

Supplementary Material

Molecular simulation study on the interaction between porcine CR1-like and C3b

A Sequence ID: **Query_46845** Length: **190** Number of Matches: **15**

Range 1: 1 to 190 [Graphics](#) [▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Method	Identities	Positives	Gaps
291 bits(746)	2e-95	Compositional matrix adjust.	142/190(75%)	154/190(81%)	1/190(0%)
Query 1175	CNAPDQFQFAKLKSTNASKFPICGTSKLYECHPEYYKMSFSIKCLENLTWSSAIDVCKRK				1234
Sbjct 1	C APD F FAKLK+QTNAS FPIGTSKLYEC PEYY FSI CL+NL WSS DVCKRK				60
Query 1235	SCETPSDPVNGMVVNTDTQFGSRINFSCNTGYRLIGHSSSTECVLSGNTVFWDAEPPICE				1294
Sbjct 61	SC+TP DPVNGMV+V TD Q GSRIN+SC TG+RLIGHSS EC+LSGN W +PPIC+				120
Query 1295	RIPCGPPPAIANGDFISSNREYFPYGTVVTYRCNLGD-RRMKFELVGSPSIYCTSKDNQV				1353
Sbjct 121	RIPCG PP IANGDFIS+NRE F YG+VVTYRCN G R FELVG PSYICTS D+QV				180
Query 1354	GIWGGPPPPQC 1363				
Sbjct 181	GIWGGP PQC 190				

B Range 2: 1 to 190 [Graphics](#) [▼ Next Match](#) [▲ Previous Match](#) [▲ First Match](#)

Score	Expect	Method	Identities	Positives	Gaps
231 bits(589)	3e-74	Compositional matrix adjust.	114/190(60%)	133/190(70%)	1/190(0%)
Query 726	CQAPRQLPFAAMPTKLIDKDEFFIGTSLKYKCRPGYYSRIFSITCQKNSFWSIPEGNCRRK				785
Sbjct 1	CQAP FA + +FPIGTSKLY+CRP YY R FSITC N WS P+ CKRK				60
Query 786	TCGTLAELVNGKMEIDKDMQFGSTVHYFCNDGYRLIGQSSATCVISGNSVIWDNDPPTCE				845
Sbjct 61	+C T + VNG + + D+Q GS ++Y C G+RLIG SSA C++SGN+ W PP C+				120
Query 846	SIPCGPPPAIANGDFISSNREYFPYGTVVTYRCNLGD-RRMKFELVGSPSIYCTSKDNQV				904
Sbjct 121	IPCG PP IANGDFIS+NRE F YG+VVTYRCN G R FELVG PSYICTS D+QV				180
Query 905	GIWGGPPPPQC 914				
Sbjct 181	GIWGGP PQC 190				

Figure S1. Sequence alignment of human CR1 SCR 15-17 fragment with the porcine CR1-like protein sequence. “Query” is porcine CR1-like protein sequence and “Sbjct” is porcine CR1-like protein sequence. (A) The fragment with the highest similarity between porcine CR1-like protein sequence and human CR1 SCR 15-17. (B) The fragment with the second highest similarity between porcine CR1-like protein sequence and human CR1 SCR 15-17.

