

Supplementary Information For:

Targeting properdin - Structure and function of a family of tick-derived complement inhibitors targeting properdin

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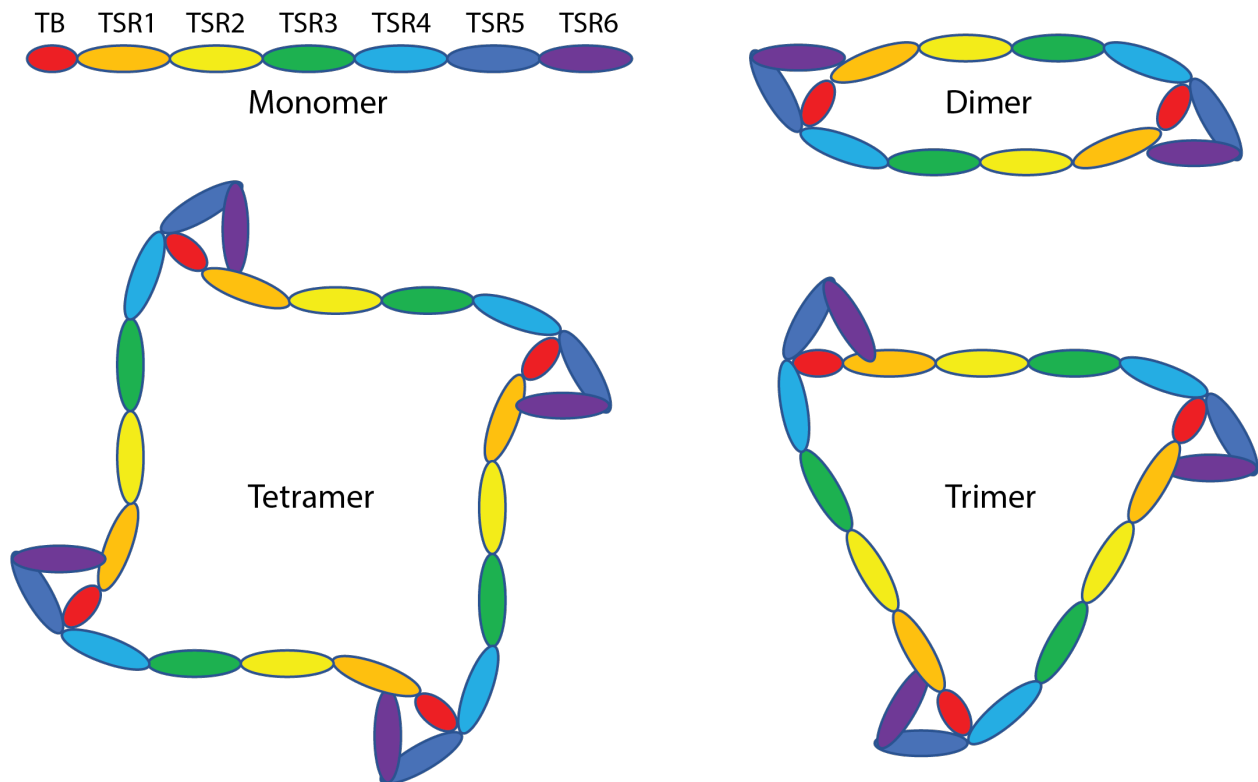
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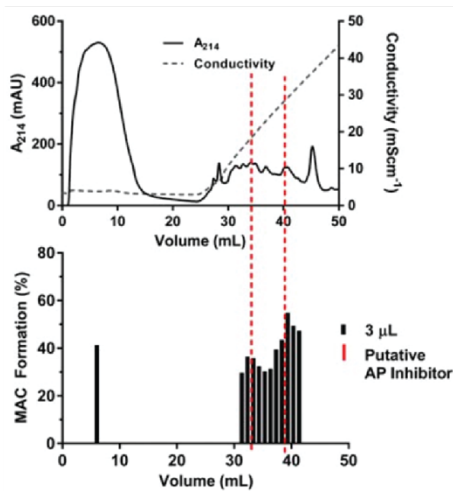
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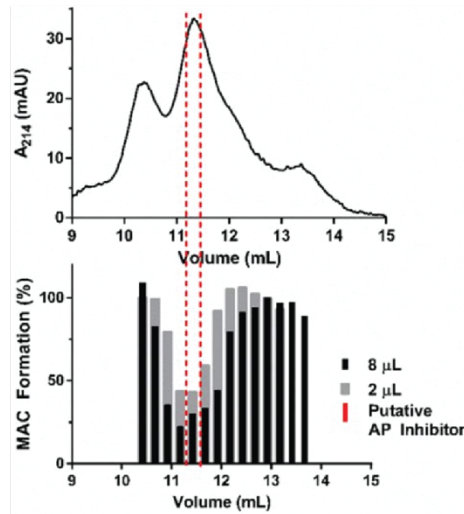
Supplementary figure 1. Properdin oligomeric states.

