



# THE HÖCKER LAB

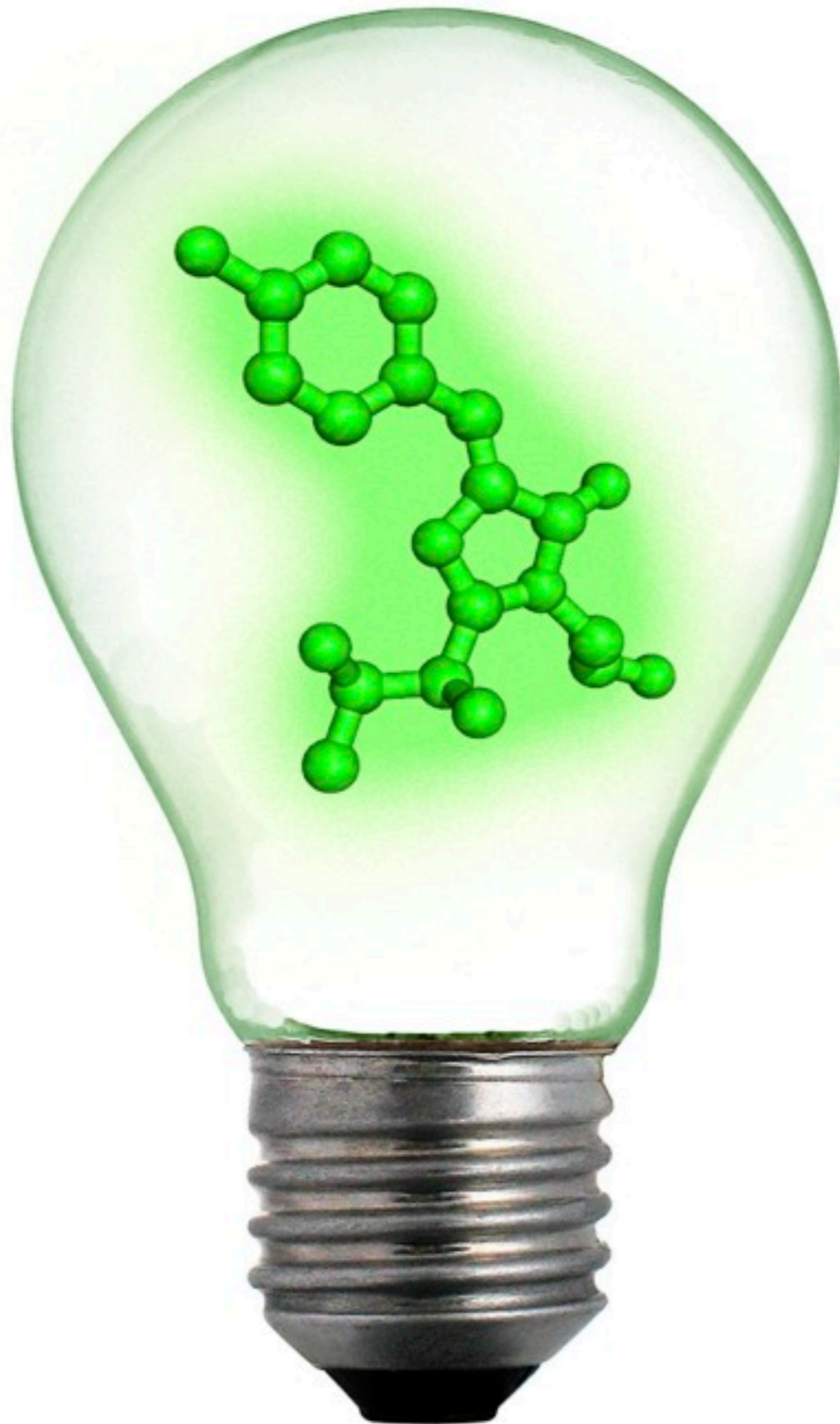


*Greetings from  
Tübingen*



MPI Developmental  
Biology





# Computational design of novel fluorescent proteins - the loooooong way towards the light

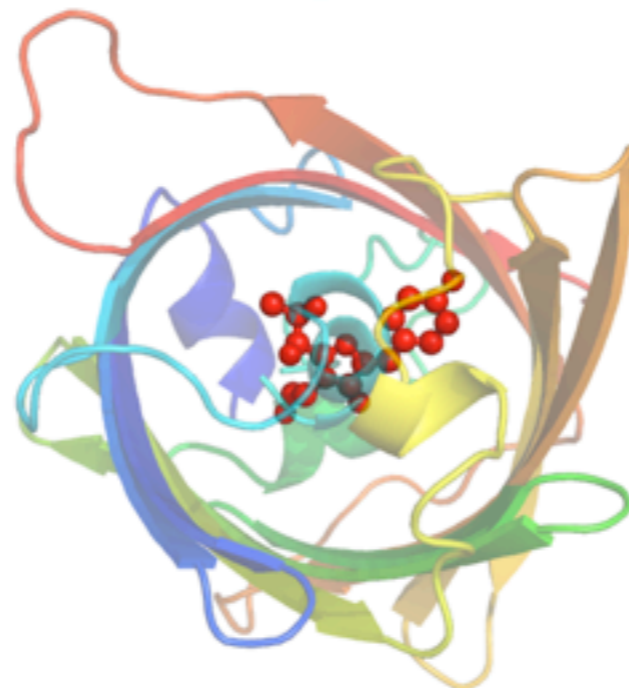
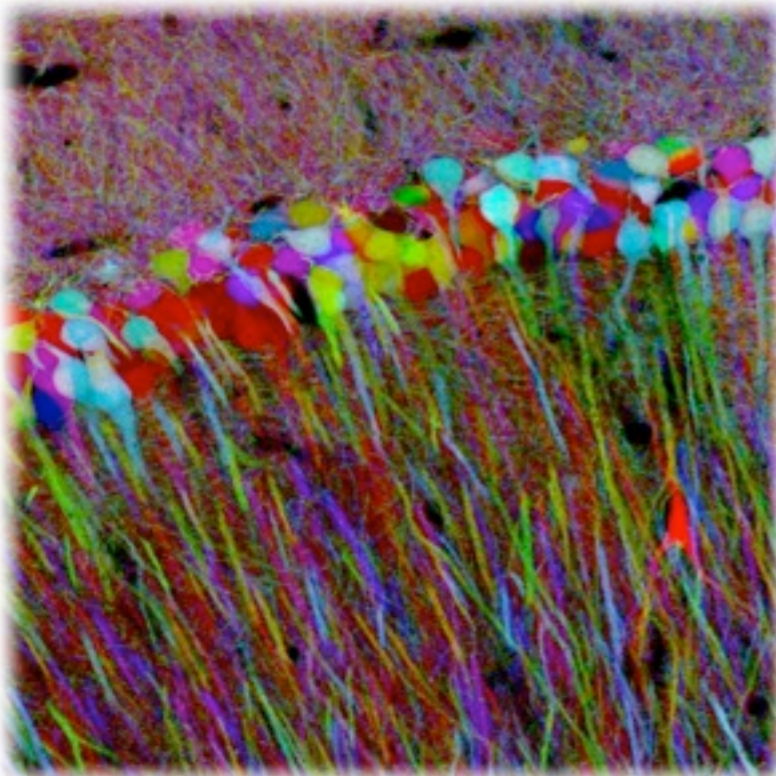
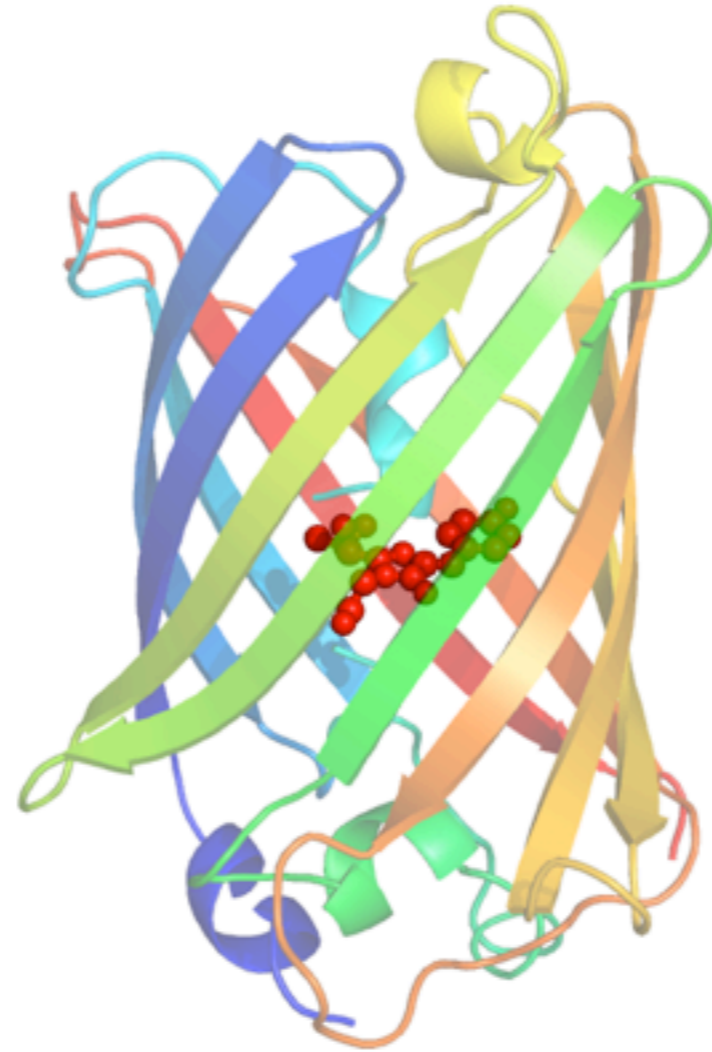
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RosettaCon2012 - Seattle, July 30th 2012

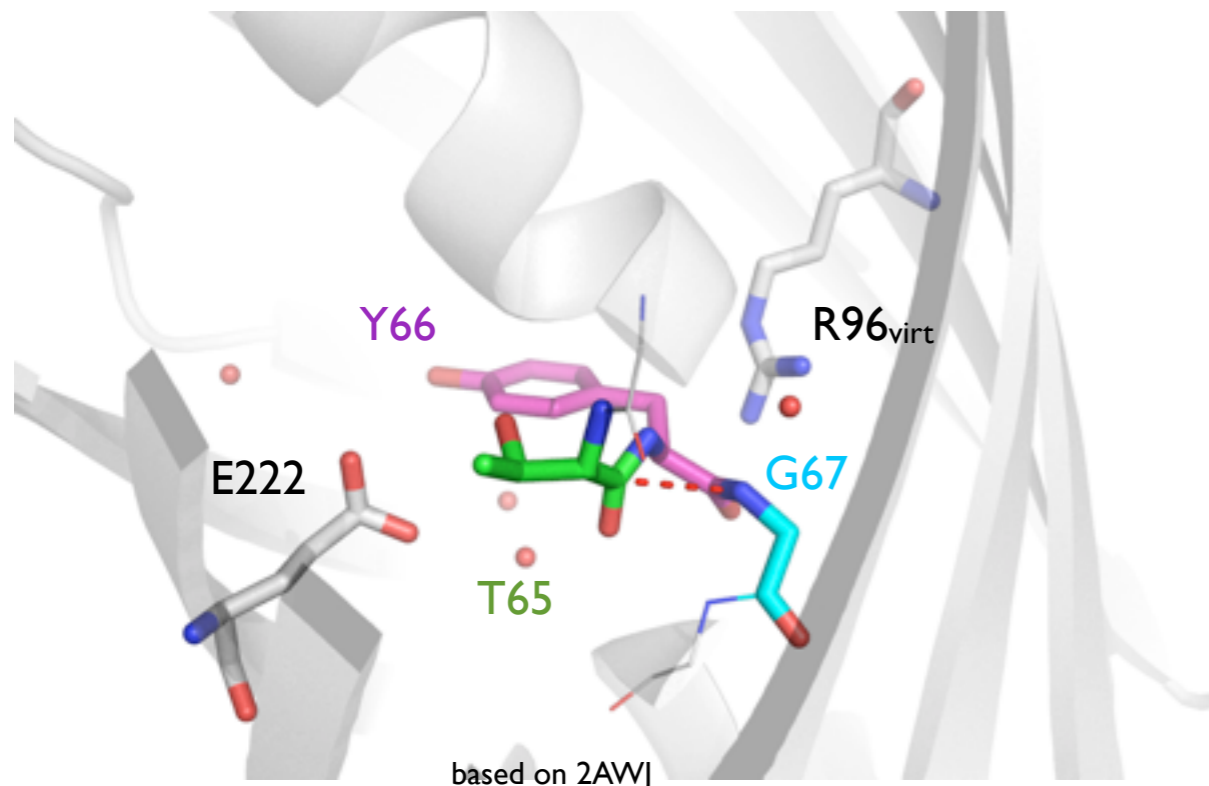
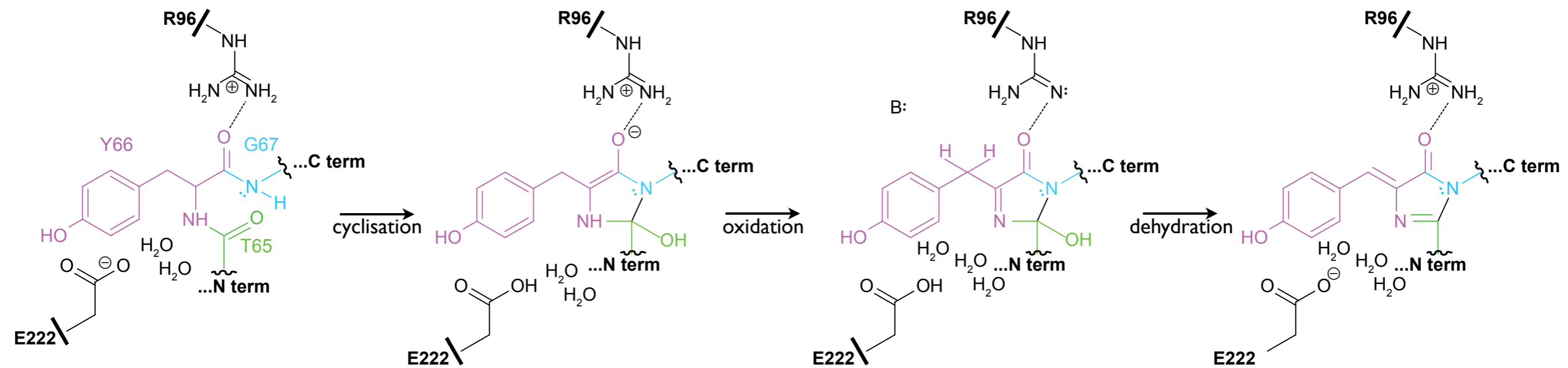
André C. Stiel - workgroup protein design,  
Max-Planck Institute for developmental biology, Tübingen, Germany



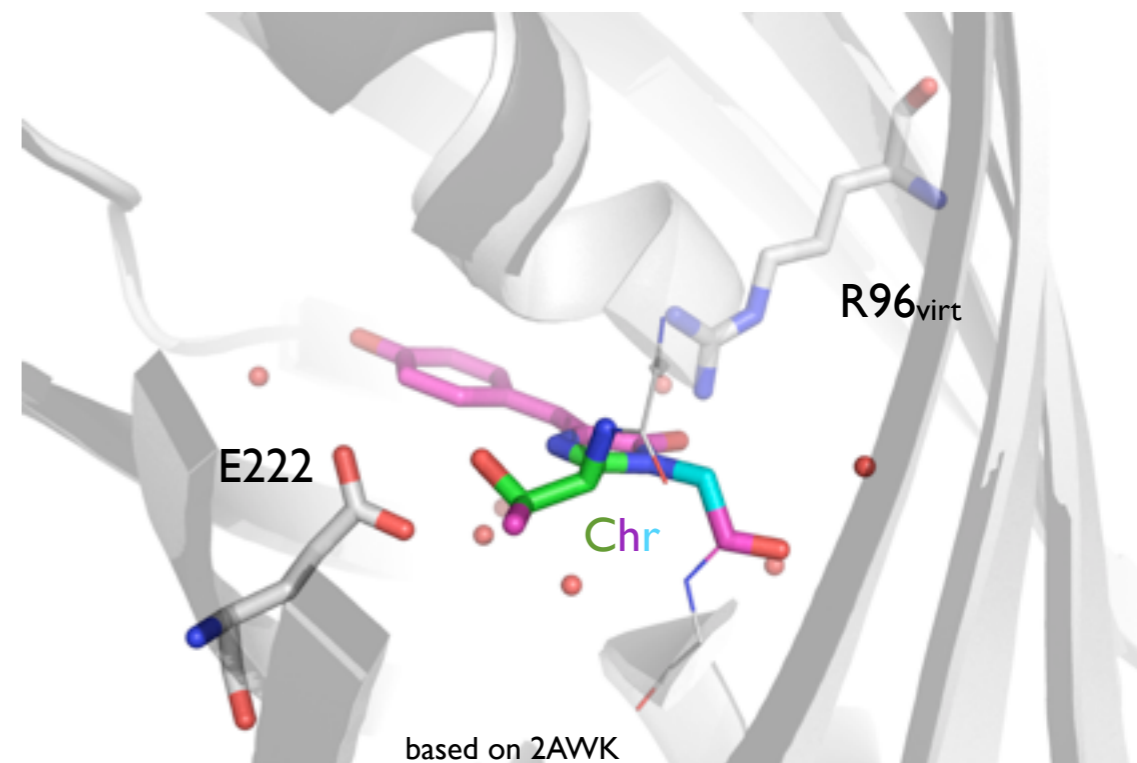
# green fluorescent proteins and friends - a most versatile class of proteins



# at the bottom of the marvel: autocatalytic chromophore formation

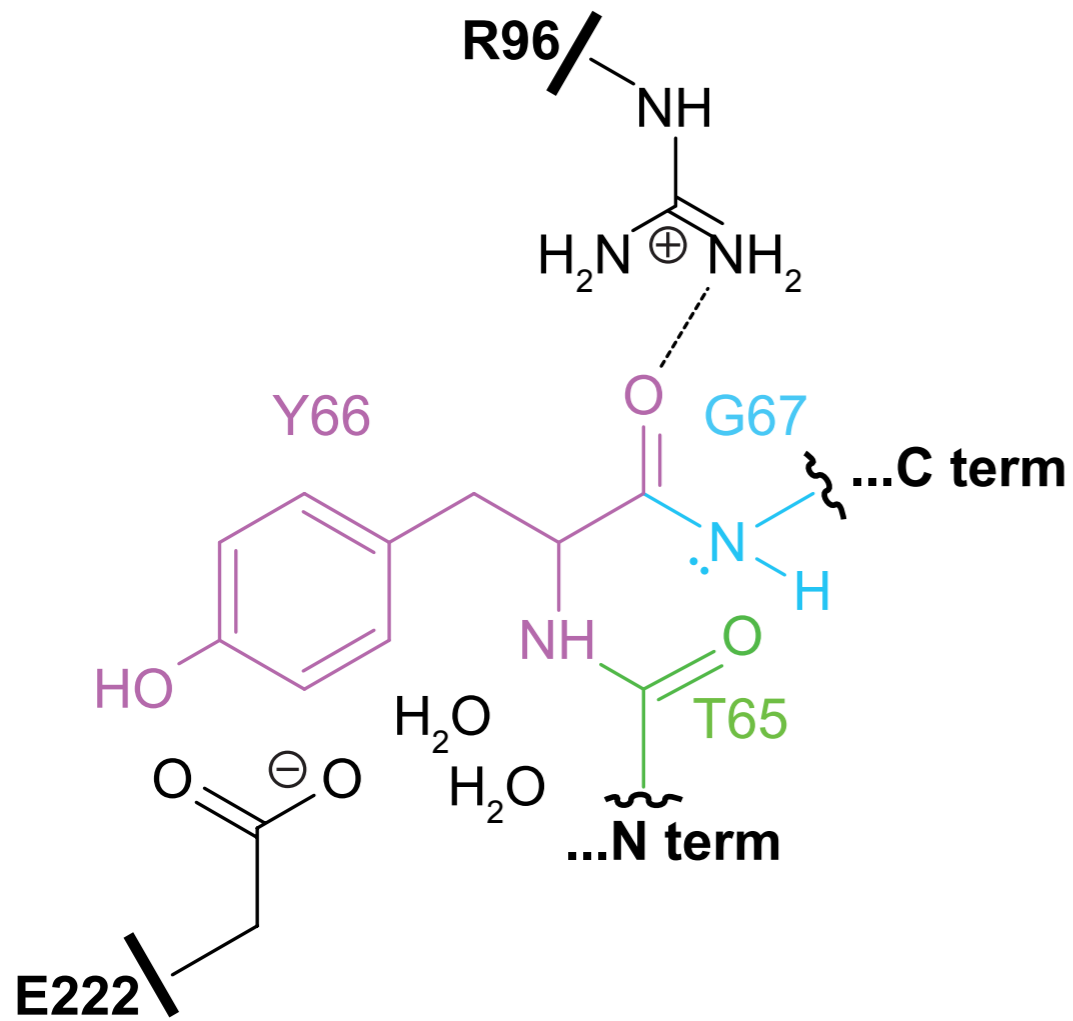


$\xrightarrow{\text{cyclisation}}$   
 $\xrightarrow{\text{oxidation}}$   
 $\xrightarrow{\text{dehydration}}$

