Single-Molecule Enzymatic Dynamics
Structure:

- The Experiment
  - Motivation
  - Enzymatic redox reaction
  - Details
  - Real reaction

- Analysis
  - Theoretical principles
  - Curve fitting
  - Static disorder
  - Dynamic disorder

- Interpretation
  - Different conformations
  - Simulation
The Experiment
Flourescence microscopy allows real-time observation of single molecules.

→ Distributions in inhomogenous systems.

→ Observe stochastical trajectories.

→ Distinguish static- and dynamic-disorder.

Here: Enzymatic turnovers of flavoenzyme molecules
• The Enzym: **Cholesterol oxidase** from Brevibacterium.

• The active site (E) contains a **flavin adenin dinucleotide** (FAD).
• Undergoes reversible redox reaction.

• Flourescent when oxidized and nonflourescent when reduced.
- 33 Molecules in agarose gel (99% water):
  - No translation
  - But freely rotating

- Substrates (Cholesterol (0.2mM) + Oxygen(0.25mM))
  free translational diffusion.

- For slow rates, replace Cholesterol with a derivative of it:
  5-pregene-3β-20α-diol
• Measurement of single molecule reveals **on-off behaviour**.  
  → The redox reaction is observed.

• Support for this conclusion:
  → No blinking without Cholesterol.
  → Turnover rate independent of excitation Intensity
  → Averaged rates match ensemble-averaged values
Analysis
• Long Trajectories:
  → Good statistics
  → Trajectory ↔ Ensemble

• Experiment:
  → No photobleaching, protection by the protein.
  → No rate decrease, high substrate concentration.
Analysis – Theoretical principles

• Poisson Process:
  → Stochastik walk
  → Markov process
  → Probability of N(t): Poisson distributed
  → Waiting time: Exponential distributed

• Experiment:
  → Reaction itself stochastic and fast
  → On-times are waiting times
• Conventional chemical kinetics:
  
  → Time-development of concentrations
  → Rates are averaged values

• Experiment:
  
  → On-times are equivalent to concentrations.
  → Different schemes may apply:
    • Reversible reaction
    • Michaelis Menten mechanism
• **Autocorrelation:**

  → Similarity of observations as a function of time between them.

• **Experiment:**

  → Analysis is based on statistical independence.
  → Needs to be tested.
• Simple reversible reaction:
  → Fails, not an exponential decay

• Michaelis Menten:
  → Can be fitted quite well.
  → Different rates for different substrate concentrations

C

\[ [S] = 0.2 \text{mM} \]
\[ \rightarrow \]
\[ k_1 = 2.9 \text{ 1/s} \]
\[ k_2 = 17 \text{ 1/s} \]

D

\[ [S] = 2.0 \text{mM} \]
\[ \rightarrow \]
\[ k_1 = 33 \text{ 1/s} \]
\[ k_2 = 17 \text{ 1/s} \]
• Easy analysis:
  
  → Use slow substrate (k1 >> k2).
  → simple exponential decay with k2.
• Comparing fits:
  → Different molecules have different rates (time averaged).

• Possible reasons:
  → Different conformations
  → Posttranslational modifications
Autocorrelation:

\[ p(x,y) \neq p(x)p(y) \]
• Autocorrelation:

  • For the backwards reaction $r(m)$ remains 0
  $\rightarrow$ no dynamic disorder in $k'1$ or $k'2$

  • No autocorrelation for the reaction when $k1$ is rate limiting
  $\rightarrow$ no dynamic disorder in $k1$

  • Spectral mean autocorrelation (no reaction) is similar to
    the autocorrelation of on-times.
  $\rightarrow$ conformational fluctuations
Analysis – Dynamic disorder

• New scheme:

\[ E \rightleftharpoons E' \]

\[ k_E \]

\[ k_{E'} \]

\[ E - \text{FAD} + S \rightleftharpoons E - \text{FAD} \cdot S \xrightarrow{k_1, k_{-1}} E - \text{FADH}_2 + P \]

\[ E' - \text{FAD} + S \rightleftharpoons E' - \text{FAD} \cdot S \xrightarrow{k_1, k_{-1}} E' - \text{FADH}_2 + P \]
Simulation of the Michaelis-Menten-Scheme via the Gillespie algorithm
Simulation of the new and more complex scheme (slide 20) via the Gillespie algorithm compared to the analytic Michaelis Menten scheme.
Exponential decay of the autocorrelation $r(m)$ with distance, reproduced via Gillespie algorithm. Simulation of the new complex scheme (slide 20)
Quellen:

• Lu, H. P.; Xun, L.; Xie, X. S. Science 1998, 282, 1877


• RCSB Protein Data Bank
  http://www.rcsb.org/pdb/explore/explore.do?structureId=3COX


• http://en.wikipedia.org/
• Intensity Autocorrelation:

→ Michaelis Mentens scheme predicts an exponential decay of the autocorrelation over time. ($<dI(t)dI(0)>$)
→ Only for ensemble-averaged.

• Not fitting:

But scrambled data!