Legends to supplementary figures

Supplementary Figure 1 Schematic representation of bovine C3, human C5, and CVF. (A) Domain structure of C3 and C5 derived from crystal structures (Fredslund et al, 2008; Janssen et al, 2005). The canonical chain names (α and β) derived from electrophoretic mobility (Nilsson et al, 1975) are indicated by colored boxes. Notice that the linker region (square marked L) and C3a/C5a (red triangle marked A) form a single continuous insert as the connecting linker is cleaved prior to secretion to generate the mature terminal β-chain in C3/C5 (blue box), while the C-terminal part of the MG6 domain belongs to the C-terminal α-chain (light grey box) in C3/C5. (B) The α-chain in C3/C5 is converted to the slightly shorter α’-chain in C3b/C5b after proteolytic removal of C3a/C5a, Nt-α’ therefore denotes the new N-terminus of the α’-chain. (C) Domain structure of CVF with the N-terminal α-chain in CVF (blue box), the central γ-chain in CVF (light grey box), and the C-terminal β-chain (yellow box). CVF processing in the venom gland removes the equivalent of C3a and the thioester domain (Vogel & Fritzinger, 2010), and as the thioester domain is an insert in the CUB domain, two chains in CVF (γ and β) are generated from the equivalent of the C3 α-chain.

Supplementary Figure 2 Conservation of regions involved in C5-CVF contacts. (A) Alignment of mammalian C5 sequences for the relevant regions within the MG4, MG5 and MG7 domains with numbering and secondary structure according to human C5. Triangles indicate residues with potential for forming CVF interactions based on a distance criteria of 4 Å. (B) As in panel A, but for mammalian C3 sequences to indicate residues in the other convertase substrate potentially in contact with the convertase. The triangles are placed according to alignment with the C5 sequences shown in panel A. Secondary structure and numbering corresponds to that of human C3. (C) Alignment of CVF and mammalian C3 sequences showing CVF residues potentially involved in contacts to C5. The corresponding C3 residues are therefore candidates for substrate residues in the alternative pathway C3- and C5-convertases. Secondary structure and numbering are according to
CVF. (D) Alignment of mammalian C4 sequences showing the conservation of residues equivalent to CVF residues potentially involved in contact to C5. The indicated C4 residues may therefore contact the substrate in the classical/lectin pathway C3- and C5-convertases. The shown secondary structure is according to CVF. Secondary structure in all four panels is derived from structures of the respective proteins determined at much higher resolution than the C5-CVF structure, and is therefore more accurate.

**Supplementary Figure 3** Stereo view of selected C5-CVF interactions and the electron density for selected regions in C5 and CVF at the intermolecular interface. (A-D) Stereo views corresponding to Figure 3 panels C-F. (E) Stereo view of a two-fold averaged 2mFo-DFc electron density contoured at 1σ showing regions of the C5 MG5 domains facing the CVF MG4 domain. (F) as in panel E, but displaying regions of the CVF MG4 domain facing the C5 MG5 domain. (G) Electron density around regions of the C5 MG7 domain facing CVF. (H) Electron density around regions of the MG6 and MG7 domains of CVF facing the C5 MG7 domain. In panels E-H, only density within a distance of 3 Å from residues shown with side chains is displayed.

