NEW COMPUTATIONAL STRATEGIES TO OBTAIN KINETIC DATA OF ENZYMATIC PROCESSES IN CROWDED MEDIA

Raquel Agraso Riobó*, Pablo M. Blanco, Francesc Mas and Sergio Madurga
Departament de Ciència de Materials i Química Física, Facultat de Química, Universitat de Barcelona
*Raquel.riobo@gmail.com

INTRODUCTION

Living cells constitute crowded cytoplasmic environment, composed of a great amount of of different biopolymers. These represent an obstacle for other biopolymers by means of non-specific interactions. In addition, they have a considerable effect in diffusional and reactivity properties that directly affect the enzymatic reactivity, protein assembly and folding, structural organization of the DNA and so on. This phenomenon is known as a *macromolecular crowding* [1].

In this scope, theoretical models that describe these processes in homogeneous environment are no longer valid and it is necessary to create new models that describe such crowded environment [2].

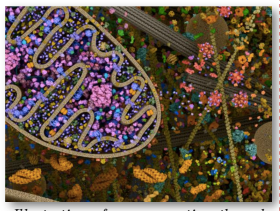


Illustration of a cross-section through a dilute cytoplasm. (Image by David S. Goodsell).

OBJETIVES

- **Simulate an *in vivo* like medium to study the crowding effect in enzymatic kinetics.** Since such simulations are very expensive, here we have developed a **Browniana Dynamics reaction-diffusion code**, which unlike previous studies, is able to obtain **realistic kinetic constants** from **shorter simulations** by means of **numerical extrapolation** procedures.
- **Study the effect of the excluded volume** for some enzymatic systems that follow the **Michaelis-Menten mechanism** [3].

THEORETICAL BACKGROUND

Diffusion

Brownian motion of the particles is described by **Langevin's equations of movement.**

$$\frac{dr(t)}{dt} = \underbrace{-\frac{\Delta t}{f}\nabla V(r,t)}_{Deterministforce} + \overbrace{\sqrt{2D\xi(t)}}^{Estocasticforce}$$

$$r(t+\Delta t) = r(t) - \frac{\Delta t}{f}\nabla V(r,t) + \sqrt{2D\Delta t}\xi(t)$$

∇V = *Potential gradient*
f = *Friction coeficient*
D = *Difusion coeficient*
ξ = *Estocastic factor*

Reacction

The reactions between the particles are given by the simplest scheme for enzymatic catalysis, the **mechanism of Michaelis-Menten:**

$$E + S \underset{k_r}{\overset{k_f}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P$$

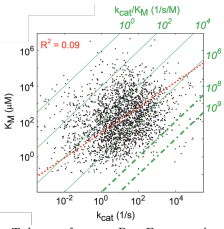
Applying the **law of mass action** to Michaelis-Menten's mechanism:

$$\frac{d[S]}{dt} = k_r[C] - k_f[S]([E_0] - [C])$$

$$\frac{d[C]}{dt} = k_f[S]([E_0] - [C]) - (k_r + k_{cat})[C]$$

$$k_M = \frac{k_r + k_{cat}}{k_f} \quad ; \quad k_f = \frac{k_r + k_{cat}}{k_M} \qquad (a)$$

$$if \quad k_r \ll k_{cat} \implies k_f \approx \frac{k_{cat}}{k_M}$$



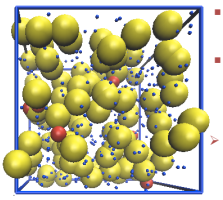
*Taken from Bar-Even, A. Biochemistry (2011), **50**, 4402.*

$$K_{bim}^{macro} = 4\pi D_{ES}\left(r_{ES} - \sqrt{\frac{D_{ES}}{K_{bim}^{micro}}}tanh\left(\sqrt{\frac{K_{bim}^{micro}}{D_{ES}}}\right)\right) \qquad (b)$$

$$p(\Delta t) = 1 - exp(\Delta t k_{micro}) \approx \Delta t k_{micro} \quad if \quad \Delta t k_{micro} \ll 1 \qquad (c)$$

K_f^{macro} $[M^{-1}s^{-1}]$	$K_f^{micro(b)}$ $[ns^{-1}]$	$K_r^{(a)}$ $[ns^{-1}]$	$K_{cat}^{(a)}$ $[ns^{-1}]$	$p_f^{(c)}$	$p_r^{(c)}$	$p_{cat}^{(c)}$
10^8	$1.77 \cdot 10^{-3}$	$3.5 \cdot 10^{-5}$	$7.1 \cdot 10^{-5}$	$1.77 \cdot 10^{-4}$	$3.5 \cdot 10^{-6}$	$7.1 \cdot 10^{-6}$

SIMULATION



Snapshot of a simulation with obstacles (yellow), enzyme (red), complex (gray), substrate (blue) and product (orange).

