

EURO^{4SEE}

Generating Mutant Protein Structures Using Advanced Techniques

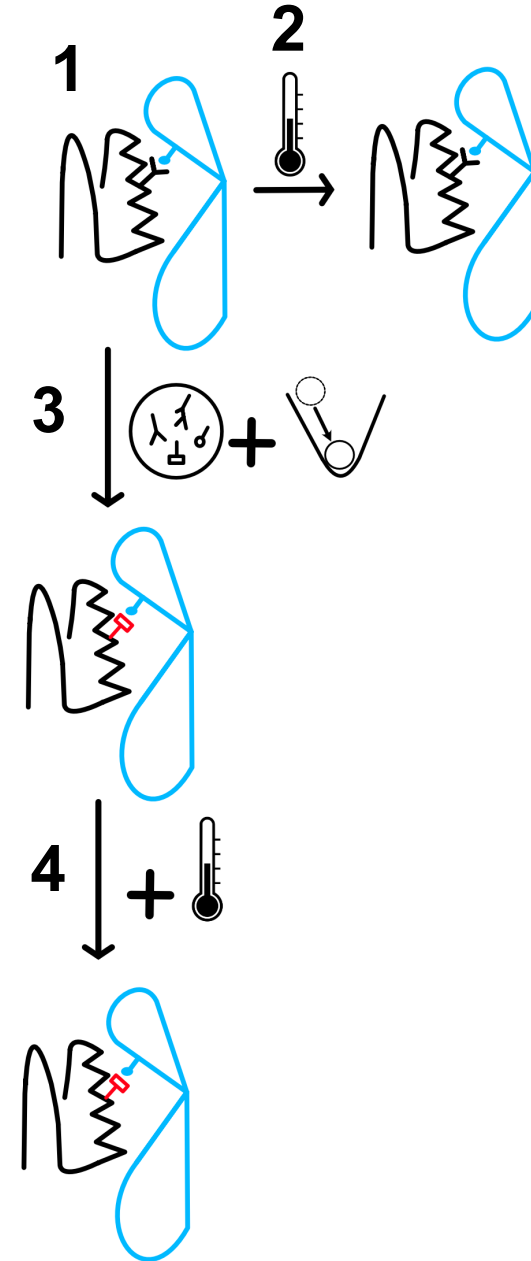
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Mutation and Minimization (MuMi)

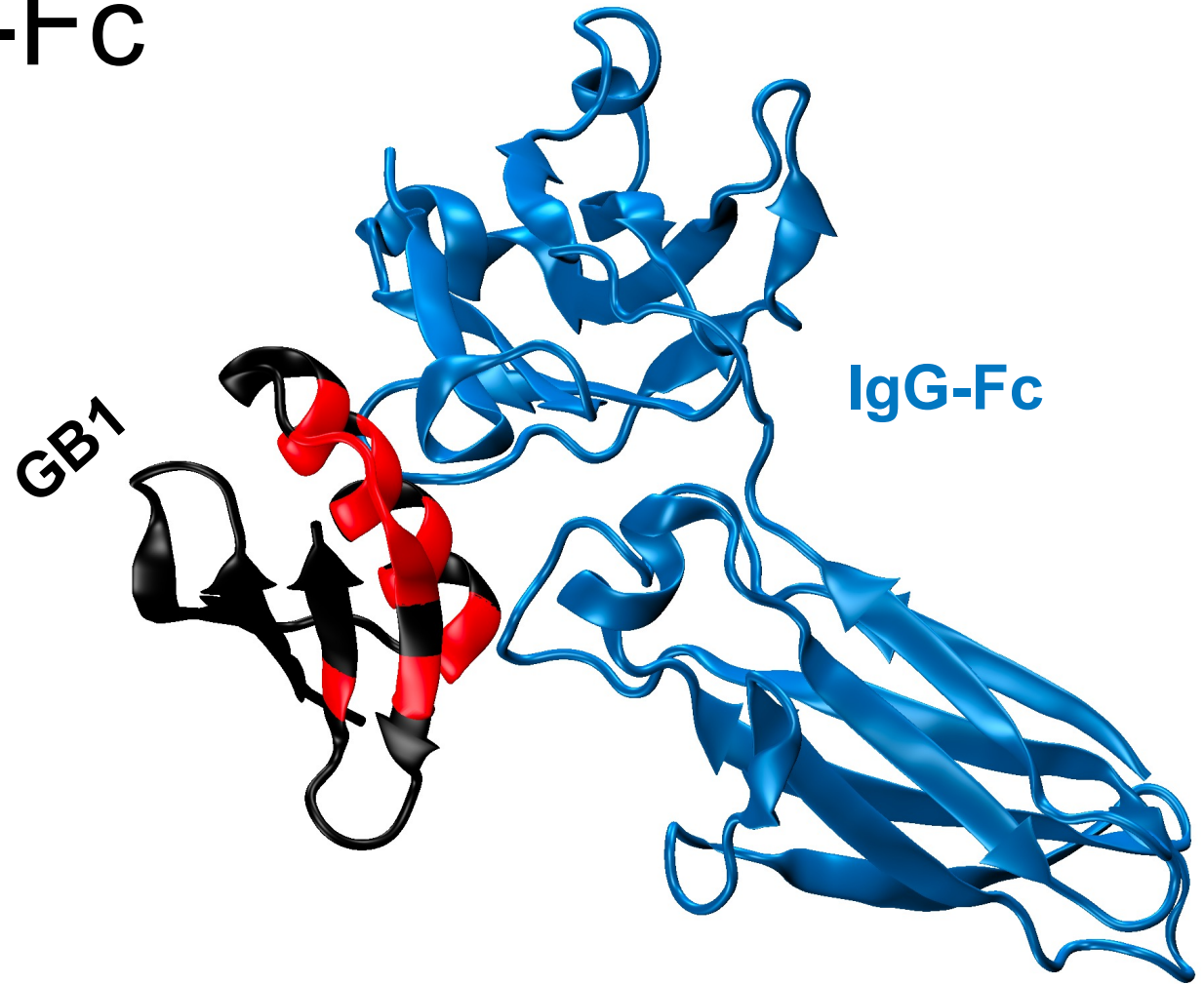
Outline

1. GB1-IgG-Fc complex
2. Molecular dynamics simulation of wild-type (**WT-MD**)
3. Mutation and Minimization (**MuMi**) scheme
4. Extension of 1-ns-long MD simulation for MuMi (**MuMi-Dyn**)



