BROWNIAN DYNAMICS SIMULATIONS OF ENZYMATIC REACTION-DIFFUSION PROCESSES IN CROWDED INTRACELLULAR ENVIRONMENTS

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INTRODUCTION

Macromolecular Crowding

The cellular cytosol is a very dense medium with huge concentrations of biological macromolecules that, by means of non-specific interactions, have a considerable effect in processes like diffusion and reactivity. This macromolecular crowding generates an **excluded volume** ϕ in the reaction media. In crowded conditions, theoretical models that describe physicochemical processes in homogeneous media are no longer valid[1,2,3].

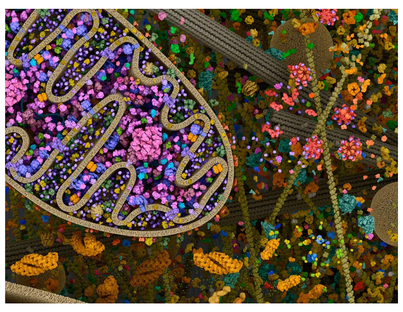
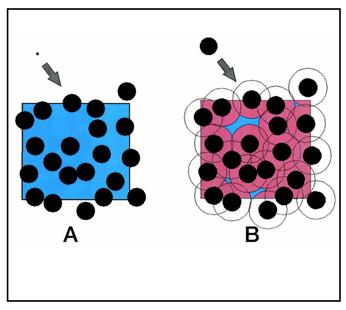


Illustration of the crowded cellular medium. The concentration of each individual biological specie is small, but overall the total concentration in the cytosol and membranes make the cellular environment an extremely crowded medium.



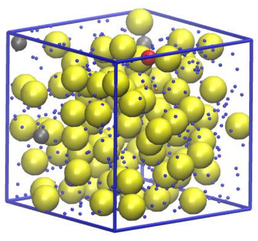
The volume available (blue areas) in a solution of macromolecules is larger for a tiny molecule (A) than for another macromolecule of similar size (B). The forbidden or excluded volume ϕ (pink areas) affect both diffusion and reactivity processes.

Brownian Dynamics

In this scope, we have developed a **Brownian Dynamics**[4] **reaction-diffusion code** to study enzyme kinetics that follow the **Michaelis-Menten mechanism**. The biomolecules are modelled explicitly as individual, non-overlapping coarse-grained hard spheres that are transported via **stochastic dynamics**. The equation of motion is the memoryless **Langevin equation**, which has a deterministic force, governed by a harmonic repulsive potential, and a stochastic force, which describes the collisions of the solvent on a Brownian particle. Species undergo reactions with other chemical species if they accomplish the **Monte Carlo criterion**, according to their respective **Poissonian reaction probability** related to the macroscopic rate constants.

$$r_{t+\Delta t} = r_t - \Delta t D \frac{\nabla V(r(t))}{k_B T} + \sqrt{2D\Delta t}\,\eta(t)$$

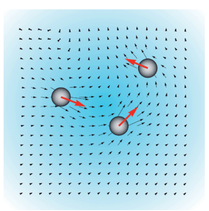
The equation of motion (the Langevin equation) for all the particles of the enzymatic Michaelis-Menten mechanism: enzymes (grey spheres), substrates (blue), products (orange), complexes (red) and obstacles or crowding agents (yellow).



Hydrodynamic Interactions

Hydrodynamic interactions (HI) are usually introduced to improve the solvent treatment. These interactions arise when a Brownian particle collides with solvent particles which, in turn, also collides with other macromolecules. This correlation of the particle's movement through the liquid leads to a decrease of the **diffusion coefficient** D of the species in the reaction medium.

The **Tokuyama model**[5] is a mean-field method and is the easiest approach to include such interactions in the system. The dilute diffusion coefficient D_0 is corrected according to the excluded volume present in the medium.



