Generating a high valency biotin binder by selecting uniform protein assemblies via crystallization

Supplementary data

Orly Avraham [1], Yael Levi-Kalisman[2] and Oded Livnah [1,*]

[1] From the [a]Department of Biological Chemistry, The Alexander Silverman Institute of Life Sciences, The Wolfson Centre for Applied Structural Biology; The Hebrew University of Jerusalem, The Edmond J. Safra Campus, Jerusalem 91904 Israel.

[2] Center for Nanoscience and Nanotechnology, and The Alexander Silverman Institute of Life Sciences, The Hebrew University of Jerusalem, The Edmond J. Safra Campus, Jerusalem 91904 Israel.

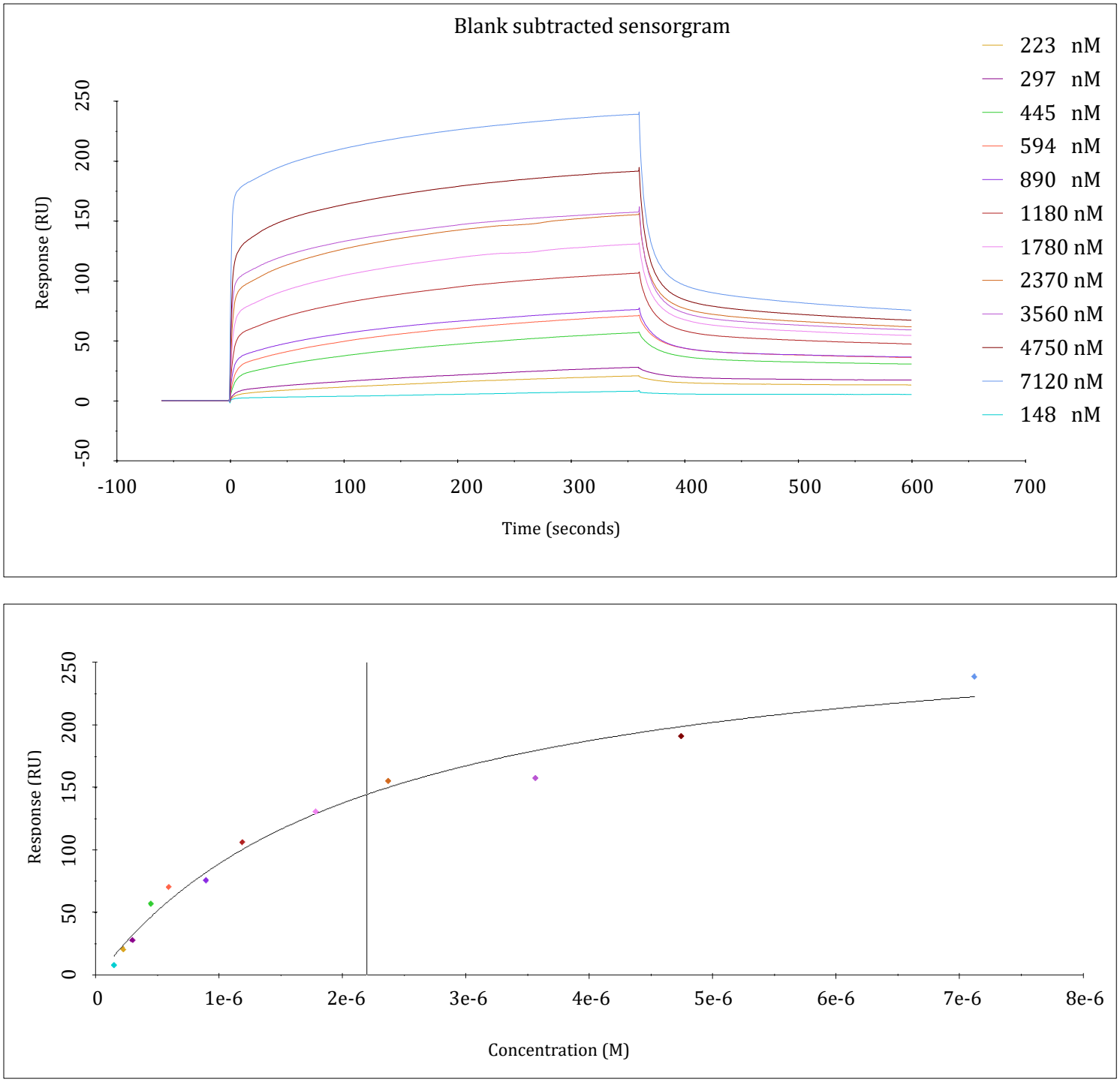
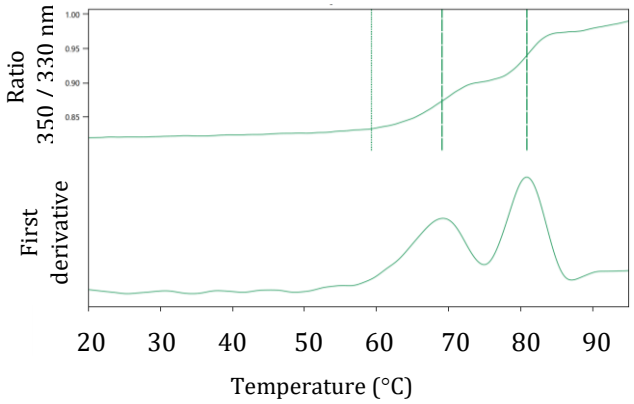


