Supporting Information

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Production, Purification, and Crystallization of C1r CUB2

The structure of a mutant form of the CUB2 domain of C1r was determined at 1.95 Å resolution and served as a reference during refinement of the two C1r-C1s structures. Four mutations (D226E, Q229P, Q230E, and H232P) were introduced to the corresponding residues at equivalent positions in MASP-1 with the aim of investigating the binding specificity of C1r for C1q over mannan-binding lectin (work ongoing). The mutant form of C1r crystallized, whereas the wild-type CUB2 domain did not. Recombinant protein was produced by cloning the mutated cDNA encoding the CUB2 domain of C1r into the NcoI and EcoR1 restriction sites of plasmid pET28a. *Escherichia coli* BL21 DE3 cells containing the resulting expression vector were grown in Power Prime broth (Molecular Dimensions), induced during mid-log phase with isopropyl β-D-1-thiogalactopyranoside (1 mM), and then harvested after growth at 37 °C for an additional 16 h. Inclusion bodies were isolated and protein refolded as described previously (1). Proteins were purified initially by ion exchange chromatography on a 10-mL Q-Sepharose column, using a 0.05–1 M gradient of NaCl in 20 mM Tris·HCl pH 8.0, followed by gel filtration on a Superdex 75 16/60 column (GE Healthcare) in 20 mM Tris pH 7.5 containing 50 mM NaCl and 2 mM $CaCl_2$. Crystals were grown by mixing equal volumes of protein with reservoir solution composed of 50 mM Tris·HCl pH 8.5 containing 25% PEG-8K, 2 mM $CaCl_2$, and 3% 1,6-diaminohexane. Data were collected and analyzed as described in the main text. Phases were solved by molecular replacement using the CUB2 domain of C1s as a search model (PDB ID code 4LOR) (2).

1. Major B, et al. (2010) Calcium-dependent conformational flexibility of a CUB domain controls activation of the complement serine protease C1r. *J Biol Chem* 285: 11863–11869.

2. Venkatraman Girija U, et al. (2013) Structural basis of the C1q/C1s interaction and its central role in assembly of the C1 complex of complement activation. *Proc Natl Acad Sci USA* 110:13916–13920.

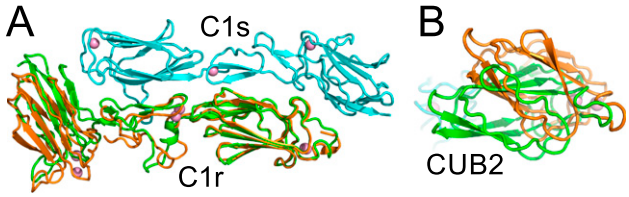

Fig. S1. Rotation at the EGF-CUB2 junction of C1r. (*A*) The C1r-C1s heterodimer overlaid with a polypeptide from the C1r homodimer. C1r is in green, and C1s is in blue. The polypeptide from the C1r homodimer is in brown. (*B*) Side view showing rotation of the CUB2 domain of C1r by ~30° in the C1r homodimer.

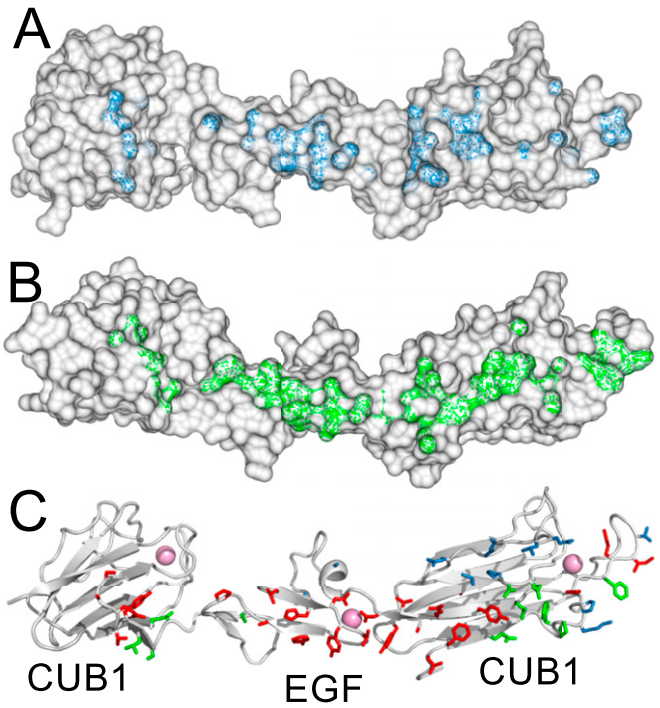

Fig. S2. Comparison of the C1r and C1r-C1s dimer interfaces. (*A* and *B*) Structures of the C1r fragment showing the interface of the C1r homodimer (*A*) and the C1r-C1s heterodimer (*B*). (*C*) Residues completely or partially buried at the interface of the homodimer (blue), the heterodimer (green), or in both complexes (red).

