strand assembly as a new approach

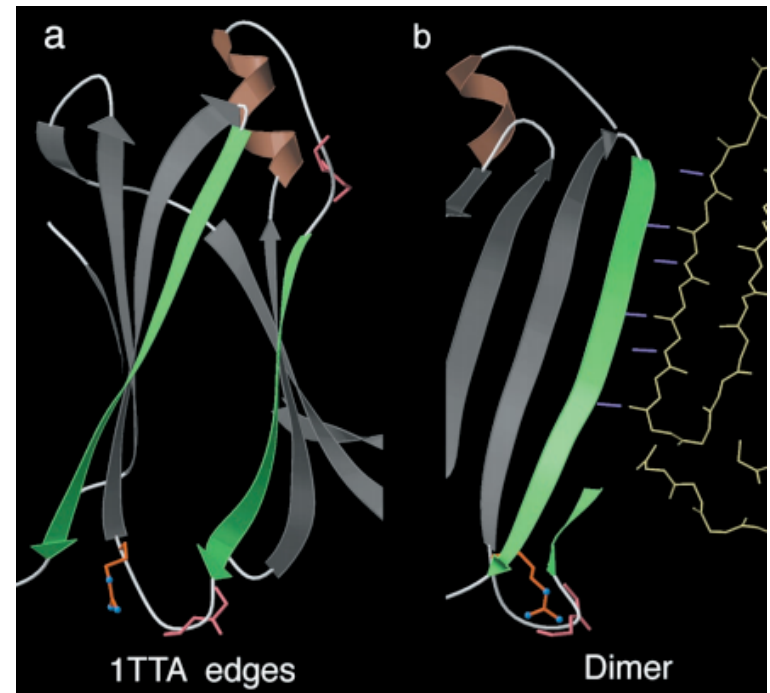


Der & Kuhlman
(2011) *Science*

β -strands are prone to assemble

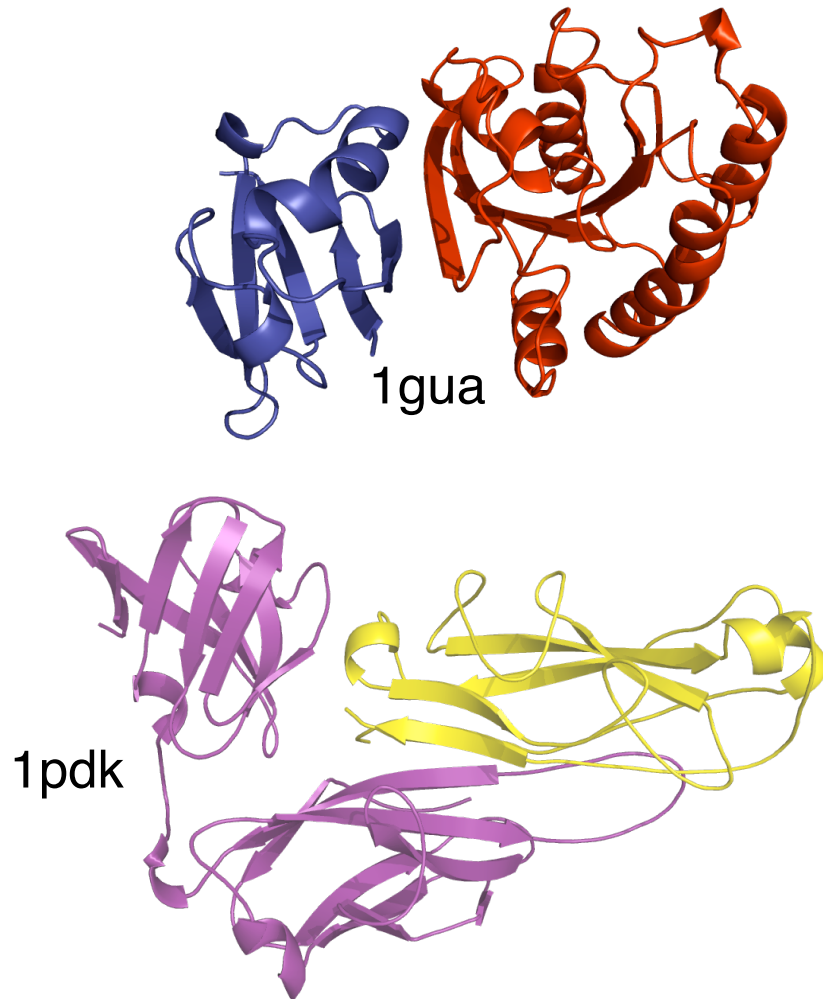


