MOLECULAR MODELING OF INTERACTIONS BETWEEN S-GATIFLOXACIN ANTIBODY AND DIFFERENT FLUOROQUINOLONES

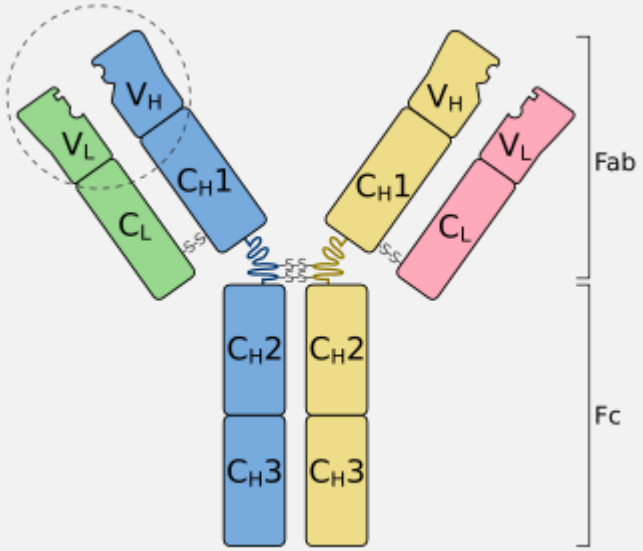
***Y. Meteleshko*, M. Khrenova, N. Byzova, A. Zherdev, B. Dzantiev,**

O. Hendrickson

Chemistry Department, M.V. Lomonosov Moscow State University, Moscow, Russia;
A.N. Bach Institute of Biochemistry, Federal Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia

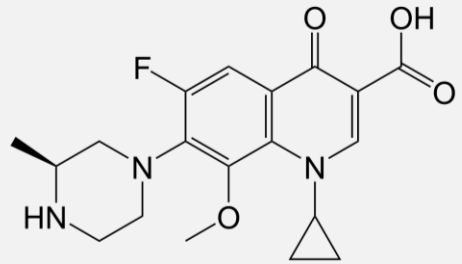
Antibodies

- **Antibody** is a large protein which is used by the immune system to identify and neutralize antigens.
- Applications: research, diagnostics, medicine
- Haptens - low-molecular-weight compounds binding with antibodies



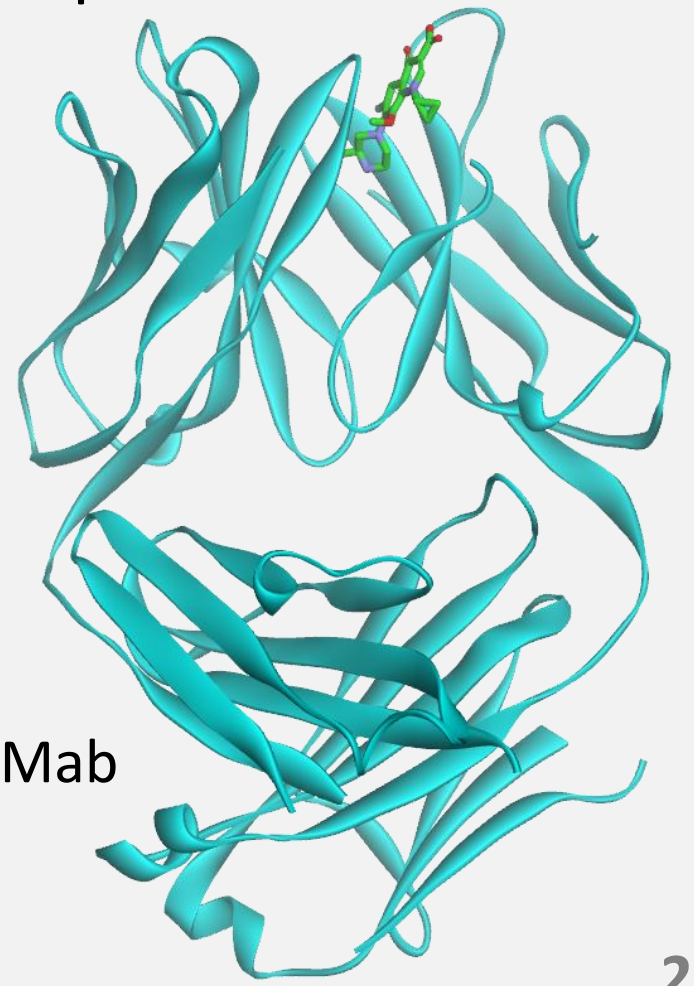
S-GAT specific Mab

- monoclonal antibody (MAb) against S-gatifloxacin
- S-gatifloxacin – fluoroquinolone antibiotic



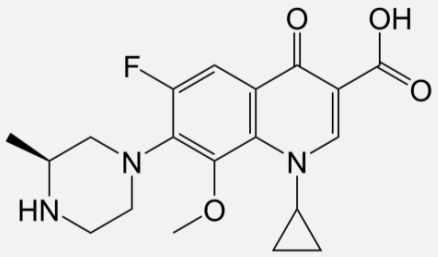
Cross-reactivity:

$$CR = \frac{IC_{50GAT}}{IC_{50cross-reactant}} \times 100\%$$

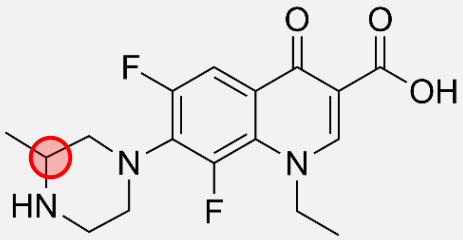


complex of S-GAT specific Mab with S-GAT (PDB ID: 7f35)

