Supporting Information

# Suppressing nonspecific binding in biolayer interferometry experiments for weak ligand-analyte interactions

*Alyssa Dubrow[1], Bryan Zuniga[1], Elias Topo[1], and Jae-Hyun Cho[1,]**

Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843, United States

*Corresponding author. Email: jaehyun.cho@agnet.tamu.edu

Figures S1 – S7.

Figure S1

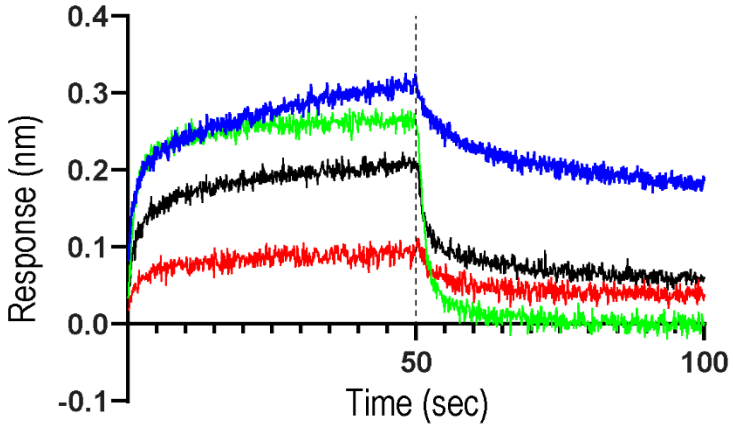


**Figure S1**. Superimposed BLI sensorgram showing NSB of p85β (40 μM) in the presence of 50mM imidazole (blue), 0.6 M trehalose (green), 0.6 M sucrose (red), and 0.6 M glucose (black). All buffers also contained 1% BSA.

Figure S2

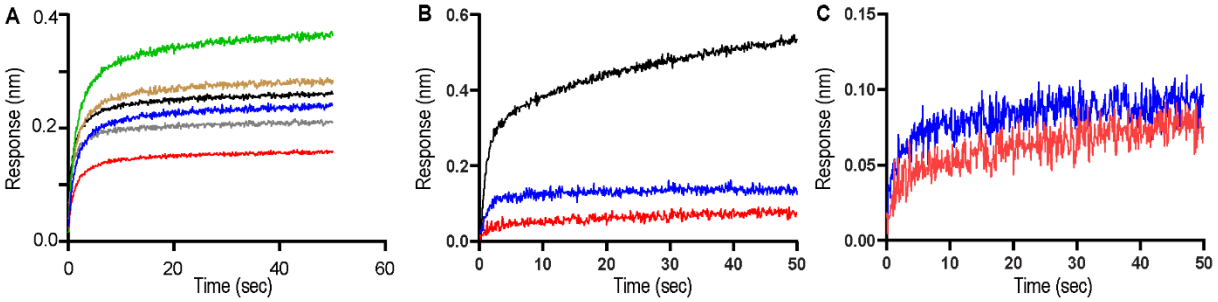


**Figure S2**. (**A**) Superimposed sensorgrams of NSB in the presence of 0.6 M sucrose (blue), 0.6 M sucorse, 20 mM imidazole (red), 0.6 M trehalose (green), 0.6 M trehalose and 20 mM imidazole (brown), 0.6 M glucose (black), 0.6 M glucose and 20 mM imidazole (gray). (**B**) Superimposed sensorgram of NSB in the presence of 1% BSA and 20 mM imidazole (black), 0.6 M sucrose, and 20 mM imidazole (blue), and of 1% BSA, 0.6 M sucrose, and 20mM imidazole (red). (**C**) Superimposed sensorgram of NSB in the presence of 1% BSA and 0.6 M sucrose (blue) and 1% BSA, 0.6 M sucrose, and 20mM imidazole (red). p85β (40 μM) is used to monitor NSB.

