Supplementary Material

Conformational adaptability determining antibody recognition to distomer: Structure analysis of enantioselective antibody against chiral drug gatifloxacin

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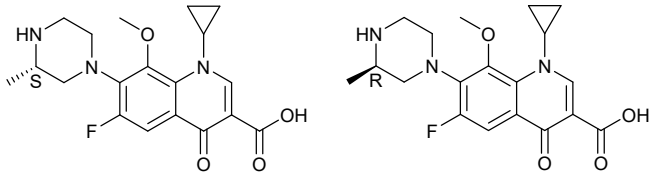


Fig. S1 Structure of gatifloxacin enantiomers.

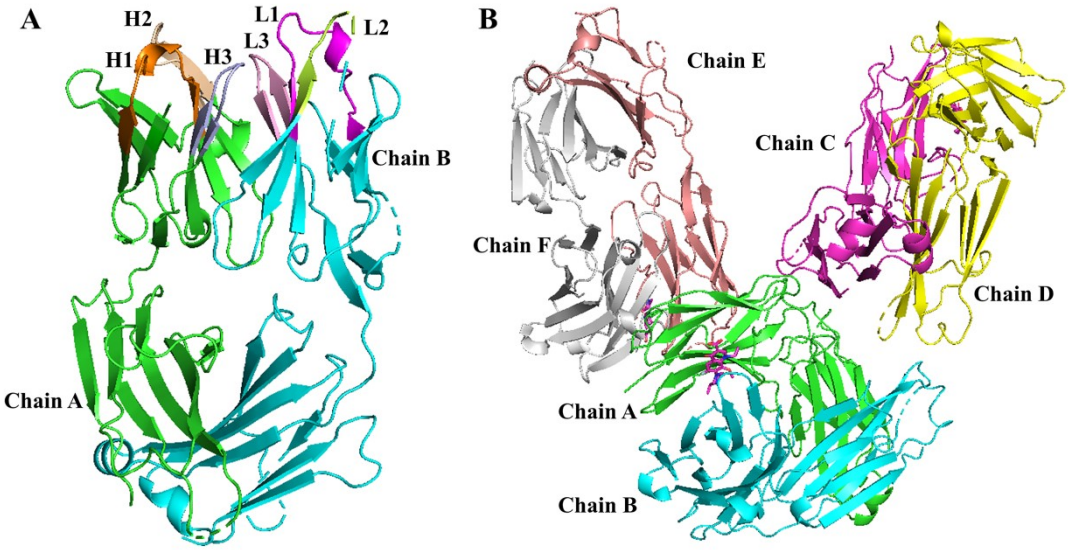


Fig. S2 Crystal structures of *S*-GAT Fab in the asymmetric unit. (A) *S*-GAT Fab monomer crystal structures (H1: H-CDR1, H2: H-CDR2, H3: H-CDR3, L1: L-CDR1, L2: L-CDR2, L3: L-CDR3). (B) *S*-GAT Fab complex crystal structures. Chain A, C and E were heavy chains and chain B, D and F were light chains. The images were drawn by PyMOL 2.5 software.

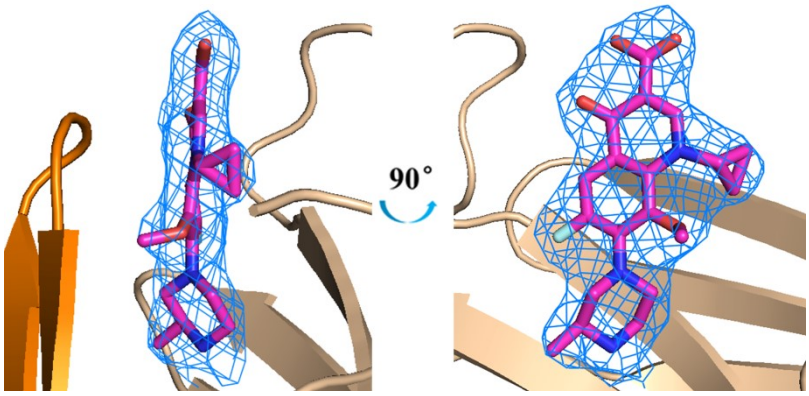


Fig. S3 Electron density of *S*-GAT ligand in *S*-GAT Fab complex structure. The colors were shown as follows: *S*-GAT ligand for magenta, light chain of *S*-GAT Fab complex for wheat color, heavy chain of *S*-GAT Fab complex for orange. The images were drawn by PyMOL 2.5 software.

