

UNDERSTANDING PROTEIN FOULING BY RESORTING TO MESO-SCALE MODELLING

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ABSTRACT

In-depth molecular scale understanding of protein adsorption on heat exchanger surfaces will inevitably lead to promising anti-fouling strategies. It is however difficult to get sufficient experimental data on protein adsorption for mechanism analyses, especially for the in-process dynamic data. Starting from the simplest biofouling system, in this work, a meso-scale modeling method developed by us was extended to investigate multi-peptide adsorption. For a system with 12-Ala hydrophobic peptides, it was found that only a small proportion of peptide-chains would keep separated while most of them would tend to aggregate together. Compared with the aggregates, those individual peptide-chains have higher flexibilities and lower environmental sensibilities, which means that they could end up with an adsorbed state very quickly. This finding implies that the adsorbed individual peptide-chains might have changed the aggregated water layers adjacent to the solid-liquid interface, which leads to barriers to the following aggregates' adsorption.

INTRODUCTION

For the pasteurization process in dairy industry, undesired protein based deposits in heat exchangers would reduce the performance of heat transfer (Georgiadis et al., 1998; Jun et al., 2005; Bansal et al., 2006; Fickak et al., 2011; Pelegrine et al., 2012; Jimenez et al., 2013). And most importantly, this kind of undesired deposits have been proved to be pathogens' excellent growing medium that would bring serious bio-pollution into dairy products (Punidadas et al., 1999; Michalski et al., 1999). A slight adjustment of surrounding environment such as ionic strengths (Christian et al., 2003; Blanpain-Avet et al., 2012; Jimenez et al., 2013) and pH (Pelegrine et al., 2012; Kröner et al., 2013) of the liquid raw material would significantly influence the adsorption rate. It can be attributed to the change of the degree of dissociation and configuration of the solute, i.e., protein in this case. When extended to new biomaterials, all operational parameters have to be resettled (Hanke et al., 2014). Although experimental methods could help us understand the influence of deposit on heat transfer at macroscale, poor understanding of molecular level adsorption mechanism could lead to long lab-working time and high production costs (Hanke et al., 2014). In order to

open the 'black-box' of adsorption mechanism, microscale computational approach is a great choice.

According to the interaction function and force fields, amino acid residues/atoms interact with surroundings via bonded and non-bonded interactions (Miller et al., 2010). Bonded interactions mainly work for the conformation change within one protein/peptide, and influence the interaction with surrounding molecules indirectly. While it is non-bonded potentials that are mainly in charge of reflecting the environment, leading to the adsorption of peptide chains to form fouling layers. Non-bonded potentials are Van der Waals potential and electrostatic potential that determine the residues' water affinities based on their hydrophobicity (Penna et al., 2014; Pandey et al., 2009). At the same time, the steric structures of amino acid residues' sidechains also play important roles in residue movements (Yu et al., 2012; Monticelli et al., 2008). Containing residues with tiny chemical groups, such as Alanine residue (Ala) and Glycine residue, a peptide would have higher backbone flexibility, which would increase its mobility in water (Yu et al., 2012; Sakiyama et al., 2006).

Considering both interaction and steric influences of ordered amino acid residues, the results from all-atom simulations demonstrated that a single peptide-chain would move from bulk water towards an uncharged solid surface and end up with an adsorbed state via a biased diffusion phase followed by an anchoring phase and a binding phase (Yu et al., 2012; Penna et al., 2014; Qiu et al., 2017). When extended to protein simulation, structure flexibility is usually not taken into account because of the low computational efficiency of all-atom simulation and the insufficient experimental support (Hagiwara et al., 2009). Rigid structure of one single protein was used to study the influence of different charge distributions and specific types of residues' distributions on its adsorption behavior (Hagiwara et al., 2009).

In industrial practice however, fouling is mainly caused by the adsorption of multi-proteins (Bansal et al., 2006; Fickak et al., 2011; Jimenez et al., 2013). Their configurations would definitely change under the influence of the environment (Christian et al., 2003; Blanpain-Avet et al., 2012; Jimenez et al., 2013). Rigid structures of all atom simulation could not deal with this important steric effect. Meanwhile, the simulation case of one single protein/peptide could not represent the real fouling system (Qiu et al., 2017).