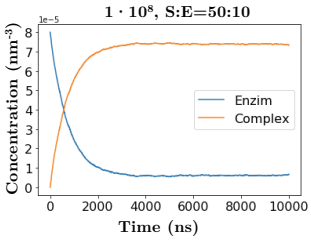
C++ code

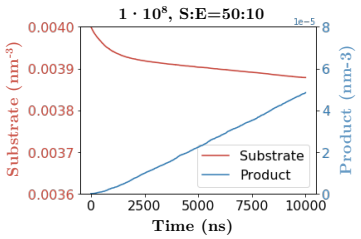
- Motion of the particles: Stochastic by means of a **Brownian motion algorithm.**
- Reactions: Stochastic processes by means of **Monte Carlo criterion.** (probabilities of reaction directly related to the kinetic constants) [3].

Specie	Radius $[nm]$	Diffusion constant$^{(d)}$ $[ns^{-1}]$
Enzyme	2.33	0.1051
Substrate	0.5	0.4901
Obstacle	4.0	0.0613

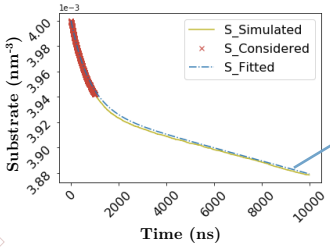
(d) Calculated from Stokes-Einstein equation

Temporary evolution of particle populations with 40% agglomeration





AJUST METODOLOGY

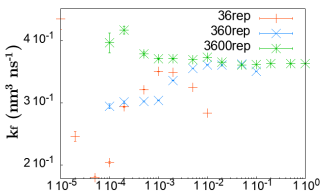


Python code: Fitting the substrate profiles using *scipy* module. *Odeint* to integrate and *curve_fit* to optimize respectively.

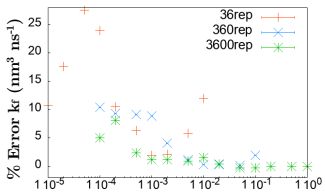
Acurate estimation at long times

✓ **From the first zone of the curve all the temporary evolution of the substrate can be reproduced.**

Variation of k_f with simulation time



Error of k_f variation with simulation time



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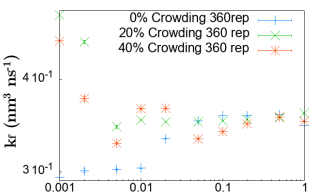

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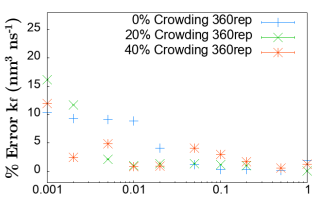

CONCLUSIONS

- The new fitting procedure allows to obtain the **bimolecular kinetic constant of the Michaelis-Menten mechanism with shorter simulation time.**
- The appropriate **estimation of k_f** needs **short but precise simulations** that require **many repetitions** to obtain accurate profiles of the evolution of the substrate.
- In **crowded systems** is also possible to **reproduce all kinetics** until achieve **times with experimental significance.**
- We anticipate our assay to be a starting point for more sophisticated simulations that allow us to **study the effect of the excluded volume for some enzymatic systems.**

REFERENCES

[1] D.Hall and A. P. Minton, "Macromolecular crowding : qualitative and semiquantitative successes, quantitative challenges", *Biochim. Biophys. Acta.*, 1649 (2003) 127–139.

[2] S. Schnell and T. E. Turner, "Reaction kinetics in intracellular environments with macromolecular crowding: simulations and rate laws", *Prog. Biophys. Mol. Biol.*, 85 (2004) 235–260.

[3] L. Pitulice, E. Vilaseca, I. Pastor, S. Madurga, J.L. Garcés, A. Isvoran, F. Mas, "Mathematical Biosciences Monte Carlo simulations of enzymatic reactions in crowded media. Effect of the enzyme-obstacle relative size", *Math. Biosci.*, 251 (2014) 72–82.