

POLITECNICO DI TORINO

Department of Mechanical and Aerospace Engineering
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Novel Concept Device for Sensing Nanomolecules Concentration by Water Diffusivity at the Nanoscale



Tutors:

Prof. Pietro Asinari
Dr. Matteo Fasano

Student:

Ruben Minoggio

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We want to know...The answer to life,
the universe, everything.

We'd really like an answer. Something
simple.

The Hitchhiker's Guide to the Galaxy

This thesis is dedicated to my family that gave me the opportunity to go all over the world to acquire as much knowledge as possible.

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LIST OF ABBREVIATIONS

CECs	Contaminants of E merging C oncern
CNT	Carbon N ano T ube
DNA	D eoxyribo N ucleic A cid
DW-MRI	D iffusion- W eighted M agnetic R esonance I maging
ESA	E uropean S ace A gency
FF	F orce F ield
GROMACS	G RONingen M Achine for C hemical S imulations
GROMOS	G RONingen M Oolecular S imulation computer pro- gram package
GUI	G raphical U ser I nterface
HWHM	H alf W idth H alf M aximum
LJ	L ennard- J ones
MD	M olecular D ynamics
MSD	M ean S quare D isplacement
NASA	N ational A eronautics and S pace A dministration
NSAID	N onsteroidal A nti- I nflammatory D rug
PDB	P rotein D ata B ank

LIST OF ABBREVIATIONS (continued)

POCT	P oint O f C are T esting
PSs	P riority S ubstances
PWD	P rint W orking D irectory
QENS	Q uasi- E lastic N eutron S cattering
QM	Q uantum M echanics
RMSD	R oot- M ean- S quare D eviation
SAS	S olvent A ccessible A rea
SPC/E	E xtended S ingle P oint C harge
SPIO	S uper P aramagnetic I ron O xide
SWCNT	S ingle- W alled C arbon N ano T ube
UWWTD	U rban W aste W ater T reatment D irective
WANA	W ater N anoconfinement evaluation package
μDADs	μ microfluidic D iatomite A nalytical D eVICES
μPBDs	μ microfluidic P aper B ased D eVICES

LIST OF SYMBOLS

$barn$	Bound scattering cross section ($10^{-24}cm^2$)
c_0	Speed of Light $2.997\,924\,58 \times 10^8\,m\,s^{-1}$
$\frac{D}{Dt}$	Lagrangian time derivative
$\frac{\partial}{\partial t}$	Eulerian time derivative
D_B	Bulk self-diffusion coefficient of water
D_C	Confined self-diffusion coefficient of water
fs	Femtosecond
g	Gravitational acceleration
k_B	Boltzmann constant $1.380\,648\,52(79) \times 10^{-23}\,J\,K^{-1}$
m	Mass
μs	Microsecond
ns	Nanosecond
ps	Picosecond
r	Interatomic distance
R_g	Radius of gyration
s	second
t	Time

LIST OF SYMBOLS (continued)

u	Velocity
U_C	Coulomb potential
U_{EFF}	Effective potential energy
U_{VDW}	van der Waals interactions included by a Lennard-Jones potential
V_{inf}	Total volume of influence of water
V_{wat}	Total volume accessible to water
$\mathbf{V}$	Potential energy function
δ	Length of nanoconfinement
ε_k	Depth of the potential
ρ	Density
ρ_{bulk}	Density of the bulk water
μ	Viscosity
σ_k	The diameter of the sphere that describe the atom
τ	Stress tensor
θ	Scaling coefficient
θ'	Scaling coefficient modified for V_{inf} overlap
∇	Nabla operator

LIST OF SUBSCRIPTS

b	Bulk
C	Coulomb
EFF	Effective
i	i-th atom
inf	influence
wat	water
VDW	van der Waals

ABSTRACT

As the concern of sustainability and countries welfare becomes bigger, the need of developing new technologies in the field of water treatments becomes fundamental. For this purpose a new field of study is gaining terrain in the field of sensing the contents of pollutants in water : Nanotechnologies. The best tool for studying the nanoscale is through Molecular Dynamics simulations. Using the powerful tool of molecular dynamics simulations, we investigated a concept type of sensor able to sense nano molecules concentration. In particular, the sensor is bioinspired by the algae called Diatoms, which are formed by a lattice structure of nanopores. The concept sensor is a silica tablet constituted by a lattice array of nanopores able to trap pollutants or biomolecules inside the pores. Once the particles are inside the pores, thanks to D-MRI, is possible to compute the self-diffusion of water and, finally, by using a scaling law is possible to compute the concentration of pollutants or biomolecules inside the pore.

In the first part of this thesis, the main concern is to show the importance that water has on our world with all its implication on the welfare of countries. In particular are shown some problems bounded with water and some field of science in which water is gaining interest.

Molecular dynamics method is presented in chapter two with the techniques used to study water diffusion at the nanoscale.

From the third chapter, we will describe molecular dynamics protocols and simulations. In particular, we will draw attention on different silica nanopores filled with nanoparticles and proteins, with the aim of studying their effect on self-diffusion coefficient of water. Finally

ABSTRACT (continued)

all the data collected during the MD simulations are used in support of a scaling law able to predict the concentration of pollutants or biomolecules in a nanopore given the measure of the self-diffusivity of the nanoconfined water molecules.

CHAPTER 1

INTRODUCTION

1.1 Water and the World

The entire history of humankind is strictly bounded with water. Water is the fundamental block of our existence; as an evidence, the amniotic fluid contained by the amniotic sac, where our existence began, is constituted of around 99% of water. Our body too is constituted by more or less 75% [2] of water, and a loss of few percentage can cause suffering of dehydration and symptoms connected to it like muscular and cerebral weakness. Moreover we can survive several days without food but not without water.

The eldest civilizations settled their empires near rivers or lakes in order to guarantee the constant presence of water in their lifetime. Nowadays, new problems involving water are arising due to climate change and overcrowded cities. Climate change is producing an increase of the temperature all around the globe causing the melting of glacier and the subsequent rising of the level of the oceans. We need only consider the example of Miami, where the rising of ocean level is producing more and more often flooding, exposing the inhabitants and the infrastructure of this coastal city to problems that has never faced [3].

Water is essential for life, as we know it. A lot of space missions of NASA or ESA are focused on searching water on exoplanets due to its implication on potential habitability of our specie [4]. An habitable world is defined as a world with liquid water at its surface [4]. This definition

can be considered without any doubt true for our life in the universe, but there can be fluids other than water as cradle of life like silicon biochemistry in liquid nitrogen [5] or complex hydrocarbon chemistry based on methane-ethane.

1.1.1 Water and the Climate Change

Renewable energies are becoming fundamental in our society. In the past decades, the energy production was based mainly on fossil fuels, but with the awareness of climate change, countries have started to look on others way of producing energy. The most common technique of producing renewable energy are: solar panels, wind turbines, tide motion devices and hydropower.

The distribution of water all around the world is not equally dispersed causing the creation of regions with different possibilities of having access to water. This effect is magnified also due to climate change.

Nowadays we are hearing more and more frequently concerning about climate change and its implication on the future. Such concerns range from desertification, floods, melting of the polar shell, to the waters war, human health, people migrations and economic stability of some regions of the world. All these problems have the recursive presence of water. So a better understanding of water properties at any level could be an Ariannes thread to face these overwhelming problems.

1.1.2 Water Treatments

An important topic that is gaining more concerns is the treatment of water. The two main treatments that the superficial water is supposed to accomplish are: waste water treatment and drinking water treatment. For what concerns the waste water treatment, it is regulated by an European directive, the so called UWWTD [6]. Urban waste water treatment directive is an European directive concerning the treatment and the release of the waste water produced by human activities, both in the urban and industrial context. The Directive was born with the aim of safeguarding the environment from improper waste water discharges [6].

The treatment of wastewater can be achieved in different ways:

1. CAS: conventional activated sludge [7]. The process provides for an aeration tank, in which the wastewater is biologically digested , and a clarifier tank, in which the sludge is removed. Finally the clarified water is subjected to disinfection treatments [8].
2. Trickling Filters: a particular biofilter [9]. This treatment is constituted by an filter covered by a biofilm, having the aim of digest organic material in wastewater, over which wastewater is continuously sprayed on.
3. Lamellar separator [10]. This process separate solid particles from water thanks to a lamellar separator.
4. Rotating Biological Contractors: this technique uses discs submerged in the wastewater to separate heavy particles from water [9].

5. Swirl-flow separator [11]. It works by injecting water tangentially inside a circular funnel tank, which produces a second radial flow that separate solid particles[11].
6. BAF: biological aerated filter [12]. The process is able to remove solid particles, ammonia and carbonaceous matter [12].
7. Membrane Processes [7].

For what concerns the treatment for the drinking water, the following operations are available::

1. Coagulation[13]. Aluminum sulfate is added to water in order to neutralize negative charges, in this way the dimensions of neutralized particles is bigger and easier to remove.
2. Flocculation[13].This is the starting point of the treatment. The addition of particular chemicals with positive charge bound with negative charged particles present in the water and together they made flocs.
3. Sedimentation: thanks to gravity, the flocs fall to the bottom of the tank[14].
4. Filtration: the remaining water pass through filters like charcoal with different pore sizes in order to remove the presence of impurities diluted in water[14].

The treatments above are the traditional ones are not foreseen any treatment regarding a systematic removal of nanoparticles or other nanopollutants diluted in water. Nanoplastic pollution, for example, is an extremely important problem that it came to light thanks to the development of nanotechnologies. This type of pollution will negatively affect human and earth wide health [15]. Processing operations with the aim of eliminate nanoparticles from

water are still elitist (in a way of highly specialized operations) and extremely expensive. So, a better understanding of water and molecular sieve are fundamental for the future spread and implementation of equipment for water treatment.

1.1.3 Water Pollutants

Another problematic concerning water is its contamination by emerging pollutants, like the abovementioned nanoplastic [15], antibiotic [16] and organic matter [17]. The spreading of the medicine, the diffusion of intensive cultivation in agriculture and the increasing welfare of peoples life, lead to the inevitable development of a lot of chemical substances use to achieve specific goals. Until now few studies have been done on the consequences of the release of these substances in water [6]. This unrestricted and uncontrolled release of emerging pollutants requires a deeper understanding in order to have a clear view of the water that surround us. Some information have been already collected for what concerns the priority substances (PSs) and the Contaminants of emerging concern (CECs) [6]. The CECs are those pollutants that European countries must control in the superficial water according to the Directive 2013/39/EU of the European Union. Moreover, the European union has also established a Watch List of Decision 2015/495/EU. The directive 2013/39/EU of the European parliament imposes a strictly control on different substances. The two most important substances that we can find in the directive are:

1. *Diclofenac*, that it belongs to the family of nonsteroidal anti-inflammatory drug NSAID and is used as a pain reliever and inflammation reduction.

2. *17 α -ethinylestradiol*, that it is a synthetic androstane steroid and it is used as hormonal contraceptives.

It is trivial that these two pharmaceutical products are widely used all over the world, so they have to be taken seriously under control. As a matter of fact, few years ago emerged a problem concerning the decline of the vulture (*Gyps Tenuirostris*) population in Asia. At first glance it would be considered as an irrelevant problem, but what was discovered was that the vultures decrease was due to a problem in the functioning of their kidney caused by eating livestock carcasses that were treated with diclofenac [18; 19]. At this point it is obvious to control and study its effect on human population.

Nanoscale water

We will investigate water as nanoconfined water. Most of the work has been done for what concern bulk water and its properties; but nowadays with the increase usage and development of nanosciences the study of nanoconfined water becomes fundamental. For example, nanotechnologies are used to create devices at the nano scale that are able of producing unidirectional spinning rotation. Such devices are nano-turbines made up of a carbon nanotube as shaft with graphene blades. In order to design and create these particular turbines, the fundamental tool for studying it is thanks to the molecular dynamics simulations. From the study of these nano-turbines emerged that their behavior, for what concerns rotation rate and fluid flow, with respect to the macroscopic one with a rotation rate 15 times smaller considering the same flow caused by the disturbance of water flow nanoconfined [20].

Due to the fact that these nano-turbines are flow-driven, it becomes fundamental the study

of the behavior of water near a carbon nanotube shaft and graphene blades which are very important for the development of nano-turbines.

1.2 Bioinspiration: Diatoms

qui devi dire che, data l'importanza di emerging pollutants e la disponibilit  di nanotecnologie che regolano le propriet  dell'acqua alla nanoscala, siamo interessati a studiare nanotech per il sieving e sensing di emerging pollutants prendendo ispirazione dalla natura...etc etc

The inalienable research on the improvement of the quality of our sick world is substained by nanotechnologies. Facing emerging pollutants can be mainly achieved by studying the nanoscale and in particular water properties at the nanoscale. We are especially interested to implement nanotechnologies for sieving and sensing purposes of emerging pollutants.

In particular we started digging into the study of nanoconfined water thanks to bioinspiration¹. As a matter of facts in nature exists algae called diatoms (Domain:*Eukaryota*, Superphylum:*Heterokonta*, Phylum:*Ochrophyta*, Class:*Bacillariophyceae*) namely unicellular microorganisms that are enveloped by a porous silica shell Figure 1 [21].

The shell is composed of a lattice structure of well ordered nanopores with diameters ranging from 3 to 65 nm [23]. These microorganisms are responsible for producing more or less 20% of the oxygen of the entire planet. Recently, diatom frustules structure have been used for different types of advanced technologies in the field of microfluidic devices; an example are the microfluidic diatomite analytical devices ($\mu DADs$). These devices use natural diatom frustules

¹Bioinspiration is a way on how humans get inspired by the surrounding nature. In this context humans try to replicate functions and materials resulting from the natural world.

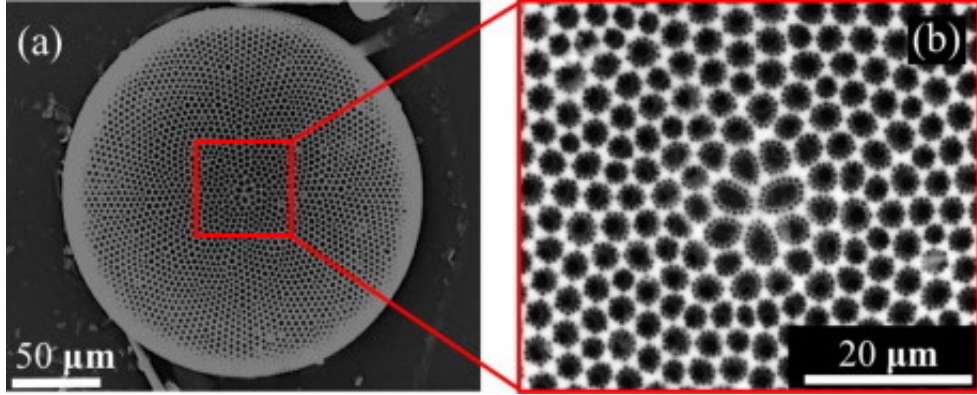


Figure 1: SEM micrograph of a diatom frustule with average pore size of $\phi = 5nm$. Figure adapted from Reference [22]

as sieve for the detection of illicit drugs [24]. This technique is able of detecting part per billion quantities of drugs, becoming an highly sensitive method of detection [24]. The advantage of $\mu DADs$ is the low cost of production and simple constructing techniques, like photolithography [25]. Another type of devices called microfluidic paper-based devices ($\mu PBDs$) are used in the clynical analysis field, in order to have an instrument easily usable in critical environment (such as in battlefield territory or in third world countries)[25]. It comes out that devices based on nanostructure, like those cited above, lay the groundwork for two big class of devices:1) biosensing; 2) POCT (Point of care testing). Diatoms can be also used for drug delivery as shown by Uthappa et al. [26]. Other scientists have used diatoms for indoor air purification[27]. In the latter case biological diatom frustules are coated by titania in order to create a device able to diminish the concentration of acetaldehyde in the air[27]. This is a case of using a nanoporous material as photocatalyst device. In general the performance of a catalyst is increased with

the increase of its surface area. Diatoms presents a high surface area over grams of material ($115 \frac{m^2}{g}$). This characteristic is of big interest in the conversion field of renewable resources into fuels[28].

In this thesis, we take inspiration from the diatoms structure to conceptualize a sieving and sensing device for emerging pollutants and/or biomolecules.

1.3 Concept Silica Sieve and Sensor

Combining the issues of monitoring and eliminating substances that can have negative effects on human body, and the bioinspired structure provided by diatoms, we came out with the idea of a nanoporous silica sieve(Figure 2). The silica sieve can be composed of nanopores with different diameters ϕ able to exclude molecules larger than the pore. This molecular sieve could be potentially used for medical purposes, like dialysis processes, or for science and engineering research field, like protein detection and water treatments (e.g. emerging pollutants).

In the following chapters we will perform molecular dynamics simulations of proteins in water and proteins inside a silica nanopore. The simulated structure is composed of a single nanopore due to the symmetry of the concept silica tablet.