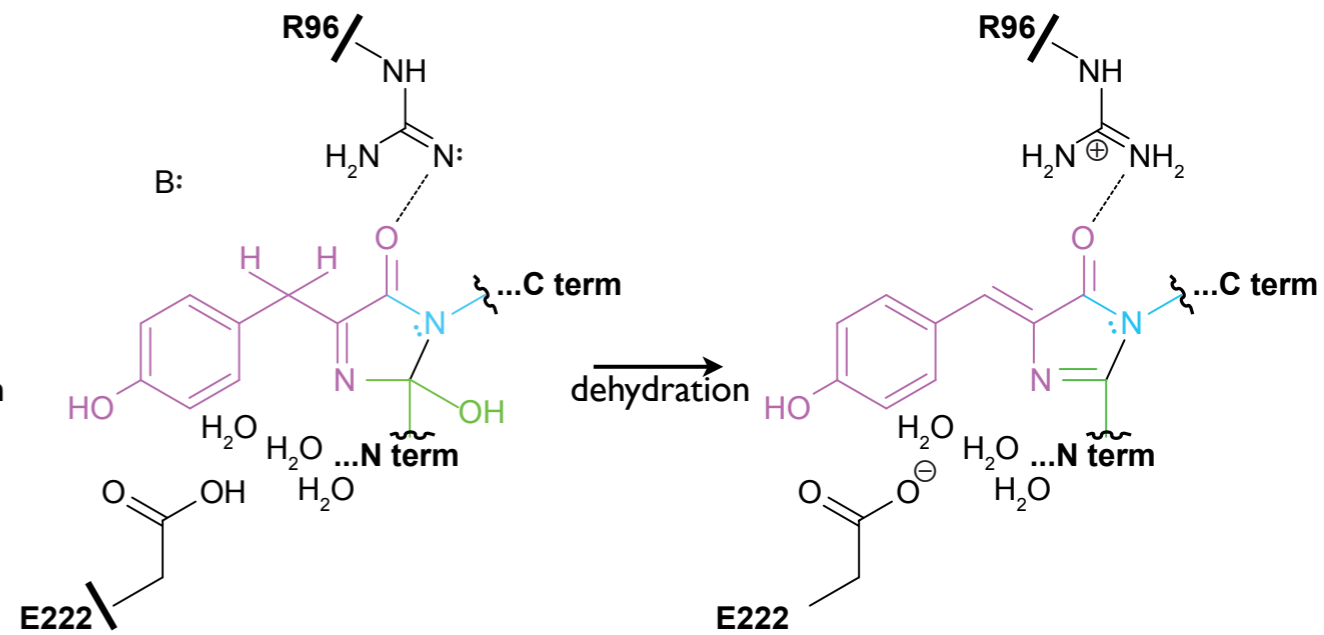




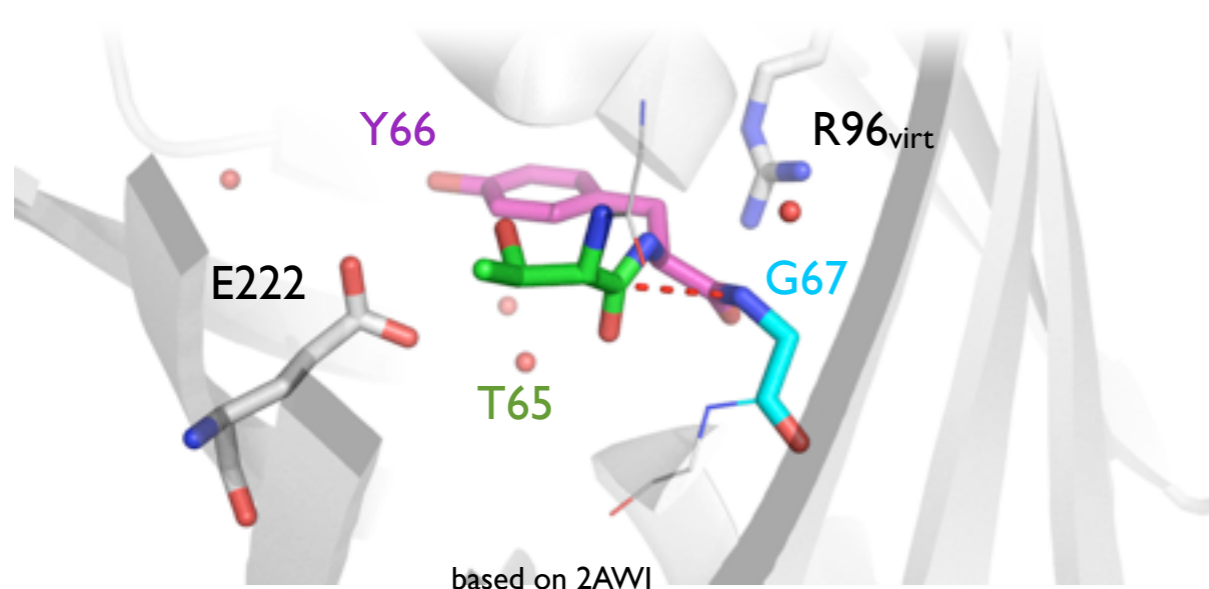
# at the bottom of the marvel: autocatalytic chromophore formation



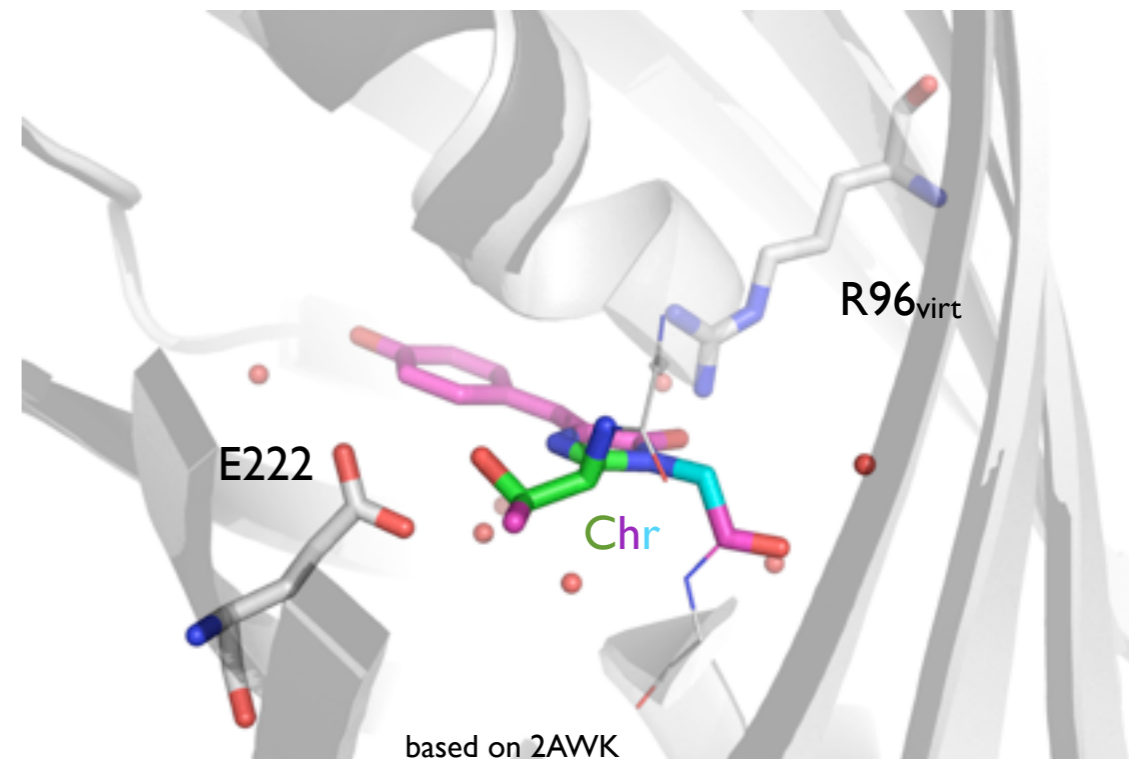
...C term  
oxidation



dehydration



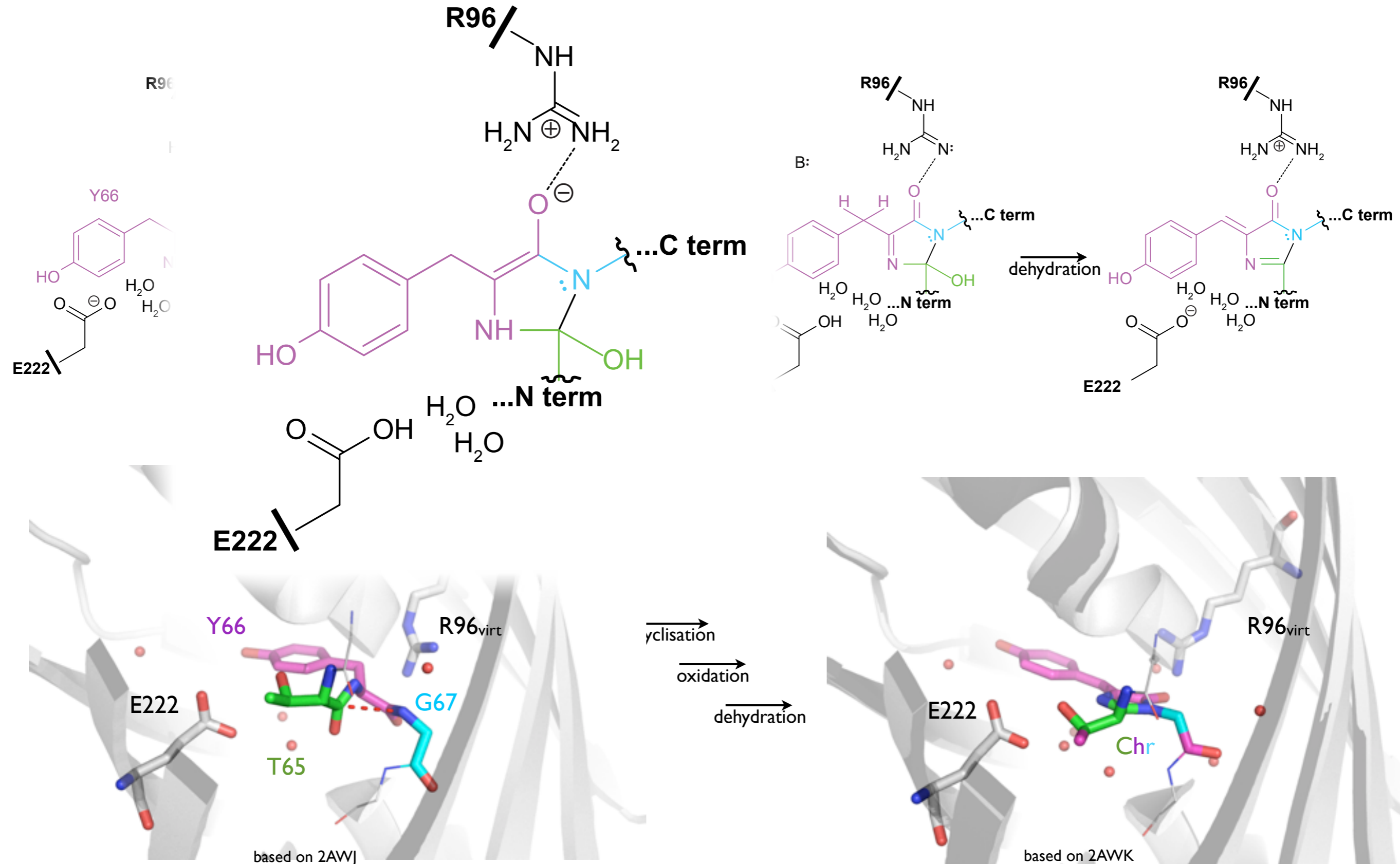
yclisation  
oxidation  
dehydration



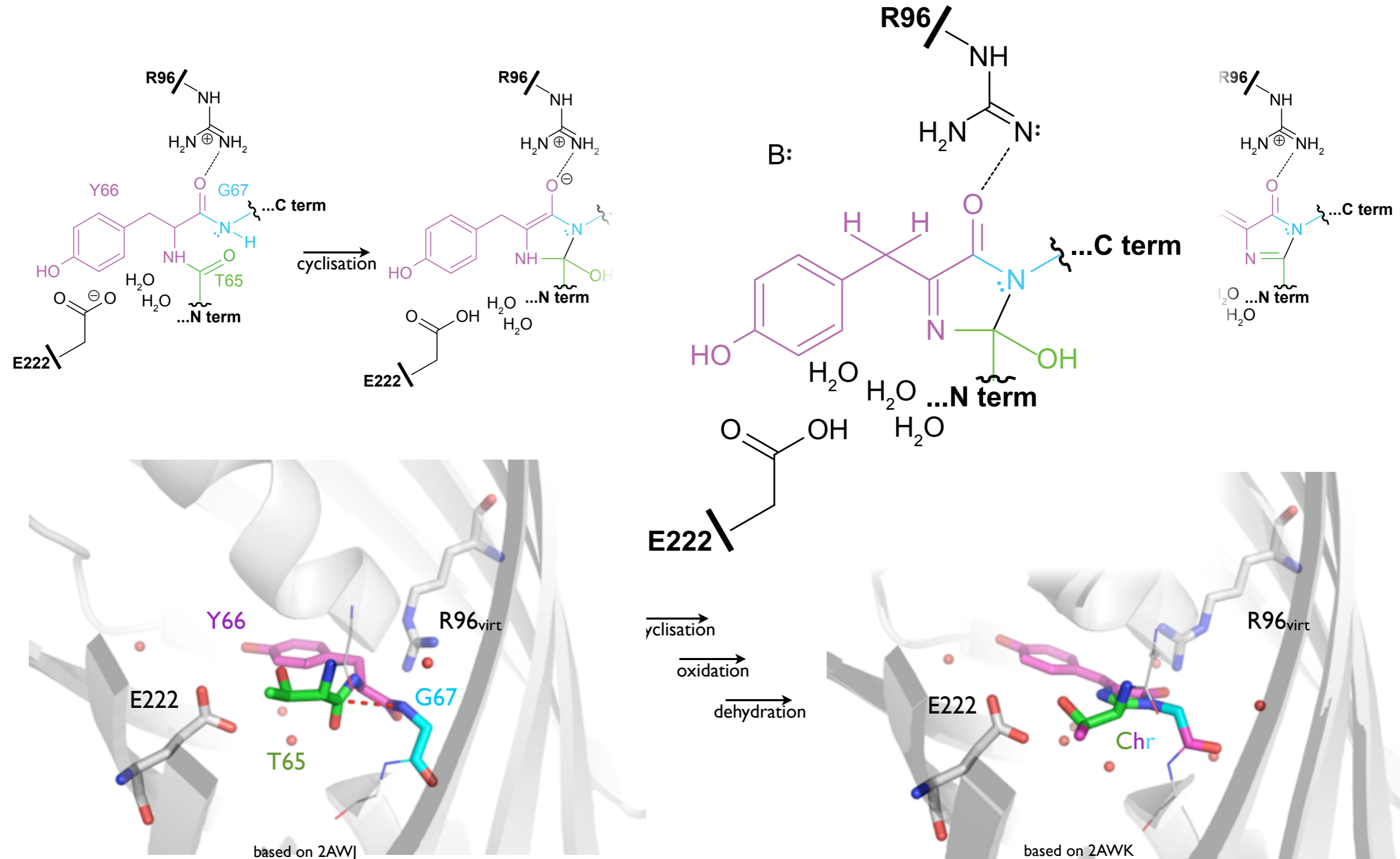
based on 2AWJ

based on 2AWK

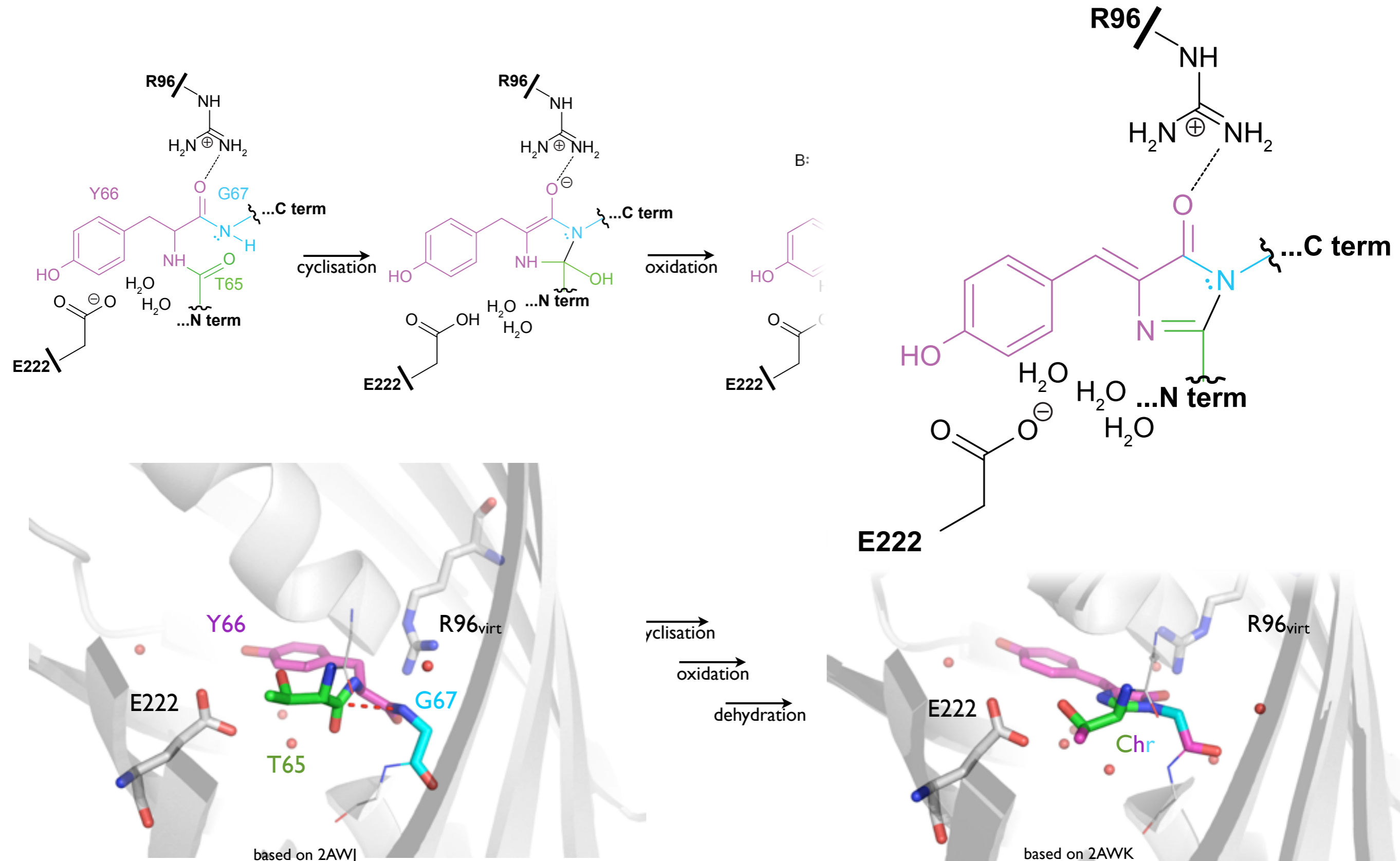
# at the bottom of the marvel: autocatalytic chromophore formation



# at the bottom of the marvel: autocatalytic chromophore formation

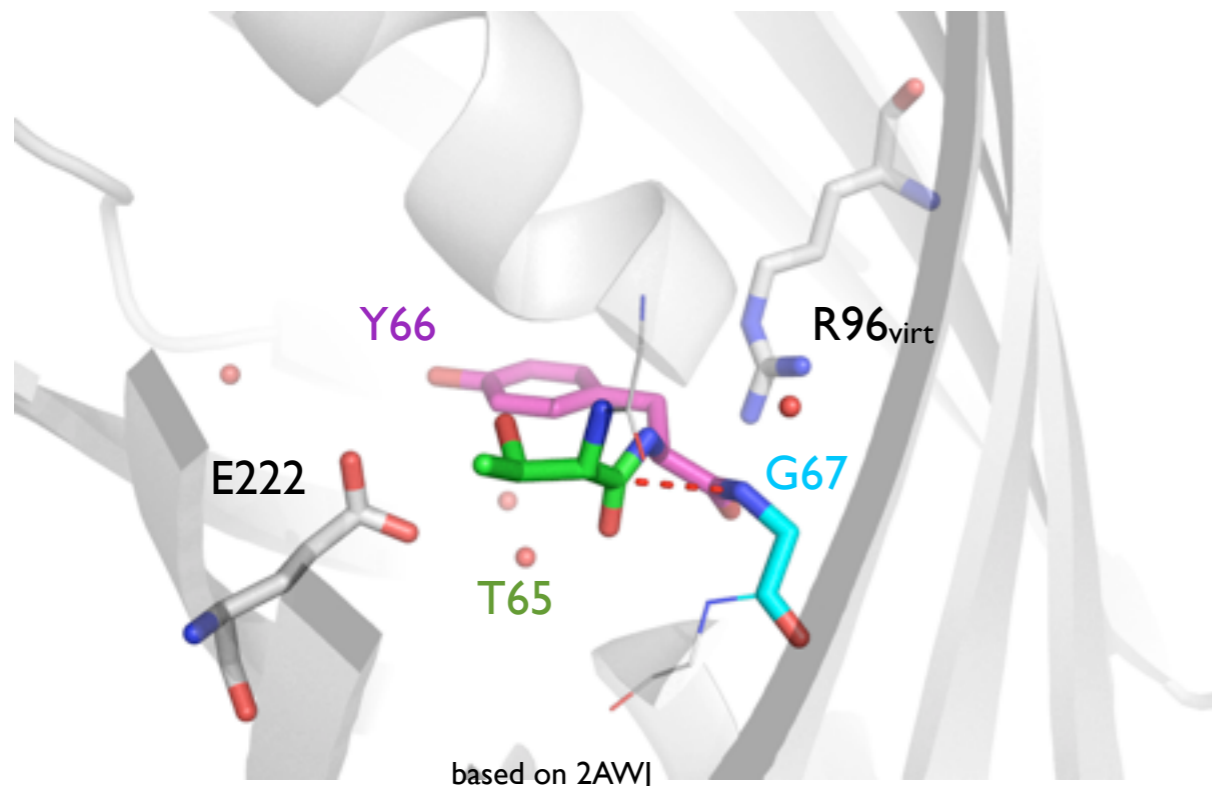
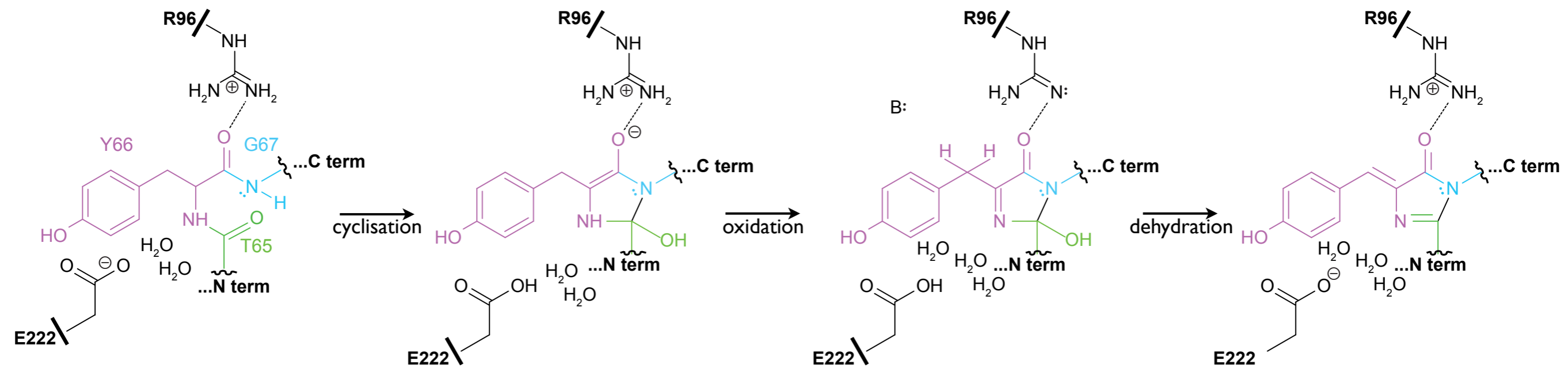


