

# Computational Screening of Fluorescent Protein Mutants

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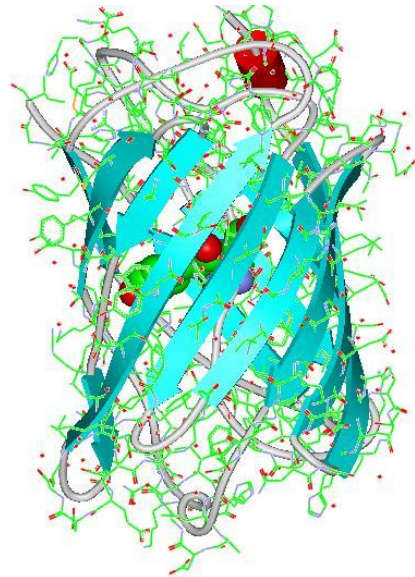
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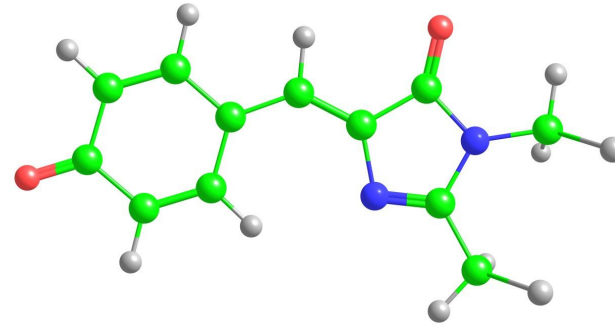
# Fluorescent proteins

Osamu Shimomura, Martin Chalfie and Roger Tsien, Nobel Prize in Chemistry 2008

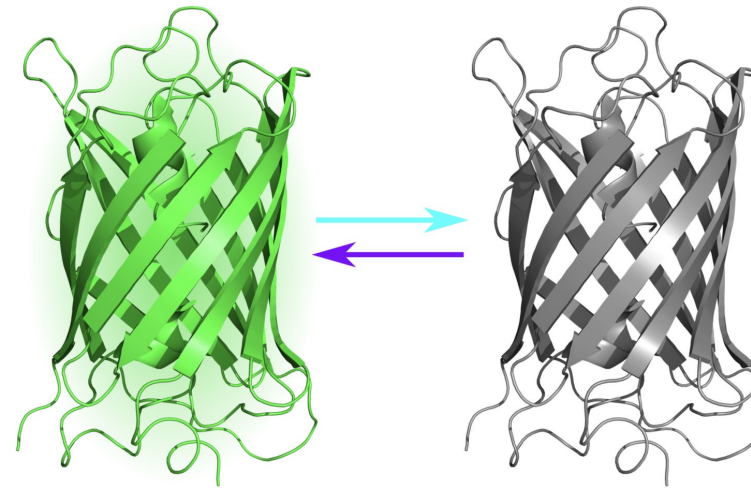
wtGFP



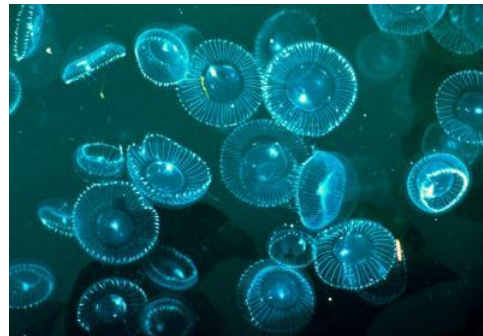
Chromophore



Could be reversibly photo-switchable

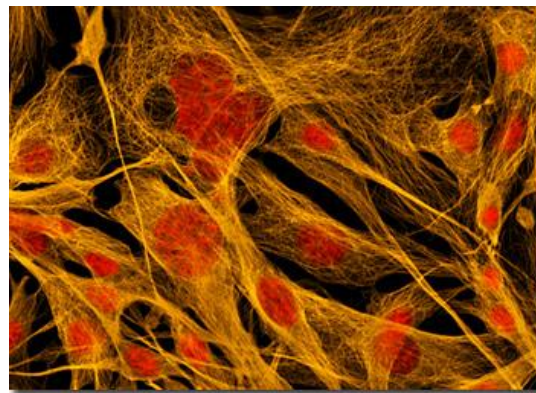
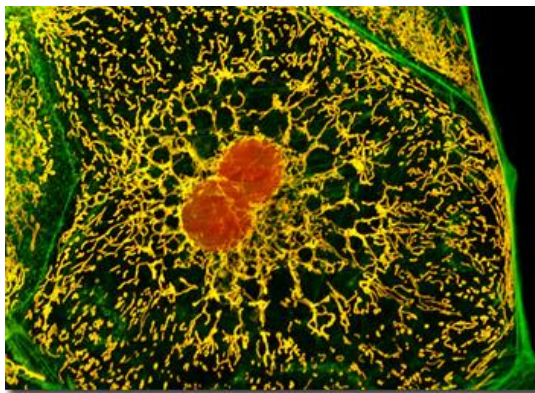
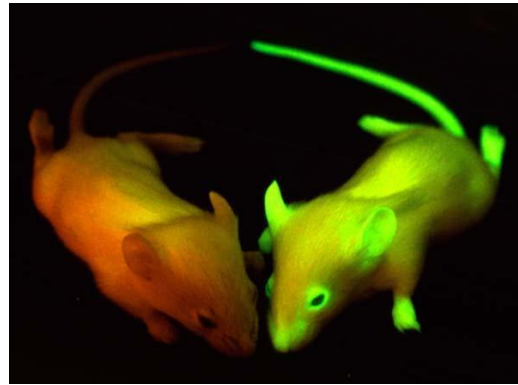
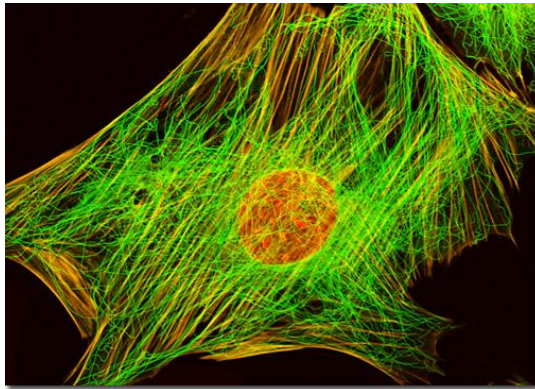


Aequorea Victoria



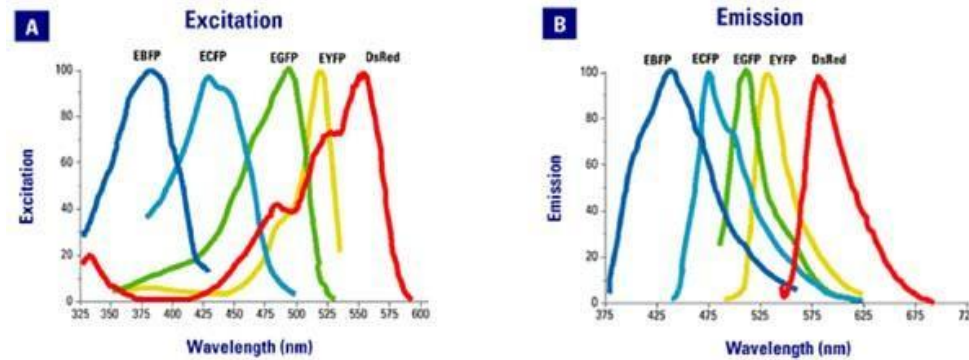
# Applications

The GFP-family proteins is widely used as a marker because of its very useful properties such as: high stability, minimal toxicity, non-invasive detection and the ability to fluoresce without specific equipment



