2P025 Single molecule force spectroscopy reveals force-enhanced binding of calcium ions by gelsolin
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Force is increasingly recognized as an important element in controlling biological processes. Forces are able to deform native protein conformations leading to protein-specific effects. Here we demonstrate that the calcium-binding affinity of the actin-binding protein gelsolin domain G6 is enhanced by mechanical force. Using a recently developed single molecule-binding assay based on atomic force microscopy, we establish that the calcium-binding affinity of G6 increases exponentially with the applied force, up to the point of G6 unfolding. This implies that gelsolin will be activated at lower calcium ion levels when subjected to tensile forces and suggests a basis for enhanced cooperativity during multi-cation induced activation.

2P026 Direct observation of the multiple sliding modes of a tumor suppressor p53
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1D sliding of a tumor suppressor p53 along DNA is an essential dynamics for its effective search of the target sequence. The 1D sliding of p53 has been assumed to be described as a single diffusive movement. However, it is conceivable that p53 has multiple sliding modes depending on the quaternary structures of the p53-DNA complex. In this study, the sliding of p53 along DNA was measured by a single-molecule fluorescence microscopy. The observed trajectories of p53 were analyzed by the change point analysis, which can detect boundaries of trajectories with different diffusion constants. Our results showed that p53 possesses at least three different diffusion modes, and further suggests that the quaternary structures of the p53-DNA complex modulate the sliding modes.

2P027 Study of a peptidase-associated domain of an aminopeptidase from thermophilic Aneurinibacillus sp. AM-1
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A thermophilic bacterium Aneurinibacillus sp. AM-1 produces an aminopeptidase (AM1AP) belonging to M28 family. The enzyme shows strong prolyl aminopeptidase activity toward prolyl-p-nitroanilide. X-ray crystal structure of AM1AP revealed that the enzyme is composed of a peptidase domain (PD, 31 kDa) and a peptidase-associated domain (PAD, 14 kDa). PD, typical for M28 family enzymes, contains an active site with two zinc atoms, whereas PAD widely occurs in peptidases/protases beyond this family. To date, the roles of PAD are undefined yet. To elucidate its roles, PD and PAD was separately expressed in E. coli, and purified, and then their molecular properties (specific activity, gel filtration, CD in the presence and absence of zinc ion, etc) were investigated.