Figure S3

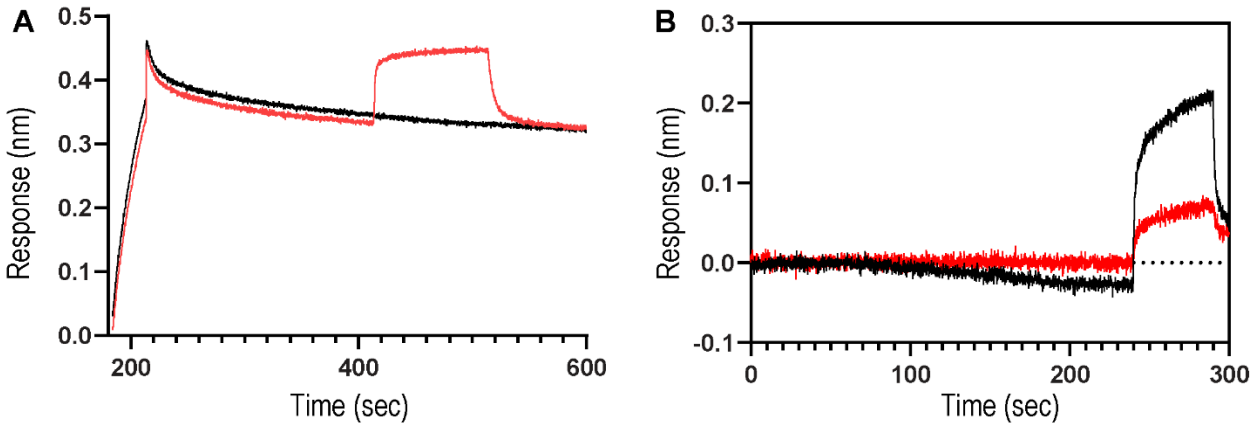


**Figure S3**. (**A**) Raw sensorgram showing the interaction between NS1(ligand) and 40 μM (red) p85β (analyte) in the presence of 1% BSA, 0.6 M sucrose, and 20mM imidazole. Reference data (0 μM p85β) is shown in black. (**B**) Raw sensorgram showing NSB of 40 μM p85β in the presence of 1% BSA (black) and in the presence of 1% BSA, 0.6 M sucrose, and 20mM imidazole (red).

Figure S4

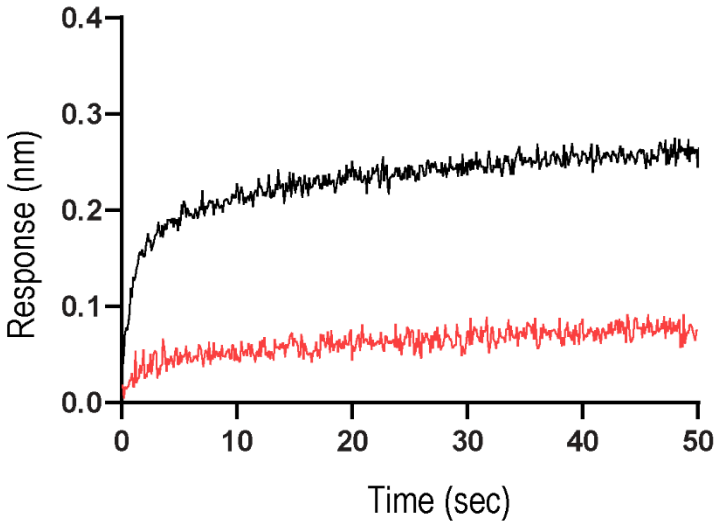


**Figure S4**. BLI sensorgram of NSB (40 μM p85β) in the presence of 0.1% BSA and 0.005% Tween (black) and 1% BSA, 0.6 M sucrose, and 20 mM imidazole (red).

