Antibody Humanization: Intra and Inter VH-VL Non-bond Energy Sort Best Humanized Antibody

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ABSTRACT

Antibody humanization of non-human derived antibody reduces immunogenicity of antibody drug. Computer aided antibody humanization has became an efficient and rapid routine process. Our computational humanization pipeline includes CDR grafting onto antibody crystal structure and humanization of CDR grafted antibody structure. Then, intra and inter VH-VL non-bond energy is calculated and sorted for selection best humanized antibody. Compare to experimental dataset, result indicate that intra and inter VH-VL non-bond energy could rank humanized antibody.

INTRODUCTION

Computer aided antibody humanization has became an efficient and rapid routine process[Tsuchiya and Mizuguchi, 2016; Nowak et al., 2016; Nguyen et al., 2017; Regep et al., 2017; Kuroda et al., 2020; Sawant et al., 2020]. Many humanized antibodies have been developed by this method[Fransson et al., 2010; Apgar et al., 2016; Margreitter et al., 2016]. Computer aided antibody humanization process include complementarity determining region (CDR) grafting and human germline framework homology modeling[Haidar et al., 2012; Hanf et al., 2014; Choi et al., 2016; Kurella et al., 2018; Chowdhury et al., 2018; Zhang et al., 2020]. In our process, the murine antibody CDR was grafted onto homology antibody structure by loop modeling and side chain packing. Then, CDR grafted antibody structure was humanized by human germline framework. All humanized antibody structure was released on website [http://120.48.17.198] and intra and inter VH-VL non-bond energy were calculated and sorted to select best humanized antibody.

METHOD

CDR region of user inputted non-human VH and VL sequence was automatically defined by program. Then, homology antibody structures were selected to build CDR grafted antibody by loop modeling and side chain packing. Energy minimized CDR grafted antibody was used to build humanized antibody by selection pair VH-VL human germline framework. Humanized antibody was optimized by loop modeling and side chain packing. Intra and inter VH-VL non-bond energy of humanized antibody was calculated and sorted to select best human germline framework. Whole humanization pipeline was built on website [http://120.48.17.198].

RESULT and DISCUSSION

1 Intra and Inter VH-VL non-bond energy of humanized antibody crystal structure

Intra and Inter VH-VL non-bond energy of humanized antibody crystal structure was calculated. In Table 1, intra VH and VL non-bond energy range from -1000kcal/mol to -1400kcal/mol , inter VH-VL non-bond energy range from -40kcal/mol to -60kcal/mol . These value are perfect well for antibody structure database(their intra VH and VL non-bond energy ranged from -900kcal/mol to -1500kcal/mol, inter VH-VL non-bond energy ranged from -25kcal/mol to -60kcal/mol).

2 Murine and other source antibody crystal structure as template of humanization

Murine or other antibody crystal structure was selected as template of humanization (CDR have already been in antibody crystal structure). Then, template structure was humanized by paired VH-VL human germline framework. Humanized antibody structure was built by loop modeling and side chain packing. Then, Intra and inter VH-VL non-bond energy of humanized antibody was calculated in Table 2. Intra VH and VL non-bond energy range from -900kcal/mol to -1400kcal/mol, inter VH-VL non-bond energy range from -40kcal/mol to -60 kcal/mol.

3 Non-human derived antibody crystal structure as template of humanization

Non-human derived antibody crystal structure was selected by sequence identity from user inputted VH and VL sequence. Non-human antibody was selected as template of CDR grafting, loop modeling and side chain packing. Optimized CDR grafted antibody structure was humanized by paired VH-VL human germline framework. Humanized antibody structure was optimized by loop modeling and side chain packing. Then, intra and inter VH-VL non-bond energy was calculated in Table 3. Intra VH and VL non-bond energy range from -900kcal/mol to 1300kcal/mol, inter VH-VL non-bond energy range from -30kcal/mol to -55 kcal/mol.

4 Best humanized antibody was ranked by template source from humanized antibody crystal structure

Top 10 VH and VL human germline framework sequence was selected by sequence identity from humanized antibody crystal structure. For example, in Table 4, VH and VL human germline framework randomly combined to build humanized antibody structure by template source from humanized antibody crystal structure. Humanized antibody structure was optimized and calculated intra and inter VH-VL non-bond energy. In Table 5-7, rank 1-9 humanized antibody was selected by intra and inter VH-VL non-bond energy.