# at the bottom of the marvel: autocatalytic chromophore formation

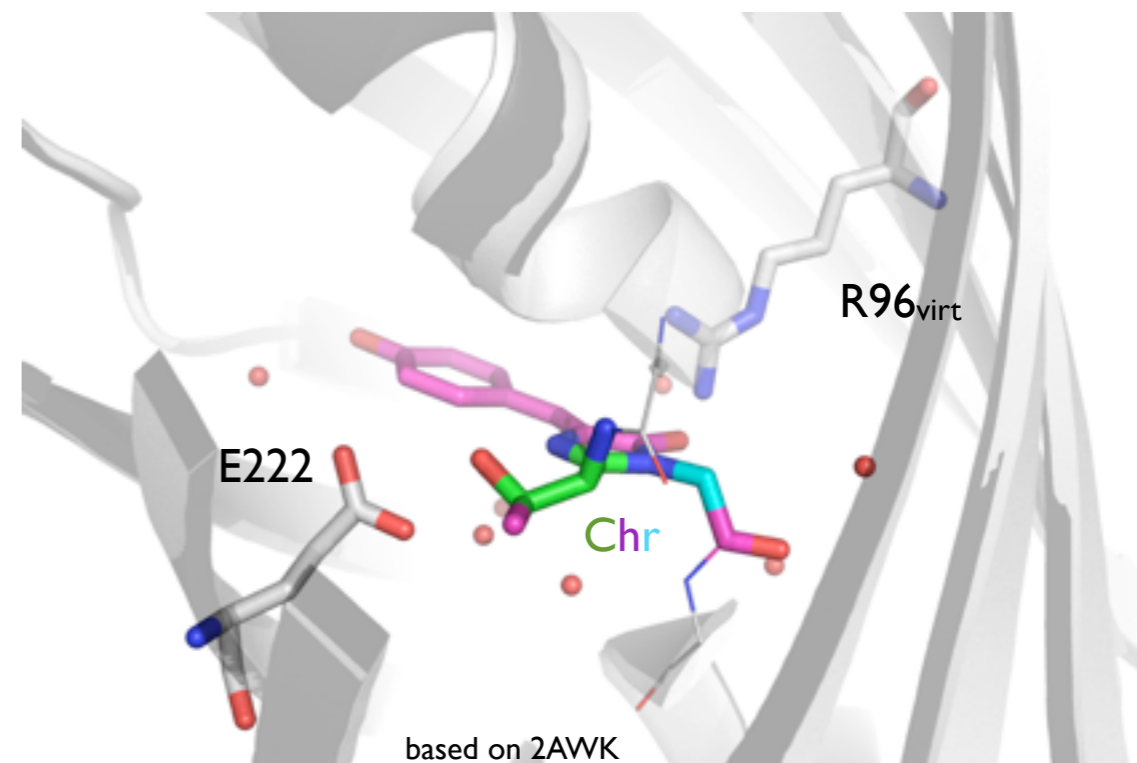




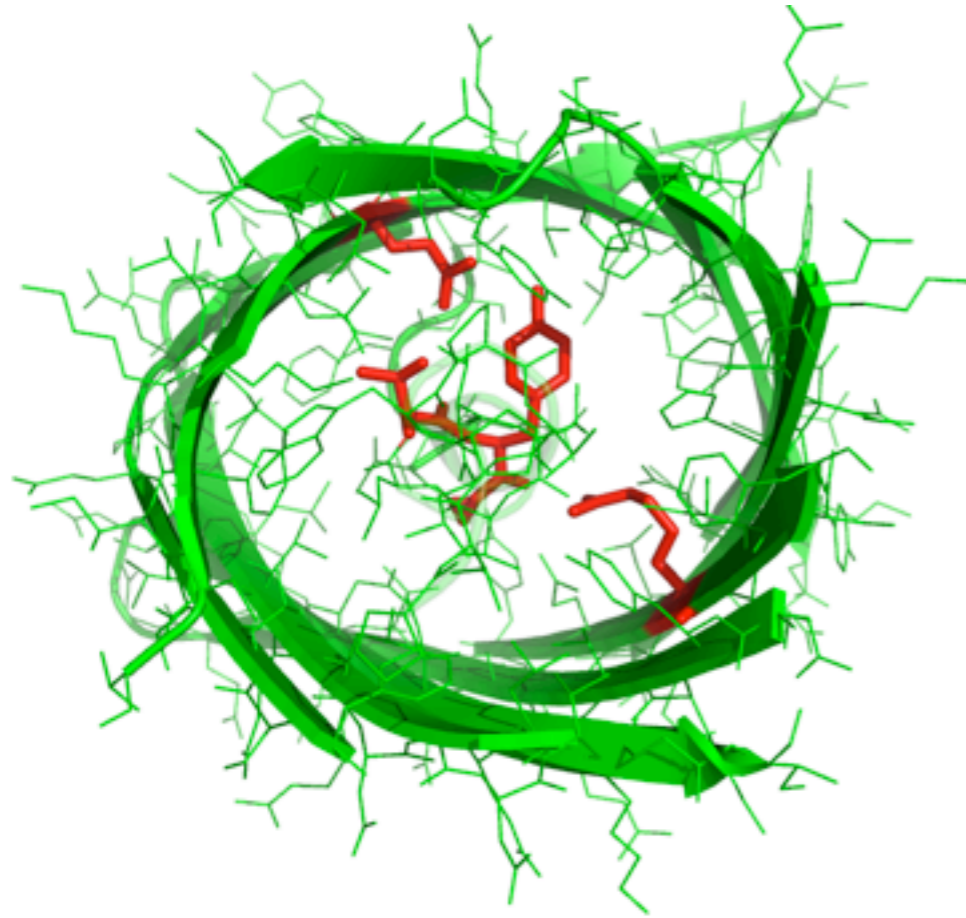
# at the bottom of the marvel: autocatalytic chromophore formation



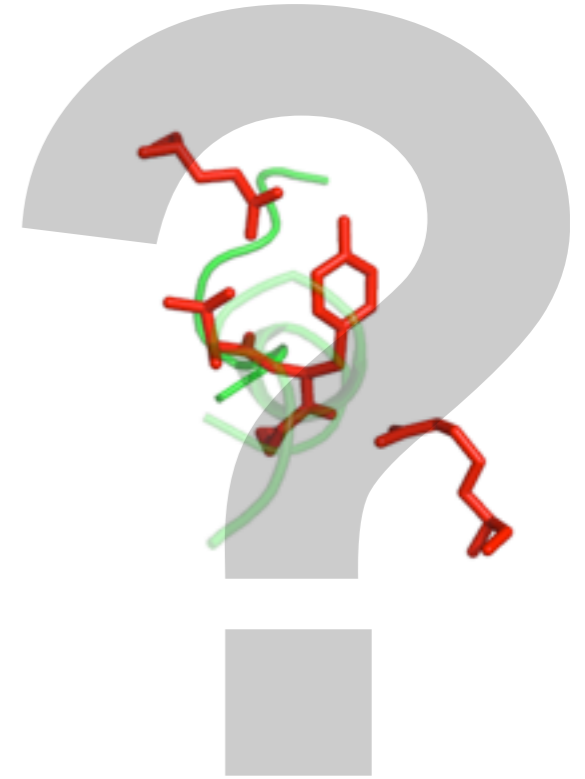
$\xrightarrow{\text{cyclisation}}$   
 $\xrightarrow{\text{oxidation}}$   
 $\xrightarrow{\text{dehydration}}$



# what if: **transfer of the autocatalysis** **to a non FP-fold**

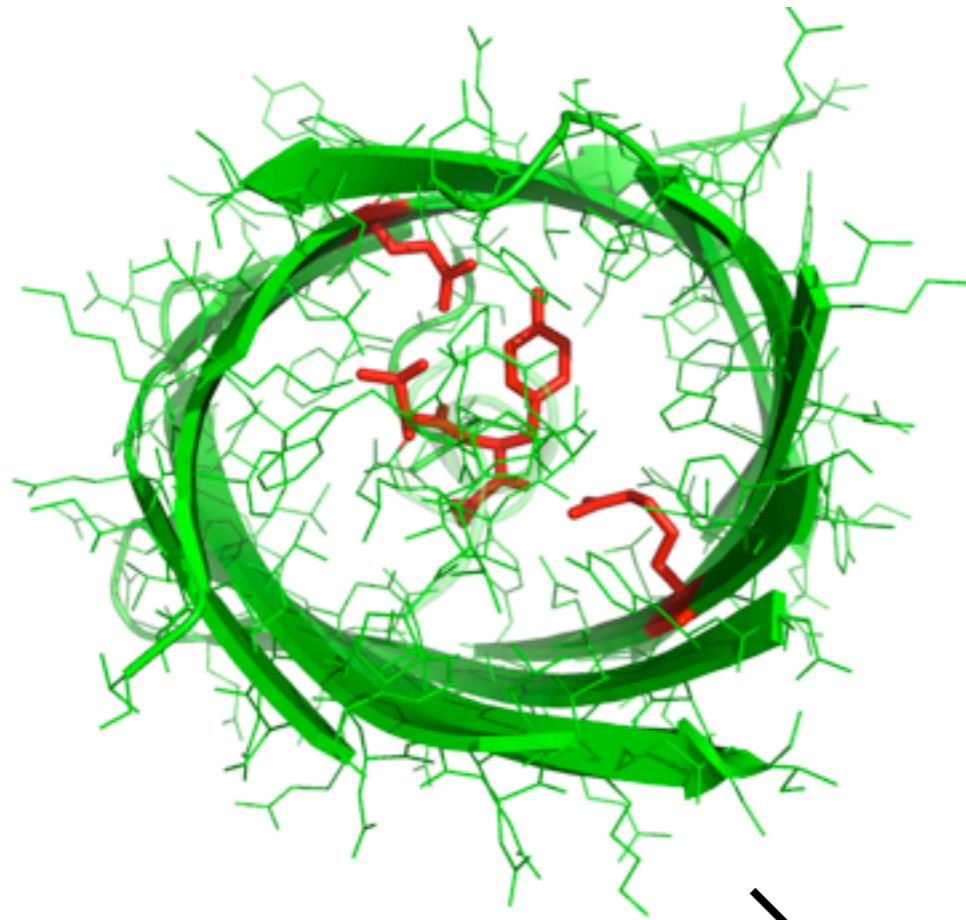


1. scaffold selection
2. design / packing





# what if: **transfer of the autocatalysis** **to a non FP-fold**

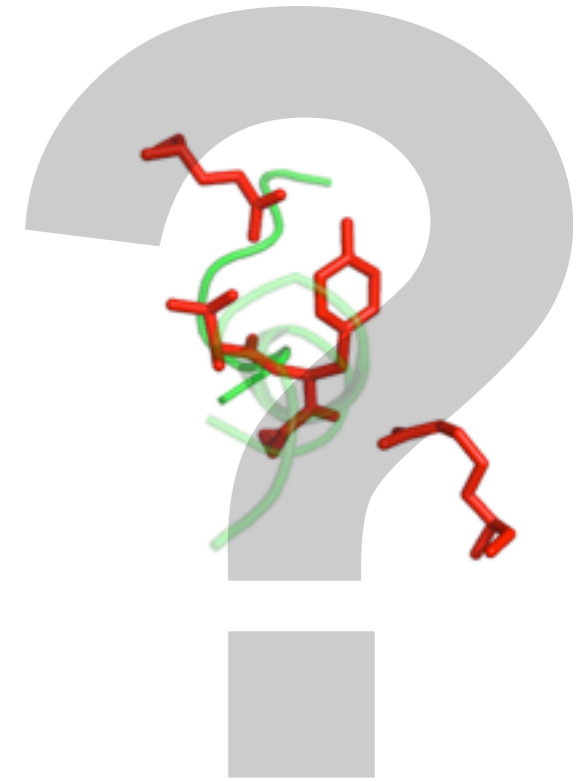


I. redesign  
**STAGE I**



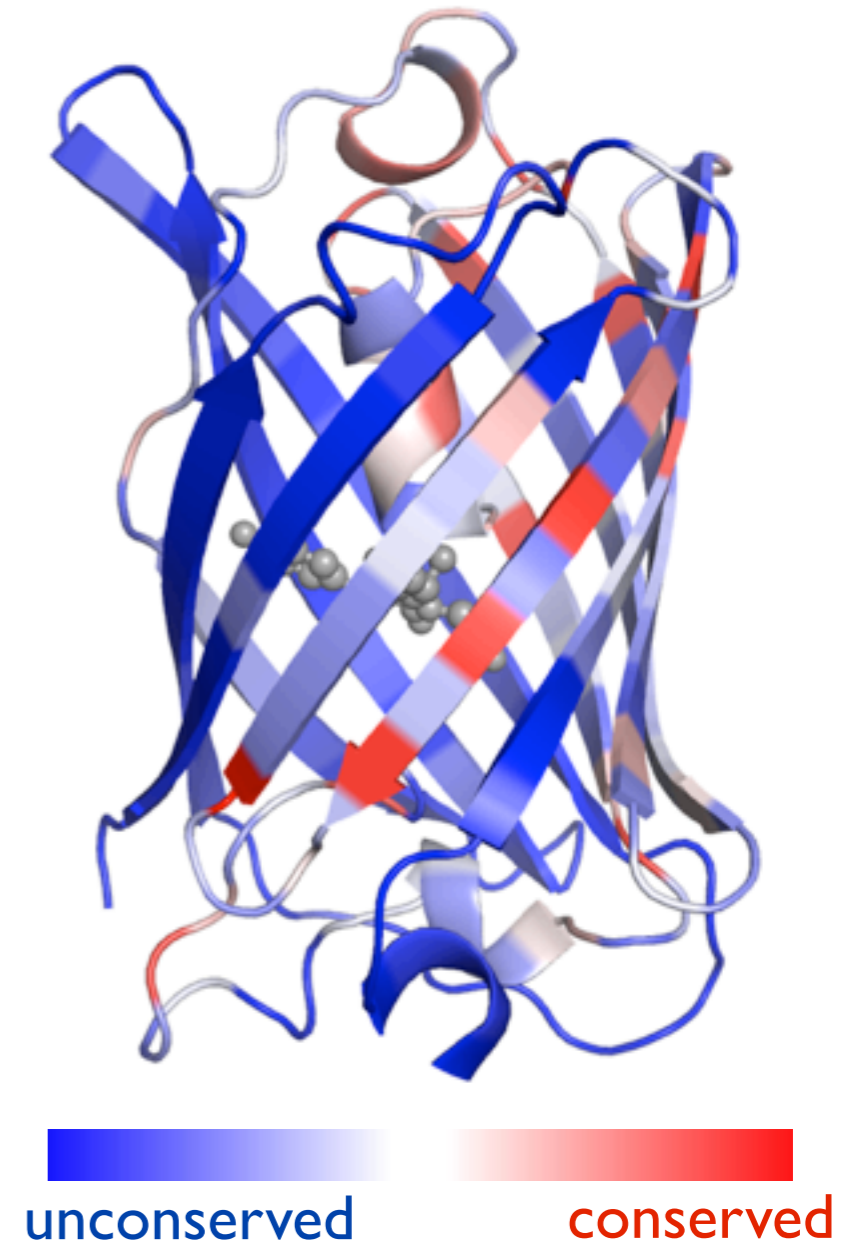
1. scaffold selection  
2. design / packing

**STAGE II**



# why it should work ;-)

- relatively low sequence conservation
- tons of variants - even compensation for key residues
- ~ 400 structures of different FPs and variants
- in total: it seems to be a very robust system





