Caratterizzazione cinetica di biosensori di DNA in presenza di effettori allosterici tramite esperimenti di singola molecola

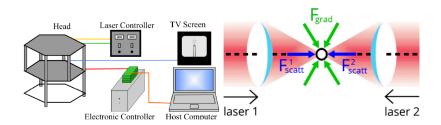
Relatrice: *Dr.ssa Annamaria Zaltron* Correlatore: *Dr. Xavier Viader Godoy*

Laureando: Riccardo Tancredi Padova, 30 settembre 2022



Optical mini-Tweezers

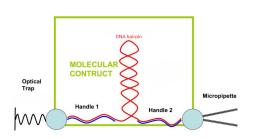


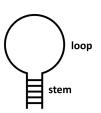


- Manipolazione di biosensori: esperimenti di singola-molecola
- Trappola ottica
- lacktriangle Duplice contributo alla forza risultante: \vec{F}_{scatt} ed \vec{F}_{grad}

Sistema biologico



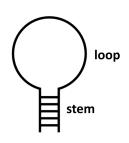


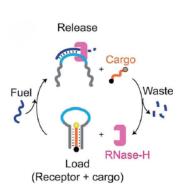


- Biglie micrometriche legate ad un hairpin di DNA
- Sequenze identiche di DNA a doppio filamento fungono da handles
- Movimentazione della trappola ottica: misure di forza e posizione

Sistema biologico





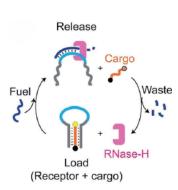


■ Studio proprietà cinetiche ed energetiche

Sistema biologico



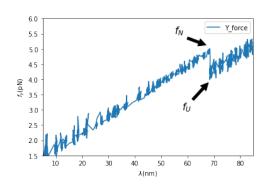




- Studio proprietà cinetiche ed energetiche
- Primo studio d'interazione con il *target*

Esperimenti di pulling

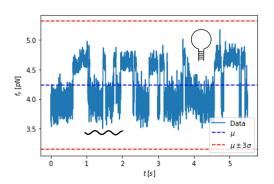




- Passaggio di configurazione come salto improvviso nella forza
- lacktriangle Due valori della forza: f_N ed f_U

Esperimenti di hopping

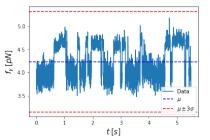


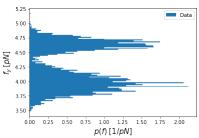


- Transizioni nel tempo tra 2 stati: native ed unfolded
- Forza di *coesistenza f_c*
- Cambiamento dei tempi di residenza totali

Fit bi-gaussiano





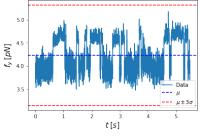


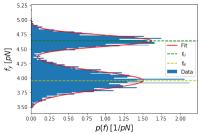
Fit bi-gaussiano



PDF gaussiana bidimensionale

$$P(f) = \frac{w_U}{\sqrt{2\pi\sigma_U^2}} \exp\left(-\frac{1}{2}\left(\frac{f - f_U}{\sigma_U}\right)^2\right) + \frac{w_N}{\sqrt{2\pi\sigma_N^2}} \exp\left(-\frac{1}{2}\left(\frac{f - f_N}{\sigma_N}\right)^2\right)$$



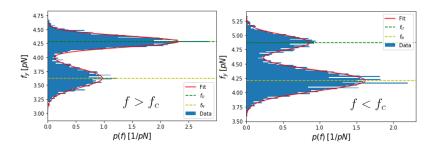


Fit bi-gaussiano



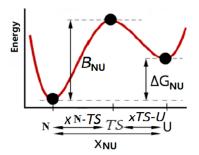
PDF gaussiana bidimensionale

$$P(f) = \frac{w_U}{\sqrt{2\pi\sigma_U^2}} \exp\left(-\frac{1}{2}\left(\frac{f - f_U}{\sigma_U}\right)^2\right) + \frac{w_N}{\sqrt{2\pi\sigma_N^2}} \exp\left(-\frac{1}{2}\left(\frac{f - f_N}{\sigma_N}\right)^2\right)$$