5 Humanized antibody was ranked by template source from murine and other antibody crystal structure

Murine and other source antibody crystal structure was selected as template to build humanized antibody (CDR have already been in antibody crystal structure). Then human germline framework in Table 4 was randomly combined for humanization. Humanized antibody was optimized by loop modeling and side chain packing. Then, optimized humanized antibody was calculated intra and inter VH-VL non-bond energy for sorting top 9 humanized antibody. Some of rank 1-9 humanized antibody VH and VL framework in Table 8-10 could be found in Table 5-7. Therefore,

intra and inter VH-VL non-bond energy could be used for selection best humanized antibody source from murine and other antibody crystal structure.

6 Humanized antibody was ranked by template source from non-human antibody crystal structure

Non-human derived antibody structure was selected by sequence identity from user inputted VH and VL sequence. Then, CDR grafted antibody was built by CDR grafting, loop modeling and side chain packing. Optimized CDR grafted antibody was humanized by human germline framework in Table 4. Humanized antibody was optimized by loop modeling and side chain packing. Then, intra and inter VH-VL non-bond energy of humanized antibody was calculated for sorting top 9 humanized antibody. In Table 11-13, some of rank 1-9 humanized antibody VH-VL gene could be found in Table 5-7. Therefore, intra and inter VH-VL non-bond energy could be used for selection best humanized antibody source from non-human crystal structure.

CONCLUSION

User inputted VH-VL sequence is used to search antibody crystal structure. These antibody structures can be used as template for CDR grafting, loop modeling and side chain packing. Optimized CDR grafted antibody is humanized by human germline framework. Humanized antibody is optimized by loop modeling and side chain packing. Then intra and inter VH-VL non-bond energy is calculated and sorted for selection best humanized antibody. Result indicate that intra and inter VH-VL non-bond energy could rank humanized antibody.

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Table 1 Humanized antibody crystal structure template calculated non-bond energy

PDBID	Humanized antibody sequence	Intra VH and VL non-bond energy kcal/mol	Inter VH-VL non-bond energy kcal/mol
1AD9	EIQLVQSGAEVKKPGSSVKVSCKASGYTFTDYYINWMRQAPGQGLEWIGWIDPGSGNTKYNEKFKGRATLTVDTSTNTAYMELSSLRSEDTAFYFCAREKTTYYYAMDYWGQGTLVTVSS	-1241.77	-53.3762
	DIQMTQSPSTLSASVGDRVTITCRSSKSLLHSNGDTFLYWFQQKPGKAPKLLMYRMSNLASGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCMQHLEYPFTFGQGTKVEVK	-1155.1	
3L7F	QVTLKESGPVLVKPTETLTLTCTVSGFSLSTYGMGVGWIRQPPGKALEWLAHIWWDDVKRYNPALKSRLTISKDTSKSQVVLTMTNMDPVDTATYYCARMGSDYDVWFDYWGQGTLVTVSS	-1244.27	-52.9754
	EIVLTQSPATLSLSPGERATLSCRASKSISKYLAWYQQKPGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQHNEYPYTFGQGTKLEIK	-1048.42	

5F3H	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSTISSGGSYTSYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKQDYAMNYWGQGTLVTVSS	-1168.4	-49.1272
	DIQMTQSPSSLSASVGDRVTITCKASQDVSTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTLTISSLNPEDFATYYCQQHYSTPWTFGGGTKVEIK	-1059.59	

Table 2 Murine or other antibody crystal structure template calculated non-bond energy