# stage I: **general approach**

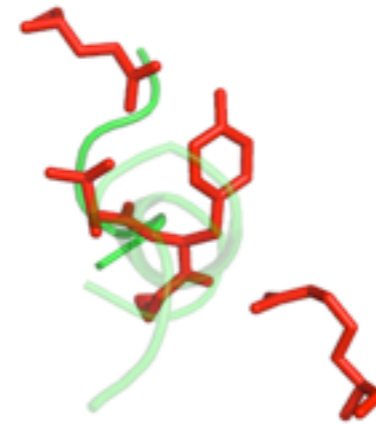
motif / template



redesign



selection ranking



# stage I: general approach

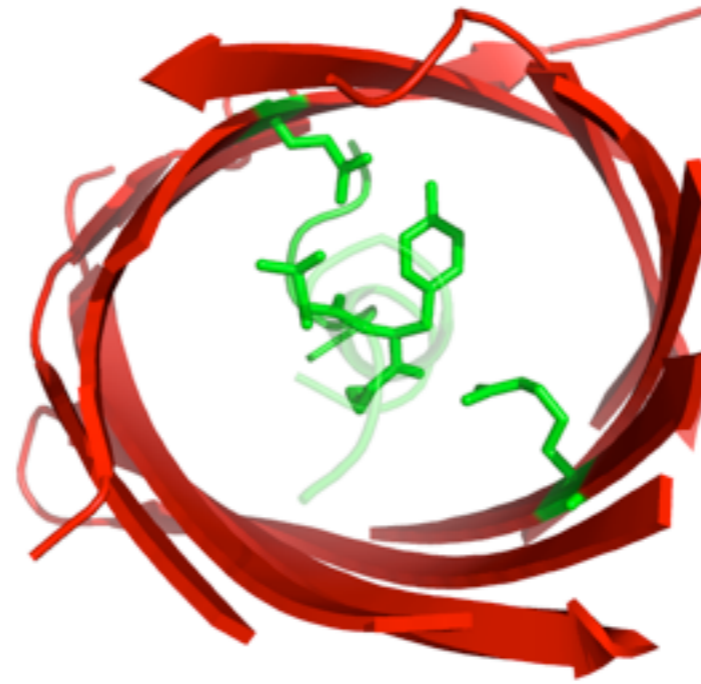
motif / **template**



redesign



selection ranking





# stage I: general approach

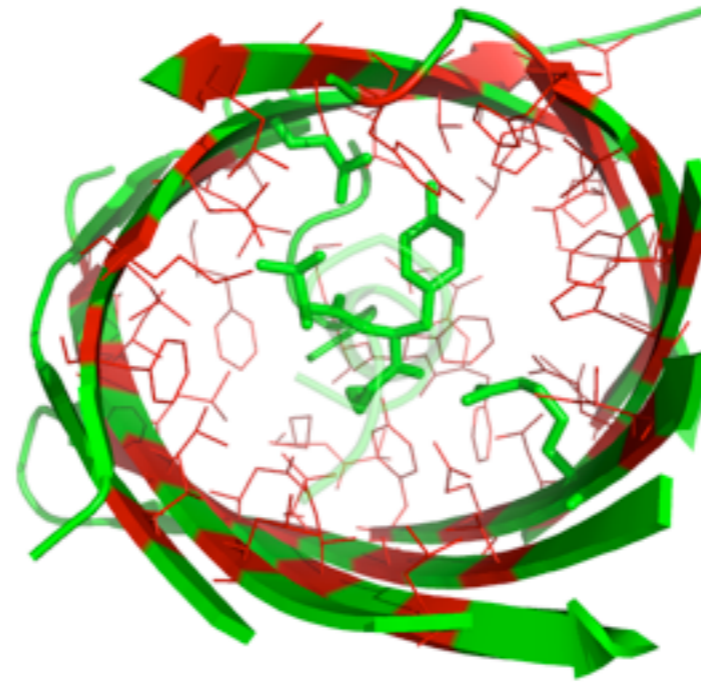
motif / template



redesign



selection ranking



# stage I: general approach

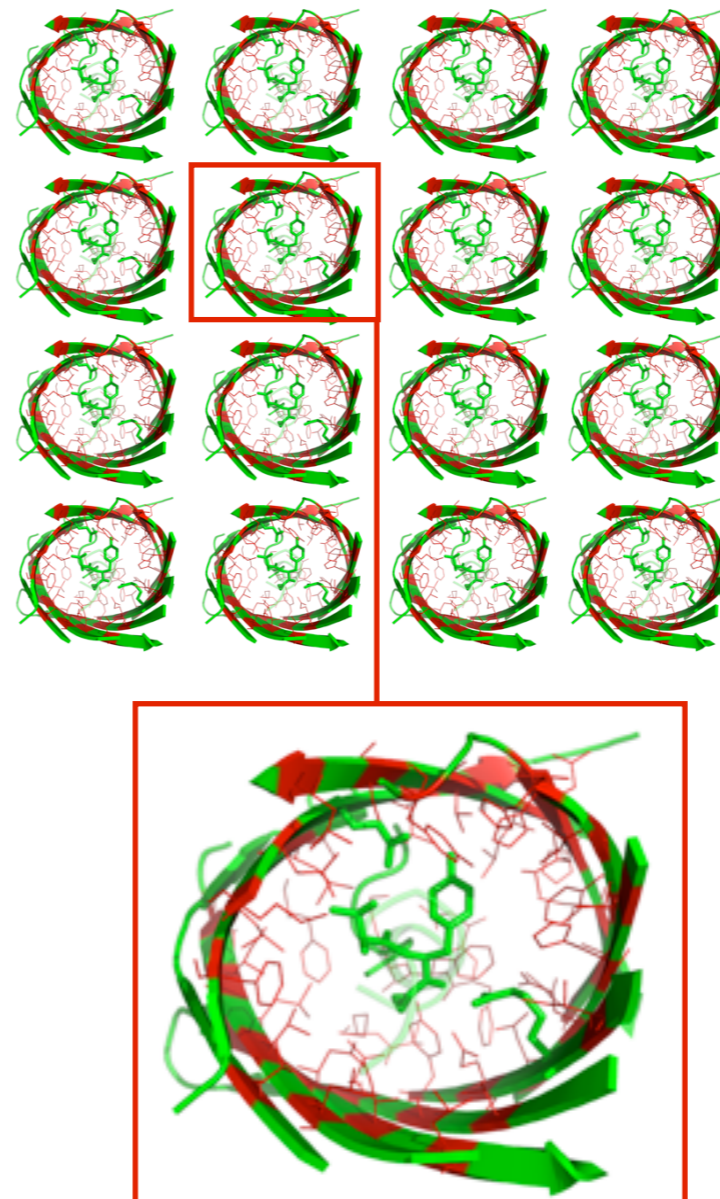
motif / template



redesign



selection ranking



motif / template



redesign



selection ranking

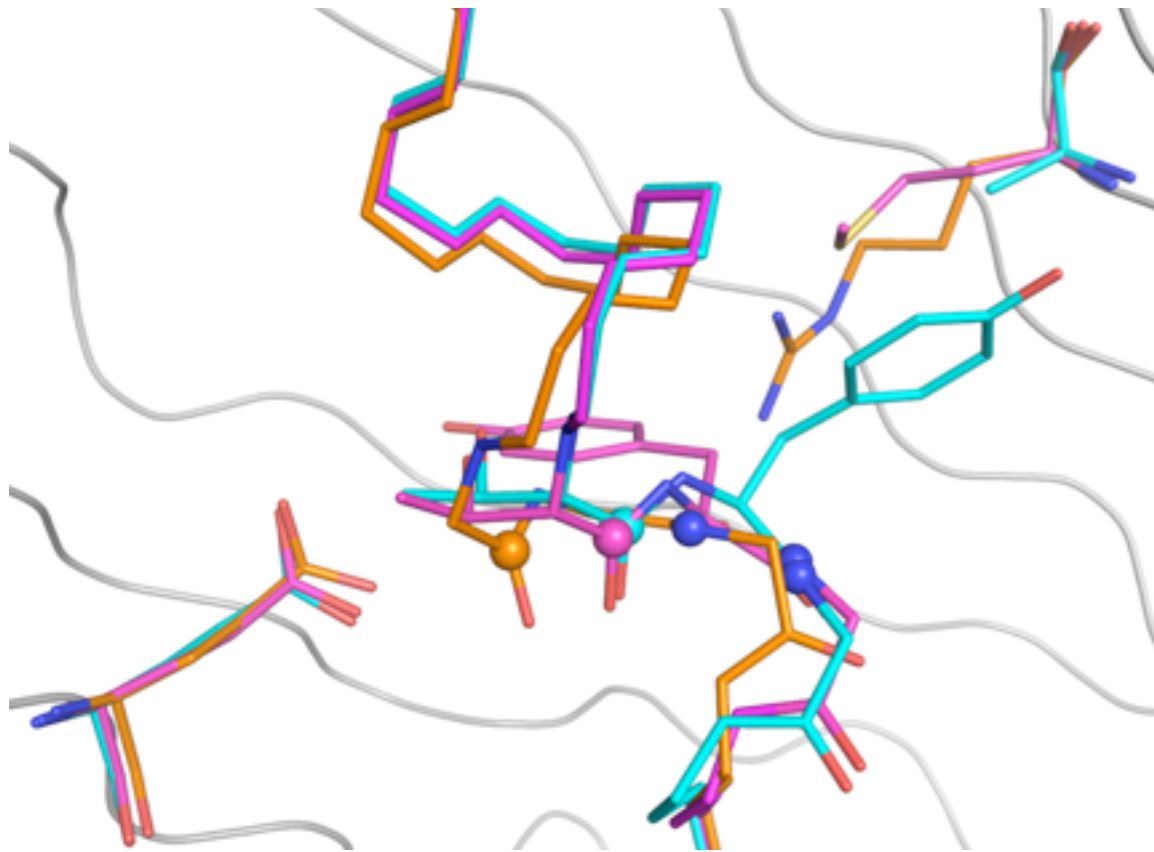
**motif**



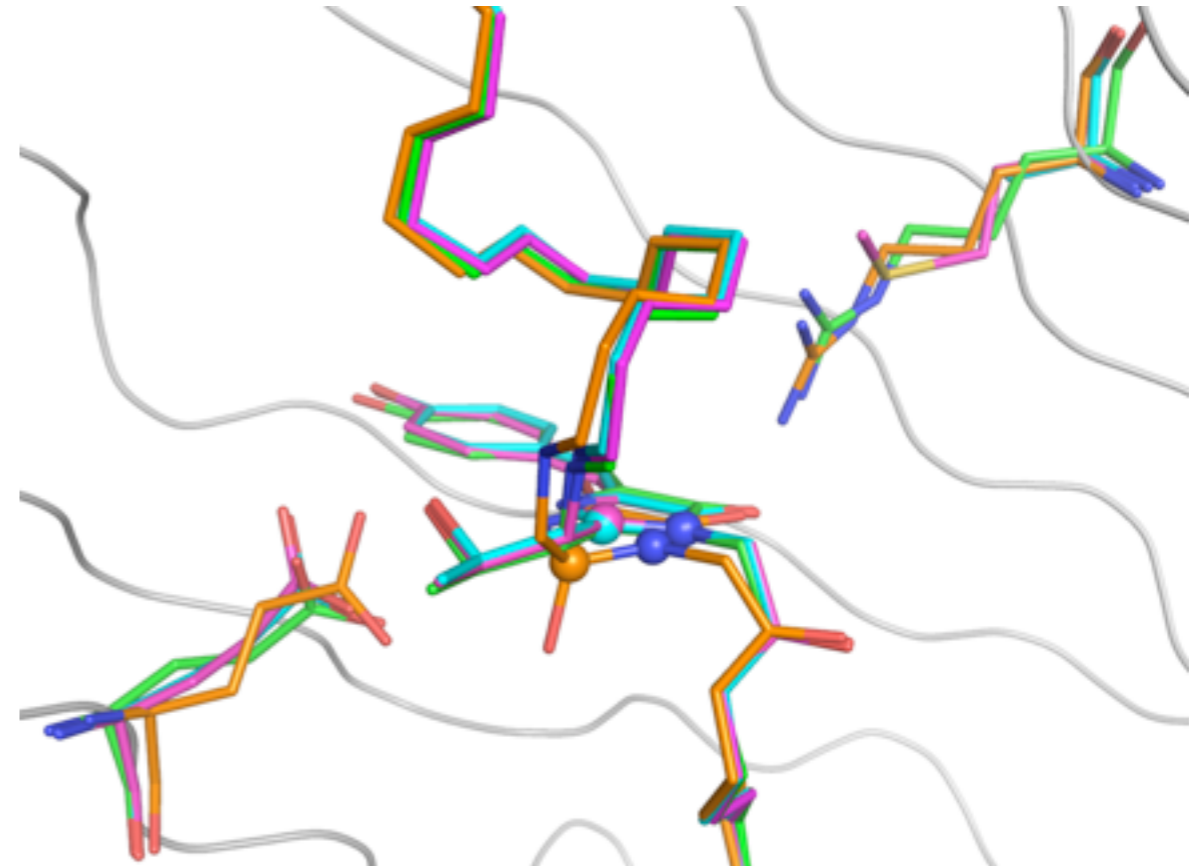


# precyclization structures - structural insight into the state before chromophore formation

## precyclized



## chromophore formed



2AWJ / 2AWK - R96M mutation - Wood, Biochemistry, 2003

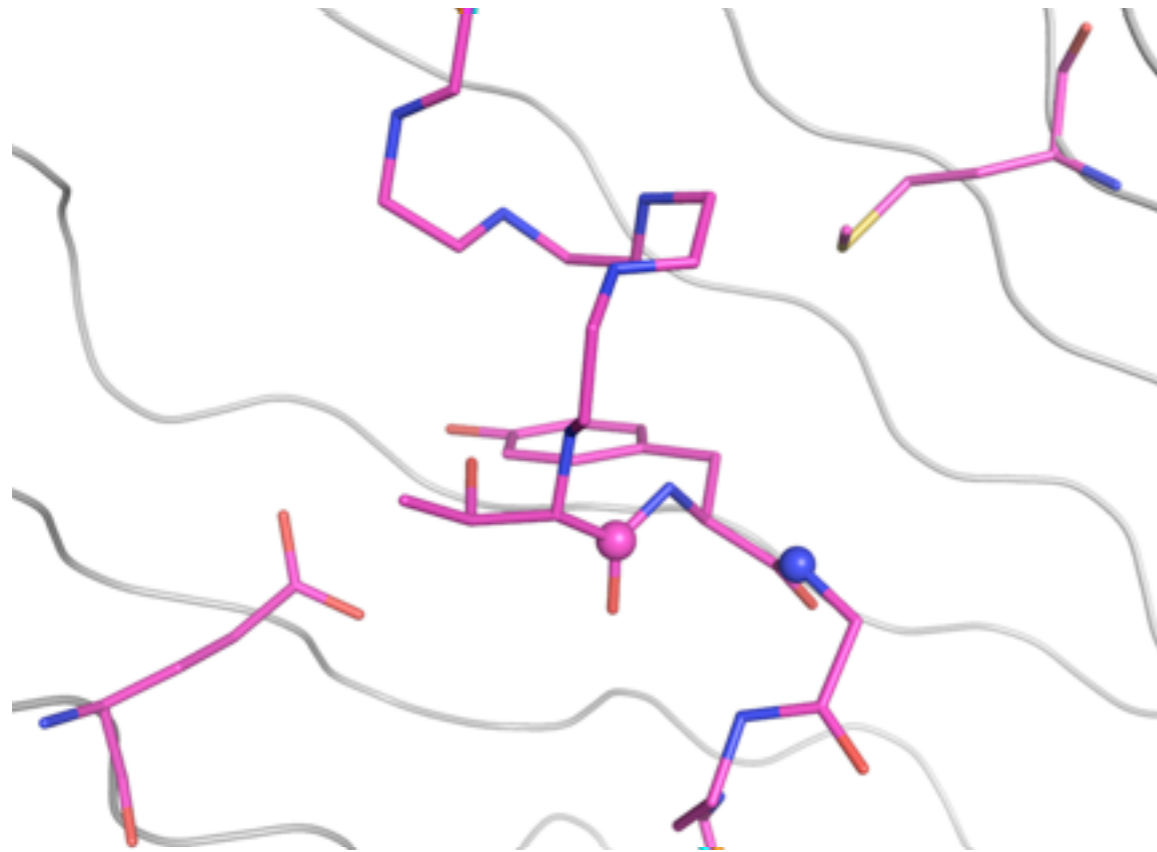
IQYO / IQYQ - GGG mutation aerob/anerob - Barondeau, PNAS, 2003

IQXT / IQYF - R96A mutation - Barondeau, PNAS, 2003

IEMA - GFP unmutated - Ormo, Science, 1996

# precyclization structures - structural insight into the state before chromophore formation

**precyclized**

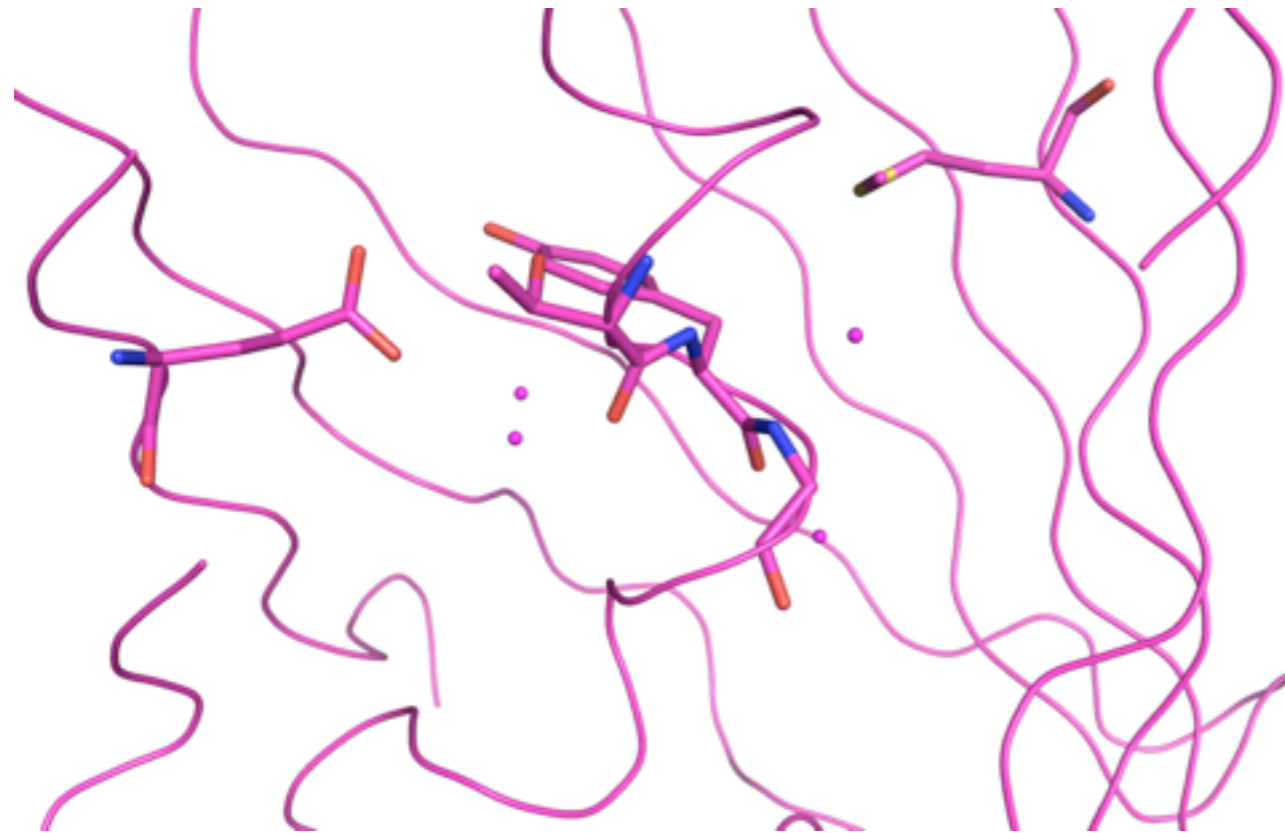


**chromophore formed**



2AWJ / 2AWK - R96M mutation - Wood, Biochemistry, 2003

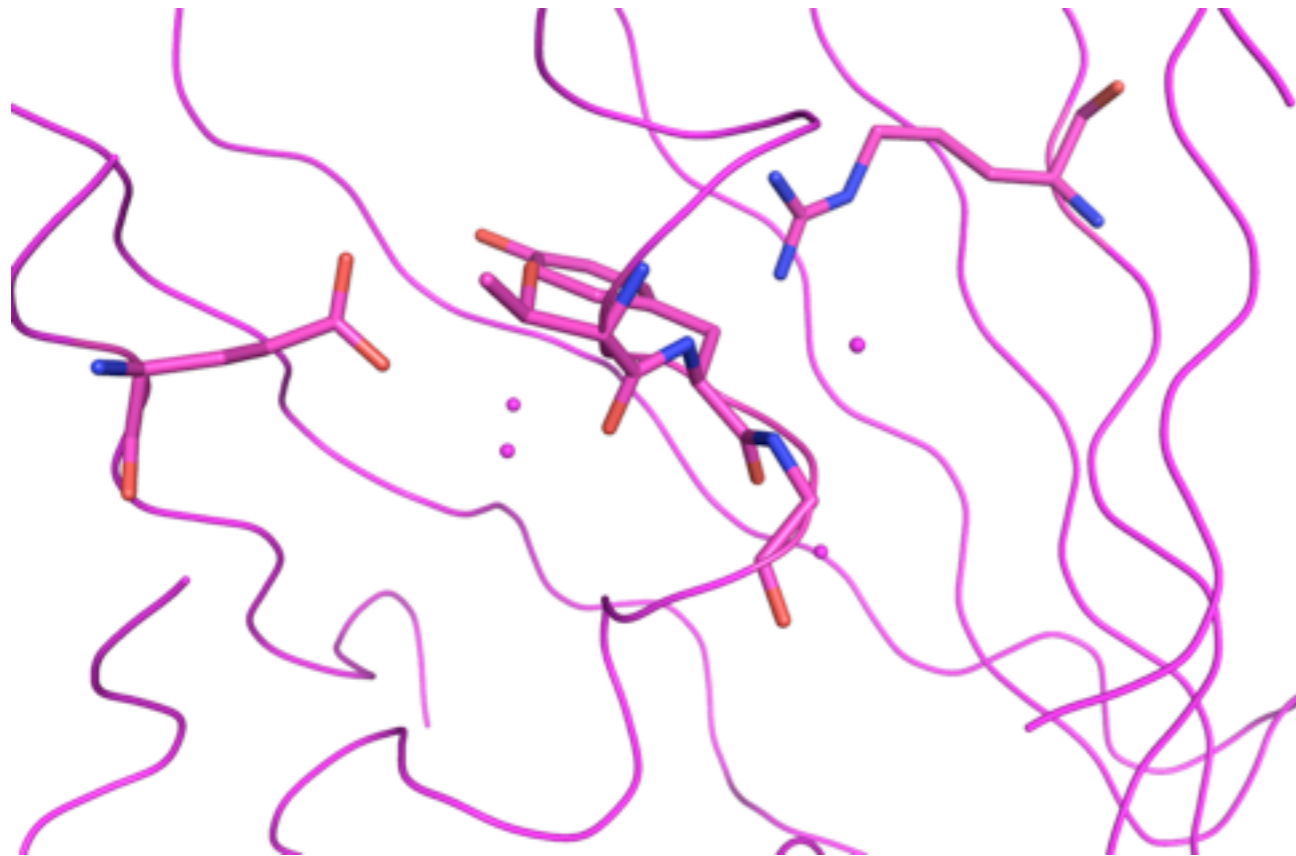
# creation of a set of **theoretical pre cyclization wt structures** using molecular dynamics



using [2AWJ](#)

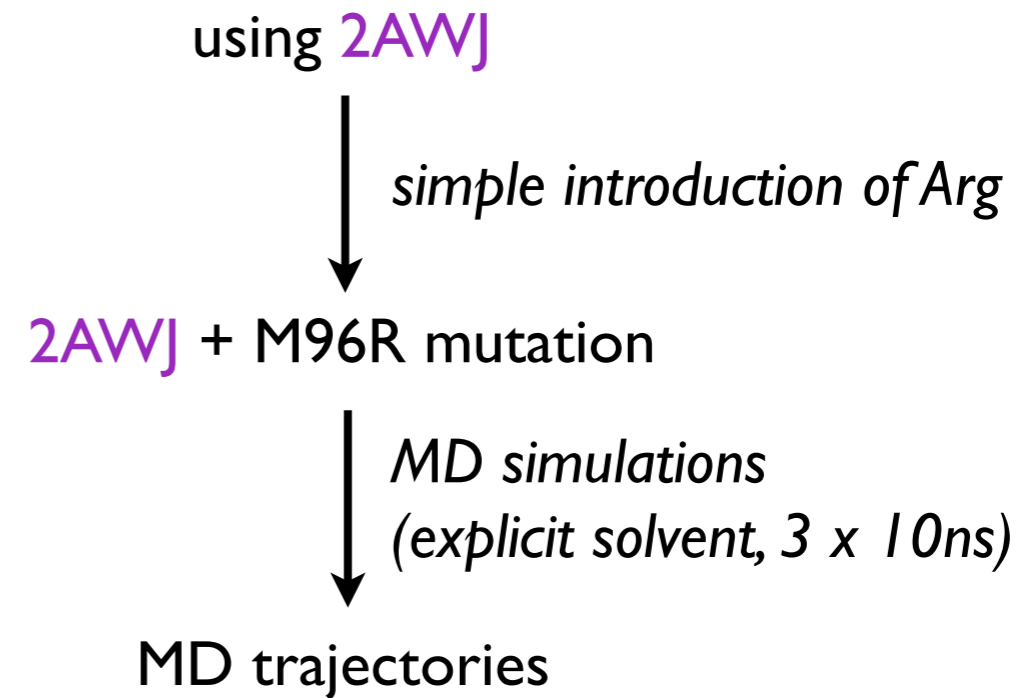
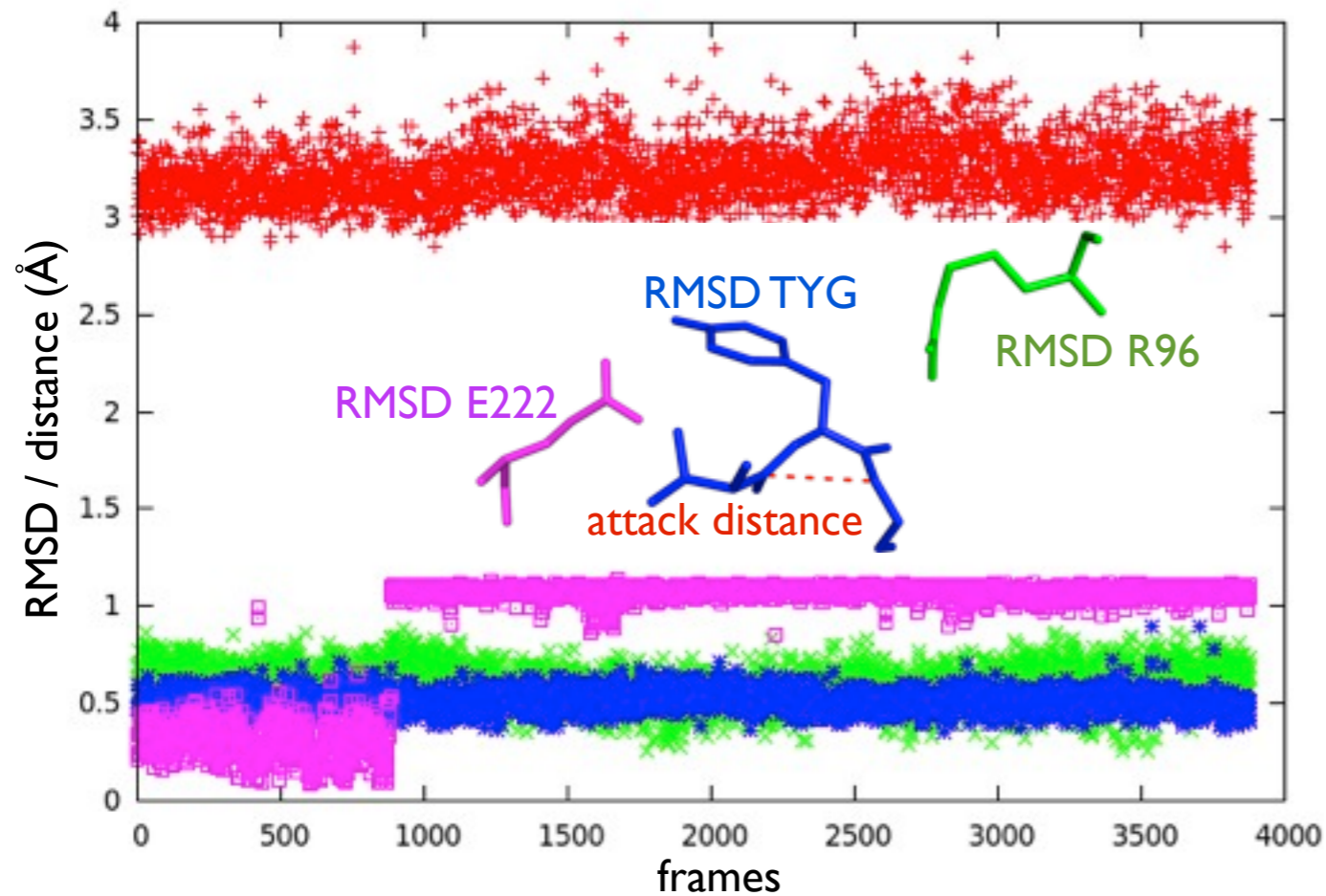