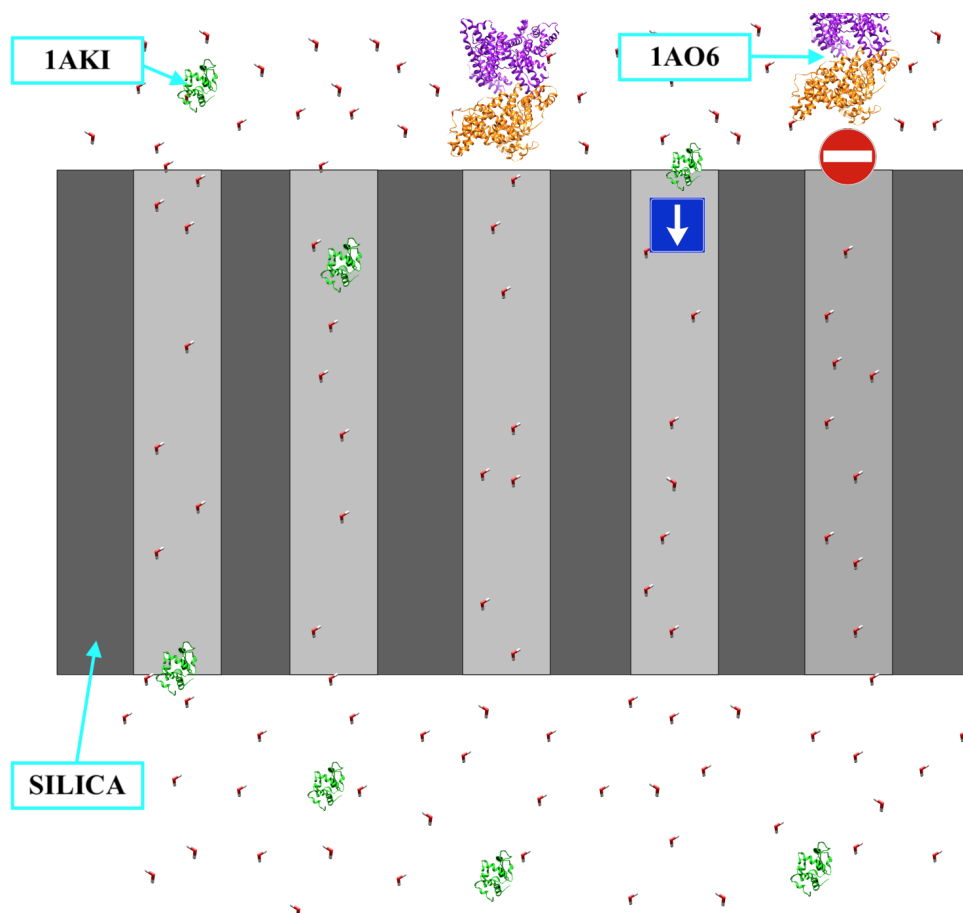


Figure 2: Silica sieve. Schematic representation of the sieve that, in this case, allows the diffusion of the 1AKI lysozyme (green) through its pores; whereas, the 1AO6 human serum albumin (magenta and orange) is excluded due to steric effects. The red and white spots represent water molecules, where proteins are solvated.

The silica sieve can be used not only for filtration issues but also as sensor. The process, as it is represented in Figure 3 starts by putting a diatom-like tablet of silica inside a becher filled with water and a certain concentration of nanoparticles (contaminants or biomolecules) to be detected. At the steady state, the nanoparticles diffuse within the pore and the tablet is removed from the becher. At this point using particular techniques like DW-MRI (*Diffusion-weighted magnetic resonance imaging*), we are able to compute the diffusivity D of water molecules nanoconfined in the silica nanopores filled with the nanoparticles, and so the concentration of nanoparticles inside the solution.

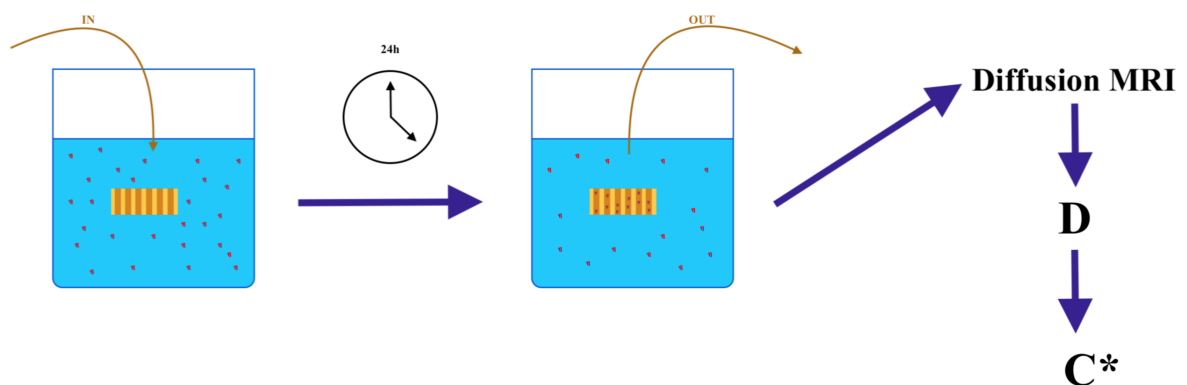


Figure 3: Concept silica sensor.

Moreover, depending on the concentration of nanoparticles in the solution, the number of nanoparticles that will diffuse inside the silica nanopores in the same span of time will be different and simply dictated by Fick's law.

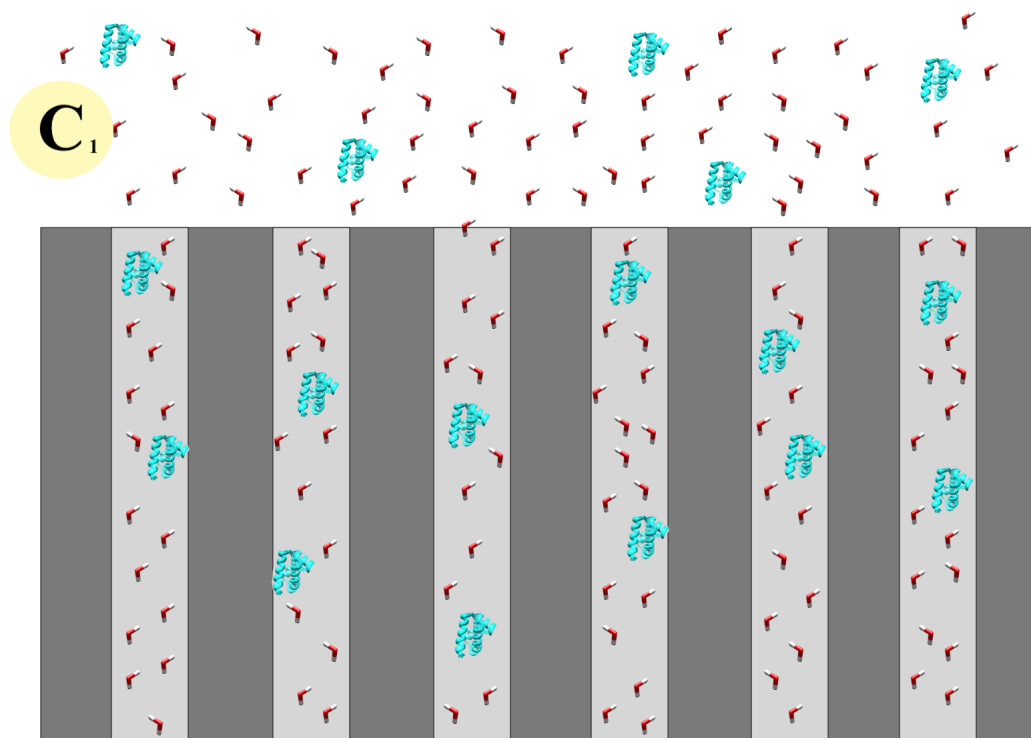


Figure 4: Low number of proteins trapped inside the pore due to a low concentration of proteins inside the solution.

Higher concentration leads to an high number of proteins trapped inside the pore, opposite for low concentration (see Figure 4 and Figure 5). Here, we employ molecular dynamics sim-

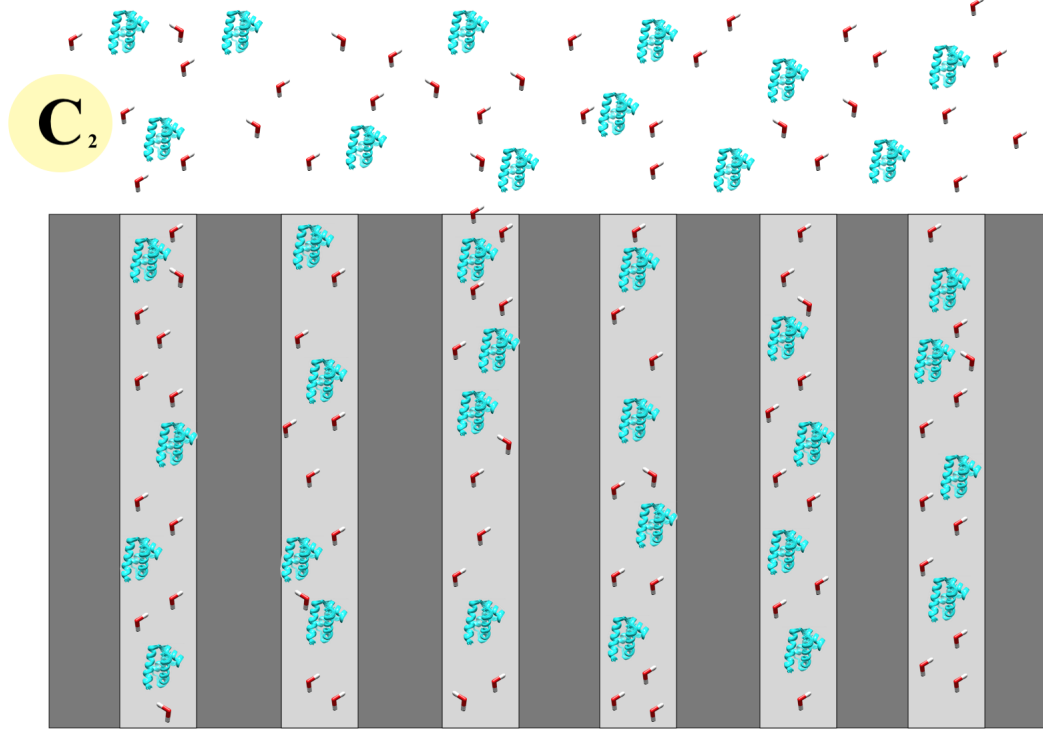


Figure 5: High number of proteins trapped inside the pore due to an high concentration of proteins inside the solution.

ulations in order to calculate the self-diffusivity of water nanoconfined in the silica nanopores filled with various concentration of nanoparticles. Such quantity can be also predicted by a scaling law, which allows to estimate D as a function of the geometrical, physical and chemical characteristics of the nanoconfined setup. The novel concept sensor is based on such scaling law for the water self-diffusivity under nanoconfined conditions, since it allows to compute the nanoparticle concentration from a simple measure of D in the filled nanopores.

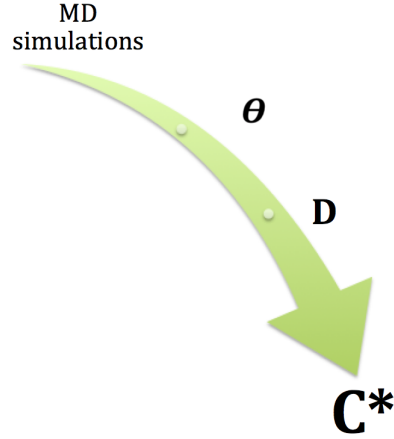


Figure 6: Flow for binding self-diffusion coefficient to concentration.

The process depicted in Figure 6 allows the creation of an archive of diffusivity values linked to a certain concentration. As a matter of fact, thanks to MD simulations we are able to compute the scaling parameter θ which is needed to compute the diffusion coefficient which, in turn, is linked to a certain concentration of molecules simulated at the beginning of the process. Then, once the scaling law is validated for all the possible cases, the need of performing MD simulations is no more necessary because we can use the plot of the scaling law in the reverse mode (i.e. with D-MRI we compute the diffusion coefficient, then from the plot we can obtain θ that with the length of nanoconfinement δ I can compute the solvent assessible area (SAS) and finally using SAS and the geometric properties I can obtain the concentration). In Figure 7, the blue dotted line represent the traditional process of finding the self-diffusion coefficient from MD simulations, while the dotted green line is the way in which we want to operate

(i.e. as abovementioned, starting from the diffusion obtained thanks to D-MRI we can obtain parameters necessary to know the concentration value of the solution).

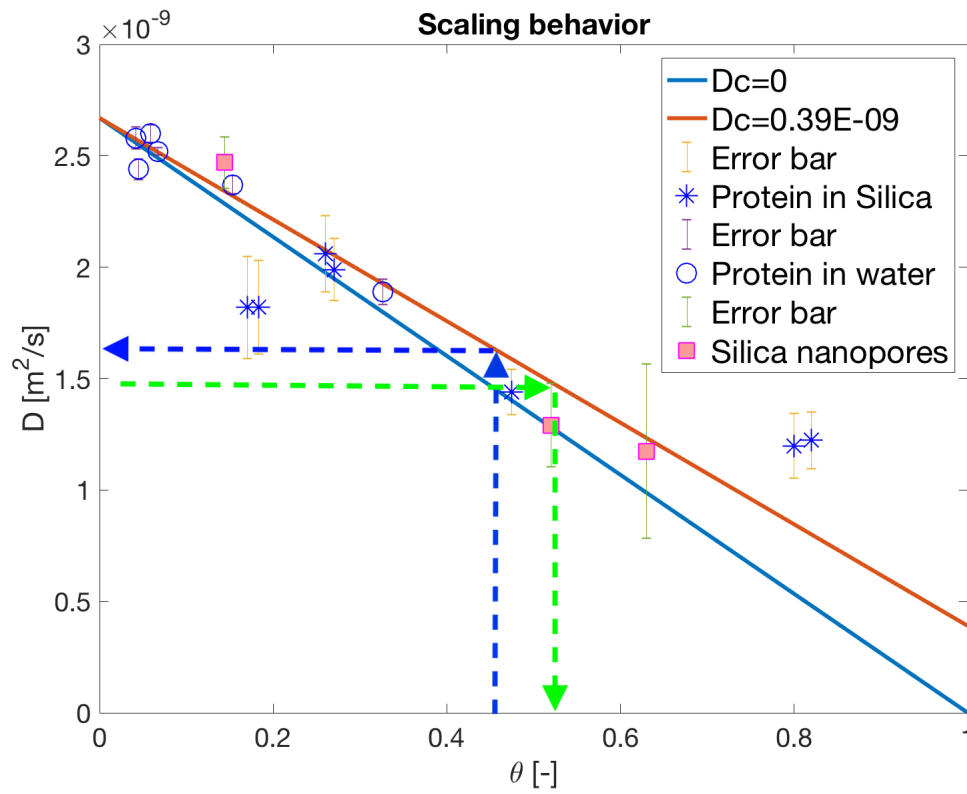


Figure 7: Plot of the scaling behaviour of self diffusion coefficient needed to obtain parameters for computing the concentration.

In this way the process of computing the concentration at the nanoscale could become quicker and less expensive in terms of computational power and time.

CHAPTER 2

THEORETICAL BACKGROUND

In this chapter we will show the benefits of modern multi-scale models and, as a consequence, our decision of exploiting molecular dynamics simulations to understand and improve our macroscale world. So, a description of molecular dynamics method is given in order to understand the main properties and its field of usage.

2.1 Multi-Scale Modelling

The entire variety of physical models can be imagined to be represented by a pyramid called "Hierarchy Pyramid of Physical Models" , as reported in Figure 8.

The way used in the past to set a physical model was that of considering just one scale. In this way, the model was based on that scale by considering the contributions of smaller and higher scales as constitutive relations. For example, structural engineer use continuum mechanics for the study of the structures and condense everything related to the atomic levels as additional constitutive equations. On the contrary, scientists that study solids at atomistic level, consider the macroscale as homogeneous.

There exist two basic ways of obtaining the constitutive relations:

1. The constitutive relations are created starting from experimental data using linearization and Taylor expansion. The implementation of this method is not always so easily achieved when we have to deal with micro or nano scales.

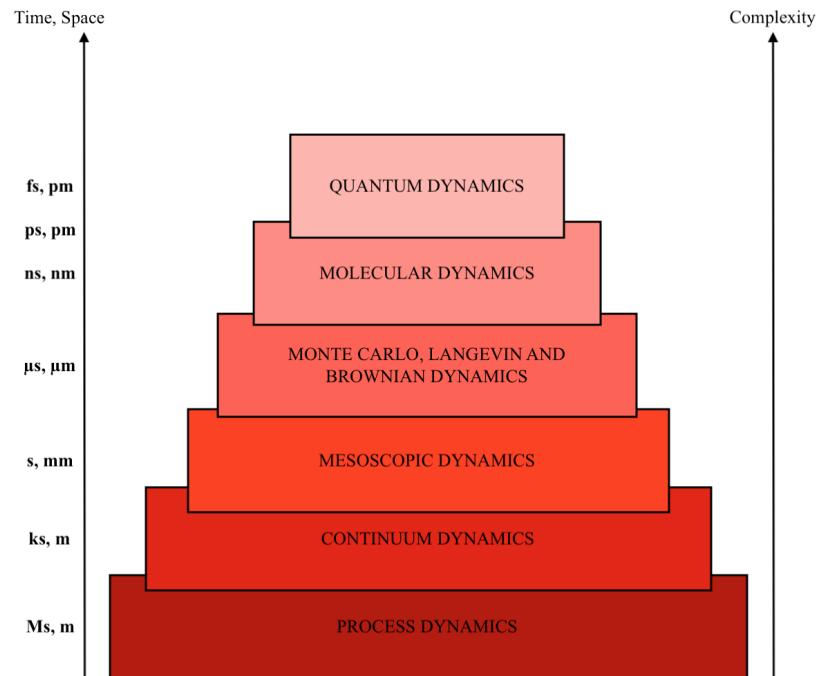


Figure 8: Hierarchy Pyramid of Physical Models

2. Using the principles of quantum mechanics. This way of proceeding is way too complicated for everyday problems.

In order to face this dilemma, multi-scale approaches hold a fundamental role. As suggested by the name, multi-scale approaches are based on combining at least two different scales, and resulting in a model that shows the benefits of both scales. An example of multi-scale model is the quantum mechanics- molecular mechanics in the field of chemistry, and the Car-Parrinello molecular dynamics [29]. The former model is used to deal with the reaction of large molecules, where the region near the reaction is modeled by means of QM, while the surrounding with a

classical representation. For what concerns Car-Parrinello model, forces of the system are not calculated using experimental data but from electronic models.

