



### Stoichiometry of the ternary complex comprising wildtype BMP-2, BMPR-IA<sub>ECD</sub> and ActR-IIB<sub>ECD</sub>

(a) Stoichiometry of the ternary complex comprising wildtype BMP-2, BMPR-IA<sub>ECD</sub> and ActR-IIB<sub>ECD</sub> in solution determined by analytical SDS-PAGE. Lane M denotes protein marker with the indicated molecular weights. Lane 1 is the ternary complex of wildtype BMP-2 as isolated from gelfiltration with 150pmol of complex loaded. In lanes 2 to 5 mixtures of BMP-2, BMPR-IA<sub>ECD</sub> and ActR-IIB<sub>ECD</sub> with defined molar ratios (as indicated) were loaded, e.g. 1:2:2 means 150pmol BMP-2, 300pmol BMPR-IA<sub>ECD</sub> and 300pmol ActR-IIB<sub>ECD</sub>. The staining intensities of the individual protein bands were integrated using the software NIH Image J version 1.37a (<http://rsb.info.nih.gov/ij/>). Calculation of the molar ratios by comparing the integrated intensities of the isolated ternary complex with those of the standard mixtures yields a molar ratio of 1.0:1.9:2.1 (BMP-2 dimer:BMPR-IA<sub>ECD</sub>:ActR-IIB<sub>ECD</sub>). The standard deviation is 5%. (b) Overlay of reversed phase HPLC chromatograms of the ternary complex (comprising wildtype BMP-2) as isolated from gelfiltration (red line) and a mixture of BMP-2, BMPR-IA<sub>ECD</sub> and ActR-IIB<sub>ECD</sub> in the molar ratio 1:2:2 (black line). The gradient of acetonitrile is indicated in blue. The peaks of the chromatogram were integrated using the software Clarity Lite (Data Apex Inc.); the areas were then divided by the extinction coefficients yielding an experimental molar ratio for the isolated ternary complex of 0.98:2.13:1.97 (BMP-2 dimer:BMPR-IA<sub>ECD</sub>:ActR-IIB<sub>ECD</sub>). The standard deviation for the molar ratios is 10%.