Supplementary Information Janssen et al.

Insights into complement convertase formation based on the structure of the factor B–CVF complex

Bert J.C. Janssen[1†], Lucio Gomes[1], Roman I. Koning[2], Dmitri I. Svergun[3], Abraham J. Koster[2], David C. Fritzinger[4], Carl-Wilhelm Vogel[4] and Piet Gros[1,#]

1. Crystal and Structural Chemistry, Bijvoet Center for Biomolecular Research, Faculty of Science, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

2. Department of Molecular Cell Biology, Section Electron Microscopy, Leiden University Medical Center, 2300 RC, Leiden, The Netherlands

3. European Molecular Biology Laboratory, Hamburg Outstation, Notkestrasse 85, 22603 Hamburg, Germany

4. Cancer Research Center of Hawaii, University of Hawaii at Manoa, 1236 Lauhala Street, Honolulu, HI 96813, USA

† Present address: Division of Structural Biology, University of Oxford, Henry Wellcome Building of Genomic Medicine, Roosevelt Drive, Oxford OX3 7BN, UK.

Supplementary Table I

Table I Data collection and refinement statistics

	2.2-Å ($C222_1$)	3.0-Å ($P2_12_12_1$)
Data collection		
Space group	$C222_1$	$P2_12_12_1$
Cell dimensions		
a, *b*, *c* (Å)	128.9, 283.5, 134.4	134.0, 137.0, 283.7
Resolution (Å)	34-2.2 (2.32-2.20)*	35-3.0 (3.16-3.00)*
R_{merge} (%)	9.3 (60.2)	9.1 (55.5)
$I/\sigma I$	11.8 (2.6)	12.6 (2.8)
Completeness (%)	99.6 (99.3)	99.7 (99.9)
Redundancy	3.8 (3.8)	3.8 (3.9)
Refinement		
Resolution (Å)	34-2.2	35-3.0
No. reflections	123939	104868
R_{work}/ R_{free} (%)	18.0 / 22.6	18.9 / 24.3
No. atoms	15938	30435
Protein	15121	30423
Ligand/ion	66	4
Water	751	8
B-factors (Å^2)		
Protein	45.1	89.1
Ligand/ion	76.4	96.0
Water	39.6	58.0
R.m.s deviations		
Bond lengths (Å)	0.007	0.004
Bond angles (°)	1.113	0.888

*Highest resolution shell is shown in parenthesis.

Supplementary Table II

Table IIA Domain rotation, translation and similarity between CVF and C3b

	Translation (Å)	Rotation (°)	RMSD Cα (Å)
CVF-C3b (2I07)			
MG1	3.4	1.2	1.0
MG2	1.4	6.3	1.5
MG3	0.9	2.5	1.1
MG4	0.4	2.3	1.0
MG5	0.1	1.9	0.8
MG6	0.6	2.7	0.7
LNK	0.6	2.5	1.0
MG7	0.6	4.3	0.7
CUB	3.6	13.3	1.1
MG8	0.6	5.9	1.1
C345c	4.2	11.3	1.3
CVF-C3b (2ICF)			
MG1	0.9	4.1	1.4
MG2	1.0	4.6	1.4
MG3	0.8	1.8	1.3
MG4	0.4	3.9	0.9
MG5	0.2	3.4	0.8
MG6	0.6	2.8	0.8
LNK	0.6	3.2	0.9
MG7	0.5	4.3	1.0
CUB	1.9	4.7	1.1
MG8	1.0	7.3	1.5
C345c	---*	---*	---*
CVF-CVF (3FRP)			
MG1	0.4	1.7	0.5
MG2	0.4	1.9	0.4
MG3	0.3	1.1	0.4
MG4	0.2	0.4	0.3
MG5	0.1	0.7	0.3
MG6	0.6	1.2	0.4
LNK	0.1	1.2	0.4
MG7	0.8	4.5	0.4
CUB	2.2	3.6	1.2
MG8	1.2	5.1	0.8
C345c	3.0	28.8	0.9

*This domain was not refined in the C3b-CRIg structure (pdb code 2ICF)