Richardson & Richardson (2002) *PNAS*

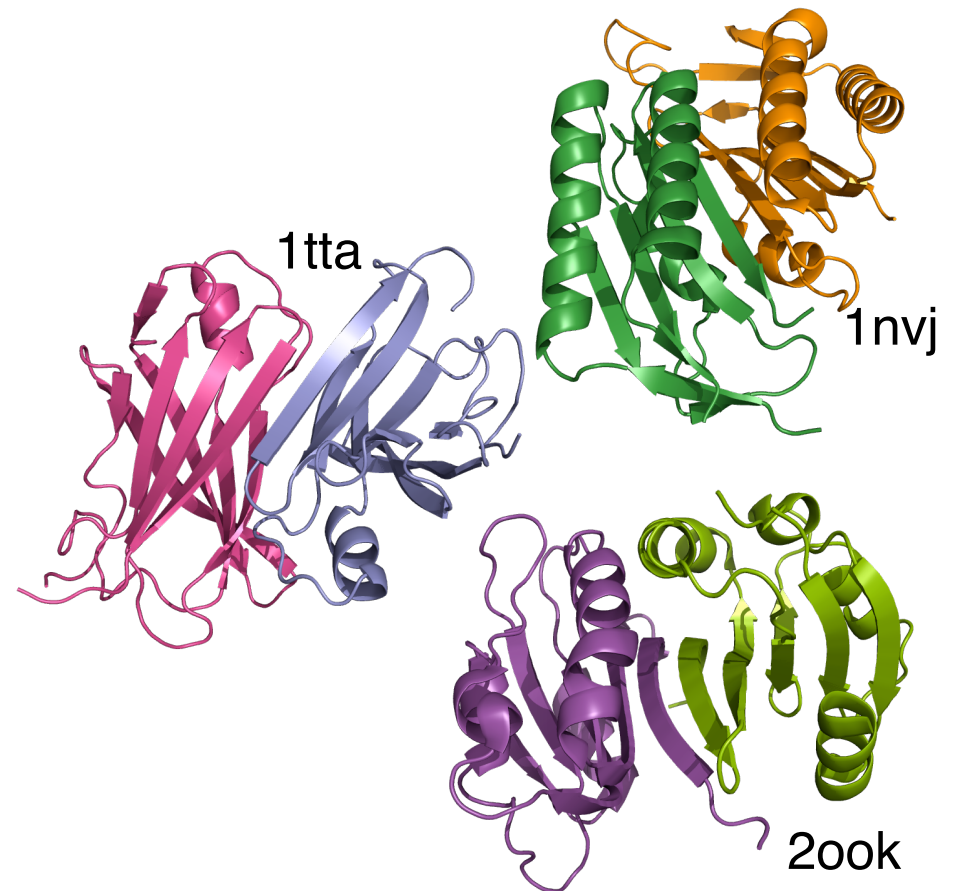


β -strands in natural interfaces

Heterodimers

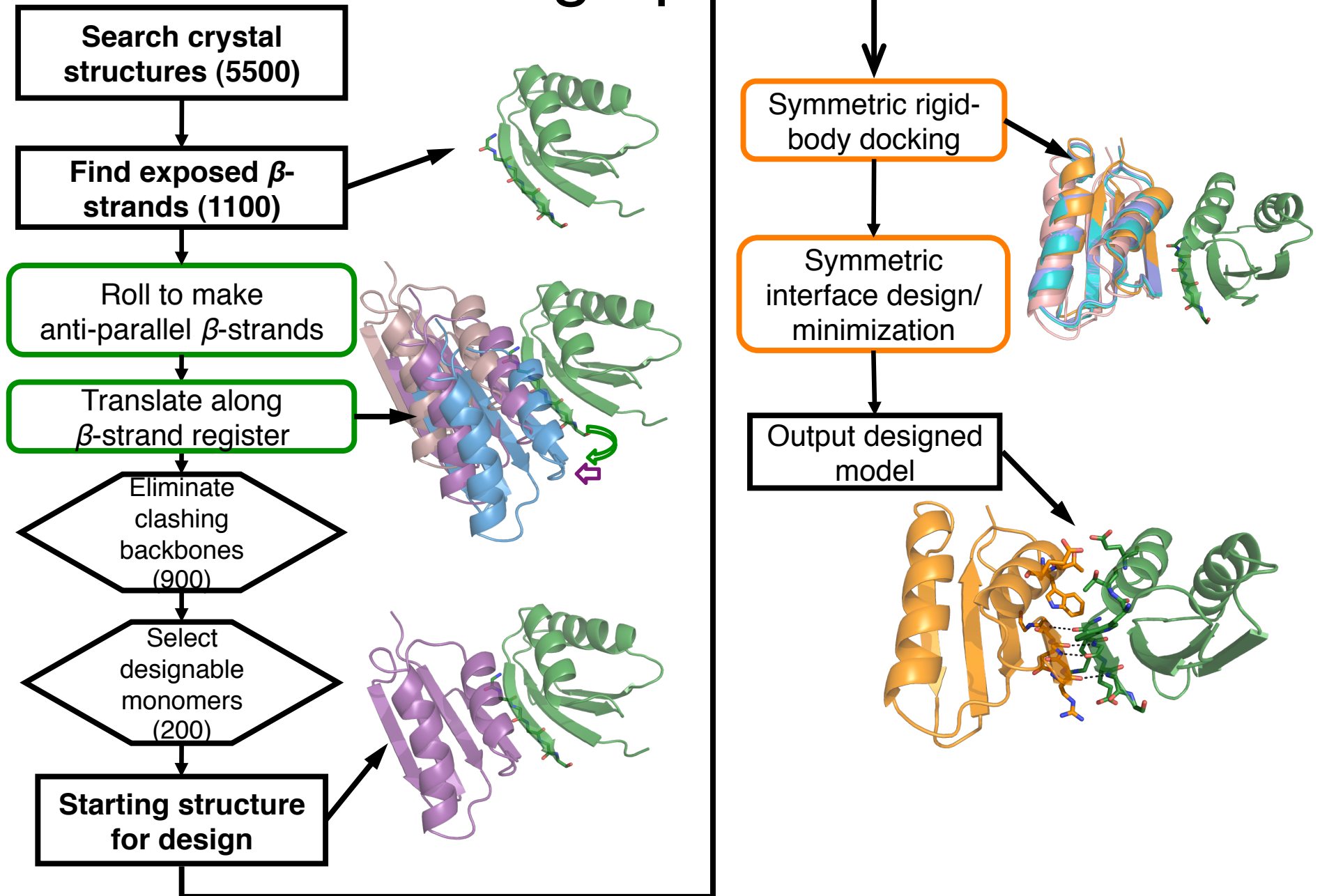