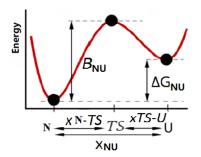


$$\blacksquare$$
 $B_{TOT} = B_{NU} - f \cdot x_{N-TS}$



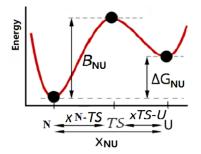


- \blacksquare $B_{TOT} = B_{NU} f \cdot x_{N-TS}$
- $k_{N \to U}(f) \equiv k^+(f) = k_m \exp[\beta(f \cdot x_{N \to TS})]$
- $k_{U\rightarrow N}(f) \equiv k^{-}(f) = k_m \exp[-\beta(f \cdot x_{TS\rightarrow U} \Delta G_{NU})]$



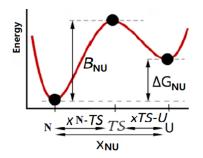


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- $k^+(f_c) \equiv k^-(f_c) \rightarrow \Delta G_{NU} = f_c \cdot x_{NU}$





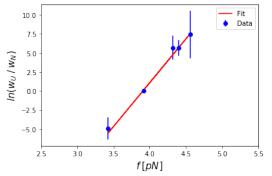
•
$$k^+(f_c) \equiv k^-(f_c) \rightarrow \Delta G_{NU} = f_c \cdot x_{NU}$$



•
$$k_B T \ln \left(\frac{w_U(f)}{w_N(f)} \right) = (f - f_c) \cdot x_{NU}$$

Fit lineare $ln(\frac{w_U}{w_N})$





$$k_B T \ln \left(\frac{w_U(f)}{w_N(f)} \right) = (f - f_c) \cdot x_{NU}$$

Forza di coesistenza

$$f_c = (4.1 \pm 0.2) \, pN$$

Estensione complessiva

$$x_{NU} = (10.6 \pm 0.4) \text{ nm}$$

Energia libera di formazione



Tramite il modello WLC:

$$\Delta G_{NU}(f) = \Delta G_0 - \int_0^f x_{ssDNA}(f')df' + \int_0^f x_d(f')df'$$

$$\Delta G_{NU}(f_c) = G_U(f_c) - G_N(f_c) = 0 \implies$$

$$\Delta G_0 = \int_0^{f_c} x_{ssDNA}(f')df' - \int_0^{f_c} x_d(f')df'$$

Energia libera di formazione

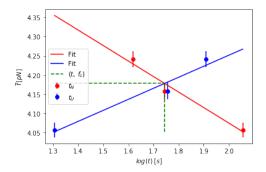
$$\Delta G_0 = (4.6 \pm 0.1) \ kcal/mol$$

Ottima compatibilità con esperimenti di pulling

Tempo di residenza totale



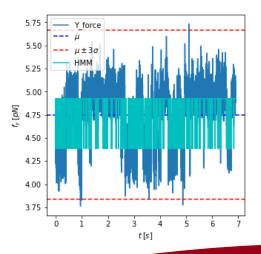
- $t^{\pm}(f) = \frac{1}{k^{\pm}(f)}$
- Metodo alternativo per ottenere informazioni cinetiche a partire dai tempi di residenza



Sequenza di stati



■ Problema: come catalogare correttamente gli stati?