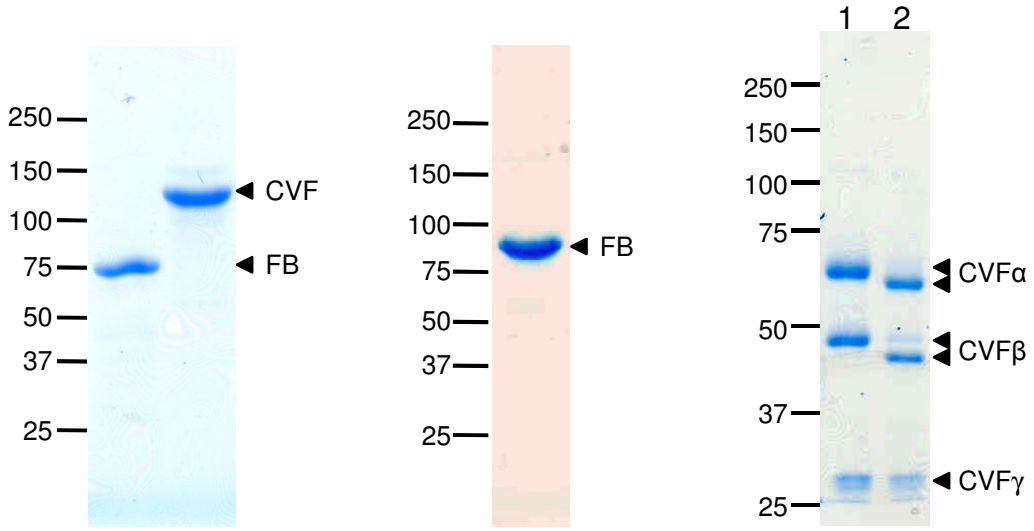
Table IIB Domain rotation and translation between FB bound to CVF and free FB

	Translation (Å)	Rotation (°)
FB(CVF) 2.2Å - FB (2OK5)		
CCP1	4.5	26
CCP2	3.8	7.7
CCP3	1.2	5.3
VWA	---	---
SP	4.7	10.4
FB(CVF) 3.0Å chain D - FB (2OK5)		
CCP1	5.0	18
CCP2	0.7	3.0
CCP3	0.5	1.8
VWA	---	---
SP	3.9	10.4
FB(CVF) 3.0Å chain I - FB (2OK5)		
CCP1	3.8	10.0
CCP2	1.2	4.4
CCP3	0.9	2.5
VWA	---	---
SP	4.0	10.4

Supplementary Table II. Comparison of CVF bound to FB with free C3b and free CVF, and FB bound to CVF with free FB.

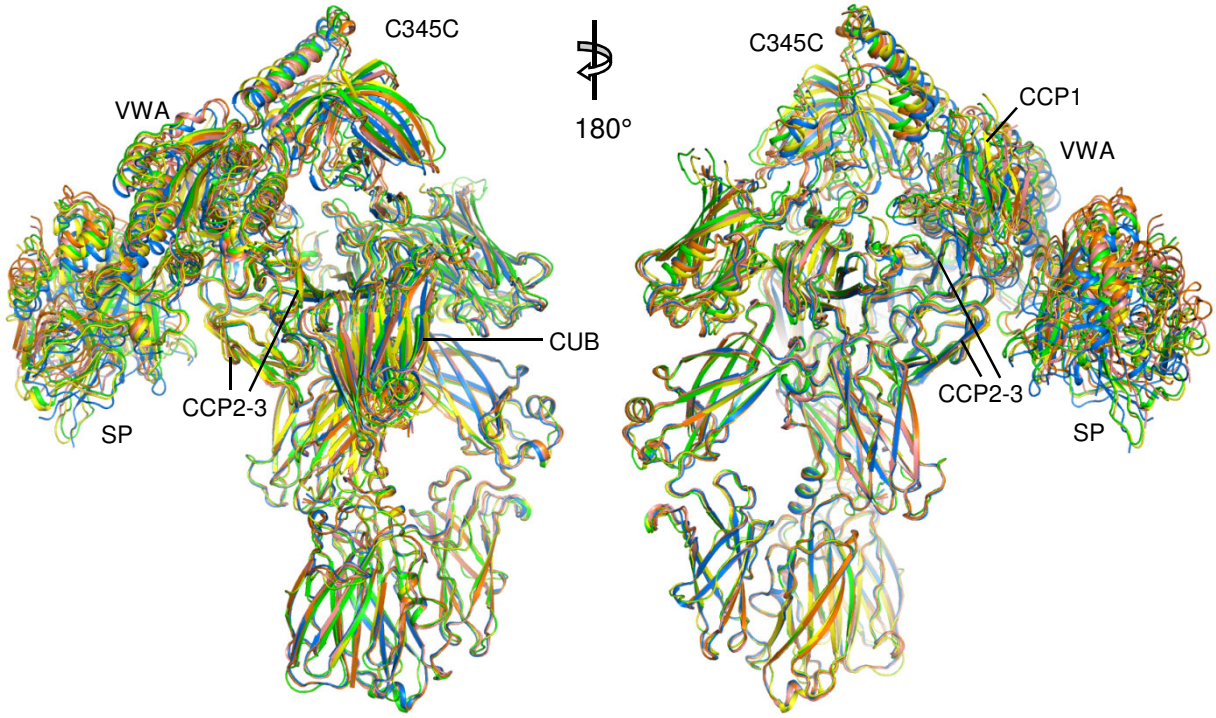
A, Domain rotation, translation and similarity between CVF bound to FB and free C3b and free CVF. CVF from the CVFB complex structure (2.2 Å) was superposed onto C3b (Janssen et al., 2006) (pdb code 2I07), C3b-CRIg (Wiesmann et al., 2006) (pdb code 2ICF) and free CVF (Krishnan et al., 2009) (pdb code 3FRP) on the basis of MG1-MG6 and LNK of the β-chain (602 residues). Using this superposition the rotation and center-of-mass translation of the domains and root-mean-square deviation (RMSD) of the Cα atoms were calculated with SUPERPOSE in the CCP4 package (CCP4, 1994). B, Domain rotation and translation between FB bound to CVF and free FB. FB from both the 2.2 Å and 3.0 Å CVFB complexes was superposed onto free FB (Milder et al., 2007) (pdb code 2OK5) on the basis of the VWA domain (200 residues). Using this superposition the rotation and center-of-mass translation of the domains were calculated with SUPERPOSE in the CCP4 package (CCP4, 1994). The orientation of CCP1 displays the largest difference. However, it also differs between the independent CVFB structures (see also SI Fig. 2 and 3). Thus, FB binding to CVF does not induce large conformational changes.

