

Supplementary information

STRUCTURE OF COMPSTATIN IN COMPLEX WITH COMPLEMENT COMPONENT C3c REVEALS A NEW MECHANISM OF COMPLEMENT INHIBITION

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Supplementary Table I, Domain rotations and translations.

a, Domain rotation and translation between the two C3c-compstatin complexes in the asymmetric unit. C3c-compstatin complex 2 (chains D,E,F,H) was superposed on complex 1 (chains A,B,C,G) on the basis of MG1, MG2, MG4-6 of the β -ring. Using this superposition the rotation and center-of-mass translation of the domains were calculated with SUPERPOSE in the CCP4 package (1).

b, Domain rotation and translation between the two C3c-compstatin complexes and two C3c molecules in the asymmetric unit (pdb entry 2A74) (2). C3c-compstatin molecule 1 consists of chains A-C, C3c-compstatin molecule 2 consists of chains D-F, C3c molecule 1 consists of chains A-C and C3c molecule 2 consists of chains D-F. C3c-compstatin molecule 2 and both C3c molecules (2) were superposed on C3c-compstatin molecule 1 on the basis of MG1, MG2, MG4-6 of the β -ring. Using these superpositions the rotation and center-of-mass translation of the domains were calculated with SUPERPOSE in the CCP4 package (1).

c, Rmsd of residues involved in compstatin binding between C3c-compstatin, C3c (pdb entry 2A74) (2) and C3 (pdb entry 2A73) (2). Molecules were first superposed on the basis of domains MG4 and MG5. Calculations were done with LSQMAN (3).

Supplementary Table II, Interactions between compstatin and C3c.

Interactions were calculated with Ligplot (4). Distances are listed for both molecule 1 (chains A, B, C and G on the left) and molecule 2 (chains D, E, F and H on the right). Residue numbering 0-14 are from compstatin, residue numbering 345-492 are from C3c.

Supplementary Figure 1, The two compstatin molecules in the asymmetric unit.

a, b, Stereo diagram of the superposition of the two compstatin molecules in the asymmetric unit shown in stick representation. **b**, The two compstatin molecules shown in the same orientation as compstatin in **Figure 1D**.

Supplementary Figure 2, Multiple sequence alignment of the compstatin binding site region, C3 domains MG4 and MG5, from different species.

The alignment was prepared with ClustalW (5). Residues involved in hydrogen bonding and van der Waals contacts are highlighted in yellow (conserved) and blue (non-conserved). The primate sequences are from *Homo sapiens*, *Pan troglodytes* and *Macaca mulatta*.

References

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2. Janssen, B. J., Huizinga, E. G., Raaijmakers, H. C., Roos, A., Daha, M. R., Nilsson-Ekdahl, K., Nilsson, B., and Gros, P. (2005) *Nature* **437**(7058), 505-511
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4. Wallace, A. C., Laskowski, R. A., and Thornton, J. M. (1995) *Protein Eng* **8**(2), 127-134
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Supplementary Table I Janssen et al.

Table Ia Domain rotation and translation between the two C3c-compstatin complexes in the asu

C3c-comp - C3c-comp	translation (Å)	rotation (°)
MG1	0.4	3.1
MG2	0.7	3.5
MG3	1.5	4.4
MG4	0.5	2.4
MG5	0.2	0.5
MG6	0.7	4.5
LNK	0.1	1.0
MG7	2.0	5.3
MG8	3.5	7.1
C345C	5.3	7.2
Compstatin	0.4	1.1

Table Ib Domain rotation and translation between the two C3c-compstatin complexes and two C3c molecules

C3c-compstatin - C3c	mol. 1 on 1		mol. 1 on 2		mol. 2 on 1		mol. 2 on 2	
	translation (Å)	rotation (°)	translation (Å)	rotation (°)	translation (Å)	rotation (°)	translation (Å)	rotation (°)
MG1	0.7	3.5	0.3	2.0	0.4	1.3	0.5	3.2
MG2	0.5	2.8	0.2	1.2	0.5	4.3	0.5	2.6
MG3	1.2	3.6	0.4	0.7	0.5	0.8	1.1	4.1
MG4	0.6	3.6	0.3	2.4	0.4	3.3	0.3	3.0
MG5	0.2	1.9	0.2	1.9	0.0	1.4	0.2	1.7
MG6	0.8	5.6	0.3	3.7	0.2	0.6	0.5	4.1
LNK	0.7	0.6	0.0	0.6	0.2	0.3	0.1	1.6
MG7	1.5	3.1	0.7	2.4	0.8	2.4	1.4	3.0
MG8	1.5	1.7	0.9	1.6	2.0	8.3	2.6	5.7
C345C	4.2	15.4	3.0	12.7	5.1	12.5	5.5	12.1

Table Ic Rmsd (Å) of the residues involved in compstatin binding

	Cα's (15 atoms)	all atoms (119, non-hydrogen)
C3c-compstatin - C3c	1.2	1.9
C3c-compstatin - C3	0.7	1.3
C3 - C3c	1.1	1.7

Supplementary Table II Janssen et al.