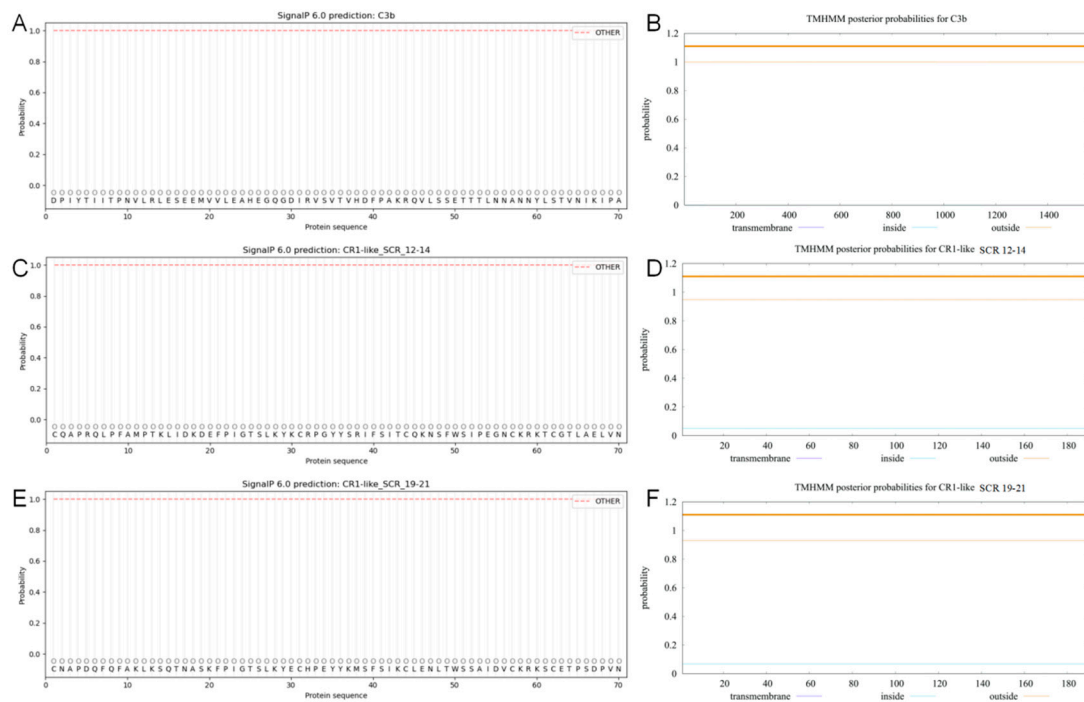


Figure S2. Analysis of the signal peptides and transmembrane regions of porcine CR1-like and C3b. (A) Signal peptide analysis of C3b. (B) Analysis of the transmembrane region of C3b. (C) Signal peptide analysis of CR1-like SCR 12-14. (D) Analysis of the transmembrane region of CR1-like SCR 12-14. (E) Signal peptide analysis of CR1-like SCR 19-21. (F) Analysis of the transmembrane region of CR1-like SCR 19-21.

[illegible]

B

MC3balphachain	SNLDEDIARENIVSRSEFPESWLNWVDELKEPPKNGISTKLMNRLKDSITTWEILAVS	60
PC3balphachain	SDIDEIIPPEIISSEFPESWLNWVDELKEPPKNGISTKLMNRLKDSITTWEILAVS	60

MC3balphachain	MSDKKIGCVADPFVETVMQDFIDRLRFPYSVVRNEQVEIRAVLYNVRQNLKVRVELLH	120
PC3balphachain	LSDKKIGCVADPFVETVMQDFIDRLRFPYSVVRNEQVEIRAILYNVREAEDLKVRVELLY	120

MC3balphachain	NPAFCSLATTKRHHQQTITIPPSSLSVPYVIVPLKTGLQEVEVKAAYVHMSDGMKKS	180
PC3balphachain	NPAFCSLATAKRHHQQTITVPKSSVPYVIVPLKTGLQEVEVKAAYVHMSDGMKKT	180

MC3balphachain	LKVVPFEGIMNKTVAVRTLDPERLGRGVQKEDIPPADLSQVDPTESETRILLQGTTPVA	240
PC3balphachain	LKVVPFEGMRVNTVVRTLDPEHKGQGVQREEIPPADLSQVDPTESETKILLQGTTPVA	240

MC3balphachain	QMTEDAVDAERLKLIVTPSGCGEQNMIGMTPTVIAVHYLDETEQWEKFGLEKRGQALEL	300
PC3balphachain	QMVEDAIDGDRKLKLIQTPSGCGEQNMIGMTPTVIAVHYLDSTEQWEKFGLEKRGQALEL	300

MC3balphachain	IKKGYTQQLAFRQSSAFAAFVKRAPSTWLTAYVVKVFLAVNLIAIDSQVLCGAVKWL	360
PC3balphachain	IKKGYTQQLAFRQKNSAFAAFQDRSLSTLLTAYVVKVFAMAANLIAIDSQVLCGAVKWL	360