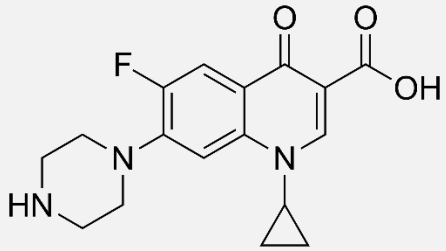
S-GAT and cross-reactive haptens



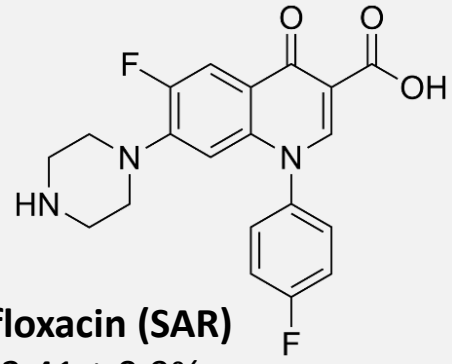
S-gatifloxacin (S-GAT)
CR 100%



Lomefloxacin (LOM)
CR 17.5 ± 0.5%

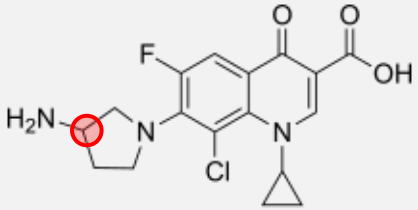


Ciprofloxacin (CIP)
CR 2.74 ± 0.08%

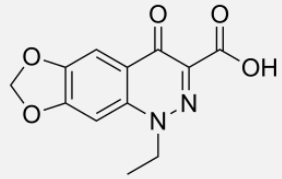


Sarafloxacin (SAR)
CR 2.41 ± 0.9%

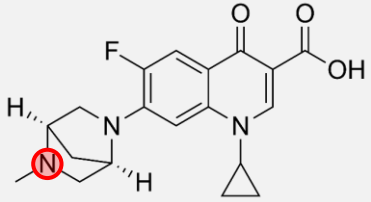
non-cross-reactive haptens



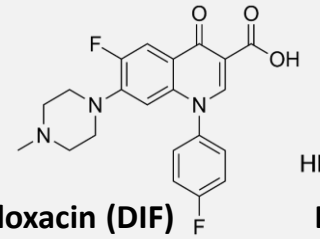
Clinafloxacin (CLI)



Cinoxacin (CIN)



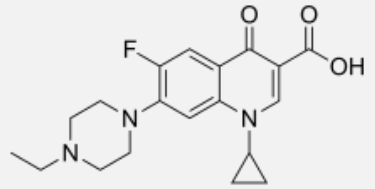
Danofloxacin (DAN)



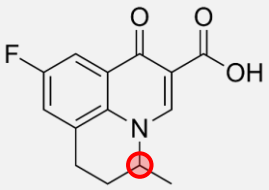
Difloxacin (DIF)



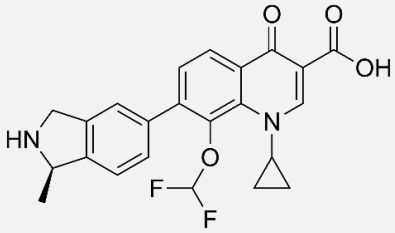
Enoxacin (ENO)



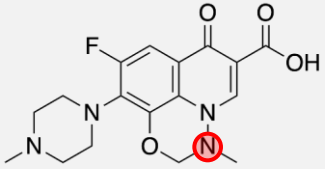
Enrofloxacin (ENR)



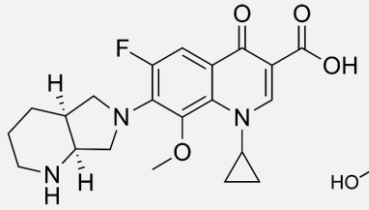
Flumequine (FLU)



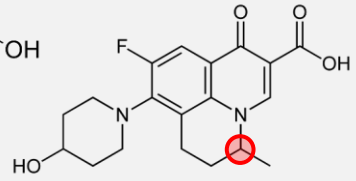
Garenofloxacin (GAR)



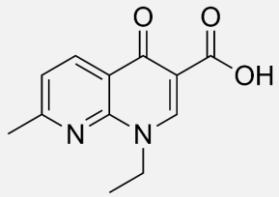
Marbofloxacin (MAR)



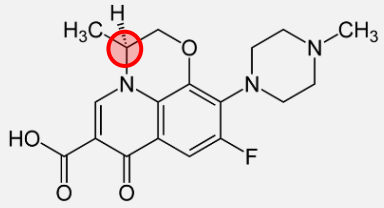
Moxifloxacin (MOX)



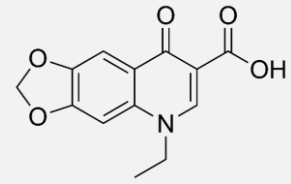
Nadifloxacin (NAD)



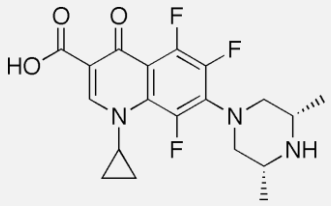
Nalidixic acid (NAL)



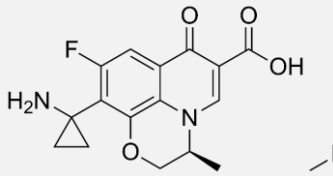
Ofloxacin (OFL)



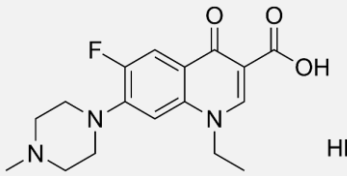
Oxolinic acid (OXO)



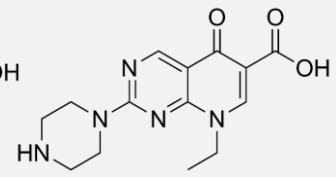
Orbifloxacin (ORB)



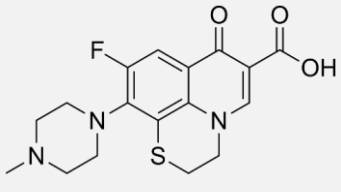
Pazufloxacin (PAZ)



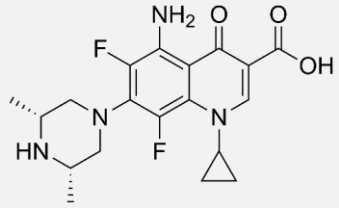
Pefloxacin (PEF)



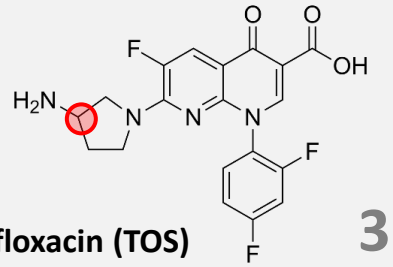
Pipemidic acid (PIP)



Rufloxacin (RUF)



Sparfloxacin (SPA)



Tosufloxacin (TOS)

