WG1 - DELIVERABLE D1.1

PROTOCOLS AND RECIPES FOR COMPUTER-ASSISTED DESIGN/OPTIMIZATION OF NEW/EXISTING CHEMICAL ENTITIES AND/OR NANOMATERIALS

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ACRONYMS

AFM – Atomic Force Microscopy
BD -Brownian Dynamics
CPU - Central Processing Unit
CD – Circular Dichroism
DFT – Density Functional Theory
DDFT- Dynamic Density Functional Theory
DPD - Dissipative Particle Dynamics
DMTA – Dynamic Mechanical Thermal Analysis
DSC – Differential Scanning Calorimetry
FEM - finite elements modelling
GPU – Graphical Processing Unit
IGC – Inverse Gas Chromatography
IR - Infrared
ITC – Isothermal Titration Calorimetry
LB - Lattice Boltzmann
MC - Monte Carlo
MD - Molecular Dynamics
MS - Mesoscale Simulation
NMR – Neutron Magnetic Resonance
QM - Quantum Mechanical
SANS – Small Angle Neutron Scattering
SAXS – Small Angle X-Ray Scattering
SEM – Scanning Electron Microscopy
SPR – Surface Plasmon Resonance
TEM – Transmission Electron Microscopy
UV – Ultraviolet
WAXS – Wide Angle X-ray Scattering

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1. Computer simulations in nanomedicine: a general perspective

Modern nanomedicine research is based on multiple synergistic stages, where success in targeting is not just about performance at the target site. There will be for instance loss of drug from the carrier by anticipated release or degradation, loss of the cargo/carrier complex through uptake into non-target sites, or reduced thermodynamic activity of the active principle once it is sequestered by proteins. The system may fail to reach the target in sufficient quantity, and payload release rate and the rate of diffusion of the free drug may be suboptimal to achieve therapeutic effects. It is one thing for a nanocarrier to reach a target tissue but another for its active cargo to be still bound to its vector and not lost *en route* or, conversely, bound to tightly that it is not released at the site of action. Recirculation of systems clearly provides further opportunity to engage with the target, but also prolongs the lifetime of the carrier in the circulation and, with most systems presently available, this increases the chances of drug leakage and premature drug loss if release is time-dependent, rather than triggered by some mechanism (e.g., pH variation or enzymatic reaction) close to the target.

With such a complex biological scenario, and with the multitude of possibilities chemists have at hand, devising new, efficient and safe nanovectors based only on empirical or semi-rational design has become a tantalizing task. Thus, accurate predictive mathematical or molecular models are fundamental in identifying those mechanisms involved in the different stages, from the design to the desired therapeutic action of a nanomedical formulation. Modeling of the relevant biophysical phenomena at different length and time scales becomes crucial for identifying the main parameters governing the spatiotemporal evolution of the system under investigation, for elucidating the role and quantifying their effects and, most importantly, for predicting the evolution of the system prior to running extensive and expensive experiments. To this end, multiscale molecular modeling can be employed to design rational experiments and to guide or inspire experimentalists. Given the complexity of biology, and the huge biological diversity among apparently similar concepts, computer-assisted multiscale molecular simulations are clearly of fundamental importance for the effective development of reliable predictive tools to be used in the design of new agents in personalized medicine.

Nanomedicine systems exhibit structural features that span several length scales, from the Å level of the individual backbone of a single molecule to the mesoscopic system morphology, reaching far into hundreds of nanometers. In addition, the time scales of the characteristic dynamic processes relevant to different nanomedicine materials properties, span a wider range, from femtoseconds to milliseconds or even seconds, hours and days (**Figure 1**). Unfortunately, no single model or simulation algorithm can cover such a vast interval of length and time scales; therefore, the seamless integration of many different models, each suitable for describing the chemistry and the physics at a given time and/or length scale, is required.

This concept constitutes the pillar of multiscale molecular modelling and simulation, that is the bridging of length and time scales by linking computational methods to ultimately predict macroscopic properties and the behavior of complex systems from fundamental molecular processes.[1] Thus, the idea of performing simulations of nanomedical systems across several characteristic length and timescales, starting from fundamental physical principles and experimental data, has an obvious appeal as a tool of potentially great effect on technological innovation and rational design.[2-7]

To sum up, the advantages of considering multiscale molecular modelling include, among others, the following:

- reduction of product development time by alleviating costly trial-and error iterations;
- reduction of product costs through innovations in material, product, and process design;
- reduction of the number of costly, large-scale experiments;
- increase of product quality and performance by providing more accurate predictions in response to material design requirements and loads.
- support in conceiving and developing entirely new materials.