MC3balphachain	LEKQKPDGVFQEDAPVHQEMIGGLRNNNEKDMALTAFLVLSLQEAKDICEEQVNSLPGS	420
PC3balphachain	LEKQKPDGVFEENGFPVHQEMIGGFKNTEEKDVSMTAFVLIALQEAKDICEFPQVNSLRS	420

MC3balphachain	ITKAGDFLEANYMNLQESYTVIAIAGYALAQMRGLKPLNKFLLTAKDKNRWEDPGKQLY	480
PC3balphachain	INKARDFLADYYLELKRPTVAIAGYALALSDKLEDFPLNKLSTAKERNRWEFPGQKLH	480

MC3balphachain	NVEATSYALLALLQINFDVPPVVRWLNQRYGGYGSTQATFMVFQALAQYQKDAFD	540
PC3balphachain	NVEATSYALLALLVQINFDVPPVVRWLNQRYGGYGSTQATFMVFQALAQYQKDVFD	540

MC3balphachain	HQELNLDVSLQPSISSKITHRMHESASLIRSEETKENEGFTVTAEGKGQGTLSVVTMY	600
PC3balphachain	HQELNLDVSLQPSISAPVRHMTLHESASLIRSEETKENEGFTLIAEGKGQGTLSVVTMY	600

MC3balphachain	HAKAKDQLTCNIGFDLKVITKPAPETEKRPQDAKNTMILEICTRYRGDQDATMSILDISM	660
PC3balphachain	HGKAKGKTTCKGFDLKVSIHPAPEPVKPKPEAKSSMVLIDICTRYLGNQDATMSILDISM	660

MC3balphachain	TGFAPDDBDLKQLANGVDRIYSKYELDKAFSDRNTLIYLDKVSHEDDCLAFKVHQYFN	720
PC3balphachain	TGFSPTEDLKLSTGVDRYISKYELNKAISNKNLTIYLDKISHTLEDICISFKVHQYFN	720

MC3balphachain	VELIQPGAVKYVAYYNLEESCTRFYHPEKEDGKLNLKLCDELRCAEENCPIQKSDDKVT	780
PC3balphachain	VGLIQPGSVKYVSYYNLEESCTRFYHPEKEDGMLNLKLCHEMRCAEENCFMHDEEEVT	780

MC3balphachain	LEERLDKACEPGVDYVYKTRLVKVLNSNDFDEYDMAIEQTIKSGSDEVQVGQRTFISPI	840
PC3balphachain	LDDRLEACEPGVDYVYKTRLLKVELSDDFDDYDMVIEQTIKSGSDEVQVGQERRFISHI	840

MC3balphachain	KCREALKLEEKHYLMWGLSSDFWGEKPNLSYIIGKDTWVEHWPEDEECQDEENQKQCQD	900
PC3balphachain	KCREALKLEGGHYLVWGVSSDLWGEKPNLSYIIGKDTWVELWPDGVCQDEENQKQCQD	900

MC3balphachain	LGAFTESMVVFGCPN	915
PC3balphachain	LANFSENMVVFGCPN	915

C

PCR112-14	CQAPRQLPFAMPTKLIDKDEFPIGTSLKYPKCPGYYSRIFSI TCQKNSFWSIPEGNCKKK	60
HCR115-17	CQAPDHFLFAKLKTQTNASDFPIGTSLKYECPPEYGRPFSTCLDNLVWSSPKDVCKKK	60
PCR119-21	CNAPDQFQFAKLKSQTNASKFPIGTSLKYECHPEYYMSFSIKCLENLTWSSAIDVCKKK	60
	*** : : * * . . : . . ***** : : * * ** *** . * . * ** ****	
PCR112-14	TCGTAEIVNGKMEIDKDMQFGSTVHYFCNDGYRLIGQSSATCVISGNSVIW DNDPPTCE	120
HCR115-17	SKTTPPDFVNGMVHVIITDIQVGS RINYSCTTGHR LIGHSSAECILSGNTAHWSTKPPICQ	120
PCR119-21	SCETPSDFVNGMVYVNTDTQFGSRINFSCNTGYRLIGHSSTECVLSGNTVFWDAEPPICE	120
	* * * : : *** : : . * * . * * : : : * * * **** : * * : : * * * : * . * * * :	
PCR112-14	SIPCGPPPAIANGDFISSNREYFPYGTVMYRCNLGDRR-MKFELVGSPSIYCTSKDNQV	179
HCR115-17	RIPCGLPPTIANGDFISTNREN FHYGSVMYRCNLGSRGRKVFELVGEPSIYCTSNDDQV	180
PCR119-21	RIPCGPPPAIANGDFISSNREYFPYGTVMYRCNLGDRR-MKFELVGSPSIYCTSKDNQV	179
	*** * : ***** : *	
PCR112-14	GIWSGPPQC	189
HCR115-17	GIWSGPAPQC	190
PCR119-21	GIWSGPPQC	189
	***** **	

