

Methods

Initial examples assume start $R = A_0 + B_0$ etc., $P = 0$
follow reaction forward → initial rate, etc. $[A]$ vs. t
 Could mix and take aliquots, quench reaction (T, pH, other perturb. . .), then use chemical analysis
Problem slow, requires a lot of material

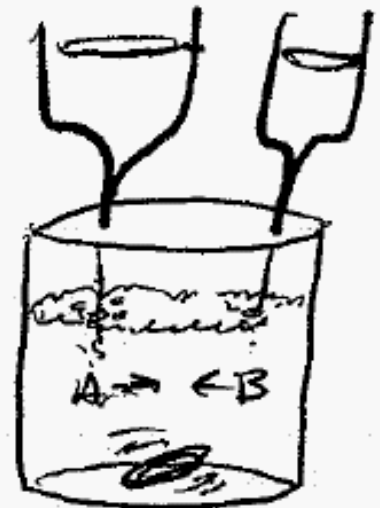
Bio models, chemical methods not work so well,
 change often is not chemically very different
 (folding, enzymology, ligand or membrane binding, . . .)
 Amounts are often very limited

Physical Methods: Monitor Absorbance, fluorescence, pH, pressure, electrochemistry, ...
 whatever is proportional to concentration

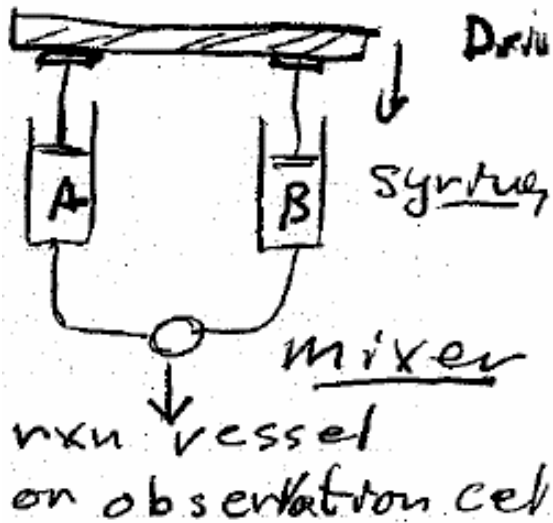
Mixing limitations:

Mix reagents in a beaker and stir
 OK for slow reaction

Slow reaction → pour together and stir
 takes time ⇒ k_2 small (seconds)



Fast reaction need - mix fast



Faster – mix in small area
rapid mixing – small mixer /
 fast flow



Stop flow

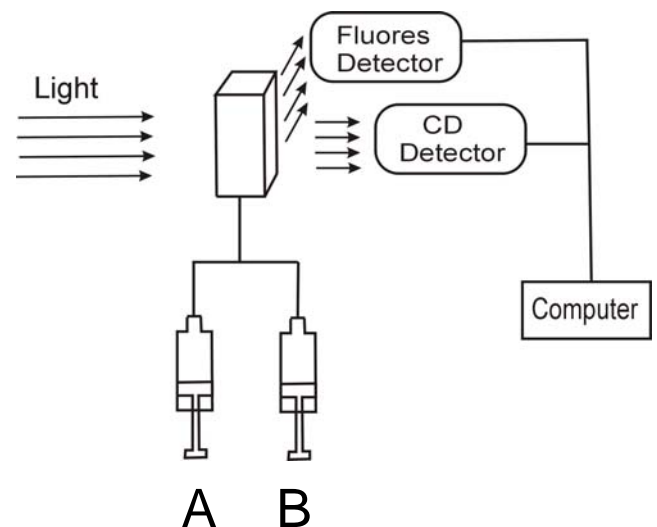
- fill cell quickly from mixer
- then stop $\Rightarrow t = 0$