Homodimers

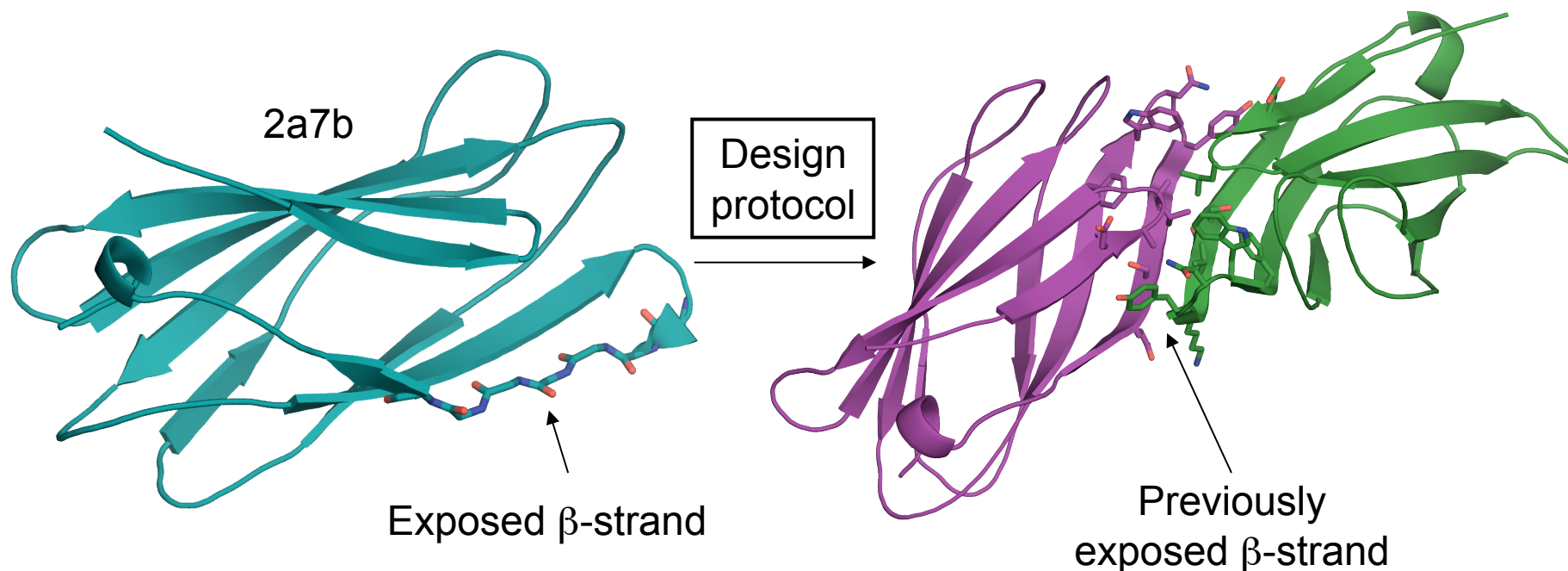


Goal: redesign a monomeric protein to form a symmetric homodimer via an intermolecular β -sheet