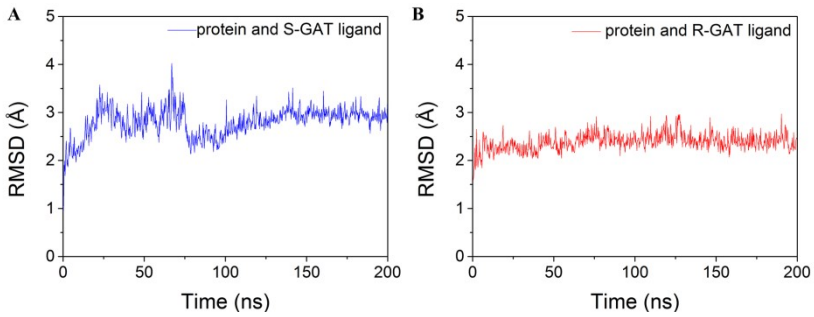


Fig. S4 RMSDs for the backbone atoms of the protein and *S*-GAT ligand (A) and *R*-GAT ligand (B) during 200 ns of simulation.

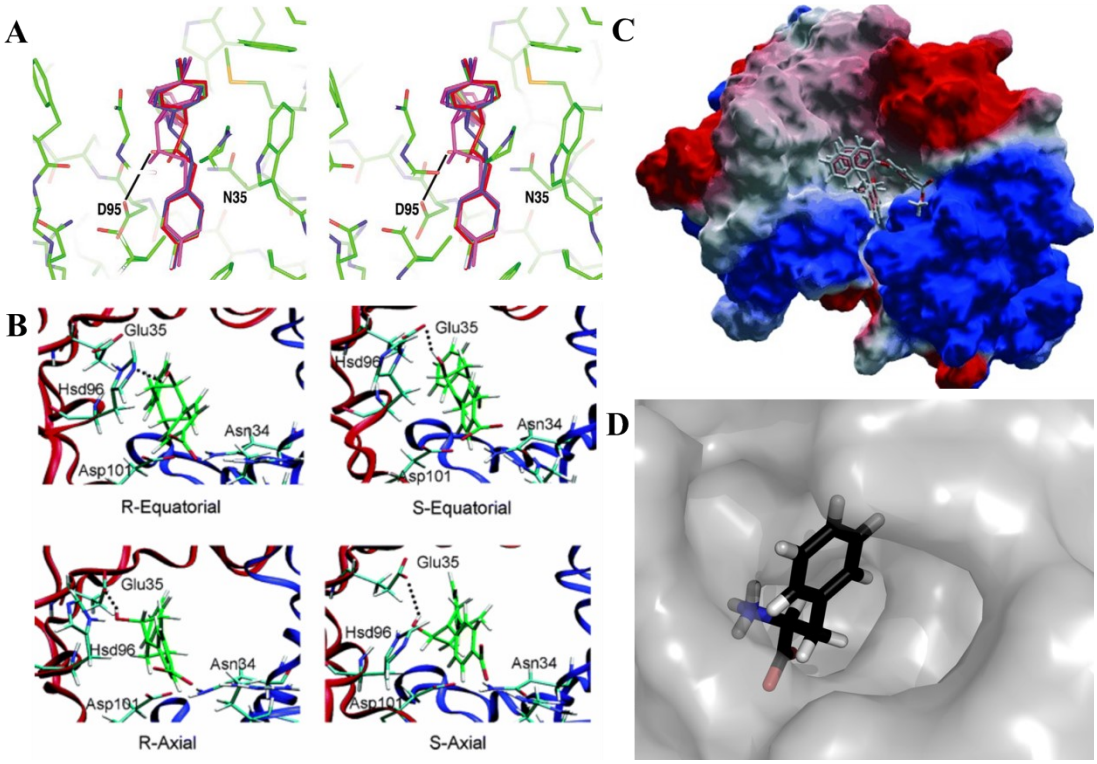


Fig. S5 Structure information of enantioselective antibody recognition in previous studies. (A) Superimposition of the binding of four different stereoisomers of the hapten in ENA11His. The observed *SR*-stereoisomer and protein is coloured according to atoms (carbon, green; oxygen, red; nitrogen, blue). The *RS*-stereoisomer is in magenta, the *RR* in blue, and the *SS* in red. The residues of Fab (Asp95 and Asn35 of the H-chain) important for stereoisomer recognition are labeled. The grey line shows the important hydrogen bond between the hydroxyl group of the SR-stereoisomer and Asp95 of the H-chain, which is suggested to be important for enantioselectivity.[1] (B) Detail of the averaged structures of the four different conformers of the TS oxy-Cope rearrangement in the active site of the matured AZ28 antibodies.[2] (C) Binding mode of *R*-BINOL derivative in antibody C2. The antibody is displayed with an electrostatic surface representation, where red shows negative charge, white neutral, and blue positive.[3] (D) Closeup of the binding pocket of anti-$_L$-

51 AA 80.1R with the docked ligand $_{L}$-phenylalanine (black) shown in a stick.[4]

Table S1. Amino acid sequence of *S*-GAT Fab. Sequence has been submitted to RCSB of Protein Data Bank along with protein structures, PDB: 7F2S and 7F35.

