Binding of the type II receptor ectodomain is not dependent on type I receptor interaction

To measure possible cooperativity, binding of the ActR-IIBECD to immobilized BMP-2 was compared to that of a binary complex consisting of BMP-2 bound to immobilized BMPRIAEC. All measurements were corrected for non-specific interactions by subtracting a control sensorgram. Apparent $K_D$ values were obtained from the dose dependence of equilibrium binding using 1, 2, 3, 5, 10, 20, and 50μM concentration of ActR-IIB ectodomain protein. Sensorgrams of the ActR-IIBEC interaction with (a) immobilized BMP-2 and (b) BMP-2 in complex with immobilized BMPRIAEC. At time -300s perfusion with 200nM BMP-2 was started to form the binary complex BMP-2:BMPRIAEC. At time 0s ActR-IIBEC was injected for 120s at above mentioned concentrations between 1 and 50μM. (c) The sensorgrams in (a) and (b) have been evaluated to show the dose dependency of ActR-IIB equilibrium binding to BMP-2 alone (o) and to a binary BMP-2:BMPRIAEC complex (□). (d) Scatchard analysis of the curves presented in c. The apparent $K_D$ values were 10μM for BMP-2 alone (o) or 9μM for BMP-2 in complex with BMPRIAEC (□).