The main goal that multi scale modeling is supposed to achieve is to understand macroscale effects from micro- or nano-scale characteristics.. Constitutive relations are based on experimental data. For example [29] if we consider the continuity equation for an incompressible fluid:

$$\nabla \cdot \mathbf{u} = 0 \quad (2.1)$$

and the conservation of momentum equation:

$$\rho \frac{\partial \mathbf{u}}{\partial t} + \rho (\mathbf{u} \cdot \nabla) \mathbf{u} = \nabla \cdot \tau \quad (2.2)$$

considering τ a function of $\nabla \mathbf{u}$ the constitutive equation of incompressible fluids is:

$$\tau = -pI + \mu \nabla \mathbf{u} + \mu (\nabla \cdot \mathbf{u})^T \quad (2.3)$$

that substituted in Equation 2.1 produces the Navier-Stokes equation:

$$\rho \frac{D\mathbf{u}}{Dt} = -\nabla p + \mu \nabla^2 \mathbf{u} + \rho \mathbf{g} \quad (2.4)$$

The abovementioned simplification of the stress tensor(puts in connection the interactions at the microscopic scale with the macroscopic one), thus leading to a relation able to model very well the behaviour of incompressible newtonian fluids.

Unfortunately, it is not always possible to obtain a constitutive equation thanks to experimental data for complex systems. A solution for this problem may be to start from quantum many-body theory and solving the Schrödinger equation. In this way it is not necessary to guess any relation between the experimental data but the computation complexity is extremely high. As we have shown, the macroscale models based on experimental data fitting, works very well for non complex systems. On the contrary models based on quantum mechanics can give a myriad of information but are way too complex to implement on everyday systems. It becomes evident the necessity of a tool that fuse together micro and macro scale: Multiscale Modeling.

2.2 Molecular Dynamics

Molecular mechanics is used to investigate the macroscale behaviour by compuring microscale simulations. As it is shown in Figure 8, we are in the field of *ns* and *nm*, where atoms are considered as single entity rigid spheres carrying a net charge and polarizability. In order to model atoms and their interactions, the properties are found both by using quantum mechanics and empirical data. In molecular dynamics, the fundamental equation that it is solved, is Newton's law:

$$\mathbf{F}_i = m_i \frac{\partial^2 \mathbf{r}_i}{\partial t^2} \quad (2.5)$$

$$\mathbf{F}_i = -\frac{\partial V}{\partial \mathbf{r}_i} \quad (2.6)$$

where m_i is the mass of the the i -th atom and $\mathbf{r}_i$ is the distance between two atoms. In Equation 2.6 V is the empirical potential energy function. This function is the Force Field

needed to carry on subsequent MD simulations. A Force field $V(r)$ is the entity that models the interactions between the atoms. The force field is constituted by:

$$V = V_{bonded} + V_{non-bonded} \quad (2.7)$$

where the first term takes into consideration the bonded interactions, while the second the non-bonded ones.

$$V_{bonded} = V_{stretch} + V_{bend} + V_{torsion} \quad (2.8)$$

$$V_{non-bonded} = V_{Lennard-Jones} + V_{electrostatic} \quad (2.9)$$

In Equation 2.8 the $V_{stretch}$ is an harmonic potential modelling the bond stretching of two atoms covalently bonded¹, V_{bend} is an harmonic potential representing the vibration between 3 atoms² and $V_{torsion}$ is defined in two planes and four atoms and it can be in turn broken down in proper³ and improper⁴ dihedral. For what concerns Equation 2.9, $V_{Lennard-Jones}$ is a potential that

¹ $V_b(r_{ij}) = \frac{1}{2}k_{ij}^b(r_{ij} - b_{ij})^2$. In which r_{ij} is the actual distance, b_{ij} is the equilibrium distance, k_{ij} is the is a fictitious spring stiffness

² $V_b(\theta_{ijk}) = \frac{1}{2}k_{ijk}^\theta(\theta_{ijk} - \theta_{ijk}^0)^2$. In which θ_{ijk} is the angle between the three atoms and θ_{ijk}^0 is the equilibrium angle

³ $V_{pd}(\phi_{ijkl}) = k_\phi(1 + \cos(n\phi - \phi_s))$. In which k_ϕ is a force constant of the cosine potential.

⁴ $V_{id}(\xi_{ijkl}) = k_\xi(\xi_{ijkl} - \xi_0)^2$. In which ξ_0 is the reference angle.

takes into consideration Van der Waals interactions¹ while $V_{electrostatic}$ it models the Coulomb interaction between two charged particles² These inter-atomic potentials are obtained both empirically and thanks to quantum mechanics.

2.3 Techniques for Nanoscale Water Diffusion

This technique is used to study how the confinement condition of a system influences its dynamics [30]. The inelastic scattering of neutrons can be described as follow:

$$\hbar\omega = \Delta E = E_i - E_f = \frac{\hbar^2}{2m} (k_i^2 - k_f^2) \quad (2.10)$$

$$\hbar\mathbf{Q} = \hbar(\mathbf{k}_i - \mathbf{k}_f) \quad (2.11)$$

being E the energy and $\mathbf{k}$ is the wavevector of the beam (f initial beam, i scattered beam) [30]. So $\hbar\omega$ refers to the energy variation of the neutron. $\mathbf{Q}$ represents the momentum transfer [30].

In general, there exists three types of neutron scattering:

1. Elastic Neutron Scattering
2. Quasi-Elastic Neutron Scattering

¹ $V_{LJ}(r_{ij}) = 4\epsilon_{ij} \left(\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^6 \right)$. In which ϵ_{ij} is the depth of the potential, σ_{ij} is the radius of the sphere that approximate the atom.

² $V_{electrostatic}(r_{ij}) = f \frac{q_i q_j}{\epsilon_r r_{ij}}$, where $f = \frac{1}{4\pi\epsilon_0} = 138.94$, ϵ_0 is the dielectric constant of vacuum and ϵ_r is the relative dielectric constant

3. Inelastic scattering

The first one is characterized by no energy exchange, which means $\hbar\omega = 0$. In the second one we have a fraction of energy exchanged. The last one is characterized by an energy exchange and so a variation of the wave vectors length of a scattered neutron [30].

We are interested in QENS because it is used to study dynamics phenomena in different field of science, in particular hydration water, protein dynamic behaviour [30]. So the quasi-elastic neutron scattering is used to study diffusion problems at the nanoscale. Moreover, this technique can individuate and separate diffusion properties from confined dynamics. As for MD simulations, the scale of investigation in terms of time and space are nanometers and nanoseconds. This peculiarity put at the same level the results coming from Molecular dynamics simulation and Quasi-elastic neutron scattering.

Going deep in the Quasi-elastic neutron scattering, the number of neutrons scattered by a sample are represented by the total scattering cross section:

$$\sigma = \frac{I_s}{I_0} = 4\pi b_i^2 \quad (2.12)$$

where I_0 is the incoming flux impinging the specimen and I_s is the number of scattered neutrons per second [30]. Due to the fact that usually samples are not made of a unique isotope, the length b_i is composed of a coherent and an incoherent part [30]. This leads to a reformulation of the total scattering cross section:

TABLE I: Neutron scattering cross sections [1]

	$\sigma_{coherent}[barn]$	$\sigma_{incoherent}[barn]$
Hydrogen	1.76	80.3
Oxygen	4.23	0.001
Nitrogen	11.01	0.500
Silicon	2.16	0.004
Carbon	5.55	0.001

$$\sigma = \sigma_{coherent} + \sigma_{incoherent} \quad (2.13)$$

For example, hydrogen has a big $\sigma_{incoherent}$ of more or less 80 barns as it is shown in Table I. This peculiarity of Hydrogen make the Quasi-elastic neutron scattering an extremely important tool to study water molecules[30].

The total scattering cross section is calculated as follows:

$$\sigma = 4\pi b_i^2 = 4\pi (b_{coherent}^2 + b_{incoherent}^2) \quad (2.14)$$

being b_i the scattering length.

TABLE II: Methods used for water diffusion analysis

Raman Spectroscopy [32]	NMR [33]	QENS	MD
-------------------------	----------	------	----

Thanks to the high value of scattering cross section of the hydrogen atoms, QENS method can be used to probe diffusive proton motion [30]. The diffusion of water for what concerns the translational motion can be expressed by the Lorentzian HWHM [31]:

$$\Gamma(Q) = \frac{D_t Q^2}{1 + D_t Q^2 \tau_0} \quad (2.15)$$

where D_t is the self diffusion coefficient.

As we have shown in the previous sections there exist different methods used to study water diffusion. Some of these techniques are listed in table Table II

2.4 Mass Transport Properties at Nanoscale

Studies and articles on nanoscale mass transfer have increased a lot in the last few years. The importance of nanoscale properties were spotted in many different fields of science, from medical (Drug delivery) [34; 35] to engineering (Mass transport for archival memory, CNT mass conveyor) [36; 37; 38] to geology [39].

Water is the so called universal solvent [40] because a lot of substances can be dissolved in it; these substances are called hydrophilic, while the substances that cannot be dissolved in

water are called hydrophobic. Moreover, due to the fact that water is an excellent solvent, is difficult to find water as a pure liquid.

The water properties in nanoconfined conditions is important for advanced technologies based on nanoscale applications.

Different behaviors of nanoconfined water have been found respect to the bulk case. For example let us consider the Wenzel equation [41] in the field of *Wetting Theory*:

$$\cos \theta_A = r \cos \theta_Y \quad (2.16)$$

where θ_A is the apparent contact angle, and

θ_Y is the Young contact angle,

r is the surface roughness.

At macroscale this theory is used to find the contact angle of a water's droplet with the surface beneath; in other words, it tells us that, at macroscale, there exist an inversely proportional correlation between the contact angle of a droplets like that of Figure 9 and the surface roughness [41]. What it comes out from the study by M.S. Ambrosia and M.Y. Ha using MD simulations is that at nanoscale roughness and contact angle are proportional.

On other studies such as that related to the nanofluidic viscosity, they found that the travelling surface wave acts on the viscosity and on the mass flow rate [42]. It is also worth to cite a recent study on capillary filling of confined water, in which they found that the viscosity

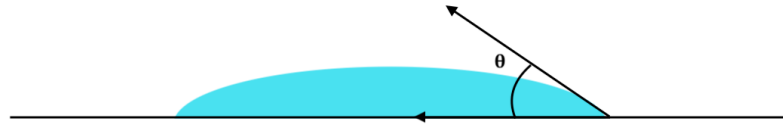


Figure 9: Droplet of water with Young angle lower than 90°

of water inside nanopores with diameters lower than 10 nm is higher compared to that of bulk water[43].

Diffusion is a phenomenon that happens always. For what concerns the situations in which gases are present in mesoporous environment, the most used diffusion mechanism adopted is the Knudsen one [44]. In this type of diffusion mechanism, the collisions take place with the wall of the nanotube. For what concerns the Brownian motion, the diffusion takes place between the molecules. There exist different type of diffusion regimes There exist also different diffusion quantities and coefficients:

1. D_t , Fick diffusion coefficient
2. D , self diffusion coefficient

Fick diffusion coefficient D_t bounds the diffusive flux¹ to the concentration. The self diffusion coefficient D will be the main parameter extrapolated from MD simulation done during this thesis work. D is a coefficient used to describe the free mixing of molecules without any concentration gradient, at equilibrium[45].

¹ $J = -D_t \frac{dc}{dx}$. The equation represents Fick's diffusion equation in which J is the diffusive flux expressed in $\frac{mol}{m^2s}$. D_t is the diffusion coefficient, c is the concentration. The equation tells that a solution constituted by higher concentration of solute will diffuse to region with lower concentration.

CHAPTER 3

SIMULATION METHODS

During this thesis work the main software that was used is **GROMACS 5.1** [46].

GROMACS stands for *Groningen Machine for Chemical Simulations* and it is a software used for molecular dynamics simulations of systems composed by thousands of atoms. The most common systems simulated using *Gromacs* are proteins and lipids. In our case, we have simulated not only proteins in water, but also protein and magnetite particles (Fe_3O_4) inside a silica nanopore (SiO_2).

As a complementary software to Gromacs for the analysis of self-diffusivity of water, we have used **WANA** routine [47].

3.1 Molecular Dynamics Protocol

Gromacs has no GUI so everything is managed via the command-line.

In Figure 10, as an example, the different command needed to perform a MD simulation carried out in this thesis are reported, starting from a PDB file to the analysis of the obtained data.

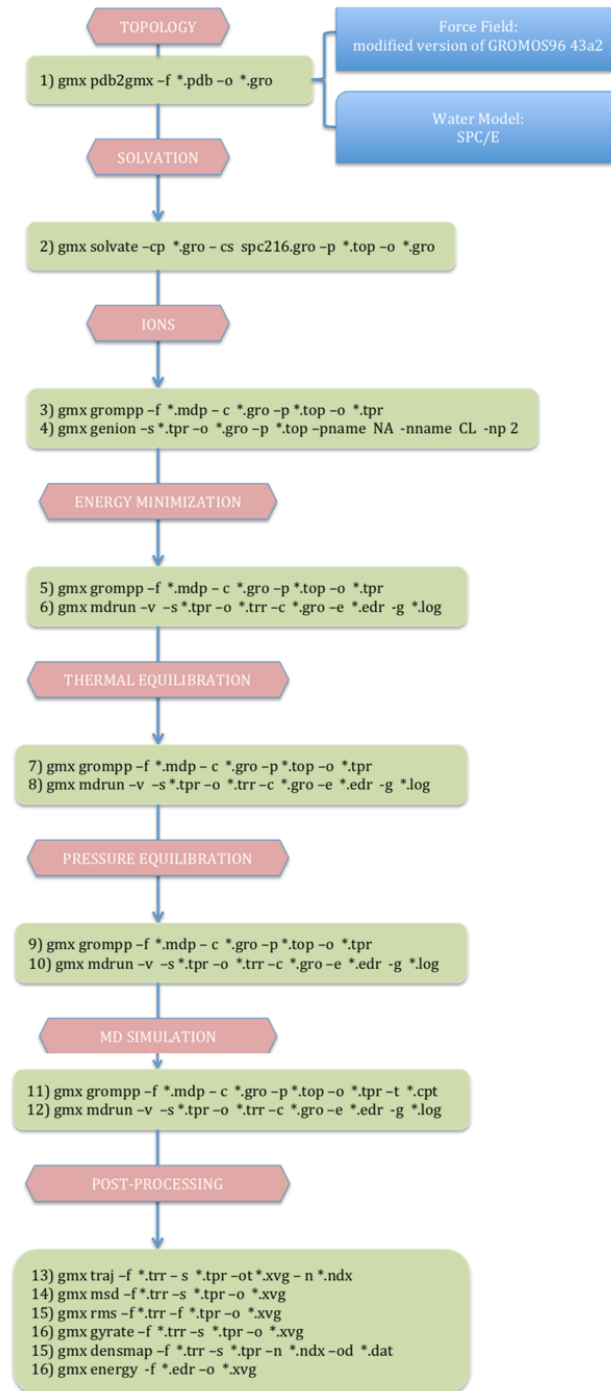


Figure 10: GROMACS simulation flow.

The first step of the simulation is the generation of topology. In this case, the proteins have been downloaded from [48] in *.pdb format; while silica nanopore have been taken from [49]. In order to create a topology file *.top in addition to the geometry file *.pdb, a force field and a water model are required. The force field used is a modified version of GROMOS96 43a2. The original GROMOS96 43a2 force field is commonly used for studying proteins in water [50],[51]. The modified version of the force field was developed by Matteo Fasano [49] for taking into account bonded and non-bonded interaction of the silica.

The second step concerns the solvation, so the addition of water molecules in the system. The outputs of this step are a new geometry file *.gro and a *.top file, both updated with the informations about the solvent.

The third step is needed only if in the system is present a net charge: in this case, we have to add ions. For doing so the first operation is to use *gmx grompp* in order to pre-process the molecular topology file and transform it into an atomic topology file *.tpr. After that, is possible to use *gmx genion* to replace water molecules with ions in order to obtain a net charge $q_{tot} = 0$.

The fourth step involves the energy minimization of the system. As before, first of all, *gmx grompp* is used, then *gmx mdrun* is applied. This step is fundamental in order to relax the system by reducing energy peaks.

The fifth and the sixth steps have both the aim of equilibrating the system for what concerns pressure and temperature. The simulation run was performed using a leap-frog algorithm in order to integrate Newton's equation of motion with a time step of 0.001 *ps*. The simulation box was

set to be periodic with periodic boundary conditions (PBC), and a $1.2nm$ cut-off distance for the short range neighbor list.