**Supplementary Figure 4** Comparison of crystal structures containing C3b and CVF. (A) Superposition of CVF (green) residues 20-1473 structure from its complex with C5, CVF bound to factor B (Janssen et al, 2009) (magenta, RCSB entry 3HRZ), isolated C3b (Janssen et al, 2006) (red, RCSB entry 2I07), and C3b from its complex with Bb and SCIN (Rooijakkers et al, 2009) (cyan, RCSB entry 2WIN). (B) As in panel A, but turned 90° and with C5 in a surface representation showing the location of the thioester domain (TED) in C3b – absent in mature CVF – relative to the bound C5. (C), As in panel B, a detailed view of the interface between the C5 MG7 domain and CVF. Notice the proximity of the Nt-γ chain in CVF and the Nt-α’ chain in the docked C3b to C5.
Supplementary Figure 5 Comparison of other C3 and C5 structures with that of C5 bound to CVF. (A) The structure of free C5 superimposed through the MG4-MG5 domains onto C5 in complex with CVF illustrates the conformational changes occurring in C5 upon binding to CVF. The C345C domain of C5 is not shown for clarity. Right part of the panel shows a close-up on the C5 MG7 domain to illustrate its resulting position relative to CVF. (B) Docking of human C3 (RCSB entry 2A73) onto CVF through superposition onto the MG4-MG5 domains of C5. As in panel A, the MG7 domain does not reach its binding pocket on CVF. (C) Docking of one copy of bovine C3 (RCSB entry 2B39) onto CVF through superposition onto the MG4-MG5 domains of C5. Right part illustrates how the bovine C3 MG7 domain is in contact with CVF as expected for a convertase substrate conformation. (D) The C5-CVF complex for comparison.

Supplementary Figure 6 Surface plasmon resonance measurements of SSL7-C5 interaction with CVF and C3b. (A) The purity of C3b, CVF and C5 used in the binding experiments examined by a reducing SDS-PAGE. The gel was stained with Coomassie blue. (B) Direct binding of CVF (dilution series 1.0 - 0.016 µM) to immobilized SSL7-C5. (C) Direct binding of C3b (dilution series 4.0 - 0.016 µM) to SSL7-C5. Dissociation constants (K_d) for CVF binding to SSL7-C5 and C3b to SSL7-C5 are indicated according to the two models used for analysis. Whereas the (SSL7-C5):C3b interaction is well explained by a simple 1:1 binding model, the (SSL7-C5):CVF interaction is significantly better described by the model assuming a conformational change in combination with 1:1 binding. This possibly reflects the observed conformational change between free C5 and CVF bound C5 (Figure 4 and S5). In contrast, C3b may not induce the complete conformational change in C5 leading to a reduced affinity (K_d=37 µM) for immobilized C5 (Rawal & Pangburn, 2001) and SSL7-C5 (K_d=70 µM, this study) compared to CVF. The K_m values for the zymosan linked AP C5 convertase C3bBb3b (Rawal et al, 2008) are very similar to that of fluid phase CVFBb (Rawal & Pangburn, 2001) with values of 0.048 µM and 0.036 µM, respectively. In terms of substrate binding.
CVF therefore compensates for the lack of the second C3b molecule. For comparison, experiments without SSL7 (Rawal & Pangburn, 2000) using immobilized C5 and CVF show $K_d=0.042$ μM and for interaction of immobilized CVF with C5 a $K_d=0.105$ μM, demonstrating that SSL7 significantly weakens the C5-CVF interaction. In contrast, interaction of immobilized SSL7-C5 with C3b is less affected ($K_d=70$ μM) compared to measurements (Rawal & Pangburn, 2000) of immobilized C5 interacting with C3b ($K_d=37$ μM).

**Supplementary Figure 7** The mechanism of convertase inhibitors as explained by the C5-CVF structure. (A) The SSL7-C5-CVF complex with the three proteins shown in cartoon representation. The grey surface indicate the modelled position of IgA Fc bound to SSL7 (Ramsland et al, 2007) showing the steric hindrance (indicated as dashed ellipse) IgA would exert on CVF trying to bind to C5, rationalizing the strong contribution of IgA to inhibition of C5 cleavage by either CVFBb or the CP and AP C5 convertases (Bestebroeer et al, 2010; Laursen et al, 2010). (B) The known inhibitory effects of three AP inhibitors S77 (Katschke et al, 2009), CRIg (Wiesmann et al, 2006), and compstatin (Janssen et al, 2007) (shown in surface representation) can be accounted for by our C5-CVF structure. These inhibitors can be predicted to interfere with substrate binding, represented by C5, to C3b containing AP convertases through steric hindrance.