Molecular docking

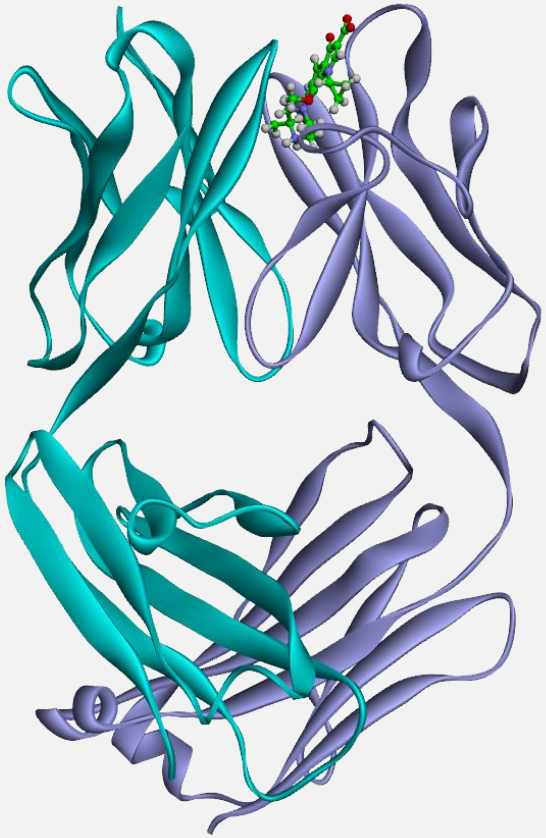
- Coordinates of heavy atoms PDB ID: 7f35
- Autodock4: Rigid-body docking, Lamarckian Genetic Algorithm
- Geometry optimization of haptens structures : ORCA, DFT/PBE0/6-31G**

Hapten	Lowest Binding energy, kcal/mol	Mean Binding Energy, kcal/mol	Clusters, %
S-GAT	-10.02	-9.98	100
S-LOM	-9.13	-9.06	100
R-LOM	-9.13	-9.04	100
CIP	-8.53	-8.5	100
SAR	-9.67	-9.62	100
CIN	-6.1	-6.08	100
DAN	-8.65	-8.63	100
DAN**	-7.52	-7.47	82
	-7.47	-7.4	4
	-7.4	-7.39	14
DIF	-8.63	-8.27	85
	-8.2	-8.17	15
ENO	-8.24	-8.12	100
ENR	-8.13	-8	38
	-7.95	-7.85	17
	-7.82	-7.75	44
	-7.31	-7.31	1
FLU	-6.68	-6.68	100
FLU*	-6.49	-6.49	100
GAR	-9.05	-8.83	92
	-8.47	-8.45	7
	-8.12	-8.12	1
CLI	-7.84	-7.79	62
	-7.67	-7.61	38
CLI*	-8.28	-7.88	100
MAR	-8.09	-8.05	100
MAR**	-8.01	-7.88	100

Hapten	Lowest Binding energy, kcal/mol	Mean Binding Energy, kcal/mol	Clusters, %
MOX	-8.43	-8.31	94
	-7.58	-7.56	6
NAD	-7.79	-7.47	97
	-7.12	-7.12	3
NAD*	-7.64	-7.41	56
	-7.49	-7.42	42
	-7.11	-7.11	2
NAL	-5.59	-5.58	100
R-OFL	-7.97	-7.93	100
S-OFL	-8.04	-8.01	99
	-7.1	-7.1	1
OXO	-6.17	-6.17	100
ORB	-8.54	-8.32	100
PAZ	-8.59	-8.5	100
PEF	-7.29	-7.24	87
	-7.06	-7.06	5
	-7.03	-7.02	8
PIP	-8.23	-8.17	100
RUF	-7.45	-7.41	17
	-7.43	-7.41	64
	-7.41	-7.38	19
SPA	-7.94	-7.87	60
	-7.55	-7.49	30
	-7.37	-7.36	3
	-7.34	-7.29	7
TOS	-8.71	-8.58	100
TOS*	-9.2	-9.15	100

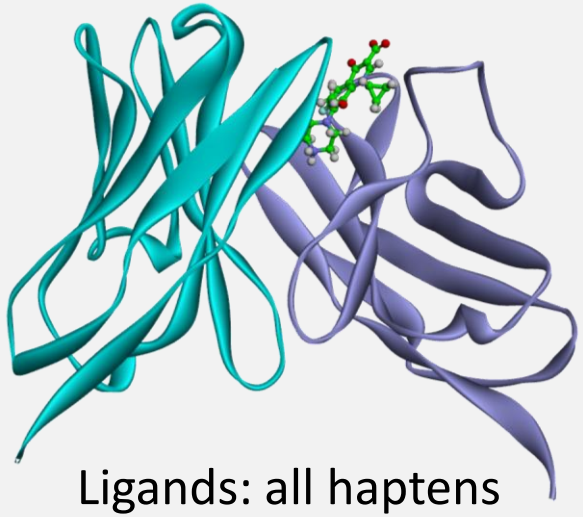
Molecular Dynamics: models and protocol

Full model (Fab)



Truncated Model

(only a variable part of Fab)