For what concerns Van der Waals interactions, a cut-off type is chosen with a $1.2 nm$ cut-off distance for Lennard-Jones and Buckingham. Due to the fact that we are dealing with a system composed by thousand of particles, a PME (Particle Mesh Ewald) method was used. This method is based on transforming the original system into a meshed one, in which every part of the lattice is characterized by a density value. The mesh is spaced by $0.16 nm$.

The same gromacs commands of the energy minimization are used, namely *grompp* and *mdrun*.

Finally, the MD simulation is performed and the data that comes out are obtained using different commands as shown in the last box in Figure 10. The output files of the post-processing phase are commonly *.xvg files that can be visualized by *xmgrace -nxy *.xvg*. Instead the *.dat files are imported in MatLab as numerical matrix in order to be better managed.

Moreover, in some cases we have extended the simulations up to $2ns$ in order to obtain a better convergence of self-diffusion values. For this aim we used two additional command right before the post processing:

```
gmx convert -tpr -s 1ns.tpr -f trajectory.trr -extend 1000 -o 2ns.tpr
```

```
gmx mdrun -v -s 2ns.tpr -o *.trr -c *.gro -e *.edr -g *.log
```

TABLE III: Gromacs extention files

<i>File extention</i>	<i>Function</i>
.gro	Molecular structure of the system (number of atoms, position of the atoms).
.pdb	Molecular structure of the protein system (position of the atoms).
.top	Topology of the system.
.mdp	Molecular dynamics parameters of the system.
.tpr ¹	Molecular structure of the system at the beginning of the simulation.
.trr	Output trajectory of MD simulation (created with <i>mdrun</i>).
.edr or .ene	Portable energy file.
.ndx	It is an index file of the whole system.
.xvg	Output file.

3.2 WANA

WANA is a software created with the aim of computing the characteristic length of nanoconfinement δ and the solvent accessible area (*SAS*). In order to let Wana computing δ and *SAS* four files are required:

- *atomSas.xvg*
- *connolly.pdb*
- *topology.top*
- *ffnonbonded.itp*

¹It is a portable binary run input file created by means of *grompp*.

The two former files are obtained using Gromacs command:

```
gmx sasa -f *.trr -s *.tpr -o sas.xvg -oa atomSas.xvg -q connolly.pdb
```

The *.xvg file contains the average area per atom.

The *.pdb file contains the informations about Connolly surface.

For what concerns the other two files, the *.top file contains the system topology and it is obtained using the first command of Figure 10. While the *.itp file contains the informations about the non-bonded interactions specified in the force field.

An exhaustive tutorial step-by-step of WANA software can be found in Reference[49].

3.3 Scaling Water Diffusivity at the Nanoscale

Water molecules display different behaviour based on where are positioned. As a matter of fact, when they are near a solid surface at the nanoscale, they are subjected to two additional constraints: 1) Coulomb, 2) van der Waals non-bonded interactions. Coulomb and van der Waals interaction will cause the layering of water at the solid-liquid interface (Figure 18), with a detrimental effect on the mobility of water[49; 43]. These additional interactions sum up creating an effective potential energy on water molecules:

$$U_{EFF}(n) = U_{VDW}(n) + U_C(n) \quad (3.1)$$

where

$$U_{VDW}(n) = \sum_{k=1}^{N_n} 4\varepsilon_k \left[\left(\frac{\sigma_k}{r_k} \right)^{12} - \left(\frac{\sigma_k}{r_k} \right)^6 \right] \quad (3.2)$$

$$U_C(n) = -E\mu_w \Gamma \left(\frac{E\mu_w}{k_B T} \right) \quad (3.3)$$

n refers to the directions perpendicular to the solvent accessible area that pass through the center of the i -th atom; while E is the electrical field strength, μ_w is the water dipole moment, k_B is the Boltzmann constant and Γ is the simplification of the Brillouin function called Langevin function $[\Gamma(x) = \coth(x) - \frac{1}{x}]$.

The characteristic distance of each atom δ_i is given by the difference between the two zeros of the equation:

$$U_{EFF}(n) + \alpha k_B T = 0 \quad (3.4)$$

where $\alpha \approx \frac{1}{4}$. In order to obtain an average characteristic length of the whole solid surface:

$$\delta = \frac{\sum_{i=1}^n \delta_i S_{local,i}}{S_{tot}} \quad (3.5)$$

where $S_{local,i}$ is the local solvent accessible area of the i -th atom, and $S_{local,i}$ is the total solvent accessible area. Such characteristic length determines the volume of water in the proximity of the solid surface whose diffusivity is significantly affected (i.e. reduced) by the non-bonded interactions with the solid.

Starting from the quantity δ , a scaling parameter θ can be then defined as:

$$\theta = \frac{V_{\text{inf}}}{V_{\text{wat}}} \approx \frac{\sum_{i=1}^N S_{\text{tot}}^i \delta^i}{V_{\text{wat}}} \quad (3.6)$$

where V_{inf} is the total volume of water whose mobility is significantly altered by solid-liquid interactions and V_{wat} is the total accessible volume of water. Moreover, in case of high nanoconfinement, we have an overlap of V_{inf} ; so in order to take it under consideration θ may be modified with the following equation:

$$\theta' = 1 - e^{-\theta} \quad (3.7)$$

θ can vary from 0 to 1. 0 is for bulk conditions while 1 is for completely confined conditions.

After having computed θ the self-diffusion coefficient of nanoconfined water can be estimated with an equation that depends only on the abovementioned scale parameter θ :

$$D(\theta) = D_B \left[1 + \left(\frac{D_C}{D_B} - 1 \right) \theta \right] \quad (3.8)$$

In Equation 3.8, D_B is the bulk self-diffusion coefficient founded in Table IV, and D_C is the self-diffusion coefficient of fully confined water.. For what concerns D_C , two values were used:

$$D_C = 0 \left[\frac{\text{m}^2}{\text{s}} \right] \text{ and } D_C = 0.39\text{E-}09 \left[\frac{\text{m}^2}{\text{s}} \right].$$

CHAPTER 4

NANOPARTICLES IN SILICA NANOPORES

In this chapter and in the following, MD simulations are carry on. Our aim is to run molecular dynamics simulations able to replicate the functioning of the concept sieve-sensor described in chapter 1. For this purpose two differents case-study are analyzed: Nanoparticles in silica nanopore (representing the emerging pollutants in water), and proteins in silica nanopore representing biological molecules that need to be detected. In particular the first simulation is focused on the computation of the self diffusion coefficient inside a silica nanopore. Silica nanopore will be used again in *Chapter 5* coupled with proteins.

2D plots are obtained using both *Matlab*®[52] or *Grace*[53]. For what concerns 3D images, they are obtained using *UCSF Chimera*[54].

4.1 Bulk Water

The main goal is to compute the self-diffusion coefficient of various system. So we started by computing the self-diffusion coefficient of bulk water at 300 K to get a reference value.

The starting point of this simulation was a *.gro file already existent in a Gromacs directory (pwd: /gromacs/share/tutor). The system that we built is a $7.0nm \times 7.0nm \times 7.0nm$ (with a total box volume of 343 nm^3) cubic box of SPC/E water. After having solvated the box, the total number of solvent molecules was 33675(Figure 11).

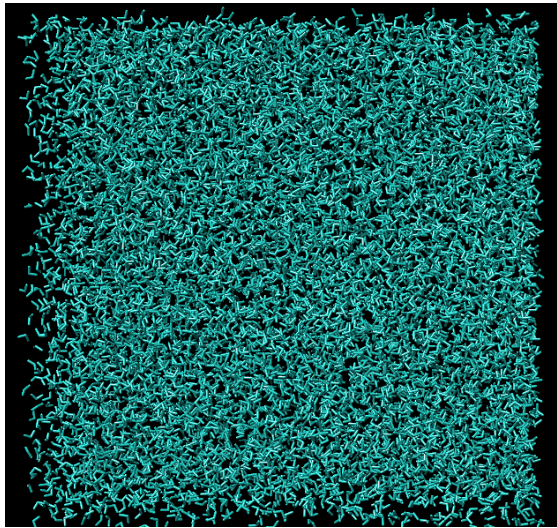


Figure 11: Water box filled with 33675 molecules.

Finally the self-diffusion coefficient that we found is $D = 2.6683(\pm 0.003) \cdot 10^{-9} m^2/s$. This value is in accordance with that found in other works [49; 55]

4.2 Water in Silica Nanopores

From now on, we will focus our attention on simulations where water molecules are nanoconfined within silica nanopores. The first simulation that was carried out has the aim of computing the self-diffusion coefficient of water in a cylindrical silica nanopore. The box dimensions used for the MD simulation are $5.41\text{nm} \times 5.11\text{nm} \times 7.03\text{nm}$.

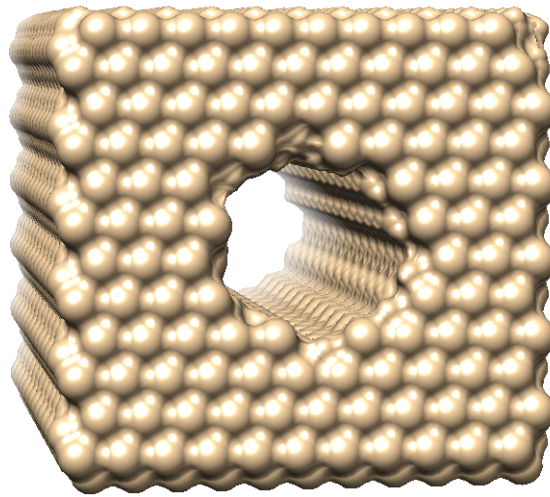


Figure 12: 2 *nm* silica nanopore.

Simulation's steps

The simulation steps to obtain the self-diffusion coefficient of water in the nanopore:

1. Creation of a simulation box.
2. Perform the energy minimization of the system.

3. Solvate the nanopore, namely introduce water molecules in the nanopore cavity.
4. Energy minimization of the system composed by nanopore plus water
5. Perform MD simulation in the NVT ensemble.
6. Extrapolation of information like: temperature, kinetic energy, potential energy, mean square displacement (MSD), mean density of water.

In detail, after having decided the simulation box of our system, the next step was solvating the pore using an SPC/E (extended simple point charge) water model, in which the water molecule is modeled as rigid entity subjected to both Coulomb and Lennard-Jones interactions.

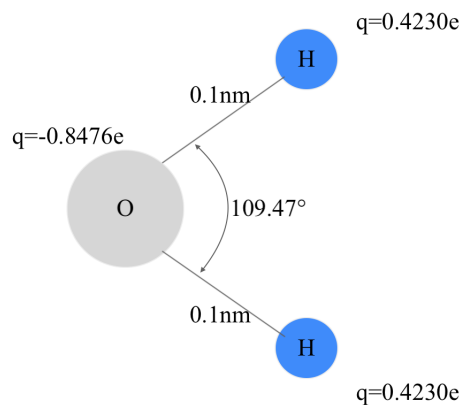


Figure 13: SPC/E water model.

Referring to the spc/e water model, the configuration of water molecule and the charges of atoms of Hydrogen and Oxygen are shown in Figure 13.

The solvation of nanopore added 518 water molecules (cyan particles in Figure 14).

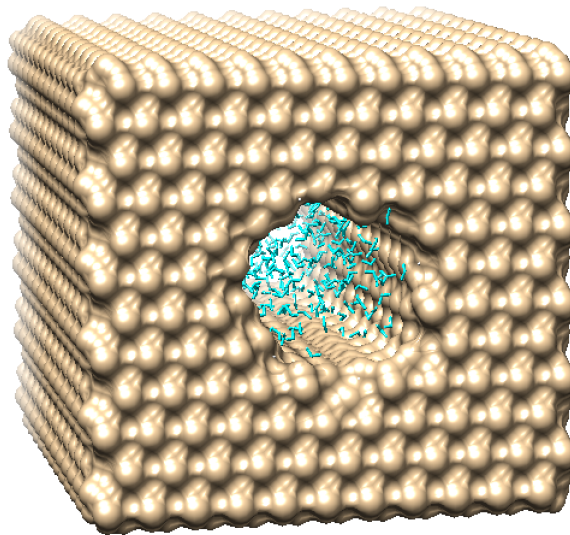


Figure 14: Silica nanopore filled with water molecules.

At the end of the simulation we obtained are presented in the following paragraphs.

The temperature trend with simulation time is represented in Figure 15, and shows that it is constant fluctuating around the value of $300K$, confirming the equilibrium state of the system.

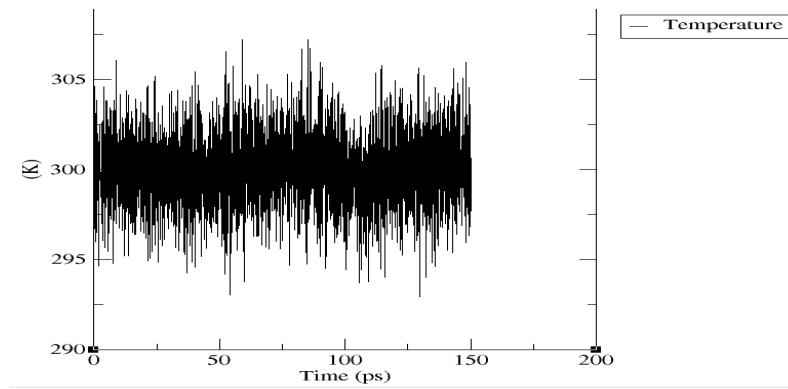


Figure 15: Temperature transient of water during the simulation.

The total energy conservation(Figure 16) of the system, results to be constant around a value of $2.53\text{e}+05 \text{ kJ/mol}$.

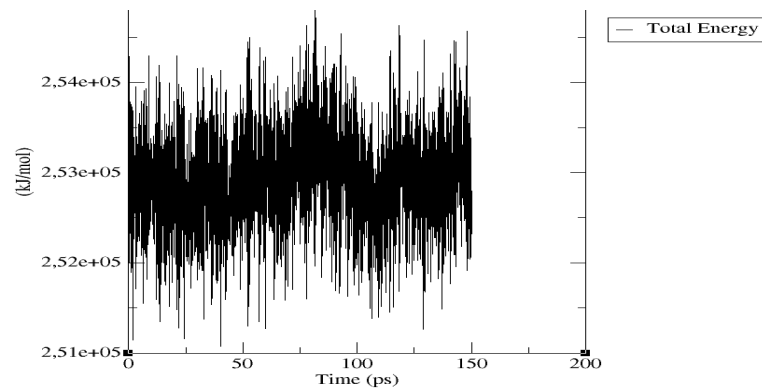


Figure 16: Total energy of the simulated system during the simulation.

For what concerns the evaluation of the mean density, the resulting plot is reported in Figure 17. From the plot we can notice an increased agglomeration of water molecules in contact with the nanopore surface (red peak region), which is a clear sign of water nanoconfinement.

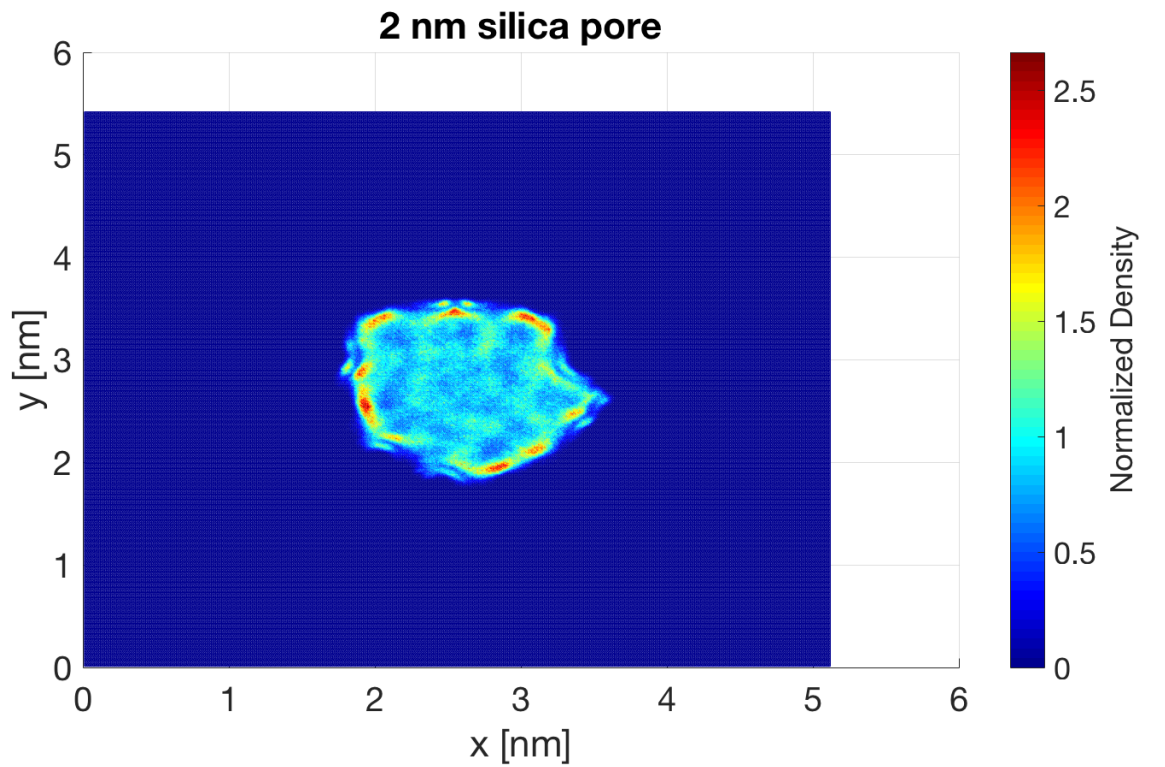


Figure 17: Mean density map of the water inside the nanopore.

