Supporting Information

Venkatraman Girija et al. 10.1073/pnas.1311113110

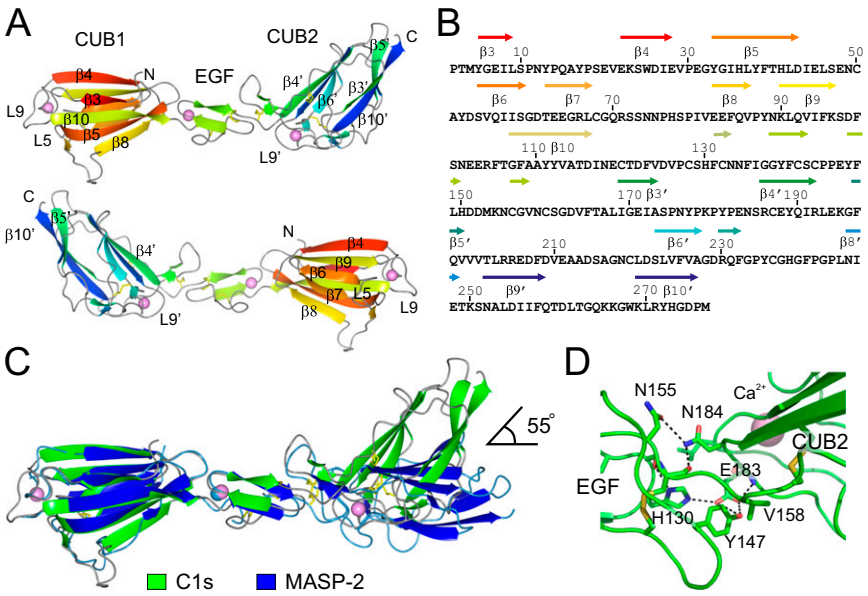

Fig. S1. Structure of the complement C1r/C1s, Uegf, and Bmp1 (CUB)1-EGF-CUB2 fragment of C1s. (*A* and *B*) Each CUB domain is a β-sandwich with eight β-strands, β3–β10, linked by loops L2–L9 (following the nomenclature for seminal plasma spermadhesins). Ca^{2+} (pink) are positioned between loops L5 and L9 and are coordinated by the side chains of Glu45, Asp53, Asp98, Asn101, the carbonyl oxygen of Ser100, and a water molecule in CUB1 and by the side chains of Glu211, Asp221, Asp260, the main chains of Thr262 and Gly263, and a water in CUB2. A disulphide bond (yellow) links loop L5 to loop L7 in both domains and CUB2 contains an extra disulphide linking loop L2 to strand β4. The Ca^{2+} in the EGF-like domain is coordinated by side chains of Asp116, Glu119, Asn134, the main chain carbonyl oxygens of Phe135 and Gly138, and a water molecule. The domain is stabilized by three disulfide bonds (yellow). (*C*) The CUB1-EGF domains of C1s (green) align closely with corresponding regions of rat mannan-binding lectin-associated serine protease (MASP)-2 (blue; PDB ID code: 1NTO; rmsd of 1.1 based on 127 C-α atoms) (1), but CUB2 is rotated by ∼55°. (*D*) The EGF-CUB2 interface of C1s is stabilized via a network of hydrogen bonds (dotted lines) and hydrophobic interactions. In particular, Tyr147 of the EGF-like domain is sandwiched between Val158 and Glu183 of the CUB, with the hydroxyl group interacting with the acidic side chain. Additional hydrogen bonds from Asn155 and Cys156 to Asn184 and between His130 and Glu183 further stabilize the domain interface.

1. Feinberg H, et al. (2003) Crystal structure of the CUB1-EGF-CUB2 region of mannose-binding protein associated serine protease-2. *EMBO J* 22(10):2348–2359.

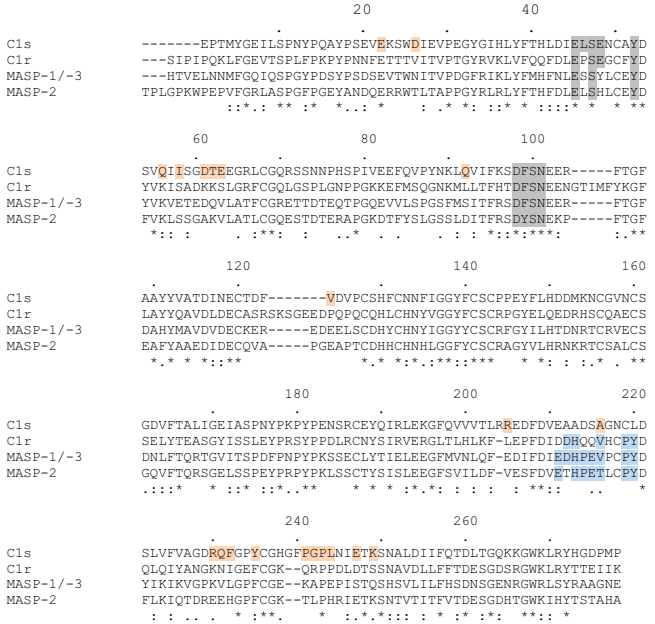

Fig. S2. Sequence alignment of the CUB1-EGF-CUB2 domains of human C1s with C1r, MASP-1/-3, and MASP-2. Residues involved in the CUB1–collagen interaction are shaded gray, the CUB2–collagen interaction in blue (from the structure of CUB2 of MASP-1 bound to a collagen-like peptide from mannan-binding lectin; ref. 1), and the C1s–C1s interface in orange. Numbering refers to C1s.