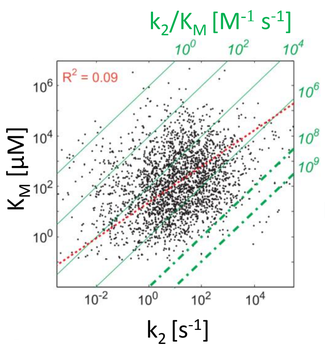
$$D^T(\phi) = \frac{D_0}{1+H(\phi)}$$

$$H(\phi) = \frac{2b^2}{1-b} - \frac{c}{1+2c} - \frac{bc(2+c)}{(1+c)(1-b+c)}$$

$$b = (9\phi/8)^{1/2} \qquad c = 11\phi/16$$

The movement of the Brownian particles generate forces that affect the neighbouring solvent particles. The Tokuyama correction to the dilute diffusion coefficient D_0 intends to include these effect in Brownian Dynamics simulations.

Reactivity



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

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Input kinetic parameters chosen according to the most common k_2 values.

Tag	k_1^{macro} [$M^{-1}\,s^{-1}$]	k_1^{macro} [$nm^3\,ns^{-1}$]	k_1^{micro} (a) [ns^{-1}]	k_{-1} (b) [ns^{-1}]	k_2 (c) [ns^{-1}]	p_1 (d)	p_{-1} (d)	p_2 (d)
1E9	10^9	1.66	1.95×10^{-2}	3.9×10^{-4}	7.8×10^{-4}	1.95×10^{-3}	3.9×10^{-5}	7.8×10^{-5}
1E8	10^8	1.66×10^{-1}	1.77×10^{-3}	3.5×10^{-5}	7.1×10^{-5}	1.77×10^{-4}	3.5×10^{-6}	7.1×10^{-6}
1E7	10^7	1.66×10^{-2}	1.75×10^{-4}	3.5×10^{-6}	7.0×10^{-6}	1.75×10^{-5}	3.5×10^{-7}	7.0×10^{-7}

(a) $k_1^{macro} = 4\pi D_{ij}\left(R_{ij} - \sqrt{\frac{D_{ij}}{k_1^{micro}}}\tanh\left(\sqrt{\frac{k_1^{micro}}{D_{ij}}}\right)\right)$

(b) $k_{-1}^{macro} = k_{-1}^{micro}$; $k_{-1} = 0.02\,k_1^{micro}$

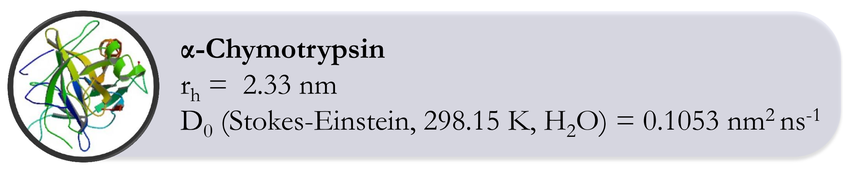
(c) $k_2^{macro} = k_2^{micro}$; $k_2 = 0.04\,k_1^{micro}$

(d) Poisson probability, $p(\Delta t) = 1 - exp(-\Delta t k_{micro})$

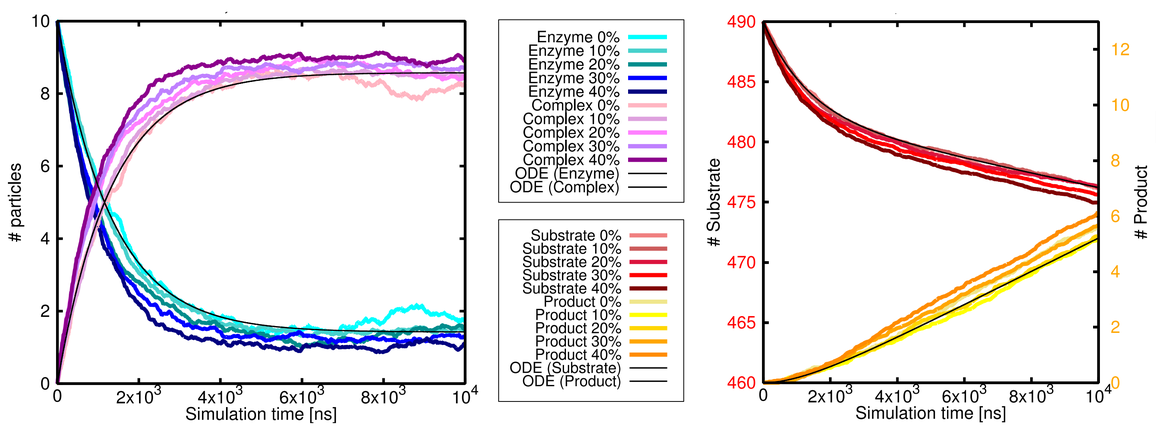
Specie	Radius [nm]	Diffusion coefficient (a) [$nm^2\,ns^{-1}$]
E and C	2.33	0.1053
S and P	0.50	0.4360
Obs	4.00	0.0311

