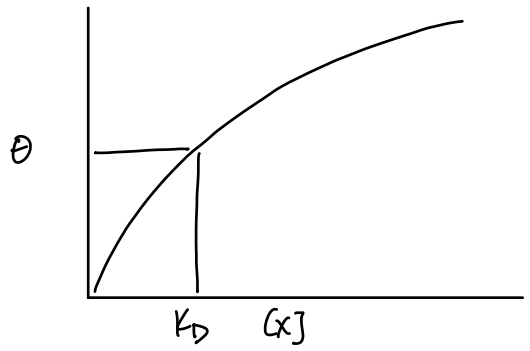
BINDING METHODS:



$$\theta = \frac{MX}{M+MX}$$

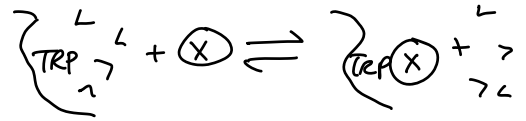
1) CHOOSE $[M]_T \ll K_D$. THIS MEANS $[X]_{TOTAL} \sim [X]$ BECAUSE (MX) IS SMALL.

2) FIND SIGNAL PROPORTIONAL TO θ

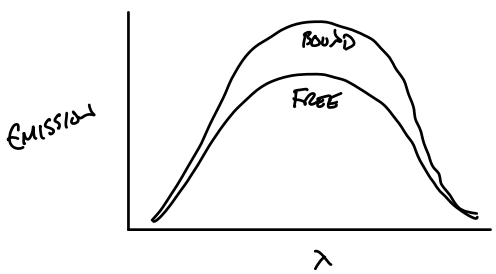
3) HOLD CONCENTRATION OF WHAT YOU MEASURE CONSTANT

1) PROTEIN SPECTROSCOPY

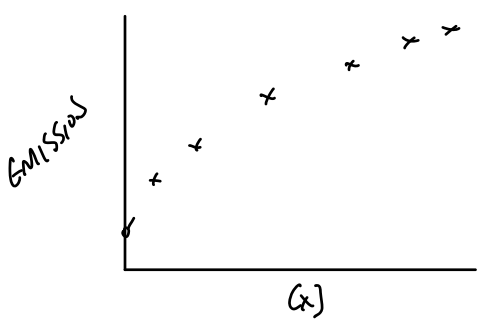
EXAMPLE:



TRP FLUORESCENCE IS SENSITIVE TO ENVIRONMENT.



FLUOROPHORE IS BRIGHT → DON'T NEED HIGH [M]



1) HOLD [M] CONSTANT AND LOW (10 nM)

2) ADD INCREASING [X]

3) TRANSFORM TO GET θ

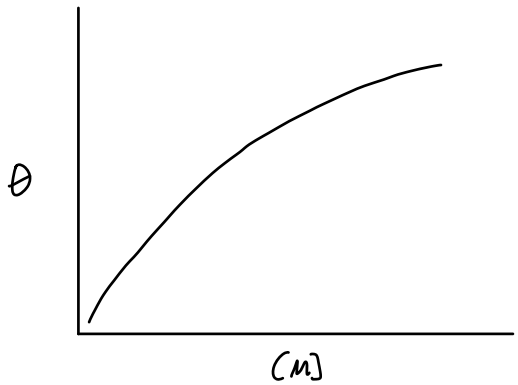
$$\frac{SIGNAL_X - SIGNAL_{SATURATING}}{SIGNAL_{SATURATING} - SIGNAL_\varnothing}$$

4) FIT BINDING MODEL TO DATA:

$$\theta \sim \frac{K_D[X]}{1 + K_D[X]}$$

CAN USE OTHER SPECTROSCOPIES: ABSORBANCE
CIRCULAR DICHROISM
NMR...

2) LIGAND FLUORESCENCE



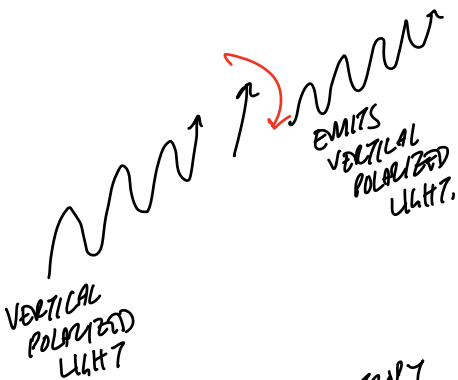
$$\theta = \frac{MX}{X + MX}$$

→ KEEP X CONC < K_D AND CONSTANT.

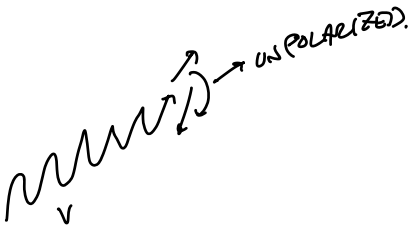
TENDS TO BE EXPENSIVE IN PROTEIN.

