Electronic Supplementary Information

Direct observation of external force mediated conformational dynamics of IHF bound Holliday junction

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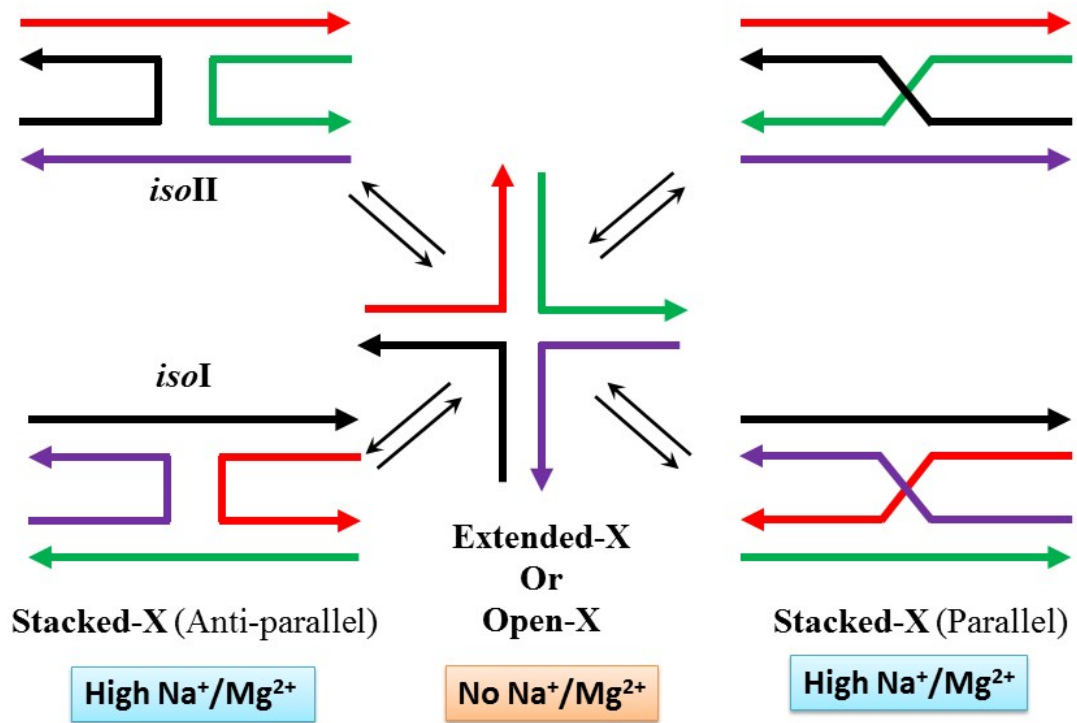[b] Plasma Physics Division, Saha Institute of Nuclear Physics, Kolkata, India, 700064

