MOTIVATION

- TB DRUG RESISTANCE (DETECT DNA, USE PCR, ON PAPER, SNPs, ..)
- CANCER CLASSIFICATION BASED ON MIRNA (ssRNA, qPCR, COMPUTATIONAL, ..
- FLU VIRUS IN CELLS (A.RAJ) (ssRNA, MICROSCOPY, ..

COMMON TO ALL : MULTIPLE INPUTS, HIGH SPECIFICITY REQUIRED, COMPLEX EXPERIMENTAL + ANALYSIS WORKFLOW.

BETTER ALTERNATIVE? MOLECULAR DIAGNOSTICS

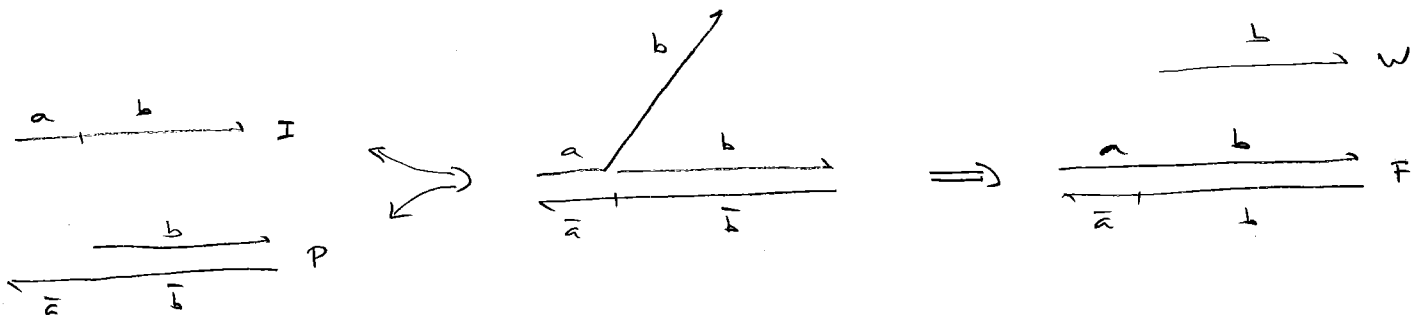
PATIENT SAMPLE

DIAGNOSTIC "CIRCUIT"

⇒ EASY TO INTERPRET DIAGNOSIS

APPROACH : DNA STRAND DISPLACEMENT

- ssDNA
- SD ANIMATION
- MODEL FOR SD : 2-STEP VS. 1-STEP (PRE-EQUILIBRIUM ANALYSIS)



$$I + P \underset{k_-}{\overset{k_+}{\rightleftharpoons}} C \xrightarrow{k_2} F + W$$

$$\frac{d[F]}{dt} = +k_2[C]$$

$$\frac{d[C]}{dt} = k_+[I][P] - (k_- + k_2)[C] \doteq 0$$

$$[C] = \frac{k_+}{k_- + k_2}[I][G]$$

$$\frac{d[P]}{dt} = \underbrace{\frac{k_2 k_+}{k_- + k_2}}_{k}[I][G] = k[I][G] \qquad (\sim \text{BIMOLECULAR})$$

STRONG TOEHOLD : $k_2 > k_-$

$$k = \frac{k_2 k_+}{k_- + k_2} \sim k_+ \Rightarrow \text{FIT DATA TO FIND } k_+!$$

(DIFFERENT FOR DIFFERENT TOEHOLDS!)

WEAK TOEHOLD: $k_2 < k_-$

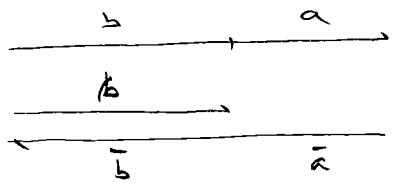
$$k \approx \frac{k_2 k_+}{k_-} = k_2 C_0 \cdot e^{-|\Delta G|/RT}$$

[CHEMICAL EQ. $K_{eq} = \frac{k_+}{k_-} = \frac{[I][G]}{[C]} = e^{\Delta G/RT}$]

ΔG IS THE FREE ENERGY OF TOEHOLD BINDING, CAN BE CALCULATED FROM FIRST PRINCIPLES.