Eric Betzig, Stefan Hell and William E. Moerner,  
Nobel Prize in Chemistry 2014

# Variety of fluorescent proteins



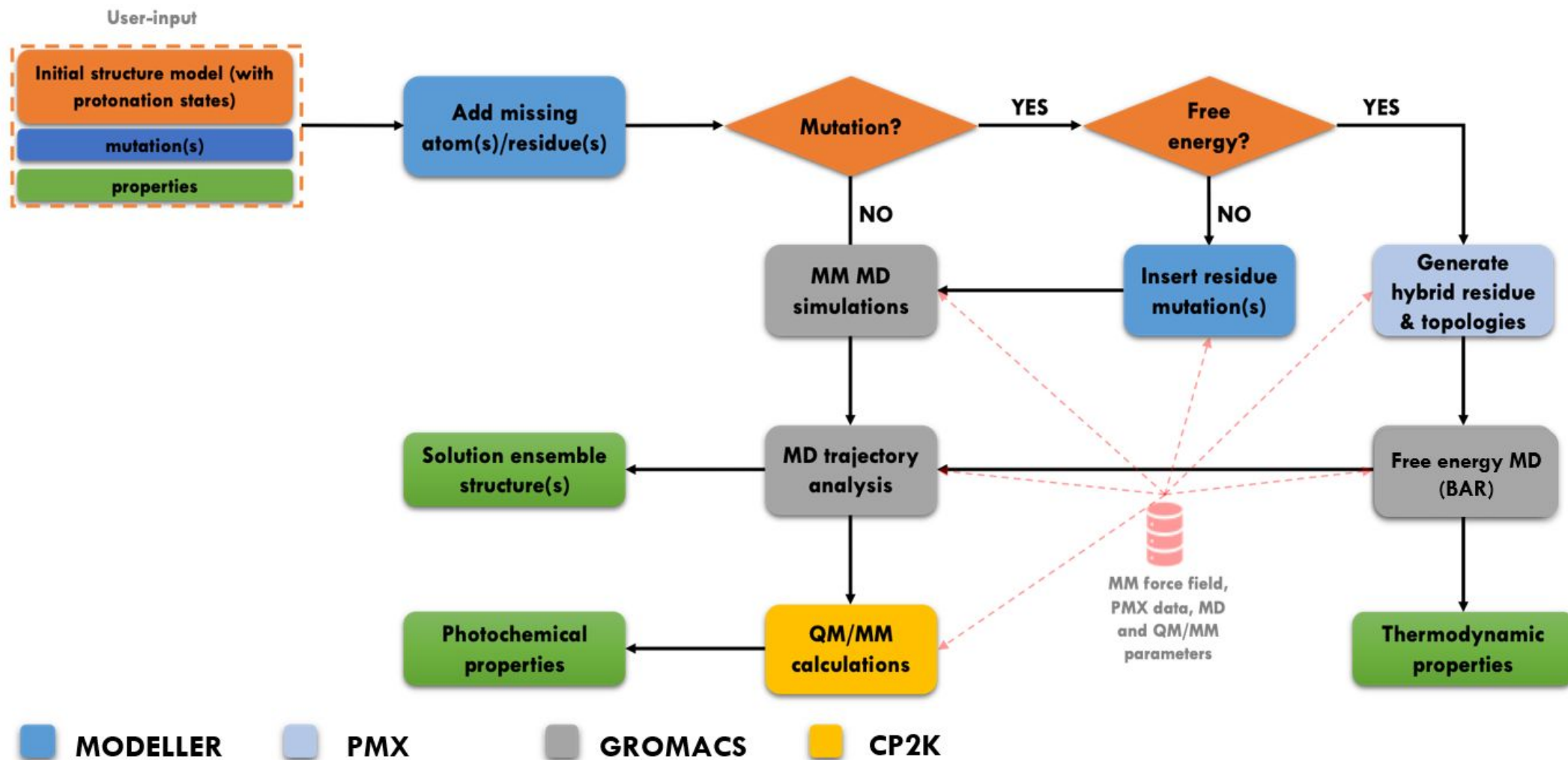
## Excitation and Emission Characteristics of Fluorescent Proteins

Fluorescent Protein	Excitation Wavelength (nm)	Emission Wavelength (nm)
wtGFP	395/475	510
ECFP	433	475
EGFP	488	507
EYFP	513	527
DsRed	558	583
B-PE	545/565	575
R-PE	410/545/565	578
APC	650	660



