Supporting Information

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SI Methods

C3 was prepared from pooled human plasma. Plasma was subjected to a 4–12% cut with PEG4000, the pellet solubilized in 10 mM Na/K phosphate, pH 7.6/5 mM NaCl, and applied to a DEAE-Sepharose anion exchange column attached to an Akta Prime (GE Healthcare). Protein was fractionated on a salt gradient to 0.5M NaCl, C3-containing fractions were identified by SDS/PAGE, dialyzed into 20 mM Na/K phosphate, pH 6/60 mM NaCl, and applied to a Source S cation exchange column (GE Healthcare). Protein was eluted with a gradient to 0.5M NaCl. C3-containing fractions were pooled, concentrated, and aggregates were removed by gel filtration on Superdex 200 preparative grade matrix in a XK16/70 column attached to an Akta Purifier (GE Healthcare).

Factor (f)B was purified from plasma of healthy volunteers homozygous for each fB variant by affinity chromatography on an anti-Bb column (mAb JC1 made in-house; 5 mL HiTrap column; GE Healthcare). Eluted fB was gel filtered on Superdex 200 to remove aggregated material and minor contaminants, and to buffer exchange into Biacore buffer (10 mM Hepes, pH 7.4/0.005% surfactant P20/1 mM Mg^{2+} with either 50 or 150 mM NaCl) for kinetic analyses, or C fixation diluent (CFD; Oxoid) for hemolysis assays. In some experiments (Fig. S1), fB_{32R} and fB_{32Q} variants were copurified by affinity chromatography from plasma of heterozygote individuals, and subsequently separated by ion exchange on either Mono S or Mono Q columns at pH 6.0. Factor B_{32R} eluted first from the Mono Q column, and last from the Mono S. Pooled protein was gel filtered into required buffers as described above. C3b was generated by incubating C3, fB, and fD in CFD until total C3 cleavage had occurred. C3b was purified from other fragments by anion exchange chromatography (Mono Q; GE Healthcare). C3b-containing fractions were pooled, concentrated, and fractionated on Superdex 200 to separate C3b monomer from dimer. To generate Ba, 19 mg methylamine-treated C3 (1) was coupled to a 5 mL Hitrap column; fB (5 mg; 2 mg/mL) and fD (100 ng/mL) were applied to the column in CFD, and incubated for 1 h at 37 °C. The column was eluted in CFD and eluate applied to the anti-Bb affinity column. Bb was retained on the column; Ba in the run-through was concentrated by ultrafiltration, and buffer-exchanged into Biacore buffer (50 mM NaCl) by gel filtration on Superdex 200.

1. Harris CL (2000) Functional assays for complement regulators. *Methods Mol Biol* 150:83–101.

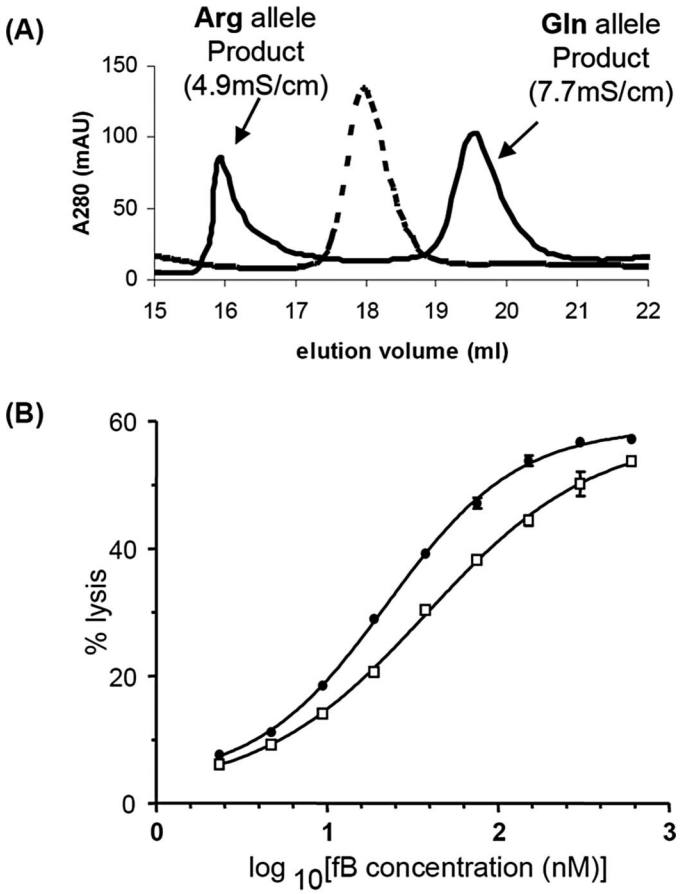

Fig. S1. Hemolytic activity of the fB variants. FB was purified from plasma of a R32Q heterozygote, the 2 fB variants were separated by ion exchange chromatography at pH 6 (*A*), and then gel filtered to remove aggregates. Solid line indicates separation of the allele products at pH 6, the dashed line indicates a single peak containing both variants at pH 7.2. (*B*) Rabbit erythrocytes were incubated with human serum that had been depleted of fB, and different concentrations of the fB variants isolated from the heterozygote individual. Lysis was developed for 45 min, and hemoglobin release was used to calculate percentage lysis; fB_{32R}, circles; fB_{32Q}, squares. Data points represent mean ± SD of 3 determinations. The $\log_{10}$ of fB concentration was plotted against percentage lysis, and curves were fitted by using nonlinear regression analysis.

