Supplementary Information for

"Ligand-dependent Folding and Unfolding Dynamics and Free Energy Landscape of Acylphosphatase"

Li Yuan,[†a] Hao Sun,[†b] Xuening Ma,[a] Yang Wang,[b] Zilong Guo,[b] Xingyu Qi,[a,b] Shimin Le,[*a] and Hu Chen,[*a,b]

[a] *Research Institute for Biomimetics and Soft Matter, Fujian Provincial Key Lab for Soft Functional Materials Research, Department of Physics, Xiamen University, Xiamen 361005, China.*

[b] *Center of Biomedical Physics, Wenzhou Institute, University of Chinese Academy of Sciences, Wenzhou 325000, China.*

[†] Equal contribution.

[*] E-mail: leshimin@xmu.edu.cn, chenhu@xmu.edu.cn.
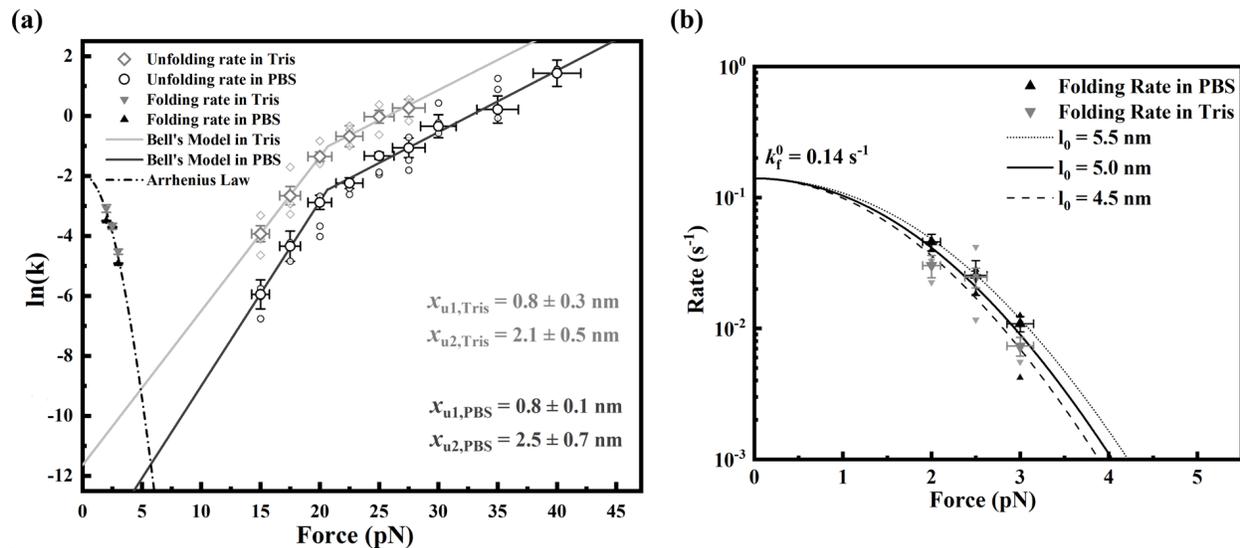
SUPPLEMENTARY FIGURES

FIG. S1. Force-dependent unfolding and folding rates in the Tris and PBS measuring buffer from nine independent protein tethers. (a) The average unfolding rates in the two solvent environments were fitted separately with Bell's model. (b) Two sets of force-dependent folding rates are fitted by Arrhenius' law to determine the size of folding transition state.

