Biolayer Interferometry of Lipid Nanodisc Reconstituted Yeast Vacuolar H^+-ATPase

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Supplementary Figures

Figure S1

Figure S2

Figure S1

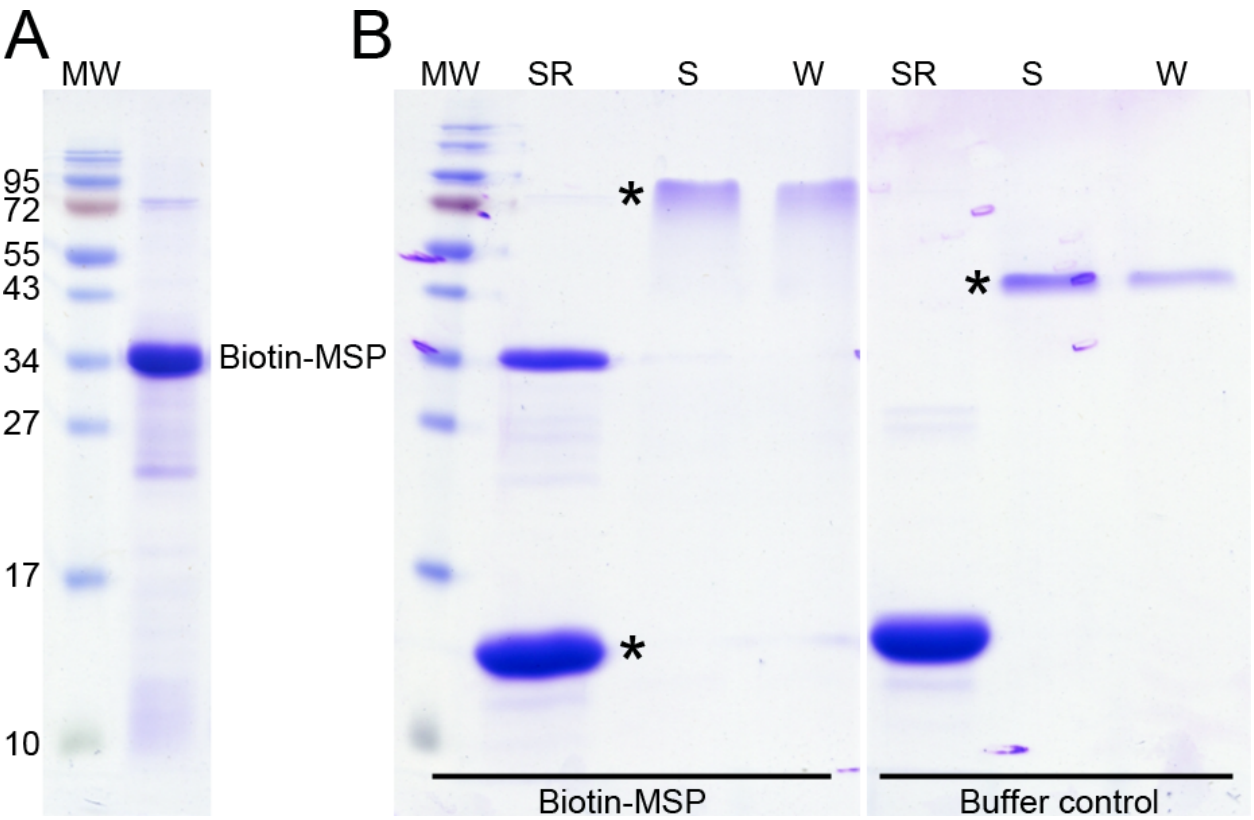

Figure S1: Purification and characterization of biotinylated MSP. (A) SDS-PAGE of purified biotinlylated MSP. (B) Pull down assay using streptactin beads: SR denotes elution from the streptactin resin, S denotes supernatant and W denotes wash. Biotinylated MSP binds to streptactin beads irreversibly while negligible amounts of MSP appear in the supernatant or wash suggestive of almost complete biotinylation. A negative control using buffer is shown on the right gel. The bands labeled by the asterisks are due to streptactin.