In Figure 18 is represented the density radial profile, averaged along the pore axis is presented. The values of water density are scaled by the bulk water density $\rho_{bulk} = 1000 \frac{kg}{m^3}$. We

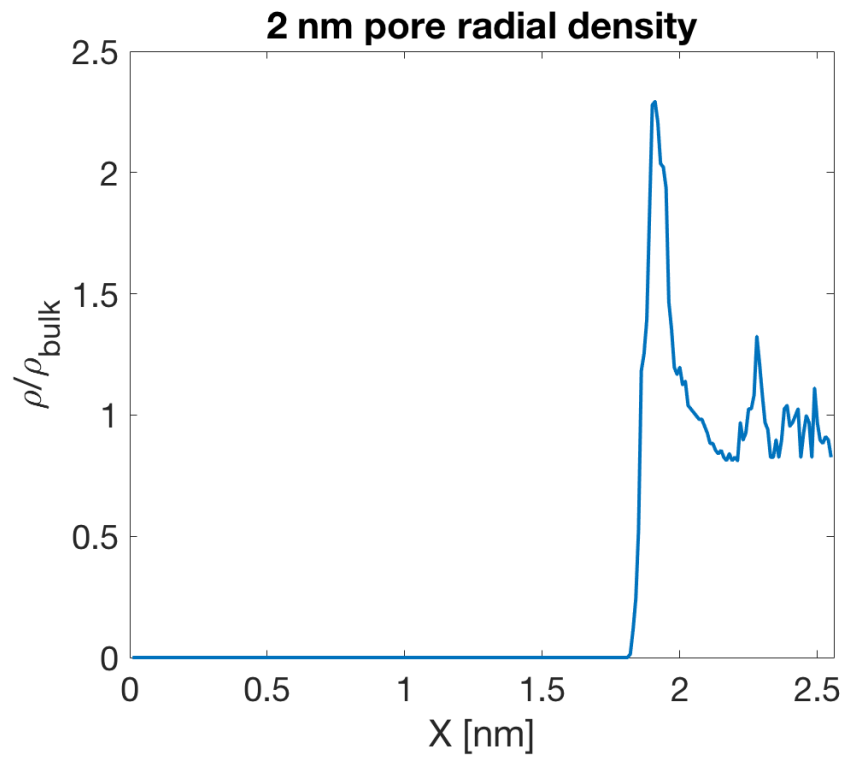


Figure 18: Radial density profile computed at the middle of the silica box of water averaged along the pore axis.

can notice a denser layer of water right near the interface with the silica wall, with a peak of $\frac{\rho}{\rho_{bulk}} = 1.36$. In Figure 18 from 0 *nm* to 2 *nm* there is the bulk part of silica box, while from 2 *nm* to 2.7 *nm* the half pore filled with water is depicted.

Finally, in order to compute the self-diffusion coefficient of water (D), the Einstein relation and the Mean Square Displacement(MSD) were used:

$$MSD = \lim_{t \rightarrow \infty} \langle \|r_i(t) - r_i(0)\|^2 \rangle_{i \in A} = 6Dt \quad (4.1)$$

The mean square displacement is used to measure the stochastic movements of molecules [56]. Molecules are subjected to a Brownian motion, that is constituted by a chaotic movement [56]. In three dimensions, the Brownian motion is represented by Equation 4.1, in which D is the diffusion coefficient and t is the lag time. For what concerns the MSD, the fitting was performed from 15 to 135 *ps*.

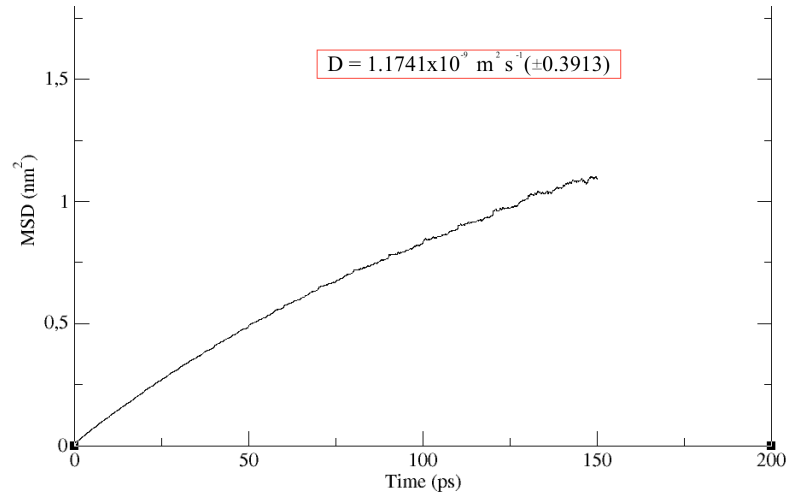


Figure 19: Mean square displacement of water.

4.3 Water in Silica Nanopores Filled with Nanoparticles

This third simulation is focused on computing the self-diffusion coefficient of water in a silica cylindrical nanopore (pore diameter equal to 8 nm) filled with 8 SPIOs (Super Paramagnetic Iron Oxide) nanoparticles. The box dimensions used for the MD simulation are 11.30 nm x 11.06 nm x 4.32 nm with a total box volume of 540.64 nm³.

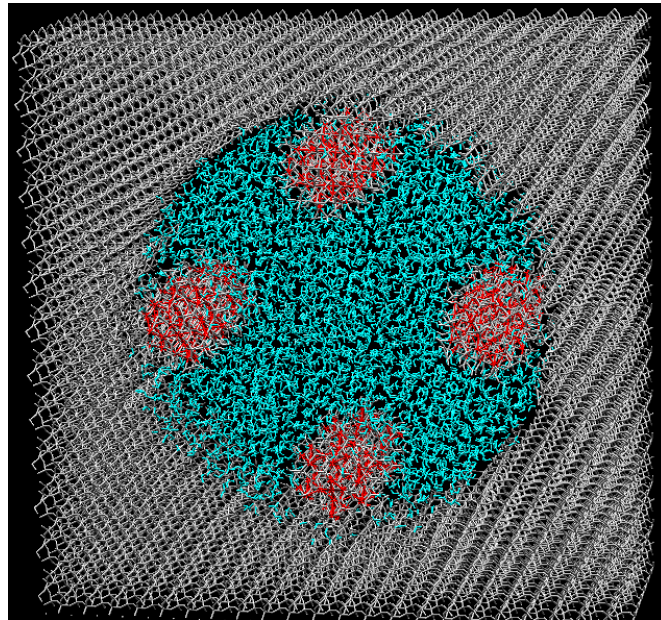


Figure 20: Silica nanopore with 8 SPIOs and nanoconfined water.

The structure of the pore is a solid crystal of silica, in which have been added 8 super paramagnetic iron oxide particles of magnetite (Fe_3O_4). The particles are positionned in the

inner diameter of the pore, 4 on the front side and 4 on the back side. The diameter of the nanopore is $\phi = 8nm$ contained in a unit cell of $11.30\text{ nm} \times 11.06\text{ nm} \times 4.32\text{ nm}$.

The simulation steps followed in order to obtain the desired self-diffusion coefficient of nanoconfined water are:

1. Creation of a simulation box.
2. Creation of the topology of the system.
3. Perform the energy minimization of the system.
4. Solvate the nanopore (and again the energy minimization).
5. Run the simulation for 400 ps.
6. Extending the simulation up to 1000 ps
7. Extrapolation of information like: temperature, kinetic energy, potential energy, mean square displacement (MSD), mean density of water.

The force field used for this simulation was a particular OPLSAA (Optimized Potentials for Liquid Simulation applied to All Atoms). For what concerns the atoms charge, in the topology file **.top* all atoms composing the bulk crystal have zero charge, while the atoms who are lying both on the surface of SPIOs and on the internal surface of the pore are characterized by a partial charge to mimic their hydrophilicity. Nevertheless the system has to be neutral with zero charge.

The simulation run was performed using a leap-frog algorithm in order to integrate Newton's equation of motion with a time step of 0.5 fs . The simulation box was set to be periodic with periodic boundary conditions (PBC), and a 1.5 nm cut-off distance for the short range neighbor list.

Due to the fact that we are dealing with a system composed by thousand of particles, a PME (Particle Mesh Ewald) method was used. This method is based on transforming the original system into a meshed one, in which every part of the lattice is characterized by a density value. The mesh is spaced by 0.12 nm .

The system is initialized at a temperature of 300 K , and the system is solvated using an SPC/E water model in which the water molecules are modeled as rigid entity subjected both to Coulomb and Lennard-Jones interactions. For what concerns the simulation that lasts 1 ns , during the solvation were added 4813 water molecules. Later we have performed another solvation of the box by adding an additional 873 water molecules to achieve the correct density of water at 300 K .

At the end of the simulation, we obtained a self-diffusion coefficient of water equal to $D = 1.2904(\pm 0.1873)10^{-9} \text{ m}^2/\text{s}$

The system temperature trend with time is represented in Figure 21, and shows that it is constant fluctuating around the value of 300 K thus confirming the equilibrium state of the system.

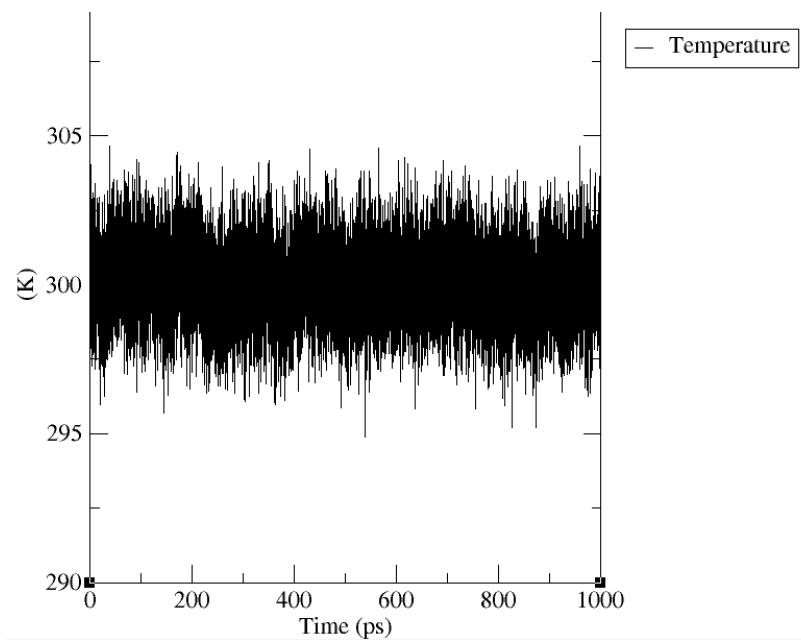


Figure 21: Constant temperature trend of the setup during the entire simulation.

The total energy conservation of the system considered on the whole simulation domain (0-1000 ps) can be considered oscillating around a value of $6.74 \times 10^5 kJ/mol$.

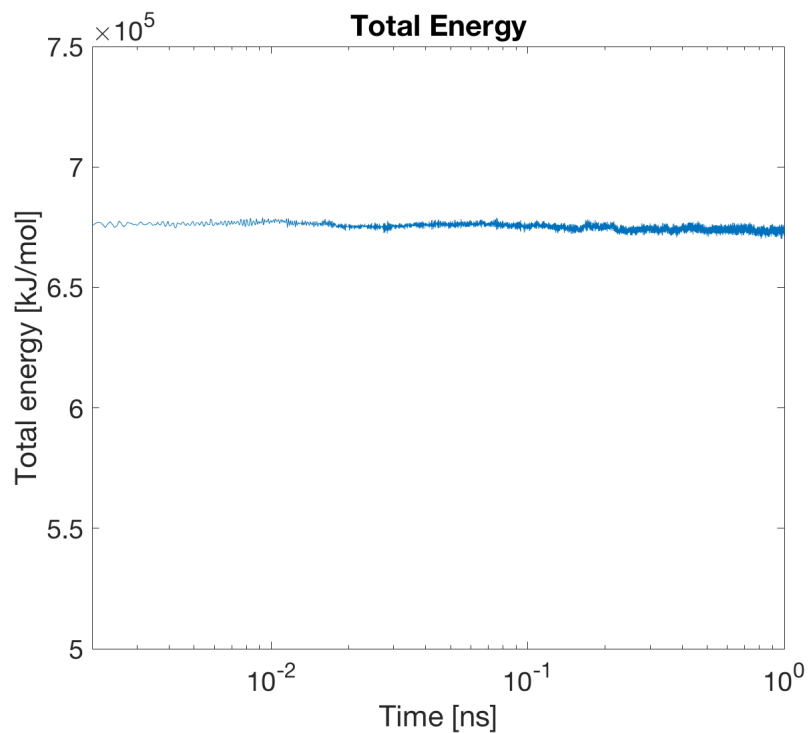


Figure 22: Total energy conservation on the whole simulation domain (0-1ns).

For what concerns the evaluation of the mean water density, the resulting plot is reported in Figure 23. From the plot we can notice an increased agglomeration of water molecules in contact with the nanopore surface (red peak region in Figure 24). This agglomeration is due to the fact that water, when is near to a solid surface, is subjected to van der Waals and Coulomb interactions.

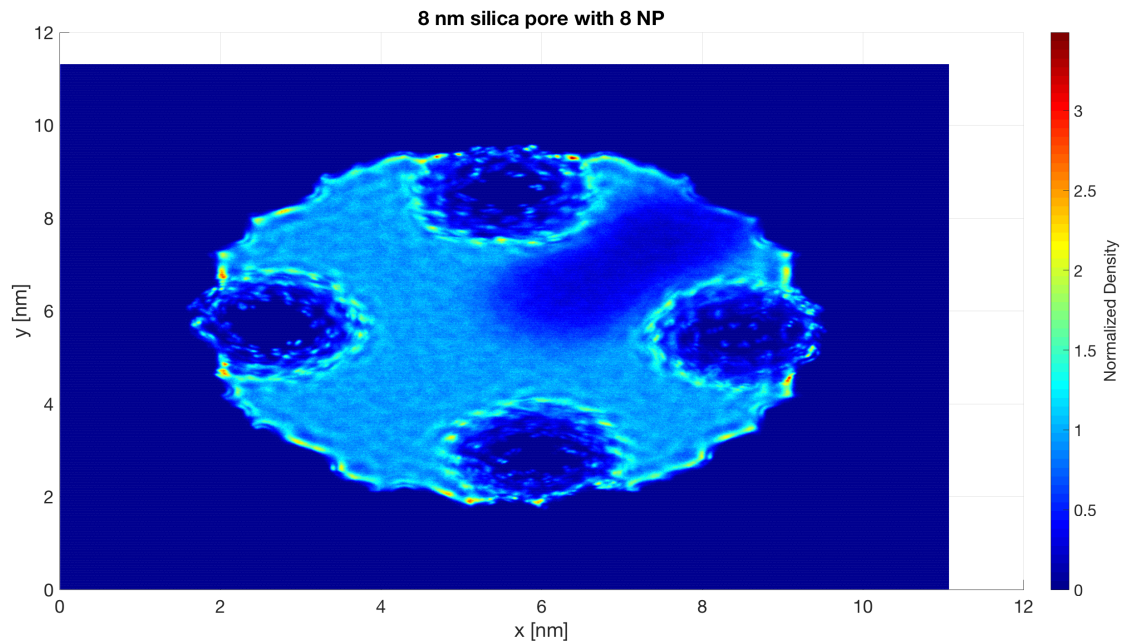


Figure 23: Density map of the system after $1ns$ simulation.

Finally, in order to compute the self-diffusion coefficient of water (D), the Einstein relation and the Mean Square Displacement(MSD) were used.

The mean square displacement's trend is linear showing that the motion is a diffusive one.

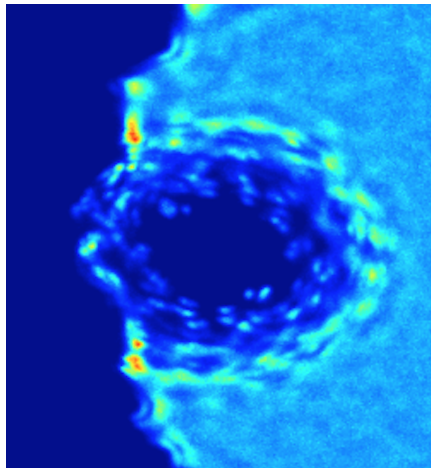


Figure 24: Zoom of the density map near the interface between the pore and the magnetite nanoparticle.