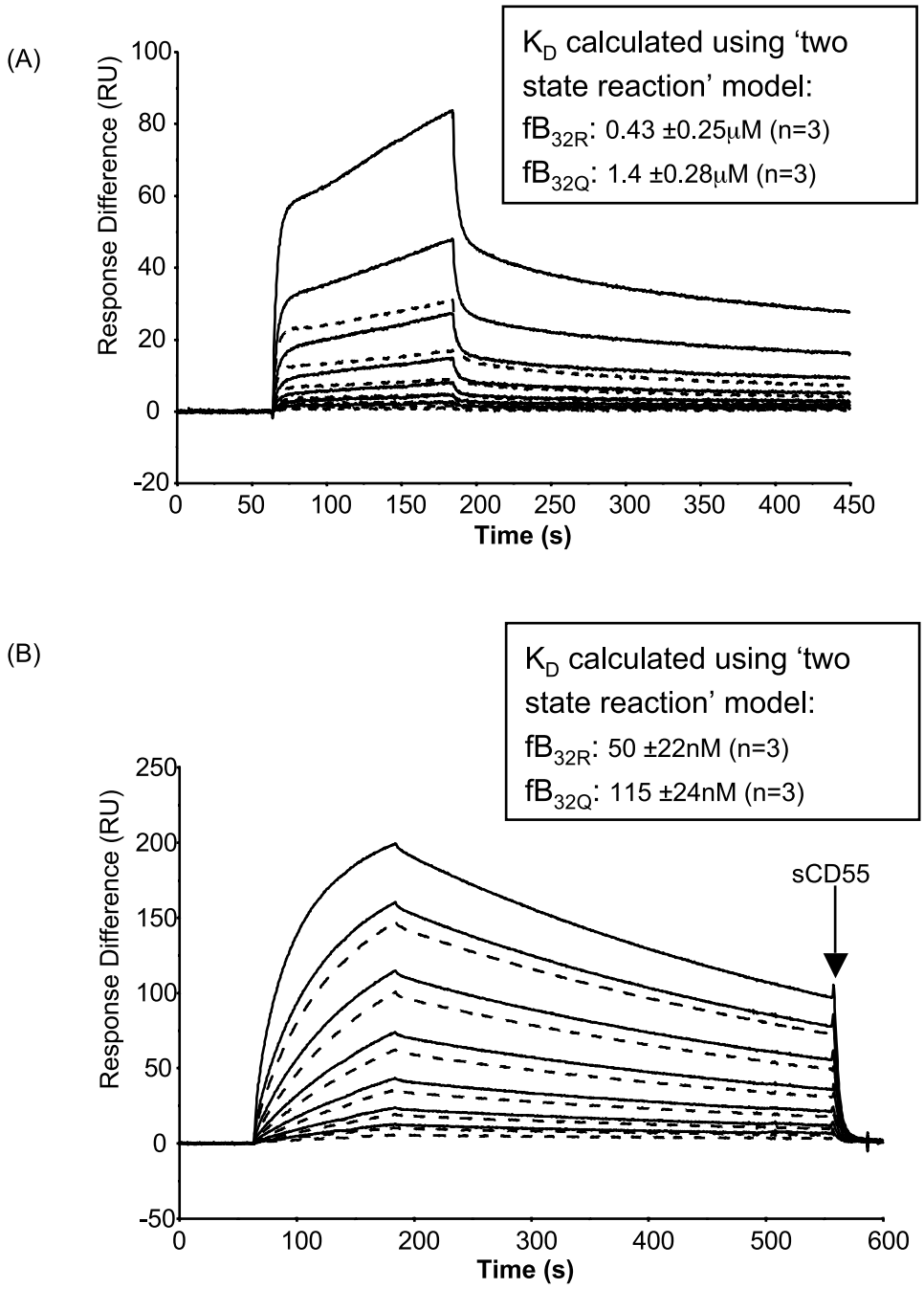

Fig. S2. SPR analysis of proenzyme and convertase formation by native fB in physiological salt concentrations. (*A*) Plasma-purified fB was flowed over the surface of the C3b-coated chip at concentrations between 1.1 μM and 18 nM in Biacore buffer containing physiological salt concentration (10 mM Hepes, pH 7.4/150 mM NaCl/1 mM $MgCl_2$/0.005% surfactant P20). Sensorgrams from fB_{32R} are solid lines, fB_{32Q} are dotted lines; identical concentrations are indicated for the 2 variants. K_D calculated using ''2-state reaction'' model: fB_{R32}, 0.43 $\pm$ 0.25 μM; and fB_{32Q}, 1.4 $\pm$ 0.28 μM. (*B*) Plasma-purified fB was flowed over the surface of the C3b-coated chip at concentrations between 1.1 μM and 18 nM in the presence of 1 μg/mL fD. K_D calculated using 2-state reaction model: fB_{R32}, 50 $\pm$ 22 nM; and fB_{Q32}, 115 $\pm$ 24 nM. Analysis using a 1:1 Langmuir model revealed similar affinities of 51 $\pm$ 21 nM (fB_{32R}) and 113 $\pm$ 26 nM (fB_{Q32}). Affinities in *A* and *B* are the means $\pm$ SD of 3 independent experiments.