Search and design protocol



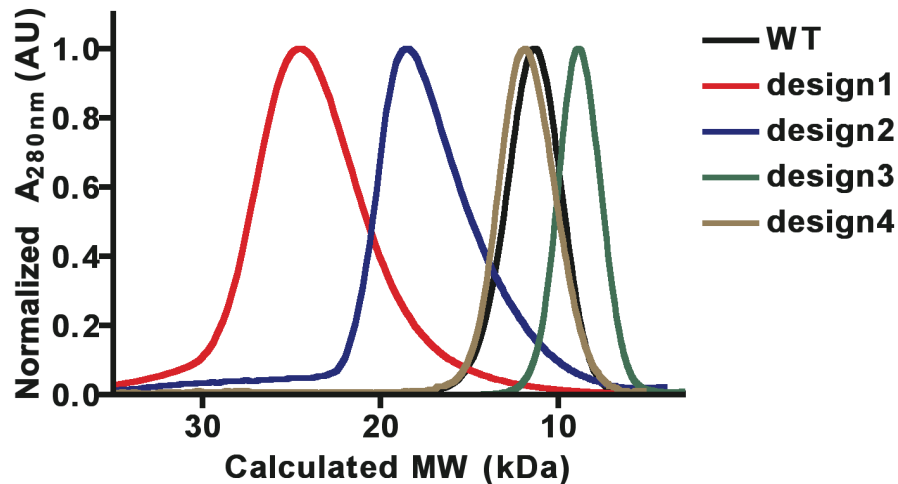
Choosing designs to express



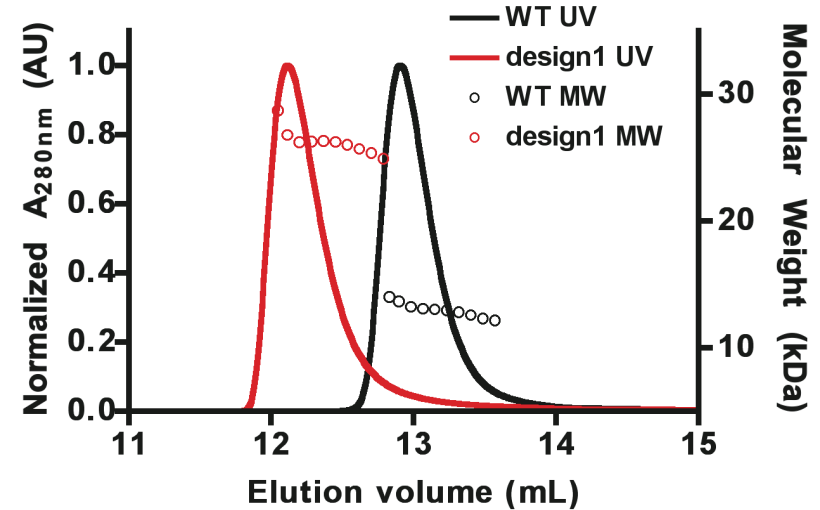
Model	# Mutations	E_{total}	ΔG_{bind}	# Buried Unsatisfied	Polar interface area %
wild type	0	-561	-13	8	48 %
design1	11	-597	-29	2	39 %
design2	7	-593	-30	0	31 %
design3	5	-596	-32	0	54 %
design4	9	-593	-27	0	46 %

