THP (25 µg/ml) was adsorbed to wells. Properdin (3 µg/ml) and 7 concentrations of THP (0-100 µg/ml) in 20mM Tris/20mM NaCl/0.05% Tween 20 (pH 7.5) were added to the THP-coated wells, and the amount of bound properdin was detected by sequential incubation with 1° and 2° Ab as described previously.

**Effect of Ionic Strength on Kₜ for THP**

**Effect of Buffer Ionic Strength**

The effect of ionic strengths on the properdin/THP interaction was assessed in the ELISA by incubating 7 concentrations of properdin (0.1 µg/ml – 50 µg/ml) in the 20mM Tris/20mM NaCl/0.05% Tween 20 buffer either with 1mM MgCl₂ and 1mM CaCl₂ or without divalent cations in THP-coated wells.

Removing divalent cations from the buffer markedly reduced the ability of properdin to bind THP, indicating that divalent cations potentiated properdin’s binding to immobilized THP.

**Ligand Blot**

Properdin (2µg), either reduced with 3% β-mercaptoethanol (lane 2) or not reduced (lane 1), was separated on a 10% SDS-PAGE gel and transferred electrophoretically to nitrocellulose.

**Effect of Buffer Ionic Strength**

The effect of buffer ionic strength on the properdin’s interaction with the THP interaction was assessed by ELISA. Properdin (0.1-25 µg/ml) diluted in 20mM Tris/1mM MgCl₂/1mM CaCl₂/0.05% Tween 20 (pH 7.5) containing either 20, 90, or 154mM NaCl was incubated in THP-coated wells.

**Properdin binds Immobilized THP in an ELISA**

THP (25 µg/ml) in 0.05M sodium carbonate-bicarbonate buffer (pH 9.6) was adsorbed to microtiter plate wells. Properdin (0.5-50 µg/ml (Complement Technology) in 20mM Tris/20mM NaCl/1mM MgCl₂/1mM CaCl₂/0.05% Tween 20 (pH 7.5) was incubated in coated wells. Goat anti-human properdin and rabbit anti-goat IgG-alkaline phosphatase in 1% milk in Tris buffer were incubated sequentially in wells. Plates were developed with 4-nitrophenyl phosphate and A₄₀₅ was measured.

**Dose dependent binding between properdin and THP was observed.** Kₜ = 3.0 × 10^4 M⁻¹.