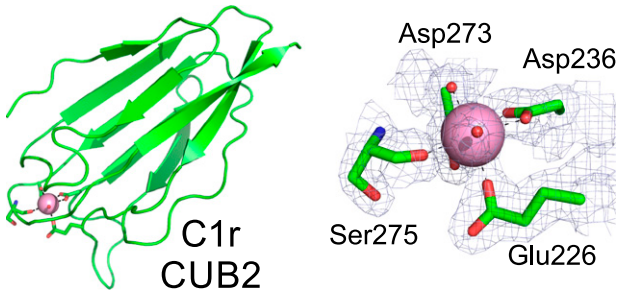

Fig. S3. (*Left*) The structure of the mutant form of the CUB2 domain of C1r served a reference during refinement of the two C1r-C1s structures. (*Right*) Close-up view of the Ca^{2+}-binding site. Ca^{2+} is shown as a pink sphere. The smaller red spheres represent water molecules.

Table S1. Data collection and refinement statistics

Parameter	C1r-C1s 1 CUB1-EGF-CUB2	C1r-C1s 2 CUB1-EGF-CUB2	C1r-C1r CUB1-EGF-CUB2	C1r CUB2
PDB ID code	6F1C	6F1H	6F39	6F1D
Data collection				
Beam line	Diamond I04-1	Diamond I02	Diamond I03	Diamond I03
Space group	P 2_1 2_1 2_1	P 2_1 2_1 2_1	C 1 2 1	P 2_1 2_1 2_1
a, *b*, *c*, Å	96.8, 123.7, 195.5	88.2 88.3 197.6	140.5 54.6 137.6	44.7, 50.5, 69.2
α, β, γ, °	90, 90, 90	90, 90, 90	90 100.2 90	90, 90, 90
Resolution, Å	97.8–4.2 (4.70–4.20)	88.3–4.5 (5.00–4.50)	68.1–5.8 (6.49–5.80)	44.49–1.95 (2.00–1.95)
$R_{sym,\ \%}$	15.3 (93.3)	16.4 (96.7)	32.4 (195.2)	7.4 (54.5)
$R_{pim,\ \%}$	8.3 (53.3)	11.4 (65.7)	11.8 (69.2)	5.4 (39.8)
CC(1/2)	0.998 (0.814)	0.994 (0.740)	0.997 (0.610)	0.998 (72.1)
$I/\sigma I$	6.6 (1.9)	5.4 (1.9)	5.1 (1.8)	13.4 (3.2)
Completeness	99.8 (99.7)	98.1 (98.5)	99.8 (100)	98.1 (99.1)
Redundancy	7.6 (7.1)	5.0 (5.3)	15.2 (16.5)	4.8 (4.8)
Resolution, Å	86.73–4.20 (4.35–4.20)	80.64–4.50 (4.66–4.50)	68.06–5.80 (6.00–5.80)	33.45–1.95 (2.02–1.95)
No. of reflections	17,763 (1,738)	9,382 (919)	2,987 (293)	11,599 (1,163)
R_{work}/R_{free}	0.247/0.305	0.300/0.339	0.315/0.333	0.173/0.202
No. of atoms	9,260	9,328	4,608	1,031
Protein	9,003	9,007	4,456	944
Ligand/ion	257	321	166	2
Water	—	—	—	85
B-factors, Å^2	226.8	254.0	354.8	34.38
Protein	225.0	253.0	353.3	33.59
Ligand/ion	286.4	281.6	395.3	23.27
Water	—	—	—	43.44
Bond lengths, Å	0.004	0.006	0.004	0.012
Bond angles, °	0.91	0.77	0.98	1.34

The highest-resolution shell is shown in parentheses.