$[E]_0 = 133$ μM, $[S]_0$ variable
Cubic simulation box of 50x50x50 nm^3
Time step of 0.1 ns

(a) Stokes-Einstein diffusion coeffients at 298.15 K in water medium, $D_i = k_B T / 6\pi\eta r_i$



α-Chymotrypsin
r_h = 2.33 nm
D_0 (Stokes-Einstein, 298.15 K, H_2O) = 0.1053 $nm^2\,ns^{-1}$



Representative case of enzyme (blue lines), complex (purple lines), substrate (red lines) and product (orange lines) particle variation with time without non-reacting obstacles (lighter lines) and with ϕ = 10, 20, 30 and 40% (darker lines) with $k_1 = 10^9$ $M^{-1}\,s^{-1}$ and a substrate:enzyme ratio S:E = 50:1. The black lines are the ordinary differential equations (ODEs), the governing equations of the species for systems in dilute conditions.

Weighted least squares fitting of k_1 into substrate's ODE:

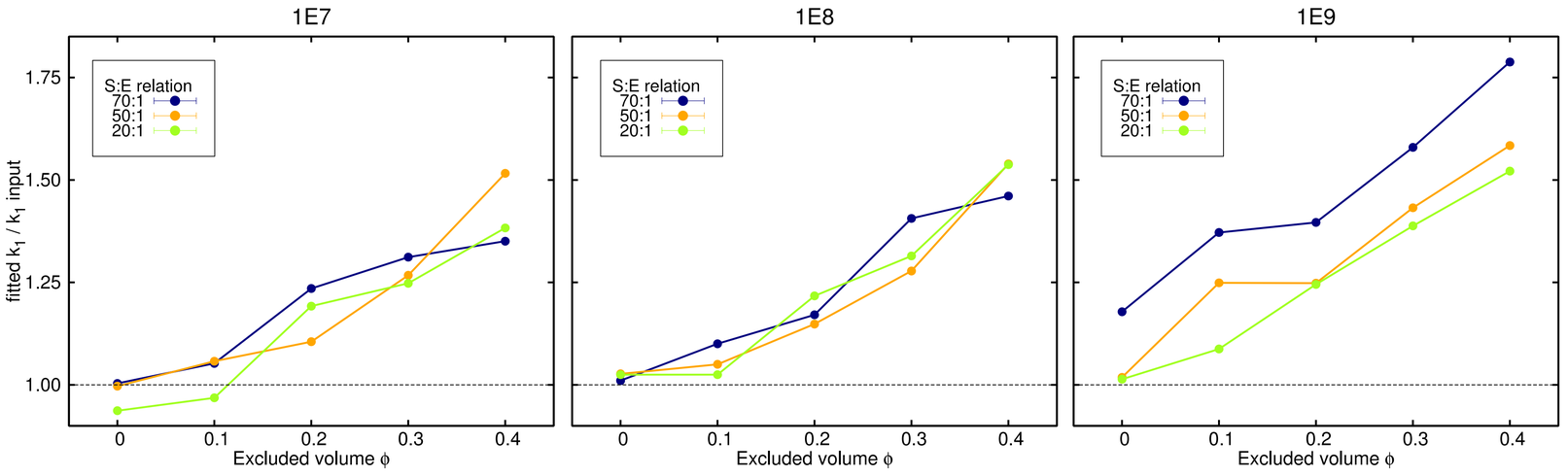
$$\frac{d[S]}{dt} = k_{-1}[ES] - k_1[S]([E]_0 - [ES])$$