Monitor absorbance, fluorescence on other properties
 proportional concentration

Stop-Flow methods

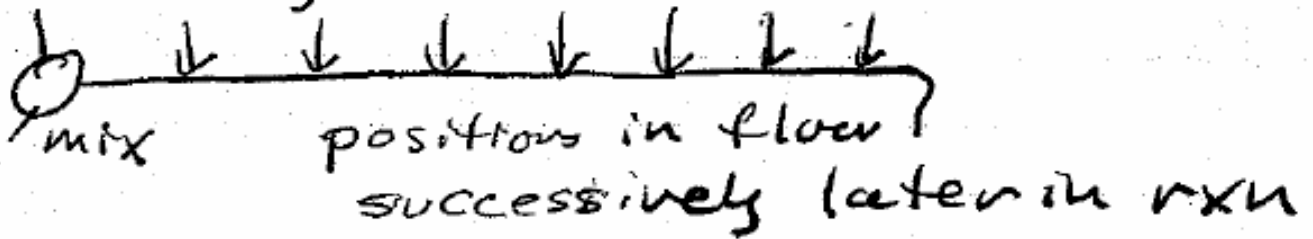
—idea is rapid mixing,
 fill/monitor small volume

Drive two components (or more) into mixing chamber and out to a cell for monitoring a physical property—typically absorbance or fluorescence



Mixing can occur in about 1ms, key is turbulence

Continuous flow – measure along flow $x \rightarrow t$



positions in flow \rightarrow successively later in reaction, $x \rightarrow t$

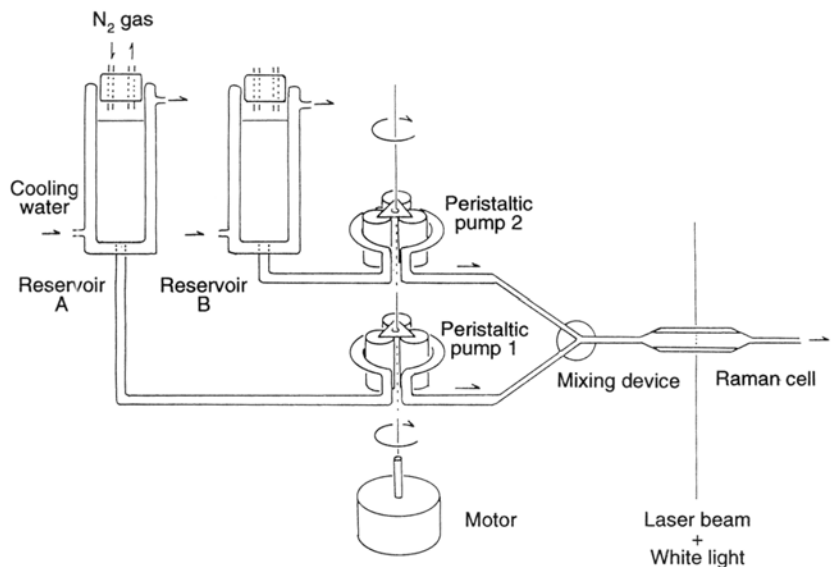
Faster

—continuous flow,
more material

Convert

time to distance

possible to get to
100 μ s or better
with micro fluidics



$x \rightarrow t$

Time scales ~ 1 ms mixing for small volume: ~ 0.1 ml

Faster \rightarrow micro mixers \rightarrow

\rightarrow micro etch channels – flow

idea – rate limit mix – diffuse A,B together

– D – different constant \sim constant

shorter distance between A,B (mixing)

Same Concept – follow conc. $[A]$ vs. t / determine rate,
vary $[A]$ / determine order

Speed up initiation—if photochemical initiate

$A \rightarrow A^*$ then this can be created in very short time

Bio-example: $Hb-CO \rightarrow Hb + CO \rightarrow$ recombine

Rhodopsin $\rightarrow Rh^*$ --conformational change

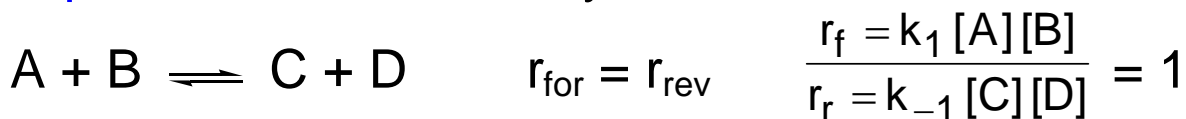
Above focus on loss of reagent (or forming product)