Figure S1: Schematic representation of interconversion of the Holliday junction conformations at different salt conditions.
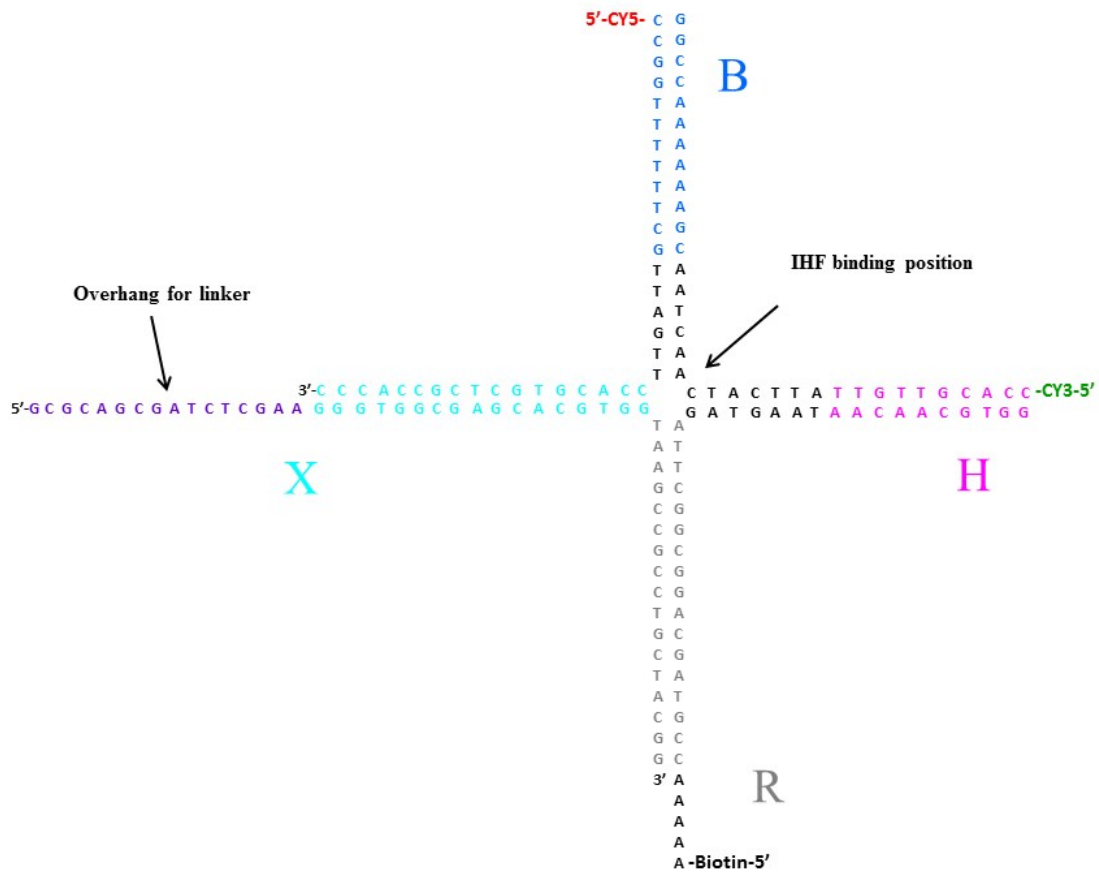
Figure S2. Structure of the 4WHJ, showing the IHF binding position (black).
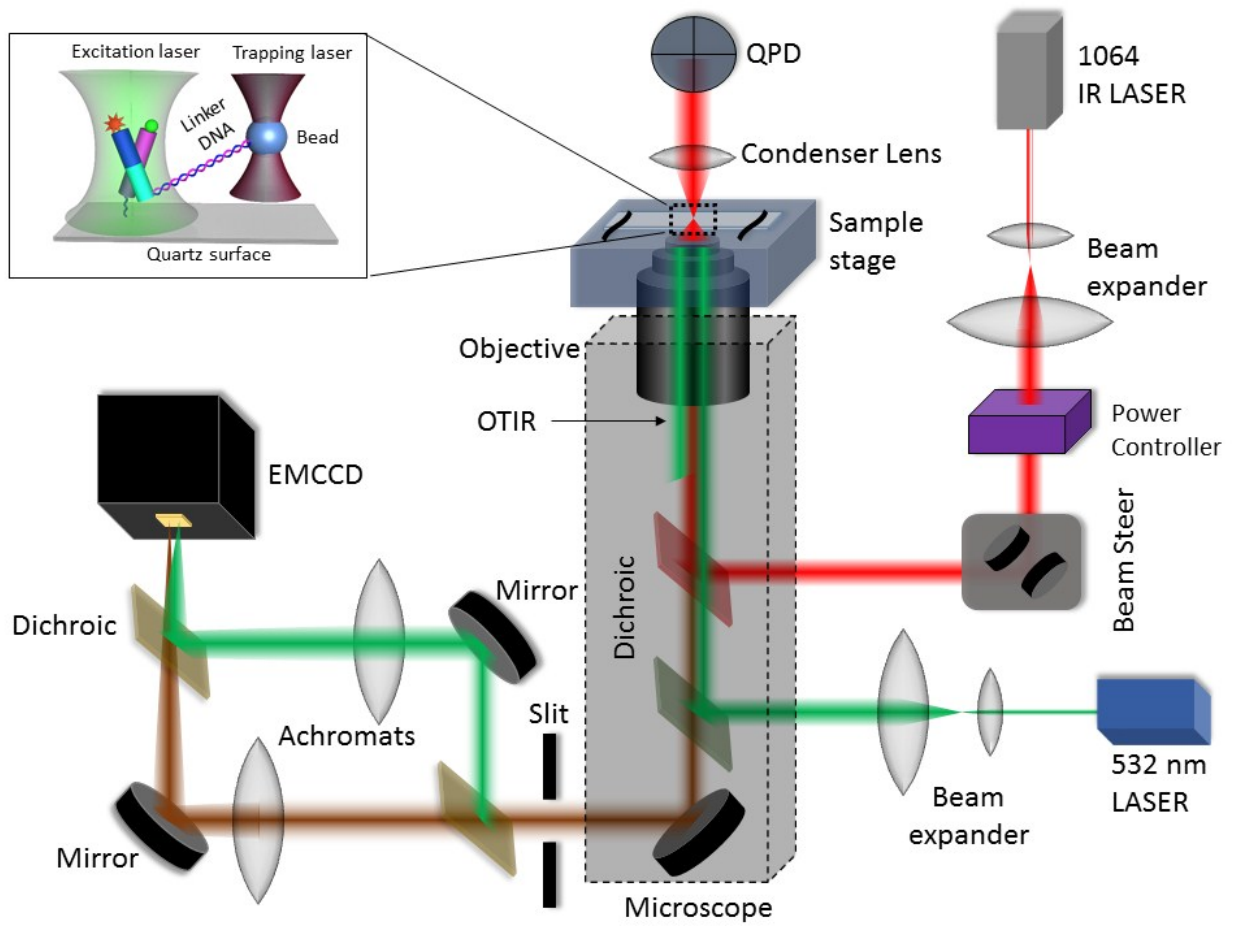
Figure S3. Schematic of smFRET-optical tweezers setup.