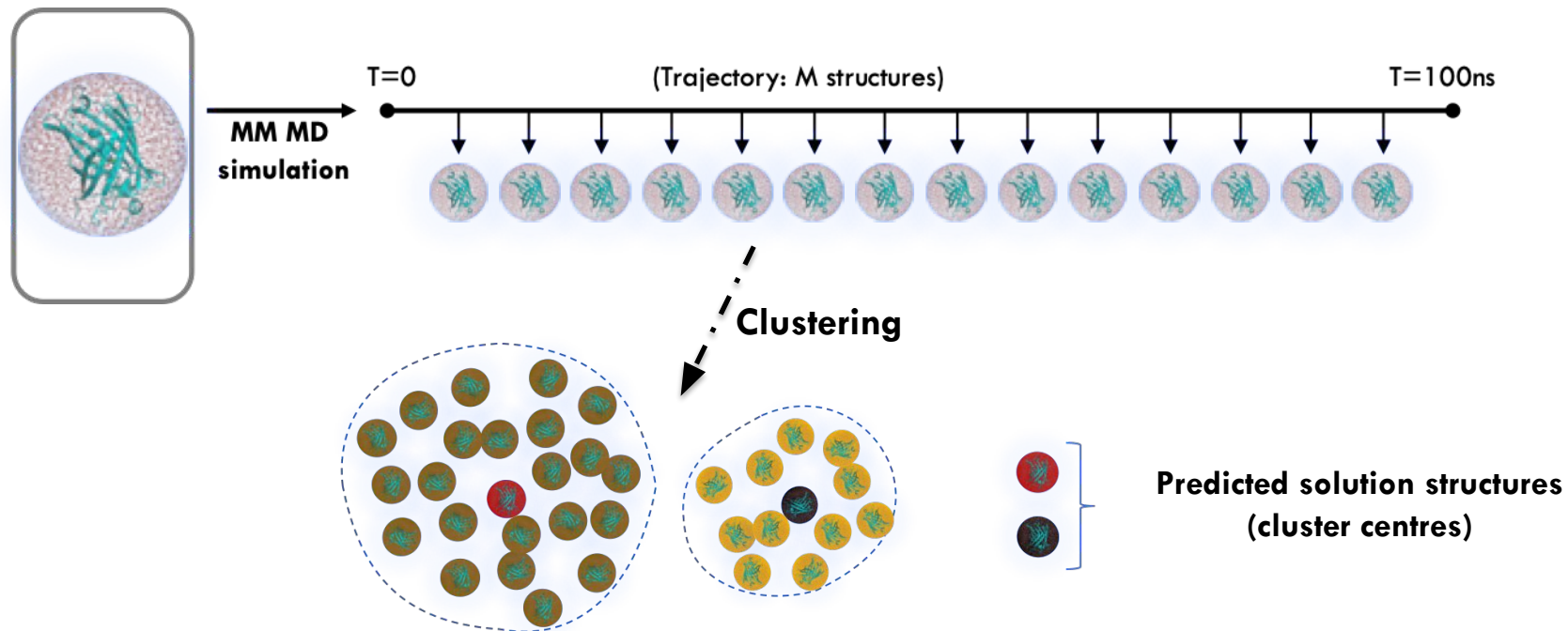
Development of task-specific FPs

# FluProCAD Workflow Overview



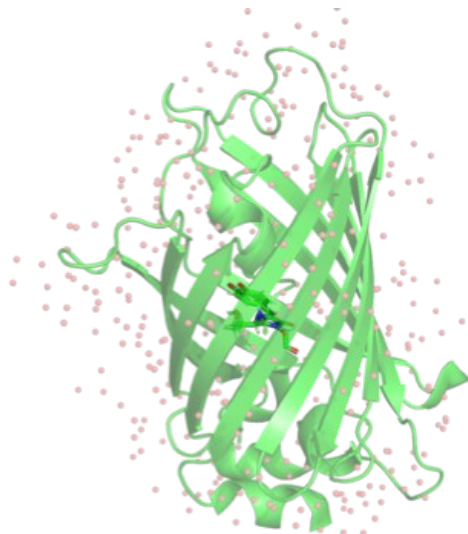
# Application I: Predicting Structures

- Pairwise RMSD for each structure
- Group neighbors within cut-off threshold
- Largest group forms a cluster and eliminated from the pool of  $M$  structures
- Iterate until pool is empty



# rsGreen0.7 protein mutants

## Starting structure



**rsGreen0.7 (eGFP variant)**

**PDB: 4XOW**

## Objectives

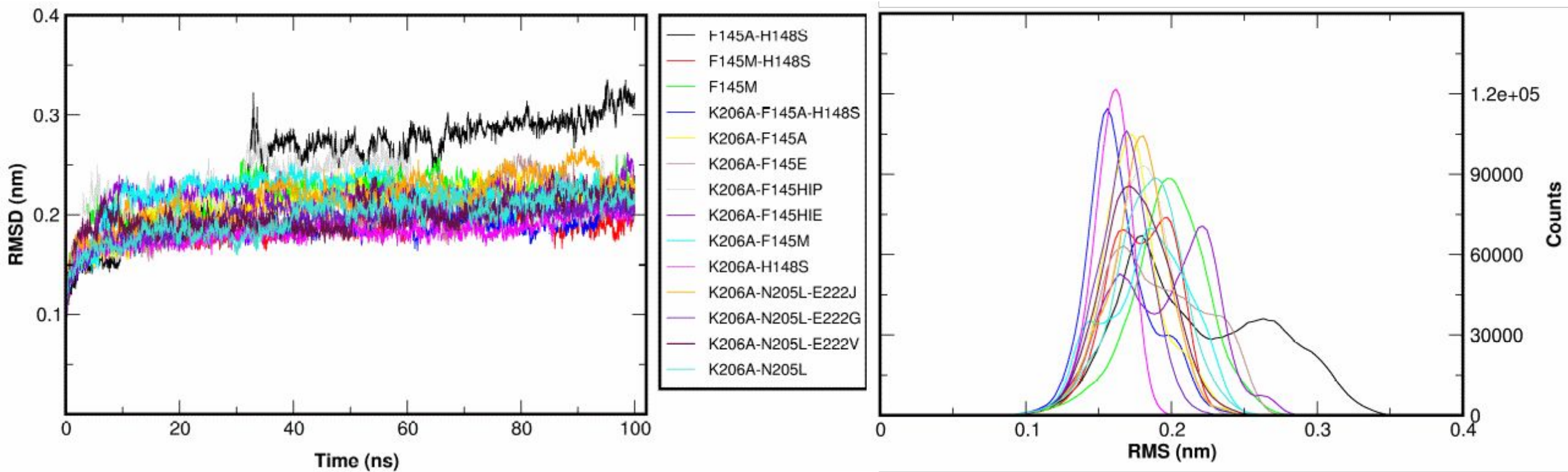
- Prepare mutant structures
- Classical MD equilibration
- Clusterization of the trajectory
- Blind check MD against crystal structures