$$\frac{d[ES]}{dt} = k_1[S]([E]_0 - [ES]) - (k_{-1} + k_2)[ES]$$

with k_{-1} and k_2 fixed:
mononuclear constants

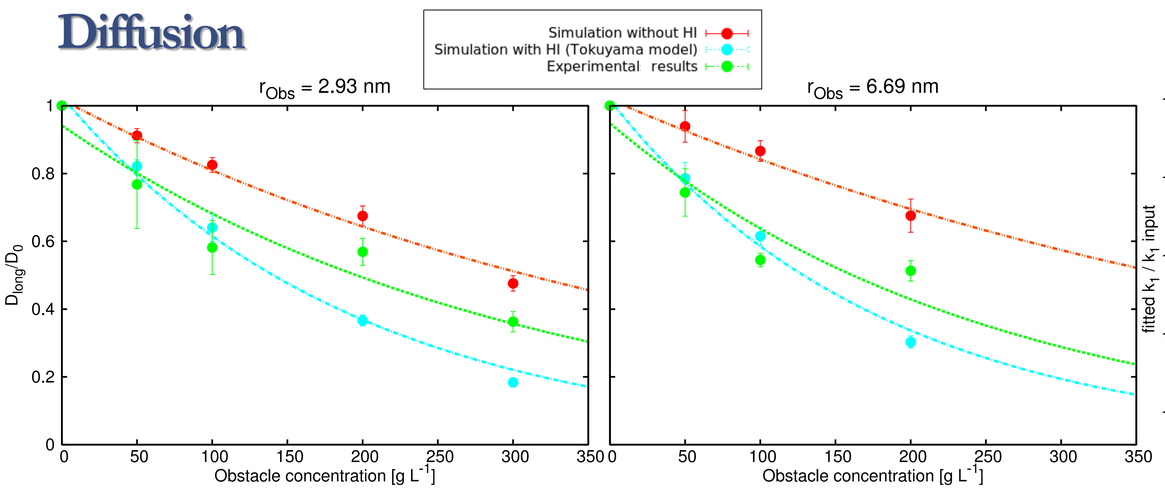
⇩

No crowding
(nor autocrowding)-dependent !



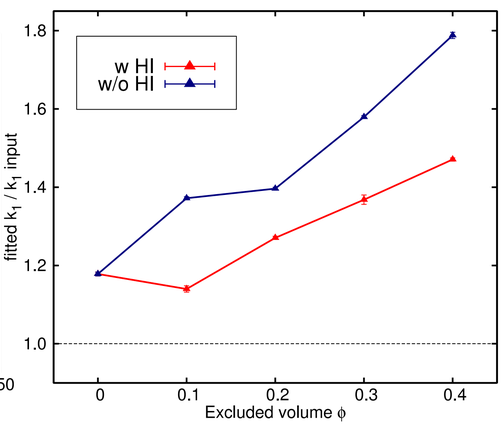
Relative values for the macroscopic bimolecular rate constant k_1 using S:E relations of 20:1 (green dots), 50:1 (orange dots) and 70:1 (blue dots) for the three kinetics studied: 1E7 (left), 1E8 (centre) and 1E9 (right).

Diffusion



Relative long-time diffusion coefficients of α-Chymotrypsin in different crowding environments, using two types of crowding agents, introducing hydrodynamic interactions, according to the Tokuyama model (red curve) and without them (blue curve). Simulation results are compared with experimental data[3].

Reaction



Relative k_1 values for reaction studied with (red dots) and without (blue dots) hydrodynamic interactions using $k_{1,input} = 10^9$ $M^{-1}\,s^{-1}$ and S:E = 70:1.

CONCLUSIONS

- The bimolecular rate constant k_1 becomes **enhanced as** the **excluded volume** of the system **increases** (macromolecular **confinement**).
- Reactions that are **diffusion-limited** (i.e. 1E9) experience **autocrowding** when the specie concentration is high (systems with S:E = 70:1). Reactions that have a **mixed control** (both by the enzyme's activity and by diffusion), like 1E7, exhibit a less stable trend.
- The **Tokuyama model** for hydrodynamic interactions **improves the diffusion description** of the species in the system, providing lower diffusion coefficients and reaction constants, closer to the experimental results.

WORK IN PROGRESS

- Reactivity has to be checked with obstacles of different sizes and with mixtures of different types of obstacles.
- The **Tokuyama model** is deduced for equal-sized systems and has to be tested with models that provide a more appropriate description for polydisperse systems.

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5) Tokuyama, M. *Phys. Rev. E* (**2011**), 83, 051402: p.1-8