PDBID	Murine or other antibody sequence	Humanized antibody sequence	Intra VH and VL non-bond energy kcal/mol	Inter VH-VL non-bond energy kcal/mol
1AE6	QIQLQQSGPELVKPGASVKISCKASGYTFTDYYINWMKQKPGQGLEWIGWIDPGSGNTKYNEKFKGKATLTVDTSSSTAYMQLSSLTSEDTAVYFCAREKTTYYYAMDYWGQGTSVTVSA	EIQLVQSGAEVKKPGSSVKVSCKASGYTFTDYYINWMRQAPGQGLEWIGWIDPGSGNTKYNEKFKGRATLTVDTSTNTAYMELSSLRSEDTAFYFCAREKTTYYYAMDYWGQGTLVTVSS	-1146.65	-46.0465
	DIVMTQAAPSVPVTPGESLSISCRSSKSLLHSNGDTFLYWFLQRPGQSPQLLIYRMSNLASGVPDRFSGSGSGTAFTLRVSRVEAEDVGVYYCMQHLEYPFTFGAGTKLELK	DIQMTQSPSTLSASVGDRVTITCRSSKSLLHSNGDTFLYWFQQKPGKAPKLLMYRMSNLASGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCMQHLEYPFTFGQGTKVEVK	-1005.98	
3L7E	QVTLKESGPGILQPSQTLSLTCSFSGFSLSTYGMGVGWIRQPSGKGLEWLAHIWWDDVKRYNPALKSRLTISKDTSGSQVFLKIASVDTSDTATYYCARMGSDYDVWFDYWGQGTLVTVSA	QVTLKESGPVLVKPTETLTLTCTVSGFSLSTYGMGVGWIRQPPGKALEWLAHIWWDDVKRYNPALKSRLTISKDTSKSQVVLTMTNMDPVDTATYYCARMGSDYDVWFDYWGQGTLVTVSS	-1160.11	-51.168

	DVQITQSPSYLAASPGETITLNCRASKSISKYLAWYQEKPGKTNKLLIYSGSTLQSGIPSRFSGSGSGTDFTLTISSLEPEDFAMYFCQQHNEYPYTFGGGTKLEIK	EIVLTQSPATLSLSPGERATLSCRASKSISKYLAWYQQKPGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQHNEYPYTFGQGTKLEIK	-1105.08	
5F3B	EVQLVESGGGLVKPGGSLKLSCAASGFTFSSYAMSWVRQTPEKRLEWVATISSGGSYTSYPDSVKGRFTISRDNAKNTLYLQMSSLRSEDTAMYYCARQDYAMNYWGQGTLVTVSS	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSTISSGGSYTSYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKQDYAMNYWGQGTLVTVSS	-1249.08	-49.0268
	DIEMTQSHKFMSTSVGDRVSITCKASQDVSTAVAWYQQKPGQSPKLLLYSASYRYTGVPDRFTGSGSGTDFTFTISSVNAEDLAVYYCQQHYSTPWTFGGGTKVEIK	DIQMTQSPSSLSASVGDRVTITCKASQDVSTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTLTISSLNPEDFATYYCQQHYSTPWTFGGGTKVEIK	-1086.49	

Table 3 Non-human antibody crystal structure template calculated non-bond energy

PDBID	Non-human antibody sequence	Humanized antibody sequence	Intra VH and VL non-bond energy kcal/mol	Inter VH-VL non-bond energy kcal/mol
1MJJ	QVQLQQPGAELVKPGASVKLSCKAS[GYTFTDYY]INWVKQRPGQGLEWIGN[IDPGSGNT]HYNEKFKNKATLTVDTSSSTAYMQLSSLTSDDSAVYYC[AREKTTYYYAMDY]WGQGTLVTVSA	EIQLVQSGAEVKKPGSSVKVSCKASGYTFTDYYINWMRQAPGQGLEWIGWIDPGSGNTKYNEKFKGRATLTVDTSTNTAYMELSSLRSEDTAFYFCAREKTTYYYAMDYWGQGTLVTVSS	-953.167	-42.319
	DIVMTQAAPSVPVTPGESVSISCRSS[KSLLHSNGDTF]LYWFLQRPGQSPQLLIY[RMS]NLASGVPDRFSGSGSGT	DIQMTQSPSTLSASVGDRVTITCRSSKSLLHSNGDTFLYWFQQKPGKAPKLLMYRMSNLASGVPSRFSGSG	-1051.16	

