Electronic Supplementary Material (ESI) for Nanoscale.
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Disease-related metabolites affect protein-nanoparticle interactions

Mahdi Tavakol[a], Abbas Montazeri[b,c], Reza Naghdabadi[a,d*], Mohammad J. Hajipour[e,f], Saeid Zanganeh[g], Giulio Caracciolo[h], and Morteza Mahmoudi[i*]

[a.] *Department of Mechanical Engineering, Sharif University of Technology, Tehran, Iran.*

[b.] *Faculty of Materials Science and Engineering, K.N. Toosi University of Technology, Tehran, Iran.*

[c.] *School of Nano-Science, Institute for Research in Fundamental Sciences (IPM), Tehran, Iran.*

[d.] *Institute for Nano-Science and Technology, Sharif University of Technology, Tehran, Iran.*

[e.] *Persian Gulf Marine Biotechnology Research Center, The Persian Gulf Biomedical Sciences Research Institute, Bushehr University of Medical Sciences, Bushehr, Iran.*

[f.] *Non-Communicable Diseases Research Center, Endocrinology and Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran.*

[g.] *Sloan Kettering Institute for Cancer Research, New York, NY, United States.*

[h.] *Department of Molecular Medicine, "Sapienza" University of Rome, Rome 00185, Italy.*

[i.] *Department of Anesthesiology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115, United States.*

* Corresponding authors: (RN) email: naghdabd@sharif.edu ; (MM) email: mmahmoudi@bwh.harvard.edu

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

Simulation Method

While all atom (AA) molecular simulations can give a detailed picture of protein/NP interactions, their inability to study large proteins for long durations of time is challenging. Coarse-Grained (CG) molecular simulations, however, try to circumvent this shortcoming by compromising some vital information. To overcome these limitations, subsequently, a multi-scale scheme composed of both of these methodologies can be devised. Such a methodology has a capacity to track the PC formation in larger time scales while maintaining information indispensable for the study of the process. The first two stages of the protein adsorption, which are the protein movement and adsorption[1], were modelled using a coarse level of simulation while the structural changes happening at the last stage[1] was studied with finer details. Recently, Ozboyaci *et al.*[2] calculated the Potential of Mean Force through Brownian dynamics for the adsorption of β-lactamase inhibitor protein on gold surface. The contact mode for the adsorption was obtained using docking, which was in accordance with the minimum of the free energy configuration[2]. Thus, in the current study, the free energy profile was benefitted for finding the adsorption mode. The umbrella sampling method was used in the coarse level of the multi-scale scheme to find the free energy profile of the PC formation[3]. The initial distance of the polymer protein was chosen as a distance much larger than the cut-off of the interatomic forces to take into account the hydrophobic interactions acting in long distances. The protein polymer distance was split into 0.1 Å windows. Also, an independent simulation was performed on each one of them. The windows were combined through the Weighted Histogram Analysis Method (WHAM) to calculate the free energy profile[4]. Then, additional windows were added. Also, the simulation length for some of the previously simulated windows was increased. These

procedures were repeated until there was enough overlap between different simulations and the probability distribution of each one was in the form of the normal distribution function. The backmapping and AAMD simulation from the minimum of the free energy profile followed the CGMD simulations to pursue the structural changes of the protein. Figure S1 of the Supporting Information summarizes the employed methodologies in this study.

CGMD Simulation

The Martini force field, in which the protein's secondary structure remains fixed, was utilized for the coarse level of the multi-scale model[3]. Disulfide bonds were taken into account because of their importance in stabilizing the protein's native structure[4]. The interaction coefficients were taken from the version 2.2 of the Martini force field[5]. The CG model for PS was based on the mapping and the parameters developed by Rossi *et al.*[6]. First, the topology of a polymer chain including the bonded interactions was constructed. During the minimization of the initial coordinates obtained from the topology, forces were faded in as recommended by Melo *et al*. [7]. Equilibration in 310 K and vacuum conditions were performed after the energy minimization. Then, a huge number of chains were randomly placed in a simulation box. Instead of equilibrating the system for several hundred nanoseconds to reach the experimentally obtained density, a simulated annealing procedure was proposed. In this protocol, the temperature of the system was linearly changed between specified minimum and maximum values in several stages, until it attained a fixed density. The density of the PS30 polymer composed of 30 styrene chains was calculated equal to the value of 938 as obtained by Rossi *et al.*[8]. To create a spherical NP from the equilibrated polymer system, those polymer chains that had at least one bead