**Supplementary Figure 8** The pseudo-symmetrical MG4-MG5 dimer in C5-CVF compared to the non-crystallographic MG4-MG5 dimer within the C3b-Bb-SCIN complex (RCSB entry 2WIN) (Rooijakkers et al, 2009). (A) Bottom view (relative to Figure 1C and D) of the MG4 and MG5 domains in C5 (blue) with their equivalents in CVF (green). (B) Same view of the C3b dimer. The MG4-MG5 domains of the red C3b molecule (convertase mimic) were superimposed onto their equivalents in CVF. (C) Overlay of panels A and B, notice that the position of the MG4-MG5 domain in the yellow C3b molecule (substrate or product mimic) is very similar to the
corresponding C5 domains, but the MG5 domain of the yellow C3b domain is rotated slightly away from the MG4 domain in the red C3b molecule.

**Supplementary Figure 9** A model of C4b in the light of the C5-CVF complex rationalises known effects of mutations in C4b. (A) The C4b model (green) in the place of CVF in its complex with C5 (blue) proposes a rough model for C5 recognition by the classical pathway C5 convertase. Side chains of a C4b region with well characterised effects on C5 convertase activity (Ebanks & Isenman, 1995) are shown in sticks. (B) Detailed view of the proposed CP convertase C4b MG5 domain interaction with the MG4 domain of C5. In particular, mutations of Arg481 and Asp487 have serious impact on CP convertase activity (Ebanks & Isenman, 1995). In the model, it seems plausible that Arg481 perhaps together with Arg484 could form salt bridges with C5 Asp414 and Asp415, while C4b Asp487 could interact electrostatically with C5 Arg412.

**Supplementary Figure 10** A putative function of the C345C domain in the C5-CP convertase complex. (A) Top view (relative to Figure 1C and D) of the modelled C5-CVFBb complex. C5 is shown in cartoon representation with the C345C domain colored pink, while the CVFBb complex is shown in a surface representation with Bb being sand colored, and CVF colored according to the standard scheme. The C5 region 1628-1633 likely to interact directly with the CP C5 convertase C4b2a3b (Sandoval et al, 2000) is indicated with a large sphere, and is located quite far away from CVFBb. (B) Upon rotation around the disulfide bridge linking the C345C to the MG7 domain to a position very similar to that observed in C5 (Fredslund et al, 2008), the C345C domain comes in contact with Bb, and the 1628-1633 loop is placed in the vicinity of the CVF Nt-γ chain. This position of the C345C domain was obtained in step 28 within a 30 step interpolation (morphing) between an initial location in the C5-CVF complex (with respect to the MG7 domain) and a final location as in free C5 (Fredslund et al, 2008). (C) Close-up of the modelled position of the C345C domain, the 1628-1633 loop is shown in dark blue and could potentially interact with both C4b and
C2a in the CP C5 convertase. Acidic side chains of the Nt-γ are shown with sticks. (D) Same view, the electrostatic potential of the C345C domain in C5 is indicated to visualize the large positively charged patch (with contributions from Lys1576, Lys1578, Lys1613, Arg1615, Arg1634, and Arg1650) in the C5 C345C domain which might interact directly with the strongly negatively charged Nt-α’ chain in C4b of the CP C5 convertase.