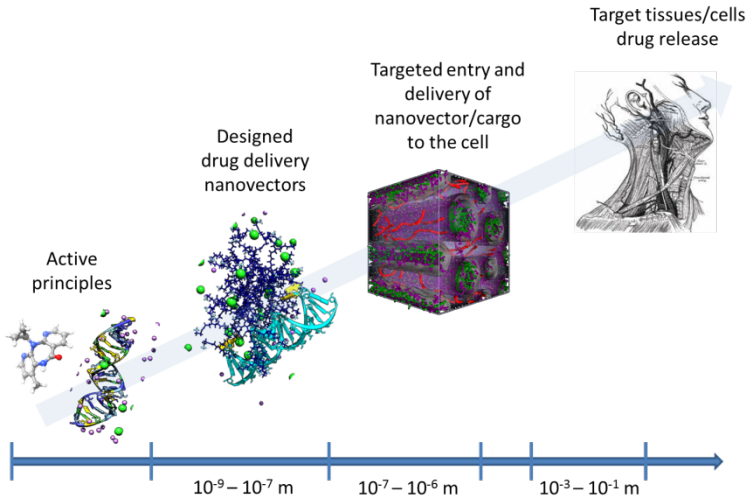

Figure 1. Typical ranges of spatial scales involved in the different physicochemical phenomena related to nanomedicine.

2. Multiscale molecular modelling: basic concepts

The last 15 years have observed a rapid expansion in the use of computer modelling techniques in both materials and life sciences, with the number of relevant articles indexed in Scopus and ISI Web of Knowledge more than tripling between 2004 and 2019 in comparison to the preceding decade. Accordingly, computational modelling is now a well-established technique in virtually all areas of mainstream nanomedicine.

Many important factors increased the use of molecular modelling and simulations in materials nanomedical research. Probably a key point is the availability of (relatively) inexpensive computational power, driven in part by the Moore's law, which, in its original form, is related to the doubling of the transistor density in integrated circuits every 18 months. In practice, this has led to a rapid decrease in the unit price of CPUs (Central Processing Units) – and today – GPUs (Graphical Processing Units), physical memory and hard disk space, as machines suitable for scientific calculations have found their way onto the mass market. Parallel to hardware improvement, a plethora of free and commercially available integrated modelling software packages now exists, among which notable examples include Gaussian® (mainly for quantum mechanics calculations), AMBER and NAMD (for atomistic simulations), Materials Studio®, Culgi, GROMACS, and LAMMPS

(for both atomistic and mesoscale simulations), and Digimat and ABAQUS® (for continuum - i.e., finite element-calculations).

By definition, multiscale molecular modelling entails the application of computational techniques at two or more different length and time scales, which are often, but not always, dissimilar in their theoretical character due to the change in scale. A distinction is made between the *hierarchical approach*, which involves running separate models with some sort of parametric coupling, and the *hybrid approach*, in which models are run concurrently over different spatial regions of a simulation. The relationships between different categories of methods commonly used in the multiscale modelling hierarchy are shown in Figure 2. Although some techniques have been known for a long time and are currently widely used (e.g., techniques in the atomistic level, including molecular dynamics (MD) and Monte Carlo (MC) methods), other, such as mesoscale simulation (MS) and some more advanced methods for accelerating atomistic simulations are not as common yet, and require extensive experience and specialized skills in the field.

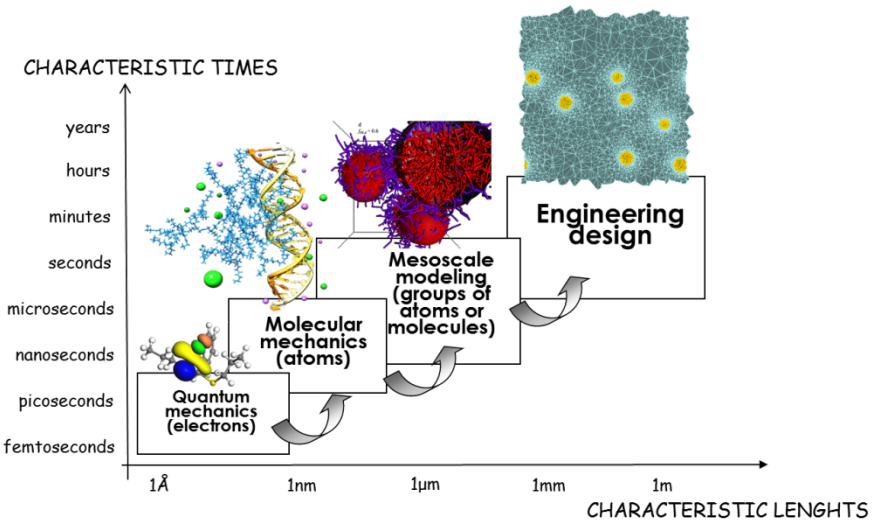

Figure 2. The multiscale molecular modeling concept: the information obtained from simulations at a given (lower) characteristic length and time scales, is used as an input for the next (upper) scale simulations.