inside the sphere were selected to be a part of the NP. As presented in Figure S2, the equilibrated Martini CG systems for the protein and the polymer were placed into a simulation box in a distance far enough so that they did not interact with each other. The glucose molecules of the CG model developed by Lopez *et al.*[9] were placed into the box with the specified concentrations. The initial positions of the glucose molecules were chosen randomly. It is noteworthy that since the contact residues obtained from the AAMD simulations can explain the adsorption and free energies correctly, the initial configuration of the metabolites does not affect the ultimate simulation outcomes. In addition, long equilibration stages were used in both the CGMD and AAMD simulations to eliminate the correlation between the initial configuration and the final simulation results. The martini ions with the concentration of 37.5 mM equal to the effective concentration of 150 mM, alongside antifreeze water molecules, were also added to the simulation box[10, 11].

The Umbrella Sampling technique was chosen to obtain the free energy profile of the PC formation. Discarding the first several nanoseconds of each window as the equilibration stage, the next 15 nanoseconds were implemented to calculate the free energy. Finally, the free energy versus the distance profile for the PC formation was obtained employing the WHAM[4]. In the CG simulation, Bussi thermostat and Parrinello-Rahman barostat were utilized with the time-step of 30 femtoseconds to mimic the system in the biologically relevant condition of 310 K and 1 bar[12]. The total accumulated CGMD simulation time was roughly 60 microseconds.

AAMD Simulation

The CG configuration with the minimum free energy determined from the previous stage was utilized as the initial system of AAMD simulations. The configuration was backmapped to the AA

resolution utilizing the protocol devised by Wassenaar *et al.*[13]. In this procedure, the projection of the CG resolution to the AA one preceded the energy minimization without non-bonded interactions. Next, the system was energy minimized before several equilibration stages where the time-step and temperature raised gradually from 0.2 femtoseconds and 50 K to 2 femtoseconds and 310 K, respectively. MD simulations were done using GROMACS with CMAP enabled version of CHARMM 36 force field[14, 15]. The energy minimization and equilibration simulations were accomplished for the backmapped system while constraining the backbone atoms of the proteins and non-hydrogen atoms of the polymer. Thereupon, AAMD simulations were pursued for 10 nanoseconds to study the structure changes of the protein. In AAMD simulations in which, Parrinello-Rahman barostat and Bussi thermostat were utilized, the time step was 2 femtoseconds.