# creation of a set of **theoretical pre cyclization wt structures** using molecular dynamics

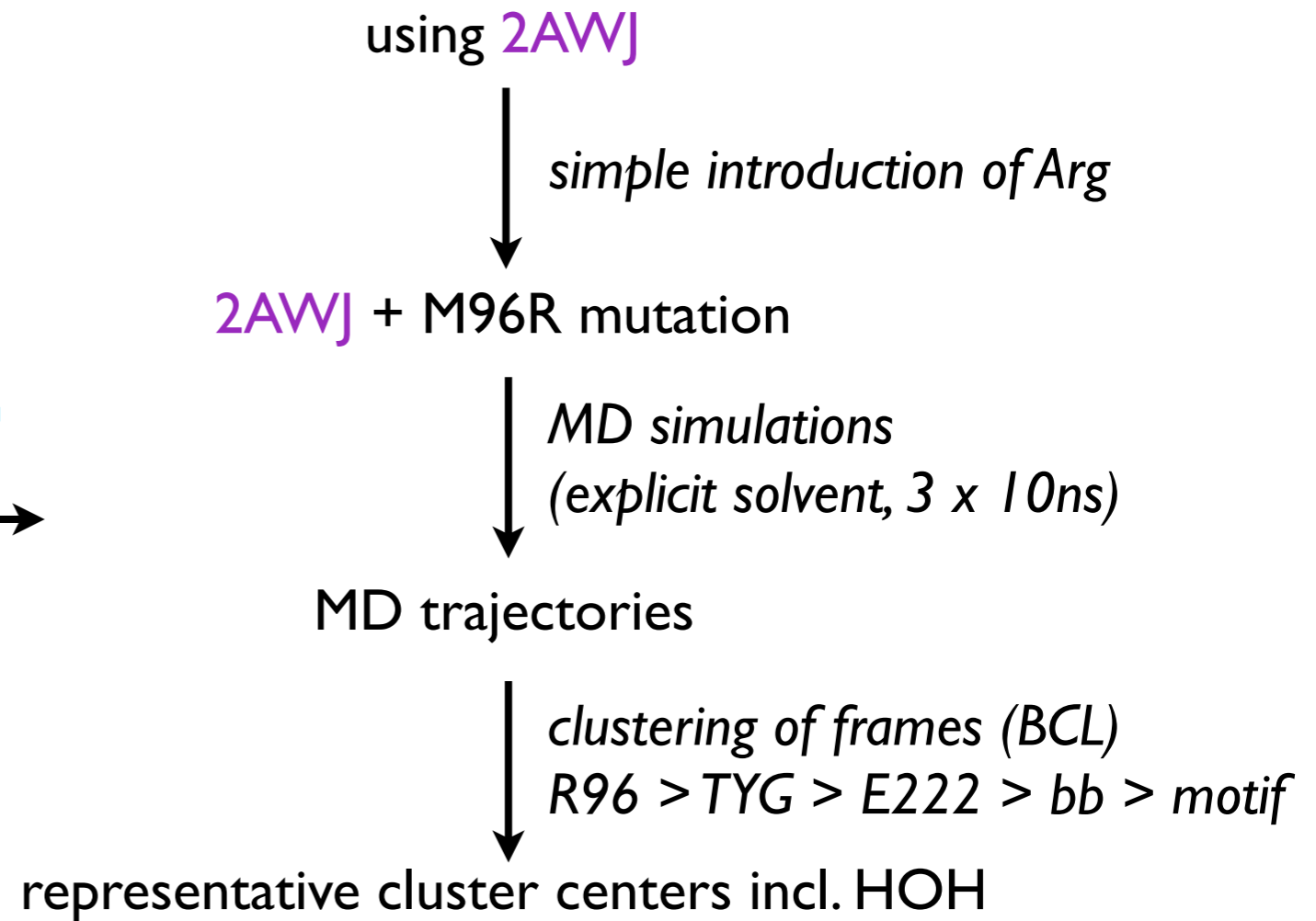
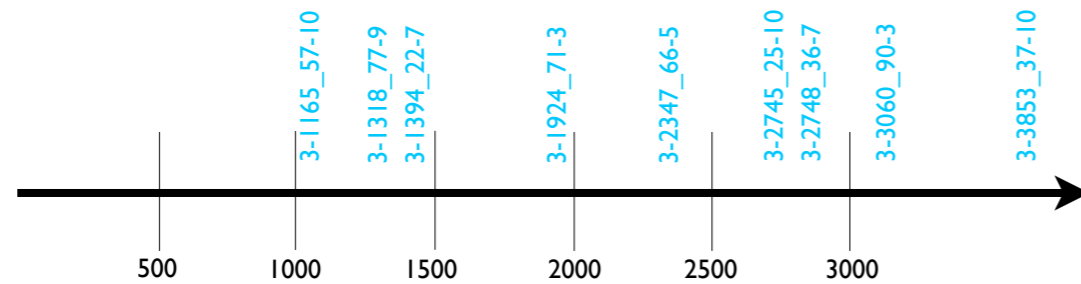


using 2AWJ  
↓  
*simple introduction of Arg*  
2AWJ + M96R mutation

# creation of a set of **theoretical pre cyclization wt structures** using molecular dynamics

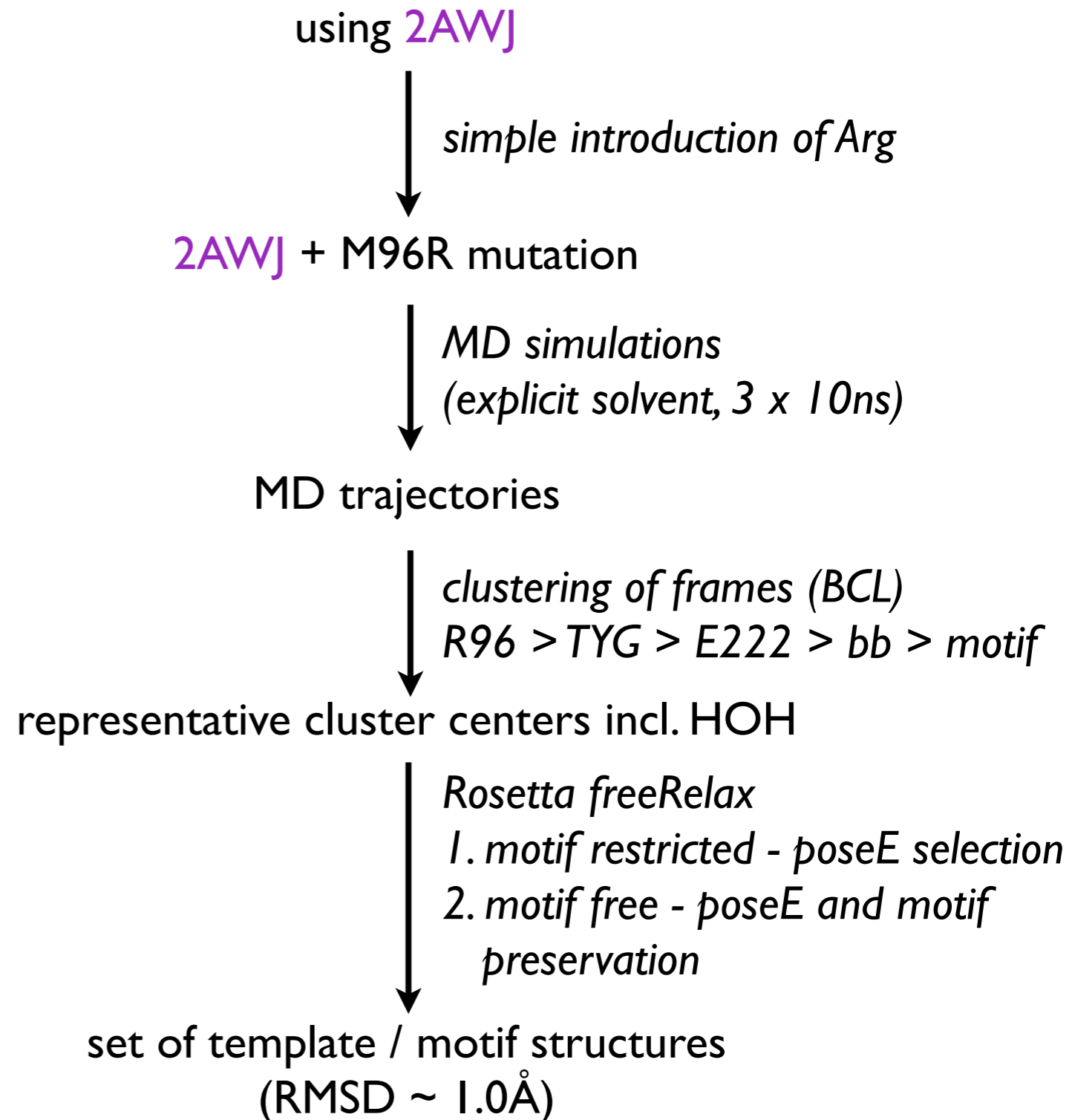
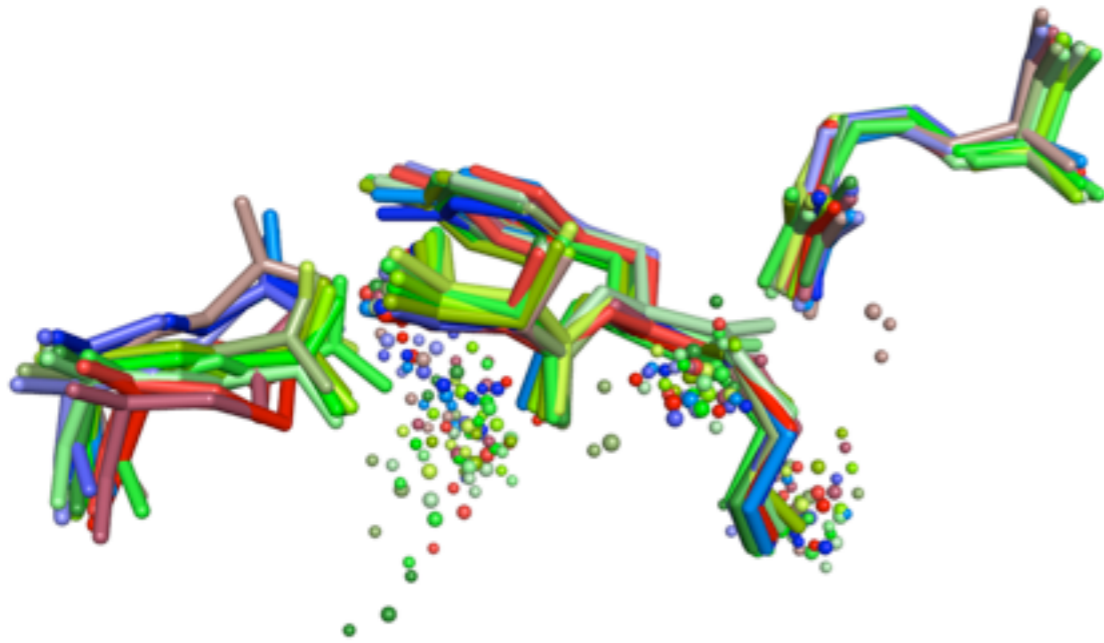


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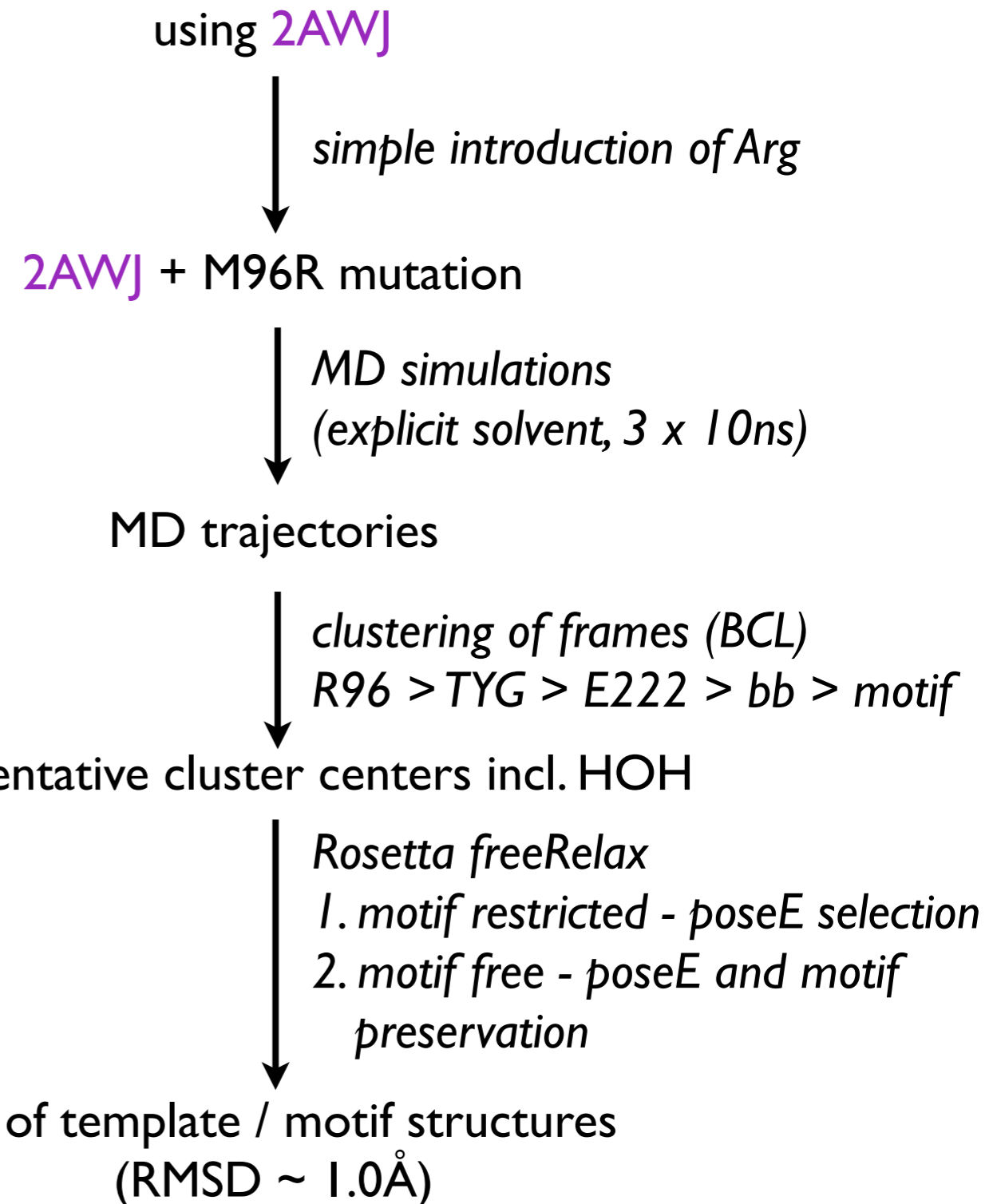
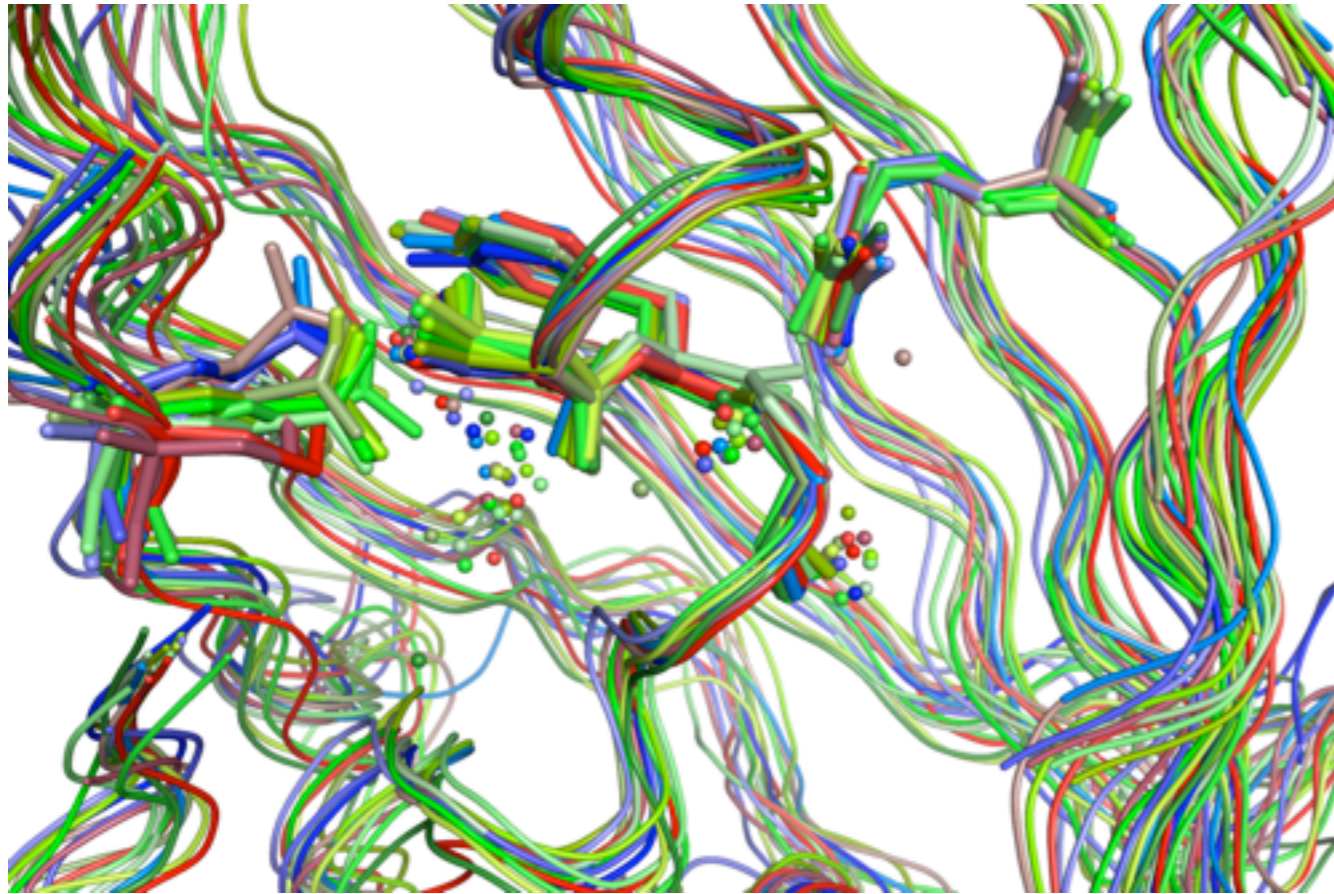




