ESF – Short Visit Grant – Applicant: Daniele Dell'Orco

Scientific Report

Project: Mathematical model of nanoparticle-protein interaction dynamics in biological fluids and environments

Purpose of the visit

When entering fluids in an ecological system or more defined systems as the bloodstream, saliva or mucus nanoparticles are immediately covered by biomolecules. Proteins, lipids, and carbohydrates create a complex corona of biomolecules around the particle. The composition and characteristics of this corona is determined by the particle surface and the chemical nature of the surroundings. Biological risks may be foreseen due to the composition of the corona, exposed epitopes in bound proteins, and/or conformational perturbations that will affect protein function. The corona will change with time until equilibrium is reached [1]. First very abundant biomolecules will bind, but with time they are replaced by less abundant biomolecules with higher affinity [1-3]. The biomolecular corona will also change as nanoparticles travel from fluid to fluid or from fluid to organisms, tissues and cells [4].

To understand the mechanisms by which nanoparticles interact with ecological and biological systems, short and long term, we proposed to model in detail the composition of the biomolecular corona as a function of time after entering a body fluid. Our long-term aim was also to study how the corona acquired in one biological fluid develops when the particle moves into a second biological fluid. In order to reach this stage it is necessary to set an initially simple dynamic model that is able to describe and predict correctly the kinetics of the interaction of few protein types with the nanoparticle. The mathematical model should contain multiple competing protein-nanoparticle interactions, each with its characteristic on-and off-rate and stoichiometry at saturation obtained from the experiments. All the rate equations and starting concentrations are finally employed to simulate the temporal development of the corona.

Description of the work carried out during the visit

An initial simple model was set up during the visit, which is intended for future extensions.
According to the experimental data available, the model aimed at simulating first the interaction between three protein types and the Co-polimer particle (70 nm diameter, 50:50 NIPAM:BAM). The proteins are all found in human blood at different concentrations, and have a different molecular size that was represented in this work by the average radius of the protein cross section, as if the protein were spherical. This simplification allowed us to evaluate the number n_i of available binding sites for protein *i* on the nanoparticle (NP) surface, by the simple relation:

$$n_i \triangleq \frac{4\pi\left(r_{NP}+r_i\right)^2}{\pi r_i^2}$$

where r_i and r_{NP} denote, respectively the protein and the nanoparticle radii. The factor n_i was used as a correction factor in the kinetic equations for the association of each protein type with the nanoparticle to account for the maximum occupancy for each protein species.

The proteins included in the first model are: high-densisty lipo-protein (HDL), human-serum albumin (HSA) and fibrinogen (Fib). The biochemical equations describing the dynamics of the system are the following:

$$NP + HSA \rightleftarrows NP \cdot HSA$$

$$NP + HDL \rightleftarrows NP \cdot HDL$$

$$NP + Fib \rightleftarrows NP \cdot Fib$$

and the ordinary differential equations employed to describe the rates of the reactions are:

$$\frac{d[NP \cdot HDL]}{dt} = n_{HDL}\, k_{HDL}^{on} [NP][HDL] - k_{HDL}^{off} [NP \cdot HDL]$$

$$\frac{d[NP \cdot HSA]}{dt} = n_{HSA}\, k_{HSA}^{on} [NP][HSA] - k_{HSA}^{off} [NP \cdot HSA]$$

$$\frac{d[NP \cdot Fib]}{dt} = n_{Fib}\, k_{Fib}^{on} [NP][Fib] - k_{Fib}^{off} [NP \cdot Fib]$$

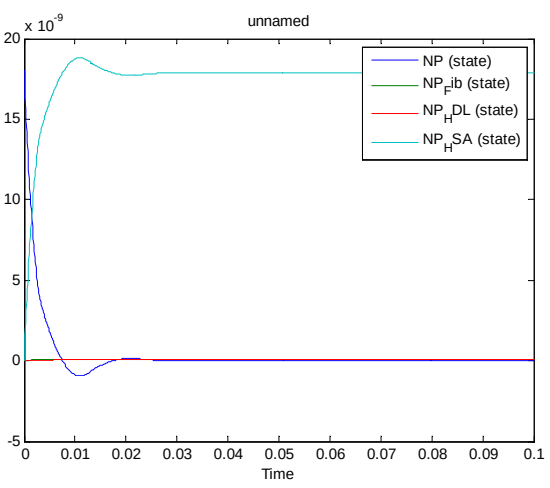
The overall dynamic model was implemented in the Systems Biology toolbox for Matlab [9,10] and the system of ordinary differential equations were numerically solved with different boundaries, corresponding to the various experimental conditions.