GB1 and its partner IgG-Fc

- GB1 is a 56 residue-long protein
- Binds to IgG-Fc
- Deep mutational scanning (DMS) experiments are extensively conducted

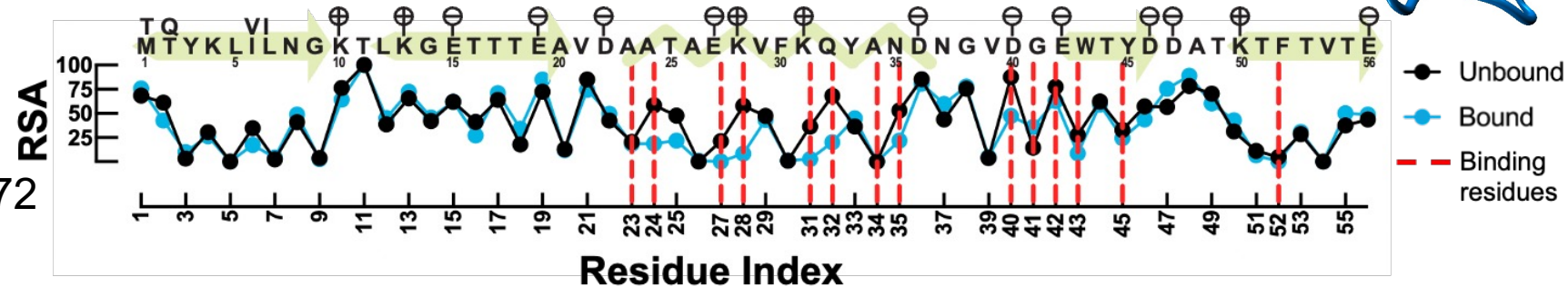
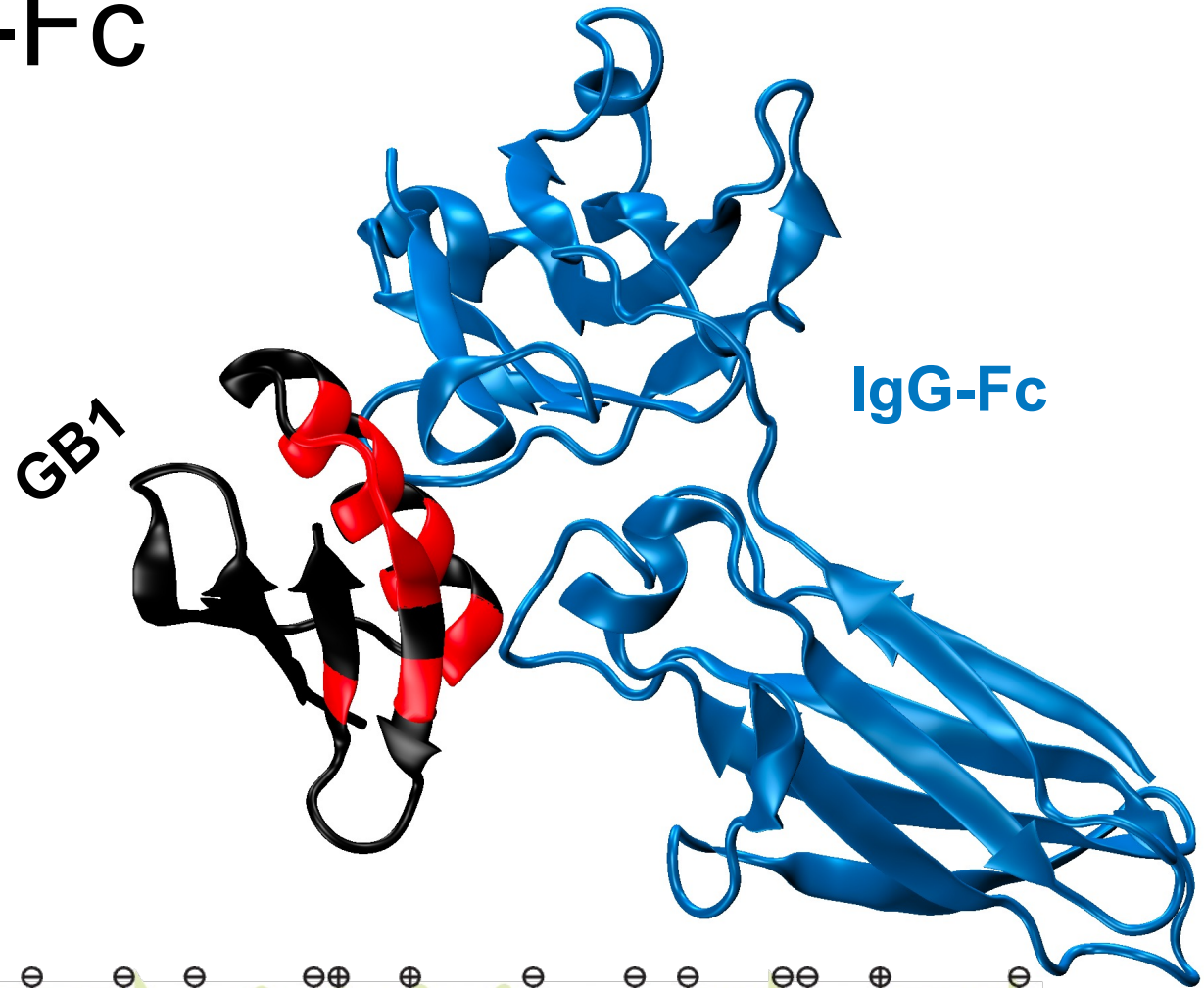


Olson *et al.*, 10.1016/j.cub.2014.09.072

Otwinowski, 10.1093/molbev/msy141

GB1 and its partner IgG-Fc

- GB1 is a 56 residue-long protein
- Binds to IgG-Fc
- Deep mutational scanning (DMS) experiments are extensively conducted
- Mutating residues at the binding cavity disrupts the binding
- Hydrogen bonds, relative solvent accessibility (RSA)?