Figure S3. Sequence alignment of human CR1-C3b binding interface with porcine CR1-like-C3b binding interface. The residues enclosed in the red box are the residues located at the interface of human CR1-C3b complex and the equivalent residues in porcine CR1-like-C3b. The order of residues corresponds to Table S1. “*” indicates positions that have a single, fully conserved residue. “:” indicates that one of the subsequent ‘strong’ groups is fully conserved. “.” indicates that one of the subsequent ‘weaker’ groups is fully conserved. “H” stands for human, “P” stands for porcine. (A) Sequence alignment of human C3b β chain (23-667) and porcine C3b β chain (23-665). (B) Sequence alignment of human C3b α chain (749-1663) and porcine C3b α chain (747-1661). (C) Sequence alignment of human CR1 SCR 15-17 (943-1132), porcine CR1-like SCR 12-14 (726-914) and porcine CR1-like SCR 19-21 (1175-1363).

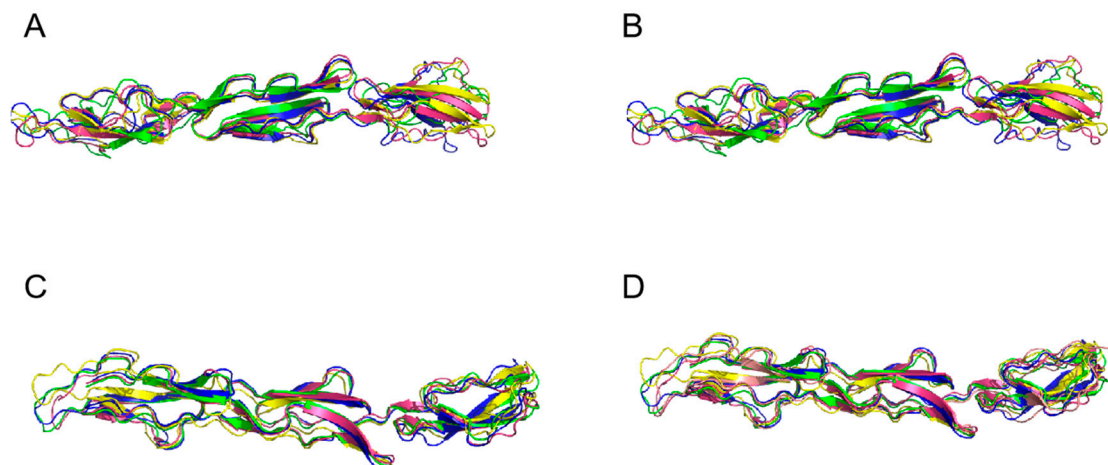


Figure S4. Structural alignment of CR1-like models. The human CR1 crystal structure is pink. ITASSER CR1-like models generated by I-TASSER, trRosetta, RoseTTAFold and AlphaFold are yellow, green, warm pink and blue respectively. (A) Structural comparison of trRosetta, RoseTTAFold and AlphaFold with ITASSER generated models (CR1-like SCR 12-14). (B) Structural comparison of models (CR1-like SCR 12-14) generated by trRosetta, RoseTTAFold, AlphaFold and ITASSER with human CR1 SCR 15-17. (C) Structural comparison of trRosetta, RoseTTAFold and AlphaFold with ITASSER generated models (CR1-like SCR 19-21). (D) Structural comparison of models (CR1-like SCR 19-21) generated by trRosetta, RoseTTAFold, AlphaFold and ITASSER with human CR1 SCR 15-17.