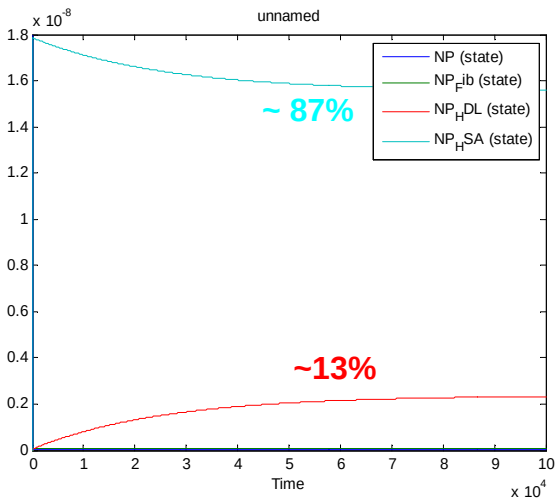
Description of the main results obtained

Starting from a set of experimentally determined boundaries, i.e. initial concentrations of the molecular species making up the system under study set equal to those in the blood, we initially tested the results of numeric simulations starting from the following parameters:

Protein	k^{on}	k^{off}	Affinity (M^{-1})	#binding sites
HDL	3.4e2	3.4e-5	1e7	256
HSA	2.4e3	2.0e-3	1.2e6	380.2
Fib	2e3	2e-3	1e6	109

The simulation of the system's time evolution when starting from the experimental concentrations and the kinetic parameters above led to the following trends:

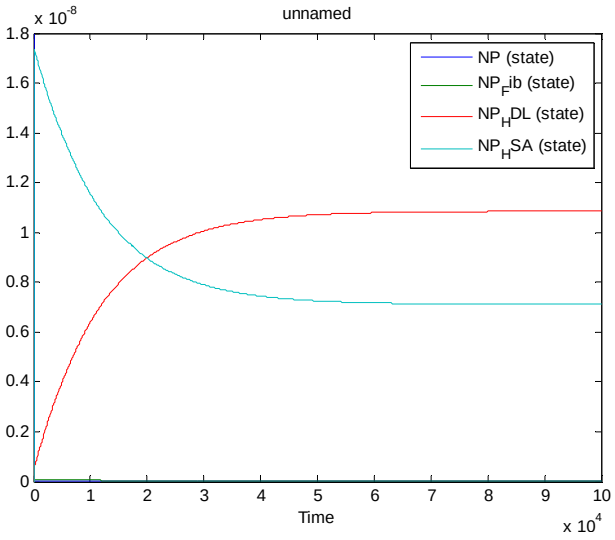




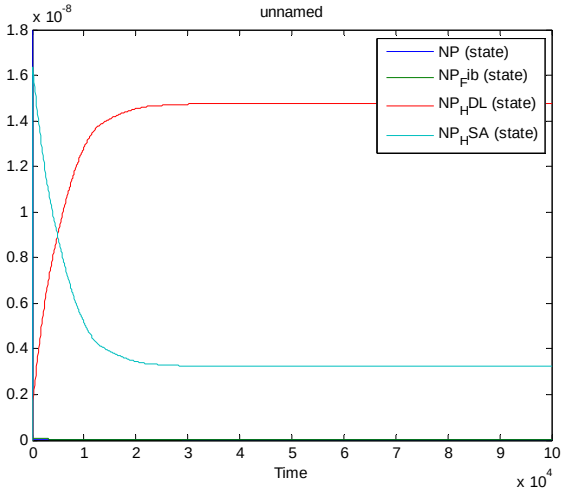
The main inference from the simulations above is that such setting implicates initially very fast events (see the panel on the left) that lead to equilibrium in about 10^5 s (~28 hours) as shown on the panel on the right. In particular, a neat prevalence of HSA is predicted (87%) to be bound to the nanoparticles at equilibrium, while the other protein species are expected to stabilize at lower levels (13% for HDL and <0.5% for Fib).
However, experimental evidence points to another equilibrium state, in which HDL prevails (~87%) with respect to other species, due to a putative higher affinity of HDL for the nanoparticle compared to that of HSA. Moreover, such equilibrium is expected to be reached faster (results not shown). In order to explore this possibility, the parameters highlighted in yellow in the table above were tuned to achieve a 10-times or 30-times higher affinity (Ka = k^{on}/k^{off}) with respect to the initial value (Ka = 10^7 $M^{-1}s^{-1}$). This was done, respectively, by acting on the association or dissociation rates individually.
The results obtained are as follows:

1) 10-times higher k^{on}_{HDL}

Increasing the association rate constant by a factor of 10 leads to a different equilibrium, which is reached faster (~16 h) than the one shown above and where the amount of HDL bound to nanoparticle (60%) prevails on HSA (40%). Neither in the above nor in this scenario does Fib contribute with significant levels at equilibrium.