3. Computational methods and relevant time- and length scales

In the context of materials simulations shown in Figure 2 , four characteristic time and length levels can be envisaged before reaching the last step, i.e. engineering design:

1. *The quantum scale* (~10^{-10} - 10^{-9} m and ~10^{-12} s), in which nuclei and electrons play the central role, and their quantum-mechanical state dictates the interactions among atoms. In quantum simulations, data describing structural and electronic features of the system can be collected, including *e.g.*, effects associated with rupture and formation of chemical bonds, changes in the electronic configurations, and other relevant phenomena (e.g., π-π interactions, hydrogen bonding etc.)

2. *The atomistic scale* (~10^{-10} - 10^{-7} m and 10^{-12} - 10^{-6} s). In atomistic simulations, all atoms are explicitly represented or in some cases, small groups of atoms are treated as single sites referred to as pseudo or united atoms. The potential energy in the system is estimated using a number of different kinds of interactions (collectively known as the force field), typically consisting of: (i) bonded interactions, including bond-length (stretch) potentials, bond-angle (bend) potentials, torsion (twist) potentials and cross-terms, and (ii) non-bonded interactions, mostly comprising Coulomb interactions and dispersion forces.
3. *The mesoscopic scale* (~10^{-9} - 10^{-3} m and 10^{-6} - 10^{1} s). In these methods, a molecule is usually treated with a field description (field-based model) or microscopic particles (particle-based model) which incorporate molecular details implicitly. Therefore, they are able to simulate phenomena on length and time scales currently inaccessible by classical atomistic approach. At the simplest mesoscopic level, a polymer system may be modelled by a phenomenological expression for the free energy (field-based approach). For example, the Flory-Huggins or Landau free energies of mixing may be used to model aspects of polymer mixtures. In such models, the details of the system are incorporated into, e.g., the Flory parameter and the monomer segment mobility. Such phenomenological expressions are equivalent to truncated expansions of a more complicated free energy expressions. On the other hand, in particle-based models the fluid is portrayed as a collection of point particles that represent lumps of fluid containing many molecules or segments of chains, termed beads. The interaction between beads is considered mesoscopic because the internal degrees of freedom of the fluid elements are ignored and only their center-of-mass motion is resolved.
4. *The macroscopic scale* (~10^{-3} - 10^{1} m and 10^{1} - 10^{3} s). At this level, constitutive laws govern the behavior of the physical system, which is considered as a continuous medium, ignoring discrete atomic and molecular details and their influence on the overall system behavior. The basic assumption thus, goes down in representing a heterogeneous material as an equivalent homogeneous one. A medium is called a continuum, if its volume contains an apparent continuity of material mass over the physical scale of the problem of interest. In general, this requires the domain of interest to be several orders of magnitude larger than the length scale of the elemental components. All mathematical functions (e.g., velocity or displacement fields) used to describe the state of the system are continuous, except possibly at a finite number of interior surfaces separating regions of continuity. Stress and strain tensors may be split into isotropic and deviatory parts, allowing to predict the behavior of the medium under both static and dynamic loading with separate descriptions of material constitutive behavior under hydrostatic and non-hydrostatic circumstances.

To elaborate more, at each length and timescale, well-established and efficient computational approaches have been developed over the years to handle the relevant, underlying phenomena. To treat electrons explicitly and accurately at the *quantum scale*, electronic models based on quantum mechanical (QM) methods can be employed. QM methods have undergone enormous advances in last decades, enabling simulation of systems containing up to several hundred atoms with good accuracy. For material properties at the *atomistic scale*, molecular dynamics and Monte Carlo simulations are usually performed employing classical interatomic potentials, which can often be derived from QM calculations. Although not as accurate as QM methods, classical MD and MC simulations are able to provide insight into atomic processes involving considerably large systems.

