

P17 – Interactions between Signal Transducing Proteins Measured by Pico-force Atomic Force Microscopy

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Pico-force atomic force microscopy (AFM) has been used to study specific interactions between signal transducing proteins, mammalian phospholipase D1 (PLD1), phospholipase C- γ 1 (PLC- γ 1), and Munc-18-1.^{1,2} To record the forces between them, the Phox homology (PX) domain of PLD1, the Src homology (SH3) domain of PLC- γ 1, and Munc-18-1 were fused with glutathione S-transferase (GST) and immobilized onto reduced glutathione (GSH) tethered surfaces. In order to enhance the recognition efficiency and avoid undesirable complications, both AFM tips and substrates were first modified with dendrons of two different sizes. Under the employed conditions, the probability of observing an unbinding event increased, most force-distance curves showed the single rupture events. To investigate dynamics of these biomolecular interactions, we measured the loading rate dependence of the unbinding forces. The unbinding forces increased linearly with the logarithm of the loading rate, indicating the presence of single potential barrier in the dissociation energy landscape. Further, we elucidated the influence of free SH3 and Munc-18-1 on the specific PX-(Munc-18-1) and PX-SH3 interaction, respectively. The unbinding force of the protein pairs in the titration curve decreased as the concentration of the competing proteins increased, providing new information on how effective the competitions were.

Reference

1. Jang, I. H. *et al. J. Biol. Chem.* **2003**, 278, 18184.
2. Lee, H. Y. *et al. J. Biol. Chem.* **2004**, 279, 16339.