Figure S5

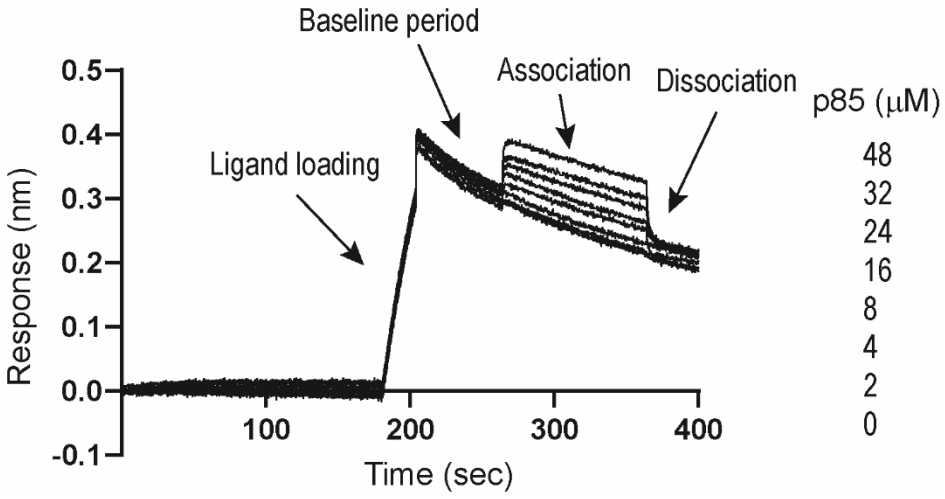


**Figure S5**. Raw sensorgram of the NS1-p85 interaction showing the process from the ligand (NS1) capture to the dissociation step in the presence of 50mM imidazole and 1% BSA.

Figure S6

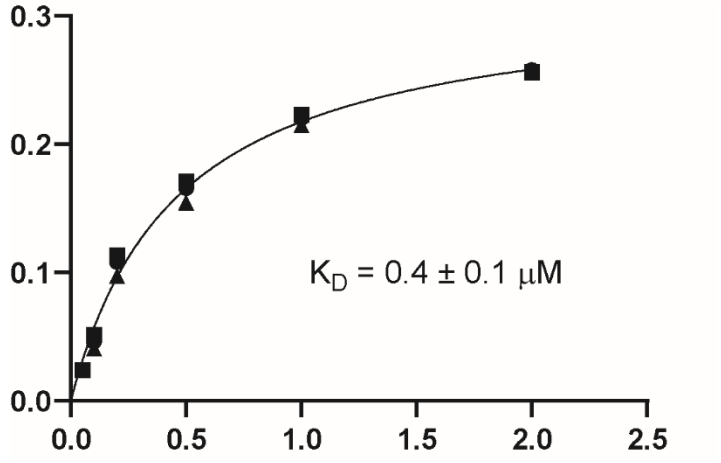


**Figure S6**. Binding isotherm of wild-type NS1 and p85β in the presence of 1% BSA, 0.6 M sucrose, and 20mM imidazole.

Figure S7

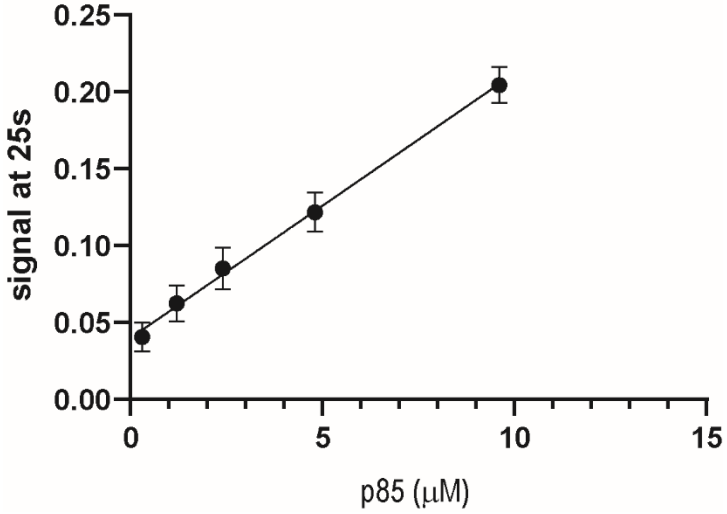


**Figure S7**. Estimation of limit of detection (LOD) and limit of quantitation (LOQ) for binding of p85 to NS1. LOD ($3.3\sigma/slope$) and LOQ ($10\sigma/slope$) were estimated to be 1.48 μM and 4.48 μM, respectively. σ and slope are the standard deviation of the response and the slope of the curve, respectively.