



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

To measure possible cooperativity, binding of the ActR-IIB<sub>ECD</sub> to immobilized BMP-2 was compared to that of a binary complex consisting of BMP-2 bound to immobilized BMPR-IA<sub>ECD</sub>. 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 50  $\mu\text{M}$  concentration of ActR-IIB ectodomain protein. Sensorgrams of the ActR-IIB<sub>ECD</sub> interaction with **(a)** immobilized BMP-2 and **(b)** BMP-2 in complex with immobilized BMPR-IA<sub>ECD</sub>. At time -300s perfusion with 200nM BMP-2 was started to form the binary complex BMP-2:BMPR-IA<sub>ECD</sub>. At time 0s ActR-IIB<sub>ECD</sub> was injected for 120s at above mentioned concentrations between 1 and 50  $\mu\text{M}$ . **(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:BMPR-IA<sub>ECD</sub> complex ( $\square$ ). **(d)** Scatchard analysis of the curves presented in c. The apparent  $K_D$  values were 10  $\mu\text{M}$  for BMP-2 alone (o) or 9  $\mu\text{M}$  for BMP-2 in complex with BMPR-IA<sub>ECD</sub> ( $\square$ ).