TREAT k_2 AS A FITTING PARAMETER.

⇒ IN CLASS WORKING SESSION: ① CALCULATE ΔG FOR DIFFERENT TOEHOLD LENGTH



n = 1	-1.52
2	-3.36
3	-4.79
4	-6.74
5	-8.1
6	

b : 5' CCTC CATT CAAT ACCC TACG

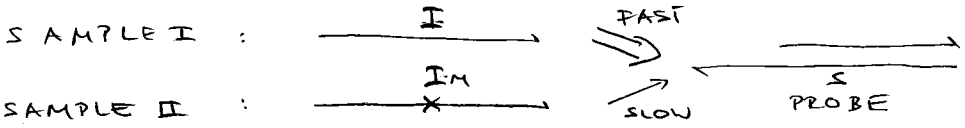
a : 5' CCACAT

② PLOT $\log_{10} k$ VS. ΔG, COMPARE TO DATA

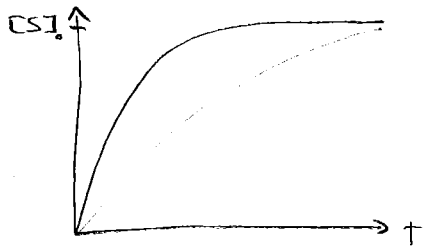
③ CONSIDER MUTATIONS CC A C A T (G G)

SNV DISCRIMINATION

WE ALREADY KNOW THAT MUTATIONS AFFECT KINETICS.
COULD THIS BE USED TO DETECT SNPs?



$[P]_0 >> [S]_0, [S_M]_0$

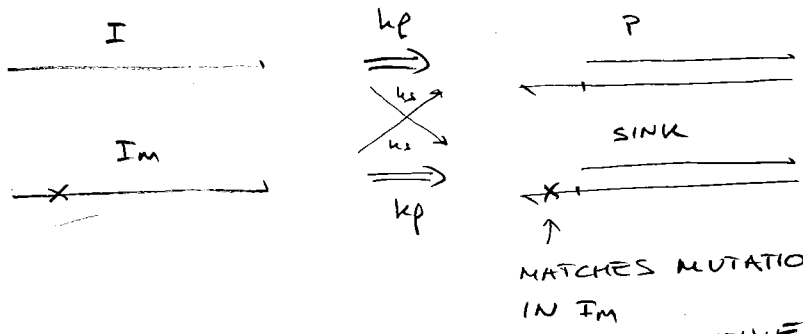


$I + P \xrightarrow{k_p} F + W$

$I_M + P \xrightarrow{k_s} F + W$

BUT
- NO CONTROL OVER CONCENTRATION
- NO DISCRIMINATION AT END POINT

USE COMPETITION



IDEAL PROBES ARE SPECIFIC + SENSITIVE

DISCRIMINATION FACTOR $DF = \frac{F}{F_M} = \frac{\text{SIGNAL WITH INPUT I}}{\text{SIGNAL WITH INPUT } I_M}$ [SAME CONC., ONLY ONE IS PRESENT]

DF IS A MEASURE OF PROBE SPECIFICITY.

YIELD $Y = \frac{[F]}{[I]_0} = \frac{\text{SIGNAL}}{\text{INPUT CONCENTRATION}}$

SINGLE SD PROPE

$DF = 1$, $\chi = 1$: NO SPE

$$\dot{[F]} = k_f [I][P] \overset{[P] \gg [I]}{\cong} k_f [P]_0 [I] = -k_f \frac{d[I]}{dt}$$

$$[I](t) = [I_0] e^{-k_f [P]_0 t} \Rightarrow [F(t)] = [I_0]\left(1 - e^{-k_f [P]_0 t}\right)$$

$t \to \infty : [F]_\infty = [I_0]$

SAME FOR $[I_m]$, JUST SLOWER

$$DF_\infty = \frac{[I]_0}{[I_m]_0} = 1 \quad : \text{NOT SPECIFIC AT END POINT}$$

$$\chi = \frac{[F]}{[I_m]_0} = \frac{[I]_0}{[I]_0} = 1 \quad : \text{NOT (VERY) SENSITIVE}$$