Supplementary Figure 1



Supplementary Figure 1. Coomassie-stained gels illustrating the purity of the FB and CVF samples. Left panel (non-reduced gel): FB (D254G/N260D) and CVF (glycosylated) used for crystallization experiments that resulted in crystals that diffracted to 8.5 Å. Middle panel (non-reduced gel): wt FB used for SAXS and EM experiments together with glycosylated CVF shown in the left panel. The FB sample in the left panel has a slightly lower molecular mass than FB in the middle panel due to mutation of one glycosylation site (N260D) and expression in GnTI deficient HEK293 cells which results in the production of shorter glycan moieties. Right panel (reduced gel) lane 1: glycosylated CVF, lane 2: deglycosylated CVF used for crystallization experiments resulting in the 3.0 and 2.2 Å datasets together with the FB (D254G/N260D) shown in the left panel. The CVF α-chain contains two N-linked glycan moieties, the β-chain contains one N-linked glycan moiety and the γ-chain contains no glycan moieties (see also Fig. 1B).

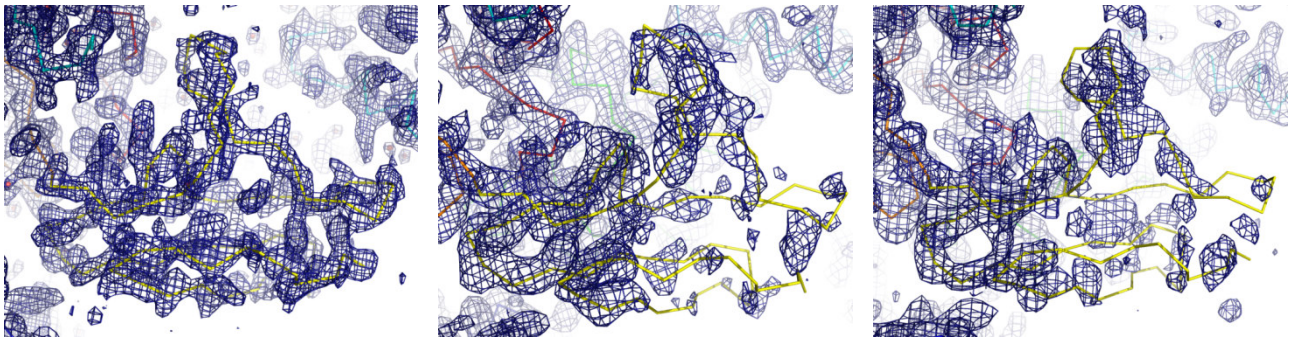
Supplementary Figure 2



Reference: CVFB (2.2Å)	CVFB-1 (3.0 Å)		CVFB-2 (3.0 Å)		CVFB-1 (8.5 Å)		CVFB-2 (8.5 Å)	
	Trans. (Å)	Rot. (°)	Trans. (Å)	Rot. (°)	Trans. (Å)	Rot. (°)	Trans. (Å)	Rot. (°)
CUB	1.0	3.2	0.7	3.8	1.0	6.3	2.0	10.6
C345C	2.6	2.6	2.9	2.0	3.3	8.5	2.4	9.9
CCP1	3.8	19	2.3	21	2.7	38	4.1	43
CCP2	0.5	1.6	0.7	2.6	0.8	4.3	2.1	5.6
CCP3	0.3	3.7	0.6	3.0	1.1	7.6	1.3	4.2
VWA	1.9	6.2	2.4	5.5	2.6	6.2	3.4	8.1
SP	5.1	8.5	7.0	12	4.0	6.7	4.8	8.5

Supplementary Figure 2. Comparison of the CVFB structures at 2.2 Å, 3.0 Å and 8.5 Å. All five CVFB complexes (colored differently) were superposed on the basis of MG1-MG6 and LNK of the β-chain using the 2.2 Å CVFB structure as reference. Using this superposition the rotation and center-of-mass translation of CUB and C345C of CVF and all FB domains were calculated with SUPERPOSE in the CCP4 package (CCP4, 1994). Except for the difference in orientation of the CCP1 domain of FB the structures of the independent CVFB complexes are very similar.