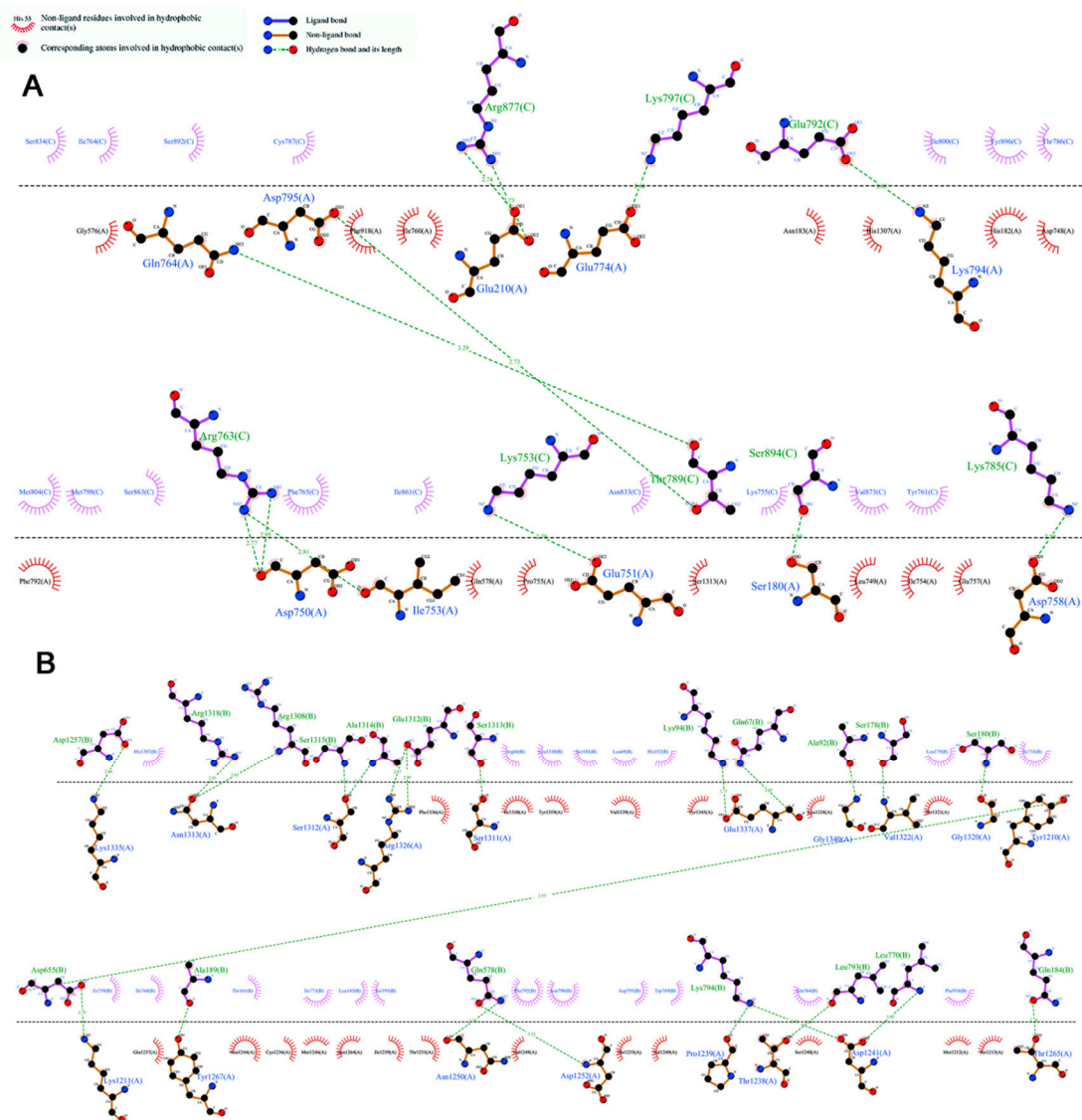


Figure S5. Analysis of hydrogen bonding and hydrophobicity at the interaction interface. (A) LIGPLOT analysis of the C3b–CR1-like SCR 12-14 complex. (B) LIGPLOT analysis of the C3b – CR1-like SCR 19-21 complex.