COMPETITION :

$$I + P \xrightarrow{k_f} F + W_1$$
$$I + S \xrightarrow{k_s} W_2$$

$$\frac{d[F]}{dt} = k_f [I][P]_0$$

$$\frac{d[I]}{dt} \cong -k_f [I][P]_0 - k_s [I][S]_0 = -[I]\underbrace{\left(k_f [P]_0 + k_s [S]_0\right)}_{\Gamma}$$

$$[I](t) = [I]_0 e^{-\Gamma t}$$

$$\frac{d[F]}{dt} = k_f [I_0][P]_0 e^{-\Gamma t}$$

$$F(t) = C_0 - \frac{k_f [P_0]}{k_f [P]_0 + k_s [S]_0} [I_0] e^{-\Gamma t}$$

$[F(0)] = 0$

$$= [I]_0 \frac{k_f [P_0]}{k_f [P]_0 + k_s [S_0]}\left(1 - e^{-\Gamma t}\right)$$

$$[F]_\infty = [I]_0 \frac{k_f [P]_0}{k_f [P]_0 + k_s [S]_0} \xrightarrow{[P]_0 = [S]_0} = [I]_0 \frac{k_f}{k_f + k_s}$$

RXN W/ I_M:

$$[F_M]_\infty = [I_M]_0 \frac{k_s [P]_0}{k_s [P]_0 + k_f [S_M]_0} \xrightarrow{[P]_0 = [S]_0} = [I]_0 \frac{k_s}{k_f + k_s}$$

$$DF_\infty = \frac{[F]_\infty}{[F_M]_\infty} = \frac{k_f}{k_s}$$

Q1: HOW BIG IS THAT NBR?

Q2: WHAT IS MUTATION IS NOT IN THE TOEHOLD?

$$x_\infty = \frac{[F]_\infty}{[I]_0} = \frac{k_f}{k_f + k_s} < 1$$

CAN WE INCREASE THE YIELD BY MAKING THE INTENDED REACTION CATALYTIC? LET'S TRY:

$$I + P \xrightarrow{k_f} I + F + W_1$$
$$I + S \xrightarrow{k_s} W_2$$

STILL ASSUME $[P]_0 = [S]_0 >> [I]$

RXNS CAN BE SOLVED ANALYTICALLY BUT HERE WE USE INTUITIVE ARGUMENT INSTEAD

$x = \frac{k_f}{k_f + k_s}$: PROB. OF SUCCESSFUL RXN IN ONE ROUND

$$[F] = [I_0] \left(x + x^2 + x^3 + \ldots \right) = \frac{x}{1-x} [I]_0$$

$$x_\infty = \frac{[F]_\infty}{[I]_0} = \frac{x}{1-x} = \frac{k_f}{k_f + k_s} \cdot \frac{k_f + k_s}{k_s} = \frac{k_f}{k_s} > 1$$

DISCRIMINATION FACTOR

HOW ABOUT I_M:

PROB OF 1ST ROUND SUCCESS: $y = \frac{k_s}{k_f + k_s} = 1 - x$

$$[F_M]_\infty = [I]_0 \left(y + y^2 + y^3 + \dots \right) = \frac{y}{1-y} = \frac{1-x}{x}$$

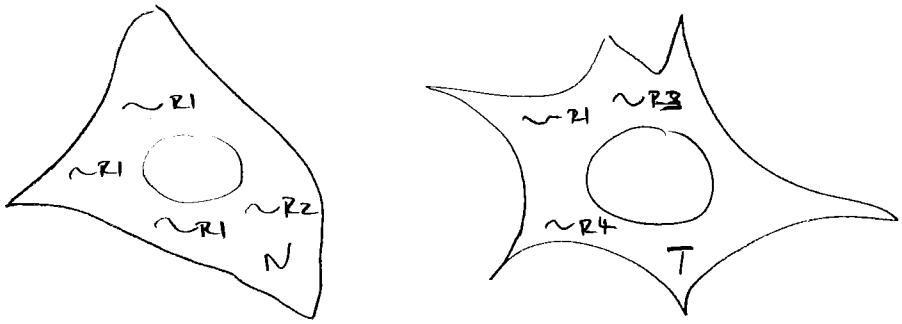
$$\underline{DF} = \frac{[F]_\infty}{[F_M]_\infty} = \left(\frac{x}{1-x} \right)^2 = \underline{(k_f/k_s)^2}$$

$\Rightarrow$ QUADRATICALLY BETTER THAN FOR COMPETITION ALONE!

