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# Interactions between proteins and poly(ethylene-glycol) investigated using molecular dynamics simulations

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## Abstract.

Poly(ethylene-glycol) (PEG) is a polymer used to coat therapeutic preparations, like drugs or drug nanocarriers, and improve their efficacy. This effect is probably due to a reduction of the interactions of the coated species with the host organism. Nevertheless, experiments show that PEGylated materials do interact with the surrounding biological milieu, and in particular with blood proteins. Here, we use atomistic molecular dynamics simulations to characterize the interactions between the polymer and several blood proteins. In these simulations, the proteins are immersed in a mixture of PEG and water molecules. We observe how PEG distributes around the protein surface and measure PEG-protein interactions in terms of preferential binding coefficients.

## 1. Introduction

Poly(ethylene-glycol) (PEG) is a non-toxic polymer highly soluble both in water and non-polar solvents and finds application in a broad variety of contexts, including for example, as dispersant in toothpastes, lubricant in skin creams and anti-foaming agent in the food industry. In biomedical applications, PEG, when attached to a therapeutic molecule or nanoparticle (PEGylation), confers them extra resistance to degradation, extended blood clearance time and, ultimately, improved therapeutic efficacy. These improvements are obtained by a combination of factors like increased overall volume, which prevents renal clearance, but also a reduction of unspecific interactions with the surrounding environment. This last phenomenon goes under the name of stealth effect, and results in the ability of PEG to screen the PEGylated species from the immune response of the host organism.

Although PEG helps reduce unspecific interactions with the surrounding biological milieu, it does not remove them completely. It has been shown that, also due the constant exposure to PEG-containing products, the organism can produce antibodies against PEG. In addition, many different proteins adsorb on PEG-coated nanoparticles, as soon as these enter in contact with blood[1]. A molecular understanding of these protein adsorption phenomena on PEG-coated surfaces and more in general of the interactions between PEG and proteins is still missing.

Atomistic molecular dynamics simulations can help to fill this gap. They have been successfully used to address protein adsorption on a large variety of surfaces [2, 3], as well as the behavior of specific blood proteins on model surfaces[4, 5]. While accurate atomistic models and force field already exist for many metallic, metal-oxides and some polymeric surfaces[3], the



surface exposed by PEGylated species poses extra challenges due to its brushy nature. These challenges have been recently addressed by simulating the proteins of interest in a mixture of water and PEG molecules[6, 7]. With this approach, which is similar in spirit to what has been done in computational drug screening exercises[8, 9], the possible binding modes of PEG with the protein surface are sampled accurately and their relative importance is determined from the simulations.

Here, we extend the approach developed in [6] to additional blood proteins and use it to determine their preferential binding coefficient for PEG.

## 2. Methods

*Molecular dynamics simulations* All the simulations were carried out using the program NAMD [10] and the charmm27 force field[11] with the extension for PEG[12]. Tip3p[13] was used as model for the explicit treatment of water. An integration time step of 1 fs was used across the simulations. Simulations were carried out using periodic boundary conditions. A pressure of 1atm and a temperature of 300K were maintained constant during the simulations using the Langevin piston algorithm and Langevin thermostat [14, 15]. A cutoff of 1.2 nm was used for the non-bonded interactions with a switch function. Long range electrostatic interactions were treated using the smooth particle mesh Ewald (PME) method [16] with a grid spacing of about 0.1 nm. To prepare the PEG-water mixture, 64 PEG molecules ( $\text{H}-[\text{O}-\text{CH}_2-\text{CH}_2]_n-\text{OH}$ , with  $n$  either 4 or 7) were placed on a 4x4x4 grid with 1.0 nm spacing between grid points. Then 1 ns high temperature (700K) simulations in vacuum with damped electrostatics interactions (dielectric constant 200) were run to randomize the initial dihedral distribution of the PEG molecules. The PEG molecules were then immersed in a box of water molecules and sodium and chlorine ions were added to reach physiological concentration (0.15 M). Mixtures with different concentrations of PEG were obtained by changing the size of the water box surrounding the PEG molecules. The prepared mixtures were then equilibrated first at high temperature (373K) for 1.0 ns and then at 300K for 1.0 ns. Several plasma proteins have been considered: human serum albumin(HSA), bovine serum albumin(BSA), transferrin(TF) and apolipoprotein A1 (ApoA1). The initial coordinates of the proteins were taken from the Protein Data Bank(PDB) (see Tab. 1 for the list of PDBids). Each protein was immersed in a box filled by replicating the coordinates of the PEG-water mixtures obtained before in the three space directions and removing mixture atoms in close contact with protein atoms. The final PEG concentration in the simulation boxes is reported in Tab. 1. The size of the boxes was large enough to leave at least 1.0 nm from each protein atom and the box boundary. In the case of HSA, larger box sizes with 1.5 and 2.0 nm distances between protein and box boundary were simulated to investigate the dependence of the simulation results upon box size. The total charge of the systems was further neutralized by changing an adequate number of water molecules into ions. The complete systems were then minimized using the steepest descent algorithm for 10000 steps with harmonic restraints on the heavy atoms of the proteins. Then the systems were equilibrated at room temperature and pressure for 1.0 ns during which the harmonic restraints were gradually removed and for 1.0 ns without restraints. Finally production runs were started with 4 or 5 replicas for each system. Length of the runs ranged from 100 to 200 ns (see tab. 1).