Molecular weight in solution

Size exclusion chromatography

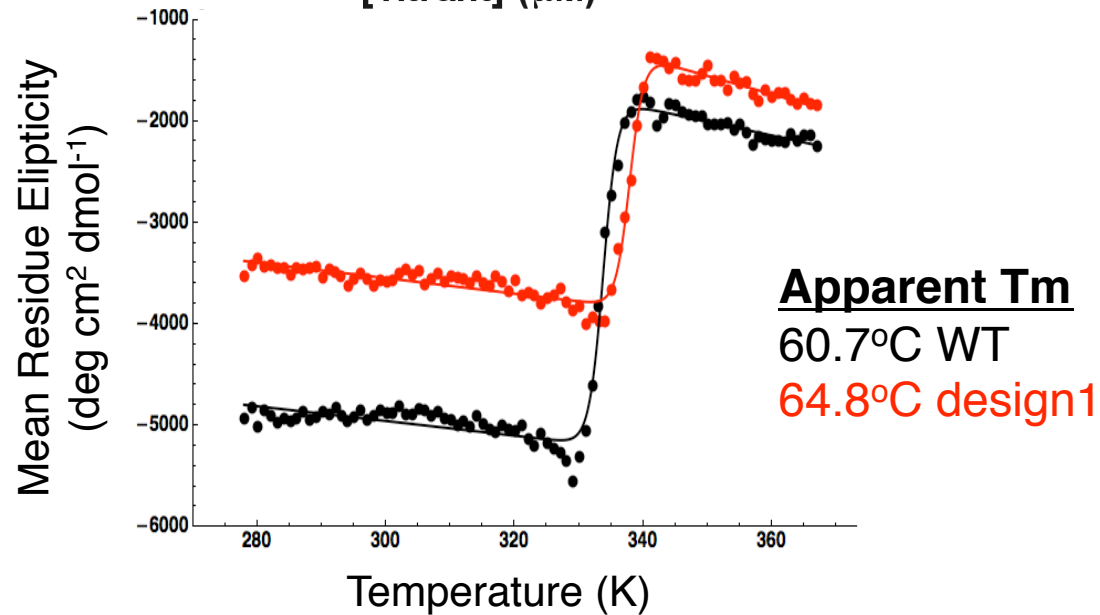
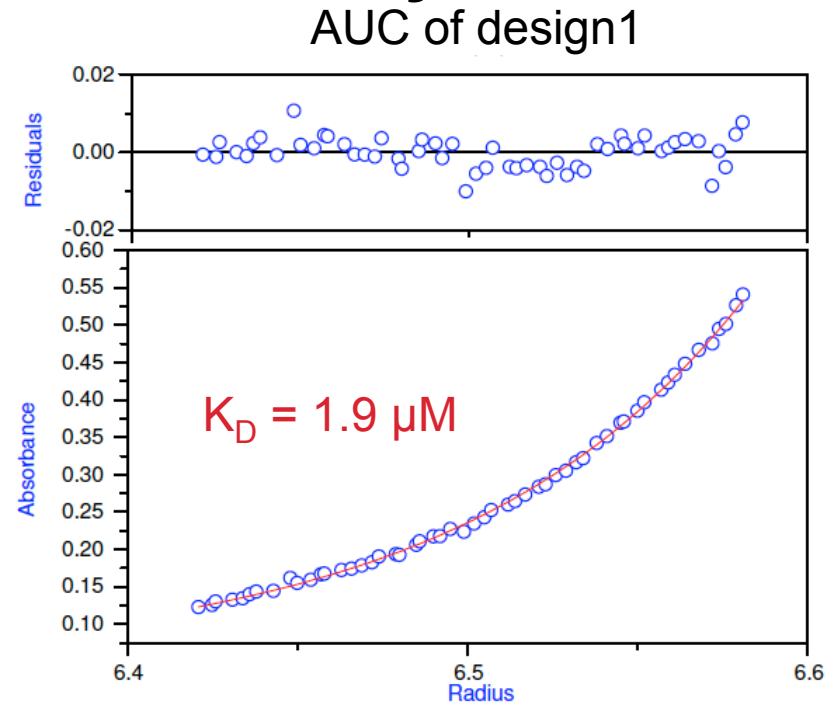
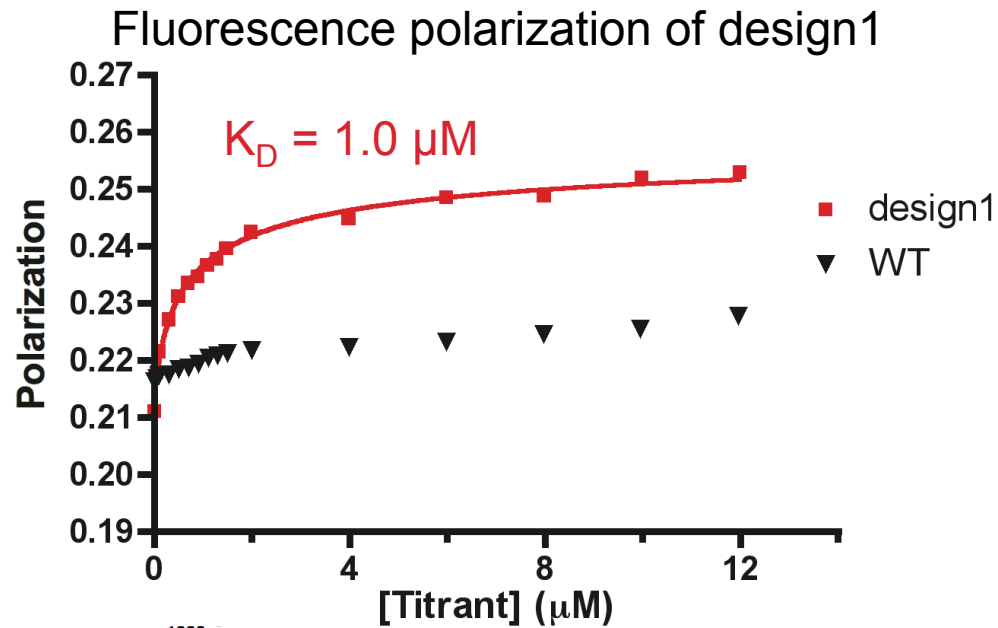