Table S1. Amino acids at the complex C3b – CR1 SCR 15-17 binding interface

Protein	Residue	Chain/domain
C3b	Arg94	C3b beta chain
C3b	Thr144	C3b beta chain
C3b	Leu146	C3b beta chain
C3b	Thr162	C3b beta chain
C3b	Met164	C3b beta chain
C3b	Gln177	C3b beta chain
C3b	Asp178	C3b beta chain
C3b	Ser179	C3b beta chain
C3b	Leu180	C3b beta chain
C3b	Ser181	C3b beta chain
C3b	Gln183	C3b beta chain
C3b	Asn184	C3b beta chain
C3b	Gln185	C3b beta chain
C3b	Asn190	C3b beta chain
C3b	Leu191	C3b beta chain
C3b	Ser192	C3b beta chain
C3b	Glu211	C3b beta chain
C3b	Gly578	C3b beta chain
C3b	Gln579	C3b beta chain
C3b	Gln580	C3b beta chain
C3b	Asp752	C3b alpha chain
C3b	Glu753	C3b alpha chain
C3b	Asp754	C3b alpha chain
C3b	Ile755	C3b alpha chain
C3b	Ile756	C3b alpha chain
C3b	Glu759	C3b alpha chain
C3b	Asn760	C3b alpha chain
C3b	Val762	C3b alpha chain
C3b	Ser763	C3b alpha chain
C3b	Ser765	C3b alpha chain
C3b	Glu766	C3b alpha chain
C3b	Trp771	C3b alpha chain
C3b	Leu772	C3b alpha chain
C3b	Trp773	C3b alpha chain
C3b	Asn774	C3b alpha chain
C3b	Val775	C3b alpha chain
C3b	Glu776	C3b alpha chain
C3b	Asn792	C3b alpha chain
C3b	Ile793	C3b alpha chain
C3b	Phe794	C3b alpha chain

C3b	Lys796	C3b alpha chain
C3b	Asp797	C3b alpha chain
C3b	Phe920	C3b alpha chain
C3b	Ser922	C3b alpha chain
C3b	Arg926	C3b alpha chain
C3b	Lys1244	C3b alpha chain
C3b	Phe1246	C3b alpha chain
C3b	Glu1291	C3b alpha chain
C3b	Arg1303	C3b alpha chain
C3b	Ser1305	C3b alpha chain
C3b	Lys1306	C3b alpha chain
C3b	Ile1307	C3b alpha chain
C3b	Thr1308	C3b alpha chain
C3b	His1309	C3b alpha chain
C3b	Arg1310	C3b alpha chain
C3b	His1312	C3b alpha chain
C3b	Glu1314	C3b alpha chain
C3b	Ser1315	C3b alpha chain
C3b	Ser1317	C3b alpha chain
C3b	Arg1320	C3b alpha chain
C3b	Glu1322	C3b alpha chain
CR1	Tyr978	SCR 15-17
CR1	Arg980	SCR 15-17
CR1	Asp997	SCR 15-17
CR1	Val998	SCR 15-17
CR1	Lys1000	SCR 15-17
CR1	Lys1002	SCR 15-17
CR1	Ser1003	SCR 15-17
CR1	Lys1005	SCR 15-17
CR1	Thr1006	SCR 15-17
CR1	Asn1007	SCR 15-17
CR1	Asn1008	SCR 15-17
CR1	Asp1009	SCR 15-17
CR1	Val1011	SCR 15-17
CR1	Asn1012	SCR 15-17
CR1	Gly1013	SCR 15-17
CR1	Met1014	SCR 15-17
CR1	Val1015	SCR 15-17
CR1	His1016	SCR 15-17
CR1	Val1017	SCR 15-17
CR1	Thr1019	SCR 15-17
CR1	Asp1020	SCR 15-17
CR1	Ile1021	SCR 15-17
CR1	Thr1032	SCR 15-17

