‘In Vivo’ transport and kinetics

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Nota: mi-am luat libertatea de a redacta paginile urmatoare in limba engleza, in ideea ca le voi putea refolosi in viitor (putin probabil insa nu se stie niciodata)

1) Experimental methods

a) Culture systems (chemostat or batch cultures)

b) Measurement approaches (direct or by extraction)

- **Flow cytometry (spectroscopic, chromatographic)**
- **Static methods**
- **Counting techniques**
- **‘Brute Force’ approach**
- **Fluxomics**

2) Physicochemical mechanisms (partially known)

a) Underlying variables span a wide range of orders of magnitude. Interplay between macroscopic and microscopic dynamics.

b) Different types of fluctuations

- **Sampling fluctuations (lottery type)**
- **Intramolecular fluctuations (local or collective variables)**
- **Cage effect, fluctuating bottlenecks**
- **Effects due to local (microscopic/macroscopic) inhomogeneities (crowding)**

3) Experiments 'in vitro' – Illustrated by single-molecule kinetics

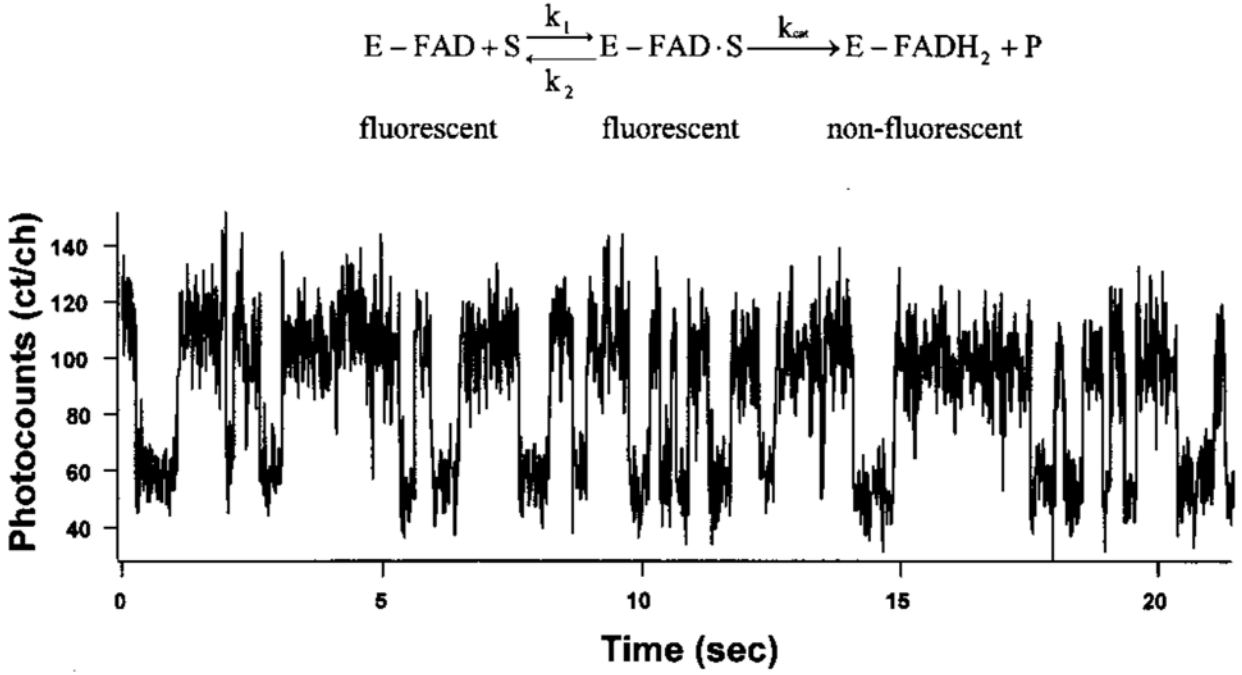
a) Confocal fluorescence microscopy

b) Enzyme immobilization, optical testing

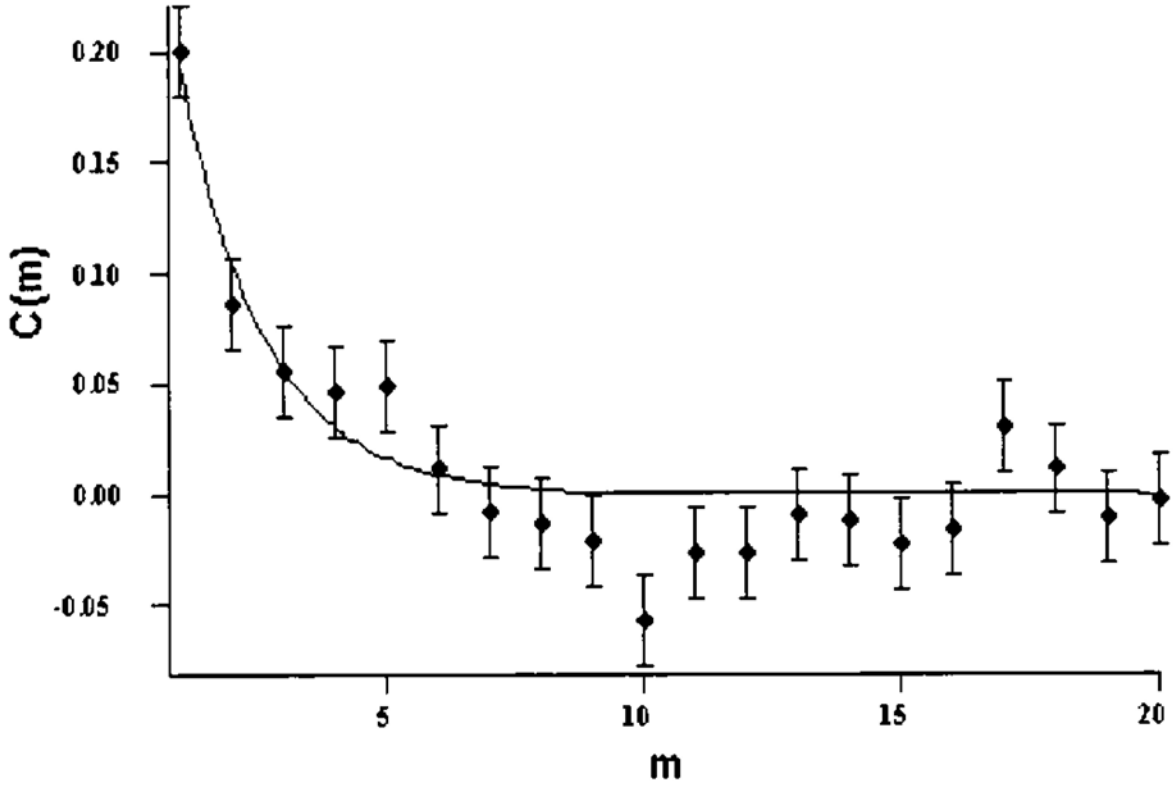
c) Collecting data

d) Experimental observables: (1) correlation functions of the fluorescent signal; (2) on/off time distributions (or correlations); (3) distribution of the reaction events

Example: Cholesterol oxidation to cholesterone by the FAD via the Michaelis-Menten mechanism (Lu, Xun, and Xie, Science 282, 1877 , 1998; J. Biol. Chem. 274, 15967, 1999; J. Chem. Phys, 117,11024 (2002))



Real-time observation of enzymatic turnovers of a single cholesterol oxidase (COx) molecule catalyzing oxidation of cholesterol molecules by oxygen. The emission from a single COx molecule as a function of time is plotted. Each on–off cycle in the emission intensity trajectory corresponds to an enzymatic turnover. The Michaelis–Menten mechanism of the enzymatic reaction is shown in the inset.



Autocorrelation function of the on-times for the same trajectory $C(m)=\langle \Delta\tau(0)\Delta\tau(m)\rangle/\langle \Delta\tau^2\rangle$, m being the index numbers of turnovers. $\Delta\tau(m)=\tau(m)-\langle\tau\rangle$. The fact that $C(m)$ is not simply a spike at $m=0$ indicates dynamic disorder of k_{cat}. The time constant of the decay gives the timescale of the k_{cat} fluctuation.

