10 jan 2019 70887 0.02V/λ × 50λ
MITOMI fitting. 0.5 1V
from Evan's email. 100

$[DNA * Pho4] = B_{max} \times \frac{[DNA_f]}{[DNA_f] + K_d}$ Fractional binding $= B_{max} \frac{[DNA_F]}{[DNA_f] + K_d}$

from my own calc_kd.R

① Read in protein raw F530.median → protein

② Read in DNA.bound.raw F685.median → DNA

③ Read in DNA free

④ Remove background.

⑤ Remove ~~unbound~~ fit correlation between protein channel and DNA6.

from an unnamed book5 ch 13.

P = TF protein. S = DNA Site

$PS \overset{K_d}{\rightleftharpoons} P + S \quad K_d = \frac{[P][S]}{[PS]}$

$[PS] = \frac{[P]\cdot[S]}{K_d} \quad [S]_t = [S] + [PS] = [S] + \frac{[P][S]}{K_d} = \frac{[S]K_d + [P][S]}{K_d}$

$\frac{[PS]}{[B]} = \frac{[P]}{K_d} \quad = \frac{K_d \cdot [S] + [P]\cdot[S]}{K_d}$

// I think we are operating in a situation of ~~TF~~ "one-component saturating" specifically, we provide sufficient amount of TF s.t. $[P] \gg K_d$.

11 jan 2019

Steps to perform MITOMI

1. ITT flow into the chip w/ all valves open except for outlet. Filling, especially the chamber.
2. Chamber valves closed (protect DNA), keep flushing.
3. Sandwich valves closed, chamber & button open. this is the reaction vessel!
4. $P + S \rightleftharpoons PS \quad K_d = \frac{[P]_f[S]_f}{[PS]}$
5. After 90 min, scan 1. → total DNA conc. $[S]_t$ // measured as median of pixel intensity in the chamber? unit $\frac{F685.median}{Pixel}$.

note: ~~the~~ concept of "concentration" here refers to total fluorescent intensity

pixel Σ Cy5 / 1 pixel area.

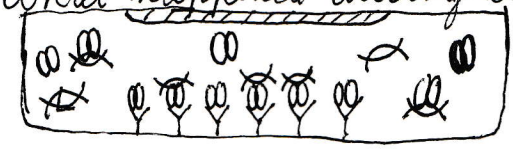
~molecule ~volume

6. button membrane closed.

11 jan 2019 MITOMI.

Continue

What happened during the incubation period.



Y surface bound α-His ab.

TF protein

DNA - Cy5.

* surface = on button membrane

Free DNA $[S_f]$

Free protein $[P_f]$ = ~~bound~~ surface bound + ~~not bound~~ in solution.

DNA in complex protein $[PS]$ = ~~bound~~ surface bound + in solution.

Assume $[PS]$ and $[P_f]$ has the same distribution between surface & solution, then one can use $\frac{[PS]_{surface}}{[P_f]_{surface}}$ as a proxy for $\frac{[PS]_{all}}{[P_f]_{all}}$.

5. after membrane is closed. ~~keep~~ open sandwich valve, close chamber valve flush

scan 2 → surface bound total protein → $[PS]_{surface} + [P_f]_{surface}$ ↳ in protein units

DNA bound to protein that is bound to surface → $[PS]_{surface}$ ↳ in DNA units

// note that $[PS] + [P_f] = [P]_{total}$ is measured in F530. protein channel

$[PS]$ → ∵ F685 (Cy5) DNA channel

311-621-5729. David Soll.

What is measured.

$$\frac{[PS]_{surface}}{[PS]_{surface} + [P_f]_{surface}} = 1 \rightarrow \text{fractional occupancy FO}$$

$[P]_{total}$ under button in F530 protein channel

notice that the ratio is not ~~unitle~~ unitless due ~~to~~ to the two different fluorofore being used to estimate $[PS]$ and $[P]_t$.