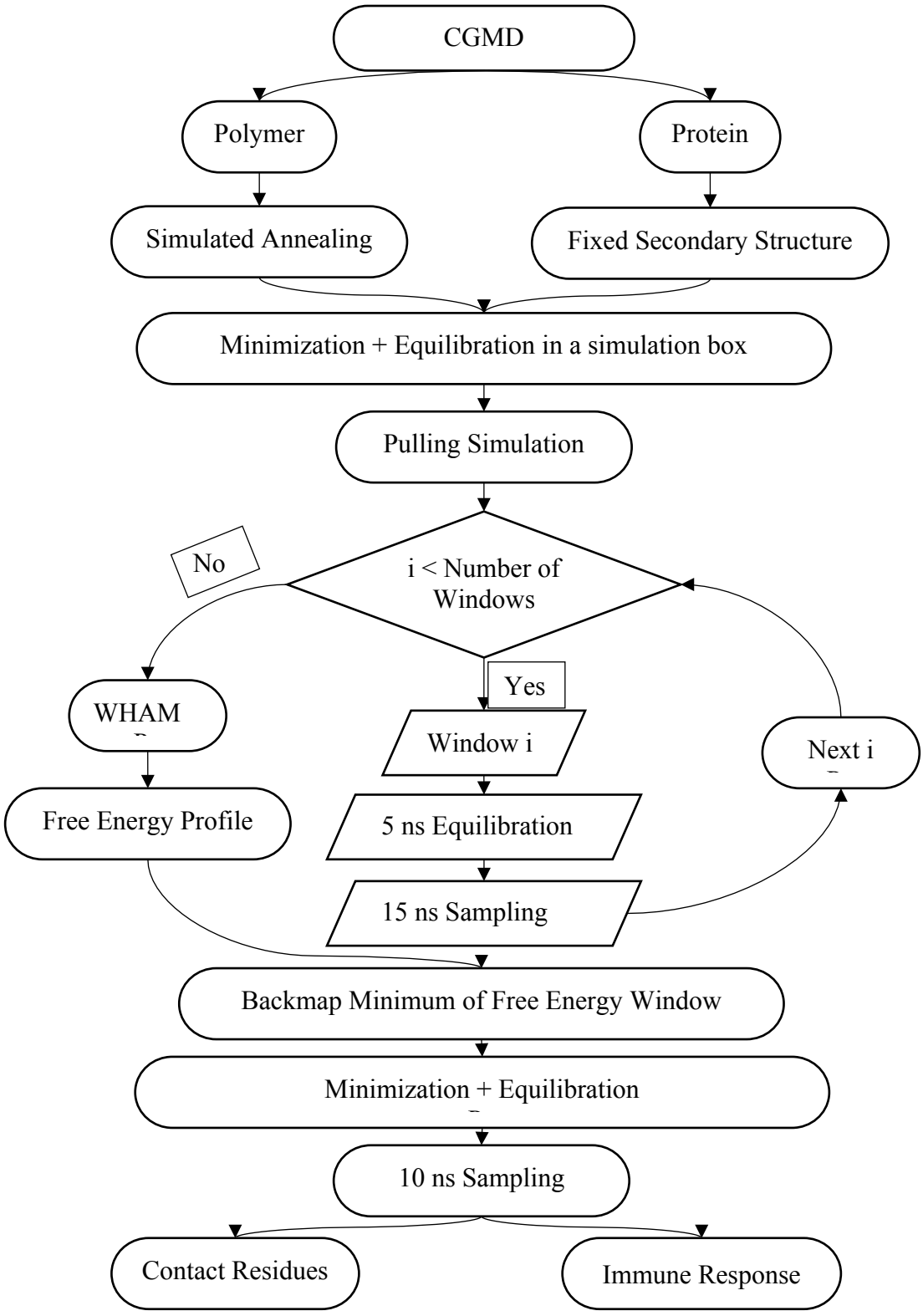

Figure S1. The multi-scale method stages.