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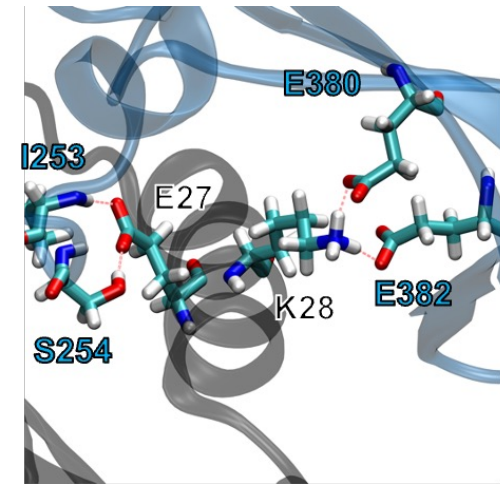
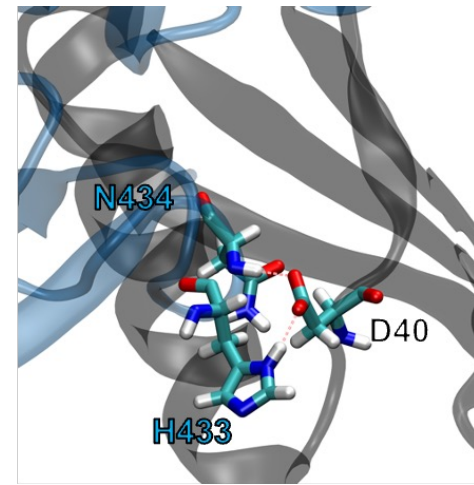
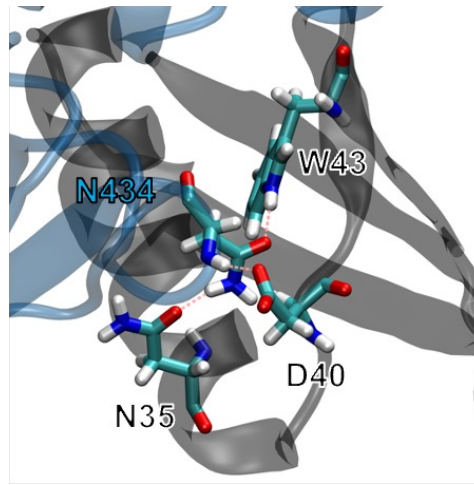
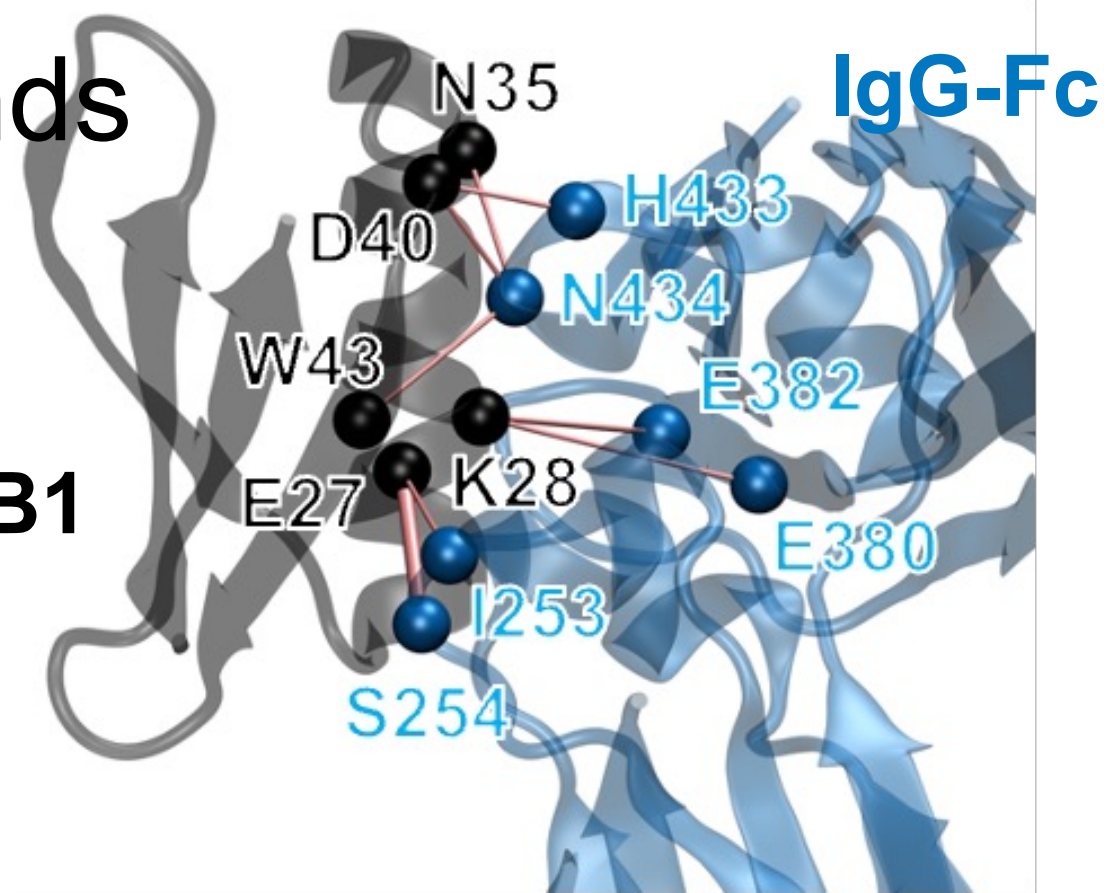
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Focusing on hydrogen bonds between GB1 and IgG-Fc