(a)

Legend:
◇ Unfolding rate at 20 pN in 0 mM Pi
○ Unfolding rate at 20 pN in 1 mM Pi
○ Unfolding rate at 20 pN in 10 mM Pi
○ Unolding rate at 20 pN in 100 mM Pi
◆ Folding rate at 2 pN in 0 mM Pi
○ Folding rate at 2 pN in 1 mM Pi
● Folding rate at 2 pN in 10 mM Pi
● Folding rate at 2 pN in 100 mM Pi
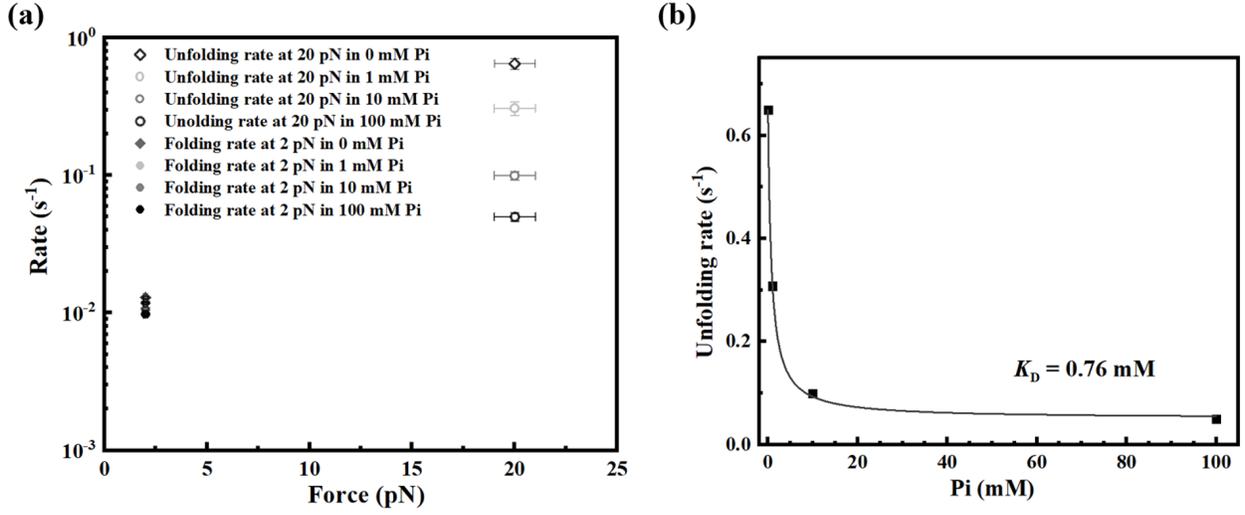
(b)

$K_D = 0.76$ mM

FIG. S2. AcP folding rate at 2 pN and unfolding rate at 20 pN of the same tether measured by force-jump experiments in buffers with various inorganic phosphate (Pi) concentrations. (a) AcP folding rate and unfolding rate in buffers with 0 mM (Tris), 1mM (0.1×PBS), 10 mM (1×PBS) and 100 mM (15.6% (w/v) Sodium phosphate monobasic dihydrate, 35.8% (w/v) Sodium phosphate dibasic dodecahydrate) Pi, PH 7.4. The folding rate remains essentially unchanged, while the unfolding rate decreases as the concentration of Pi increases. (b) AcP unfolding rate in buffers with 0 mM Pi (Tris), 1mM Pi, 10 mM Pi and 100 mM Pi and dissociation constant K_D of AcP with Pi. The black curve represents the fitting curve to equation $k_u(C) = (k_{AcP}K_D + k_{AcP,Pi}C)/(K_D + C)$, where C is the concentration of Pi, k_{AcP} and $k_{AcP,Pi}$ are the unfolding rates of AcP and AcP-Pi complex, respectively (Chiti et al, 1998).
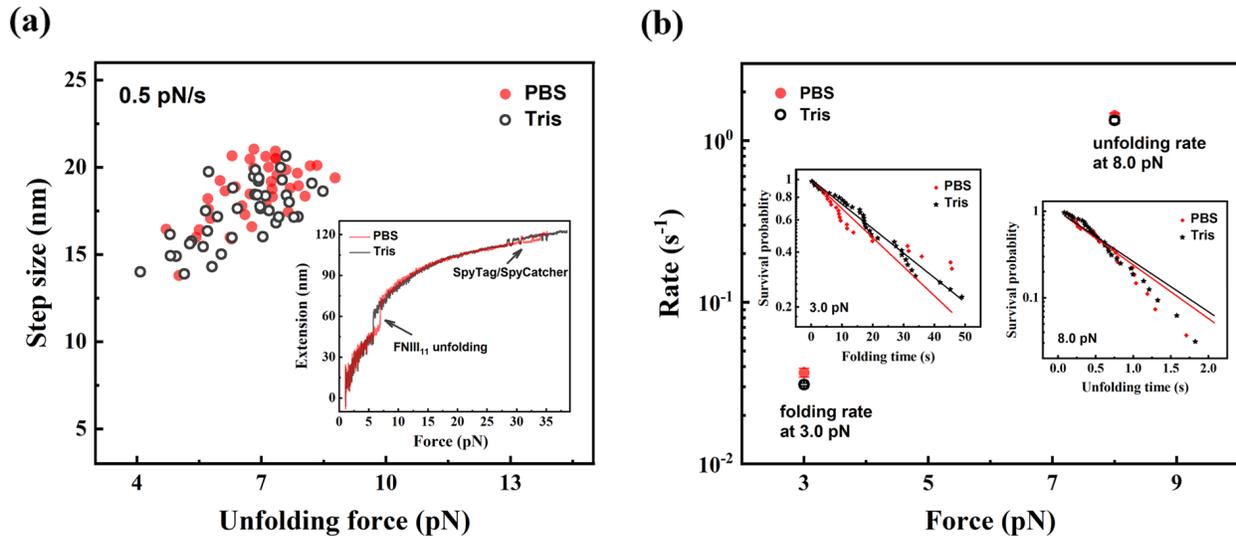
(a)