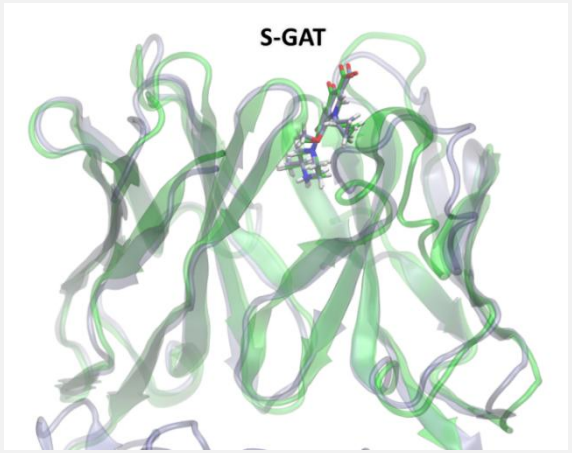


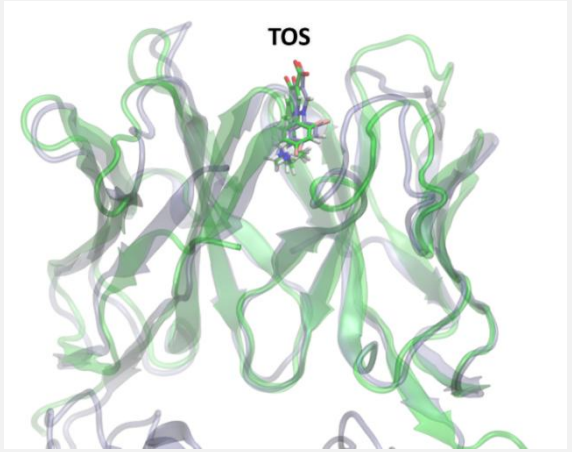
Ligands: all haptens

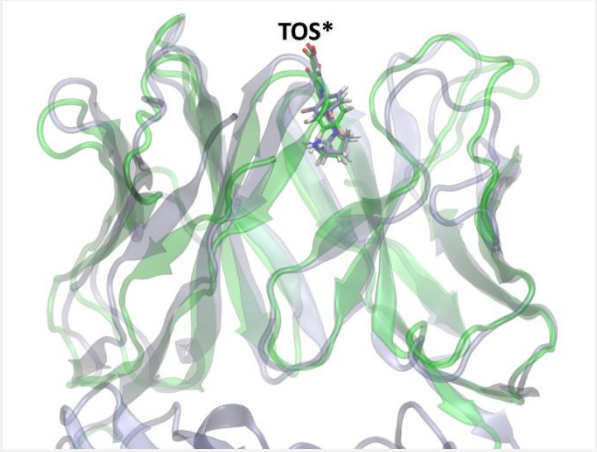
- NAMD
- coordinates of heavy atoms PDB ID: 7f35
- CHARMM36 Force Field for protein, TIP3P model for waters, CGenFF for ligands
- NPT ensemble, temperature 300 K, pressure 1 atm
- integration step 2 fs
- trajectories length 200 ns

Ligands:

- S-gatifloxacin (S-GAT)
- Tosufloxacin (TOS, TOS*)