# creation of a set of **theoretical pre cyclization wt structures** using molecular dynamics



# creation of a set of **theoretical pre cyclization wt structures** using molecular dynamics



motif / template

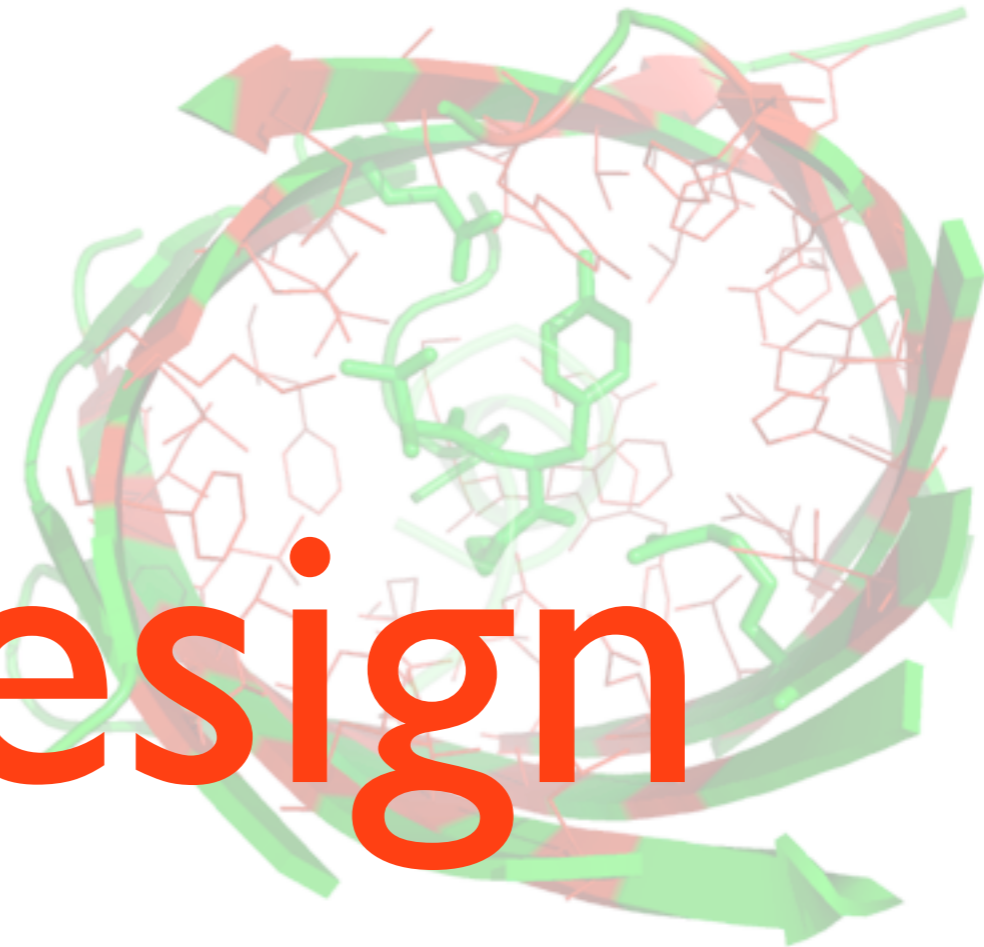


redesign



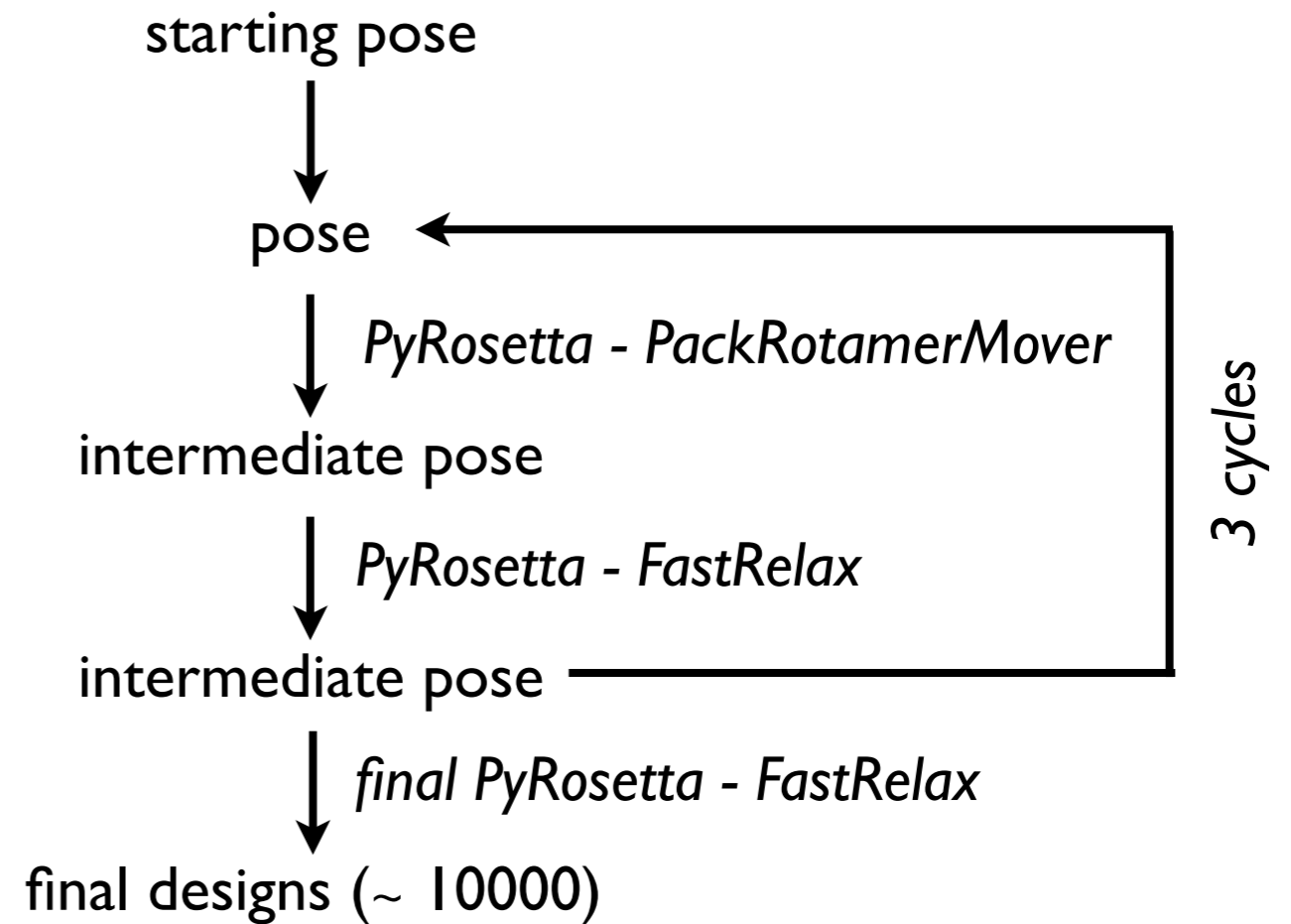
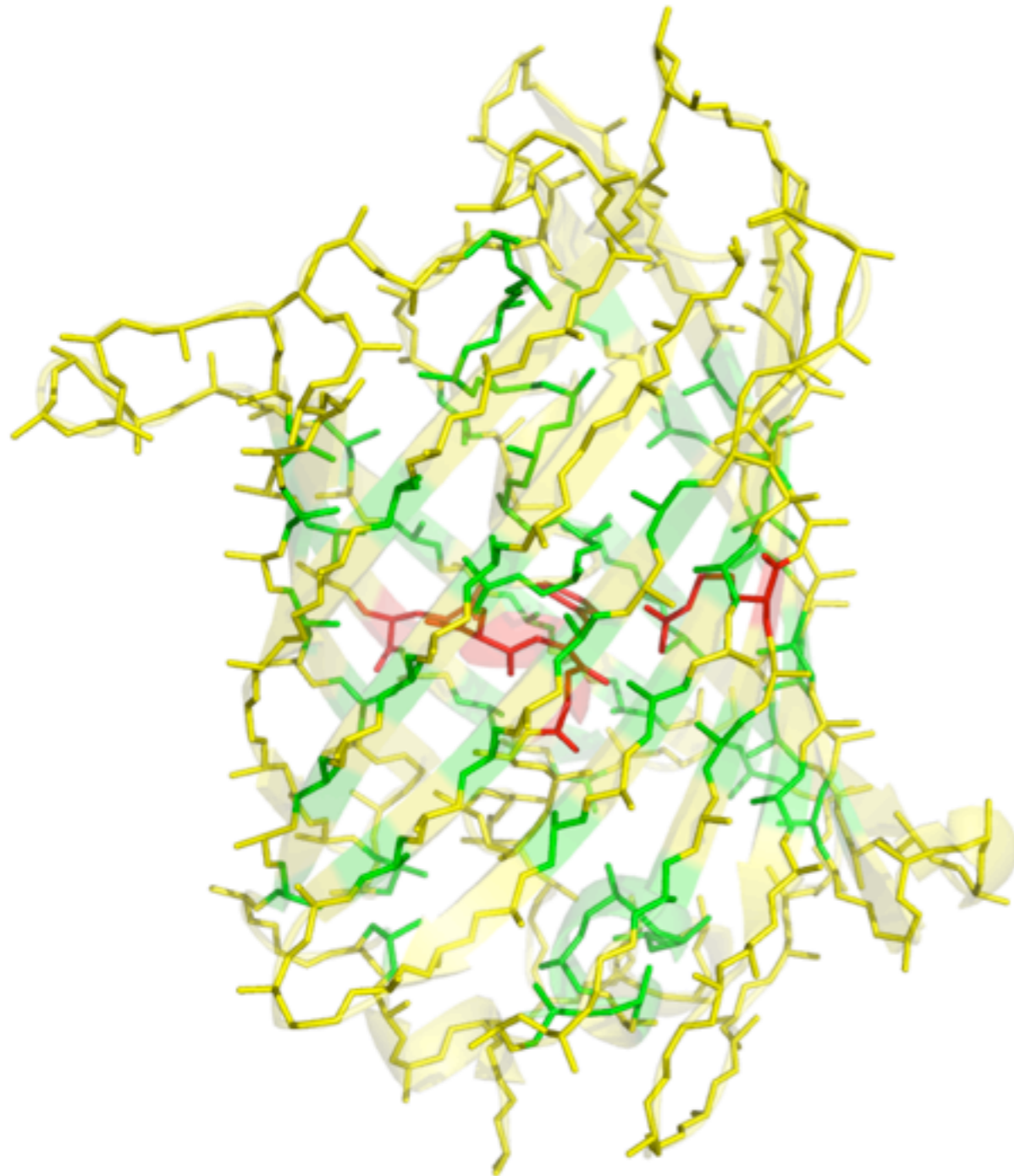
selection ranking

# redesign





# Rosetta Redesign on all templates/motifs



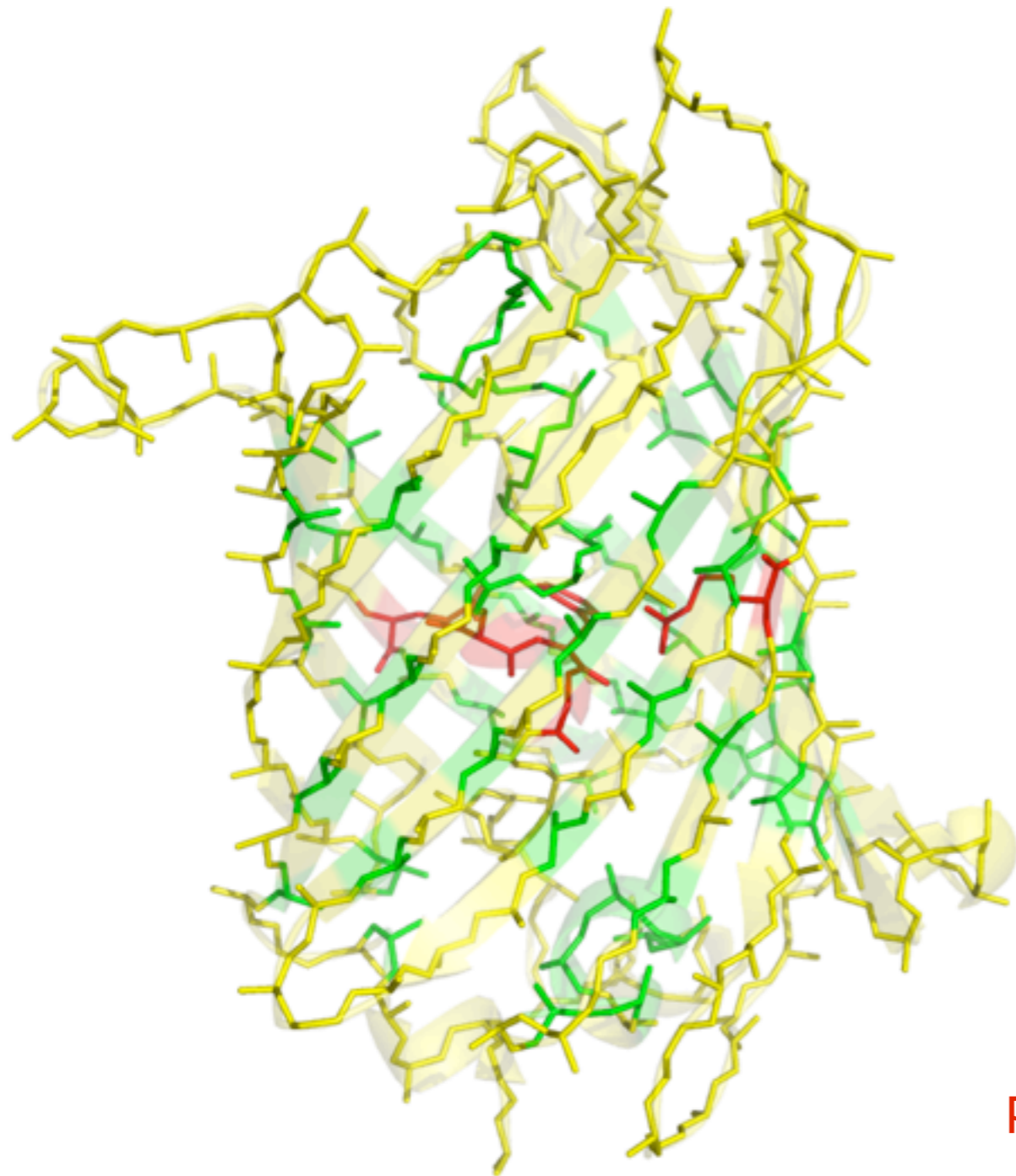
fixed rotamer = the motif

only rotamer movements allowed =  
solvent exposed / loop regions / (motif-) helix

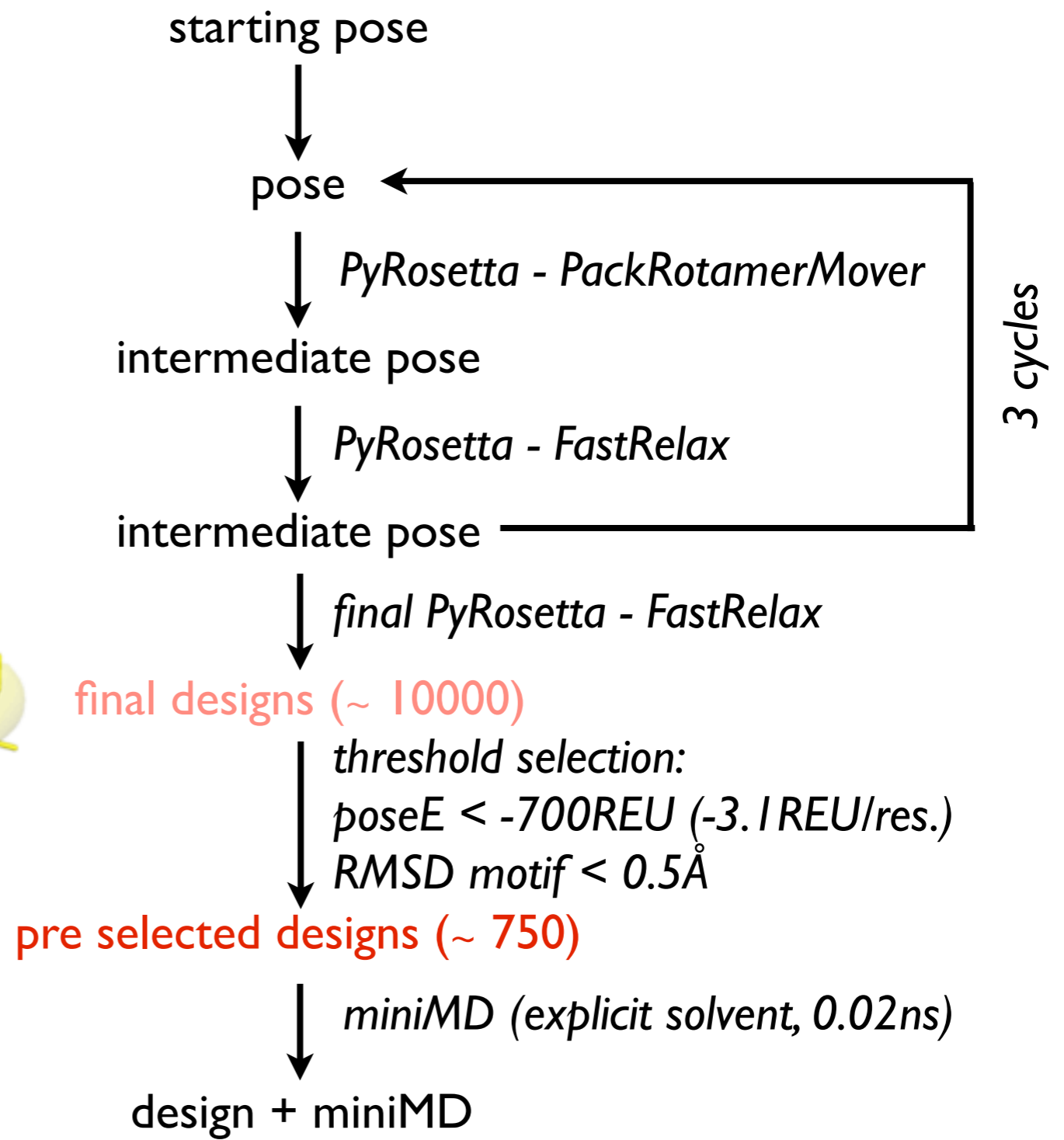
(fully) mutable = barrel interior = ~ 50 mutable residues



# pre selection - on motif RMSD and poseE

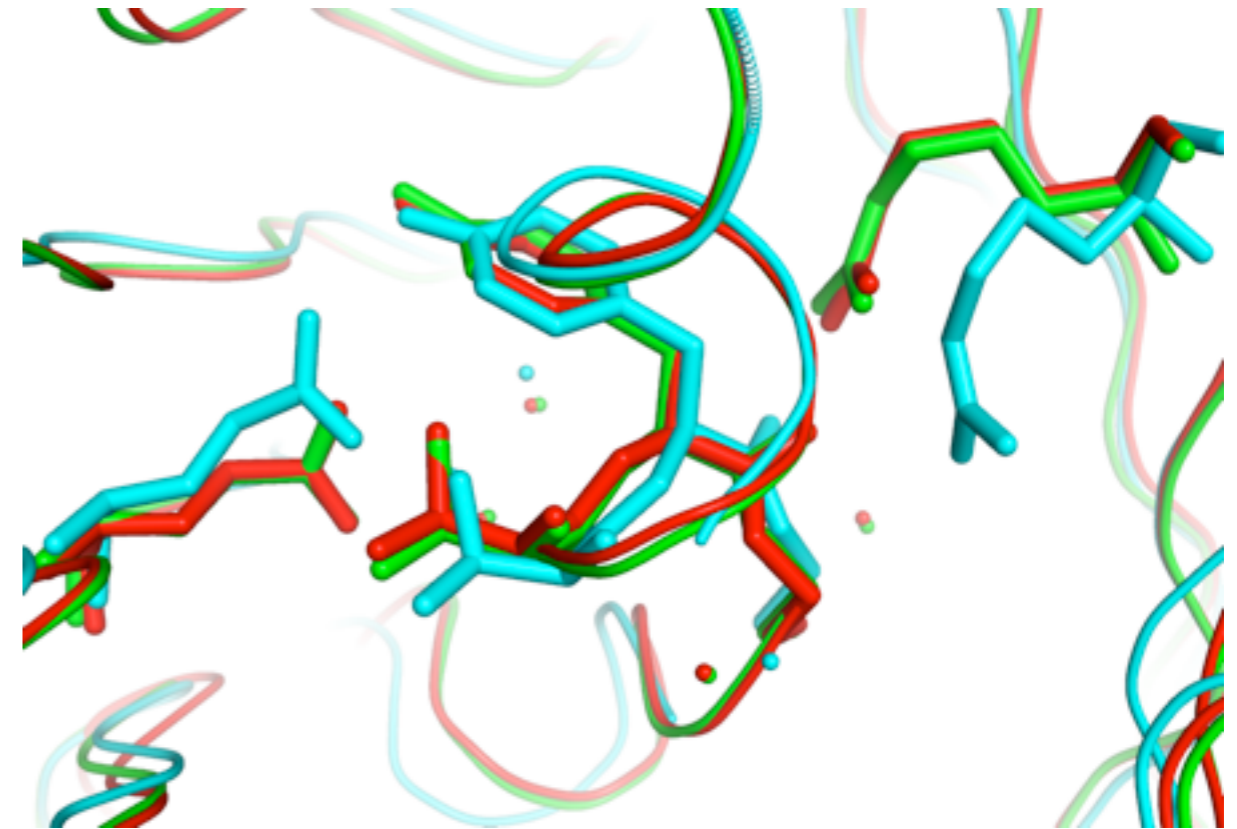
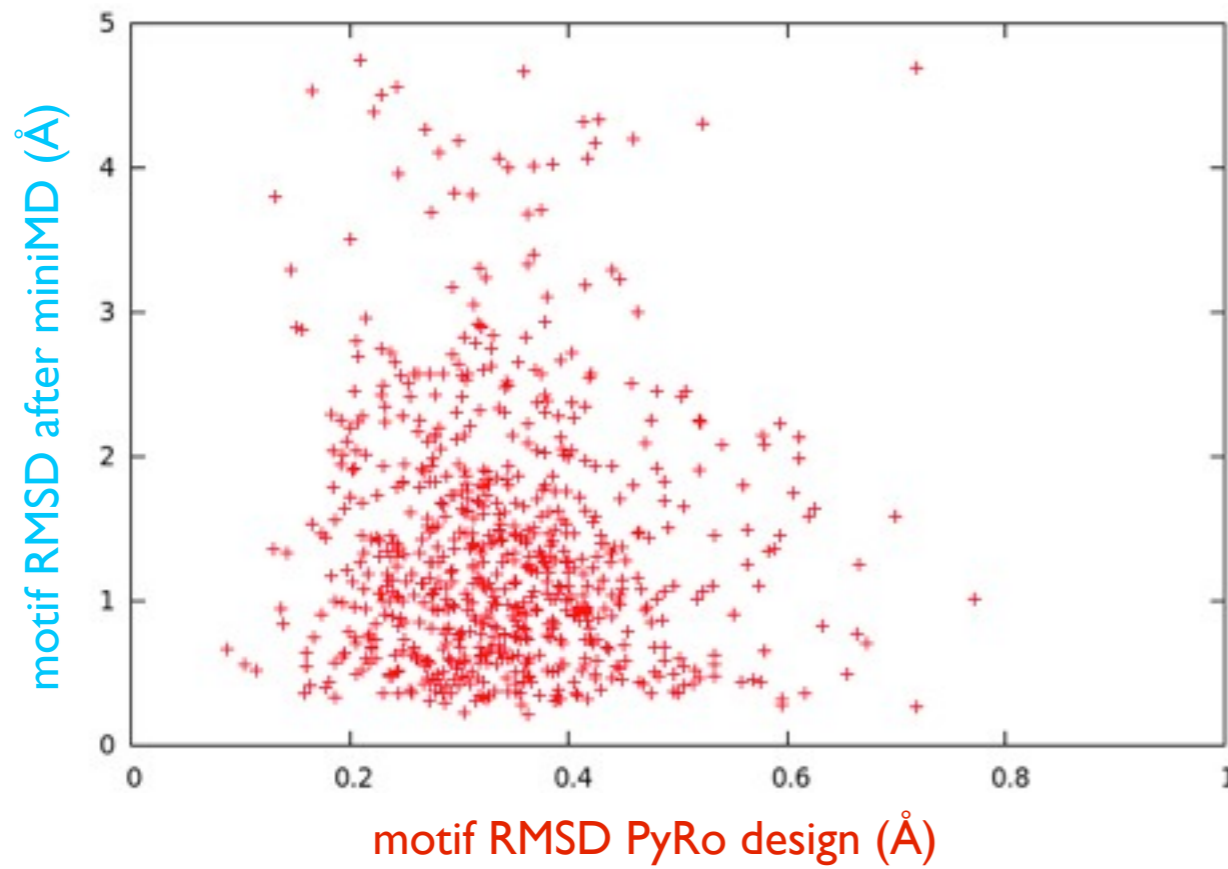


fixed rotamer = the motif  
only rotamer movements allowed =  
solvent exposed / loop regions / (motif-) helix  
(fully) mutable = barrel interior