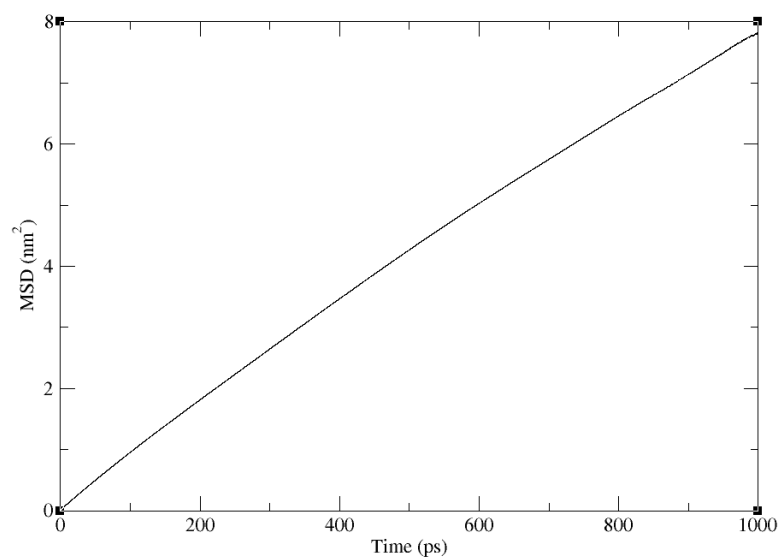


Figure 25: MSD fitted from 100 to 1000 ps.

CHAPTER 5

BIOMOLECULES IN SILICA NANOPORES

This chapter is focused on molecular dynamics simulations of proteins in a water box and, in particular, inside a silica nanopore. Water has a key role in what concerns protein behavior. As a matter of fact, depending on the content of water, proteins act in different ways[57]. Proteins are molecules embedded in living organisms with fundamental roles such as DNA replication and mean of transport of other molecules. Their structure is characterized by the presence of amino acid residues chains.

5.1 1UBQ Protein in Water

1UBQ is a protein with different roles in the dynamics of many cellular events, such as innate immunity.

The simulation's steps used for each protein solvated in water are:

1. Definition of the simulation box.
2. Production of a topology file¹.
3. Solvation of the box.
4. In some cases, addition of ions if the structure has a non-zero total charge.
5. Energy minimization, in order to relax the system.

¹As shown in Figure 10, a *.pdb is needed in order to obtain a topology file. In the *.pdb file that we used, we removed the crystal water to be able to completely solvate the system.

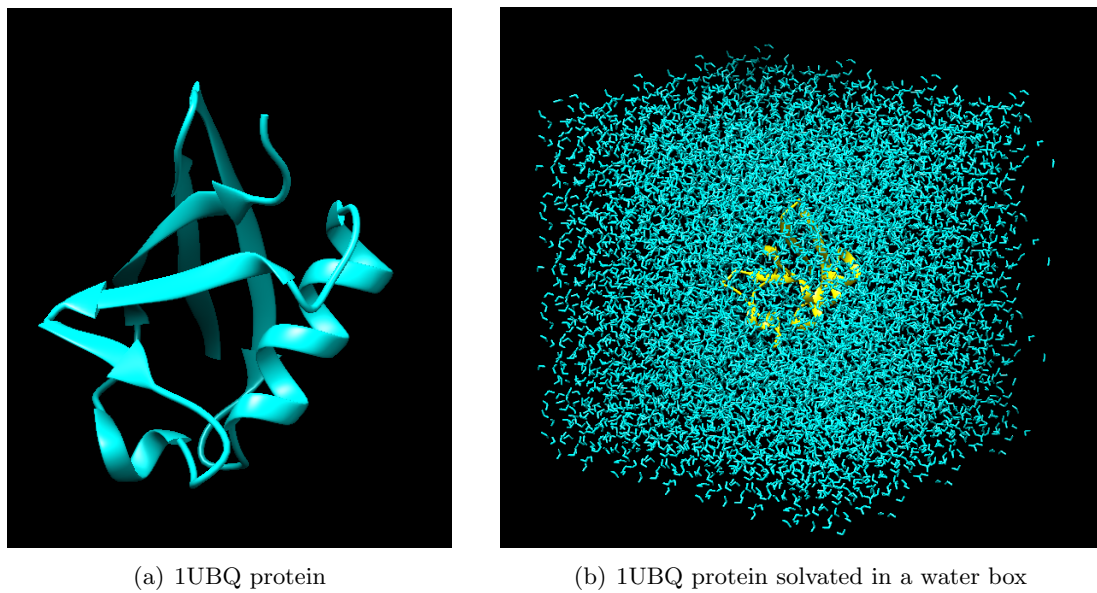


Figure 26: 1UBQ protein simulated by MD.

6. Equilibration of the solvent using a constant number of particles, volume and temperature (NVT).
7. Equilibration of the solvent using a constant number of particles, pressure and temperature (NPT).
8. Molecular dynamics simulation.
9. Post-processing of the MD trajectories.

Finally, in order to compute the self-diffusion coefficient of water (D), the Mean Square Displacement (MSD) ?? was used.

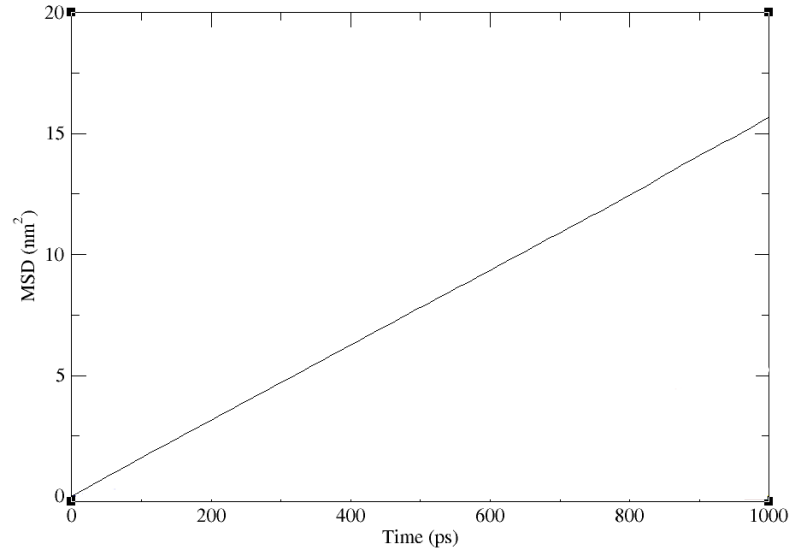
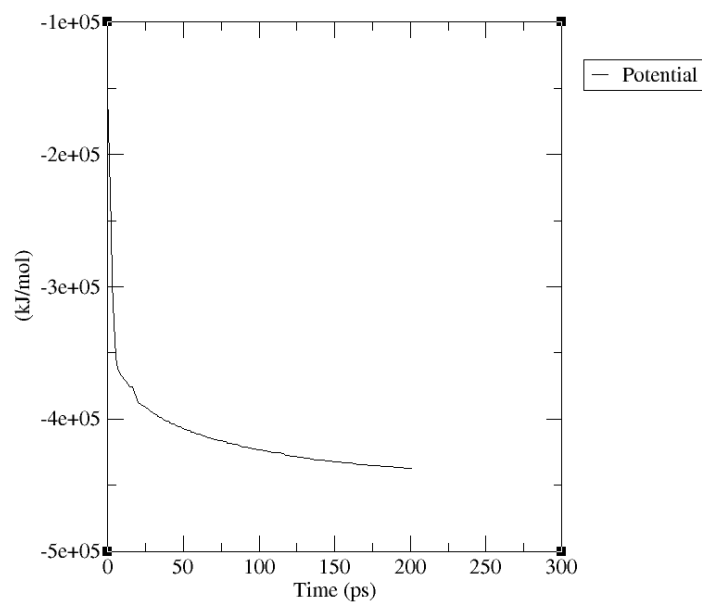


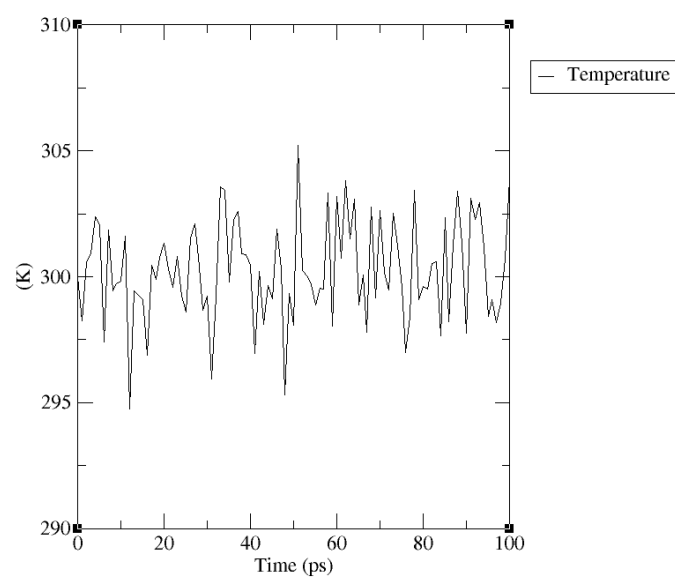
Figure 27: MSD fitted from 100 to 900 ps.

The mean square displacement's trend is linear (see Figure 27) showing that the motion is a diffusive one. The diffusivity value is $D = 2.5907(\pm 0.0175)10^{-9}m^2/s$. This value just diminish of 3% from that of bulk water. This result can be explained by the big box of water, which has a 6.49 nm of edge.

The temperature trend with time and the potential energy of the system are depicted in Figure 28. The temperature fluctuates around the value of $300K$, with an average value just above 300 K . For what concerns the potential energy, we can state that the energy minimization step was well performed with a decreasing trend until $-4.5 \cdot 10^5 \frac{kJ}{mol}$.



(a) 1UBQ protein potential energy after energy minimization.



(b) 1UBQ protein temeprature trend

Figure 28: 1UBQ potential energy and temperature trend.

5.2 1UBQ Protein in a Silica Nanopore

The step after having completed the simulation of 1UBQ in water was the simulation of the same protein inside a silica nanopore.

The dimensions of silica box are: $11.03\text{ nm} \times 11.03\text{ nm} \times 7.03\text{ nm}$, with a pore size of $\phi = 8\text{ nm}$.

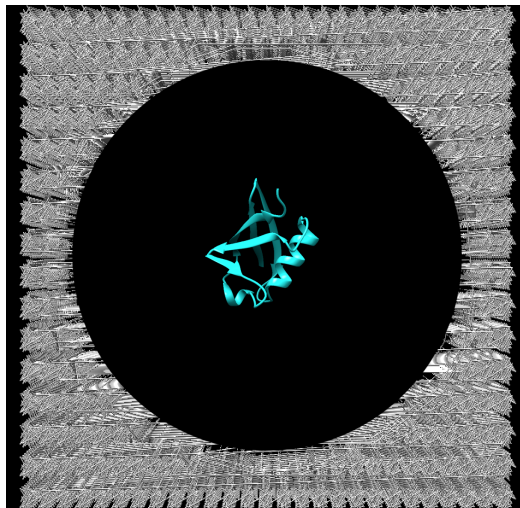


Figure 29: 1UBQ protein in a silica nanopore before solvation.

In this case, the first step was that of combining the topology of both silica pore and the protein; then was performed an energy minimization of the dry system Figure 30. At this point we proceeded with the solvation of the pore and another energy minimization. The energy minimizations have the goal of relaxing the system toward steady state conditions. Finally,

the equilibration steps include: The first one performed at a reference temperature of 300 K , Berendsen thermostat is applied to the water and the protein separately with a time constant $\tau_t = 0.1$; The second one performed with a Nosé-Hoover thermostat, at reference temperature of 300 K and time constant $\tau_t = 0.2$, attached to the protein.

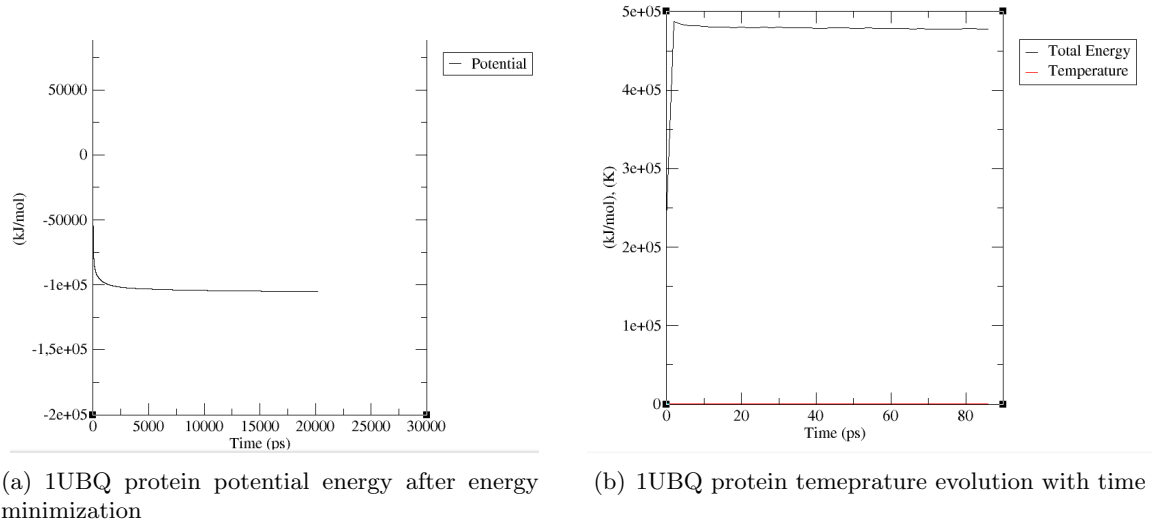


Figure 30: 1UBQ in silica pore: Total energy and temperature profile

As we can see from Figure 30 on the right hand side, the average total energy is 473841 kJ/mol and the mean temperature is 300.125 K . Finally, we have found a value of diffusivity of $D = 1.8235(\pm 0.2289)10^{-9}m^2/s$ fitted from 110 to 988 ps (Figure 31). So, we have a decrease

of 30% of D compared to the case in which the protein was in bulk water, and a decrease of 17% with the case of 8 nm silica nanopore filled just with water.

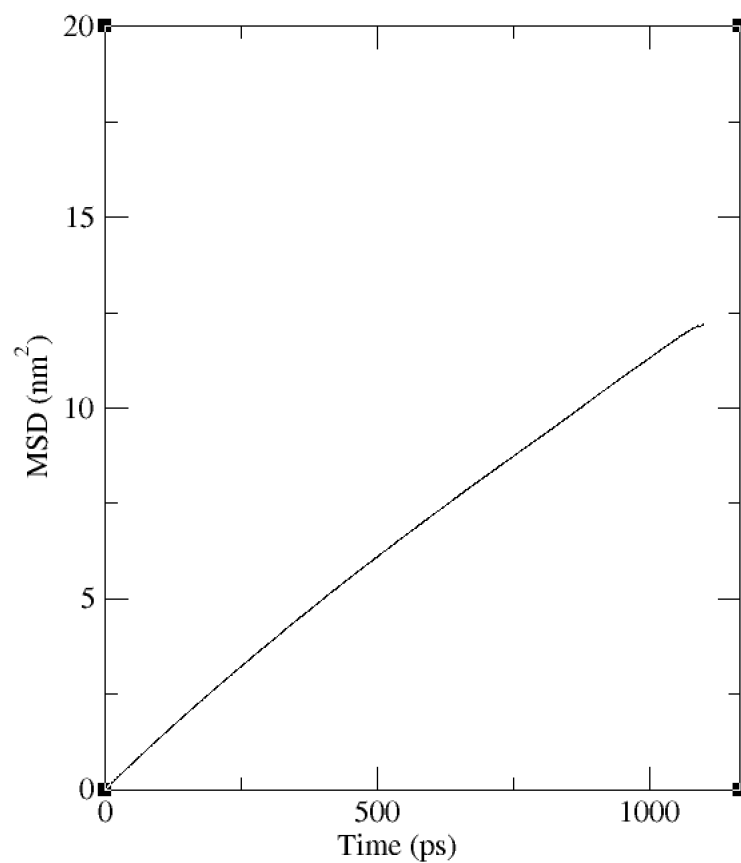


Figure 31: MSD of 1UBQ protein in a silica nanopore.

5.3 1AKI Enzyme in Water

1AKI is the code name of the orthorhombic form of Hen Egg-White lysozyme[48]. The lysozyme is an antimicrobial enzyme fundamental for the immune system of animals. So, as the 1UBQ, the lysozyme is part of the innate immune system.

The dimension of the simulation box are: $7.01\text{ nm} \times 7.01\text{ nm} \times 7.01\text{ nm}$. After the solvation, the number of solvent (SPC/E water) molecules is 10636. Due to the fact that the system, in this case the sole protein, presented a total net charge of $q_{TOT} = +8e$, we add 8 Cl ions in order to obtain a neutral system.

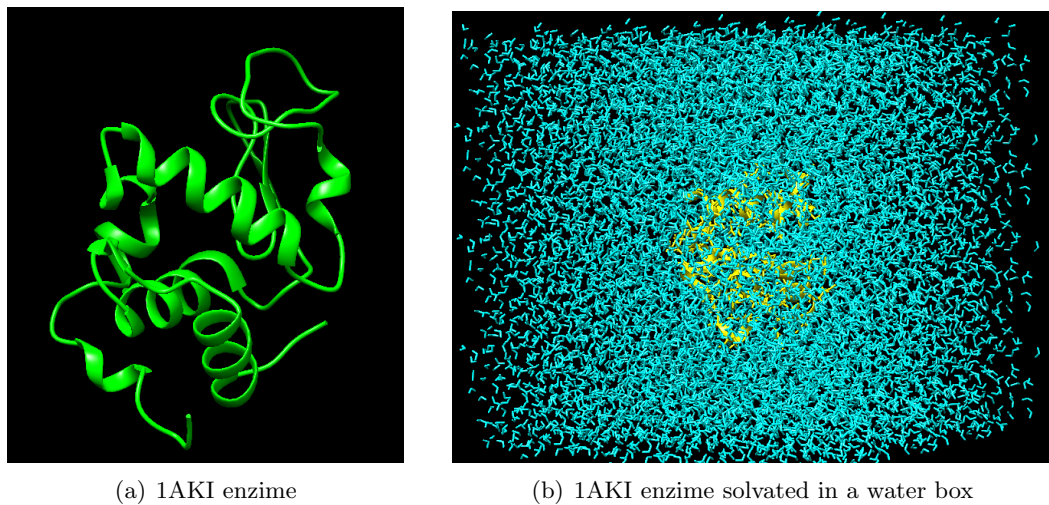


Figure 32: 1AKI simulated setup.