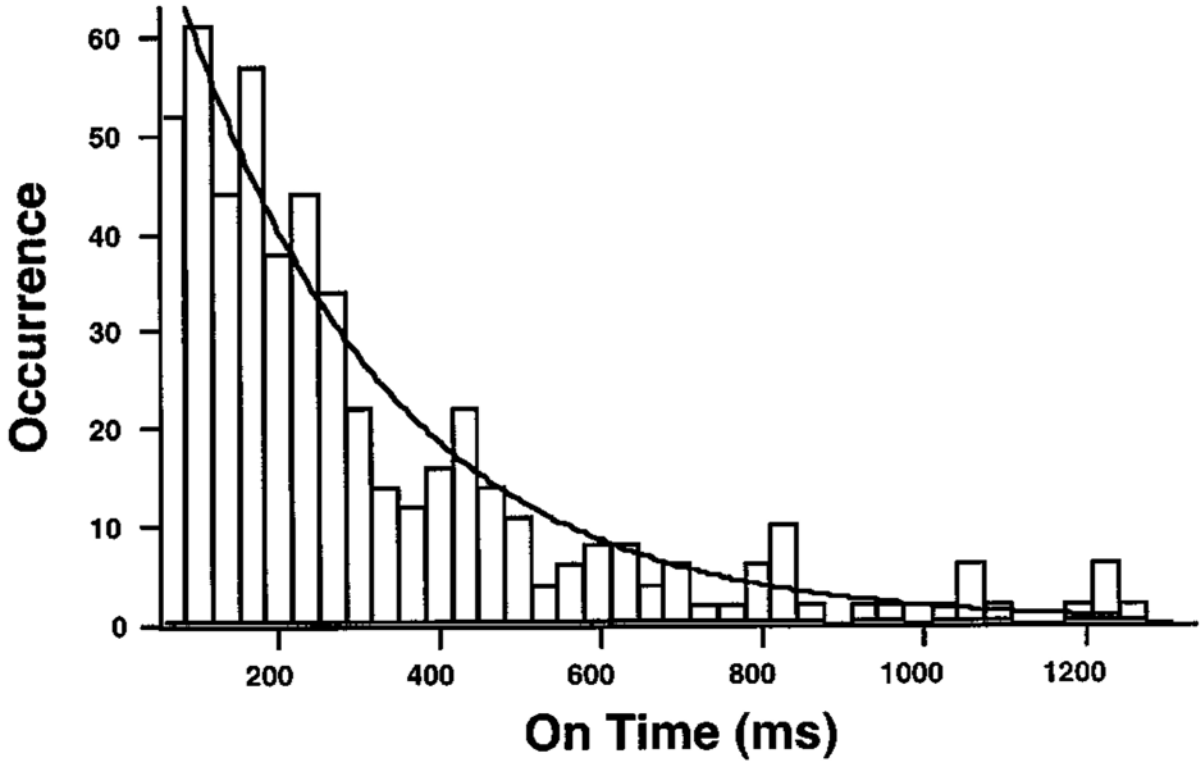


FIG. 8. Histogram of the on-times of a COx fluorescence intensity trajectory taken at a high substrate concentration at which k_{cat} is rate-limiting. The solid line is a single exponential fit with $k_2 = 3.9 \pm 0.5\ s^{-1}$.

4) Possible theoretical interpretation (Vlad, Schneider, Moran, Sanchez, PNAS, Physical. Review. E 65, 061110. (1-17) (2002)., (PNAS) vol. 99, no. 20 pp. 12548-12555 (2002). Chemical Physics 287, 83–90 (2003).

a) Intramolecular dynamics (density operator, quantum Liouville equation)
b) Collective variables + intrinsic degrees of freedom (not unlike in nuclear physics) + a few chemical states
c) Projection operator techniques in 'lambda time squared limit' lead to a master equation with fluctuating rate coefficients:

$$\partial_t \mathbf{P}(t) = \mathbf{K}(t)\mathbf{P}(t),$$

$$\mathcal{G}\left[\mathbf{q}(t')\right] = E\left\{\exp\left[\int \mathbf{K}(t') \bullet \mathbf{q}(t')dt'\right]\right\}$$

5) Proposal for a research project (suggested by Alexandru Corlan)

a) **Consider an organ made up of a large number of cells (for example liver)**
b) **In each cell takes place a reaction which involves small numbers of molecules (stochastic kinetics).**
c) **Investigate under what circumstances a set of classical kinetic laws can describe adequately the evolution of the reaction for the whole organ.**

6) Conclusion: we know almost nothing, and are far away from fully understanding the physics, chemistry and biology of ‘in vivo’ intracellular processes. What we lack is physical and biological insight, not computer power or help from mathematicians/computer engineers or scientists (although they certainly may help).