## Mutants

1. K206A
2. K206A/F145H
3. K206A/F145M
4. K206A/H148G
5. K206A/E222G
6. K206A/E222V

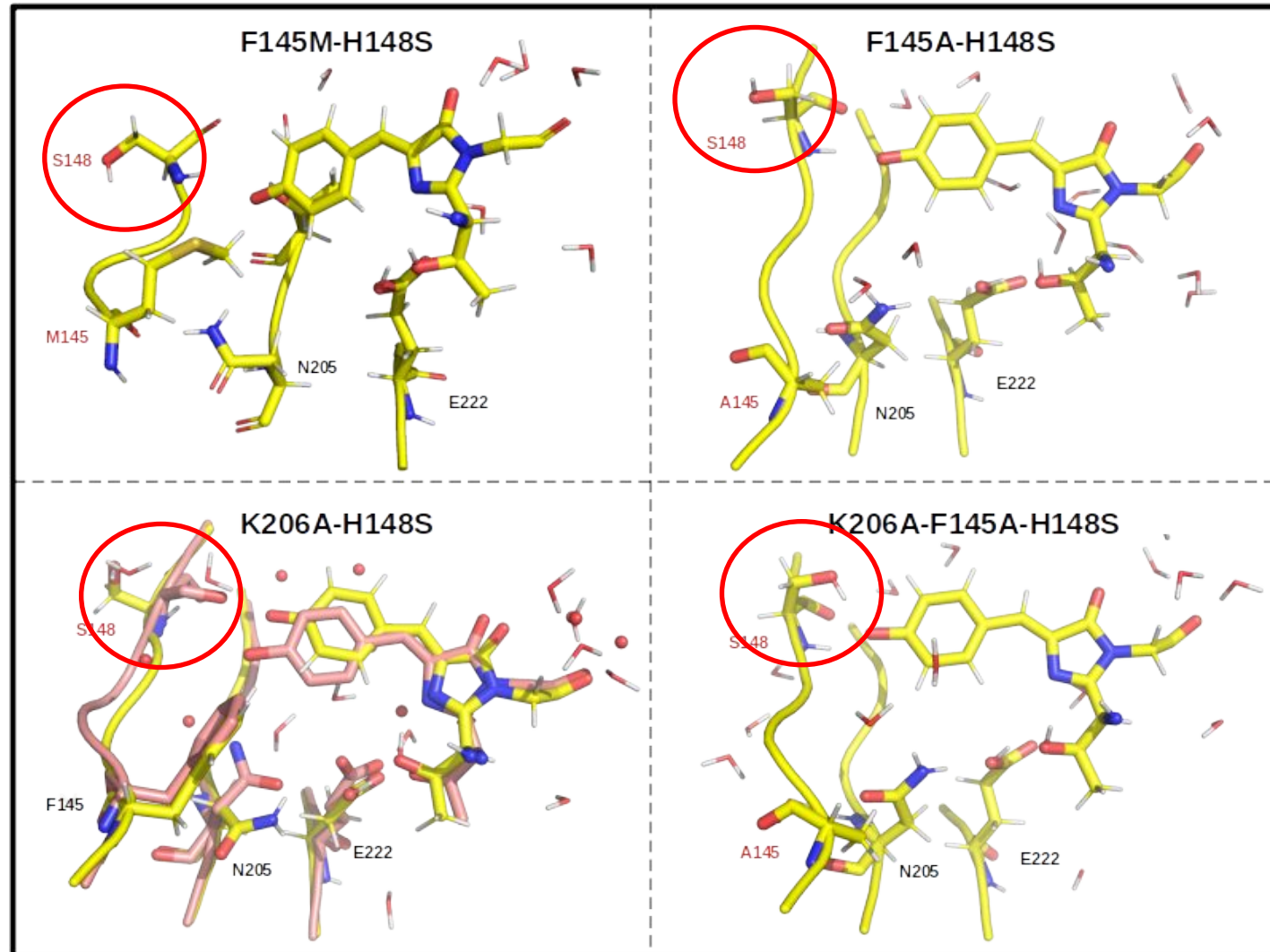
In total 14 mutants have been modelled

# Stability of the structures

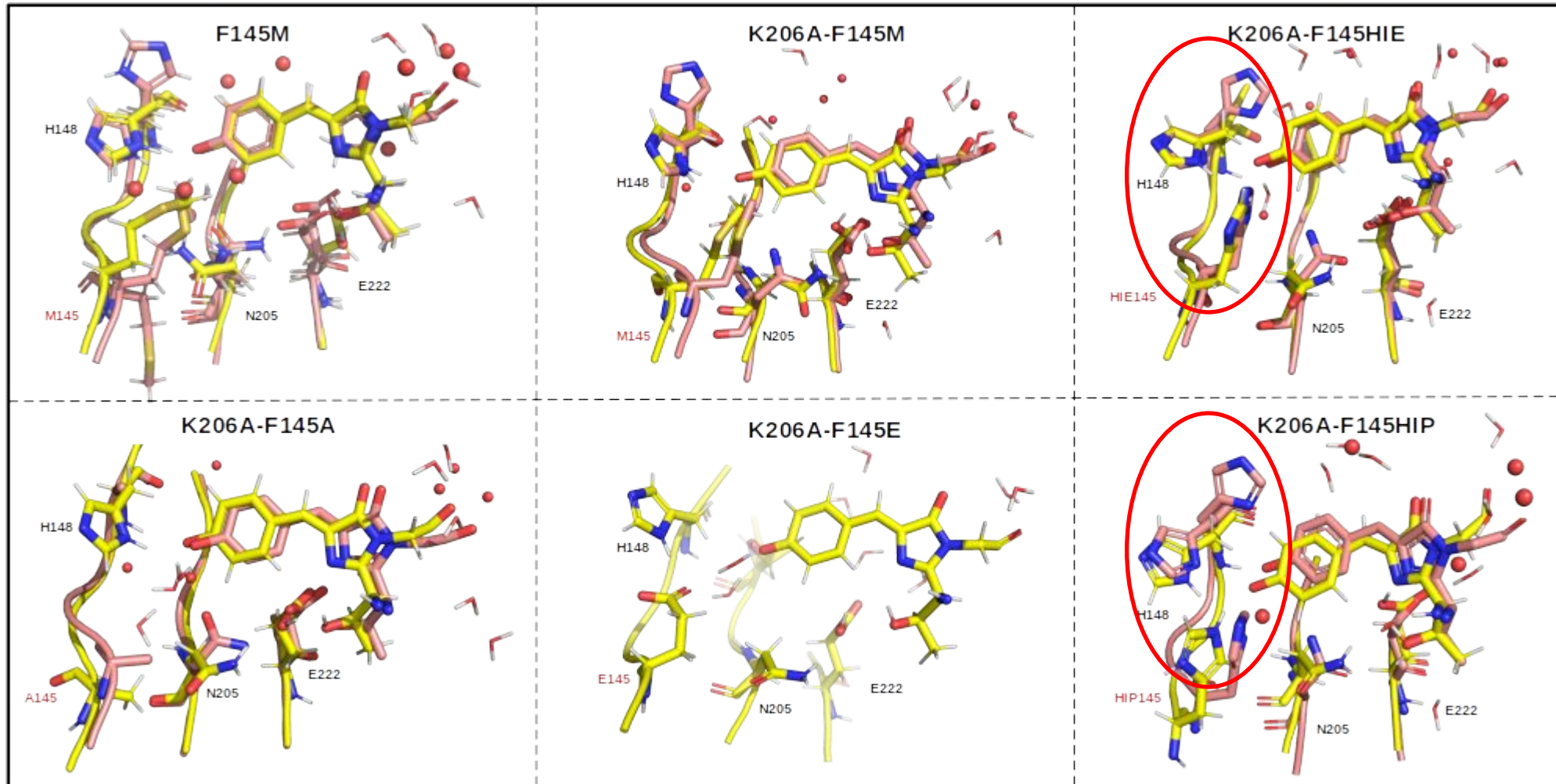




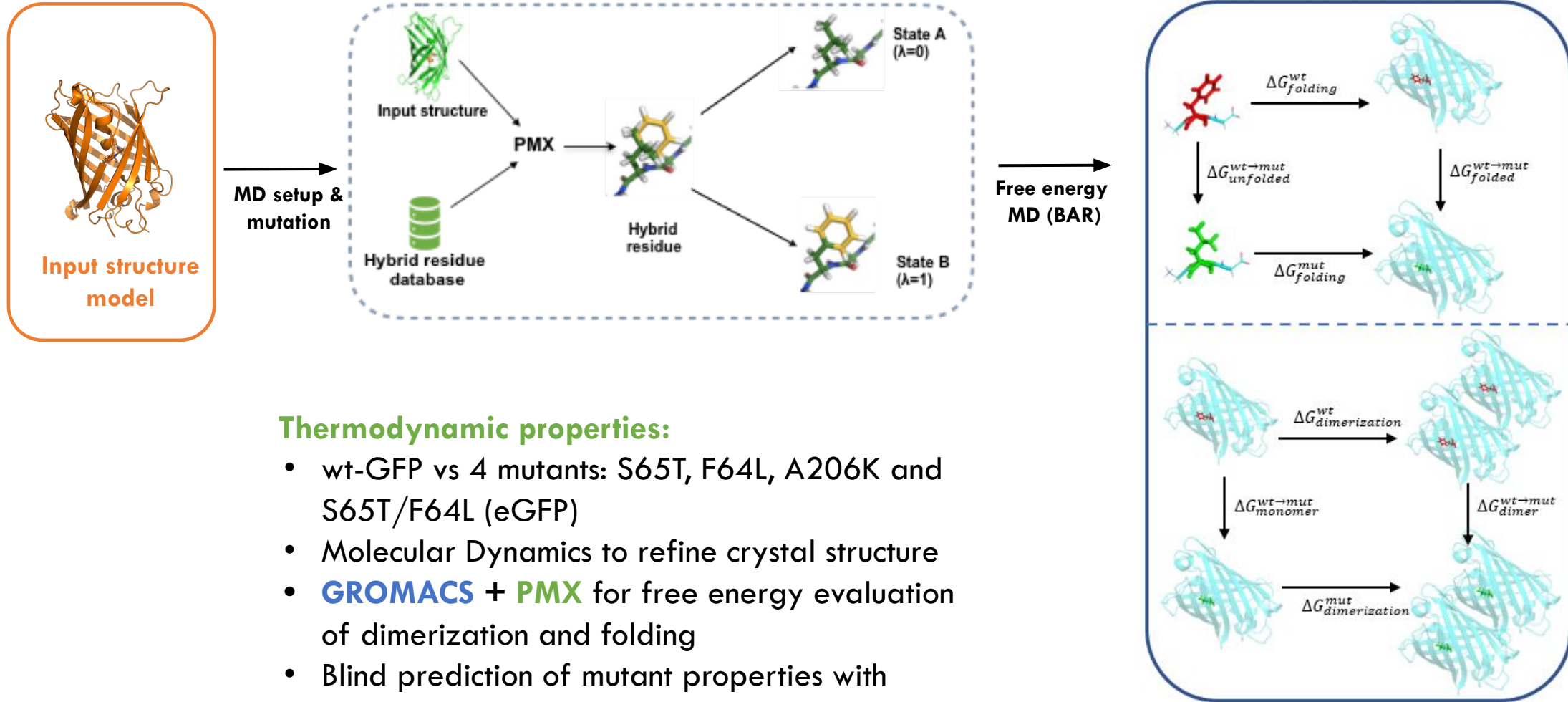
# Stability of the structures



# Stability of the structures



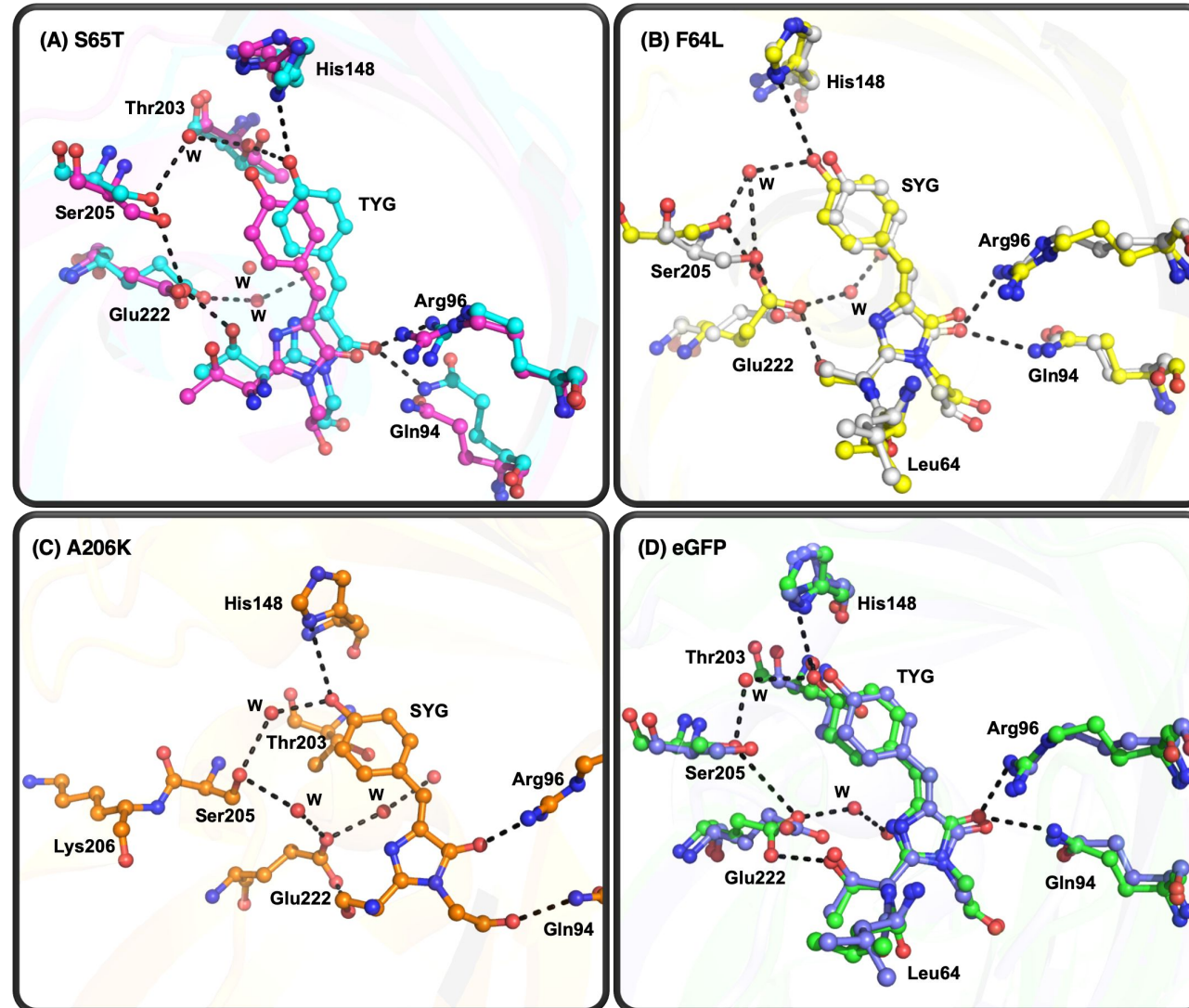
# Application II: Thermodynamic properties prediction



## Thermodynamic properties:

- wt-GFP vs 4 mutants: S65T, F64L, A206K and S65T/F64L (eGFP)
- Molecular Dynamics to refine crystal structure
- **GROMACS** + **PMX** for free energy evaluation of dimerization and folding
- Blind prediction of mutant properties with unknown structure