*Analysis of trajectories* The direct PEG-protein interactions along the simulations were measured using the NAMD pair-interaction utility. The time series along the trajectories of the number of PEG and water heavy atoms found within a distance  $d$  of the protein or of each amino acid type was determined using the "pbwithin" selection command of VMD. The ratio of these numbers was compared to the ratio between all the PEG and water heavy atoms in the simulation box (bulk ratio). A PEG/water ratio larger than bulk for an amino acid implies the presence of an effective attractive interaction between PEG and the amino acid. On the other

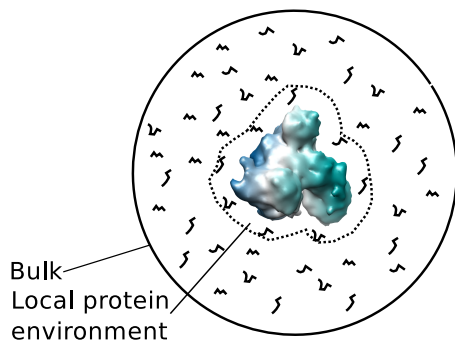
**Table 1.** List of the simulations performed

System	Box size (Å)	N. Atoms	PEG length	[PEG] (g/ml)	Simulation time (ns)
HSA+lipids	103	114000	4	0.13	4 x 105
HSA	98.8	100881	4	0.08	4 x 100
	98.2	99301	4	0.11	4 x 100
	108.6	134134	4	0.12	4 x 125
	118.2	172541	4	0.12	5 x 100
BSA	103.6	116489	4	0.11	4 x 100
Trasferrin	108	132009	4	0.11	5 x 200
	108.7	134663	4	0.07	5 x 200
	108.4	133303	7	0.07	5 x 200
	108.8	134372	7	0.04	5 x 200
ApoA1	192	739653	4	0.13	4 x $\approx$ 100

hand, amino acids with a PEG/Water ratio smaller than bulk exert an effective repulsion for PEG. For the residue specific PEG/Water ratio calculations we used  $d = 5.0\text{\AA}$ . The preferential binding coefficient measures the excess of PEG found in the local environment of the protein, i.e., in its vicinity (Fig. 1) and was defined as:

$$\Gamma = [PEG]_L - [H_2O]_L \frac{[PEG]_B}{[H_2O]_B} \quad (1)$$

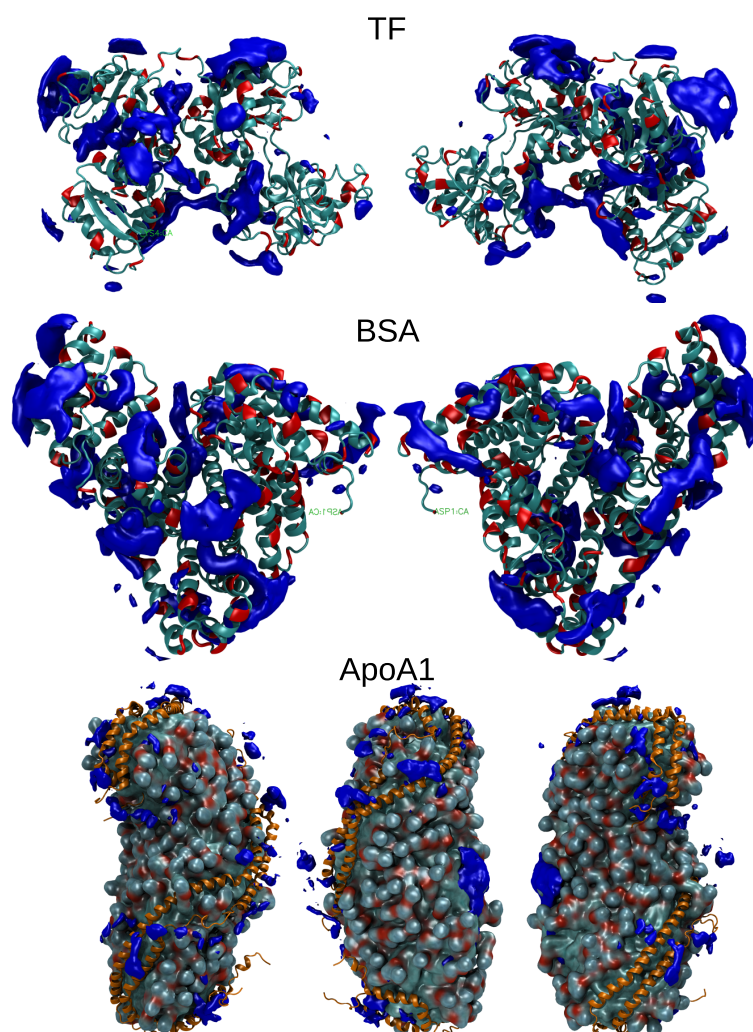
where  $[\cdot]_L$  is the concentration of PEG or water in the vicinity of the protein and  $[\cdot]_B$  the concentration in the bulk, that is, away from the protein. The local environment of the protein is defined as a shell of thickness  $d$  around the protein.  $\Gamma$  converges for  $d \geq 9.0\text{\AA}$ , which is the value we used for these calculations.



