

FIG. 3. Interactions between EAP20 and CHMP6. (A) Yeast two-hybrid mapping of the interaction sites between EAP20 and CHMP6. (B) GST pull-down experiment showing a direct interaction between EAP20 and the N-terminal half of CHMP6. The proteins present in each incubation mixture are given above lanes 2 to 5. Pure recombinant EAP20 is shown in lane 1 for reference. MW, molecular weight.

adopt autoinhibited “foldback” conformations (45). In this regard, it is interesting that several different CHMP proteins can be posttranslationally modified and that the unmodified CHMP proteins preferentially localize to the cytoplasm (68, 83). The posttranslational modifications might therefore destabilize autoinhibitory conformations, thereby enhancing the

protein-protein interactions that help dictate ESCRT-III protein localization.

EAP45-ubiquitin interactions. The yeast ESCRT-II complex helps sort ubiquitylated protein cargoes and binds ubiquitin through the second NZF motif (NZF-C) within the N-terminal GLUE domain of Vps36p (1). Mammalian EAP45 proteins

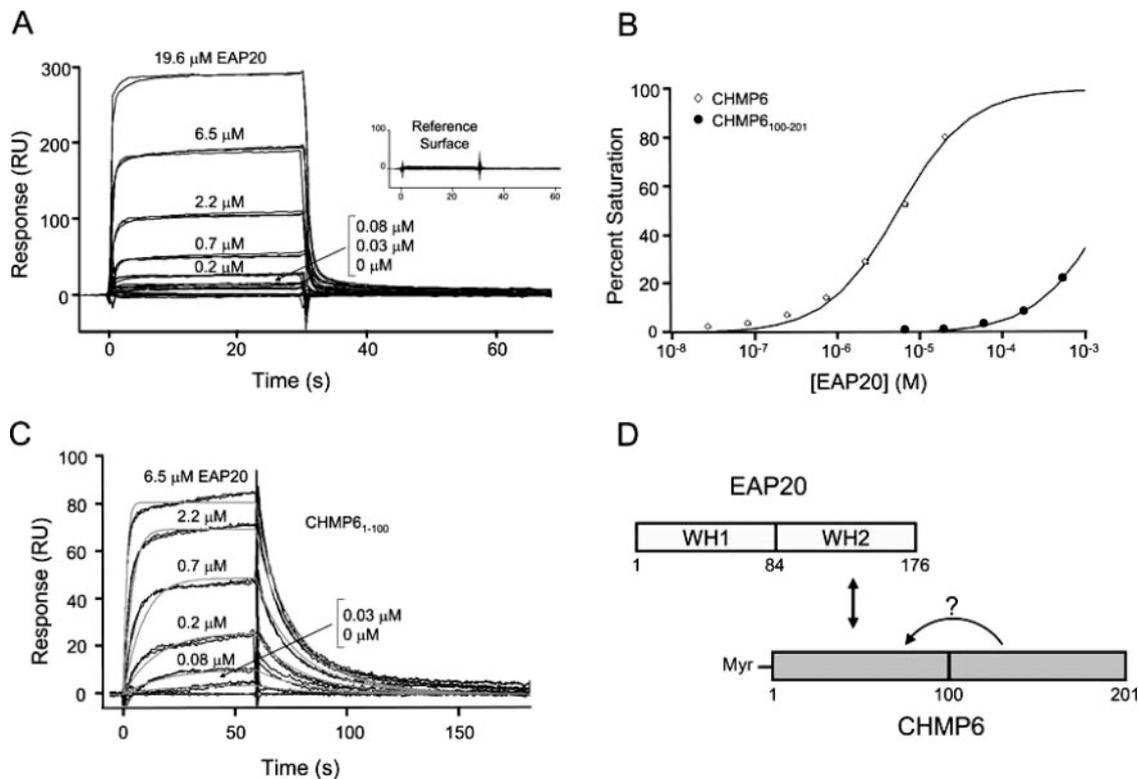


FIG. 4. Biosensor quantification of EAP20 binding to full-length and truncated CHMP6 proteins. (A) Sensorgrams showing different concentrations of EAP20 binding to full-length, immobilized GST-CHMP6. The inset shows EAP20 binding to a control GST surface. (B) Binding isotherms for EAP20 binding to full-length GST-CHMP6 ($K_D = 5.3 \pm 0.2 \mu\text{M}$) and GST-CHMP₁₀₀₋₂₀₁ ($K_D > 1 \text{ mM}$). Dissociation constants and errors were derived by fitting simple 1:1 models to the equilibrium binding data (panel A and data not shown). (C) Sensorgrams showing different concentrations of EAP20 binding to GST-CHMP₁₋₁₀₀. The association and dissociation phases were globally fitted to simple 1:1 interaction models. The light-gray lines show global fits with the following parameters: association rate constant $k_a = (1.46 \pm 0.04) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, dissociation rate constant $k_d = 0.088 \pm 0.003 \text{ s}^{-1}$, and $K_D = 601 \pm 9 \text{ nM}$. (D) Schematic summary of the interaction sites between EAP20 and CHMP6. The arrow with the question mark illustrates a possible “autoinhibitory” interaction between the N- and C-terminal regions of CHMP6.