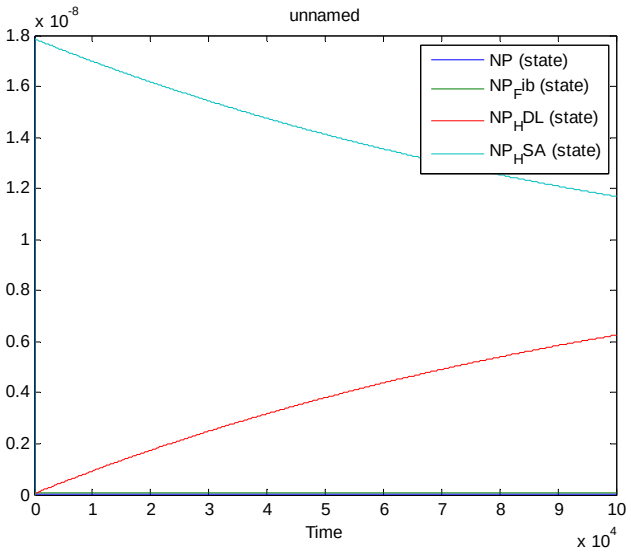
2) 30-times higher k^{on}_{HDL}



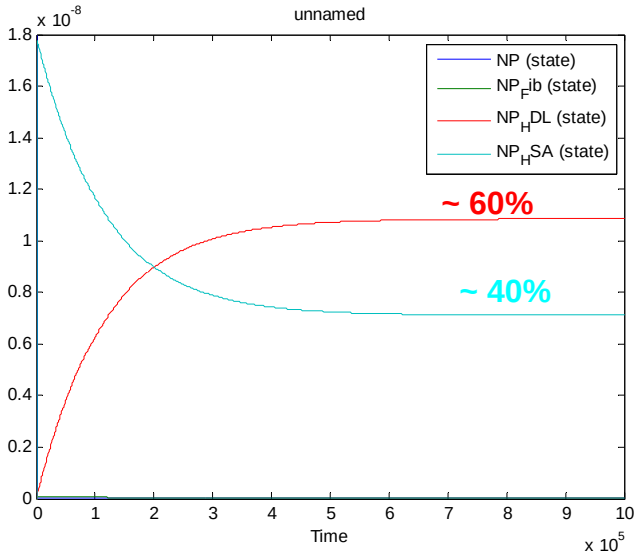
When k^{on} is further increased to 30-fold the initial value, the equilibrium is reached even faster (~5.5 h) and the relative proportion of HDL and HSA bound to NP (82% and 17% respectively) approaches the experimental values.
Tests were done also neglecting the correction factors arising from the number of binding sites, resulting in similar outputs (results not shown) indicating that these are not critical for the present setting.

3) 10 or 30 -times lower k^{off}_{HDL}

The affinity of protein-protein interactions can be changed also acting on the k^{off}, thus leaving the k^{on} unaltered. We hence compared the predictions from simulations when the same final affinity for HDL-NP binding is achieved by lowering the dissociation constant.
When k^{off} is reduced ten times with respect to the initial value, the following trends are observed:

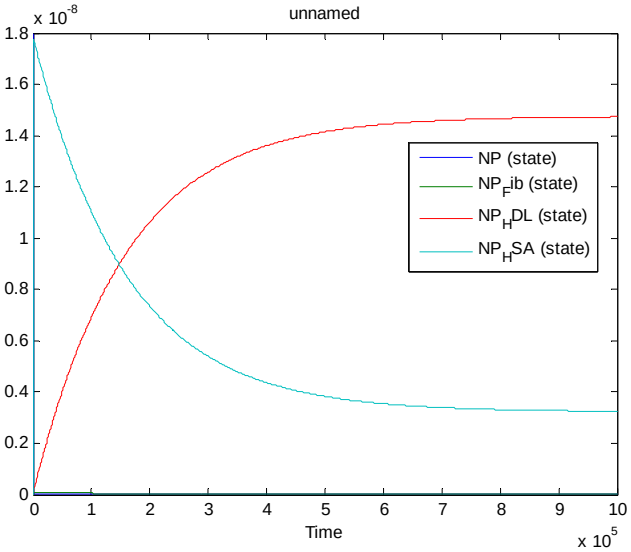


t = 10^5 s (27.8 hours)



t = 11 days

We point out that reaching a similar equilibrium as with ten times higher k^{on} requires however considerably longer times. The same is observed when decreasing k^{off} of a factor 30:



Hence, we conclude from this preliminary modeling the following:

- According to the tested settings, Fib is expected to play a minor role in the NP-binding compared to HSA and HDL

- For the same protein-nanoparticle equilibrium state, very different kinetics is expected depending on the perturbation of the association or dissociation rates, which reflects in very different times required to reach the actual equilibrium
- Further experimental data both at equilibrium levels and monitoring the time-evolution of the different species are required. The realization of such experiments can be aided by the results of this simple but effective model.

Future collaboration with host institution

The results from this simple initial model are realistic and promising. A future collaboration between the visitor and the group of Prof. Sara Linse is expected aimed at building more complex and complete models to simulate the corona dynamics. This will be achieved by iteratively updating the model with newly collected experimental parameters.

Projected publication

In the context of future collaborations, publication of the final outcome of the joint experimental-modeling efforts is expected.

References

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