See Bin He notes on 5th mai 2009 (paper)

$[P]_t \cdot C_p$, $[S]_t \cdot C_s$, $[PS] \cdot C_s$

$$y = \frac{[PS] \cdot C_s}{[P]_t \cdot C_p} = \frac{C_s}{C_p} \frac{[PS]}{[P]_t} = \frac{C_s}{C_p} \frac{[S]_f \cdot C_s}{C_s K_d + [S]_f \cdot C_s}$$

$$K_d = \frac{[P_f][S_f]}{[PS]}$$ all in nM units

$$FO = \frac{[PS]}{[P_f] + [PS]} = \frac{[PS]}{\frac{K_d[PS]}{[S_f]} + [PS]} = \frac{[S_f]}{K_d + [S_f]}$$

During fitting, however, $[S]_t$ is used in place of $[S]_f$.

→ what's desired? $[S]_f$

what's used/measured? $[S]_t \cdot C_s$.

Can we use $[S]_t$ to approximate $[S]_f$?

12 Jan 2019.

* Various approximations

(See also my paper notebook on 5 mai 2009).

☐ organize reading list for Zoheb / Thomas

1. For strong (eg. consensus site), we should be able to reach

0.1 ~ 0.01 μm. 100 ~ 10 nM.

if Kd for CACGTG is ~ 10 nM, and the spotted DNA is ? (can't use the [DNA] in spotting to detect, as volume changes).

Scl hot → CACGTG. Kd ~ 11.1 nM (Maerkl & Quake 2007)

for consensus site, we will reach the plateau. that is, $[S]_t$ will be greater than Kd ($[S]_t \gg Kd$), such that the protein is saturated!

$$\frac{[PS]}{[P]_t} \to 1 \quad \text{or} \quad \frac{C_S \cdot [PS]}{C_P \cdot [P]_t} \to \frac{C_S}{C_P} = B_{max}$$

[PS]

at lower $[S]_t$, ($[S]_t < Kd$), if $[P]_t \ll Kd$, then $\frac{[PS]C_S}{[P]_t C_P} = B_{max} \frac{[S]_f \cdot C_S}{Kd + [S]_f \cdot C_S}$ *

* $\approx B_{max} \frac{[S]_f \cdot C_S}{Kd}$ (linear) in this case.

Kd \ DNA $[S]_t$	low	high
low (consensus)	Fractional Occupancy is low (in linear range) assume $[P] \ll Kd_{(consensus)}$ $\frac{[PS]}{[S]_t} = \frac{[P]_f}{Kd + [P]_f} \approx \frac{[P]_f}{Kd} \ll 1$ $[S]_f \approx [S]_t$.	If we assume $[P] \ll Kd_{(consensus)}$ then regardless of $[S_t]$. $\frac{[PS]}{[S]_t} = \frac{[P_\beta]_f}{Kd + [P]_f} \approx \frac{[P]_f}{Kd} \ll 1$ $\Rightarrow [S]_f \approx [S]_t$.
high (weak sites)		

Table. 分情况讨论. Can $[S]_t \approx [S]_f$.

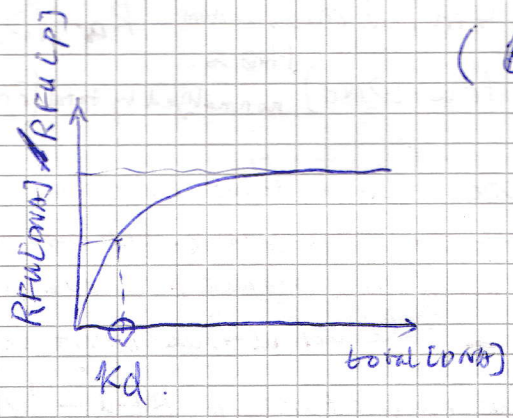
* Protein-Ligand binding affinity [WIKI - Kd. affinity]

$C \rightleftharpoons P + L$

$K_d = \frac{[P][L]}{[PL]}$

Kd has molar units (M), which corresponds to the [conc] of Ligand [L] at which the binding site on a particular protein is half occupied.