# miniMD - the **bright** side (see poster for dark side)

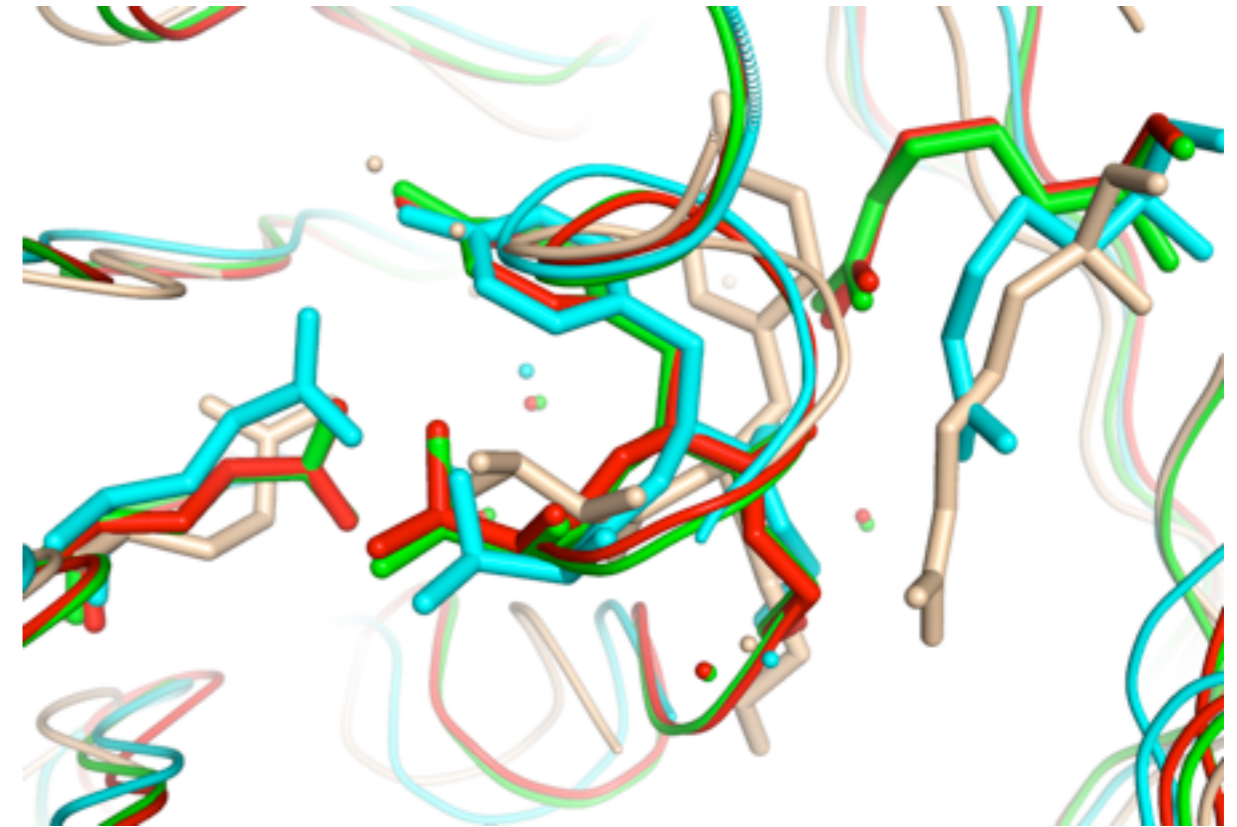
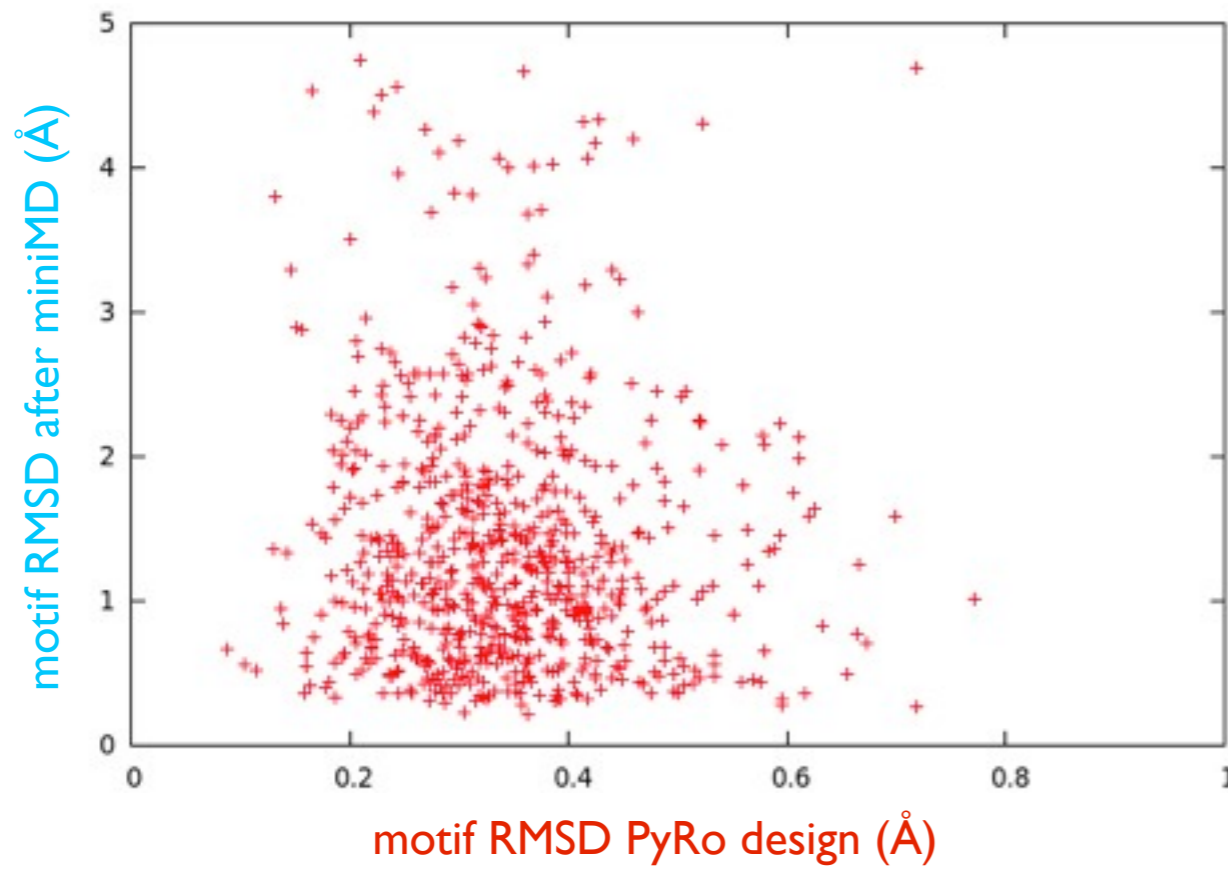
R96 RMSD PyRo design vs. after MD



template (starting pose)  
design  
avg. last frames miniMD

# miniMD - the **bright** side (see poster for dark side)

R96 RMSD PyRo design vs. after MD

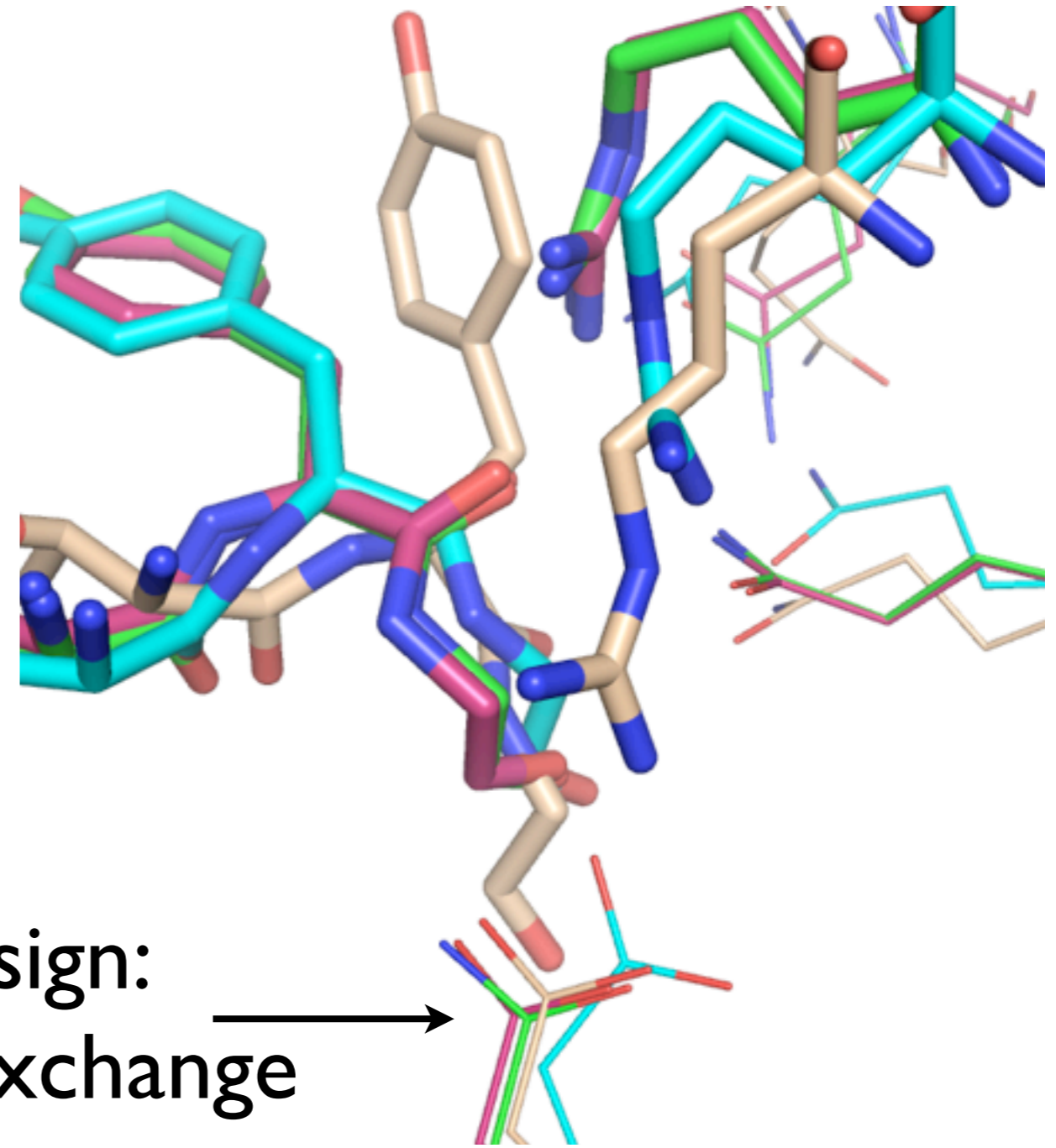
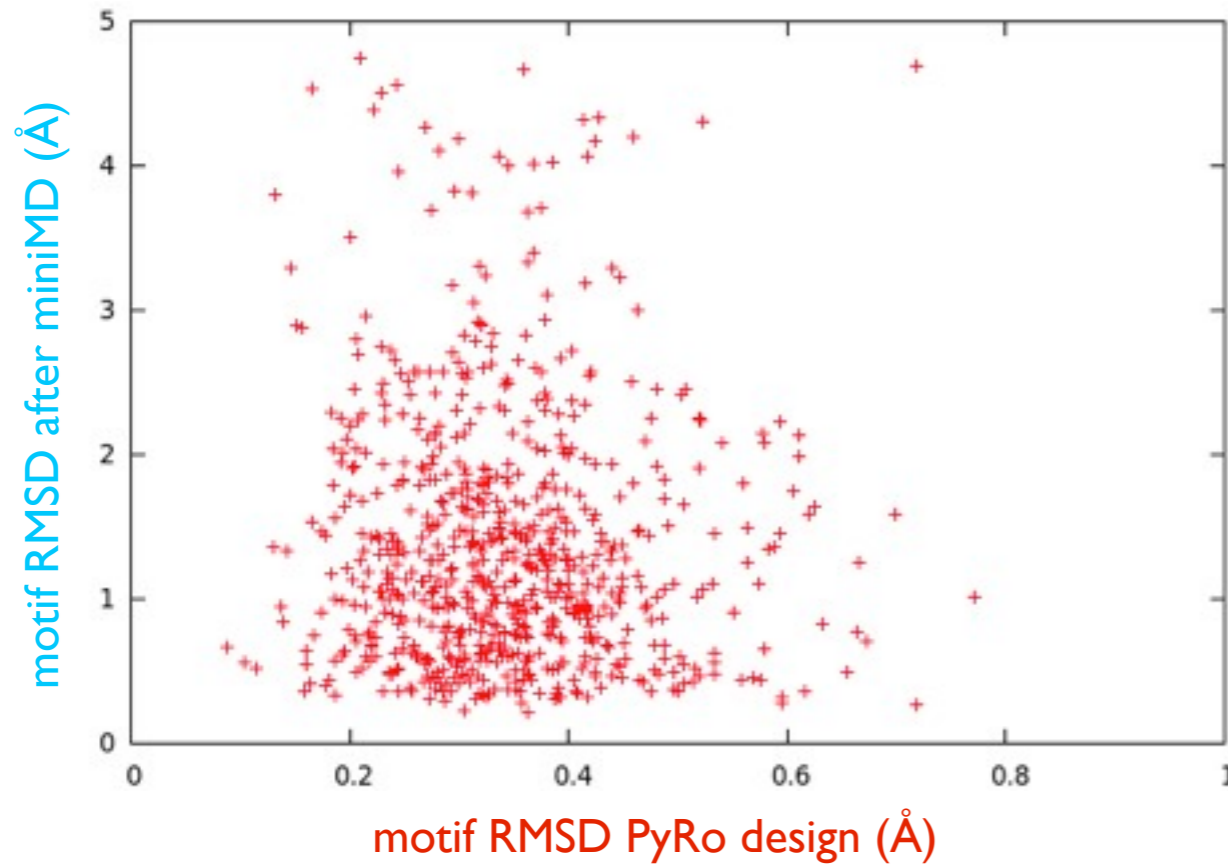


template (starting pose)  
design  
avg. last frames miniMD  
avg. frames after 8ns MD



# miniMD - the **bright** side (see poster for dark side)

R96 RMSD PyRo design vs. after MD



design:  
N/D exchange

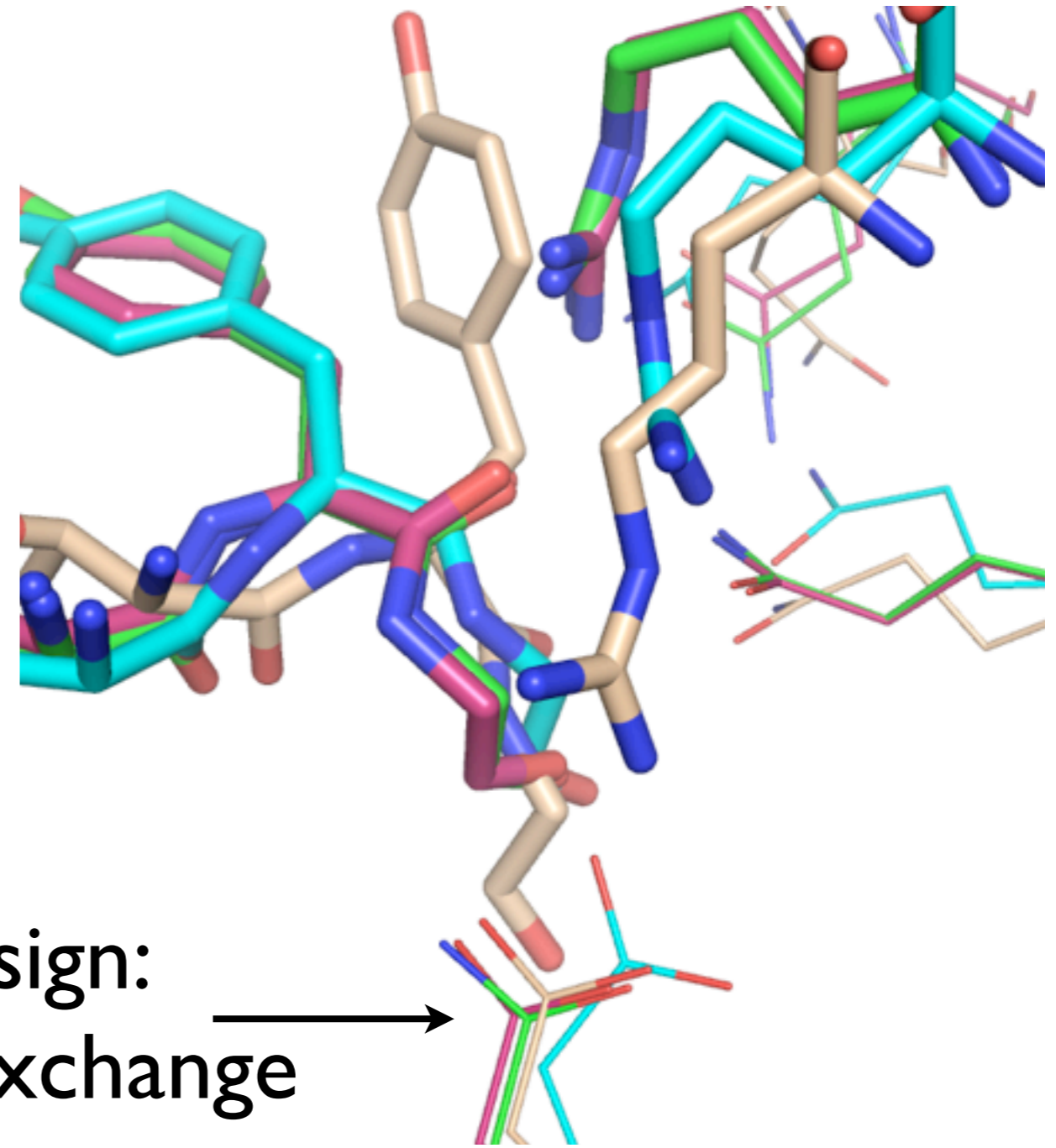
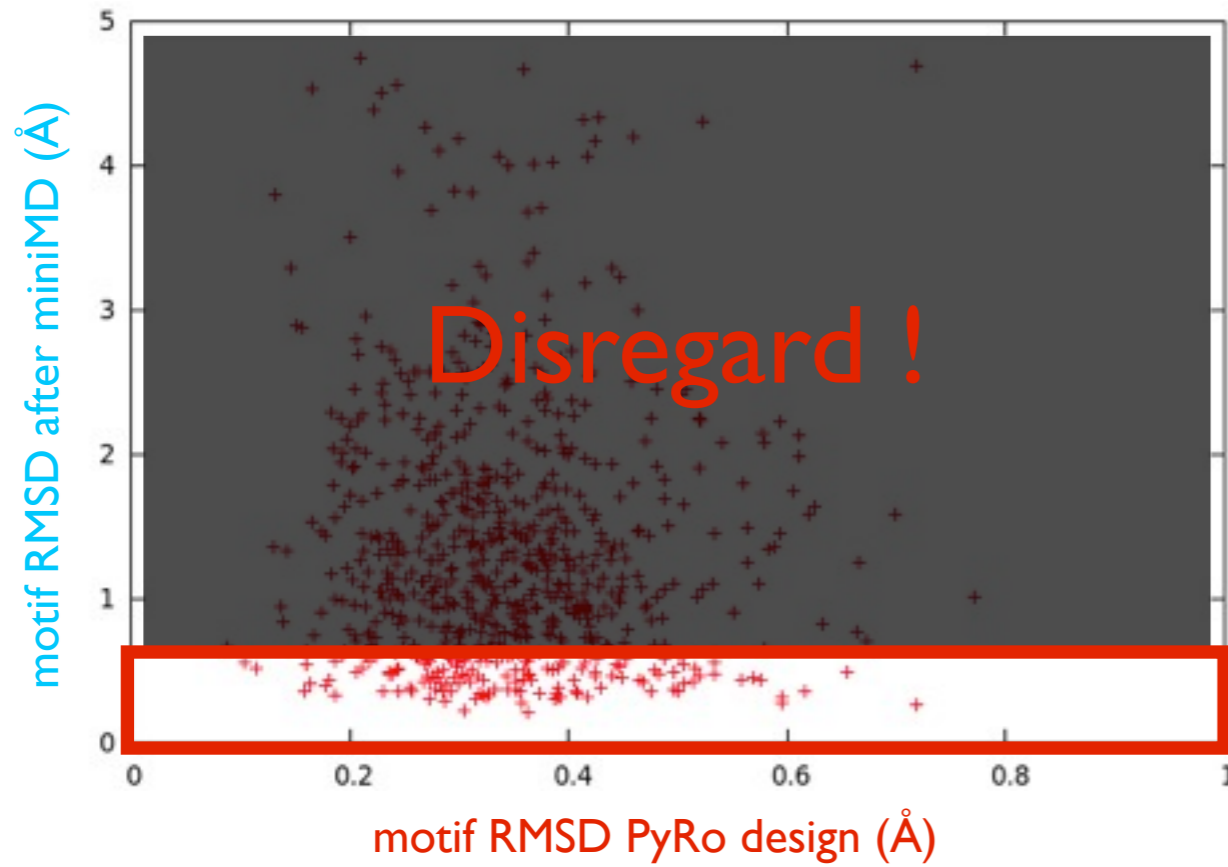


template (starting pose)  
design  
avg. last frames miniMD  
avg. frames after 8ns MD