HOW CAN WE MAKE A CATALYST? $\Rightarrow$ DNA STRAND DISPLACEMENT CASCADES AND SEESAW AMPLIFIERS

MULTI-ANALYTE DETECTION

GOAL: BUILD MOLECULAR CLASSIFIER

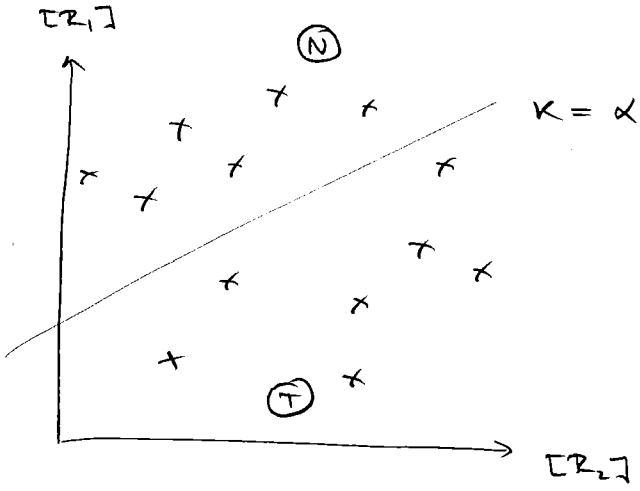


BOOLEAN CLASSIFIER:

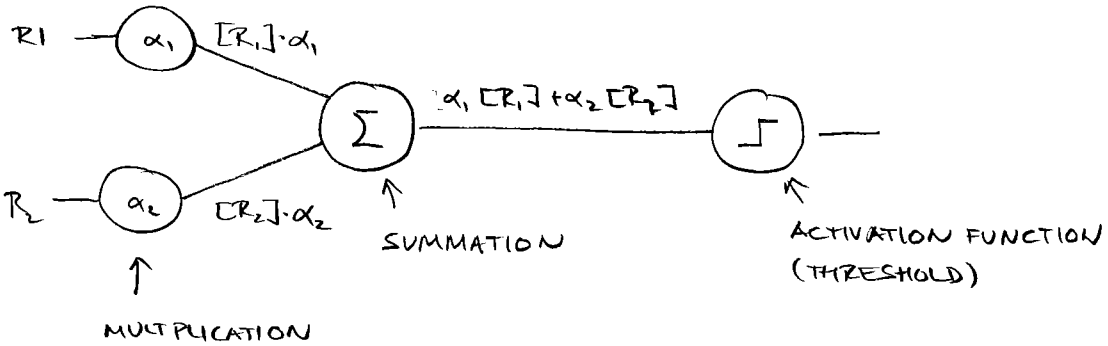
N: R_1 AND R_2

T: R_3 OR R_4

MORE COMMON: LOW/HIGH RATHER THAN ABSENCE/PRESENCE



ANOTHER WAY TO REPRESENT THIS



CONSIDER MULTIPLICATION FIRST.

LET'S REVISIT COMPETITIVE INHIBITION!

BUT THIS TIME ASSUME $k_P = k_S = k$, $[P]_0 \neq [S]_0$, $[P]_0, [S]_0 \gg [I]_0$

$$I + P \xrightarrow{k} F + W_1 + I \quad \text{(CATALYTIC)}$$

$$I + S \xrightarrow{k} W_2$$

PROB. OF SUCCESS FOR ONE ROUND:

$$x = \frac{k[P]_0}{k[P]_0 + k[S]_0} = \frac{[P]_0}{[P]_0 + [S]_0}$$

TOTAL FLUORESCENCE AFTER MANY ROUNDS:

$$\underline{[F]} = [I]_0 \left(x + x^2 + \ldots \right) = [I]_0 \frac{x}{1-x} = [I]_0 \underbrace{\frac{[P]_0}{[S]_0}}_{\alpha} = \underline{\alpha [I]_0}$$

MULTIPLICATION W/ ARBITRARY α CAN BE REALIZED BY SETTING THE INITIAL CONCENTRATIONS OF $[P]_0$, $[S]_0$ ACCORDINGLY!