# Structures of the mutants



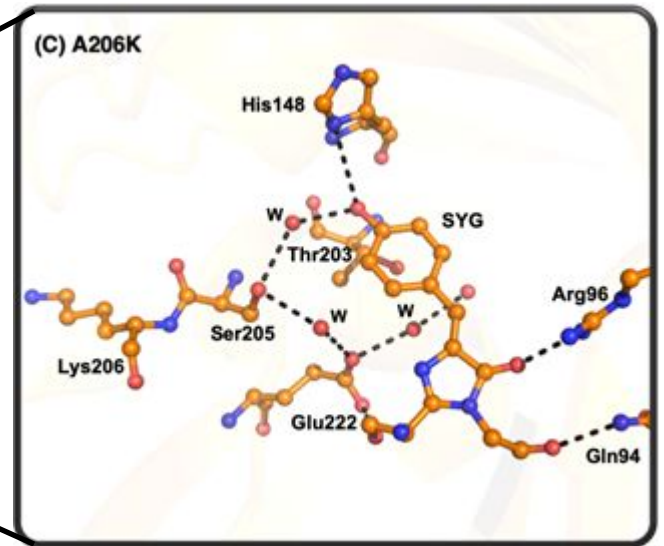
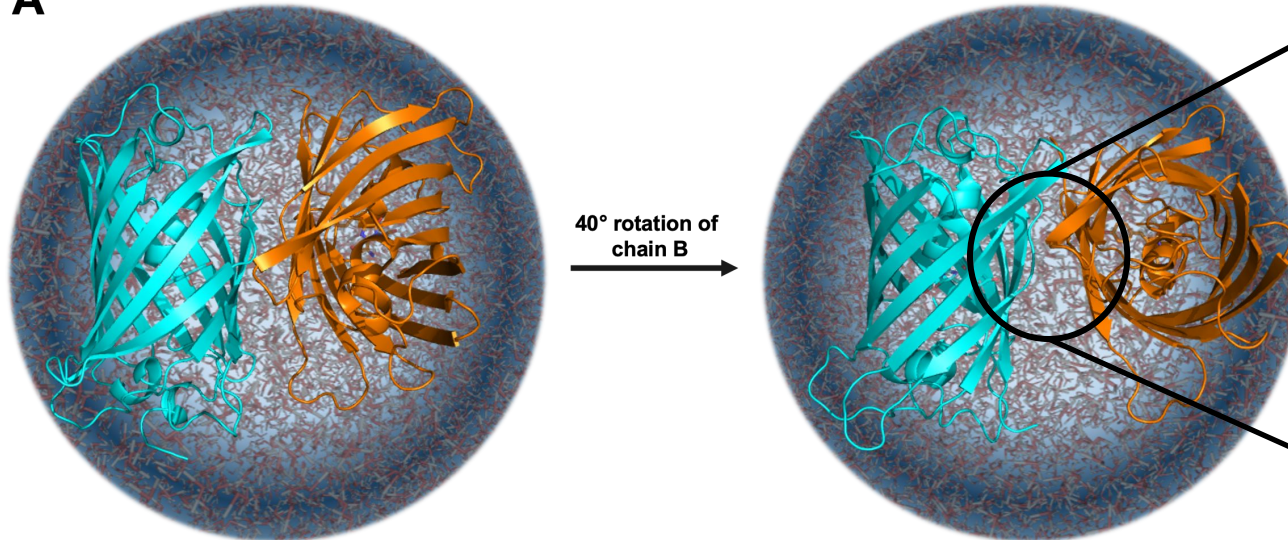
# Free energies of dimerization and folding

GFP MUTANTS	$\Delta\Delta G$ (kcal/mol)	
	Folding	Dimerization
S65T	6.399 ( $\pm 0.8393$ )	-8.241 ( $\pm 0.9198$ )
F64L	<b>-3.726</b> <b>(<math>\pm 0.2041</math>)</b>	-1.955 ( $\pm 0.4097$ )
A206K	3.457 ( $\pm 0.1529$ )	<b>10.481</b> <b>(<math>\pm 0.5241</math>)</b>
eGFP (F64L/S65T)	10.125 ( $\pm 0.2927$ )	-4.789 ( $\pm 0.6471$ )

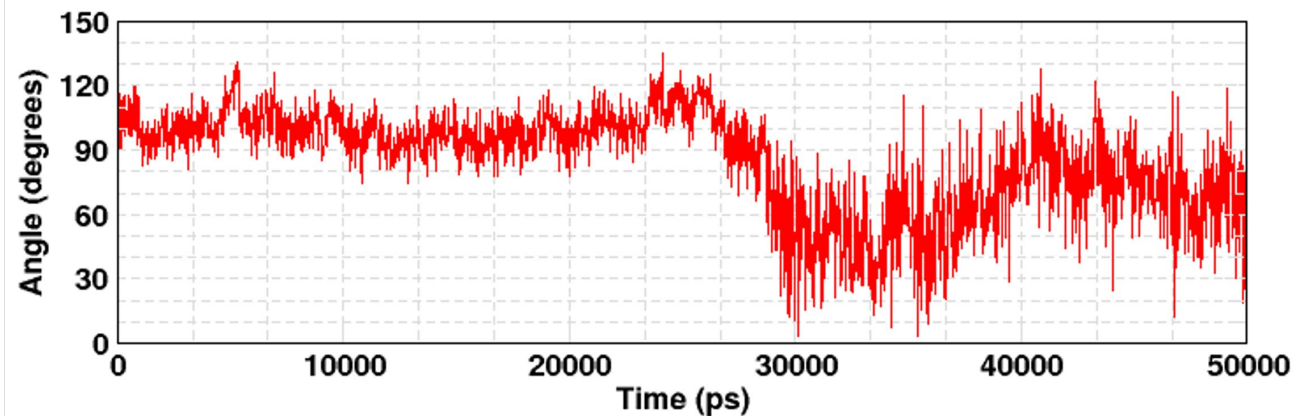
- [F64L](#): improved stability
- [A206K](#): Prefers to be in monomeric form
- Validated MM-MD models against known crystal structures.
- Predict solution structures for (un)-known mutants with no crystallographic data (K206A).