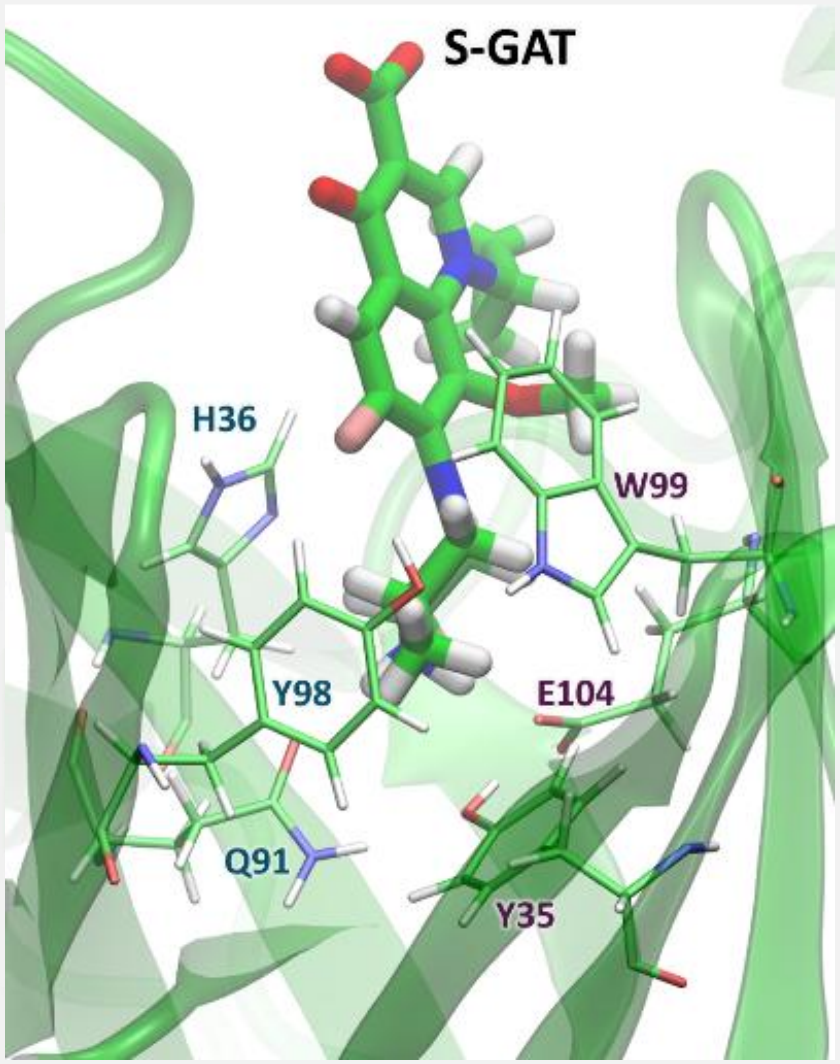


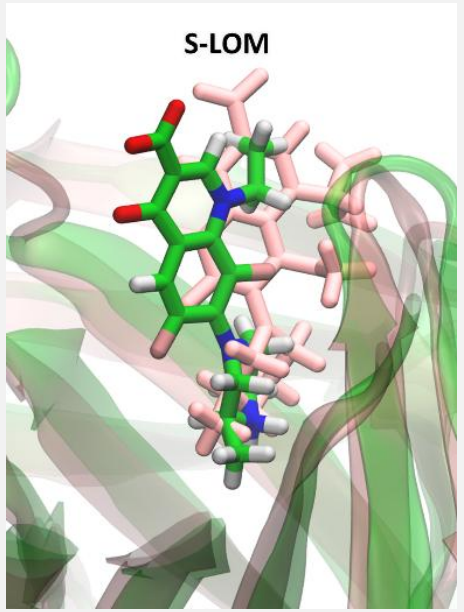


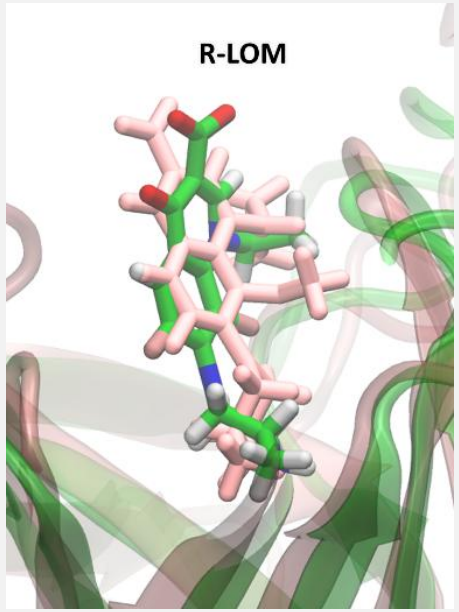


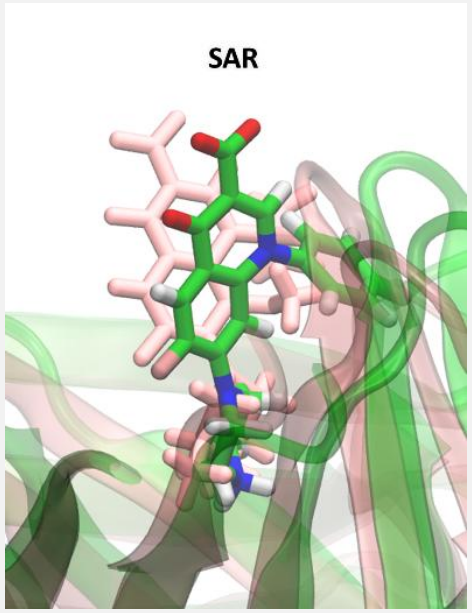
Full and truncated Fab models at 200 ns

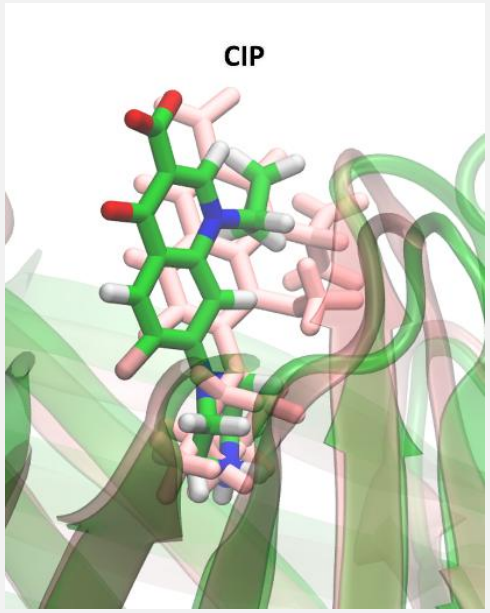
MD trajectories: S-GAT and cross-reactive haptens





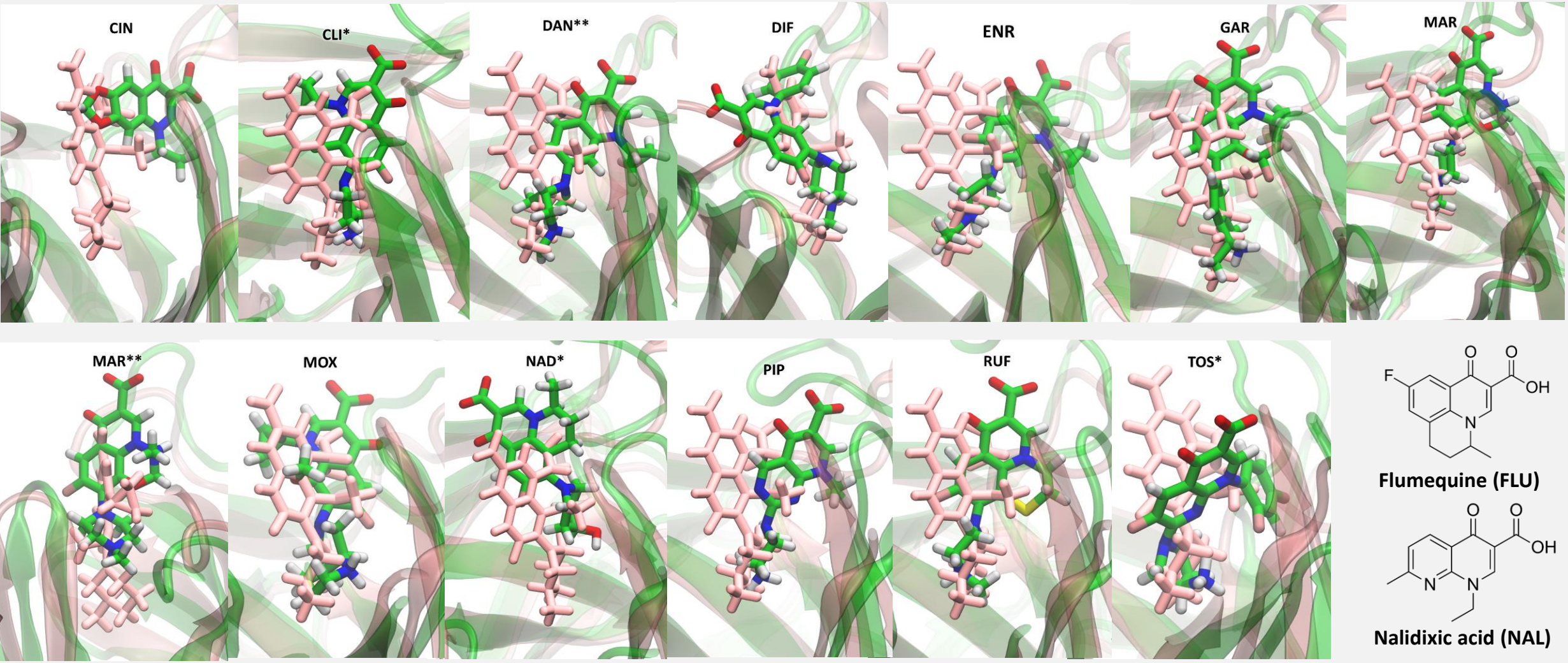






binding site configurations at 200 ns

MD trajectories: non-cross-reactive haptens

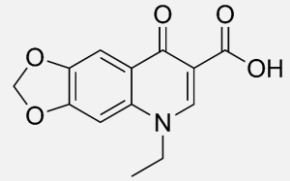


binding site configurations at 200 ns

FLU*, NAL and OXO completely left the binding site

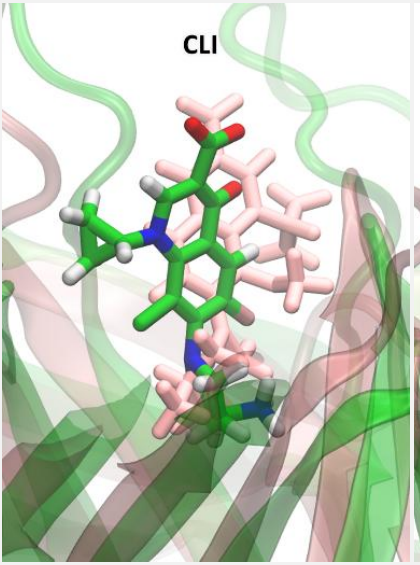
Flumequine (FLU)