Table II Interactions between compstatin and C3c

A: Hydrogen bonds between compstatin and C3c

Donor	Acceptor		Distance (Å)	
Ace0 O	Asn390	OD1	2.95	
Ile1 N	Asn390	OD1	2.88	2.54
Trp4 N	Gly345	O	3.05	2.98
Gln5 NE2	Leu455	O	2.66	
Gln5 NE2	Asp491	OD2	2.72	3.26
Trp7 NE1	Met457	O	2.63	2.57
His10 N	Asp491	OD1	2.81	2.61
Arg456 NE	Trp4	O	3.06	
Met457 N	Gln5	OE1	2.85	

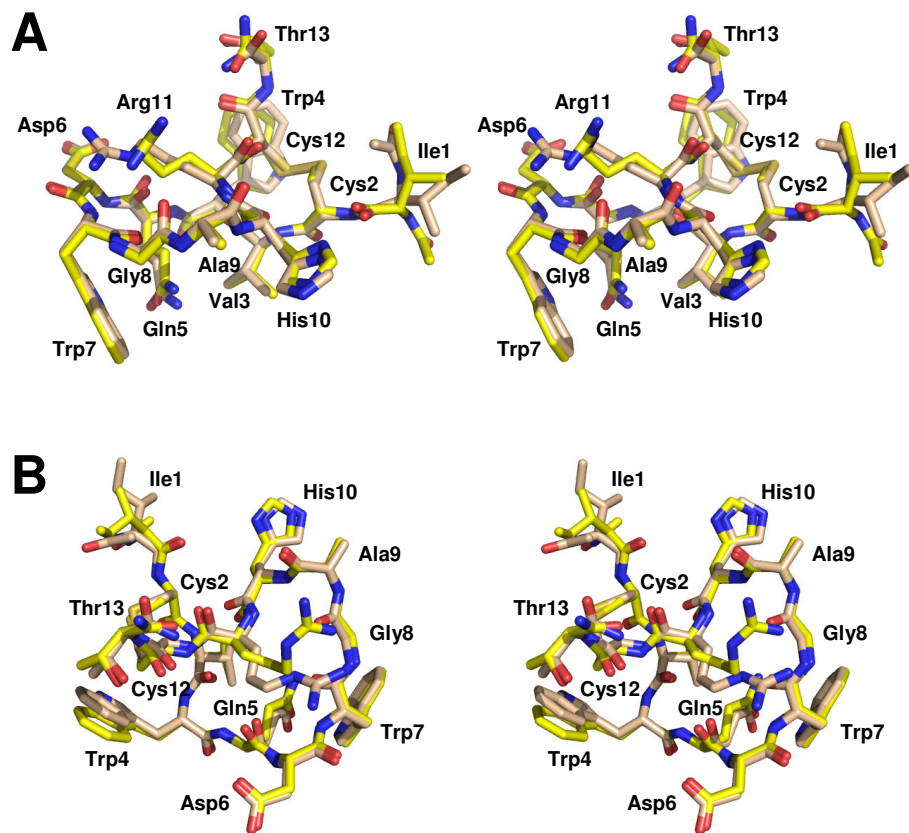
B: Hydrogen bonds within compstatin

Donor	Acceptor		Distance (Å)	
Val3 N	His10	O	3.02	2.99
Gln5 N	Val3	O	2.94	3.03
Cys12 N	Val3	O	3.01	
Thr13 OG1	Cys12	O	2.95	
NH ₂ 14 N	Cys12	O	2.48	

C: Hydrophobic interactions between C3c and compstatin

Compstatin	C3c		Distance (Å)	
Cys2 SG	Asn390	CG	3.84	
Val3 CG2	Met346	CE	3.88	
Val3 CG2	Pro347	CD	3.84	
Val3 CG2	Leu454	CD2	3.79	3.82
Val3 CG2	Leu454	CG	3.70	3.81
Val3 CG1	Arg456	CB	3.84	3.74
Val3 CG1	Arg456	CD	3.46	3.90
Trp4 CB	Pro393	CG	3.59	
Trp4 CZ2	His392	CE1	3.71	3.68
Gln5 CD	Arg456	CA	3.88	
Trp7 CH2	Leu455	CD2	3.89	
Trp7 CD1	Arg459	CA	3.70	3.79
Trp7 CD1	Arg459	CB	3.83	
Trp7 CE2	Glu462	CG	3.81	
Trp7 CE2	Glu462	CB	3.85	
Trp7 CZ2	Glu462	CB	3.66	3.66
Trp7 CZ3	Gly489	C	3.56	3.71
Gly8 C	Asp491	CG	3.80	
Gly8 CA	Asp491	CG	3.50	3.68
His10 CD2	Asp491	CB	3.86	3.43
His10 CD2	Leu492	CB	3.70	

Supplementary Figure 1 Janssen et al.



Supplementary Figure 2 Janssen et al.