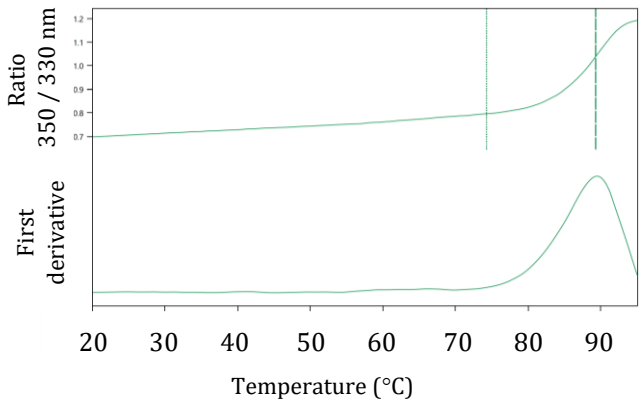
Figure S1: *SPR sensorgram.*
Upper figure shows the SPR sensorgram used to calculate the affinity towards 2-iminobiotin.
The lower figure shows the fitted curve and the derived Kd.

P61C hoefavidin

Apo form

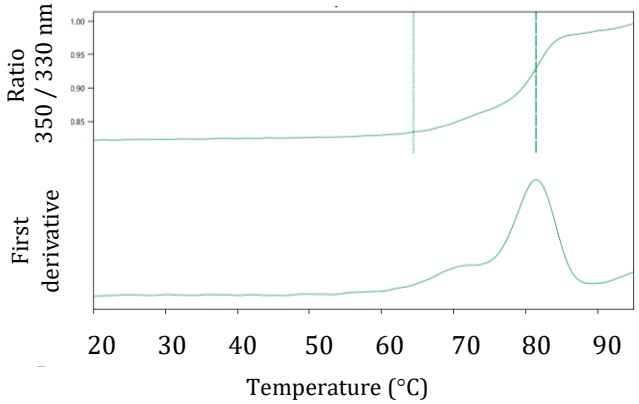


Biotin Complex

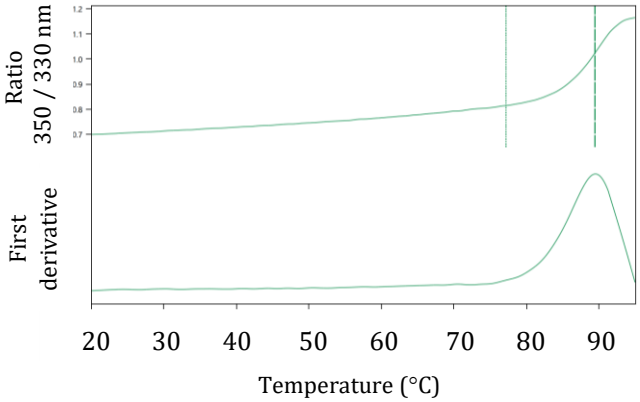


P61C hoefavidin dissolved crystals

Apo form

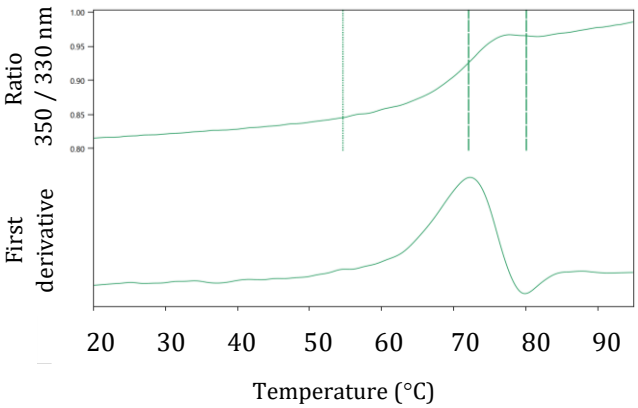


Biotin Complex



Wild type hoefavidin

Apo form



Biotin Complex

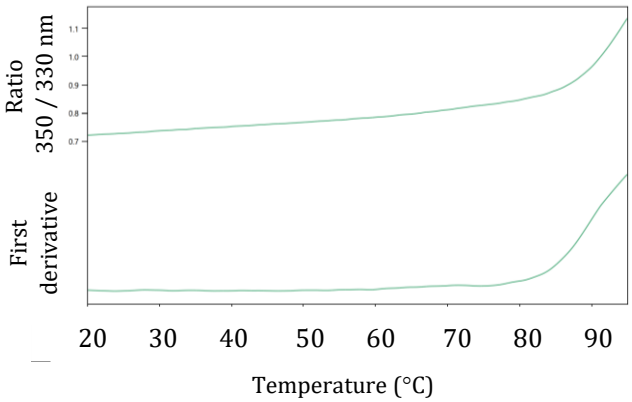


Figure S2: *DSF traces of P61C hoefavidin before and after crystallization and of wt hoefavidin.*

In the apo forms of the P61C and wt hoefavidin, two peaks are present for *Tm* calculation, indicating the heterogeneity of the samples that includes several species (e.g. octamers and dimers) suggesting that the lower temperature peak corresponds to the dimer and the higher to the octamer. Upon biotin binding there is a notable increase in the Tm as previously analyzed for other avidins.