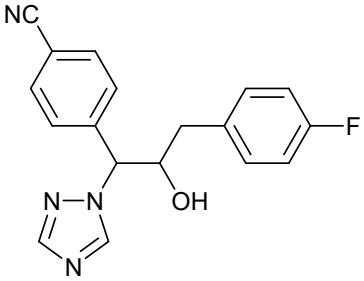
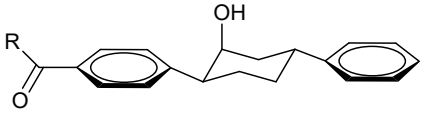
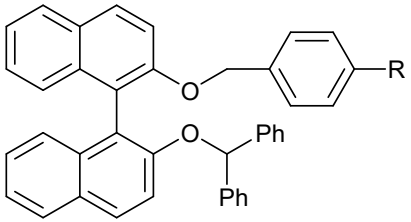
Chain	Sequence
Heavy chain [1]	EIQLQQSGPELVKPGTSVKVSCKASGYALTSYTMYWVKQ
	SHGKSLEWIGYIDPYNGGTSYNQKFKGKATLTVDKSSSTA
	YMHLNSLTSEDSAVYYCAGWNRYDEDWGQGTTLTVSSA
	KTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVT
	WNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETV
	TCNVAHPASSTKVDKKIVPRDC
Light chain	DIVLTQSPASLAVSLGQRATISCRTSETIDSYGNSFMHWY
	QQKPGQPPKLLIYRASNLKSGIPARFSGSGSRTDFTLTINPV
	EADDVATYYCQQTNEVMYTFGGGTKLEIKRADAAPTVSI
	FPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQN
	GVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEA
	THKTSTSPIVKSFNRNEC

[1] CDR sequences were highlighted.

Table S2. Molecular docking models of *R*-GAT ligand docking to *S*-GAT Fab complex crystal structure.

No.	Total score	Crash	Polar	Similarity	CSCORE
1	5.5597	-1.0407	1.0235	0.822	3
2	5.1187	-0.7578	0.7830	0.507	0
3	4.9639	-1.0200	0.0045	0.535	0
4	4.8742	-0.9572	0.0568	0.779	4
5	4.6340	-2.0474	0.9702	0.615	0
6	4.4314	-0.9298	1.1385	0.500	4
7	4.3706	-1.0167	0.1027	0.732	1
8	4.3388	-0.4013	1.3328	0.615	1
9	4.3357	-0.9867	0.1121	0.738	3
10	4.3352	-1.2577	1.0207	0.811	0

58 **Table S3.** Summarization of enantioselective antibody recognition information in previous studies.

Antibody	Ligand	Ligand structure	Enantioselectivity	Conformational adaptability	Reference
ENA11His	finrozole		The affinity of *SR*- and *SS*-enantiomers was twice that of *RR*- and *RS*- enantiomers.	The conformation adjustment of the flexible chain made the position of the three rings stationary.	[1]
AZ28	transition state analogue (TSA) of the oxy-cope reaction from the substituted hexadiene		The free energy barriers for the *S*-axial, *R*-axial, *S*-equatorial and *R*-equatorial were 26.8, 24.9, 24.2 and 24.4 kcal·mol^{-1}, respectively.	The positions of two benzene rings were relatively fixed, and the hydroxyl groups were combined with different residues because of the different orientations.	[2]
C2	BINOL derivative		Antibody could not recognize *S*-enantiomer.	*S*-enantiomer could not dock into antibody binding cavity.	[3]

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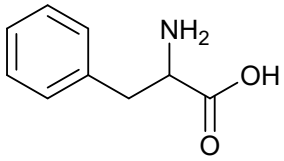
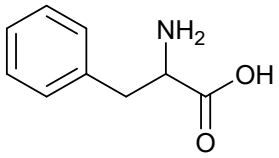
Antibody	Ligand	Ligand structure	Enantioselectivity	Conformational adaptability	Reference
anti-$_{L}$-AA 80.1R	phenylalanine		Antibody could not recognize $_{D}$-phenylalanine	Clashes occurred between benzene ring of $_{D}$-phenylalanine and the antibody	[4]
anti-$_{D}$-AA 67.36	phenylalanine		Antibody could not recognize $_{L}$-phenylalanine	Clashes occurred between benzene ring of $_{L}$-phenylalanine and the antibody	[5]

References

1. T. Parkkinen, T. K. Nevanen, A. Koivula and J. Rouvinen, *J. Mol. Biol.*, 2006, **357**, 471–480.

2. S. Martí, J. Andrés, V. Moliner, E. Silla, I. Tuñón and J. Bertrán, *J. Phys. Chem. A*, 2006, **110**, 726–730.

3. B. S. Rasmussen, J. M. Pedersen, J. Sørensen, J. Egebjerg, B. Schiøtt, K. K. Mortensen and T. Skrydstrup, *Chembiochem*, 2007, **8**, 1974–1980.

4. D. I. Ranieri, H. Hofstetter and O. Hofstetter, *J. Sep. Sci.*, 2009, **32**, 1686–1695.

5. D. I. Ranieri, D. M. Corgliano, E. J. Franco, H. Hofstetter and O. Hofstetter, *Chirality*, 2008, **20**, 559–570.