	AFTLRISRVEAEDVGVYYC[MQHLEYPFT]FGAGTKLELK	SGTEFTLTISSLQPDDFATYYCMQHLEYPFTFGQGTKVEVK		
3L5W	QVTLKESGPGILQPSQTLSLTCSFS[GFSLSTYGMG]VGWIRQPSGKGLEWLAH[IWWDDVK]RYNPALKSRLTISKDTSGSQVFLKIASVDTSDTATYYC[ARMGSDYDVWFDY]WGQGTLVTVSA	QVTLKESGPVLVKPTETLTLTCTVSGFSLSTYGMGVGWIRQPPGKALEWLAHIWWDDVKRYNPALKSRLTISKDTSKSQVVLTMTNMDPVDTATYYCARMGSDYDVWFDYWGQGTLVTVSS	-1226.97	-50.4224
	DVQITQSPSYLAASPGETITLNCRAS[KSISKY]LAWYQEKPGKTNKLLIY[SGS]TLQSGIPSRFSGSGSGTDFTLTISSLEPEDFAMYFC[QQHNEYPYT]FGGGTKLEIK	EIVLTQSPATLSLSPGERATLSCRASKSISKYLAWYQQKPGQAPRLLIYSGSTLQSGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQHNEYPYTFGQGTKLEIK	-1106.68	
5XS7	EVQLVESGGGLVKPGGSLKLSCAAS[GFTFSSYA]MSWVRQTPEKRLELVAH[ISSGGSYT]YYPDTVKGRFTISRDNAKNTLYLQMSSLKSEDTAMYYC[ARQDYAMNY]WGQGTTLTVSS	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSTISSGGSYTSYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKQDYAMNYWGQGTLVTVSS	-1085.16	-33.7364
	DIVMTQSHKFMSTSVGDRVSITCKAS[QDVSTA]VAWYQQRPGQSPKLLIY[SAS]YRYTGVPDRFTGSGSGTDFTFTISSVQAEDLAVYYC[QQHYSTPWT]FGGGTKLEIK	DIQMTQSPSSLSASVGDRVTITCKASQDVSTAVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTLTISSLNPEDFATYYCQQHYSTPWTFGGGTKVEIK	-931.264	

Table 4 Top 10 VH and VL human germline framework

Rank	1AD9		3L7F		5F3H	
1	1AD9H	1AD9L	3L7FH	3L7FL	5F3HH	5F3HL
2	IGHV1-3*01	IGKV1-5*01	IGHV2-26*02-IGHV2-2	IGKV3-11*01	IGHV3-23*03	IGKV1D-12*02-IGKV1D-12*01-

			6*01			IGKV1-12*02-IGKV1-12*01
3	IGHV1-18*04-IGHV1-18*01	IGKV1-5*03	IGHV2-26*03	IGKV3D-7*01-IGKV3/OR2-268*02-IGKV3/OR2-268*01	IGHV3-23*05	IGKV1-6*02-IGKV1-6*01
4	IGHV1-69D*01-IGHV1-69*13-IGHV1-69*12-IGHV1-69*01	IGKV1-5*02	IGHV2-5*02	IGKV3D-11*03	IGHV3-23*04	IGKV1D-13*02-IGKV1D-13*01-IGKV1-13*02
5	IGHV1-69*05	IGKV1-17*01	IGHV2-5*01	IGKV3D-11*01	IGHV3-23D*01-IGHV3-23*01	IGKV1D-39*01-IGKV1-39*01
6	IGHV1-69*16	IGKV1-16*01	IGHV2-5*09	IGKV3D-11*02	IGHV3-53*02	IGKV1-39*02
7	IGHV1-69*15	IGKV1-NL1*01	IGHV2-5*06-IGHV2-5*05	IGKV3-11*02	IGHV3-23*02	IGKV1-NL1*01
8	IGHV1-69*11	IGKV1-17*03	IGHV2-5*04	IGKV3D-20*02	IGHV3-66*04-IGHV3-66*02-IGHV3-66*01	IGKV1-5*01
9	IGHV1-69*17	IGKV1-9*01	IGHV2-5*08	IGKV3-20*01	IGHV3-66*03	IGKV1-5*03
10	IGHV1-69*14-IGHV1-69*06	IGKV1-17*02	IGHV2/OR16-5*01	IGKV3D-15*01-IGKV3-15*01	IGHV3-53*01	IGKV1-27*01

Table 5 Top 9 humanized antibody template with 1AD9

Rank	HL Gene	H intra kcal/mol	L intra kcal/mol	HL inter kcal/mol
1	IGHV1-3*01-1AD9L	-1264.06	-1174.53	-52.1395
2	IGHV1-3*01-IGKV1-NL1*01	-1273.36	-1133.73	-51.4032
3	IGHV1-69*11-1AD9L	-1234.91	-1170.49	-50.8565
4	IGHV1-69*15-1AD9L	-1234.91	-1170.49	-50.8565
5	1AD9H-1AD9L	-1241.77	-1155.1	-53.3762
6	IGHV1-69*05-1AD9L	-1218.29	-1171.59	-50.3606
7	IGHV1-69*16-1AD9L	-1218.29	-1171.59	-50.3606
8	1AD9H-IGKV1-NL1*01	-1249.31	-1135.32	-52.2981
9	IGHV1-69D*01-IGHV1-69*13-IGHV1-69*12-IGHV1-69*01-1AD9L	-1215.11	-1171.07	-50.4236