# miniMD - the **bright** side (see poster for dark side)

R96 RMSD PyRo design vs. after MD

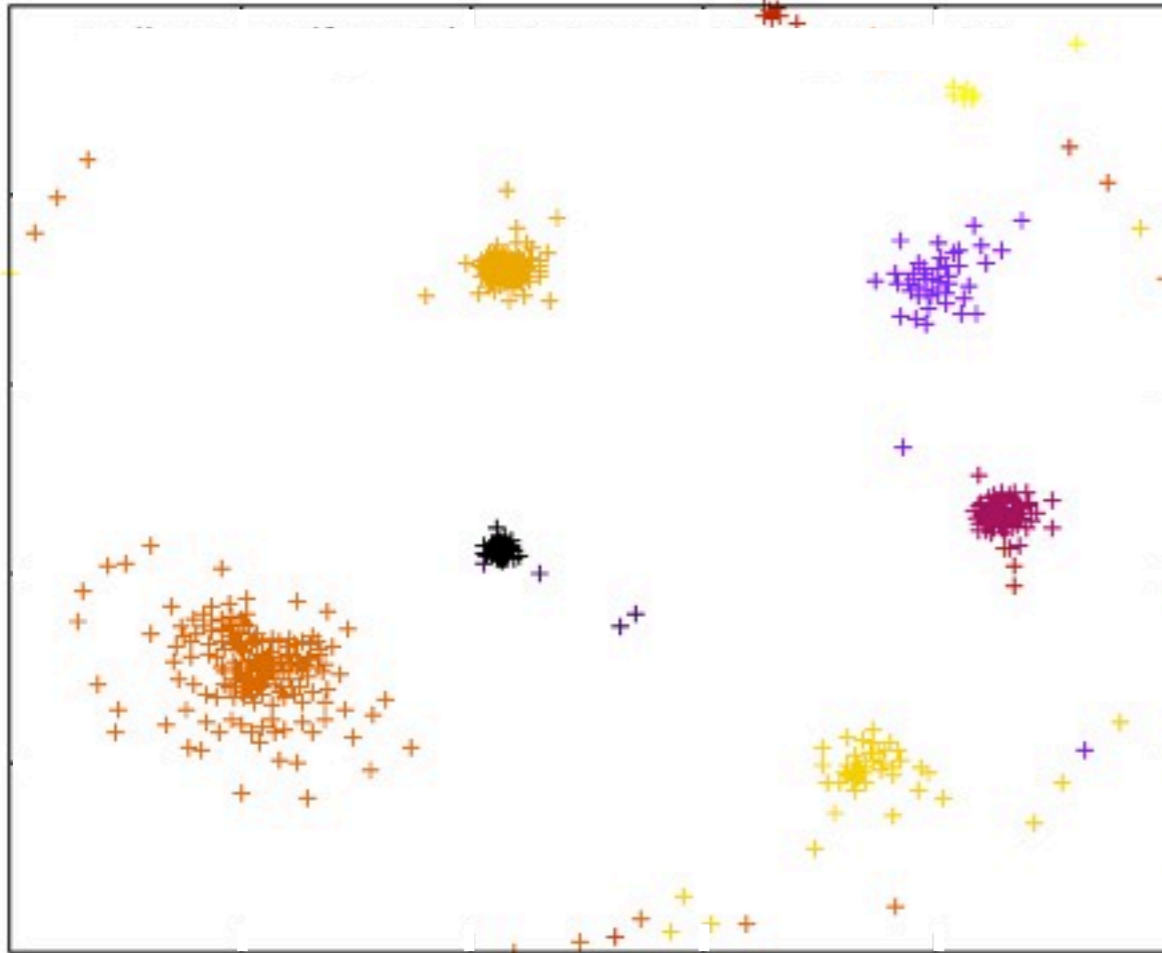


design:  
N/D exchange

template (starting pose)  
design  
avg. last frames miniMD  
avg. frames after 8ns MD

# different templates show different behavior - despite their same sequence

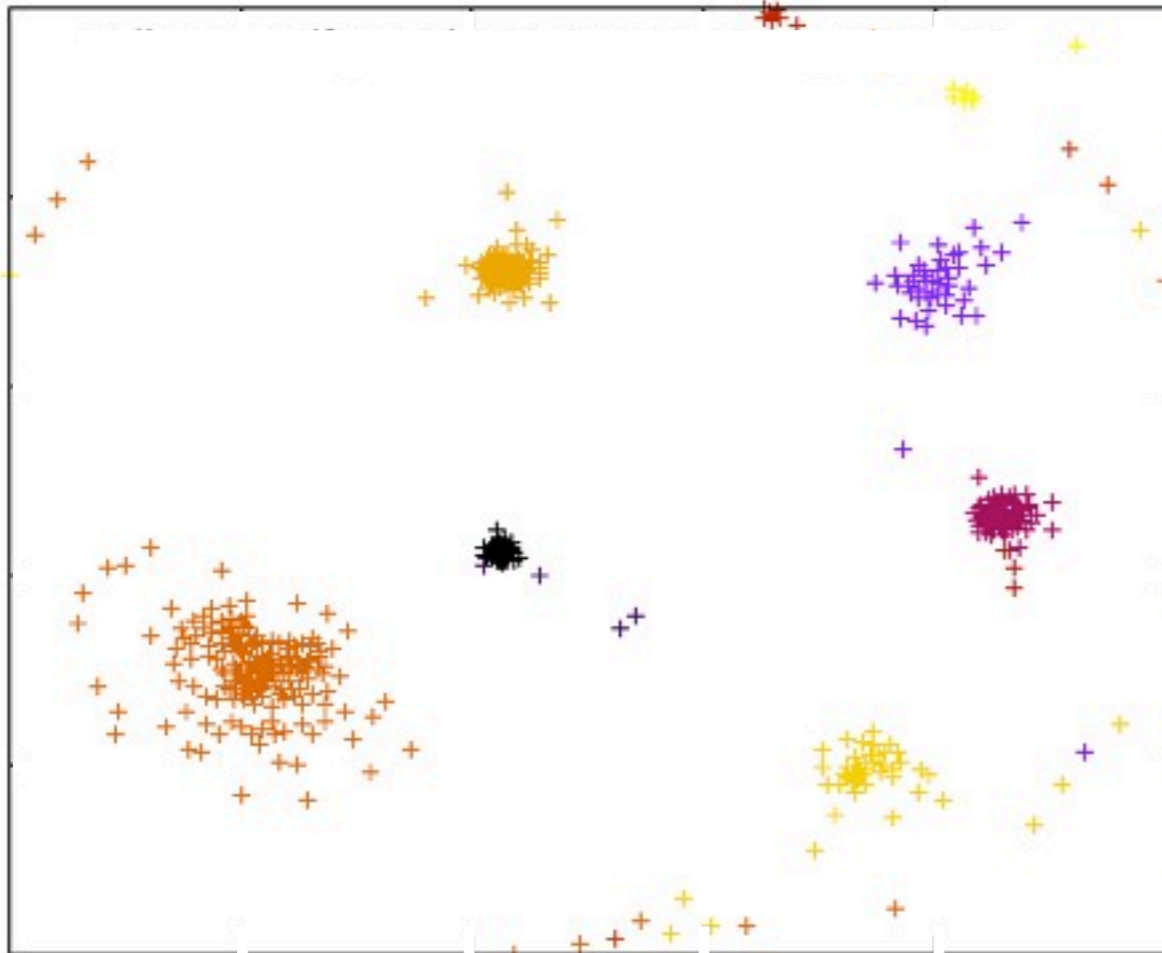
sequence cluster, coloring according to template



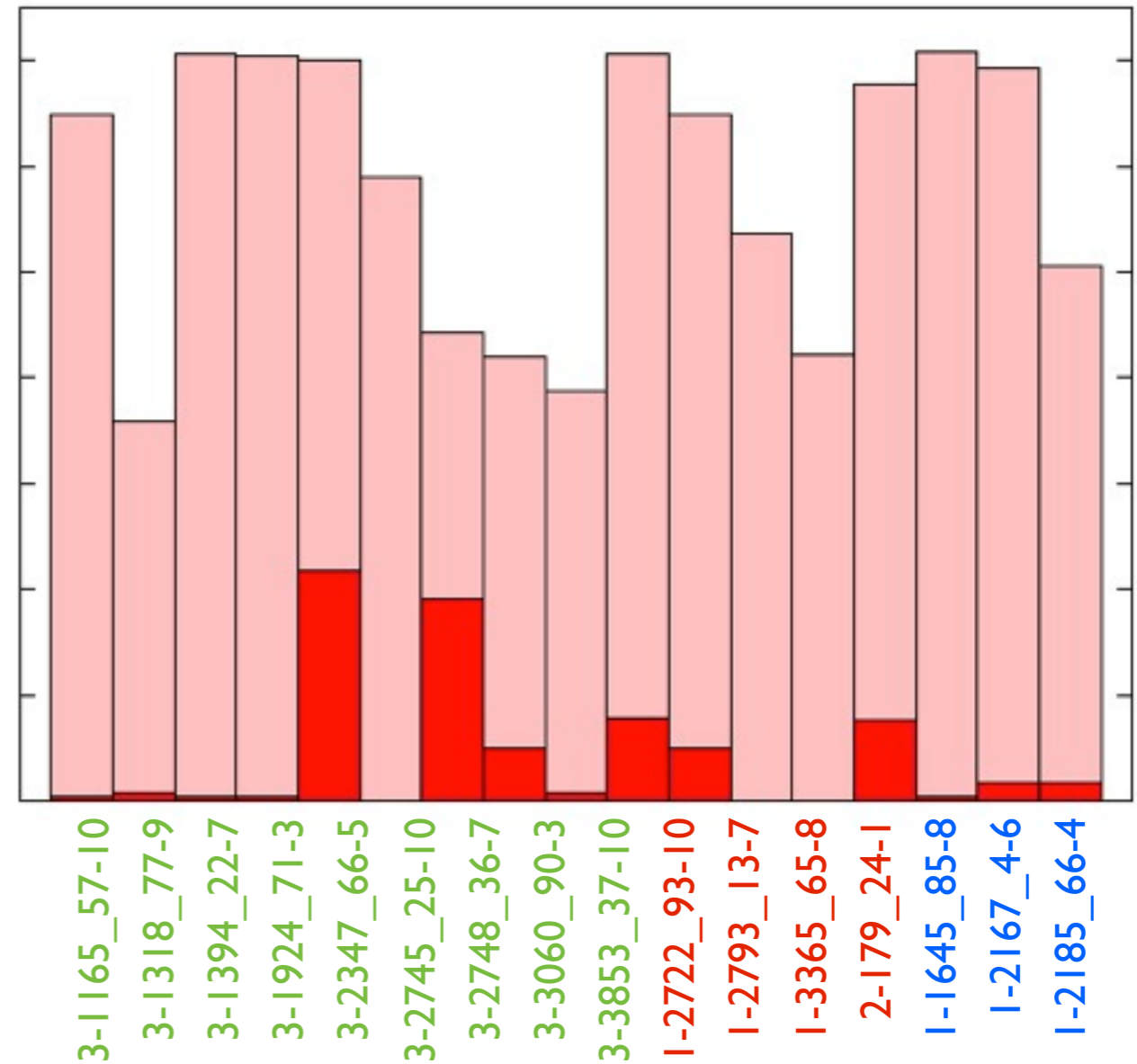
clustering of preselected designs  
using clans (Frickey, Bioinformatics, 2004)  
p-value = 1.0 E-19

# different templates show different behavior - despite their same sequence

sequence cluster, coloring according to template **all designs** vs. **met preselection criteria** (-700REU ...)



clustering of preselected designs  
using clans (Frickey, Bioinformatics, 2004)  
p-value = 1.0 E-19



designs starting from same the template seem to follow similar pathways

motif / template



redesign



selection ranking

# selection / ranking





# final selection - regarding all data

I. weighted data PyRo:

- emphasizing poseE
- importance of attack distance and R96
- neglect aaE

II. weighted data miniMD:

- only look at RMSDs and attack distance

III. visual inspection ;-)

IV. try to cover template & sequence space

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long MDs on roughly 30 designs

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30 designs for final selection

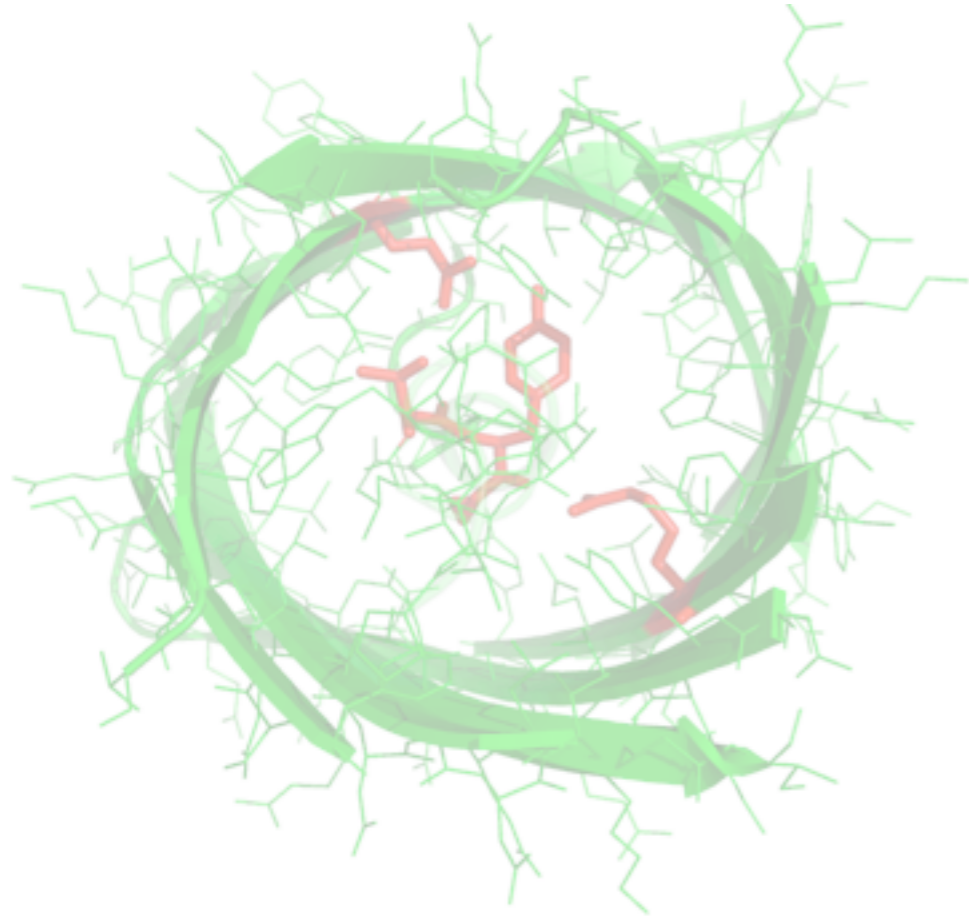
6 ordered

**final selection** - regarding all data



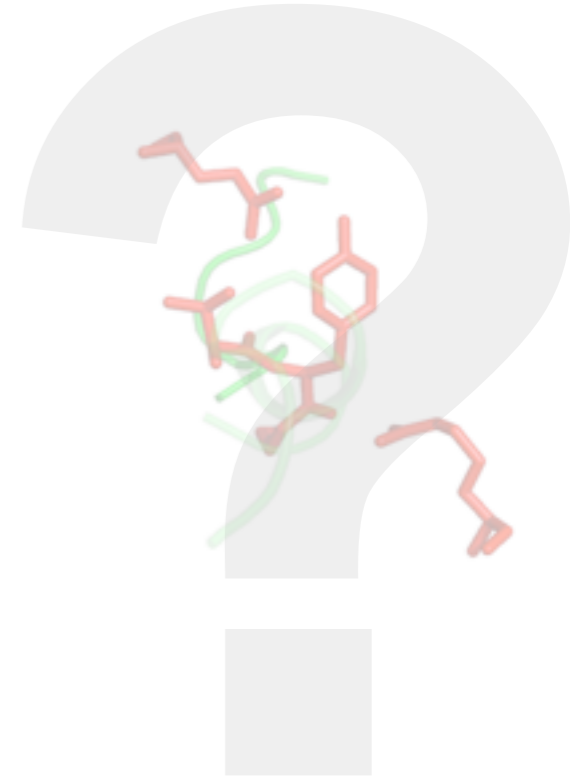
so far: all expressed designs do not fold properly

# the pie in the sky - potential **approaches stage II**

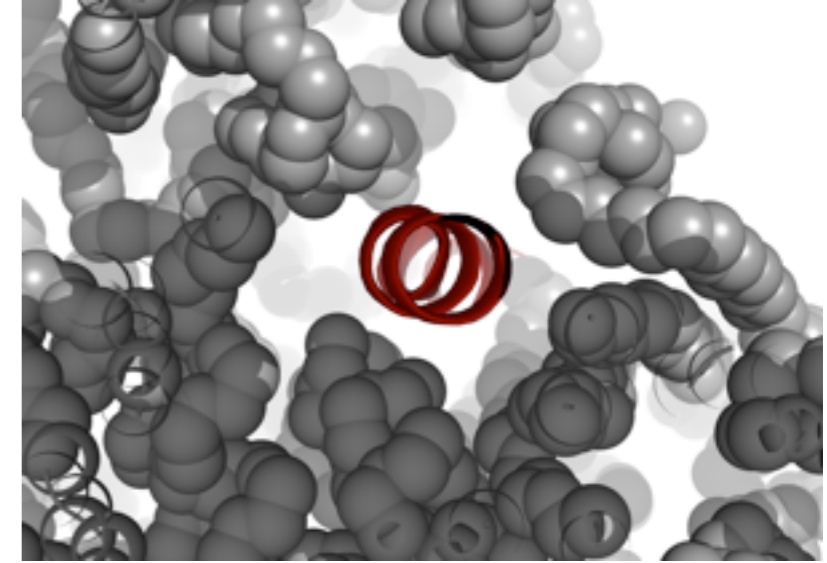
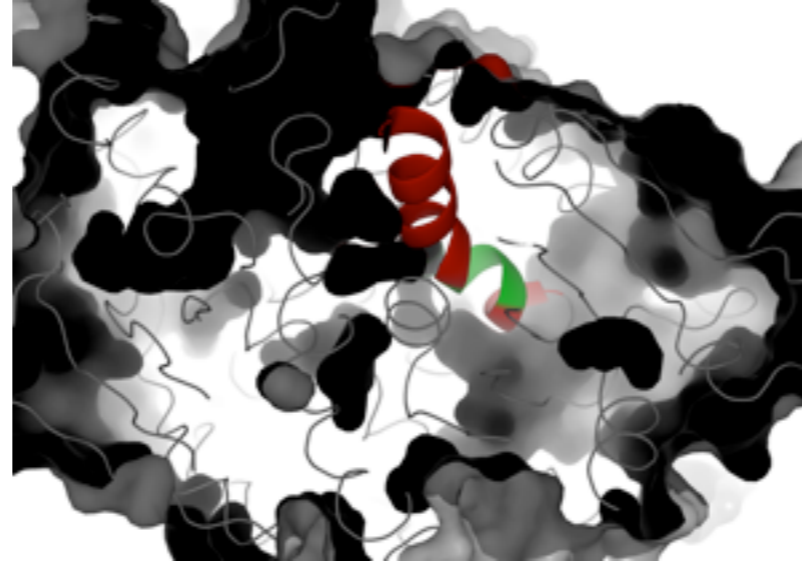
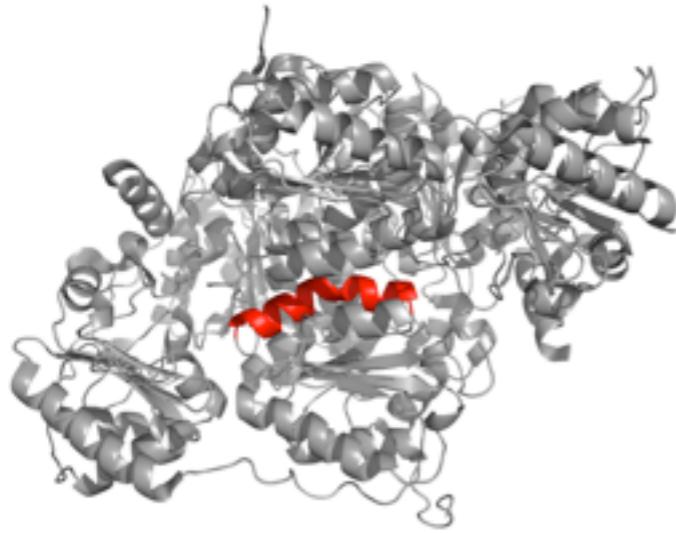


1. scaffold selection
2. design / packing

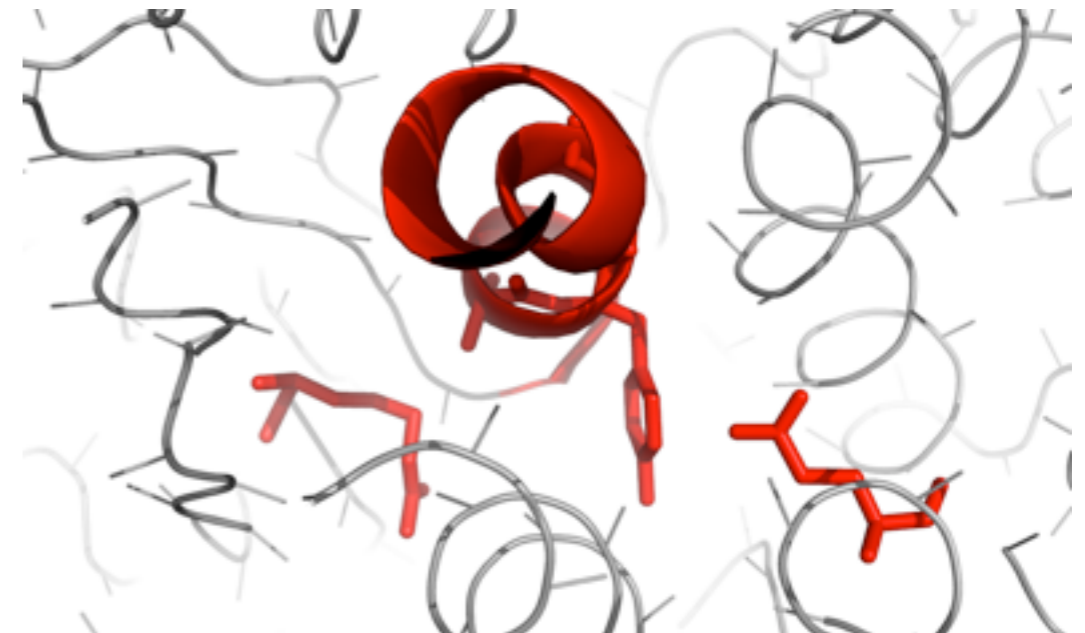
**STAGE II**



# the pie in the sky - potential **approaches stage II**



- pre search for: kinked helix, buried ...
- ScaffoldSelection (Höcker Lab, Malisi, Proteins, 2009)
- RosettaMatch?





# take this home ...

## **the approach:**

- transplantation of unique autocatalytic chromophore formation: worth the effort!
- use of MD to generate motif geometry
- stage I: redesign of the barrel, stage II: new scaffold

## **three interesting points:**

- miniMD might give valuable hints on problematic residues
- even without sequence differences, templates show different behavior
- the folding problem: too much changed?

# take this home ...

## the approach:

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- use of MD to generate motif geometry
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## three interesting points:

- miniMD might give valuable hints on problematic residues
- even without sequence differences, templates show different behavior
- the folding problem: too much changed?

... many thanks to

Birte Höcker

Sooruban Shanmugaratnam

Christoph Malisi

Nils Wötzel



more details  
on the poster!