(b)

FIG. S3. Force response of the protein structure of AviTag-FH1 linker-FNIII$_{11}$-FH1 linker-SpyTag. (a) Step size of FNIII$_{11}$ measured by 0.5 pN/s loading rate experiments. Inset shows the force-extension of the protein construct. (b) Folding rate at 3.0 pN and unfolding rate at 8.0 pN of FNIII$_{11}$ measured by force-jump experiments in Tris and PBS buffers. Insets show the exponential fitting of survival probability to obtain the folding and unfolding rates.
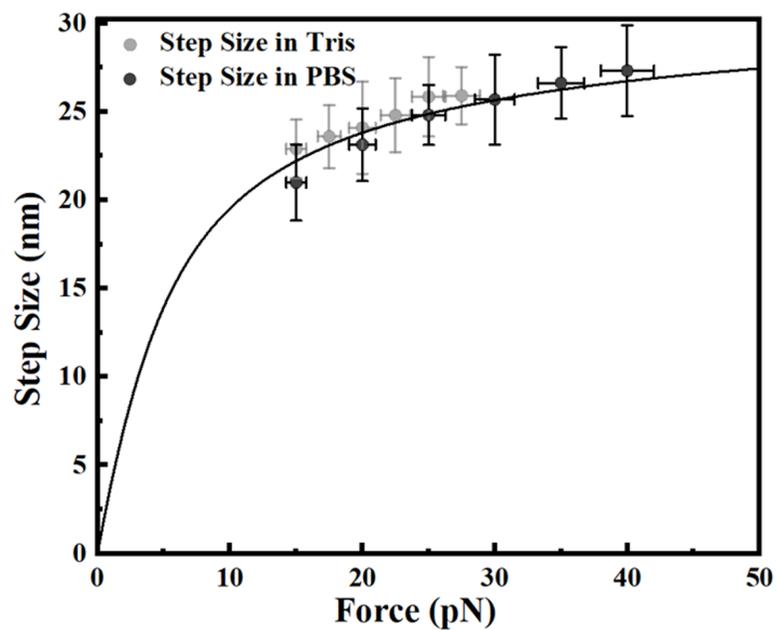
FIG. S4. The average unfolding step sizes of AcP in Tris and PBS buffers were obtained from force-jump measurement. The black curve represents the extension difference between native AcP and the Unfolded peptide.