Schematic representation of the major properdin oligomeric states, as predicted by the crystal structures of monomerized properdin. Domains are color-coded as described for the monomer.

a) MonoQ fractionation



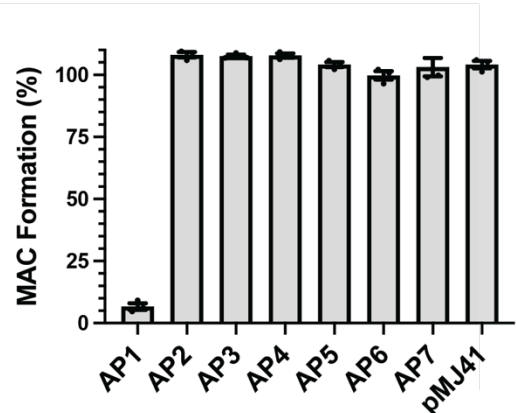
b) SEC fractionation



c)

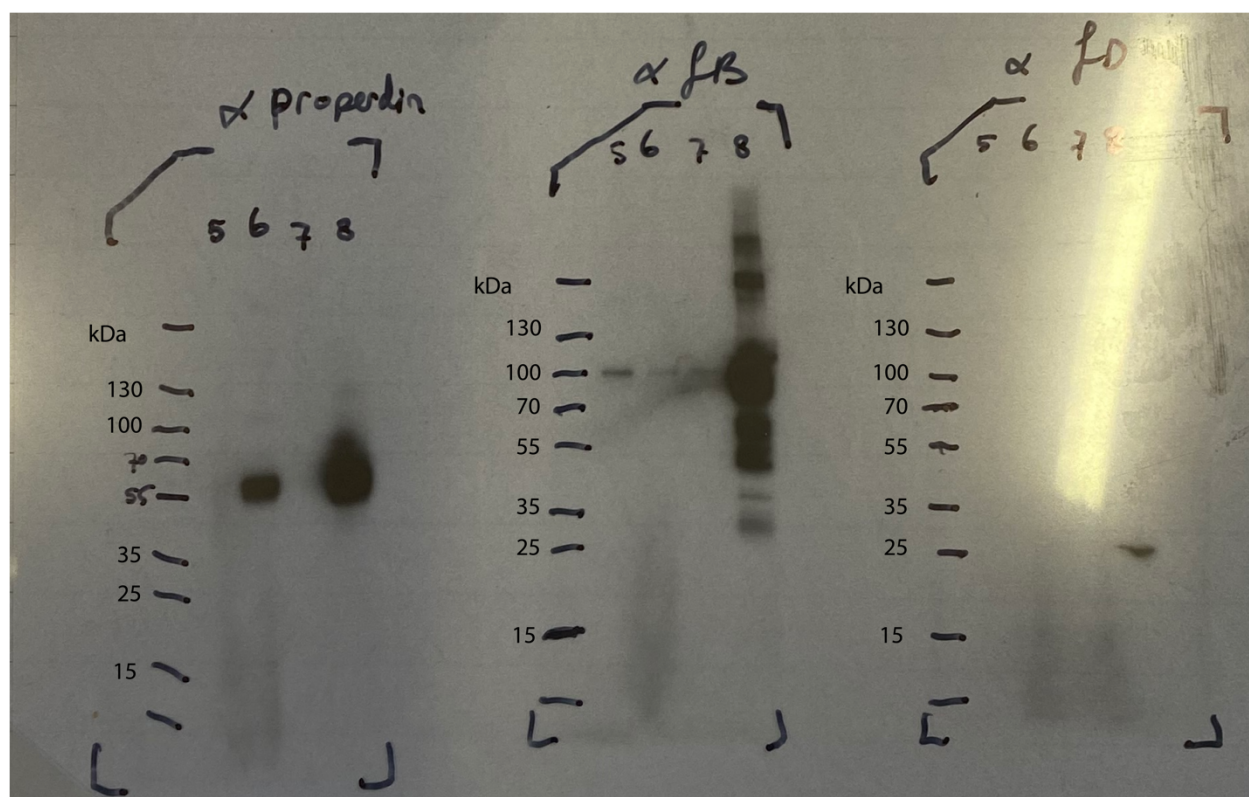
Accession No.	Re-name	Score	Mature Protein Mass (Da)	pI	BLAST Top Hit	BLAST E score	BLAST RefSeq
comp52_seq0	AP1	239	20068	4.67	None		
comp1106_seq1	AP2	79	19433	5.97	Salivary lipocalin (A. variegatum)	1.00E-13	DAA34698.1
comp3626_seq0	AP3	130	45968	5.16	Hypothetical Protein, lscW, lxxodes scapularis	0	XP_002409462.1
comp1215_seq0	AP4	110	38706	6.15	Proliferation-associated Protein 2G4	1.00E-157	KDR23244.1
comp8435_seq1	AP5	81	22430	4.89	None		
comp5629_seq0	AP6	71	24630	8.79	None		
comp4_seq0	AP7	36	21995	5.38	Lipocalin	5.00E-4	ABI52661.1
Rplx75-921729	-	612	22957	5.21	Salivary lipocalin (A. variegatum)	3.00E-11	DAA34698.1
RpSigP-58530	-	24	21995	5.38	N(G),N(G)-dimethylarginine dimethylamino-hydrolase	3	WP_011213937.1