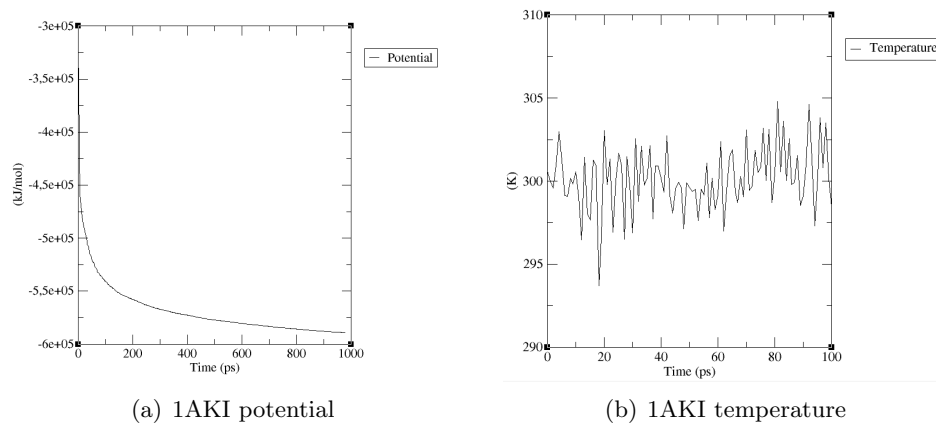


Figure 33: 1AKI Potential energy and temperature trend with time.

After the MD simulations we analyzed also the radius of gyration of the protein (Figure 34).

Data are obtained using the gromacs command:

```
gmx gyrate -f trajectory.trr -s structure + mass.tpr -o output.xvg
```

The radius of gyration analysis is used to understand if the protein unfold or not. If it unfolds, the radius of gyration varies over the simulation time, if not it remains stable. In our case the R_g is very stable around the value $R_g = 1.4$, meaning that the protein doesn't unfold and confirming the stability of the system.

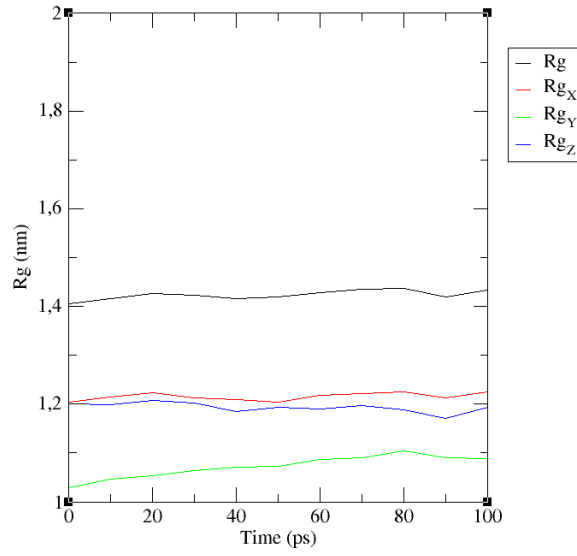


Figure 34: 1AKI radius of gyration.

In Figure 34, we report not only the R_g but also all its components along the 3 axis X, Y and Z.

A further analysis done in order to verify the stability of the system was done by evaluating the rmsd as shown in Figure 35.

Finally we have found a value of diffusivity $D = 2.6031(\pm 0.0385) \cdot 10^{-9} m^2/s$. This value is just 2.5% lower than that of bulk water, meaning that nanoconfinement of water is not so significant.

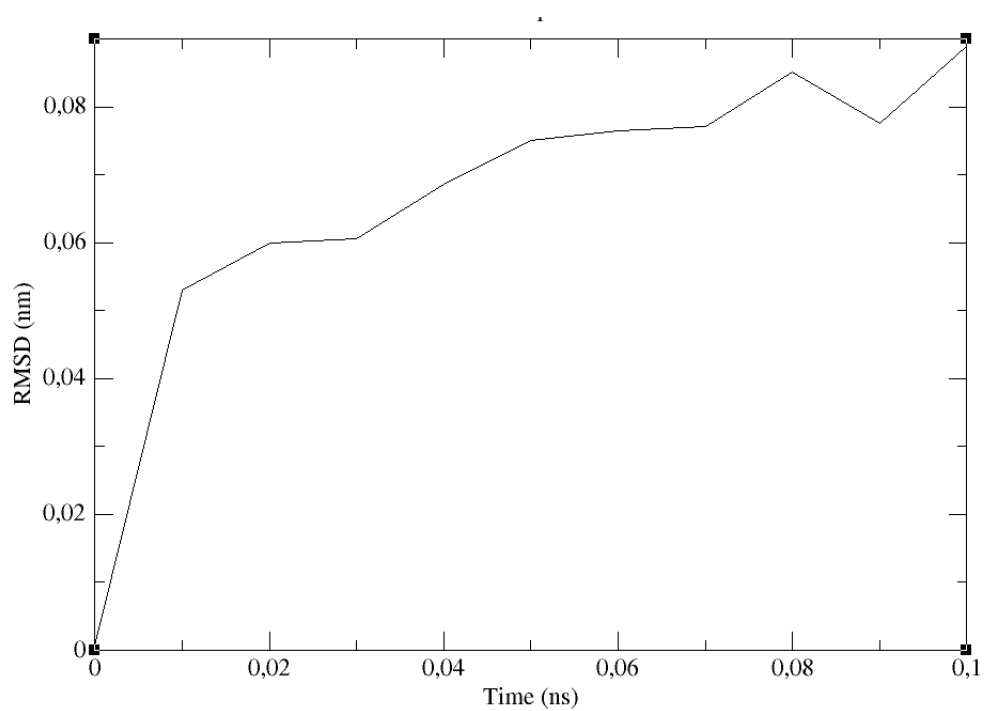


Figure 35: 1AKI rmsd.

5.4 1AKI in a Silica Nanopore

As done for the protein 1UBQ, also in this case we performed a simulation of the protein inside a silica nanopore with diameter of $\phi = 8nm$. The simulation steps are that listed in section ???. In Figure 36, we can see that the rmsd of the system is very stable with an average value of 2 Å.

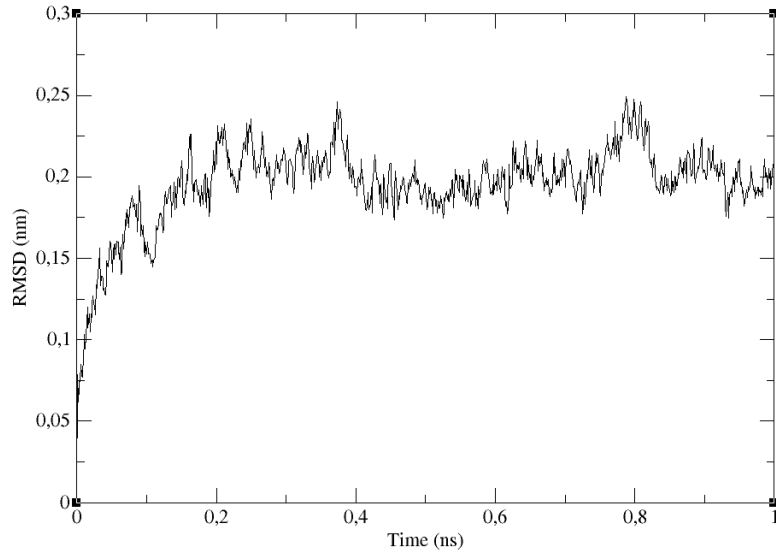
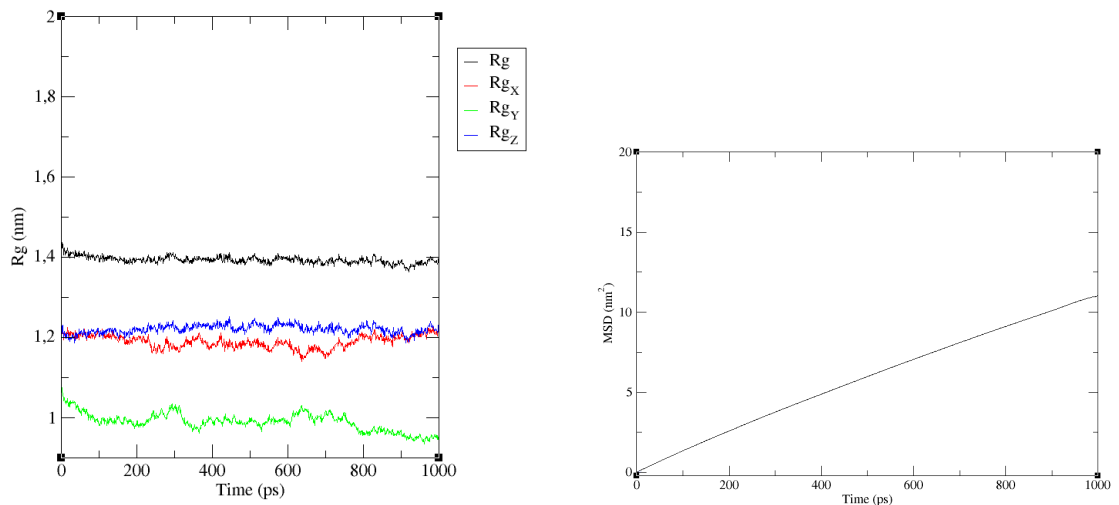


Figure 36: RMSD of 1AKI protein inside a silica nanopore.

The radius of gyration of the protein is analyzed in ???. The mean square displacement shown in 37(b) is linear, confirming the diffusive regime of the system. Finally the self-diffusion coefficient is $D = 1.8155(\pm 0.2093)10^{-9}m^2/s$ fitted from 100 to 900ps. In this case we have a

decrease of 32% compared to bulk water and 30% compared to silica 8 *nm* nanopore filled with water.



(a) Radius of gyration of 1AKI protein inside a silica nanopore. (b) MSD of 1AKI protein inside a silica nanopore.

Figure 37: 1AKI radius of gyration and mean square displacement

5.5 1MNH Protein in Water

The last protein simulated was 1MNH myoglobin. Myoglobin is a protein in the muscle tissue responsible for the oxygen storage and able to release it in cases of hypoxia and anoxia [58].

The procedure that we adopted for running the simulation is the same of previous cases. The average temperature is around 300 K and the potential energy have a decreasing trend during the energy minimization step, stabilising right below the value of $-6 \cdot 10^5 \frac{kJ}{mol}$.

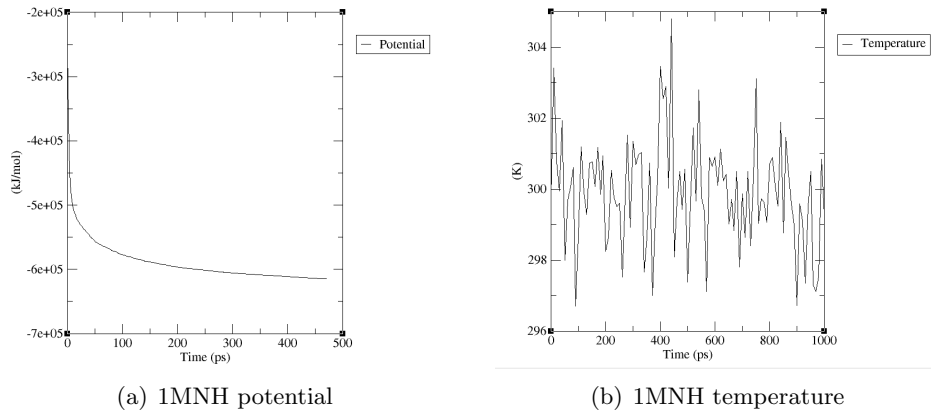


Figure 38: 1MNH Potential energy and temperature trend during the simulation

Finally we found the self-diffusion coefficient $D = 2.5611(\pm 0.0803) \cdot 10^{-9} m^2/s$ from 100 to 900ps. The simulation results show a decrease of 4% compared to bulk water. This weak decrease in D value is common over all the simulated cases of protein inside a water box.

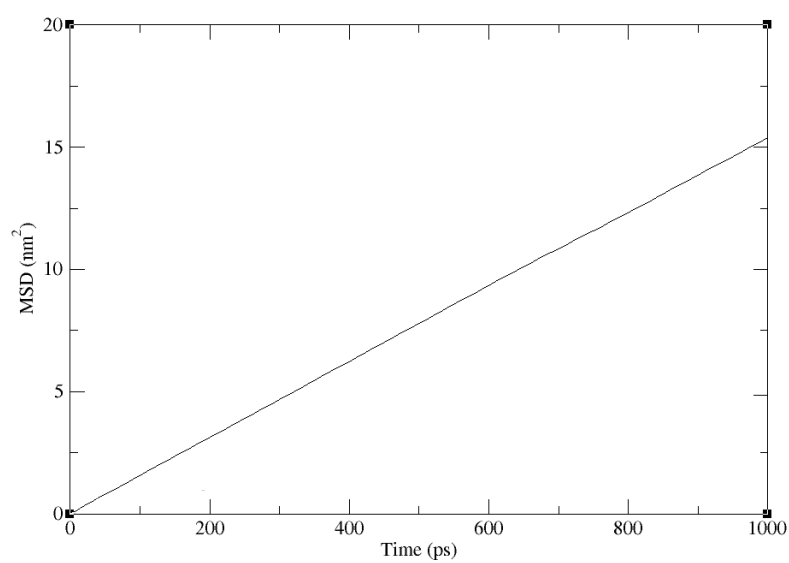


Figure 39: MSD of 1MNH protein inside a water box.

Also in this last simulation, as we can see from Figure 39 the MSD is linear confirming the diffusive regime of water.

5.6 Multiple Proteins in Silica Nanopore

This section is dedicated to the simulations of different concentrations of the lysozyme 1AKI in a silica nanopore of diameter $\phi = 11nm$ solvated with spc/e water model.

The first step of these simulations, as always, was that of creating the system topology. To do so, we combine the topology of the silica nanopore with that of the 1AKI both created with the modified force field gromos96-43a2. For what concerns the *.gro file of the system, it was created using the following gromacs command:

```
gmx insert - molecules - f SiPore.gro - ci 1AKI.gro - o 1AKI&Si.xvg - nmol *1
```

where *nmol* is used to insert the desired number of lysozyme molecules.

Then we performed the energy minimization of the dry system. Next we solvated the system (16463 molecules of water for the system composed of 9 Lysozymes, 19777 for the system with 3 lysozymes and 20331 for 2 lysozymes)

Due to the fact that the system composed by silica nanopore, water and lysozymes is not neutral, we add respectively 16, 24 and 72 *Cl*⁻ ions to the system composed by 2,3 and 9 lysozymes. In this way, the net charge of the resulting systems was $q_{tot} = 0$.

¹In the case where we added 9 molecules, another option was included in *gmx insert-molecules*: "-try *". With this option we were able to try to insert molecules 100000 times before stopping the command. With the option "-try 10000" the maximum number of 1AKI inside the $\phi = 11nm$ silica nanopore was 9.

In Figure 40 and in Figure 41, all the graphs show a stable trend of the total energy; this analysis is done to control the stability of the system.

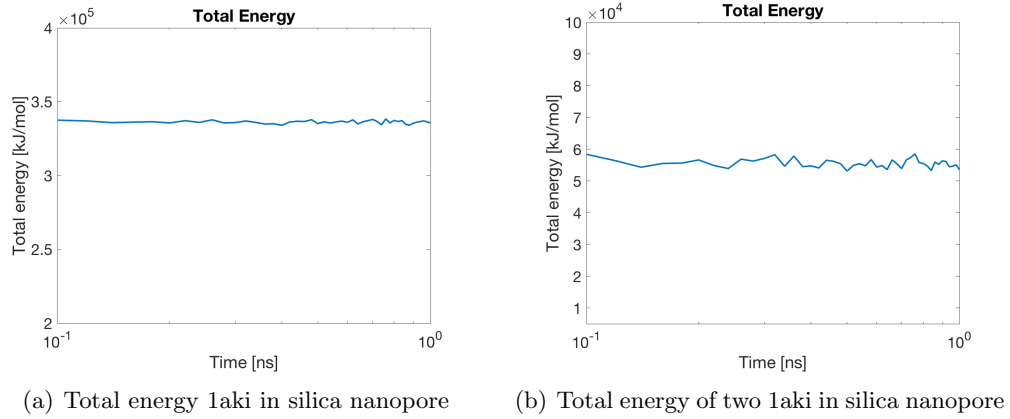


Figure 40: 1AKI total energy

In Figure 42 is represented the trend of density taken as a slice in the middle of the simulation box, averaged along the pore axis z . From $0nm$ to $1nm$ and from $12nm$ to $13nm$ the density is zero, because these regions include the bulk silica. At the interface of solid nanopore and water we have a peak in density. The two depression zones at $3nm$ and $9nm$ are due to the presence of proteins. In the middle of the pore we don't have the presence of proteins, and the average density fluctuates around the value of $1000 \frac{kg}{m^3}$ (on the graph the density is normalized by the bulk water density). Moreover other two proteins were simulated: 1POU and 2A3D. The former is a DNA binding protein present on *homo sapiens* organisms, while the latter is a

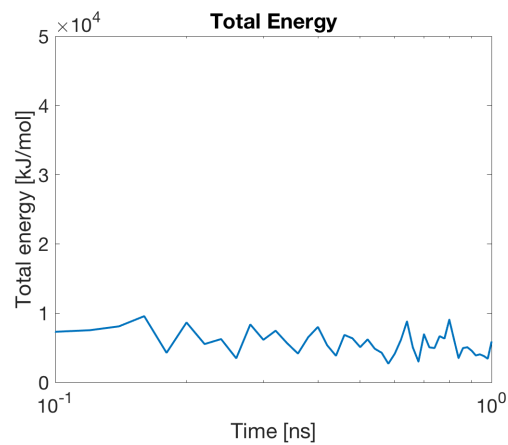


Figure 41: Total energy of nine laki in silica nanopore

de novo designed three-helix bundle protein. Both the abovementioned proteins were simulated in 11 *nm* silica nanopore with a maximum concentration of proteins inside the pore equal to 17.