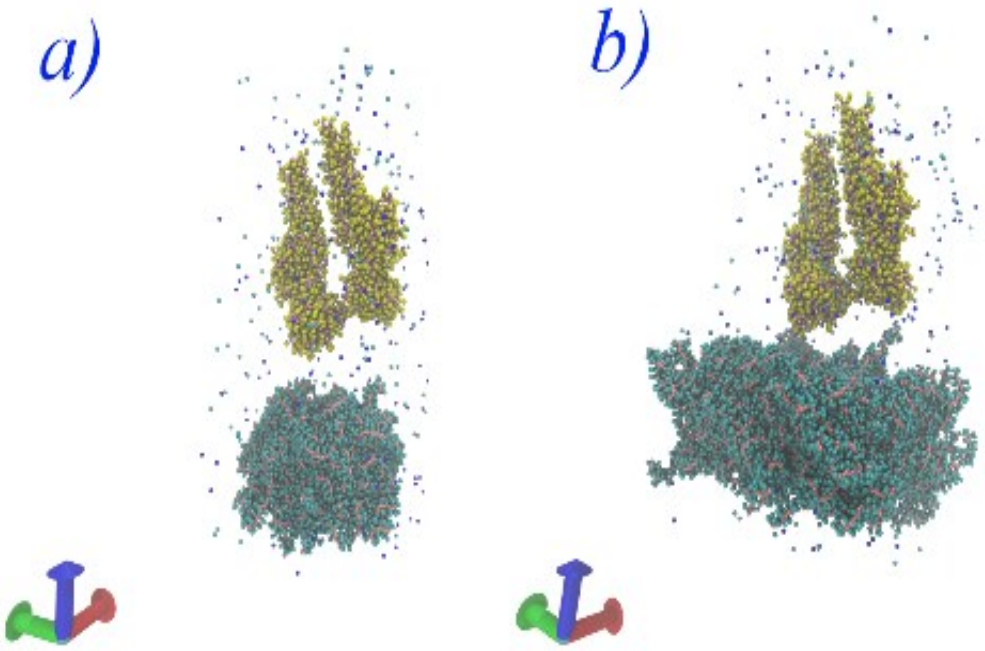

Figure S2. The initial configuration for the coarse-grained simulation of FIB adsorption on a) spherical PS30 b) flat PS30. Water and antifreeze particles were removed for clarity.

Table S1. Contact residues for adsorption of FIB segment D on the surface of flat PS30 in the presence and absence of the metabolite

No Glucose			4 mM Glucose			12 mM Glucose		
Residue	Number	Percent	Residue	Number	Percent	Residue	Number	Percent
PHE	11	13.75	ALA	12	15.00	PRO	11	11.11
ALA	10	12.50	PHE	9	11.25	LYS	10	10.10
LYS	8	10.00	PRO	6	7.50	PHE	9	9.09
PRO	7	8.75	ARG	6	7.50	ALA	7	7.07
THR	6	7.50	GLY	5	6.25	ILE	7	7.07
TYR	5	6.25	ASP	5	6.25	GLY	7	7.07
GLY	4	5.00	LEU	5	6.25	ASP	6	6.06
ILE	4	5.00	LYS	5	6.25	LEU	5	5.05
LEU	3	3.75	SER	4	5.00	GLU	5	5.05
SER	3	3.75	THR	4	5.00	SER	5	5.05
ARG	3	3.75	GLU	4	5.00	MET	4	4.04
ASP	3	3.75	ILE	3	3.75	GLN	4	4.04
GLN	3	3.75	TYR	3	3.75	TYR	4	4.04
MET	3	3.75	MET	2	2.50	THR	4	4.04
GLU	3	3.75	ASN	2	2.50	ARG	3	3.03
ASN	3	3.75	GLN	2	2.50	ASN	3	3.03
VAL	1	1.25	TRP	2	2.50	TRP	2	2.02
HIS	0	0.00	VAL	1	1.25	VAL	2	2.02
CYS	0	0.00	CYS	0	0.00	HIS	1	1.01
TRP	0	0.00	HIS	0	0.00	CYS	0	0.00
Total	80	100	Total	80	100	Total	99	100

Table S2. Contact residues for the adsorption of FIB on the surface of flat PS NP in the presence of healthy and hypercholesterolemia concentrations of cholesterol.

5.2 mM cholesterol concentration			6.2 mM cholesterol concentration		
Residue	Number	Percentage	Residue	Number	Percentage
PHE	8	10.67	PHE	13	12.04
GLY	7	9.33	LYS	12	11.11
ASP	7	9.33	PRO	11	10.19
ALA	6	8.00	ILE	8	7.41
LEU	6	8.00	LEU	7	6.48
PRO	6	8.00	SER	7	6.48
GLN	6	8.00	ALA	6	5.56
LYS	5	6.67	GLN	6	5.56
TYR	4	5.33	ARG	5	4.63
GLU	4	5.33	ASN	5	4.63
ARG	3	4.00	GLY	5	4.63
MET	3	4.00	THR	5	4.63
THR	3	4.00	ASP	4	3.70
ILE	2	2.67	TYR	4	3.70
SER	2	2.67	MET	3	2.78
ASN	1	1.33	VAL	3	2.78
VAL	1	1.33	GLU	2	1.85
HIS	1	1.33	HIS	2	1.85
CYS	0	0	CYS	0	0
TRP	0	0	TRP	0	0
Total	75	100	Total	108	100

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