($[L] = K_d \Rightarrow \frac{[P]}{[P]_0} = 1$.)



Saturation : $[\text{Protein:Ligand complex}] = \frac{B_{max}[\text{Ligand}]}{K_d + [\text{Ligand}]}$

where B_{max} is the maximum

[Theory III - Finale!)

What I measure

$S_1 [D]_{free} \cdot C_{CONA}$ — before wash, outside the Button.

$S_2 [PD] \cdot C_{CONA}$ — after wash.

$S_2 [P]_t \cdot C_P$ — after wash

where S_1, S_2 are the size of the detection area.

C_{CONA} is the constant that translates $\frac{[D]}{RFU}$ to RFU. determined by the standard curve

VAB.

$$K_D = \frac{[P][D]}{[PD]} \Rightarrow [PD]K_D = [P][D] = ([P]_t - [PD])[D]$$

$$\Rightarrow [PD](K_D + [D]) = [P]_t \cdot [D]$$

$$\Rightarrow \frac{[PD]}{[P]_t} = \frac{[D]}{K_D + [D]} \qquad Y = B_{max}\frac{X}{X + K_D} \qquad \frac{1}{Y} = \frac{1}{B_{max}}\left(1 + \frac{1}{X}K_D\right)$$

$$\frac{S_2[PD] \cdot C_{DNA}}{S_2[P]_t \cdot C_p} \cdot \frac{C_p}{C_{DNA}} = \frac{C_{DNA}}{C_p}\frac{[D]}{K_D + [D]}$$

Y ; $\frac{C_{DNA}}{C_p} \rightarrow B_{max}$.

Standard RFU curve

~~X~~ = RFU = 28.147 [DNA] (nM).

or $[DNA]_{(nM)} = .0.0355\,RFU$

Let

$$\frac{1}{Y} = \frac{1}{B_{max}} \cdot \frac{1}{X} \cdot K_D + \frac{1}{B_{max}}$$

Let $y = \frac{1}{Y}$ $x = \frac{1}{X}$.

$y = 470.39x + 0.841$ $R^2 = 0.9336$ → for HB consensus

$B_{max} = 1.189$

$K_D = 559.322$ (RFU) ≅ 19.85 nM

For hb 18:

$y = 2797x + 11.961$

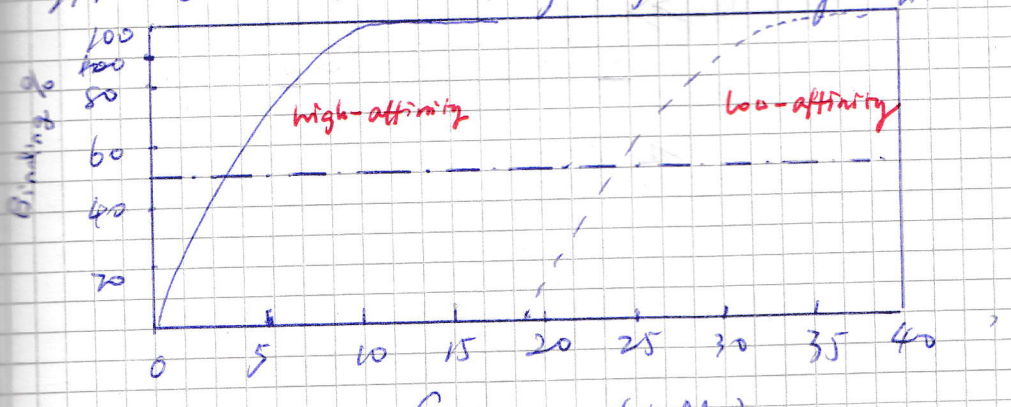
$B_{max} = 0.0836\ldots$

If use consensus B_{max}

$K_D = 2339.186 \times 0.0355$

$= 83.041$ nM.

11. What's the range of binding affinities



Another hb seq: $K_D = 4807.2$ ≅ 170.656 nM

Get pretty low binding for bcd.