- Two 1- μ s-long MD simulation of bound form of WT GB1(WT-MD) using 1fcc PDB coded structure
- We found **9 dominant hydrogen bonds** with high occupancy between GB1 and partner
- Further, we search for these specific hydrogen bonds in single mutants of bound GB1 by using **MuMi**

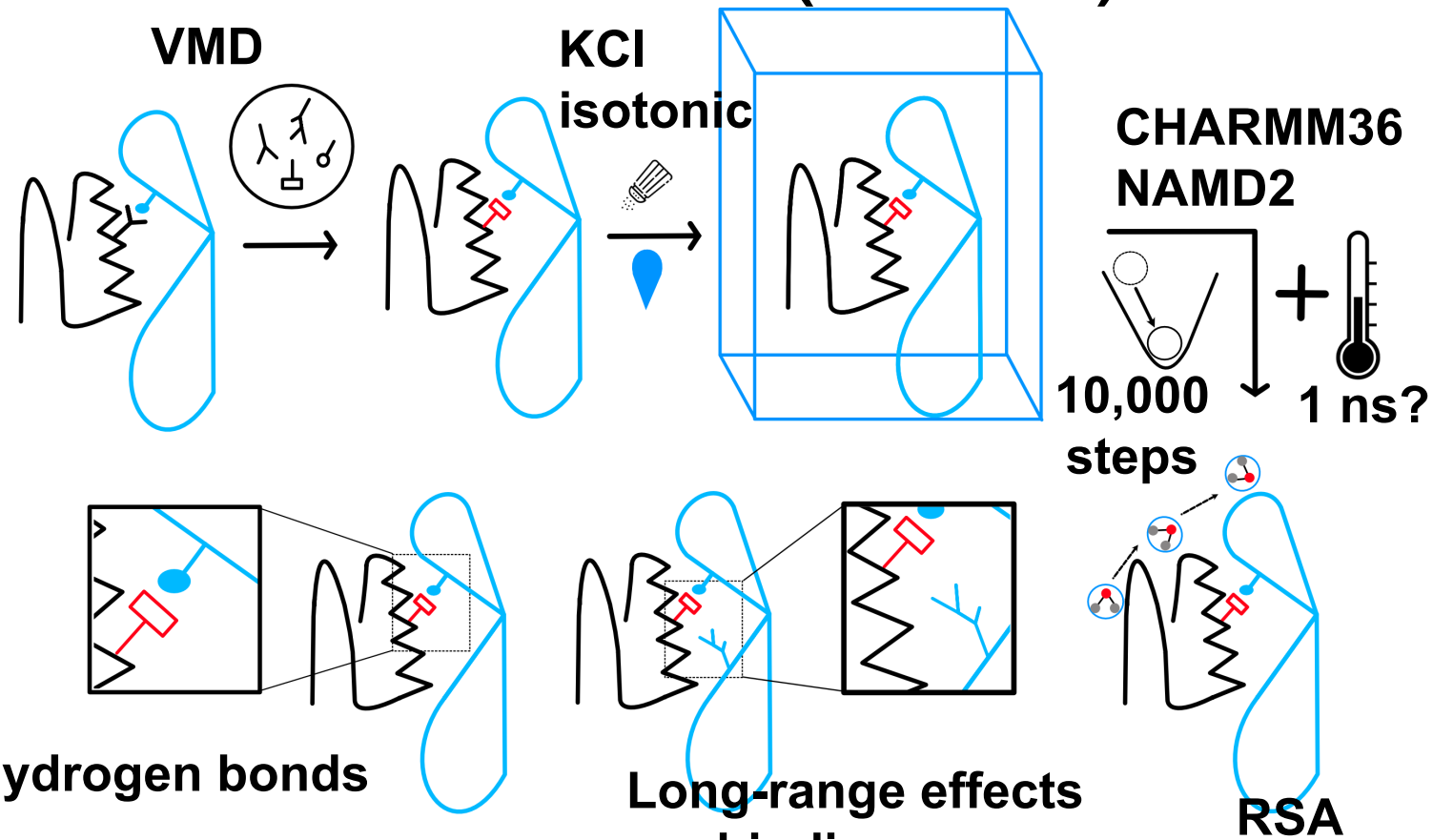
GB1

IgG-Fc



Mutation and Minimization scheme (MuMi)

- Starting from WT structure we generated all possible **single mutations** ($56 \times 19 = 1064$), solvate, ionize and perform minimization



Search for WT dominant hydrogen bonds in mutant structures to explain mutational landscape of binding