Table 6 Top 9 humanized antibody template with 3L7F

Rank	HL Gene	H intra kcal/mol	L intra kcal/mol	HL inter kcal/mol
1	3L7FH-IGKV3D-20*02	-1245.37	-1068.45	-52.9509
2	3L7FH-IGKV3-11*02	-1246.3	-1064.82	-52.8501
3	3L7FH-IGKV3-11*01	-1246.2	-1065.4	-49.4023
4	3L7FH-IGKV3D-15*01-IGKV3-15*01	-1245.52	-1061.7	-49.9812
5	3L7FH-IGKV3-20*01	-1246.05	-1054.99	-52.8499
6	3L7FH-3L7FL	-1244.27	-1048.42	-52.9754
7	3L7FH-IGKV3D-7*01-IGKV3/OR2-268*02-IGKV3/OR2-268*01	-1245.91	-1040.16	-50.3278
8	IGHV2-5*09-IGKV3D-20*02	-1068.69	-1075.64	-51.972
9	IGHV2-5*09-IGKV3-11*02	-1068.45	-1074.8	-52.1395

Table 7 Top 9 humanized antibody template with 5F3H

Rank	HL Gene	H intra kcal/mol	L intra kcal/mol	HL inter kcal/mol
1	IGHV3-23*03-IGKV1-5*01	-1188.43	-1065.99	-49.6727
2	IGHV3-23*03-IGKV1-5*03	-1188.43	-1065.99	-49.6727
3	IGHV3-66*04-IGHV3-66*02-IGHV3-66*01-IGKV1-5*01	-1184.17	-1066.22	-49.1262
4	IGHV3-66*04-IGHV3-66*02-IGHV3-66*01-IGKV1-5*03	-1184.17	-1066.22	-49.1262
5	5F3HH-IGKV1-5*01	-1179.68	-1065.45	-49.9844
6	5F3HH-IGKV1-5*03	-1179.68	-1065.45	-49.9844
7	IGHV3-23*05-IGKV1-5*01	-1179.95	-1065.56	-49.4334
8	IGHV3-23*05-IGKV1-5*03	-1179.95	-1065.56	-49.4334
9	IGHV3-53*01-IGKV1-5*01	-1177.03	-1066.15	-49.2809
22	5F3HH-5F3HL	-1168.4	-1059.59	-49.1272

Table 8 Top 9 humanized antibody template with 1AE6

Rank	HL Gene	H intra kcal/mol	L intra kcal/mol	HL inter kcal/mol
1	IGHV1-3*01-1AD9L	-1127.61	-1047.12	-53.0664
2	1AD9H-IGKV1-5*01	-1143.12	-1032.38	-48.7873
3	1AD9H-IGKV1-5*03	-1143.12	-1032.38	-48.7873
4	1AD9H-IGKV1-5*02	-1140.71	-1029.61	-50.2803
5	IGHV1-3*01-IGKV1-5*01	-1125.35	-1033.61	-52.8156
6	IGHV1-3*01-IGKV1-5*03	-1125.35	-1033.61	-52.8156
7	1AD9H-IGKV1-17*02	-1143.97	-1026.23	-46.9193
8	1AD9H-IGKV1-17*01	-1147.72	-1022.63	-46.7637
9	IGHV1-3*01-IGKV1-5*02	-1128.03	-1031.66	-52.033

Table 9 Top 9 humanized antibody template with 3L7E

Rank	HL Gene	H intra kcal/mol	L intra kcal/mol	HL inter kcal/mol
1	IGHV2-5*08-IGKV3D-20*02	-1185.35	-1115.58	-47.9664
2	IGHV2-5*08-3L7FL	-1182.2	-1108.66	-50.4048
3	IGHV2-5*08-IGKV3-11*02	-1185.55	-1107.84	-48.2813
4	IGHV2-5*08-IGKV3-20*01	-1185.31	-1107.56	-48.0529
5	IGHV2-5*04-IGKV3D-20*02	-1175.21	-1114.91	-48.0891
6	IGHV2-5*01-IGKV3D-20*02	-1172.76	-1115.2	-48.1578
7	IGHV2-5*02-IGKV3D-20*02	-1172.76	-1115.2	-48.1578
8	IGHV2-5*08-IGKV3-11*01	-1185.57	-1102.47	-48.0565
9	IGHV2-5*04-IGKV3-11*02	-1176.39	-1109.7	-48.1239