d)



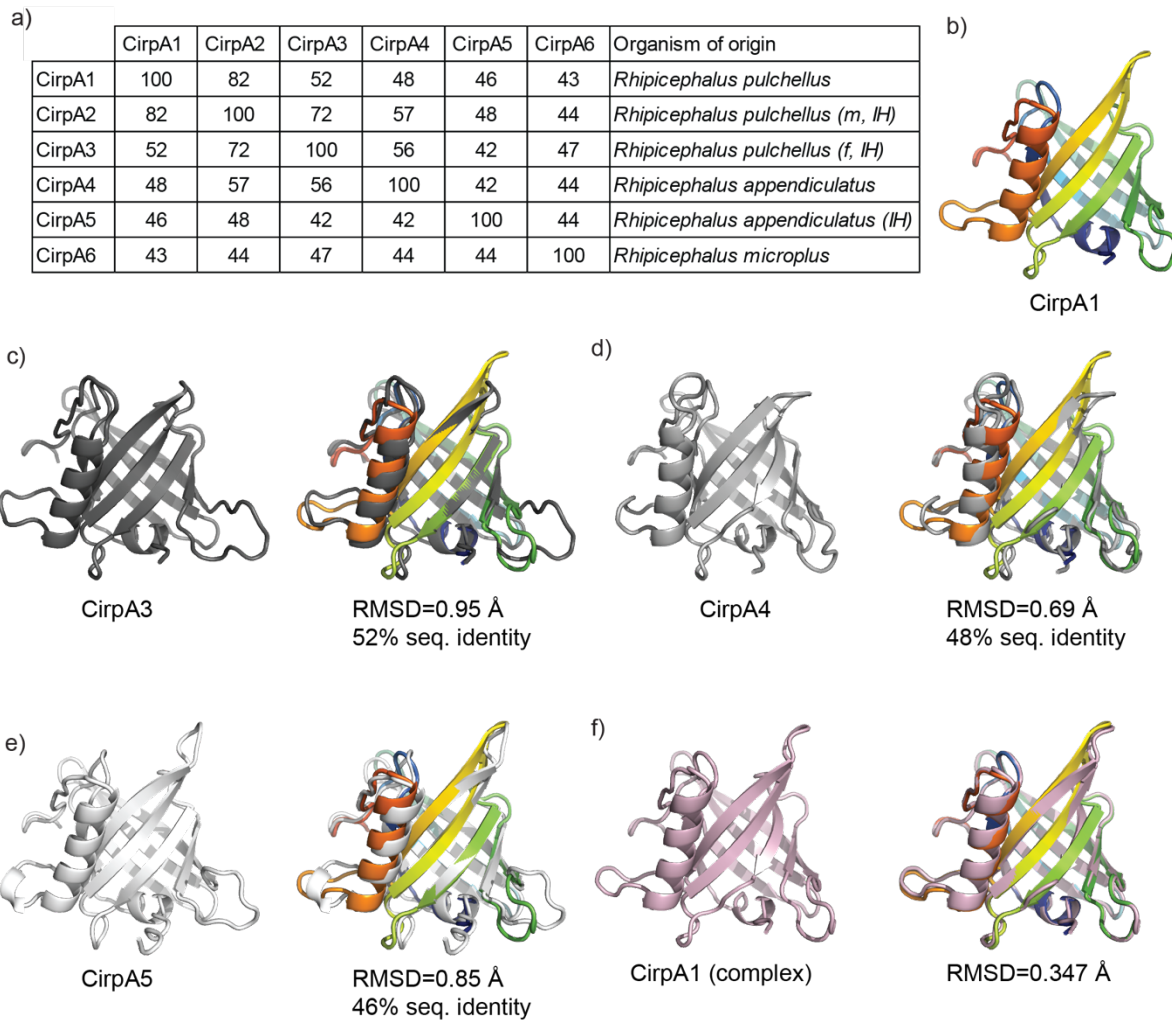
Supplementary figure 2. Fractionation of alternative pathway inhibitory activity.