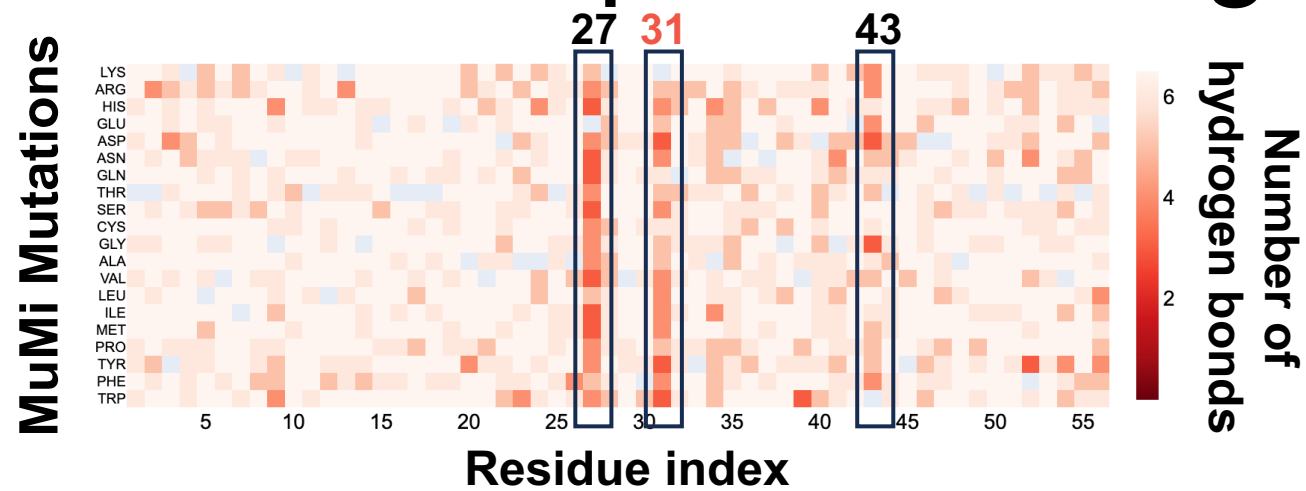
Find if other residues are important for binding in WT-MD or MuMi

Compare the structures

https://github.com/midstlab/MuMi_scheme

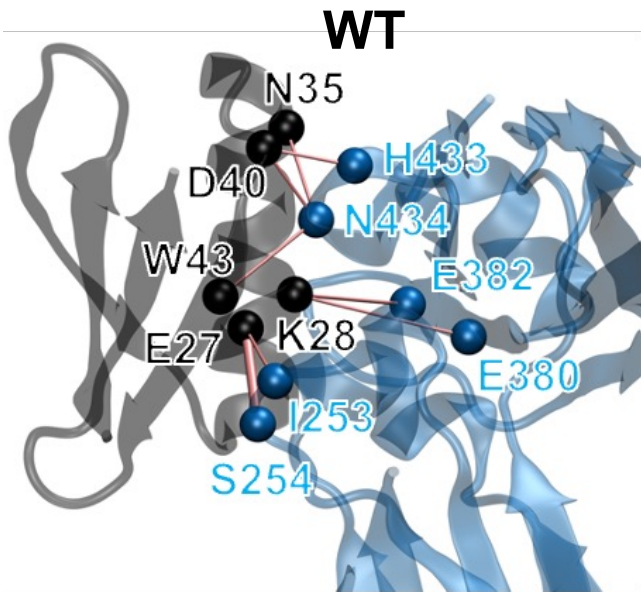
MuMi explains the fitness landscape of binding

The number of dominant WT hydrogen bonds between GB1 and IgG-Fc varies between 3-8 in single-mutants.



Hydrogen bond occupancies of WT-MD

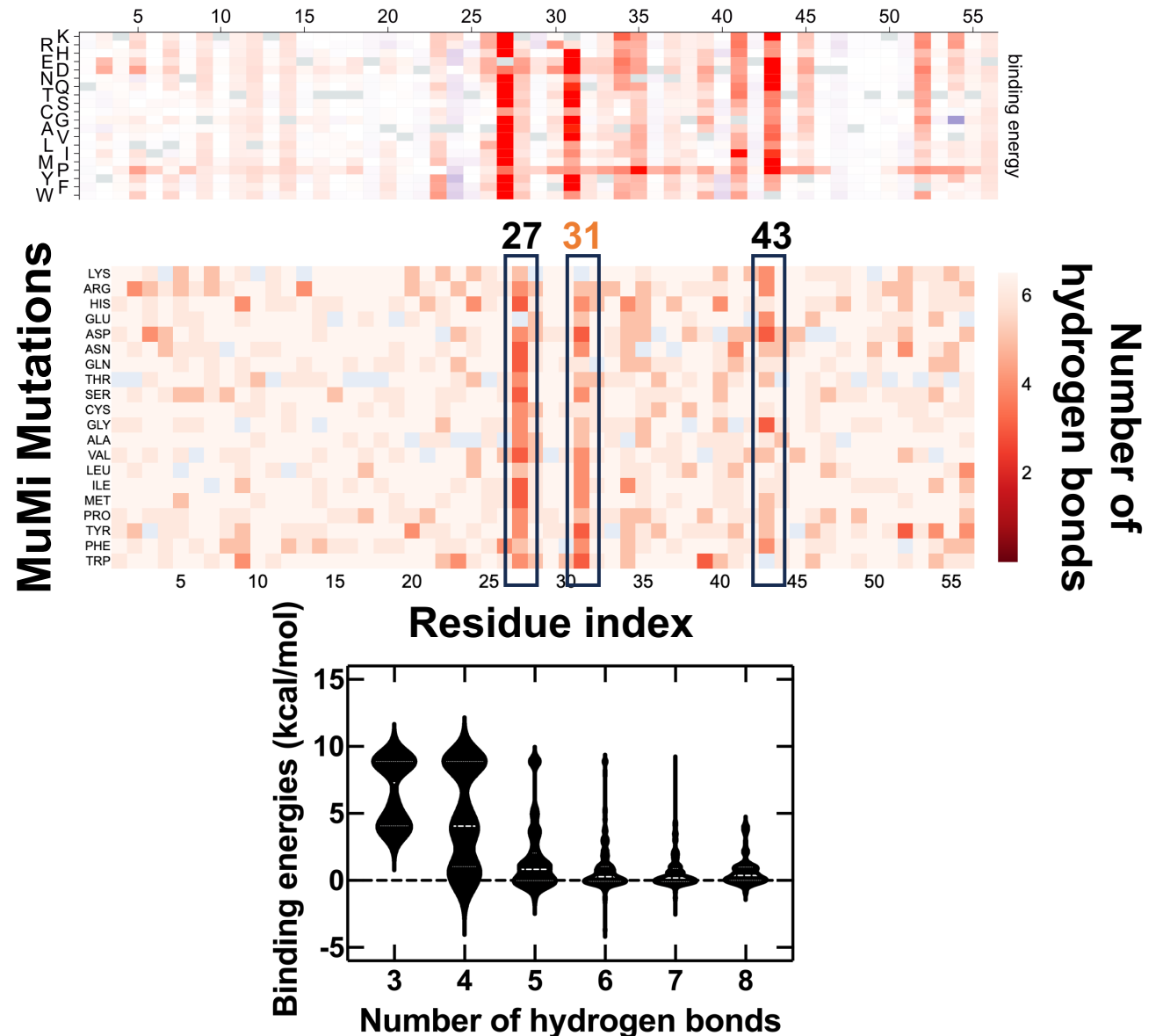
WT-MD		
GB1	IgG-Fc	Occupancy
E27-SC	I253-BB	39%
E27-SC	S254-SC	74%
E27-SC	S254-SC	43%
K28-SC	E380-SC	59%
K28-SC	E382-SC	54%
N35-SC	N434-SC	23%
D40-SC	H433-SC	38%
D40-SC	N434-BB	37%
W43-SC	N434-SC	44%



