**SUPPLEMENTARY INFORMATION**

# A peptide tag system for facile purification and single-molecule immobilization

Jin Huang, Stas Nagy, Akiko Koide, Ronald S. Rock* and Shohei Koide*

## Experimental Methods

**Protein preparation**. The genes for an ePDZ-b1 variant containing a Cys residue at the C-terminus, erbin PDZ domain containing a C-terminal Cys residue and C-tagged yeast SUMO were constructed using standard PCR methods and cloned in an expression vector, pHFT2 (*15, 16*). These proteins were expressed as $His_{10}$-tag proteins in *E. coli* and purified using nickel affinity chromatography as described previously (*7*). Typically, ~50 mg of a purified affinity clamp protein was obtained from a liter of *E. coli* culture.

The gene for C-tagged myosin X was constructed in pBiEx-3 (Novagen) for transient transfection in Sf9 insect cells. We replaced the multiple cloning site (MCS) of pBiEx-3 between the SacI and XhoI sites with the MCS of pBluescript KS+ from the SacI to XhoI sites by digestion and ligation of the appropriate fragments. The start codon found within the leading NcoI site of pBiEx-3 was removed by quickchange mutagenesis. The MCS of this modified vector, pBiEx3BS, was verified by sequencing. We then subcloned our previously described myosin X HMM-GCN4-GFP-FLAG construct (*13*) into pBiEx3BS by digestion and ligation from pBluescript, using the SpeI and HindIII sites. From this vector we used overlap-extension PCR to obtain fragments of the myosin X tail that encode a FLAG-tag-C-tag (DYKDDDDKRGSIDTWV) immediately following the GCN4 sequence (omitting the GFP). Here, we omitted the thrombin recognition site to minimize the total lengths of the two tags. These fragments were TOPO-cloned, digested from BstBI to SalI, and inserted into a similarly cut myosin X/pBiEx3BS vector. This construct was verified by sequencing.

**Affinity purification using immobilized PDZ resin**. The erbin PDZ domain containing a Cys residue was immobilized to SulfoLink Coupling Resin (Thermo Scientific) following the manufacturer's instructions. The resin was packed into a PD-10 column (Amersham) for coupling and protein purification. Affinity purification of C-tagged SUMO was performed as follows.

*E. coli* lysate containing C-tagged SUMO was prepared as described previously (*7*) and applied to the resin. The column was then washed with 5 gel-bed volume of TBS (pH7.4). The bound protein was eluted with TBS containing 20 μM elution peptide. The fractions containing purified C-tagged SUMO were pooled and dialyzed against thrombin cleavage buffer (20 mM Tris pH 8.4, 150 mM NaCl). The affinity resin was regenerated by first washing with 10 gel-bed volume of 8M urea or 6M guanidine hydrochloride with 1 mM DTT and then with 10 gel bed volume of TBS (pH 7.4).

$CaCl_2$ was added at a final concentration of 2.5 mM before adding thrombin (Novagen) to the solution. Thrombin cleavage was performed at room temperature for 16 hours. Purification of cleaved product was performed as described above.

Sf9 insect cells were transiently transfected with the myosin X/pBiEx3BS expression vector (500 mL at 2 x $10^6$ cells/mL). The cells were harvested after 3 days, and a half of the cells were purified as previously described using the FLAG-resin/FLAG-peptide (*13*). The second half was purified in the same manner except that we used the ebrin PDZ affinity resin/elution peptide.