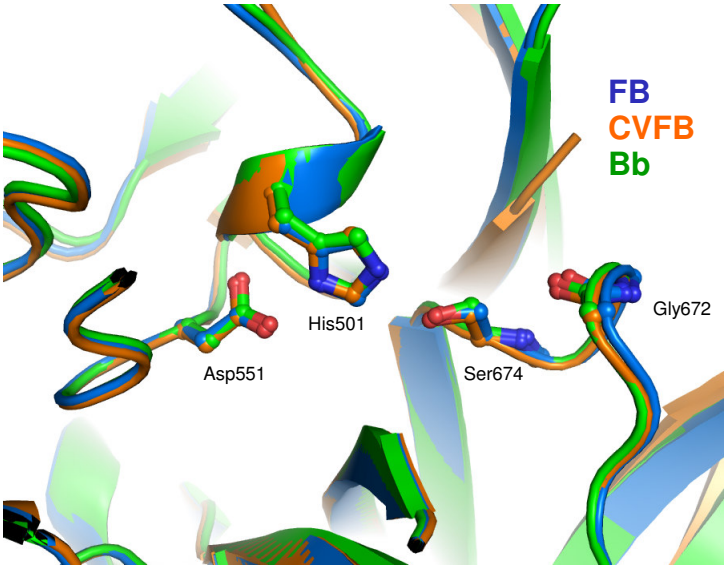
Supplementary Figure 3



CCP1 at 2.2 Å | CCP1 at 3.0 Å mol. 1 (chain D) | CCP1 at 3.0 Å mol. 2 (chain I)

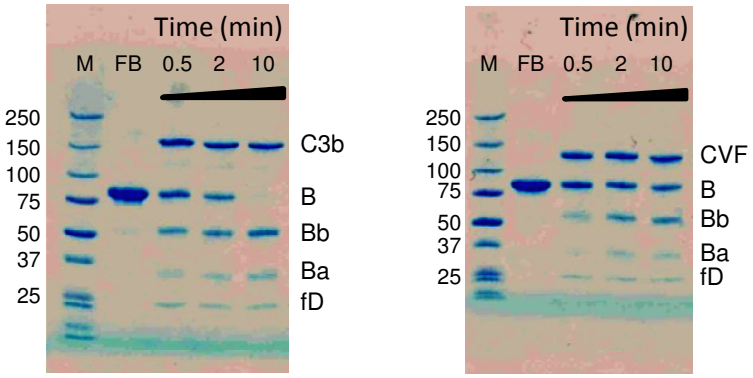
Supplementary Figure 3. Electron density for the CCP1 domain of FB.
Electron density (using coefficients 2Fo-Fc) of CVFB contoured at 1σ (blue wire frame), CCP1 in wire representation (yellow). Whereas CCP1 is ordered in the 2.2 Å structure it is partially disordered in both complexes in the 3.0 Å structure and thus likely moves as a rigid body.

Supplementary Figure 4



Supplementary Figure 4. The serine protease domain catalytic center.
The catalytic center of FB (Milder et al., 2007) (pdb code 2OK5), CVFB and Bb (Ponnuraj et al., 2004) (pdb code 1RRK) is indicated. The catalytic triad (His501, Asp551 and Ser674) is in a characteristic active and identical conformation in all three forms. The oxyanion hole (amide of Ser674 and Gly672) is in an identical, near-active conformation with an outward orientation of the glycine amide in all three forms.

Supplementary Figure 5



Supplementary Figure 5. Activation rate of FB by FD in CVFB is slower than in C3bB.
Analysis of FB activation by FD checked by non-reduced gel electrophoresis. C3b (left panel) or CVF (right panel) and FB were pre-incubated for 15 minutes at room temperature in PBS and 5 mM $MgCl_2$. Subsequently, FD was added to obtain final concentrations of C3b (2.4 µM) or CVF (2.4 µM), FB (3.0 µM) and FD (1.2 µM) and incubated for different time periods at room temperature. To stop the reaction samples were boiled immediately in sample buffer and subjected to gel electrophoresis. FB is fully activated into Bb and Ba within 10 min. when incubated with C3b (left). In contrast only a part of FB is processed within 10 min. when incubated with CVF (right). Lanes: marker (M, with sizes in kDa indicated left), purified FB (FB), samples at three different time points (0.5, 2 and 10 min.).