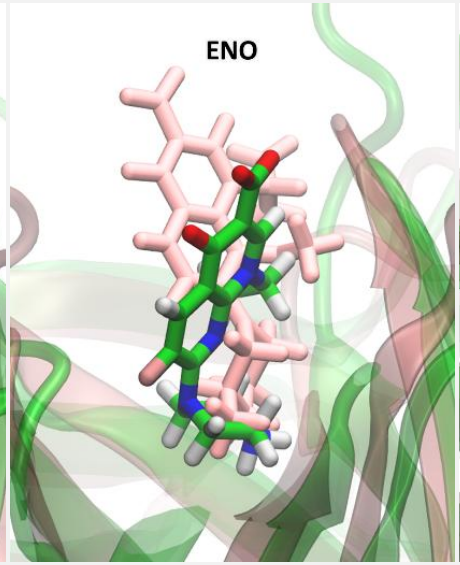
Nalidixic acid (NAL)

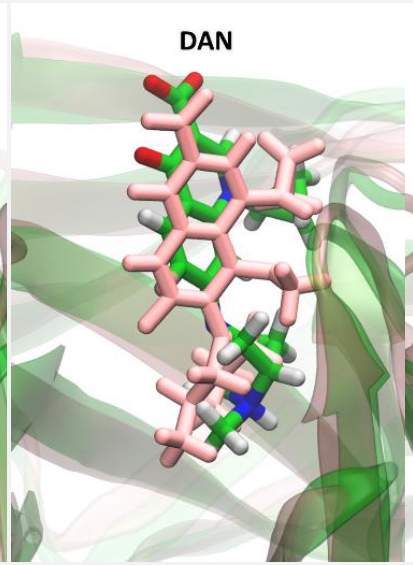


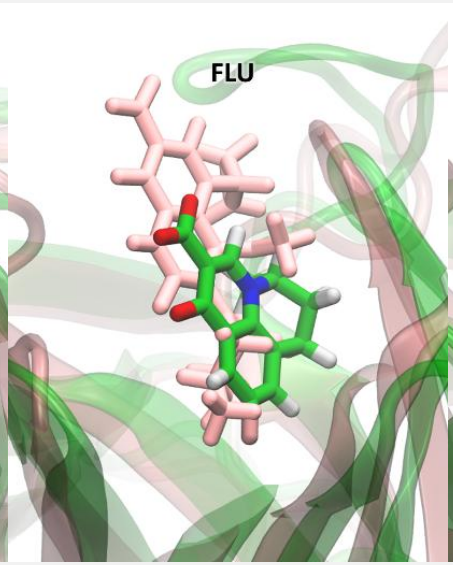
Oxolinic acid (OXO)

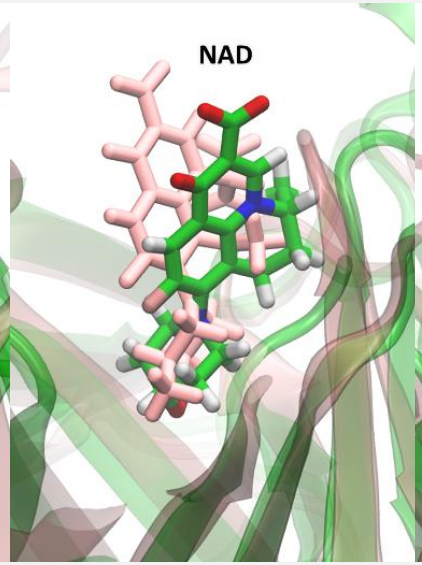
MD trajectories: non-cross-reactive haptens