At the *mesoscopic scale*, the atomic degrees of freedom are not explicitly treated, and only large-scale entities are modelled (that is, agglomeration of atoms called *beads*, obtained through a coarse-graining procedure, *vide infra*). Mesoscale models are particularly useful for studying the behaviour of polymers and soft materials. They can model even larger molecular systems, but with the commensurate trade-off in accuracy. Typical results of mesoscale simulations are morphologies of matter in the range of nm - mm range at specific conditions of temperature, composition, and shear. Various simulation methods have been proposed to study the mesoscale structures in polymer-based materials, the most common being Brownian Dynamics (BD), Dissipative Particle Dynamics (DPD), Lattice Boltzmann (LB), time-dependent Ginzburg–Landau theory, and Dynamic Density Functional Theory (DDFT). Eventually, it is possible to transfer the simulated mesoscopic structure to finite elements modelling (FEM) tools to calculate macroscopic properties for the systems of interest.

Whatever multiscale protocol is developed, it is important to be able to compare the calculated results with experimental findings at each scale a computation is performed. Fortunately, the experimental methods available nowadays, in most of the cases allow such comparison to be made, along the entire length- and timescale interval.

In summary, the ultimate goal of a multiscale modelling is the prediction of the macroscopic behavior of an engineering process from first principles, by adopting a sequential simulation pathway, by collecting information at a smaller (finer) scale and pass it to a model at a larger (coarser) scale. This procedure disregards (i.e. coarse grains) all the degrees of freedom pertaining to the immediately smaller scale, which is considered to be in equilibrium.

4. Link between in silico and experimental methods

The main advantage of using computational methods in nanomedicine, is their ability to predict and/or interpret structural, physicochemical and mechanical/transport properties associated with the materials involved. It is therefore very informative to establish a link between the in-silico approaches and the outcomes of the pertinent experimental techniques, based on the properties that can be computed and measured, respectively.

Table 1 outlines a general correspondence of the simulation methods and experimental techniques employed in materials research.

Table 1. Correspondence of simulation methods with experimental techniques based on the computed and measured properties.

Scale	Typical Simulation Methods	Predicted Property	Experimental Technique

Electronic	Self-Consistent DFT	NMR, IR, UV spectra	NMR, IR, UV
	Car-Parinello (ab initio) Molecular Dynamics	Molecular geometry	X-ray, TEM, AFM
	Ab initio or semiempirical methods	Electrostatic potential, charge distribution	High resolution X-ray
Atomistic	Molecular Mechanics and Dynamics	Equilibrium and non-equilibrium structural properties	x-ray diffraction, SEM, TEM, SAXS, SANS, AFM, CD, laser speckle spectroscopy
		Transport, thermal and mechanical properties	DLS, thermal conductivity methods rheology, NMR
		Thermodynamic properties	ITC, DSC, IGC, SPR
		Electric properties	Cyclic voltammetry, dielectric spectroscopy, zeta potential
		Process kinetics	Stopped flow techniques, SPR
Mesoscopic	Coarse-grained models	Supramolecular morphology	AFM, TEM, SEM, SAXS, SANS, WAXS
		Transport properties	Rheology, DLS
Macroscopic	Computational Fluid Dynamics	Macroscopic flow	Rheology
	Finite elements	Thermomechanical and transport properties	Rheology, DMTA

To focus more on the physical behavior associated with the different stages encountered in nanomedical processes, we can summarize the properties which can be calculated by means of the computational techniques at different time and length scales as described before.

i) Structural properties

For a single molecular species

a) *at the molecular level*: molecular structure, molecular self-assembly;

b) *at the supramolecular level*: morphology of the assemblies and characteristics associated with presence of long-range order.

In the presence of carriers

a) *at the single complex level*: morphology and characteristics of the formed complex (e.g., micelle, liposome, aggregation number)

b) *at the supramolecular assembly level*: formation of structures with a higher degree of order where the single complexes act as the elementary building blocks (e.g., ordering of micelles, physically associated nanoparticles, physical networks etc.)

ii) Physicochemical properties

For a single molecular species

at the molecular and the supramolecular level: solubility, spectroscopic properties.

In the presence of carriers

at the single complex and at the supramolecular assembly level: degree of loading, release profiles, binding energy, binding kinetics, degree of thermodynamic stability, identification of self-assembly driving forces, nature and intensity of the interactions with the environment, critical micelle concentration

iii) Mechanical and transport properties

For all systems: diffusion (equilibrium and non-equilibrium), response under the application of external fields (electromagnetic, mechanical).

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