3) WHAT IF YOU DON'T WANT TO FIND SIGNAL THAT CHANGES ON BINDING?

FLUORESCENCE ANISOTROPY.



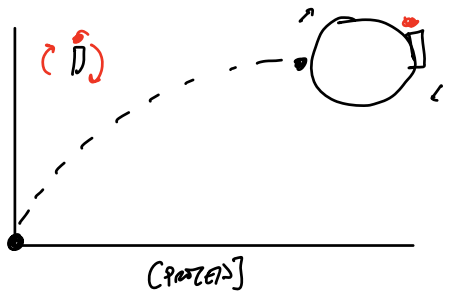
IF TUMBLES FASTER THAN FLUORESCENCE, SMEARS OUT POLARIZATION.

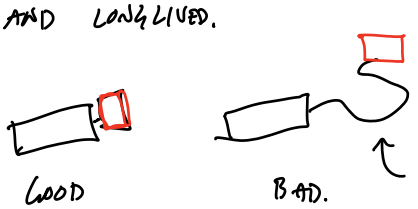


- ANISOTROPY

SHINE VERTICAL POLARIZED, MEASURE V AND H @ 90°.

1) KEEP LABELED BIT CONSTANT
2) TITRATE IN LARGER BIT.
3) MAKE SURE FLUOROPHORE IS RIGID AND LONG LIVED.

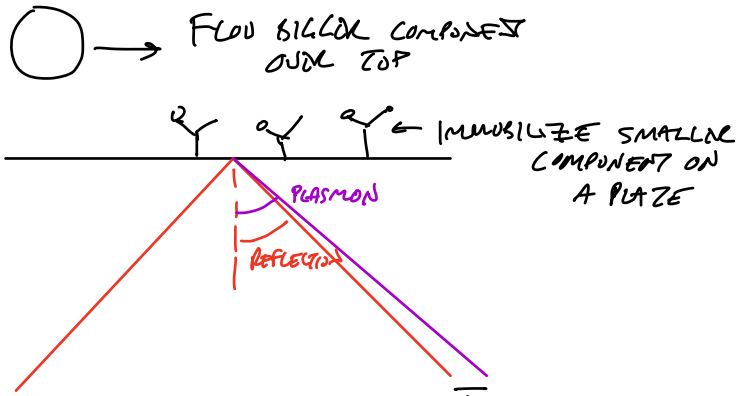




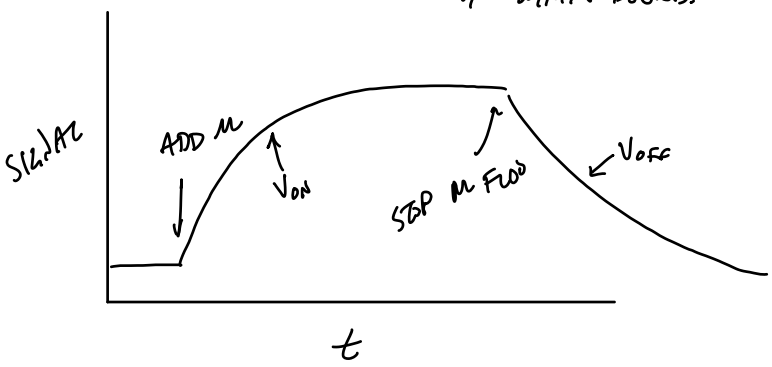
ANISOTROPY DOES NOT CHANGE ON BINDING!

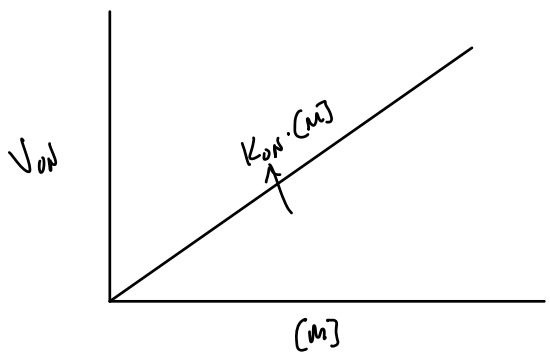
4) CHIP BASED METHODS

SURFACE PLASMON RESONANCE
BIOLAYER INTERFEROMETRY



- CHIP W/ MOLECULE
- FLOW CELL MOVES MOLECULES OVER CELL.





$$K_D = \frac{k_{off}}{k_{on}}$$

5) FRET (FÖRSTER RESONANCE ENERGY TRANSFER)

$$E = \frac{1}{1 + (r/R_0)^6}$$