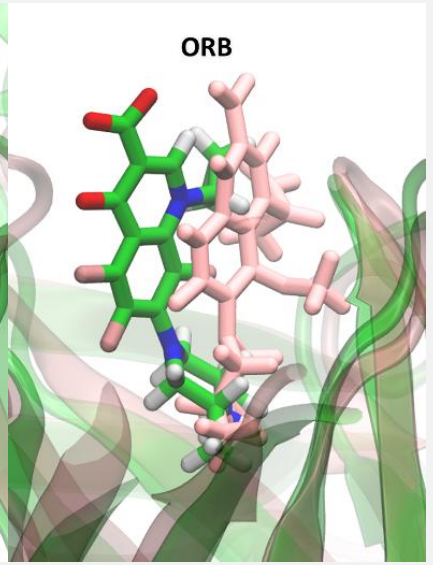


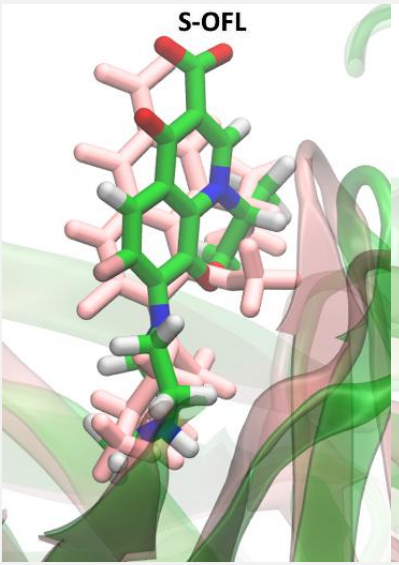


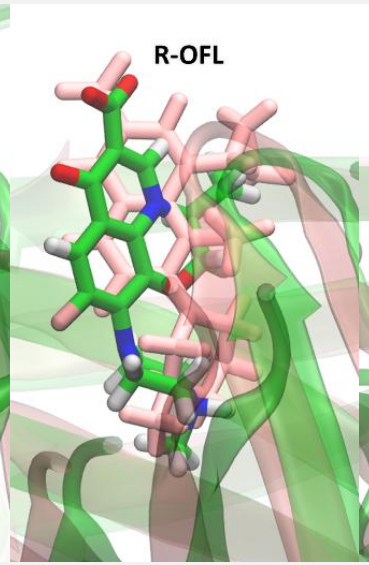


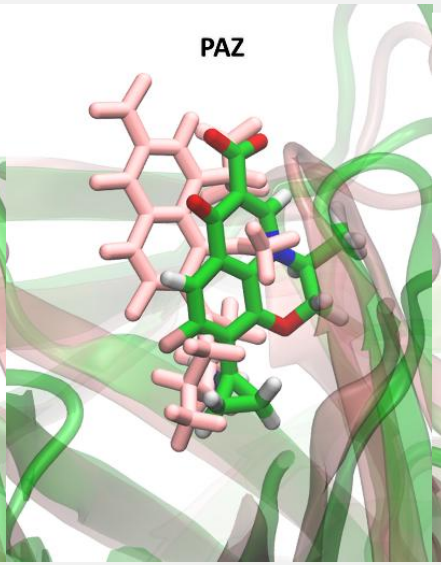


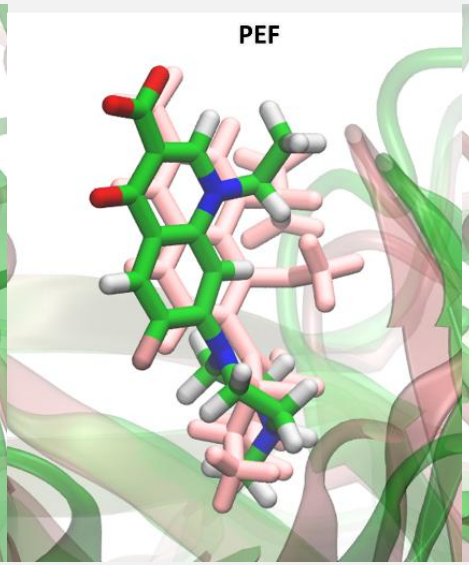


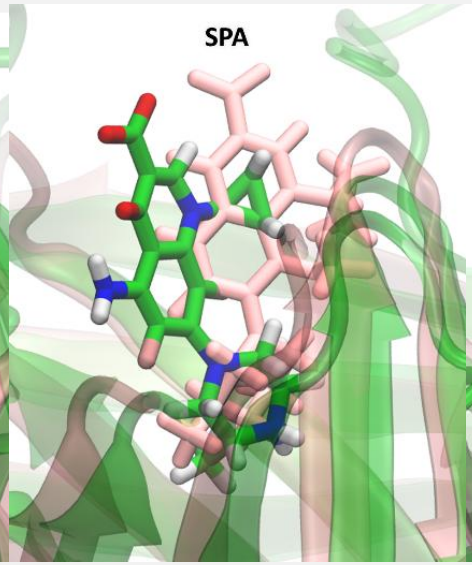


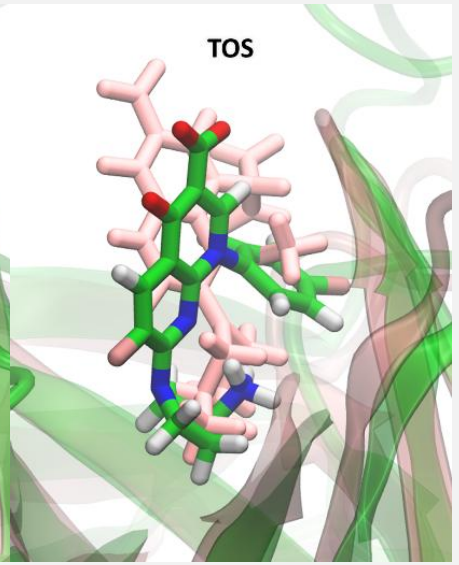








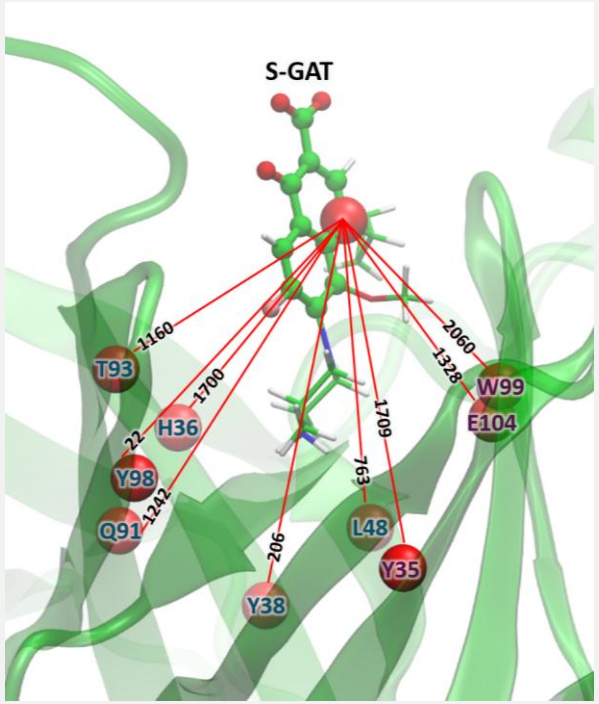


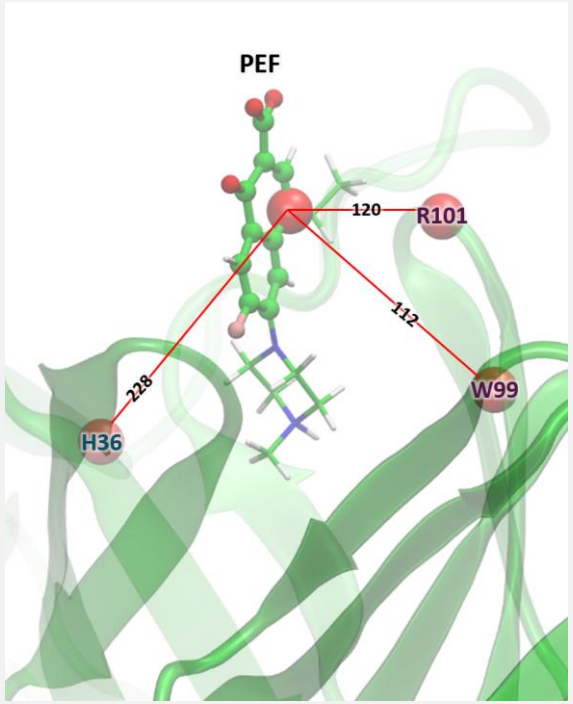


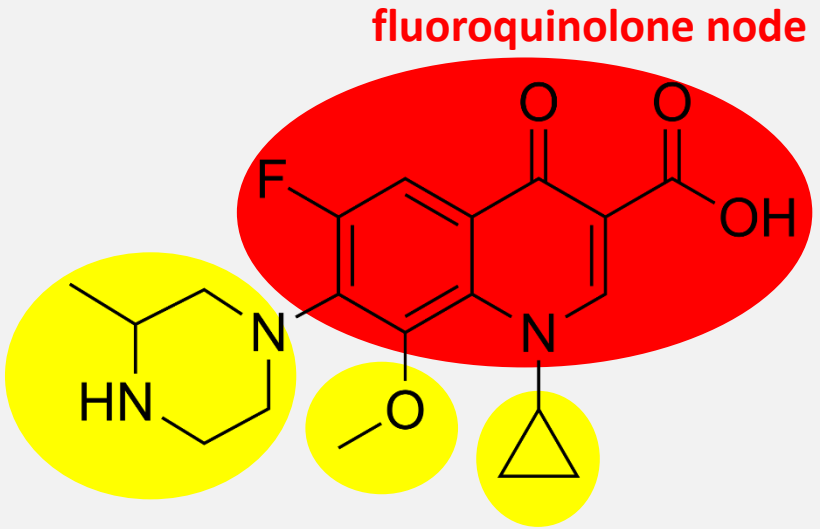
Dynamical Network Analysis

studies correlation of representative atoms (nodes) movements and determines if there are interactions between different parts of system.

- Division into the nodes:
  - antibody – 1 node per amino acid
  - ligands – 3-4 nodes (fluoroquinolone ring and radicals)
- Betweenness centrality measures the importance of a node in the whole network.



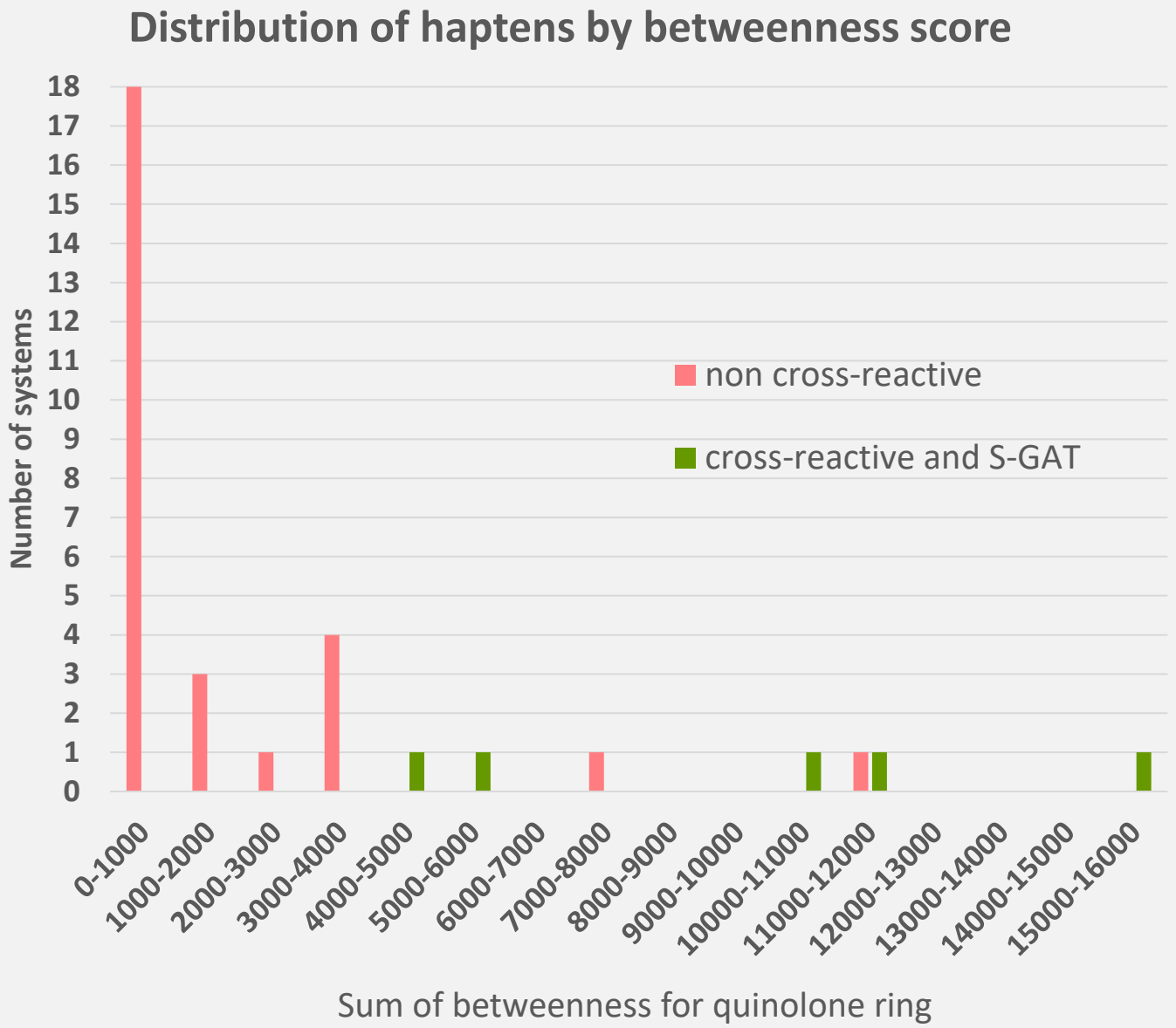




Sum of betweenness for fluoroquinolone node

Hapten	Betweenness
S-GAT	**10189**
S-LOM	**15231**
R-LOM	**11178**
CIP	**4169**
SAR	**5028**
CIN	450
CLI	735
CLI*	851
DAN	3252
DAN**	2167
DIF	7905
ENO	3232
ENR	462
FLU	456
FLU*	0
GAR	710

Hapten	Betweenness
MAR	462
MAR**	462
MOX	1362
NAD	1062
NAD*	882
NAL	0
R-OFL	3227
S-OFL	211
ORB	11033
OXO	0
PAZ	1840
PEF	460
PIP	3339
RUF	644
SPA	858
TOS	628
TOS*	462



Conclusions

- For complexes of S-GAT specific Mab with different fluoroquinolones we demonstrate that utilization of conventional MD simulations does not discriminate between cross-reactive and non-cross-reactive compounds.
- Further analysis of MD trajectories allows us to suggest a binary classifier that correlates with the experimentally observed binding potency.
- Betweenness centrality was found to be the most efficient descriptor for estimating the binding of fluoroquinolone molecules with the antibody. For cross-reactive compounds, the betweenness centrality is higher than for non-cross-reactive compounds.