Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information (SI)

Manipulating and Probing Enzymatic Conformational Fluctuations and Enzyme-Substrate Interactions by Single-Molecule FRET-Magnetic Tweezers Microscopy

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S1. Experimental details

In our experiment, we chose fluorescence dye labeled 6-hydroxymethyl-7,8-dihydropterin pyrophospho- kinase (HPPK) as a model system to measure the FRET and magnetic tweezers manipulations in the solutions with and without enzymatic substrates. The fluorescent dyes Cy3 (donor)/Cy5 (acceptor) was labeled to the mutated amino acid residue 48 on loop 2 and residue 151 close to the active site of the enzyme, respectively, shown in figure S1. HPPK molecules were bound to the glass cover slip at one end by 3-triethoxysilylpropylamine-Dimethyl Suberimidate links and attached to a super-paramagnetic bead at the other end by biotin-streptavidin links. The HPPK molecule was labeled at residue position 48-151 with *Cy3* and *Cy5* dye molecules respectively.

All the attachment to HPPK molecule, either biotin or DMS, can only be fulfilled via connection with lysine in the amino acid sequence. Hence there are five possible positions in an HPPK molecule to allow attachment of magnetic particle via biotin or attachment to cover glass via DMS. As a result, there are five possible positions on one HPPK molecule available to attachment to either coverslip or magnetic bead. In the reaction two of these five positions will be occupied by attachment to either coverslip or magnetic bead. The combination brings multiple different possible types for the relative position of chromospheres and the lysine

attached to glass or bead on a single HPPK molecule. Although from the crystal structure we can preclude some tethering conditions that are less possible: for example, in figure 1A we can see that it is essentially impossible for two linkers to consecutively tether on residue 154 and residue 157, we still are not able to pinpoint one deterministic specific amine residue pair for protein tethering. On the other hand, during our FRET measurement, we focused on one certain HPPK molecule, no matter what certain type it is. If we can observe its reproducibly FRET change under periodically applied magnetic field, we are able to say conformational manipulation is achieved via magnetic tweezers, although we do not know which certain two of the five lysine on the HPPK protein is attached.[1-3]

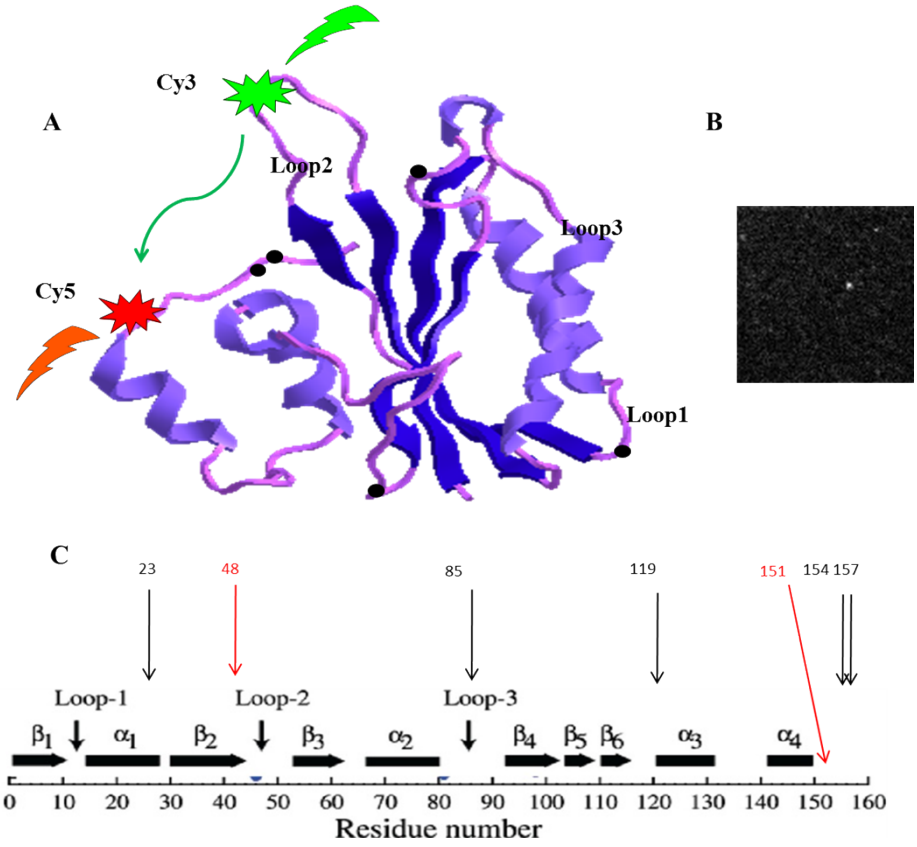

Figure S1. (A) Crystal structure of HPPK molecule from protein databank (PDB ID: 1HKA). The mutated dye-labeled residue and Lysine residue has been pointed out (black spots on the molecule). The HPPK molecule was labeled at residue position 48-151 with *Cy3* and *Cy5* dye molecules which are the green and red spot in the scheme respectively. **(B)** An example of single molecular image of the HPPK molecule observed by confocal microscope. **(C)** Full sequence of HPPK protein.