# Why A206K prefer to be monomer?

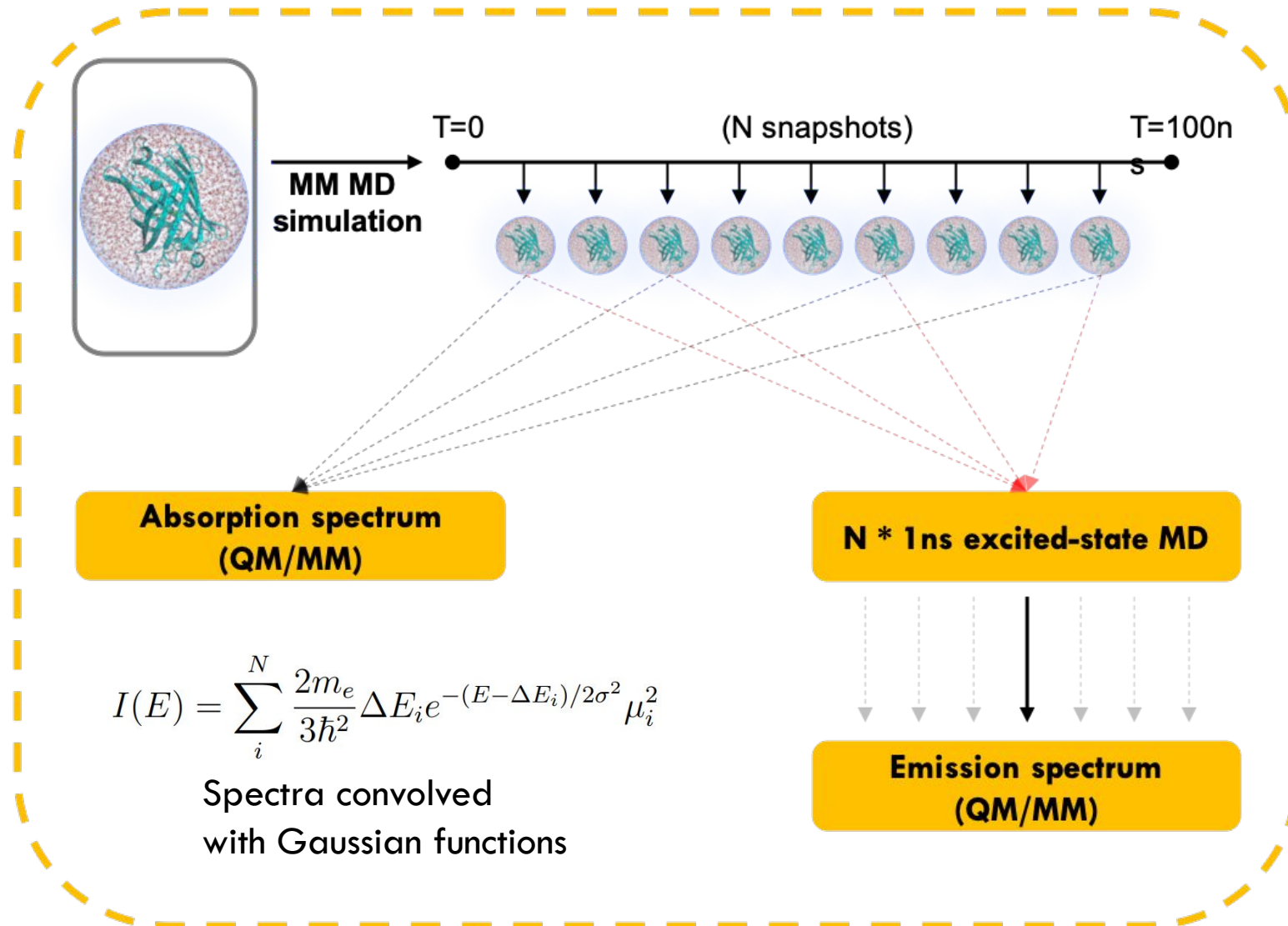
**A**



**B**



# Application III: Photochemical Properties



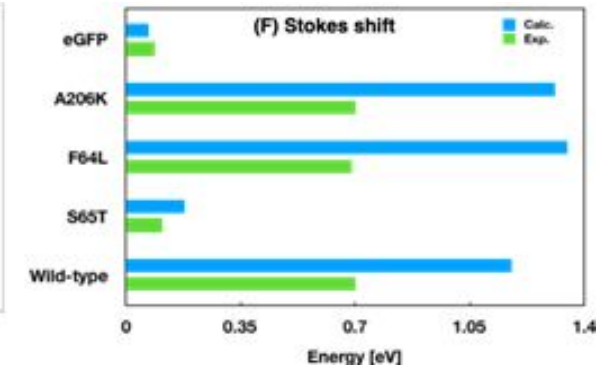
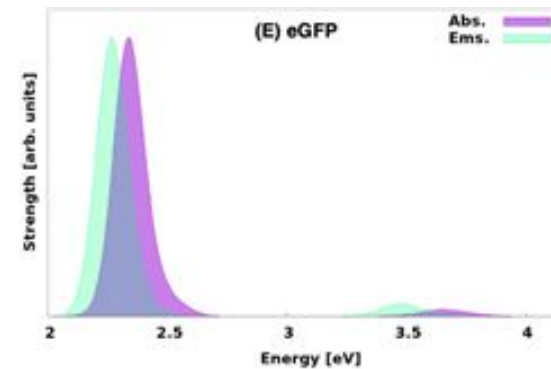
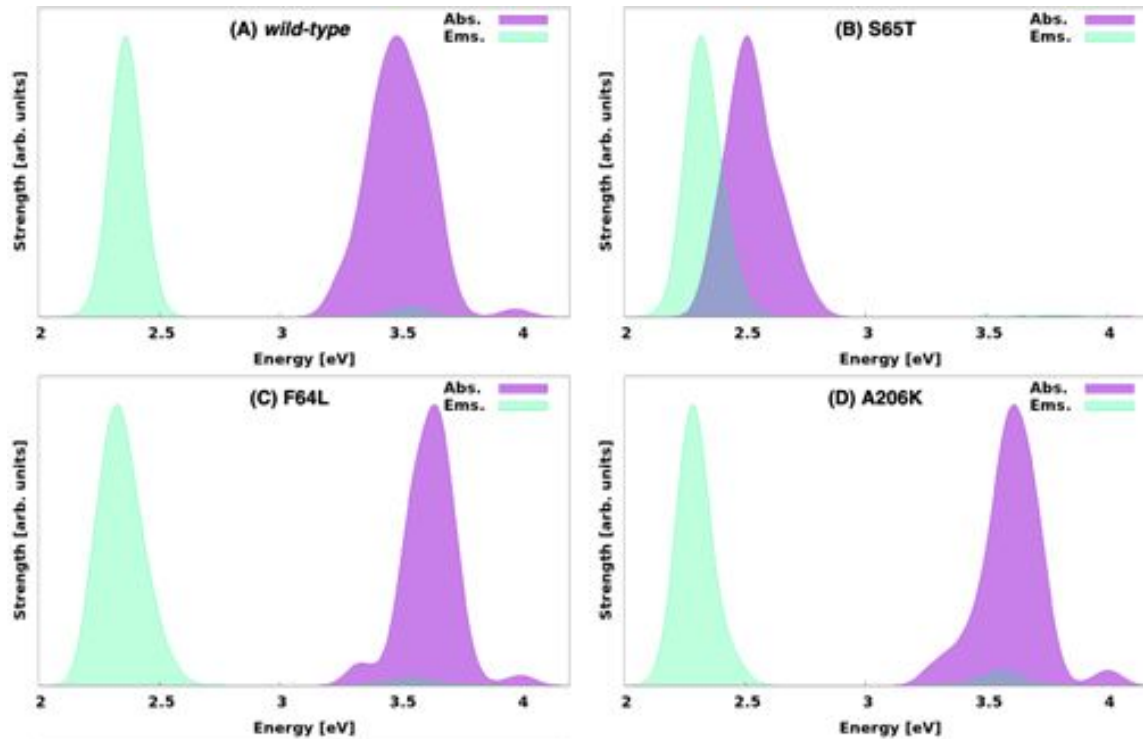
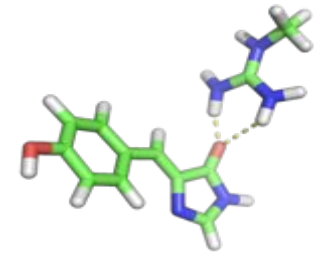
# Spectra of *wt*GFP and 4 mutants