**Figure 1.** The concentration of PEG in the local environment of the protein and in the bulk solution are used for the measure of the preferential binding coefficient (Eq. 1).

### 3. Results and Discussion

Four different plasma proteins have been simulated immersed in a PEG/water mixture, as reported in section 2. Three of them (HSA, BSA, TF) are relatively abundant in plasma but not on the surface of PEGylated nanoparticles and one (ApoA1) is abundant on the nanoparticle [1]. The simulations were performed at different PEG concentrations to verify a possible dependence of the results on this parameter. Additional simulations explored the role of PEG length and box size. Each simulation was repeated 4 to 5 times to improve the statistical accuracy of the results. Only short PEG molecules were considered (4 or 7 monomers), which diffuse fast and allow for a thorough exploration of the possible binding modes with the protein. This insures



**Figure 2.** Cartoons of three different simulated proteins (cyan) where regions with PEG density larger than twice as in the bulk have been highlighted (dark blue). Left and right and center pictures represent different views of the proteins. Negatively charged residues are highlighted in red. In the case of ApoA1, the lipids present in the apolipoprotein are also rendered as a continuous surface.

that the observed quantities converge in the accessible simulation times (100ns-200ns). It is worth noting that, since the persistence length ( $3.8\text{\AA}$ ) of PEG is comparable with the distance between monomers, longer PEG chains are expected to explore the same binding modes as the shorter chains simulated here. The simulations show that the protein structures are not affected by the presence of PEG. The average root mean square deviation with respect to the starting structure or, in the case of ApoA1 to a control run in pure water, is in agreement with native state fluctuations of proteins of similar size ( $3\text{-}5\text{\AA}$  for HSA, BSA and TF).

PEG molecules in the simulations reach and leave the surface of the protein continuously, in a rather dynamical equilibrium. However, some regions of the surface are more frequently populated by PEG (Fig. 2).

We, then, analyzed in more detail the possible origin of the observed uneven distribution of



particular they measure the excess number of cosolvent molecules in the vicinity of each protein molecule, thus they are positive in case of a preferential binding of the cosolvent to the protein, and negative otherwise. Preferential binding coefficients are thermodynamic parameters that can be accessed experimentally through high precision densitometry experiments[18], differential scanning calorimetry[19] and vapor pressure osmometry[20]. In the present case, they report the affinity of each protein for the cosolvent (PEG) *versus* the solvent (water) and can be approximated using eq. 1[21]. The measurements we have performed using the simulation data (Tab. 2) show in most cases positive coefficients very close to zero, indicating a very weak preference of the analyzed proteins for PEG.

**Table 2.** Preferential binding coefficients measured along the simulations

System	Box size (Å)	PEG length	[PEG] (g/ml)	$\Gamma$ (n.mol.)
HSA+lipids	103	4	0.13	3.4 (1.2)
HSA	98.8	4	0.08	5.0 (0.8)
	98.2	4	0.11	7.0 (1.3)
	108.6	4	0.12	2.9 (0.9)
	118.2	4	0.12	-0.1 (1.1)
BSA	103.6	4	0.11	0.0 (1.0)
Trasferrin	108	4	0.11	4.8 (0.9)
	108.7	4	0.07	7.5 (1.0)
	108.4	7	0.07	4.7 (0.7)
	108.8	7	0.04	2.9 (0.7)
ApoA1	192	4	0.13	-1.2 (1.6)

#### 4. Conclusions

Here we show how atomistic molecular dynamics simulations can be used fruitfully to assess the interaction of plasma proteins with PEG. The simulations reveal that the interaction patterns with surface amino acids are similar across the different proteins and the different simulation conditions analyzed here. This fact has allowed for building simplified models of protein interactions with PEG[6]. The simulations provide also direct measurements of the preferential binding coefficients which could be assessed experimentally to test and help improve the available force fields. The approach used in the present work is rather general and could be extended to evaluate interactions of proteins with other polymers that, like PEG, are highly soluble in waters and do not induce large conformational changes to proteins. Indeed, large efforts are being made to identify polymers that could replace PEG in biomedical applications and solve the issues related to its possible long-term toxicity and nonbiodegradability. We expect the techniques developed here to help in that direction.

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