	330	340	350	360	370	380
Homo sapiens	SPYQIHFTKTPKYFKP	MPFDLMVFVTNPDGSPAYRVPVAVQGEDTVQSLTQGDGVAKLS				
Pan troglodytes	SPYQIHFTKTPKYFKP	MPFDLMVFVTNPDGSPAYRVPVAVQGEDTVQSLTQGDGVAKLS				
Macaca mulatta	SPYQIHFTKTPKYFKP	MPFDLMVFVTNPDGSPAYRVPVAVQGEDAVQSLTQGDGVAKLS				
R. norvegicus	SPYQIHFTKTPKFFKP	MPFDLMVFVTNPDGSPARRVPVVTQGSN-AQALTQDDGVAKLS				
Bos taurus	SPYQIHFTKTPKFFKP	MPFDLMVYVTNPDGSPARHIPVVTQGSN-VQSLTQDDGVAKLS				
Mus musculus	SPYQIHFTKTPKFFKP	MPFDLMVFVTNPDGSPASKVLVVTQGSN-AKALTQDDGVAKLS				
Canis familiaris	SPYQIHFTKTPKFFKP	MPFDLMVFVTNPDGSPAPHVVPVGIQNYR-VQALTQKQDGVAKLT				
Guinea pig	SPYQIHFTKTPKYFKP	MPFEIMVLVTNPDGSPAPHVVPVVTQGSN-VQSLTQADGVARLS				
Sus scrofa	SPYQIHFTKTPKFFKP	MPFDLMVYVTNPDGSPARHIPVVTEDFK-VRSLTQEDGVAKLS				

MG4

	390	400	410	420	430	440
Homo sapiens	INTHPSQKPLSITVTRTKKQELSEAEQATRIMQALPYSTVGNSSNNYLHLSVLRTELRPGET					
Pan troglodytes	INTHPSQKPLSITVTRTKKQELSEAEQATSTMQALPYSTVGNSSNNYLHLSVPRTELRPGET					
Macaca mulatta	INTHPSQKPLSITVTRTKKQELSEAEQATRIMEAQPYSTVGNSSNNYLHLSVPRAELRPGET					
R. norvegicus	VNTPNNRQPLTITVSTKKEGIPDARQATRIMQAQPYSTMHNSNNYLHLSVSRVELKPGDN					
Bos taurus	INTQNKRDPLTITVTRTKKDNIPESRQATRIMQALPYNTQGNSSNNYLHLSVPRVELKPGET					
Mus musculus	INTPNSRQPLTITVTRTKKDTLPESRQATKTMEAHPYSTMHNSNNYLHLSVSRMELKPGDN					
Canis familiaris	INTPDSKPLHITVSTKKEGILESRAQATRIMEVQPYNTIGNSRNYLHLSVPRMELKPGET					
Guinea pig	INTPNTRQPLSVTVQTKKGGIPDARQAINTMQALPYTMYNSNNYLHLSMPRTELKPGET					
Sus scrofa	INTPDNRNSLPITVTRTEKDGIPAARQASKTMHVLPYNTQGNSSNNYLHLSLPRVELKPGEN					

	450	460	470	480	490	500
Homo sapiens	LNVNELLRMDRAHEAKIRYYTYLIMNKGKLLKAGRQVREFGQDLVVLPLSITITDFIPSF					
Pan troglodytes	LNVNELLRMDRAHEAKIRYYTYLIMNKGKLLKAGRQVREFGQDLVVLPLSITITDFIPSF					
Macaca mulatta	LNVNELLRMDRTQEAQIRYYTYLIMNKGKLLKAGRQVREFGQDLVVLPLSITITDFIPSF					
R. norvegicus	LNVNELLRTDAGQEAQIRYYTYLVMNKGKLLKAGRQVREFGQDLVVLPLSITITDFIPSF					
Bos taurus	LNVNELLRTDPGGEAKIRYYTYMIMNKGKLLKAGRQVREFGQDLVVLPLSITITDFIPSF					
Mus musculus	LNVNELLRTDPGHEAKIRYYTYLVMNKGKLLKAGRQVREFGQDLVVLPLSITITDFIPSF					
Canis familiaris	LNVNELLRTDPSKEAQIRYYTYLIMNKGKLLKAGRQVREFGQDLVVLPLSITITDFIPSF					
Guinea pig	INVNELLRSDPNQEAQIRYYTYLIMNKGKLLKAGRQVREFGQDLVVLPLSITITDFIPSF					
Sus scrofa	LNVNELLRTDPGYQDKIRYYTYLIMNKGKLLKAGRQVREFGQDLVVLPLSITITDFIPSF					

MG5

	510	520	530
Homo sapiens	LVAYYTLIGASGQREVVADSVWVDVK		
Pan troglodytes	LVAYYTLIGASGQREVVADSVWVDVK		
Macaca mulatta	LVAYYTLIGANGQREVVADSVWVDVK		
R. norvegicus	LVAYYTLIGANGQREVVADSVWVDVK		
Bos taurus	LVAYNTLINAKGQREVVADSVWVDVK		
Mus musculus	LVAYYTLIGASGQREVVADSVWVDVK		
Canis familiaris	LVAYYTVIGSSGQREVVADSVWVDVK		
Guinea pig	LVAYYTLIGASAQREVVADSVWADVR		
Sus scrofa	LVAYYTLIAANGQREVVADSVWVDVK		