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Ultra-stable Biomembrane Force Probe to Characterize Strong Protein-Protein Interactions on a Living Cell

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Abstract: Biomembrane force probe (BFP) is a single-molecule biomechanical technique that has been widely used to characterize protein dynamics (e.g., protein-protein interactions and protein conformational changes), especially suitable for measuring force-regulated receptor-ligand binding kinetics *in situ*[1-4]. Integrated with various force spectroscopies, such as lifetime assay, it has become a powerful platform to systematically characterize many force-regulated receptor-ligand dissociation of great biological significance, which cannot be done with traditional solution based assays (e.g., surface plasma resonance) [5].

Even though the BFP has been quite successful in characterizing binding kinetics of weak and transient molecular interactions, it is still incapable of stably clamping the loading force and measuring force-dependent strong molecular bond with long lifetimes (e.g., >10s) [2], which impedes our further understanding of the biological significance behind some strong interactions, especially during antibody screening. It is mainly because current BFP system lacks force feedback control such that any environmental perturbations could induce force drifting, which in turn affects the accuracy and stability of long bond lifetimes at constant force.

To overcome this drifting problem, we integrate new experimental setup, tracking strategy, and computer controlled feedback program to systematically enhance the clamping force stability when measuring bond lifetimes. We firstly correct the micropipette drifting by adopting double-edge tracking to replace the one edge tracking of BFP bead. To achieve clear and stable double-edge tracking, another glass bead is attached to the probe micropipette via "molecular glue" to offer a sharp edge for precise and steady tracking. This bead edge represents probe micropipette's position. By tracking the distance between these two edges, we can stably characterize the deformation of the RBC in real-time, even in the presence of probe micropipette's drifting. Secondly, to further eliminate force drifting from the target pipette, we embed a force feedback control algorithm into BFP control program. This algorithm compensates force drfiting through controlling the motion of piezo stage where the target pipette is mounted. To ensure feedback efficiency and avoid computer's memory overloading, the sampling rate is kept highest while the data saving rate is reduced by 20 times in order to steadily record tracking data of an event with the bond lifetime longer than 200s.

To validate the stability of this new BFP system in measuring long bond lifetimes in ultra-slow dissociation event, we performed force-clamped lifetime assay to measure force-dependent bond lifetimes of single PD-1 antibody binding with PD-1 expressed on a living Jurkat T cell. This new BFP system significantly outperforms the older version in measureing ultra-slow dissociation kinetics. With this new system, we obtained the mean bond lifetime of ~37s at 5pN, five times longer than ~7s with the older BFP system. The base-line driftings from micropipette in new BFP system were mostly confined within 2pN, much more stable than the old system which easily drifts over tens of piconewtons. Moreover, when clamping force at 10pN, the force drifting frequency (the ratio of lifetimes whose mean force deviated more than 3pN) reduced from 33.5% to 1.7%, greatly enhancing the quality and efficiency of single-molecule lifetime data collection.

Taken together, this newly developed BFP is much more stable, competent, accurate and efficient in characterizing strong molecular interaction with ultra-slow dissociation kinetics. It would become a useful platform to *in-situ* screen most effective therapeutic antibodies for immunotherapy.

Keywords: Ultra-stable biomembrane force probe; force clamp; feedback

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References

1. Lou J, Yago T, Klopocki AG, Mehta P, Chen W et al. Flow-enhanced adhesion regulated by a selectin interdomain hinge. *Journal of Cell Biology* **2006,** 174(7): 1107-1117.
2. Chen W, Lou JZ, Zhu C. Forcing Switch from Short- to Intermediate- and Long-lived States of the alpha A Domain Generates LFA-1/ICAM-1 Catch Bonds. *Journal of Biological Chemistry* **2010,** 285(46): 35967-35978.
3. Huang J, Zarnitsyna VI, Liu B, Edwards LJ, Jiang N et al. The kinetics of two-dimensional TCR and pMHC interactions determine T-cell responsiveness. *Nature* **2010,** 464(7290): 932-936.
4. Liu BY, Chen W, Evavold BD, Zhu C. Accumulation of Dynamic Catch Bonds between TCR and Agonist Peptide-MHC Triggers T Cell Signaling. *Cell* **2014,** 157(2): 357-368.
5. Liu BY, Chen W, Zhu C. Molecular Force Spectroscopy on Cells. *Annual Review of Physical Chemistry* **2015,** 66: 427-451.