Single Molecule Kinetics

- Consider radioactive decay:

$$ {}^{32}_{15}P \rightarrow {}^{32}_{16}S^{+1} + e^{-} + \bar{\nu}_e $$

- Questions:
  - How does one phosphorus molecule know when to decay?
  - Do molecules have watches?

What Does *k* Mean?

- **One view:** Rate at which radioactive molecules decay (N molecules per second)

- **Valid Alternative:** Probability that one molecule will decay per unit time (N transitions per second)

Algorithmic Thinking

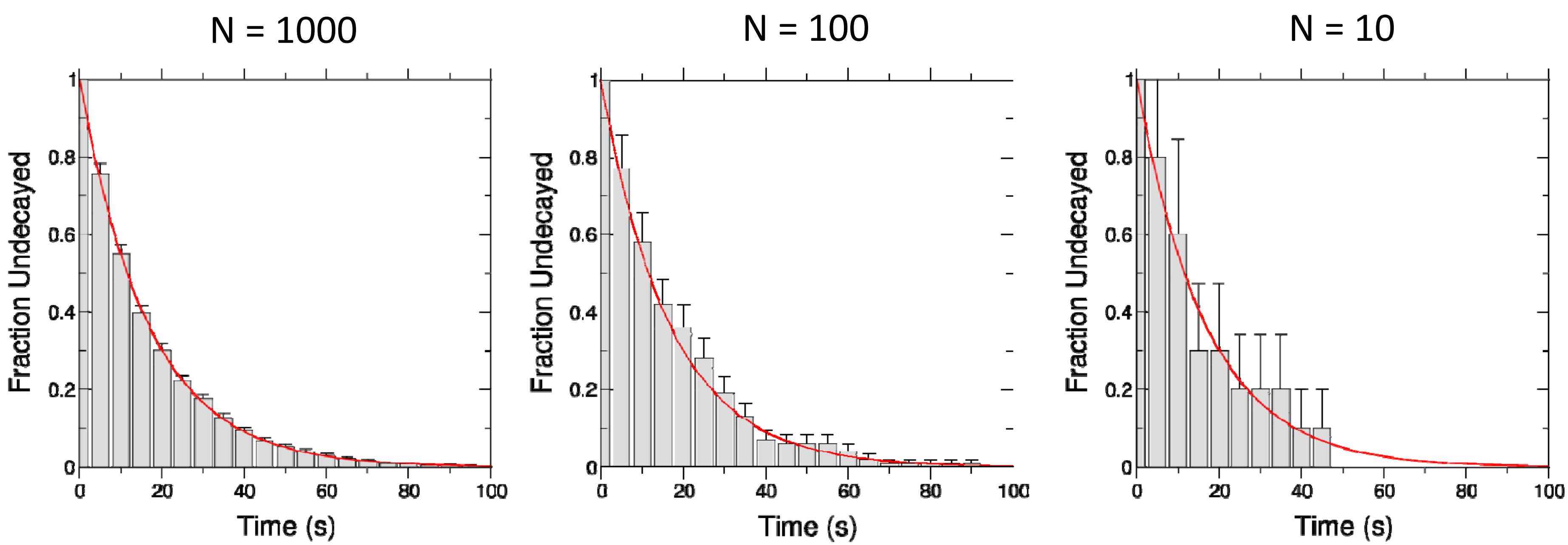
- **Consider:** Ensemble of N radioactive ^{32}P atoms, rate constant k.

- In period Δt, the likelihood of decay for one atom is:

$$\left(k\,\frac{\text{transitions}}{\text{s}}\right)\left(\Delta t\ \frac{\text{s}}{\text{period}}\right) = k\Delta t\,\frac{\text{transitions}}{\text{period}}$$

- For each of N molecules, generate a random number r between 0 and 1:
  - If $0 \leq r < k\Delta t$, decay to $^{32}S^{+} + e^{-}$
  - Otherwise, don't decay this period

Simulation Results



- Rate constant is 0.06 s^{-1}, time step is 1 s.
- Red line is $e^{-0.06t}$.

(Python simulation code available upon request.)