(a) Salivary gland extract from 250 pairs of salivary glands, diluted with 2mM $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$, pH 7.0, was applied to a MonoQ 5/50 GL column (GE) and eluted with a 0-0.5 M NaCl gradient. Complement inhibitory fractions were pooled and further purified by HIC (Fig. 1b). (b) Size exclusion chromatography of the complement inhibitory fraction from HIC. Sample was run on a Superdex75 10/300 column (GE). (c) Seven protein sequences identified by mass spectrometry from the SEC peak were analyzed by BLAST. (d) Supernatants from S2 cell cultures expressing the seven hits identified by MS (plus an empty vector control) were used in a complement inhibition ELISA assay. Error bars are Mean with s.e.m. for three technical replicates (n=3). Only AP1 demonstrated complement inhibition activity.



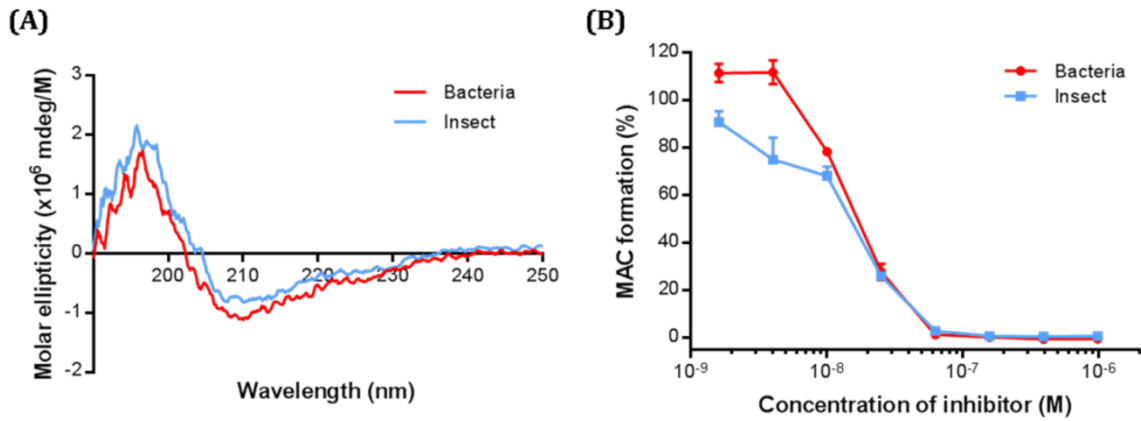
Supplementary figure 3. Alternative complement pathway pull-down analysis.