**Supplementary Figure 11** Implications of the substrate-convertase model for the reactive thioester in nascent C3b and the CP C5 convertase. (A) The enzyme-substrate complex with an uncleaved C3 is shown in the left panel, while the middle panel displays an intermediate conformational state of nascent C3b with the reactive thioester (red sphere) and the future nucleophile (grey sphere). In the right panel, the final C3b conformation has been obtained, and the product has dissociated from the convertase. The convertase bound conformation of C3 in the left panel was derived by superposition of the individual C3 domains to their equivalent in the CVF bound conformation of C5 (see also Supplementary animation 2). (B) The role of C3b in the cross-linked CP C5 convertase. Under the assumption that the C3b cross-linked to C4b adopts a conformation similar to that of known structures of C3b, simple distance considerations propose that mainly the MG7, MG8 and the C345C domains of the C3b molecule will be able to directly contact C5.
**Supplementary animation 1** The conformational change occurring between free C5 and C5 bound to CVF. To the right, CVF is shown in green. In C5, the MG7 and MG3 domains are colored orange and grey, respectively. C5a and Nt-α’ are colored red and yellow, respectively, while the rest of C5 is blue. The C345C domain of C5 is not shown for clarity. The slight conformational changes seen in CVF are caused by the interpolation procedure used for creating the animation.

**Supplementary animation 2** The conformational change in nascent C3b bound to a convertase oriented roughly perpendicular to the surface. To the right is shown a model of the AP C3 convertase with C3b (green) in complex with Bb (sand colored) and covalently linked to the surface through the C3b thioester (red sphere). To the left a nascent C3b (blue) is undergoing the conformational change from a model of C3 bound to the convertase (see legend to Supplementary Figure 11A) to the C3b conformation, while it remains interacting through its MG4 and MG5 domains with the convertase. The highly reactive thioester that ends at the surface in nascent C3b is marked with a large blinking sphere.
Structure of the C5-CVF complex – Supplemental information

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**Supplementary Table I** Breakdown by resolution of statistics for the structure determination of the C5-CVF complex after scaling of diffraction data with XSCALE and refinement with PHENIX.REFINE. Overall statistics are shown in Table I.
Supplementary Table II Breakdown by resolution of statistics for the structure determination of the SSL7-C5-CVF complex after scaling of diffraction data with XSCALE and refinement with PHENIX.REFINE. Overall statistics are shown in Table I.
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**Supplementary Table III.** Interface areas calculated by PISA (Krissinel & Henrick, 2007) between C5 and CVF in the structure of C5-CVF and SSL7-C5-CVF, and between C5 and SSL7 in the structure of SSL7-C5-CVF. The two interface areas in C5 and CVF are compared with the total interface area as indicated. The interface area for C5-SSL7 (RCSB entry 3KLS, resolution 3.6 Å) is shown for comparison due to uncertainty caused by the low resolution of the two structures.
References to supplemental material


Structure of the C5-CVF complex – Supplemental information


Supplementary figure 1

A

MG1 MG2 MG3 MG4 MG5 MG6 MG7 MG8 C345C

β-chain

α-chain

bovine C3: 23-665
human C5: 19-673

bovine C3: 670-1661
human C5: 678-1676

B

MG1 MG2 MG3 MG4 MG5 MG6 MG7 MG8 C345C

β-chain

α'-chain

bovine C3: 23-665
human C5: 19-673

bovine C3b: 747-1661
human C5b: 752-1676

C

MG1 MG2 MG3 MG4 MG5 MG6 MG7 MG8 C345C

α-chain

γ-chain

β-chain

CVF: 23-649
CVF: 733-984
CVF: 1264-1642
Possible interactions with CVF

Supplementary figure 2 p1
Possible interactions with CVF for equivalent C5 residue

Supplementary figure 2 p2
Possible interactions with C5 by equivalent residue in CVF

Supplementary figure 2 p4
Supplementary figure 3 p1
Supplementary figure 4
Supplementary figure 5
Supplementary figure 6
Supplementary figure 7
Supplementary figure 8
Supplementary figure 9
Supplementary figure 10
A

C3 bound to convertase

Conformational change of nascent C3b

C3b membrane anchored

B

C345C CUB TED MG1 MG2 MG3 MG4 MG5 MG6 MG7 MG8

C3b

C5

C4b

C2a

Ser1236

C3b thioester

Supplementary figure 11