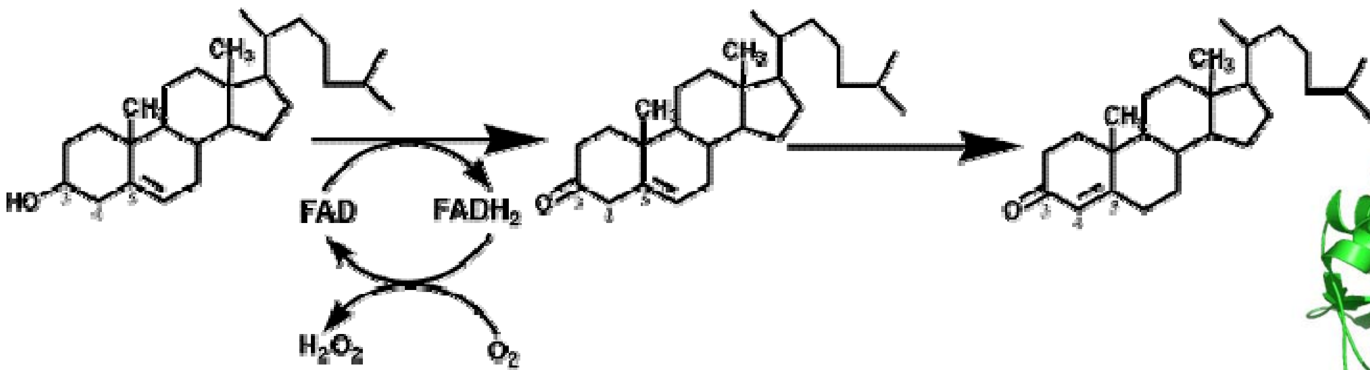
The Master Equation

- **Paradigm Shift:** Instead of a single rate law, imagine a set of transition probabilities from one state (^{32}P) to another (^{32}S)
  - Works for more complicated systems, too

- The *master equation* allows us to integrate probabilities and determine population change over time

- Well beyond the scope of this course!

Example: Cholesterol Oxidase



- Mechanism:

$$E \cdot FAD + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} (E \cdot FAD) \cdot S \xrightarrow{k_2} E \cdot FADH_2 + P$$

Fluorescent

Not Fluorescent

Source: http://crystal.bcs.uwa.edu.au/px/alice/projects/SCOA.html

Example: Cholesterol Oxidase

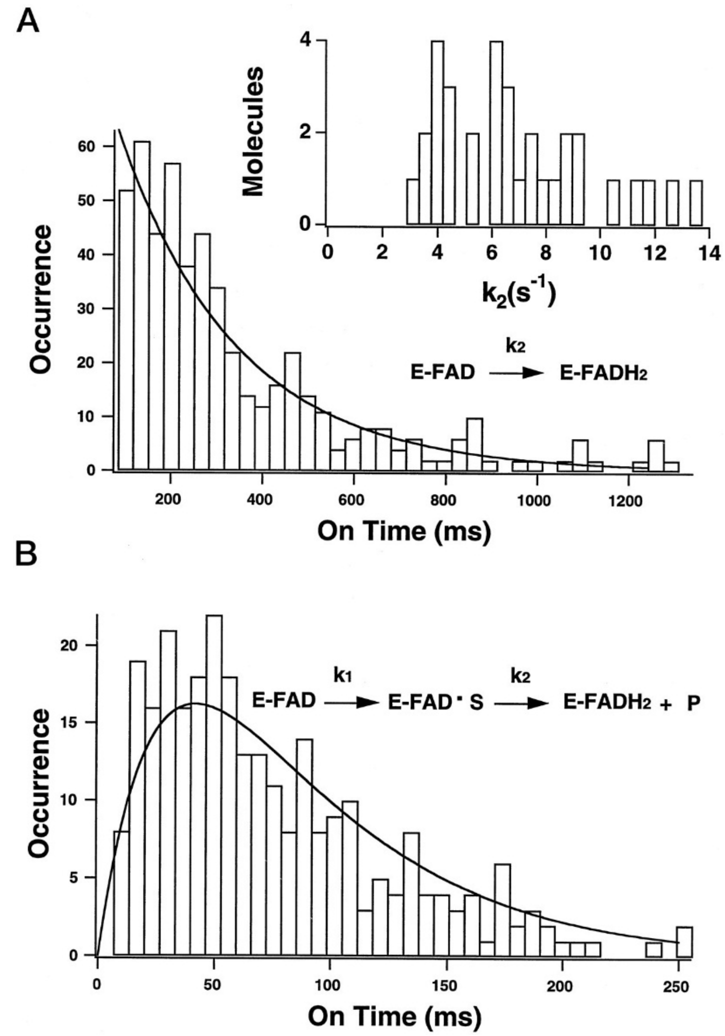
- Trap enzymes in a rigid matrix, observe individual molecules in a fluorescence microscope

- Measure lifetimes of hundreds of cholesterol oxidase molecules

- Form a histogram: how many molecules had a lifetime of x ms?

Single Molecule Kinetics: Takeaways

- Individual molecules have a distribution of lifetimes
- Average behavior closely follows Michaelis-Menten kinetics
- **New insight:** If step 1 is slow, is step 2 also slow? <u>*Single molecule dynamics!*</u>



Xie, S. X. and Lu, H. P. (1999) *J. Biol. Chem.* **274**: 15967.

Final Exam Details

- Tuesday, Dec. 9 from 12:00 – 3:00 pm
  - Location is in our usual classroom
- Total of 200 points (20% of your grade):

Topic	Book Chapters	Weeks	Points
Protein/DNA Structure and Thermodynamics	1-3, *MoL* Chapter 1	1-4	55
Equilibrium, Binding, and Statistical Mechanics	4, 5, 7, and pp. 206-213	5-9	55
Chemical Kinetics	9	10-13	60
Enzyme Kinetics, Protein Folding,	10	15-16	30