Towards a realistic modelling of protein diffusion in polymer crowded media

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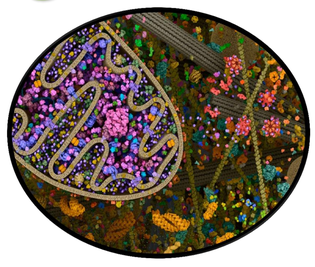
What does macromolecular crowding mean?

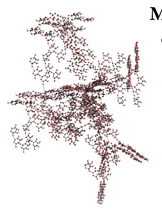
Biological media is known to contain a wide variety of macromolecular species. The total macromolecular concentration can dramatically increase leading to highly crowded systems (*e.g.* 200-300 g/L in cell cytosol).



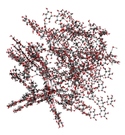
Artistic representation of cell cytosol.

Macromolecular crowding is the inert macromolecular co-solutes present in biological reactions[1]. However, crowding interacts with the reactants by means of non-specific interactions[2-5]. As result, thermodynamic and transport properties are altered resulting a crucial factor in biochemical reactivity.

Crowding effect on diffusion → Enhancing of Macromolecular folding



Macromolecular concentration increase

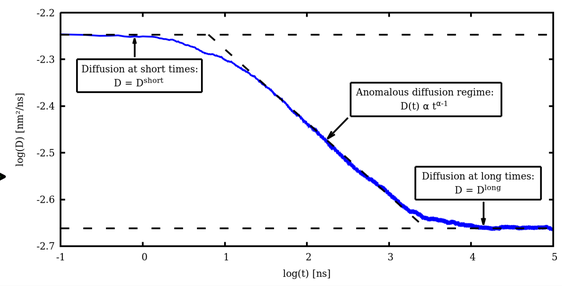


Graphical representation of a model of D50 dextran macromolecule in two different states: unfolded (left) and folded (right). The macromolecular crowding increase enhances the folded conformations. In consequence, the macromolecular diffusion and the excluded volume of the system are altered.

Anomalous diffusion:

Diffusion coefficient changes over time!

Brownian dynamic simulation of the diffusion of a tracer protein in a system with 350 g/L macromolecular concentration.



Modelling diffusion in crowded media

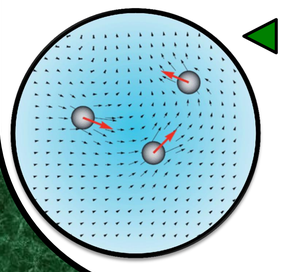
Macromolecular crowded solution contains a really high number of atoms. As a result, computational cost of detailed simulation techniques (*e.g.* Molecular Dynamics) becomes rather expensive.

Decreasing the degrees of freedom

Implicit solvent: Brownian Dynamics[6]

Correction ↓

Including hydrodynamic interactions: Tokuyama method[7]

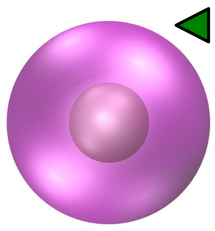


When a macromolecule diffuses , it creates a solvent flow altering the other macromolecules motion. These solvent-mediated correlations are called hydrodynamic interactions. Tokuyama method allow to effectively take into account this effect.

Macromolecules: Coarse-graining

Correction ↓

Including conformational changes: Continuous Shouldered potential

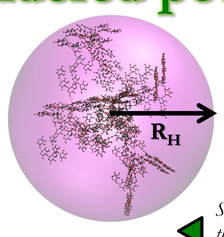


Coarse-graining the macromolecule leads to the loss of their conformational properties. Macromolecular folding is essential to properly describe the excluded volume changes with macromolecular concentration. The Continuous Shouldered potential causes a two-state behaviour, allowing a transition from the unfolded to the folded state as the concentration increases.

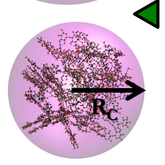
Coarse-graining: Continuous Shouldered potential

Macromolecule characteristic radius[8]

Dilute solution: Hydrodynamic radius (R_H)



Crowded solution: Compact radius (R_C)



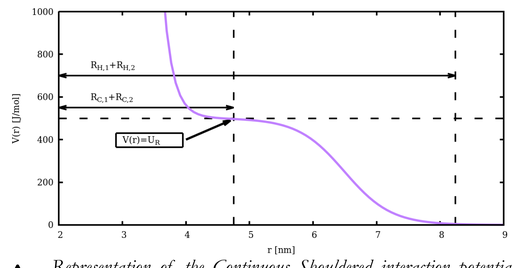
Schematic representation of the coarse graining of the unfolded (Upper) and folded (Down) states of D50 dextran.

Continuous Shouldered potential:

The Continuous Shouldered potential V(r) is a pairwise repulsion potential acting between the macromolecules. It has two states of minima potential gradient at distances equal to the sum of the R_C (S_C) and the R_H (S_H) of the interacting macromolecules.

$$V(r) = \left(\frac{S_c}{r}\right)^{24} + \left(\frac{-U_r}{2}\right)\tanh\left(\frac{S_c}{S_H - S_C}\left(r - \frac{S_H + S_C}{2}\right)\right) + \frac{U_r}{2}$$

$S_H \equiv R_{H,1} + R_{H,2}$
$S_C \equiv R_{C,1} + R_{C,2}$
$U_r \equiv$ Energetic penalty of the macromolecular folding (500 J/mol)



Representation of the Continuous Shouldered interaction potential between Streptavidin and D50 dextran macromolecule.