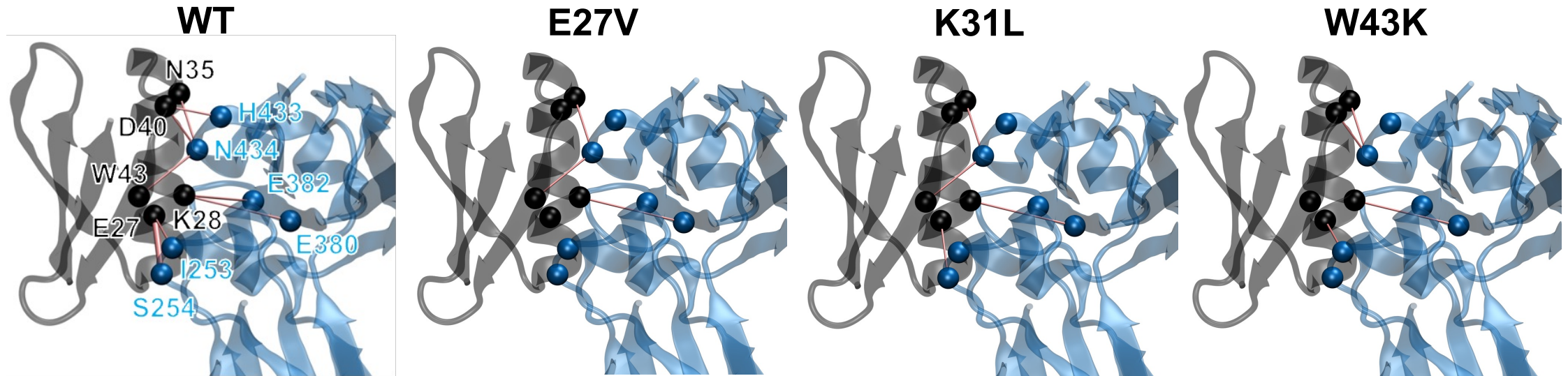
MuMi explains the fitness landscape of binding

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- The number of dominant WT hydrogen bonds between GB1 and IgG-Fc varies between 3-8 in single-mutants.
- Comparing hydrogen bonds with the experimental binding energies shows the similarity