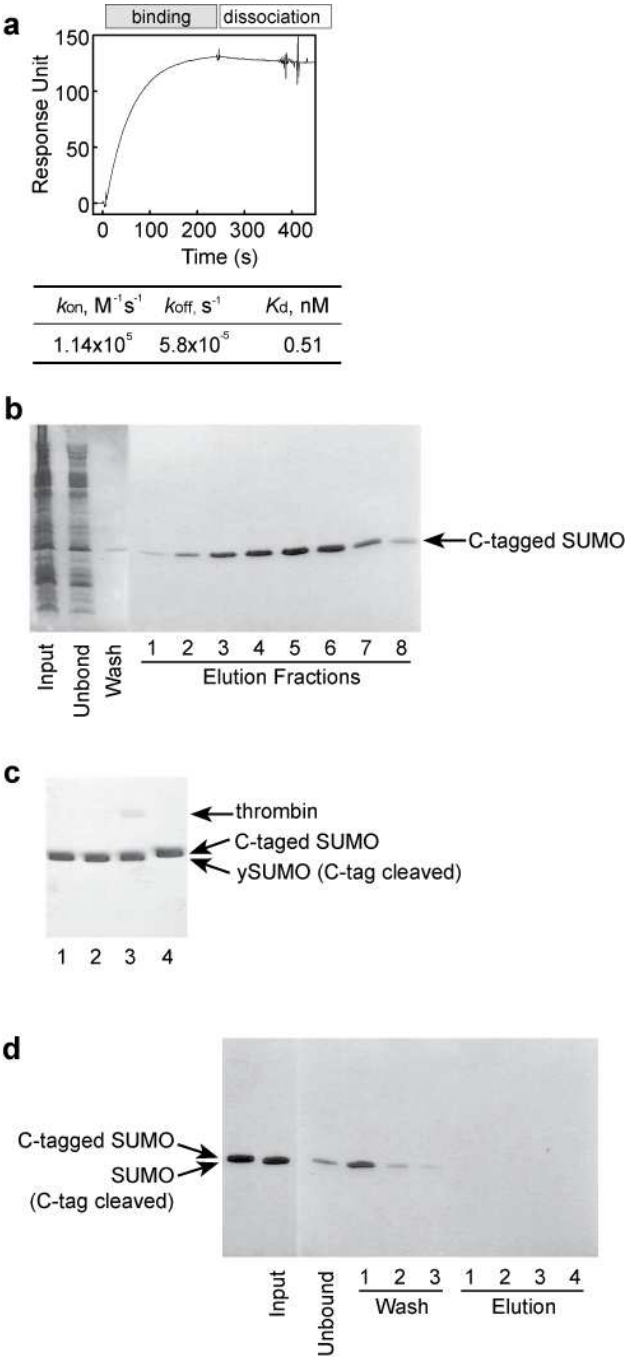
Single molecule assays on myosin X were performed as previously described (*13, 17*), with the modifications described in the main text. For immobilization of motors in gliding filament and optical trapping assays, the ePDZ-b1 protein replaced the monoclonal anti-GFP antibody (3E6) in the procedures. We applied ePDZ-b1 at 2.7 μM for 2 min to coverslip surfaces. For labeling motors with fluorophores for TIRF motility assays, the Cy3-labeled ePDZ-b1 protein replaced the calmodulin exchange procedure described in Nagy et al(*13*). Motors were labeled at a 1:1 molar ratio of heavy chain to ePDZ-b1.

## References for Experimental Methods

15. Huang, J., Koide, A., Nettle, K. W., Greene, G. L., and Koide, S. (2006) Conformation-specific affinity purification of proteins using engineered binding proteins: Application to the estrogen receptor, *Protein Expr Purif 47*, 348-354.

16. Koide, A., Gilbreth, R. N., Esaki, K., Tereshko, V., and Koide, S. (2007) High-affinity single-domain binding proteins with a binary-code interface, *Proc Natl Acad Sci U S A 104*, 6632-6637.

17. Rock, R. S., Rief, M., Mehta, A. D., and Spudich, J. A. (2000) In vitro assays of processive myosin motors, *Methods 22*, 373-381.




**Supplementary Figure 1.** Purification of C-tagged SUMO expressed in *E. coli* using the immobilized PDZ resin. **a) Surface plasmon resonance analysis of C-tag/ePDZ-b1 interaction.** A sensorgram of C-tagged SUMO interaction with immobilized ePDZ-b1 is shown. The binding and dissociation phases of the experiment are indicated. The association and dissociation rates as well as the dissociation constant derived from the measurement are listed. **b) Single-step purification of C-tagged SUMO.** SDS-PAGE of samples taken from different stages of purification is shown. Input, the soluble fraction of crude cell lysate; Unbound, the unbound (flow-through) fraction; Wash, wash with TBS pH7.4 ; and Elution, elution fractions using 20 μM peptide. The arrow indicates the position of the C-tagged SUMO protein. Note that the results shows were obtained with the resin that had been regenerated with 6 M GdHCl and 1 mM DTT three times. **c) Thrombin cleavage of C-tagged SUMO**. The protein was cleaved with 1, 10, 100 units per mg C-tagged SUMO for 16 hours (lanes 1, 2 and 3, respectively). The positions of thrombin, C-tagged SUMO and cleaved SUMO are indicated. **d) Confirmation of thrombin-cleavage of C-tagged SUMO using the affinity resin**. The cleaved product appeared in the unbound and wash fractions. No SUMO protein was detectable in the elution fractions, indicating essentially complete cleavage of C-tagged SUMO.

## Movie Legends

**Supplementary Movie 1. Gliding filament motility assays in which myosin X was immobilized through the C-tag/affinity clamp linkage.** We observe smooth and continuous movement of actin filaments. The movie images were collected at 2 fps and are displayed at 20 fps.

**Supplementary Movie 2. TIRF motility assays of C-tagged myosin X labeled with Cy5-affinity clamp.** Single fluorescent myosin X motors (green) move along fascin-actin bundles (red). The movie images were collected at 5 fps and are displayed at 20 fps.