1. Gingras AR, et al. (2011) Structural basis of mannan-binding lectin recognition by its associated serine protease MASP-1: Implications for complement activation. *Structure* 19(11): 1635–1643.

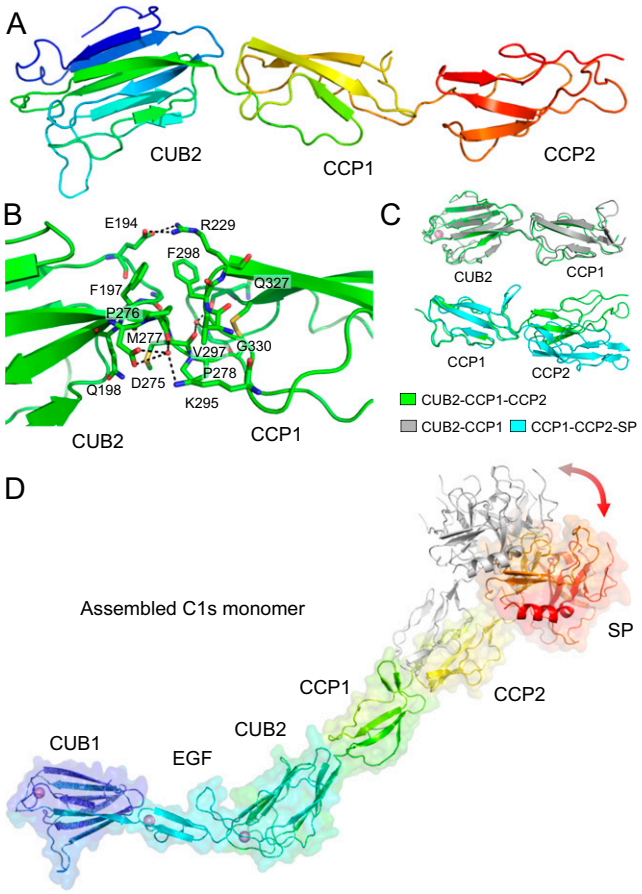

Fig. S3. Structure of C1s. (*A*) The CUB2-complement control module (CCP)1-CCP2 structure. Although crystallized in the presence of Ca^{2+}, no Ca^{2+} was bound to the CUB2 domain probably due to citrate in the crystallization buffer, which chellates Ca^{2+}. (*B*) The CUB2-CCP1 interface from the CUB2-CCP1 structure. (*C Upper*) The CUB2 and CCP1 domains overlay in the CUB2-CCP1-CCP2 (*green*) and CUB2-CCP1 structures (gray) with a rmsd of 1.6 Å over 183 C-α atoms. Ca^{2+} (pink sphere) was observed in the shorter fragment only. (*C Lower*) A difference in the position of the CCP2 domains in the CUB2-CCP1-CCP2 (*green*) and CCP1-CCP2-SP structures (blue; PDB ID code: 4J1Y) (1) suggests potential flexibility at the CCP1-CCP2 junction of C1s. (*D*) Assembled C1s monomer showing the alternative position of the CCP2-SP domains (gray), arising from flexibility at the CCP1-CCP2 junction.

1. Perry AJ, et al. (2013) A molecular switch governs the interaction between human complement protease C1s and its substrate, complement C4. *J Biol Chem* 288(22):15821–15829.

Table S1. Data collection and refinement statistics

	C1s collagen complex	C1s tetramer	C1s CUB2-CCP1	C1s CUB2-CCP1-CCP2
Data collection				
Beam line	Diamond I04-1	Diamond I04-1	Diamond I04-1	Diamond I04
Space group	C 1 2 1	P 1 2_1 1	P 2_1 2_1 2_1	C 1 2 1
a, *b*, *c*, Å	71.3, 71.2, 98.4	67.9, 71.7, 157.7	66.5 69.2 76.7	143.0 58.6 51.2
α, β, γ, °	90, 111.1, 90	90, 95.9, 90	90, 90, 90	90 91.1 90
Resolution, Å	48.57–2.50 (2.59–2.5)	67.51–2.92 (3.03–2.92)	30.0–1.97 (2.12–1.97)	54.22–2.92 (2.95–2.92)
R_{sym}	8.2 (48.9)	12.3 (51.4)	2.7 (30.9)	8.4 (32.9)
$I/\sigma I$	12.8 (2.7)	9.60 (2.1)	33.3 (5.6)	5.69 (2.1)
Completeness	93.2 (82.1)	98.9 (94.2)	99.3 (97.2)	94.8 (94. 9)
Redundancy	3.9 (3.6)	6.6 (3.9)	6.4 (6.6)	2.8 (2.6)
Refinement				
Resolution, Å	48.57–2.50	67.51–2.92	28.49–1.97	54.22–2.92
No. of reflections	14,978 (1,572)	32,700 (3,003)	24,501 (2,328)	9,504 (1,368)
R_{work}/R_{free}	0.195/0.251	0.208/0.252	0.179/0.217	0.271/0.293
No. of atoms	2,703	8,768	1,610	1,908
Protein	2,653	8,752	1,463	1,908
Ligand/ion	5	16	1	—
Water	47	—	146	—
B actors, $Å^2$	48.4	46.50	52.00	132.20
Protein	48.2	46.50	51.70	132.20
Ligand/ion	45.3	—	38.80	—
Water	43.4	—	55.40	—
rms deviations				
Bond lengths, Å	0.011	0.003	0.007	0.004
Bond angles, °	0.86	0.77	1.12	0.96

The highest resolution shell is shown in parenthesis.