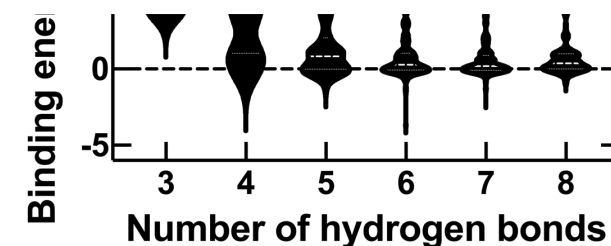


MuMi explains the fitness landscape of binding



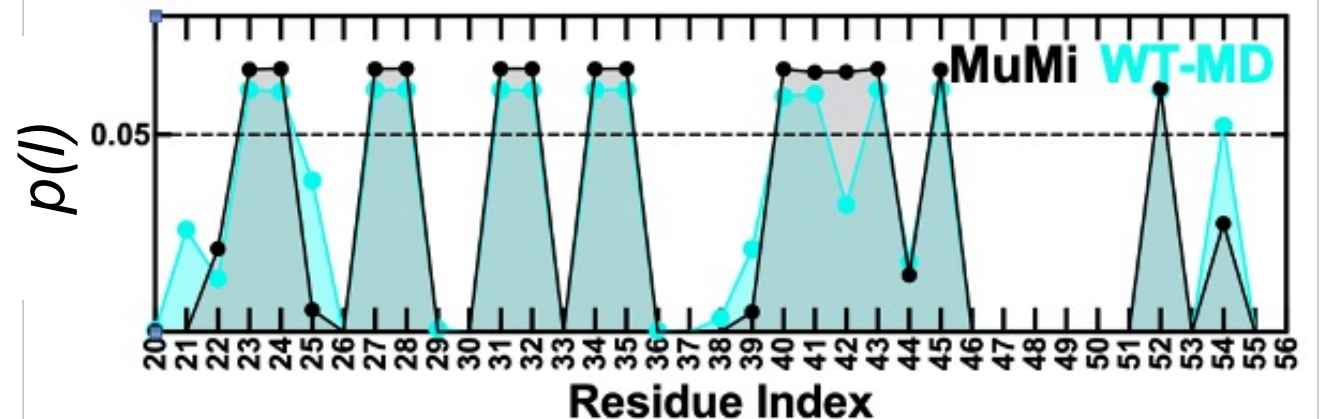
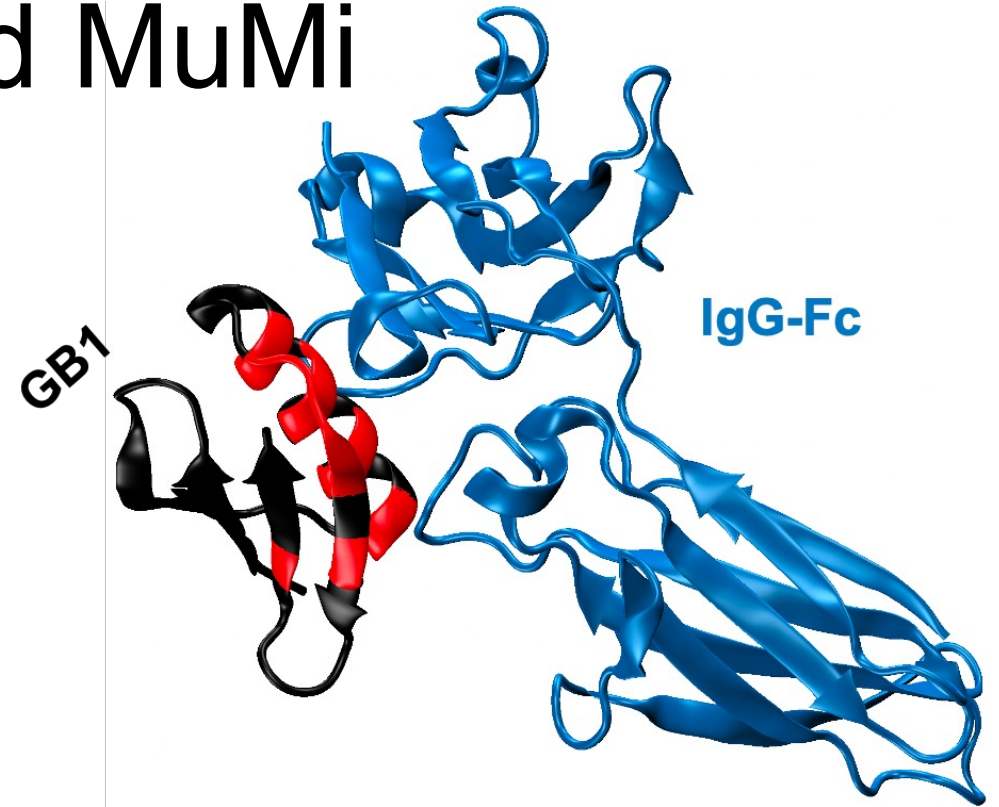
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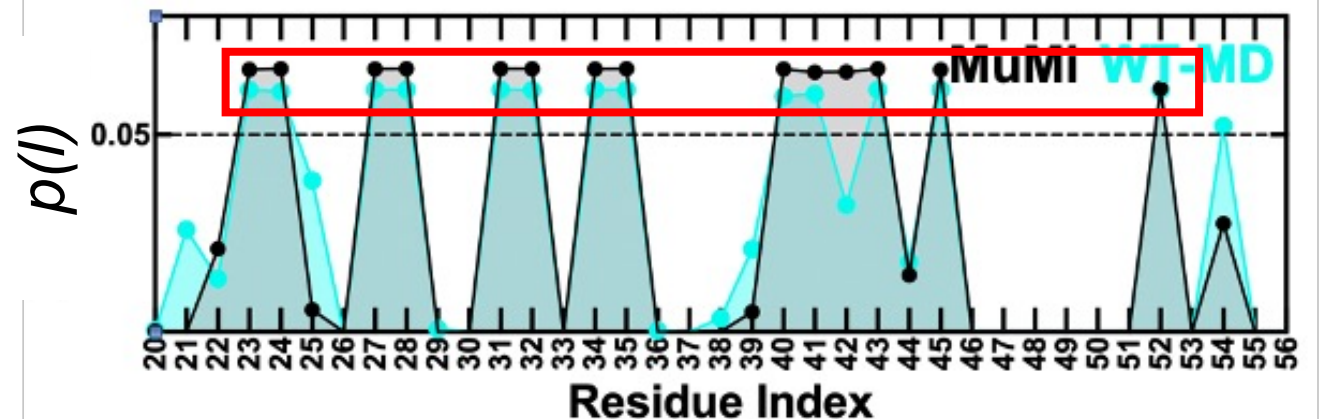
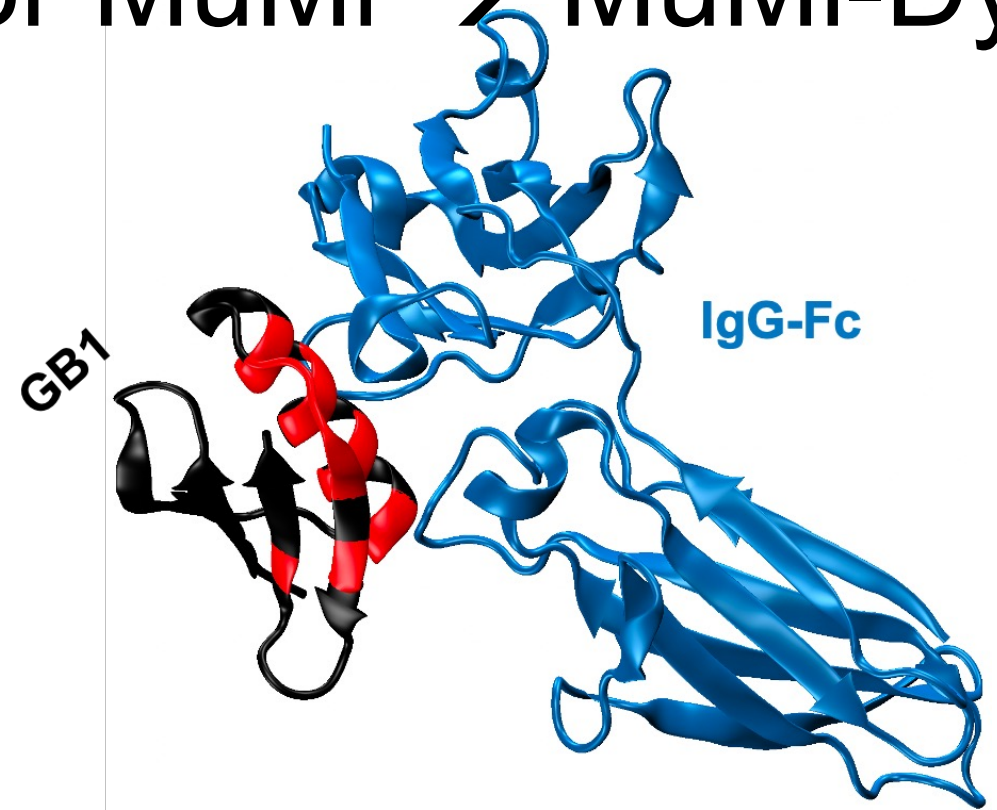
Residues at the binding region do not differ significantly in WT-MD and MuMi