Brownian Dynamics algorithm

$$\mathbf{r}(t+\Delta t) = \mathbf{r}(t) + \underbrace{\frac{\Delta t D}{k_B T}\nabla \mathbf{V}(r)}_{\text{Interaction between macromolecules}} + \underbrace{\sqrt{6D\Delta t}\boldsymbol{\xi}(t)}_{\text{Solvent interaction}}$$

$D \equiv$ Diffusion coefficient
$\boldsymbol{\xi} \equiv$ Random number with Gaussian distribution

Tokuyama method:

$$D(\phi) = \frac{D_0}{1 + H(\phi)} \qquad H(\phi) = \frac{2b^2}{1-b} - \frac{c}{1+2c} - \frac{bc(2+c)}{(1+c)(1-b+c)} \qquad b = \sqrt{\frac{9}{8}\phi}$$

$D_0 \equiv$ Diffusion coefficient at dilute solution $\quad \phi \equiv$ Excluded volume $\quad c = \frac{11}{16}\phi$

Excluded volume calculation:

The macromolecular conformational changes and entanglement make the excluded volume computation not straightforward. Tokuyama theory for D^{long} and experimental data have been used to find an empiric expression able to calculate the excluded volume.

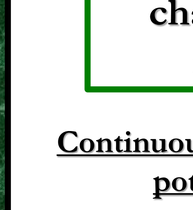
$$\phi = \frac{K\left(\nu \cdot c \cdot \frac{R_C}{R_H}\right)^{\beta}}{1 + K\left(\nu \cdot c \cdot \frac{R_C}{R_H}\right)^{\beta}}$$

$K = 6.6 \pm 0.8$
$\beta = 1.43 \pm 0.08$
c = Concentration [g/L]
ν = Specific volume [L/g]

Results

System of study:

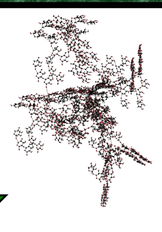
Tracer: Streptavidin protein



M_W = 52.8 kDa
R_H = 3.04 nm
R_C = 2.45 nm
D_0 = 0.081 nm²/ns

Graphical representation of a crystallographic structure of Streptavidin protein.

Obstacle: Dextran macromolecules

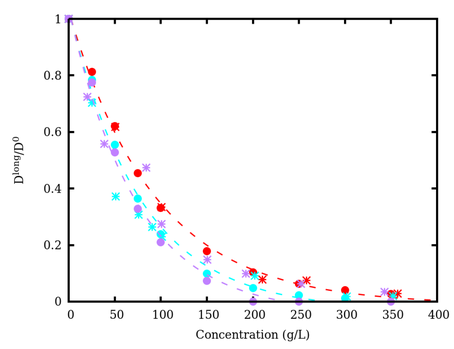


Graphical representation of a model of D50 dextran.

Dextran	D50	D400	D700
M_W [kDa]	48.6	409.8	667.8
R_H [nm]	5.2	13.5	16.8
R_C [nm]	2.3	4.7	5.5
D_0 [nm²/ns]	0.047	0.018	0.015

Radial distribution function

D^{long} study

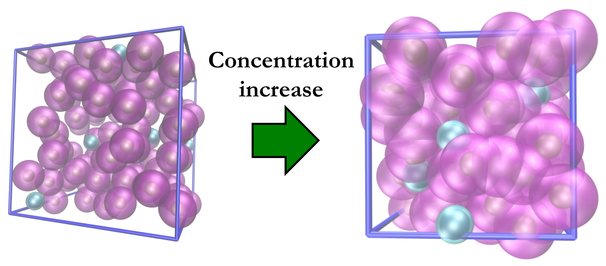


Normalized D^{long} simulation results for Streptavidin diffusing among D50 (red circles) , D400 (cyan circles) and D700 (purple circles) dextran obstacles at different macromolecular concentrations. Experimental results obtained for the same systems in Ref [2] are also plotted as squares. The displayed lines are only a guide of the simulation tendency. The simulation results exhibit very good agreement with the experimentally reported. The results show a clear D^{long} decay with Dextran size.

Radial distribution function between Streptavidin and D50 particles obtained in BD simulations with macromolecular concentration ranging between 25 to 350 g/L. As the macromolecular concentration increases, the folded state become more populated allowing the macromolecular diffusion in highly crowded media.

Conclusions

- The computational model proposed successfully reproduces the experimental behaviour of macromolecular diffusion in crowded media.
- The Continuous Shouldered potential provides a good description of the macromolecular folding expected in a crowded solution.
- Macromolecular conformational changes are found crucial to properly describe the excluded volume and macromolecular diffusion in crowded media.
- The simulation results show a clear decay of D^{long} with obstacle size. It is caused because excluded volume is a function not only of concentration but of obstacle size.



Snapshot of the simulation box at two different macromolecular concentration : 50 g/L (Left) and 250 g/L (Right).

Bibliography

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