Purified CirpA1 was covalently coupled to NHS-activated magnetic beads (ThermoFisher) and incubated with 50 μ l human serum, followed by removal of excess serum and washing in PBS. Beads were run on SDS-PAGE and proteins visualized on Western blot using anti-properdin, anti-Factor B or anti-Factor D antibodies. Lane 5=Empty bead control. Lane 6=CirpA1 coated beads. Lane 7=Salp20 coated beads. Lane 8=Purified properdin, Factor B, Factor D mix. Representative result of n=2.



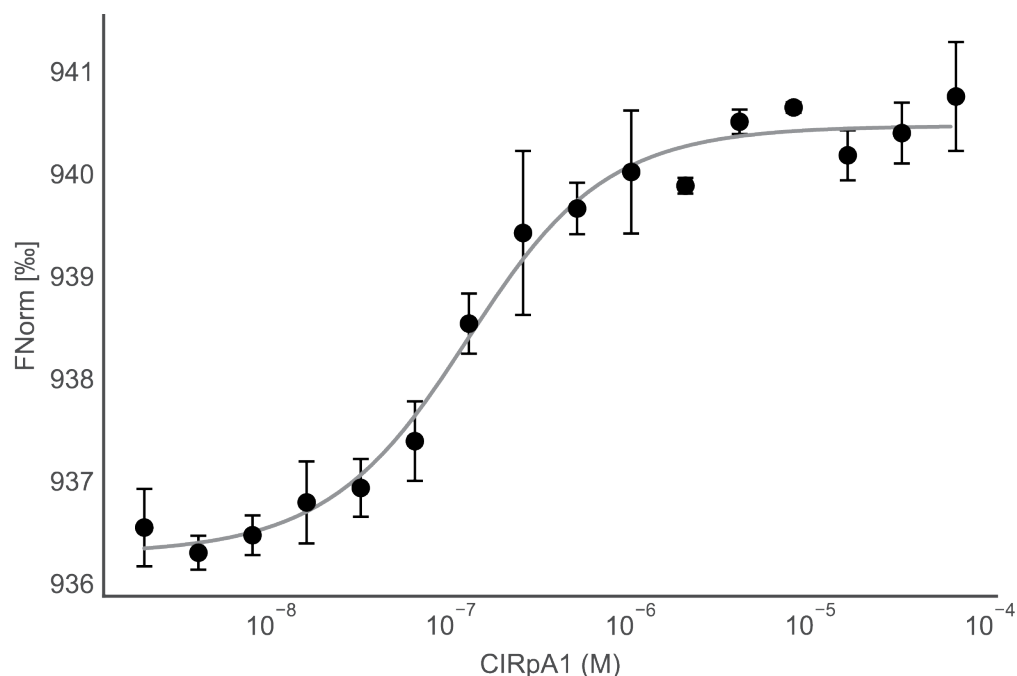
Supplementary figure 4. Sequence and structure analysis of CirpA homologues.

(a) Pairwise sequence identity of the CirpA family. (b) Structure of CirpA1 colored from N (blue) to C (red) terminus. (c) Structure of CirpA3 (grey) overlaid with CirpA1 (rainbow). (d) Structure of CirpA4 (grey) overlaid with CirpA1 (rainbow). (e) Structure of CirpA5 (grey) overlaid with CirpA1 (rainbow). (f) Structure of CirpA1 (pink) from the CirpA:Properdin complex overlaid with free CirpA1 (rainbow).



Supplementary figure 5. Comparison of CirpA1 produced from insect and bacterial cells.

(A) Circular dichroism spectroscopy of CirpA1 produced from S2 cells (blue) and refolded from *E. coli* inclusion bodies (red). (B) Alternative pathway inhibition of CirpA1 produced from S2 cells (blue) and refolded from *E. coli* inclusion bodies (red). Error bars are Mean with s.e.m. for three technical replicates.



Supplementary figure 6. Micro-scale thermophoresis of the CirpA1:Properdin interaction.

Labelled, FPΔ2,3 (50 nM) was incubated for 5 min with unlabeled CirpA1 at concentrations between 2 nM and 64 μM. MST measurements were performed on a Monolith NT.115 at 20% LED power and low MST power. An MST on time of 2.5 s was used for analysis via the MO.Affinity Analysis 2.3 Software, and a K_d of 97 nM $\pm$ 18 nM was derived for the interactions (n=3 independent measurements, error bars represent the standard deviation of the mean).