**GROMACS + CP2K**

**QM subsystem : CRO + Arg96**

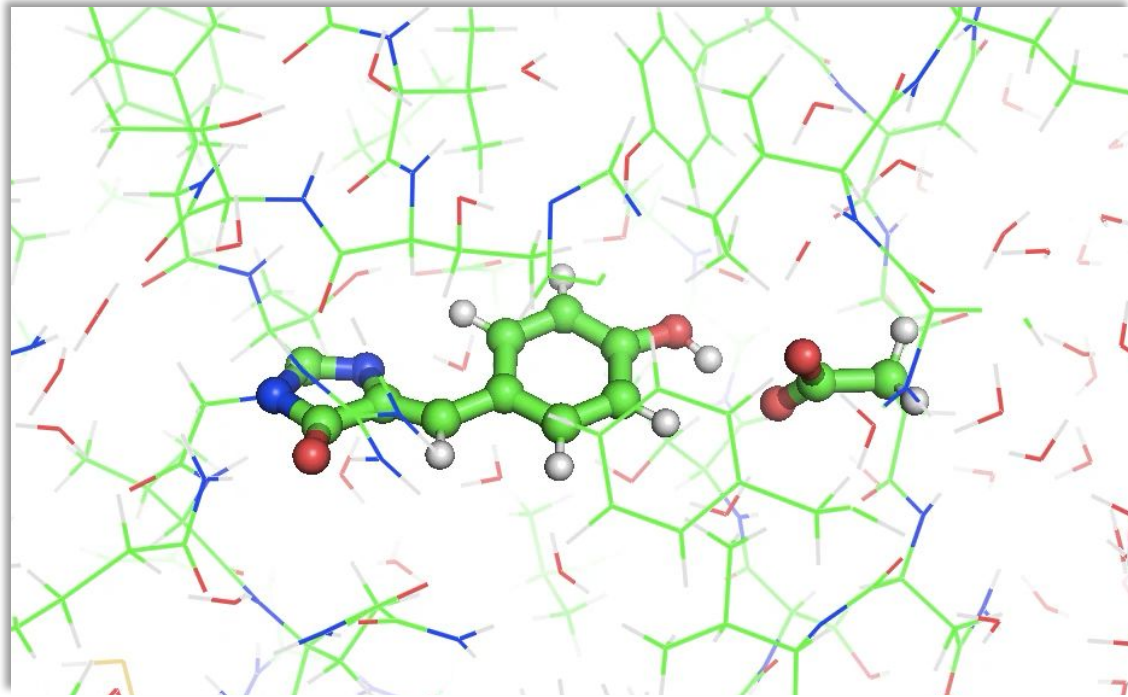
**Functional: BLYP**

**Basis: DZVP-MOLOPT-GTH**





# Outlook: excited state reactions



## **GROMACS + CP2K**

Ultra-fast proton transfer in  
GFP mutant S65T/H148D.

**QM subsystem** : Chromophore + Asp148

**Method**: TD-DFT

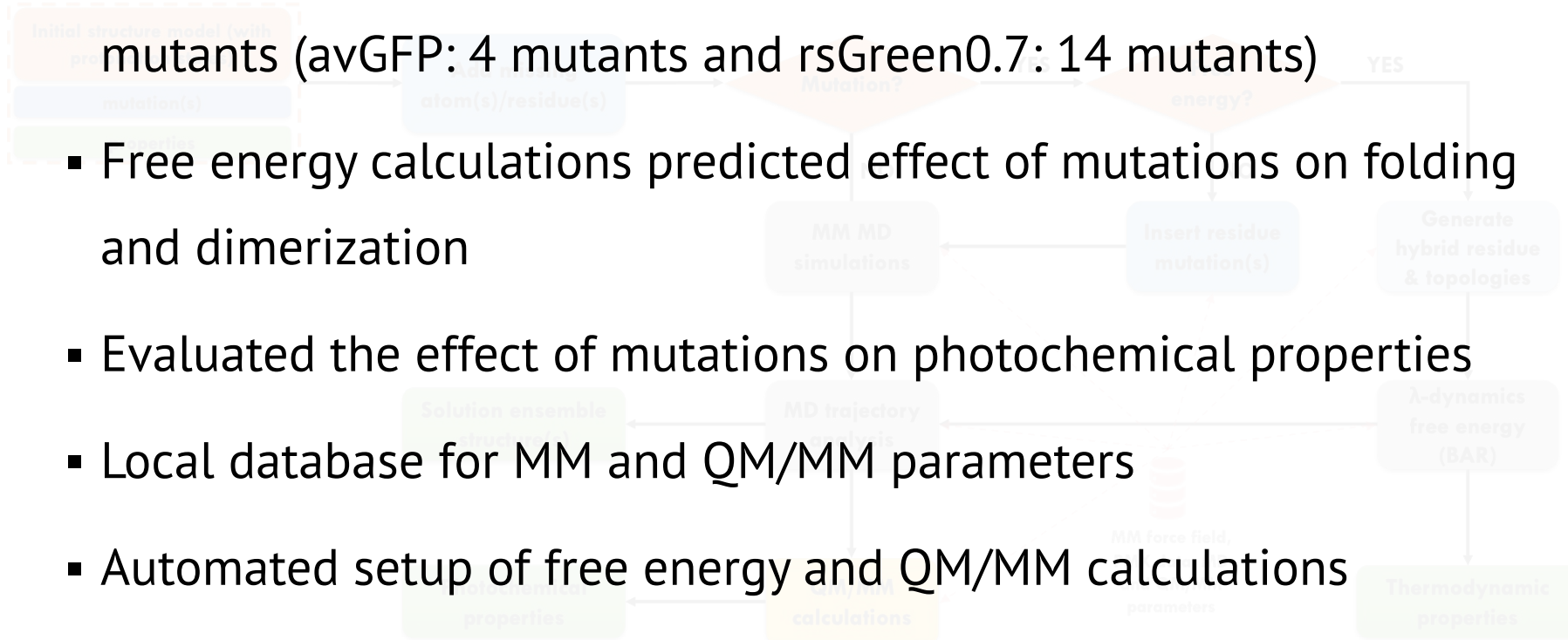
**Functional**: PBE

**Basis**: DZVP-MOLOPT-GTH

**Software**: GROMACS-CP2K

# Summary

- MD simulations + Clustering predicts solution structures for FP mutants (avGFP: 4 mutants and rsGreen0.7: 14 mutants)
- Free energy calculations predicted effect of mutations on folding and dimerization
- Evaluated the effect of mutations on photochemical properties
- Local database for MM and QM/MM parameters
- Automated setup of free energy and QM/MM calculations
- Computational mutagenesis protocol for fluorescent proteins



# Acknowledgements



## Collaborators:

Prof. Peter Dedecker

Dr. Elke De Zitter

Prof. Jeremy Harvey



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