Figure S2

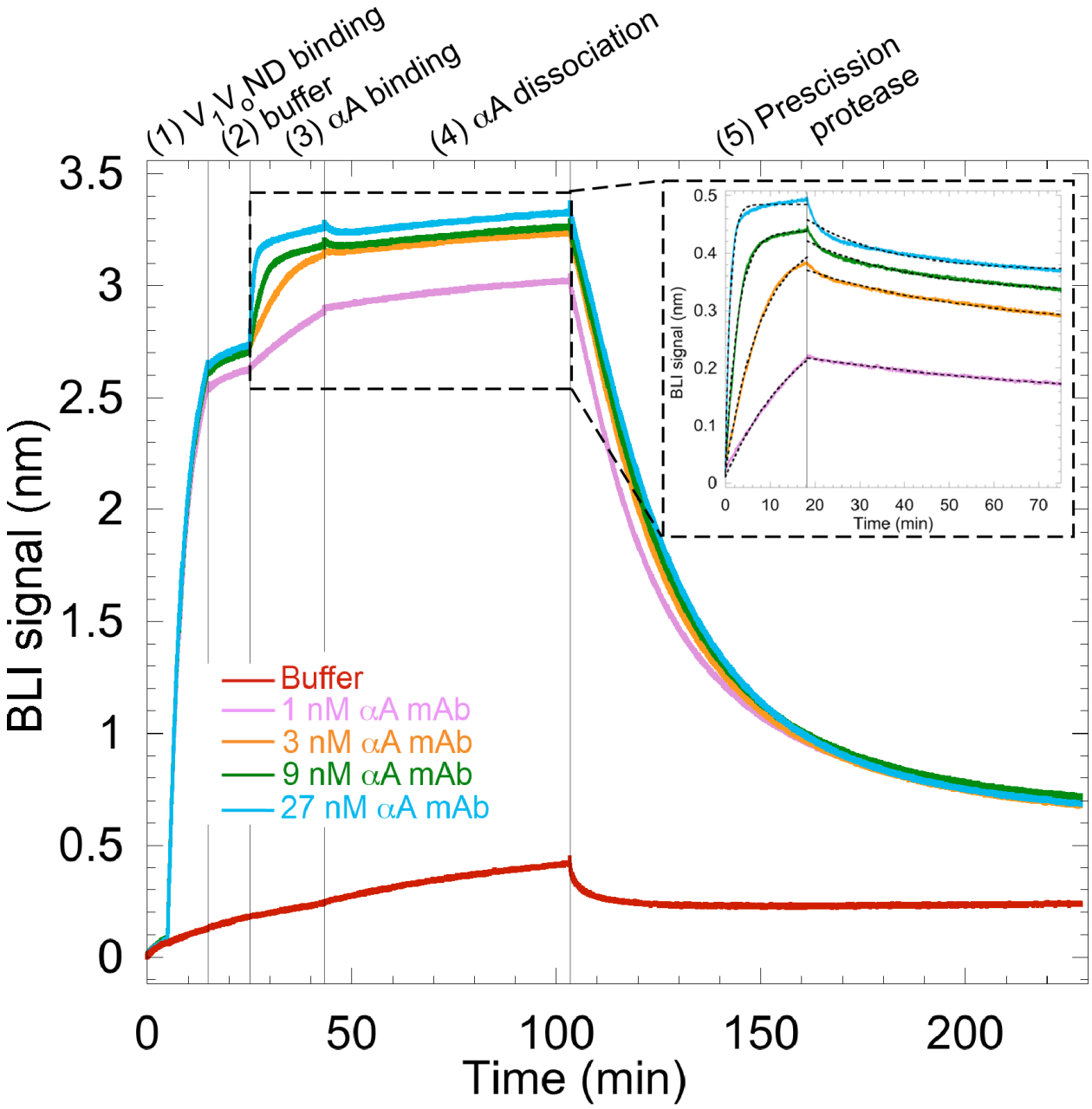

Figure S2: Biolayer interferometry of V_1V_oND with anti-subunit A mAb: V_1V_oND was immobilized at 5 µg/ml (step 1) and the BLI sensors were then dipped in wells containing buffer (step 2) followed by increasing concentrations (1, 3, 9, 27 nM) of a monoclonal antibody against subunit A (αA mAb; 8B1) to measure association rates (step 3). All the sensors were then dipped into wells containing buffer to measure dissociation rates (step 4). Sensors were then dipped into wells containing 0.2 U/µl of Prescission protease (step 5). αA mAb showed concentration dependent binding to V_1V_oND (enlarged box) as evident from the association and dissociation phases. The

signal obtained in buffer (red) was subtracted from the αA mAb signal and the resultant curves were fitted to a local partial 1:1 binding model using the FortéBio data analysis software (black dotted traces). An observed on rate of ~ $6 \cdot 10^5 \pm 1.12$ X 10^{-5} s^{-1} and off rate of $5.7 \cdot 10^{-4} \pm 3.6$ X 10^{-6} s^{-1} with a resultant K_d of ~ 0.9 nM was obtained. K_ds in the high pM to low nM range have previously been reported for the interaction of Protein A with human IgG using BLI as described[1]. Representative experiment from two repeats is shown.

Bibliography

1. Wilson, J.L., Scott, I.M. & McMurry, J.L. Optical biosensing: Kinetics of protein A-IGG binding using biolayer interferometry. *Biochem Mol Biol Educ* **38**, 400-7 (2010).