At the end of the simulation, we have controlled also the temperature stability. As we notice from Figure 43 the trend is constant, and the average temperature during the entire simulation is $T = 300.3K$

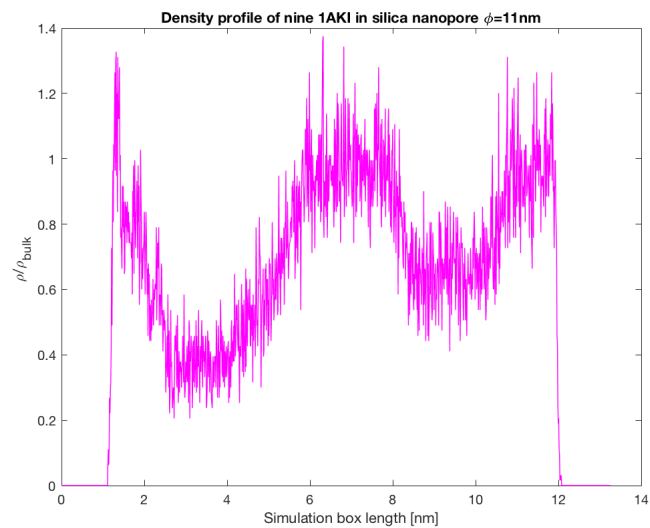


Figure 42: Density profile of water in the box made of nine AKI proteins inserted in the 11 nm silica nanopore.

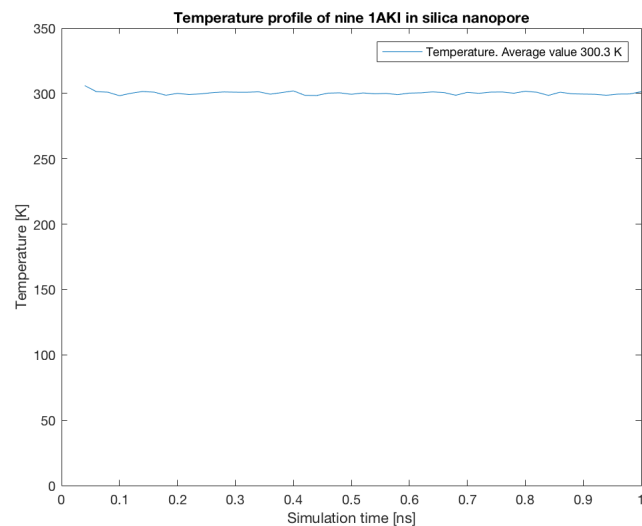


Figure 43: Temperature evolution of the system with nine 1aki in Silica nanopore.

5.7 Scaling the Simulated Self-Diffusion Coefficients

Different configurations and geometry have been studied so far. First of all, in Table IV is presented the self- diffusion coefficient of bulk water as a mean of comparison with all the others values.

TABLE IV: BULK WATER

	<i>System</i>	$V[nm^3]$	$D[\frac{m^2}{s}]$	$\pm$	θ
1	Bulk water	343	$2.67 \cdot 10^{-9}$	$0.003 \cdot 10^{-9}$	0

In Table V are summarized the results obtained for the cases of proteins solvated in SPC/E water. As we can see, the results of D are close to that of bulk water, meaning that the proteins have no such an infleuence on water diffusion in a large box.

It is worth to note that both 1UBQ and 1AKI are small-sized proteins composed of a single amino acids chain, while 1MNH is a bigger protein and that reflects the lower self-diffusion coefficient D .

TABLE V: PROTEINS IN WATER

	<i>System</i>	$V[nm^3]$	$D[\frac{m^2}{s}]$	$\pm$	$\delta[nm]$	SAS [nm^2]	θ
2	1UBQ	267.5	$2.60 \cdot 10^{-9}$	$0.0385 \cdot 10^{-9}$	0.303	49.16	0.060
3	2A3D	364.4	$2.58 \cdot 10^{-9}$	$0.0487 \cdot 10^{-9}$	0.291	49.9	0.042
4	1POU	330.94	$2.44 \cdot 10^{-9}$	$0.045 \cdot 10^{-9}$	0.300	47.37	0.045
5	1AKI	344.5	$2.52 \cdot 10^{-9}$	$0.0174 \cdot 10^{-9}$	0.313	69.03	0.067
6	2 1AKI	330.94	$2.37 \cdot 10^{-9}$	$0.041 \cdot 10^{-9}$	0.313	69.03	0.153
7	9 1AKI	709.01	$1.89 \cdot 10^{-9}$	$0.056 \cdot 10^{-9}$	0.313	69.03	0.394

In Table VI are shown the results of proteins inside a silica nanopore.

TABLE VI: PROTEINS INSIDE A SILICA NANOPORE

	<i>Protein</i>	ϕ Pore [nm]	$V[nm^3]$	$D[\frac{m^2}{s}]$	$\pm$	δ_S^1 and $\delta_P^2[nm]$	θ
8	1UBQ	8	854.9	$1.82 \cdot 10^{-9}$	$0.229 \cdot 10^{-9}$	$\delta_S : 0.331 \quad \delta_P : 0.303$	0.170
9	1AKI	8	854.9	$1.81 \cdot 10^{-9}$	$0.209 \cdot 10^{-9}$	$\delta_S : 0.331 \quad \delta_P : 0.313$	0.183

We can see that the self-diffusion coefficients in Table VII are drastically reduced, thus indicating a high nanoconfinement of water.

¹**S** stands for *Silica SAS*. This notation is the same also for the tables that will follow.

²**P** stands for *Protein SAS*. This notation is the same also for the tables that will follow.

TABLE VII: SILICA NANOPORES AND WITH NANOPARTICLES

	<i>System</i>	$D[\frac{m^2}{s}]$	$\pm$	$\delta[nm]$	θ
10	SILICA 2nm PORE	$1.17 \cdot 10^{-9}$	$0.3913 \cdot 10^{-9}$	0.38	0.63
11	SILICA 4nm PORE	$1.57 \cdot 10^{-9}$	$0.222 \cdot 10^{-9}$	0.333	0.37
12	SILICA 8nm PORE	$2.20 \cdot 10^{-9}$	$0.101 \cdot 10^{-9}$	0.331	0.20
13	SILICA 11nm PORE	$2.53 \cdot 10^{-9}$	$0.128 \cdot 10^{-9}$	0.331	0.25
14	SILICA + 8SPIOs	$1.2904 \cdot 10^{-9}$	$0.1873 \cdot 10^{-9}$	0.33-0.49	0.52
15	SILICA + 4SPIOs	$1.91 \cdot 10^{-9}$	$0.101 \cdot 10^{-9}$	0.33-0.49	0.36
16	SILICA + 2SPIOs	$2.07 \cdot 10^{-9}$	$0.141 \cdot 10^{-9}$	0.33-0.49	0.28

Finally the last simulations that we have done are that of multiple lysozymes 1AKI and proteins in a silica nanopore of diameter $\phi = 11nm$.

TABLE VIII: MULTIPLE PROTEINS IN SILICA NANOPORE

	<i>System</i>	# Prot.	$D[\frac{m^2}{s}]$	$\pm$	$\delta[nm]$	SAS [nm^2]	θ
17	SI+1AKI	2	$2.06 \cdot 10^{-9}$	$0.171 \cdot 10^{-9}$	$\delta_S : 0.331 \delta_P : 0.313$	238.1-289	0.26
18	SI+1AKI	3	$1.93 \cdot 10^{-9}$	$0.140 \cdot 10^{-9}$	$\delta_S : 0.331 \delta_P : 0.313$	207.1-289	0.27
19	SI+1AKI	9	$1.44 \cdot 10^{-9}$	$0.102 \cdot 10^{-9}$	$\delta_S : 0.331 \delta_P : 0.313$	621.0-289	0.65
20	SI+1POU	17	$1.32 \cdot 10^{-9}$	$0.124 \cdot 10^{-9}$	$\delta_S : 0.331 \delta_P : 0.313$	243.8-289	0.80
21	SI+2A3D	17	$1.33 \cdot 10^{-9}$	$0.086 \cdot 10^{-9}$	$\delta_S : 0.331 \delta_P : 0.313$	250.1-289	0.83

For what concerns the cases of seventeen 1POU and seventeen 1AKI in silica nanopore, the simulations were extended to 2ns in order to have a better evaluation of the self-diffusion coefficient.

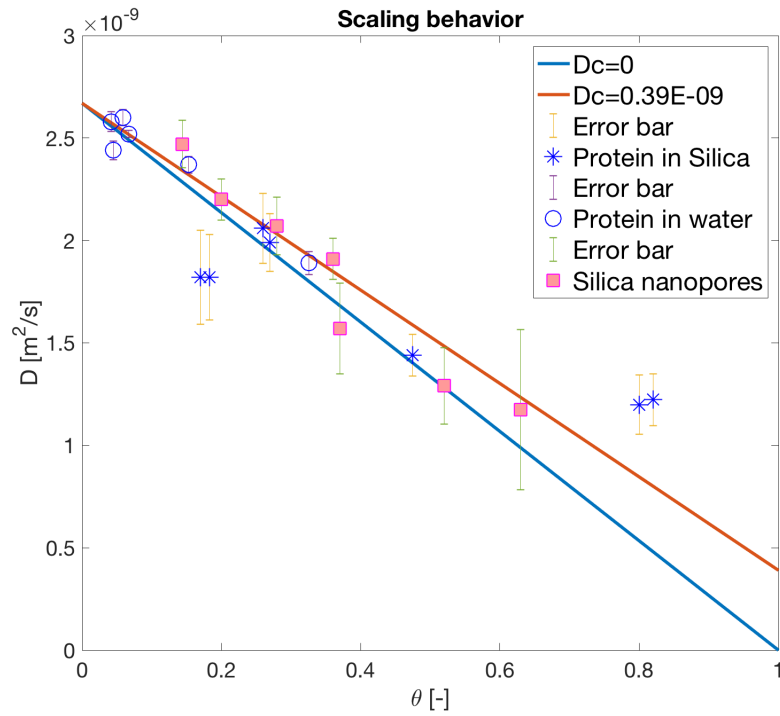


Figure 44: Scaling behaviour of self-diffusion coefficient.

In Figure 44 the two solid lines blue and red represent respectively equation Equation 3.8 computed for value of $D_C = 0 \left[\frac{m^2}{s} \right]$ and $D_C = 0.39 \cdot 10^{-9} \left[\frac{m^2}{s} \right]$. The vertical bars represent

the possible error on the evaluation of the self-diffusion coefficient by means of mean square displacement.

The cyan spots in Figure 44 represent the cases of proteins inside 11nm silica nanopore. The cases refers to 1AKI, 1POU and 2A3D proteins (the main properties are shown in Table IX).

TABLE IX: PROTEINS PROPERTIES

Case	Atoms	Residues	Protein chains
1AKI	1001	129	1
1POU	577	71	1
2A3D	572	73	1

Figure 45 helps to better understand the dependence of the self-diffusion coefficient compared to the number of proteins inside the silica nanopore. Both the simulations of 1POU and 2A3D are done by putting 17 proteins inside the pore. Moreover the number of atoms and residue of the two proteins are similar (see Table IX). This leads to similar results in term of self-diffusion coefficient. For what concerns 1AKI, if we take apart the self-diffusion coefficient of the empty nanopore, the other results lay on a straight line that linearly decreases with the increase of the number of 1AKI inside the nanopore.

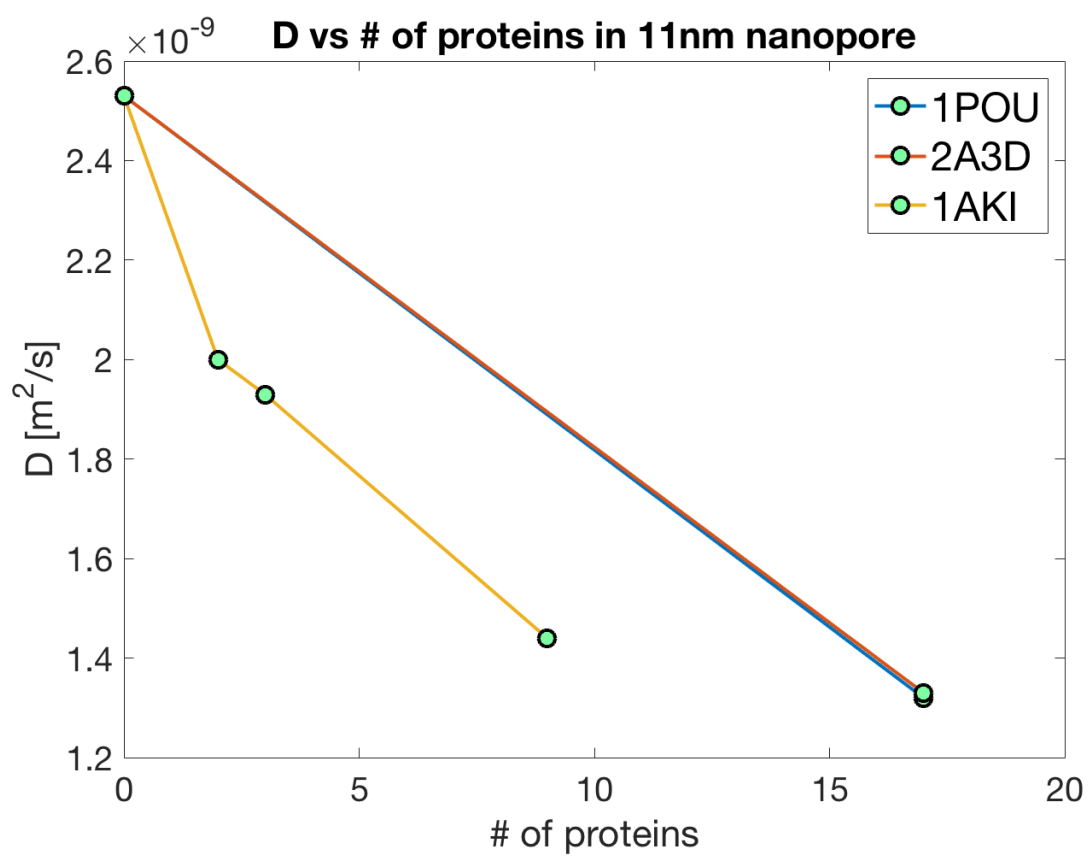


Figure 45: Self-diffusion coefficient vs number of proteins.

CHAPTER 6

CONCLUSION

The thesis objective was to study the diffusivity of water under nanoconfined conditions, with the aim to conceptualize a novel sensor for detecting the concentration of nanoparticles with simple water diffusivity measures. To do so, MD simulations have been used to obtain self diffusion coefficients. Simulations have highlighted and proved that transport phenomena at nanoscale are extremely bounded with non-bonded interactions between the solvent (i.e. water) and the solid surface. For example, the self-diffusion coefficient of water inside a silica nanopore of 11nm of diameter is two times that of water inside a 2nm silica nanopore.

We obtained as results that the self-diffusion coefficient D strongly varies from bulk water to nanoconfined conditions (i.e inside a silica nanopore) with a trend that can be predicted by an analytical scaling law recently presented in the literature.

The resulting concept sensor device could become an important and ready-to-use tool to know the concentration of biomolecules or emerging pollutants inside water. As a matter of fact by knowing the diffusivity of water thanks to D-MRI it is possible to use the plot in Figure 44 to find θ . Knowing θ is possible to compute how many proteins there are inside the pore and, consequently, the concentration of nanoparticles in the solution.

Obviously, methods based on molecular dynamics simulations may have some intrinsic limitations due to the fact that long molecular dynamics simulations are mathematical ill-conditioned.

For what concerns future developments, an interesting idea can be that, on one hand, to create an archive of different proteins and pores data by performing molecular dynamics simulations; on the other hand, to develop better and simpler sensor not only for protein detection but also for other water pollutants (e.g. pharmaceutical or pesticides). Taking as reference carbon nanotube arrays sieves that have the properties to finely tune the water diffusivity value thanks to the application of an electrical field [49], the same can be applied to silica sieve.

This thesis has, on the one hand, pointed out problems that surround humankind every day, e.g. pollutants or diseases detection, and on the other hand has given alternative, not conventional, approach on how these problems can be tackled: understanding the infinite small is the key to improve our macroscale world.

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