Size exclusion chromatography /
Multi-angle light scattering

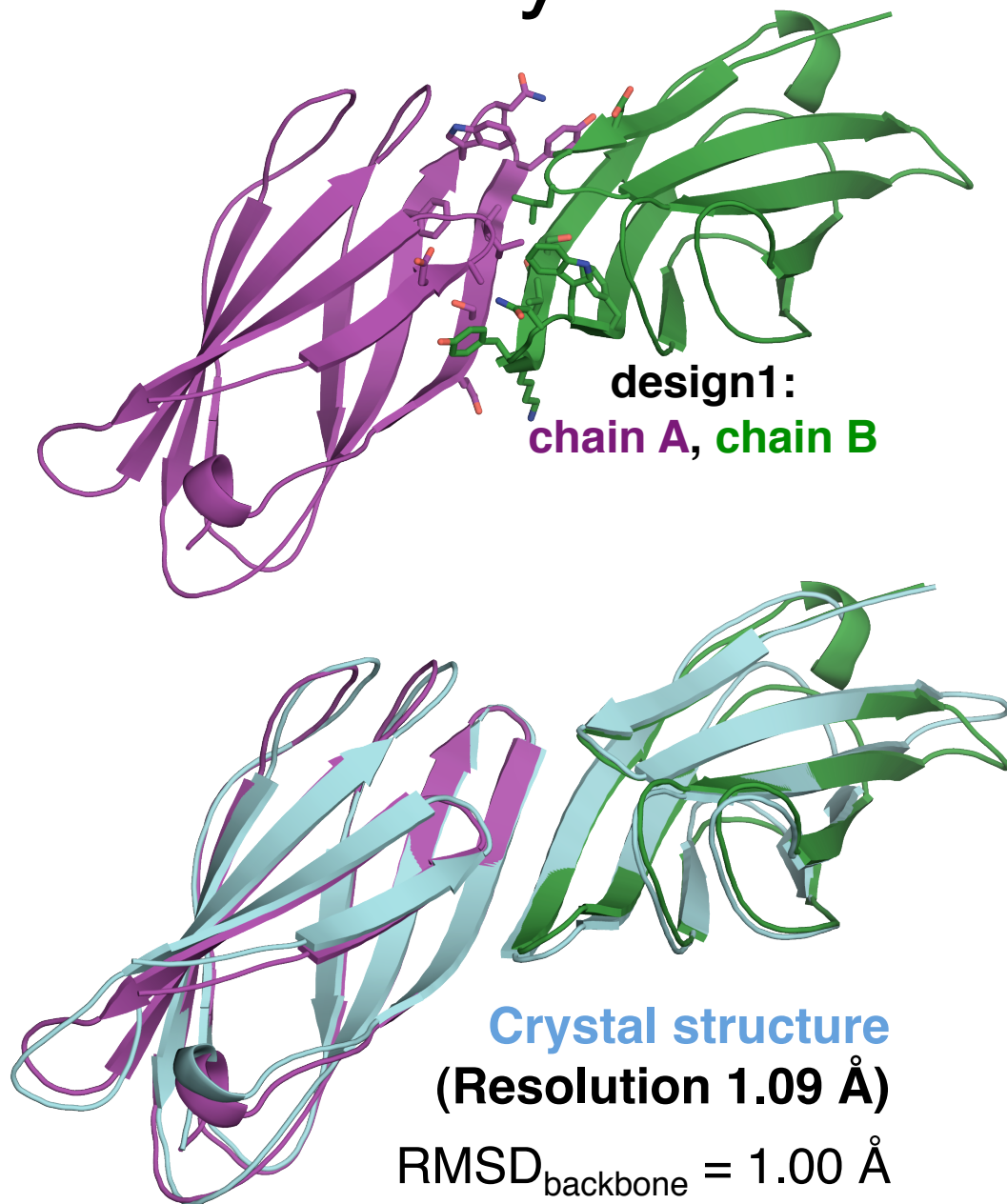


Protein	Monomer MW (kDa)	Size Exclusion MW (kDa)
wild type	13.4	11
design1	13.4	26
design2	13.5	21
design3	13.5	10
design4	13.4	14