If reverse reaction or alternate steps important

Need to monitor other species (intermediates)

At equilibrium rates change depend on: R & P

Equilibrium, if elementary



$$K_e = k_1/k_{-1} = \frac{[C]_e [D]_e}{[A]_e [B]_e}$$

Disturb equilibrium (relaxation) – perturbation-relaxation

- change T, P, pH, ... how? \rightarrow discharge capacitor

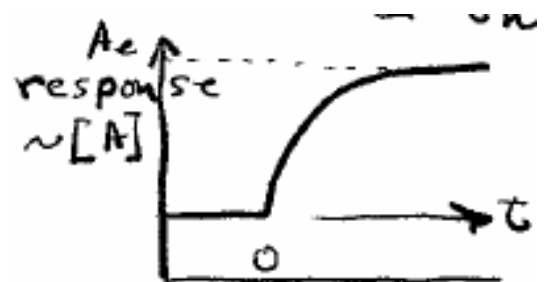
(T), shock wave (P), Laser flash-(pH precursor) or

T-jump (laser absorb by solvent)

- system relax to new equilibrium

- reaction must go forward/reverse

to new state

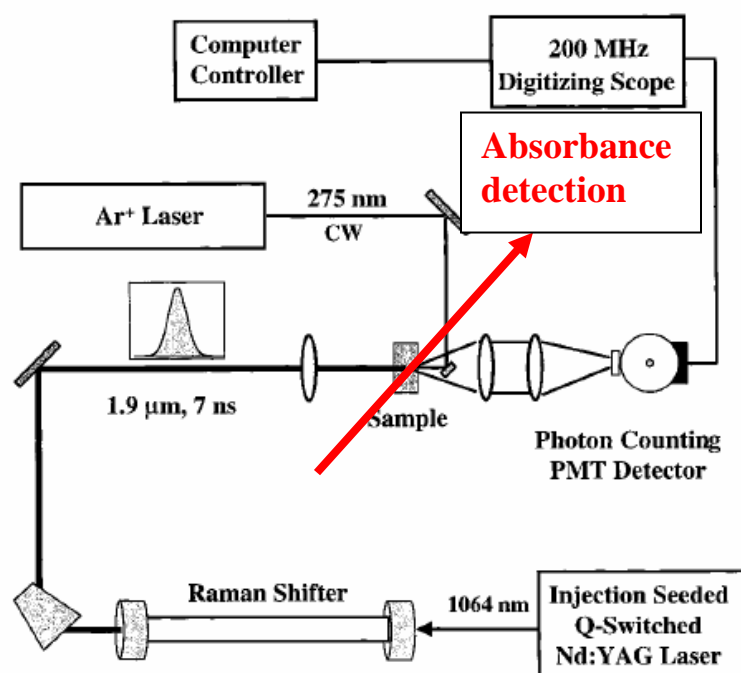


x = departure from equilibrium at time = t , new T or P

$x = x_e e^{-t/\tau}$ τ relaxation time: $1/\tau = k_1 + k_{-1} = k_{\text{rel}}$

Note: relaxation faster if either k_1 or k_{-1} fast
 (see Derivation 7.2 in Atkins, Table 7.4 in Tinoco
 or Engel Ch. 25.11)

Since $K_{eq} = k_1/k_{-1}$ get both values k_1, k_{-1} from τ & K_{eq}



Example—see
 Engle p.687 – RNA
 conformation

Faster—Dyer and co-
 workers protein and
 peptide folding

See slides - Webpage

FIGURE 2: Schematic of the fluorescence T-jump spectrometer. See the text for details.