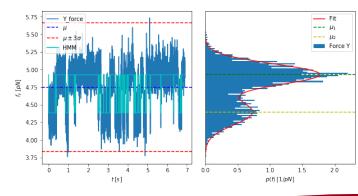


Hidden Markov Model



■ 3 algoritmi distinti per risolvere:

- evaluation problem
- uncovering problem
- *learning* problem

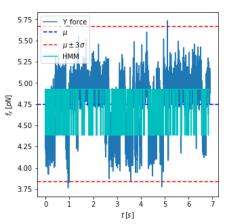


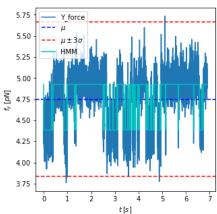
Hidden Markov Model



Hidden Markov Model



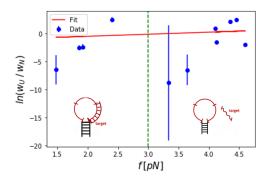




Oligo-nucleotide



- Legame tra l'hairpin e il target
- Misure di *hopping* con una concentrazione di 0.5 *nM* di *target* nel campione



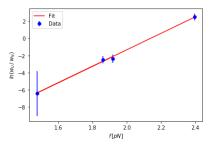
Oligo-nucleotide



Forza di coesistenza

$$f_c = (2.1 \pm 0.3) \, pN$$

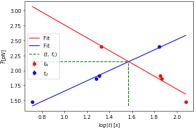
■ Legame tra l'hairpin e il target



Energia libera di formazione

$$\Delta G_0 = (1.6 \pm 0.1) \, kcal/mol$$

■ Abbassamento di f_c post-oligo



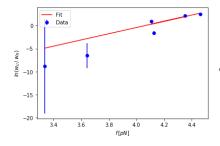
Oligo-nucleotide



Forza di coesistenza

$$f_c = (4.1 \pm 0.8) \, pN$$

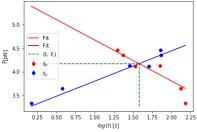
■ Legame tra l'hairpin e il target



Energia libera di formazione

$$\Delta \textit{G}_0 = (4.6 \pm 0.6) \; \textit{kcal/mol}$$

Compatibilità risultati pre-oligo



Conclusione



- Esperimenti di singola molecola su hairpin di DNA con Optical mini-Tweezers
- Misure di *hopping* e modello Bell-Evans
- $f_c = (4.1 \pm 0.2) \, pN$
- $lacktriangledown \Delta G_0 = (4.6 \pm 0.1) \ kcal/mol$
- lacktriangle Caratterizzazione dei tempi di residenza totali ightarrow HMM
- lacktriangle Legame di un oligo nucleotide: abbassamento di f_c



Grazie dell'attenzione



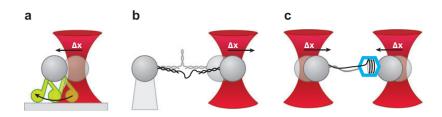


Figure: Geometrie d'intrappolamento ottico possibili



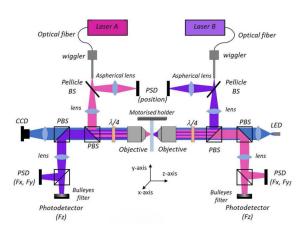


Figure: Schema del percorso ottico



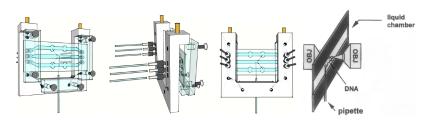


Figure: Montaggio della cameretta e posizionamento della camera tra i due obiettivi



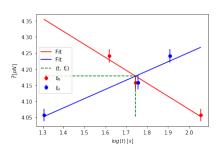
■
$$ln t_U(f) = m_1 f + q_1$$
, con $q_1 = -ln k_m$ ed $m_1 = -\beta \cdot x_{N \to TS}$

■
$$ln t_N(f) = m_2 f + q_2$$
, con $q_2 = q_1 - \beta \Delta G_{NU}$ ed $m_2 = \beta \cdot x_{TS \to U}$

Eseguendo un ulteriore fit lineare si può poi trovare $f_{\rm C}=rac{q_1m_2-q_2m_1}{m_2-m_1}$



■ Confrontando w_N e w_U :



■ Hidden Markov Model:

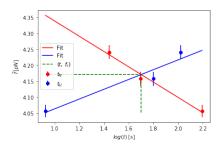




Table: Stime di f_c attraverso w_N , w_U

N° molecola	$f_c \pm \sigma_{f_c} [pN]$	$x_{NU} \pm \sigma_{x_{NU}} [nm]$	$\Delta G_{NU} \pm \sigma_{\Delta G_{NU}} [pN \cdot nm]$	$\Delta G_0 \pm \sigma_{\Delta G_0} [pN \cdot nm]$
2	4.3 ± 0.5	10.5 ± 0.9	45 ± 34	35 ± 3
2 (cp)	3 ± 1	6 ± 2	20 ± 5	24 ± 6
5 (cp)	3.9 ± 0.7	12 ± 2	45 ± 6	30 ± 4
6	4.4 ± 0.9	9 ± 1	39 ± 6	36 ± 6
6 (cp)	4 ± 2	15 ± 4	60 ± 18	33 ± 9
14	5 ± 3	14 ± 6	70 ± 30	43 ± 16
14 (cp)	5 ± 1	23 ± 4	110 ± 20	41 ± 6
15	4.0 ± 0.7	11 ± 1	42 ± 5	31 ± 3
18	3.9 ± 0.6	13 ± 1	49 ± 5	30 ± 3
19	4.2 ± 0.5	5.5 ± 0.5	23 ± 2	33 ± 3
19 (cp)	4.2 ± 0.5	9.5 ± 0.8	39 ± 3	33 ± 3
20	3.5 ± 0.7	8 ± 1	30 ± 5	25 ± 4
20 (cp)	4 ± 1	12 ± 2	41 ± 9	25 ± 5
21	4.3 ± 0.6	3.2 ± 0.3	14 ± 2	35 ± 3
22	4.5 ± 0.6	7.5 ± 0.7	34 ± 3	37 ± 3
22 (cp)	4 ± 3	13 ± 6	60 ± 30	36 ± 15



Table: Stime di f_c attraverso t_c

N° molecola	$f_c \pm \sigma_{f_c} [pN]$	$t_c \pm \sigma_{t_c} [s]$	$\Delta G_{NU} \pm \sigma_{\Delta G_{NU}} [pN \cdot nm]$	r_{f_c}	$r_{\Delta G_{NU}}$
2	4.306 ± 0.008	5.06 ± 0.07	45 ± 4	0.0	0.0
2 (cp)	3.48 ± 0.01	4.73 ± 0.05	21 ± 5	0.5	0.1
5 (cp)	3.90 ± 0.01	4.83 ± 0.07	45 ± 6	0.0	0.0
6	4.44 ± 0.07	5.7 ± 0.4	39 ± 6	0.0	0.0
6 (cp)	4.21 ± 0.08	5.6 ± 0.9	60 ± 20	0.1	0.0
14	4.918 ± 0.009	2.29 ± 0.03	70 ± 30	0.0	0.0
14 (cp)	4.78 ± 0.02	3.9 ± 0.2	110 ± 20	0.2	0.0
15	4.01 ± 0.01	$\textbf{4.14} \pm \textbf{0.05}$	43 ± 5	0.0	0.0
18	3.914 ± 0.006	3.03 ± 0.03	49 ± 5	0.0	0.0
19	4.153 ± 0.005	3.64 ± 0.03	23 ± 2	0.1	0.0
19 (cp)	4.185 ± 0.009	3.61 ± 0.06	40 ± 4	0.0	0.0
20	3.587 ± 0.009	3.25 ± 0.04	30 ± 5	0.1	0.0
20 (cp)	3.56 ± 0.01	4.05 ± 0.05	41 ± 8	0.4	0.0
21	4.37 ± 0.01	3.08 ± 0.01	14 ± 1	0.1	0.0
22	4.446 ± 0.005	2.47 ± 0.02	33 ± 3	0.1	0.2
22 (cp)	4.50 ± 0.03	3.1 ± 0.2	60 ± 30	0.2	0.0