Ans (a).

According to Generalized version of CFT ⇒

$$\frac{P_F(+w)}{P_R(-w)} = e^{+w} \quad \text{—— (1)}$$

w is entropy production of the driven system measured over some time interval.

$$w = -\beta \Delta F + \beta W \quad \text{—— (2)}$$

Whenever eq. (1) is valid →

$$\langle e^{-w} \rangle = \int_{-\infty}^{+\infty} P_R(-w)\, dw = 1$$

So following relation holds →

$$\langle e^{-w} \rangle = 1$$

Sustitute the value of w from eq. (2) ——

$$\langle e^{-(-\beta \Delta F + \beta W)} \rangle = 1$$

$$\langle e^{\beta \Delta F - \beta W} \rangle = 1$$

$$\because e^{a+b} = e^{a} \cdot e^{b}$$

$$\langle e^{\beta \Delta F} \cdot e^{-\beta W} \rangle = 1$$

free energy difference is a state function, and can be moved outside the average.

$$e^{\beta \Delta F} \langle e^{-\beta W} \rangle = 1$$

$$\langle e^{-\beta W} \rangle = 1/e^{\beta \Delta F}$$

$$\boxed{\langle e^{-\beta W} \rangle = e^{-\beta \Delta F}}$$

The above relation is known as Jarzynski equality.

Ans (b).

- The vertical axis of the graph PU(W) denote the probability distribution of the values of the work performed on the molecule in an infinite number of pulling experiments along the unfolding (U) process, and define PR(W) analogously for the reverse (R) process.

- The unfolding and the refolding work distributions cross at a value of the work ΔG. The significance of the line is that the work done doesn't depend on pulling speed and the distributions also satisfy the CFT and they can be fitted to guassian distributions.

- Irreversibility increases with the pulling speed and unfolding–refolding work distributions become progressively more tcyeseparated. however, that the unfolding and the refolding distributions cross at a value of the work ΔG.

Ans ©.

- The folding energy for wild type is approximately 154.1 ±0.4kBT while for mutant type it is around 157.9 ±0.2 kBT.
  So, the difference between these two is approximately 3.8 ±0.6kBT.

- the slope of the inset curve in figure 3 (upper righthand corner, small graph) being 1.06 tells us that these molecules are ideal to test the validity of CFT equation in far-from equilibrium regime.
  Following is the math behind it -

According to CFT ⇒

$$\frac{P_U(W)}{P_R(-W)} = \exp\left(\frac{W-\Delta G}{K_B T}\right)$$

in given graph of $\log\left\{\frac{P_U(W)}{P_R(-W)}\right\}$ v/s $\frac{W}{K_B T}$

slope $\simeq 1.06 \simeq 1$

$$\log\left\{\frac{P_U(W)}{P_R(-W)}\right\} = 1 \times \frac{W}{K_B T} + C$$

$$\log\left\{\frac{P_U(W)}{P_R(-W)}\right\} = \frac{W}{K_B T} + C$$

$$\frac{P_U(W)}{P_R(-W)} = \exp\left(\frac{W}{K_B T} + C\right)$$

Assuming $C = \frac{-\Delta G}{K_B T}$

$$\frac{P_U(W)}{P_R(-W)} = \exp\left(\frac{W}{K_B T} + \left(\frac{-\Delta G}{K_B T}\right)\right)$$

$$\boxed{\frac{P_U(W)}{P_R(-W)} = \exp\left(\frac{W-\Delta G}{K_B T}\right)}$$

So, overall idea is simple that slope is nearly equal to one which proves the CFT.