Table 10 Top 9 humanized antibody template with 5F3B

Rank	HL Gene	H intra kcal/mol	L intra kcal/mol	HL inter kcal/mol
1	IGHV3-53*01-5F3HL	-1270.01	-1086.12	-47.9012
2	IGHV3-66*03-5F3HL	-1270.01	-1086.12	-47.9012
3	IGHV3-66*04-IGHV3-66*02-IGHV3-66*01-5F3HL	-1268.52	-1085.73	-48.038
4	IGHV3-23*03-5F3HL	-1266.15	-1086.16	-48.9234
5	IGHV3-53*02-5F3HL	-1262.08	-1086.12	-48.8344
6	IGHV3-23*03-IGKV1-5*01	-1265.61	-1084.3	-46.7451
7	IGHV3-23*03-IGKV1-5*03	-1265.61	-1084.3	-46.7451
8	IGHV3-66*04-IGHV3-66*02-IGHV3-66*01-IGKV1-5*01	-1267.16	-1084.06	-45.8075
9	IGHV3-66*04-IGHV3-66*02-IGHV3-66*01-IGKV1-5*03	-1267.16	-1084.06	-45.8075

Table 11 Top 9 humanized antibody template with 1MJJ

Rank	HL Gene	H intra kcal/mol	L intra kcal/mol	HL inter kcal/mol
1	IGHV1-3*01-1AD9L	-957.923	-1049.05	-47.2008
2	IGHV1-3*01-IGKV1-5*02	-953.934	-1043.96	-50.0913
3	IGHV1-3*01-IGKV1-16*01	-954.108	-1044.86	-49.342
4	IGHV1-3*01-IGKV1-9*01	-950.754	-1046.37	-48.7738
5	IGHV1-3*01-IGKV1-5*01	-953.907	-1040.09	-49.9078
6	IGHV1-3*01-IGKV1-5*03	-953.907	-1040.09	-49.9078
7	1AD9H-1AD9L	-953.167	-1051.16	-42.319
8	1AD9H-IGKV1-9*01	-951.375	-1050.56	-41.6717
9	1AD9H-IGKV1-5*01	-950.974	-1041.92	-46.0397

Table 12 Top 9 humanized antibody template with 3L5W

Rank	HL Gene	H intra kcal/mol	L intra kcal/mol	HL inter kcal/mol

1	3L7FH-IGKV3-11*02	-1220.15	-1120.73	-54.1098
2	3L7FH-IGKV3-11*01	-1218.71	-1110.03	-55.5043
3	3L7FH-IGKV3D-20*02	-1224.81	-1113.4	-49.979
4	3L7FH-3L7FL	-1226.97	-1106.68	-50.4224
5	3L7FH-IGKV3-20*01	-1224.62	-1102.52	-52.3325
6	IGHV2-5*09-IGKV3-11*02	-1198.87	-1122.39	-53.9434
7	IGHV2-5*01-IGKV3-11*02	-1197.92	-1122.18	-53.0765
8	IGHV2-5*02-IGKV3-11*02	-1197.92	-1122.18	-53.0765
9	IGHV2-5*04-IGKV3-11*02	-1194.83	-1122.41	-53.9548

Table 13 Top 9 humanized antibody template with 5XS7

Rank	HL Gene	H intra kcal/mol	L intra kcal/mol	HL inter kcal/mol
1	IGHV3-23*04-5F3HL	-1104.9	-929.997	-36.4064
2	IGHV3-23D*01-IGHV3-23*01-5F3HL	-1103.05	-931.231	-36.5693
3	IGHV3-23*05-5F3HL	-1101.42	-932.443	-36.3889
4	IGHV3-23*02-5F3HL	-1100.79	-929.255	-36.3646
5	IGHV3-53*01-5F3HL	-1097.44	-931.202	-36.9109
6	IGHV3-66*03-5F3HL	-1097.44	-931.202	-36.9109
7	IGHV3-66*04-IGHV3-66*02-IGHV3-66*01-5F3HL	-1095.53	-929.957	-37.0653
8	IGHV3-53*02-5F3HL	-1095.15	-930.529	-36.868
9	IGHV3-23*03-5F3HL	-1093.12	-932.089	-36.8587