S2. Sample preparation

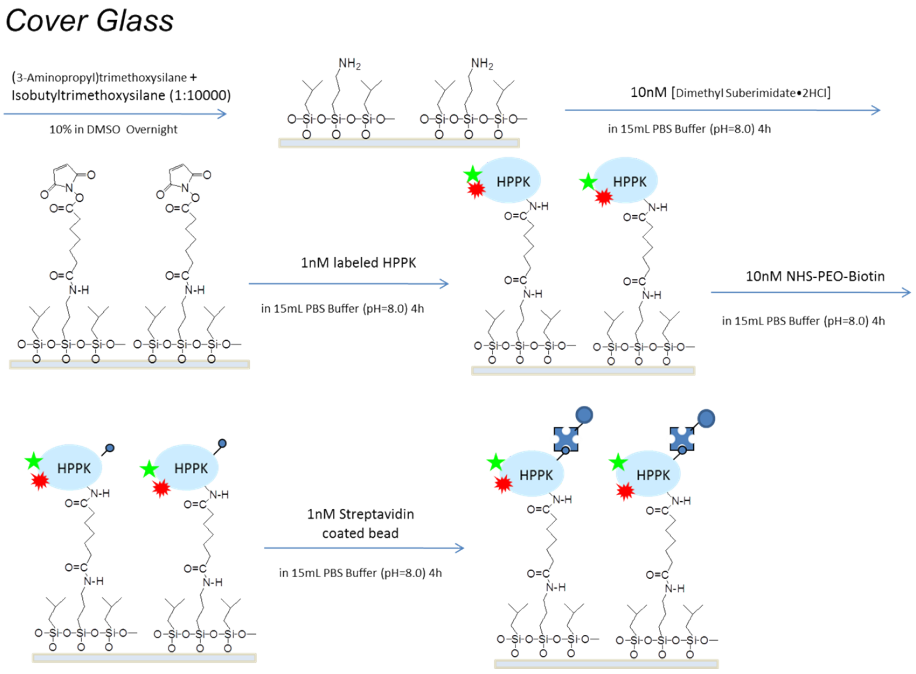

Figure S2. We tethered protein molecules at one end to the coverslip by Dimethyl suberimidate-2HCl (DMS-2HCl) and at the other end to a 1 µm size paramagnetic bead by biotin-streptavidin bonding. Force was implied by adding external magnetic field and hence the molecule could feel it through the beads.

S3. The correlated single-molecule FRET-magnetic tweezers microscopy setup

We correlated magnetic tweezers with single-molecule FRET imaging to achieve mechanical manipulation and simultaneous optical observation of protein conformation. Details of the FRET imaging part is the same as that described in our previous publication.[4] We have used the single-molecule photon stamping approach to record the single- molecule FRET fluctuation time trajectories photon by photon for both the donor and acceptor simultaneously. The fluorescence images and photon-counting trajectories are acquired with an inverted confocal microscope (Axiovert 200, Zeiss). The excitation laser (532 nm CW, Crystal laser) confocal beam is reflected by a dichroic beam splitter (z532rdc, Chroma Technology) and focused by a high- numerical-aperture objective (1.3 NA, 100×, Zeiss) on the sample at a diffraction limited spot of ~300 nm diameter. In order to obtain the fluorescence images and intensity trajectories, the emission signal is split by using a dichroic beam splitter (640dcxr) into two color beams c entered at 570 nm and 670 nm representing Cy3 and Cy5 emissions, respectively. The signals from two channels are detected by a pair of Si avalanche photodiode single photon counting modules (SPCM-AQR-16, Perkin Elmer Optoelectronics). Typical images (10 μ m × 10 μ m) are acquired by continuously raster-scanning the sample over the laser focus with a scanning speed of 4ms/pixel, with each image of 100 pixels × 100 pixels. The fluorescence intensity trajectories of the donor (Cy 3) and accepter (Cy5) are recorded by a two-channel Picoharp 300 (PicoQuant) photon-stamping set-up. Typically, the fluorescence intensity trajectories are collected for about 100s. A permanent magnet is connected on an independent z-axis stage to control its distance to the sample glass coverslip, while X-Y direction in-plane movement is controlled by the tuning the two-layer sample stage by computer.

Reference

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