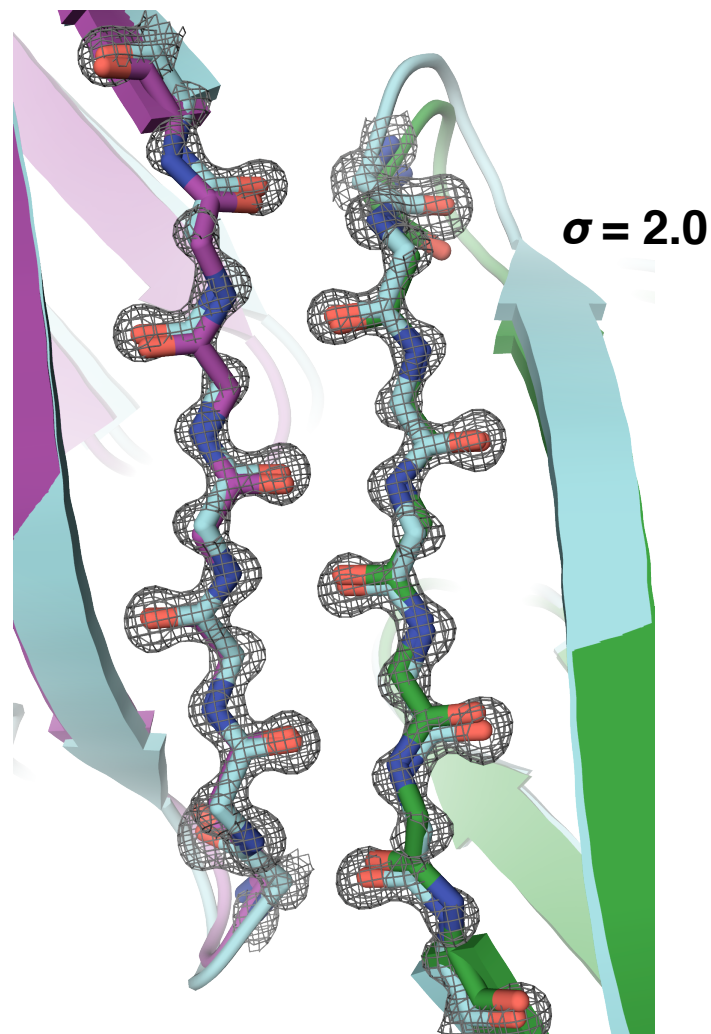
design1 stability and self affinity



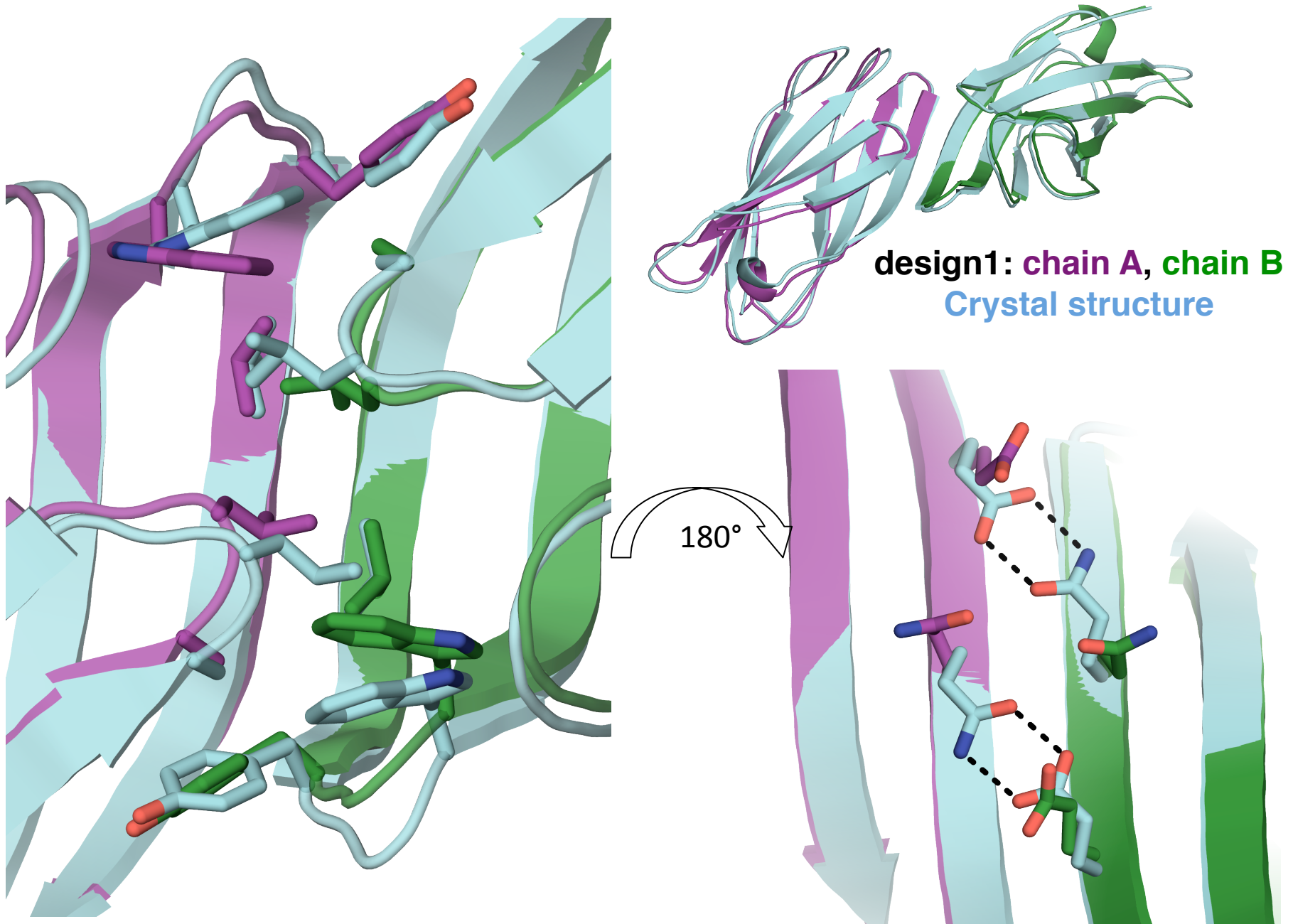
Model to crystal structure comparison



Designed β -strand
pairing is preserved

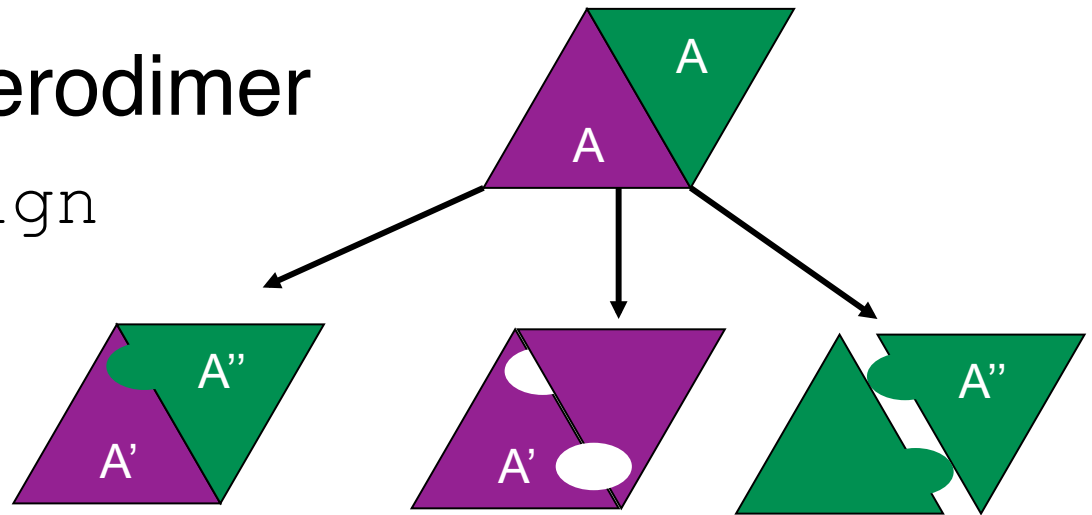
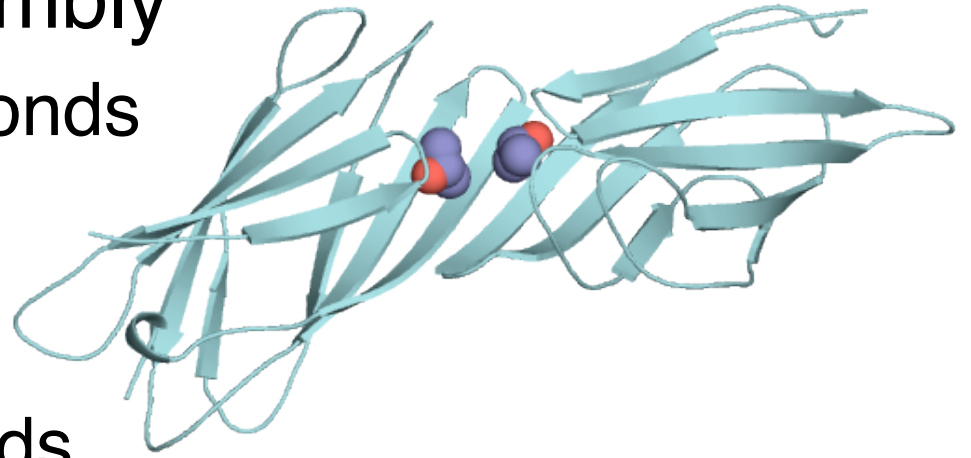


A closer look at the interface



What we learned & looking forward

- Fine control over assembly
 - Can get packing + H-bonds
 - Create new scaffolds
- Pitfalls
 - Hard to find open strands
 - Aggregation
- Homodimer to heterodimer
 - `MultiStateDesign`



Acknowledgements

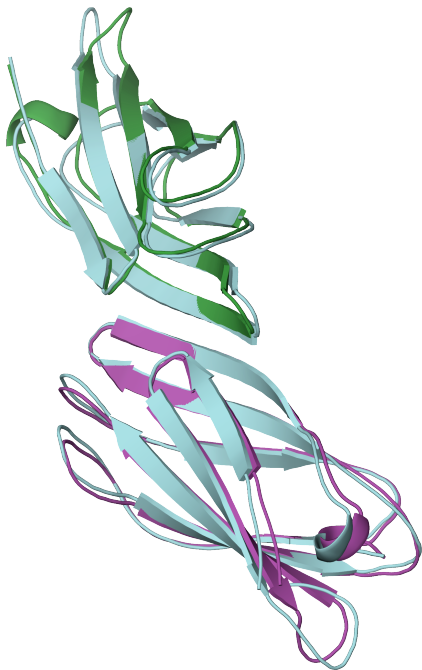
- Kuhlman Lab
 - Brian Kuhlman
 - Bryan Der
 - Ron Jacak
 - Andrew Leaver-Fay
 - Steven Lewis

- UNC folks
 - Ashutosh Tripathy
 - Mischa Machius
 - Mike Miley
- Rosetta Community
 - Sarel Fleishman
 - Ingemar André



What we learned

- β -strand pairing helps orient interface
- Can design hydrophobic packing well
- Problems remain designing H-bonds



Protein	Design character	Forms homodimer?	Structure known?
wild type	none	nope	yes
design1	Hydrophobic packing	YES!	YES!
design2	Hydrophobic packing	Probably!	not enough protein
design3	Hydrogen bonds abound	nope	nah, boring
design4	Hydrogen bonds everywhere	nope	nope

Why does WT not homodimerize?