- We investigated the residues of GB1 that have at least one heavy atom close ($< 5 \text{ \AA}$) to the partner for WT-MD (2,000 frames) and MuMi (1064 mutant structures)
- The probability of being located at binding region shows that general behavior does not change drastically



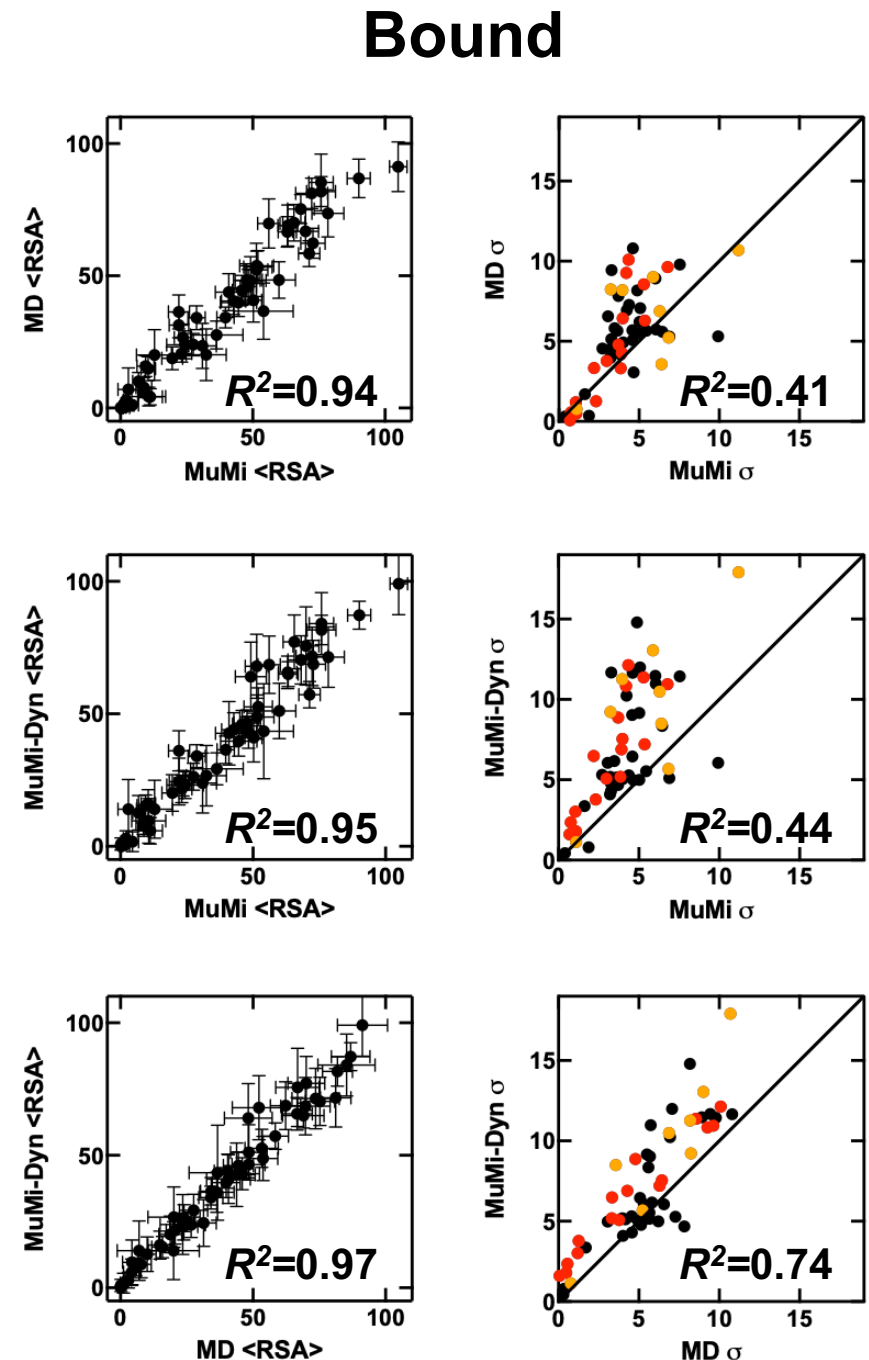
1-ns-long MD extension for MuMi → MuMi-Dyn

- For these 14 positions ($14 \times 19 = 266$) at the binding region, we performed 1-ns-long MD simulation to assess the thermal fluctuations
- We compared WT-MD, MuMi and MuMi-Dyn generated conformations by using relative solvent accessibility (RSA)



Comparison of WT-MD, MuMi and MuMi-Dyn

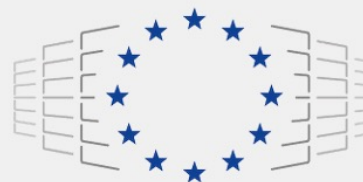
- Our results show that MuMi-generated conformations are in agreement with the WT-MD and MuMi-Dyn
- Although overall solvent accessibility does not change, slight changes in hydrogen bonding between GB1 and IgG-Fc affects the optimum (WT) binding specificity



Thanks!



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