CR1	Thr1033	SCR 15-17
CR1	His1035	SCR 15-17
CR1	Asn1050	SCR 15-17
CR1	Thr1051	SCR 15-17
CR1	Asp1076	SCR 15-17
CR1	Ile1078	SCR 15-17
CR1	Ser1079	SCR 15-17
CR1	Thr1080	SCR 15-17
CR1	Asn1081	SCR 15-17
CR1	Arg1082	SCR 15-17
CR1	Glu1083	SCR 15-17
CR1	Asn1084	SCR 15-17
CR1	His1086	SCR 15-17
CR1	Tyr1087	SCR 15-17
CR1	Gly1088	SCR 15-17
CR1	Ser1089	SCR 15-17
CR1	Val1090	SCR 15-17
CR1	Thr1092	SCR 15-17
CR1	Arg1094	SCR 15-17
CR1	Ser1099	SCR 15-17
CR1	Arg1100	SCR 15-17
CR1	Ser1112	SCR 15-17
CR1	Tyr1114	SCR 15-17
CR1	Thr1116	SCR 15-17

Table S2. Hotspot residues at the complex C3b – CR1 SCR 15-17 binding interface

Protein	Residue	Chain/domain	KFC	PredHS	DrugScore ^{PPI}
C3b	Asp178	C3b beta chain	+	-	X
C3b	Ser179	C3b beta chain	+	-	X
C3b	Leu180	C3b beta chain	+	+	X
C3b	Ser181	C3b beta chain	-	+	X
C3b	Gln185	C3b beta chain	-	+	X
C3b	Val762	C3b alpha chain	+	-	X
C3b	Trp773	C3b alpha chain	+	+	X
C3b	Phe794	C3b alpha chain	+	-	X
C3b	Lys796	C3b alpha chain	+	-	X
C3b	Phe920	C3b alpha chain	+	-	X
C3b	His1309	C3b alpha chain	+	-	X
C3b	Arg1320	C3b alpha chain	+	+	X
CR1	Tyr978	SCR 15-17	+	-	+
CR1	Arg980	SCR 15-17	-	+	-
CR1	Thr1006	SCR 15-17	+	-	-
CR1	Asp1009	SCR 15-17	+	-	-
CR1	Asn1012	SCR 15-17	-	+	-
CR1	Met1014	SCR 15-17	-	+	-
CR1	Val1017	SCR 15-17	+	-	-
CR1	Thr1080	SCR 15-17	+	+	-
CR1	Arg1082	SCR 15-17	+	-	+
CR1	Val1090	SCR 15-17	+	+	+
CR1	Tyr1114	SCR 15-17	-	-	+

‘+’ indicates a positive result, ‘-’ indicates a negative result, ‘X’ indicates that no analysis was performed.

Table S3. Confidence assessment of CR1-like models generated by I-TASSER.

Model	C-score	TM-score	Estimated RMSD
SCR12-14	-1.13	0.57±0.14	7.7±4.3Å
SCR 19-21	-0.42	0.66±0.13	6.1±3.8Å

Table S4. CR1-like model quality assessment.

Model	Tools	Ramachandran plot (%)				Verify 3D (%) residues score>0.2%
		Favoured	Allowed	General	Disallowed	
CR1-like ₍₁₂₋₁₄₎	I-TASSER	85.7	12.3	1.9	0.0	81.5
CR1-like ₍₁₂₋₁₄₎	trRosetta	89.0	9.7	0.6	0.6	28.0
CR1-like ₍₁₂₋₁₄₎	RoseTTAFold	88.3	10.4	0.6	0.6	32.8
CR1-like ₍₁₂₋₁₄₎	AlphaFold	90.3	9.7	0.0	0.0	31.2
CR1-like ₍₁₉₋₂₁₎	I-TASSER	88.0	10.8	1.3	0.0	89.4
CR1-like ₍₁₉₋₂₁₎	trRosetta	89.2	10.1	0.0	0.6	25.4
CR1-like ₍₁₉₋₂₁₎	RoseTTAFold	88.0	10.8	0.0	1.3	35.5
CR1-like ₍₁₉₋₂₁₎	AlphaFold	87.3	12.7	0.0	0.0	29.6