Theory of fluorescence polarization study of FRET acceptor

A formula to estimate a timescale of the local motion between a FRET pair based on the fluorescence polarization of a FRET acceptor is presented here. The two major approximations made in the following equations are 1), that emission dipoles of the dyes are parallel to their absorption dipoles; and 2), that anisotropy decay can be described as a single exponential decay. The first approximation would not discount the validity of internal comparison between systems with the same fluorophores. A thorough description of the anisotropy decay behavior of a charged macromolecule (e.g., DNA) in an aqueous buffer solution would require impractically laborious modeling and analyses.

Fluorescence polarization decay of a parallel aligned fluorescence emission dipole "a" can be expressed as below, considering the rotational diffusion to follow a single exponential decay with a rate constant k_{Ra}:

$$P(t) = \frac{I_{a,\parallel}(t) - I_{a,\perp}(t)}{I_{a,\parallel}(t) + I_{a,\perp}(t)} = e^{-k_{Ra}t}$$

The denominator of the equation should be substituted with $I_{a,\parallel}(t) + 2I_{a,\perp}(t)$ for anisotropy, which is a more appropriate measure of polarization in a typical fluorimeter setup.

Another assumption in the above equation is that the fluorescence decay is much slower than the rotational diffusion of the dipole. With a slowly diffusing dipole, however, this assumption may not be valid. For a fluorescence emission dipole with an emission decay rate of k_a, the probability of emitting a photon at time t (t=0 is defined as the moment of excitation) is defined by $k_a e^{-k_a t}$. Therefore, the observable fluorescence polarization at time t can be corrected as follows for a slowly diffusing dipole.

$$P(t) = k_a e^{k_a t} e^{-k_{Ra} t}$$

Integrating the equation yields the total fluorescence polarization from a dipole collected over a long period of time during which the dipole experiences many excitation/emission cycles.

$$P = \dfrac{\displaystyle\int_0^\infty e^{-kat}}{e^{-kRat}}\,dt$$

When the fluorescence is due to FRET, we need to consider the rotational diffusion of the donor and the acceptor during the energy transfer. If the donor diffusion is much slower than the acceptor diffusion, the relative rotational diffusion of the acceptor to the donor can be approximated to the absolute acceptor diffusion with a rate k_{Ra}. The fluorescence decay rate should be replaced with FRET decay rate k_f Polarized FRET from the donor to the acceptor is then as follows.

$$P_{FRET}(t') = k_f e^{-kft'} e^{-kRat'}$$

This equation gives the amount of polarized FRET at time t' some of which will be emitted polarized from the acceptor. The total observable polarized emission from the acceptor due to the polarized FRET at time t' is then as follows:

$$P_{acceptor}(t') = k_f e^{-kft'} e^{-kRat'}\,\dfrac{\displaystyle\int_0^\infty kae^{-kat}}{e^{-kRat}}\,dt$$

Integration of the equation over time t' yields the total fluorescence polarization from the acceptor during the entire FRET decay time window.

$$P = 1/2\,\dfrac{\displaystyle\int_0^\infty k_f e^{-k_f t'}}{e^{-k_{Ra}t'}}\,\dfrac{\displaystyle\int_0^\infty k_f e^{-k_a t}}{e^{-k_{Ra}t}}\,dtdt' = \dfrac{1}{2}\,\dfrac{k_f}{k_f + K_{Ra}}\cdot\dfrac{k_a}{k_f + K_{Ra}}$$

The factor of ½ is to incorporate the equation to consider the absorption by dipoles that are not perfectly parallel to the excitation polarization. Effect of FRET between imperfectly aligned donor-acceptor is ignored, as the resulting excited acceptor will be randomly aligned to the external excitation polarization and consequently can increase or decrease the acceptor emission polarization. Therefore, the resulting average polarization vs. the signal integration time should not be affected by this factor.

SmFRET experiment setup

The donor (Cy3) is exited using a 532 nm laser. The donor and accepter (Cy5) fluorescence signal are then divided into two corresponding spectral regions using a dichroic mirror (640DCXR, Chroma technology, USA). An EMCCD camera (Ixon 897, Andor Corporation, USA) is used to capture the time traces of three different polarization components. The data are captured using a Visual C++ based code and analyzed using codes written on IDL.

Nonlinear dynamics analysis

Power spectrum

This method is very useful to check whether a noisy signal possesses any periodic nature or not. To do that the noisy/fluctuating time series data is analyzed in frequency domain or vice versa. In this regards Fourier transform (FT) is a common way to do such analysis. The FT of a function $x(t)$ can be expresses as

$$\tilde{x}(f) = \frac{1}{\sqrt{N}} \sum_{n=1}^{N} x_n e^{2\pi i k n/N}$$

Where $f_k = k/(n\Delta t)$, $k = -N/2, \ldots, N/2$ and Δt is the sampling interval. A highly periodic or nearly periodic signal gives sharp peaks while measurement noise adds a continuous floor to the spectrum. In case of chaotic signal this continuous floor decay exponentially.

Lyapunov exponents

This describes the rate of convergence or divergence of nearby trajectories onto the attractor in the phase space

$$\lambda = \lim_{t \to \infty,\, d_0 \to 0} \frac{1}{t} \log \left(\frac{d_t}{d_0} \right)$$

Where d_0 and d_t are separations of initial and evolved trajectories.