

**S14 Fig. SPR - Numerical simulation of SPR binding curves.**

Build-up of analyte concentration in an SPR chamber was simulated under different conditions (S1 Text and S1 Table). (A-B) Comparison of analyte build-up in the presence and absence of surface ligand – i.e. (+) and (-) adsorption (ads). (A) It takes ~2-3 s to fully replace the SPR chamber volume. (B) Expansion of the first 0.5 s of (A) shows an additional mixing lag in the presence of surface ligand relative to the bare surface. Comparisons of (C-D) analyte build-up and (E-F) bound analyte at different flow rates. Even at flow rates that are an order of magnitude faster than those used in our SPR experiments (i.e., 100  $\mu\text{l}/\text{min}$ ) and with smaller model proteins that diffuse faster than those used in our experiments, the simulations indicate mixing lags approaching a few tenths of a second. This time scale is orders of magnitude larger than the time scale of FG-Kap95 interactions, suggesting that mass transport limitation was inevitable with our SPR setting. The reported values are averaged concentration values at the outlet of the SPR chamber for (A-D) and the total amount of analyte bound over the lower SPR reaction surface for (E-F) (see model description). (B), (D) and (F) are magnifications of (A), (C) and (E), respectively.

