C-reactive protein (CRP) aptamer binds to monomeric but not pentameric form of CRP

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Methods: C1q binding measurements

Binding of different isoforms of CRP to immobilized C1q were performed as described previously [1]. C1q (1 μg/mL) was immobilized on high binding ELISA plates (Santa Cruz Biotechnology, Inc. CA) in coating buffer (30 mM Na₂CO₃, 70 mM NaHCO₃, pH 9.6) and incubated overnight at 4°C. The wells were washed three times using 0.05% Tween-20/PBS and once with PBS before blocking with 3% BSA for 2 hrs at room temperature. Following the Tween-20/PBS and PBS washes, various forms of CRP were diluted in 1% BSA and added to the wells and incubated for 2 hrs, followed by the incubation of a polyclonal anti-CRP antibody (1:5000) for 1 hr at room temperature. After further washes, the wells were incubated with streptavidin-HRP conjugate (1:10,000, Pierce Thermo Fisher Scientific, IL) for 30 min. TMB substrate (100 μL) (1-Step Slow TMB, Pierce Thermo Fisher Scientific, IL) was added to each well and the reaction was stopped by the addition of 0.5 M H₂SO₄ once the desired color development was achieved. The absorbance was measured at 450 nm on a plate reader (Multiskan Ascent, Thermo Fisher Scientific, IL) with background subtracted at 620 nm.
Fig. S1. Binding of different CRP isoforms to immobilized C1q. ELISA wells were coated with C1q (1μg/mL) and incubated with various modifications of CRP at increasing concentrations. The degree of CRP binding was detected using biotinylated polyclonal anti-CRP antibody (1:5000) with a streptavidin-HRP conjugate (1:10,000) and the absorbance of TMB substrate was measured on a plate reader at 450 nm with background subtraction at 620 nm. mCRP<sub>SDS</sub> was formed by heating with 0.1% SDS.