Correction: A small-molecule competitive inhibitor of phosphatidic acid binding by the AAA+ protein NSF/Sec18 blocks the SNARE-priming stage of vacuole fusion

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In the original article the "Experimental procedures" section on *Surface plasmon resonance* was written in a way that was confusing to readers. For clarification, the authors provided the corrected *Surface Plasmon Resonance* section below. The authors state that their corrections do not affect the results and conclusions of the paper.

Experimental procedures

Surface plasmon resonance

SPR measurements for IPA binding were performed on a Biacore T200 instrument equipped with either an Ni-NTA chip or an L1 chip (1). For direct binding measurements of IPA to Sec18-His$_8$ monomer, full length Sec18-His$_8$ was attached to an Ni-NTA chip following activation with capture level of approximately 9000 response units (RU) using 100 µM NiSO$_4$ flowed at 10 µl/min followed by NHS/EDC ethanolamine to covalently immobilize the octahistidine tag (2). Injections of IPA were performed in HBS buffer at a flow rate of 30 µl/min with an association time of 90 s and dissociation time of 300 s, and binding was measured in RU as described (3). IPA was injected using a series of 1:1 dilutions from the highest concentration and steady state binding values were obtained using GE BIAcore T200 evaluation software version 3.0 (BIAevaluate), exported into GraphPad Prism 7.00 for Windows (GraphPad Software, La Jolla, CA) and fit using a one-site–specific binding model.

For binding of IPA to Sec18 domains either D1, D2, or D1-D2 were non-covalently attached to a Ni-NTA chip using 100 µM NiSO$_4$ flowed at 10 µl/min with capture time of 600 s followed by blank buffer injections of HBS buffer prior to each sample injection. Regeneration of the chip was done with 100 µM EDTA at a flow rate of 30 µl/min for 120 s following each injection where each domain was then recaptured prior to each injection of IPA. Approximate capture levels for each injection of IPA were 2500 RU for D1, 4500 RU for D2, and 1500 RU for D1-D2, respectively. IPA was injected using a series of 1:1 dilutions with at least one concentration from each titration run in duplicate at 30 µl/min with an association time of 90 s and a dissociation time of 120 s. Kinetic fits were obtained using a 1:1 binding model on BiaEvaluate and sensorgrams were generated by exporting data into GraphPad.

For SPR using attached liposomes an L1 liposome chip was used with liposomes attached to a sample flow cell and no liposome to the reference flow cell. Liposomes were attached after conditioning the chip with two injections of 20 mM CHAPS over both flow cells for 30 s at 30 µl/min on each flow cell. Each liposome capture was regenerated when a different protein was flowed using 30-s injections of CHAPS at 5 µl/min to clean the sensor chip. Proteins were attached freshly for each type of Sec18, where proteins were titrated in the presence and absence of IPA yielding IC$_{50}$ and K_D values. K_D values to liposomes were determined at 30 µl/min with association of 70 s and disassociation of 300 s for D1, 60 s association and 300 s dissociation for Sec18 monomer and hexamer, and 60 µl/min for D2 with association 75 s and disassociation of 225 s. IC$_{50}$ values were calculated in the presence of IPA titrations with 40 s association and 40 s and disassociation of 40 s to Sec18 domains and Vam7 as a control. Results were exported from BiaEvaluate into Graphpad and fit via one-site–specific binding model for each protein saturation curve yielding K_D values for different Sec18 constructs and log-inhibitor v. response 3 parameter equation for competition experiments involving IPA inhibition of Sec18 domain binding to liposomes.

References

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