In the liquid phase, most single proteins would gather and form much larger aggregates that affect the moving trajectories of each other (Bansal et al., 2006; Blanpain-Avet et al., 2012; Jimenez et al., 2013; Bouvier et al., 2014). These experimental results show the limitation of all-atom method and the requirement in the development of a mesoscale coarse-grained method.

By now, only a few papers have reported the simulations of multiple peptides. Pandey *et al.* (2009, 2010 and 2012) has contributed greatly in the simulation of multiple peptides with a unique all-residue Monte Carlo model. The adsorption density, binding energy and supramolecular assembly of multiple peptides were investigated. The adsorption preferences on different solid surfaces which have been reported in experimental studies were reproduced, which demonstrates the validity of the coarse-grained Monte Carlo model (Pandey et al., 2009; Eby et al., 2010). With further consideration of the root mean square displacement of the center of mass and the radius of gyration for single long peptide-chains, potential applications of this method to much more complex systems have been proposed (Foo et al., 1998, 1997; Pandey et al., 2012; Pandey et al., 2010; Pandey et al., 2009).

The present study focuses on quantitative and mechanistic analyses of the adsorption behavior of 12-Ala hydrophobic peptide-chains. This module peptide was designed with the hydrophobic and denatured characters that most proteins have. The studies on peptide aggregation in bulk water and peptide adsorption on the surface can shed light on fundamental fouling mechanisms.

MODELING METHODOLOGY

In this study, a hybrid coarse-grained lattice Monte Carlo model was used to investigate peptides' adsorption on Au (111) surface (Qiu et al., 2017). The movement of bead is partially accepted according to the Metropolis Algorithm. This model has been used in our previous research. It can reproduce the water distribution on the liquid-solid interface, and also multiple adsorption phases that were reported in other molecular dynamics simulation results (Qiu et al., 2017). The simulation box of $7.50 \times 7.50 \times 7.50 \text{ nm}^3$ was constructed from identical cubes, whose sizes were $0.125 \times 0.125 \times 0.125 \text{ nm}^3$ and could only host one coarse-grained bead to occupy its eight vertexes (Rouault et al., 1995; Carmesin et al., 1988; Xiao et al., 2009). Periodic boundary conditions were applied to the x and y directions. For the vertical direction (z) of the simulation box, within its bottom and top 1.2 nm space (equal to the cut-off radius) were filled with surface beads (providing the solid phase) and unmovable water beads (providing the bulk liquid phase), respectively.

The Coarse-Grained (CG) beads, which were coarse-grained based on a four-to-one mapping scheme (Monticelli et al., 2008), can be divided into four types, i.e., polar, charged, uncharged and water beads according to specific distributions of charged sites (Monticelli et al., 2008; Yesylevskyy et al., 2010; Zhang et al., 2011; Jong et al., 2012; Qiu et al., 2017).

Because most proteins are overall hydrophobic, a linear 12-Ala (AAAAAAAAAAAA) CG peptide-chain was used in

this study. According to the CG map (Jong et al., 2012), the Ala residue is only represented by one P4 type CG bead that could remove the steric influence. Meanwhile, the CG bead type of Ala residues is the same as water beads, which means that the interaction parameters between water-surface and Ala-surface are the same. Without the influence from different potentials and specific sidechain steric hindrances, 12-Ala peptide-chain could allow us to focus more on the process of aggregation and adsorption.

Gold surface is the most stable and well-studied metal surface (Heinz et al., 2008; Pandey et al., 2009; Yu et al., 2012; Penna et al., 2014). Using this surface could provide us with model validations and result comparisons (Yu et al., 2012; Penna et al., 2014). The adjusted Au surface structure was the one used in Heinz *et al.* (2008). The coordinates were (0.00, 0.00, 0.00), (0.125, 0.250, 0.00), (0.00, 0.125, 0.250), (0.125, 0.375, 0.250), (0.250, 0.125, 0.500) and (0.00, 0.375, 0.500) nm in an Au crystal cell with the lattice at (0.250, 0.500, 0.625) nm.

The potential of a CG bead $U(r_{ij})$ summarized from all non-bonded interactions among its neighbors within the cut-off radius at 1.20 nm (Equation 1). The bonded interaction among one peptide-chain was replaced by Bond-Fluctuation Model (Carmesin et al., 1988; Xiao et al., 2009). The non-bonded interaction includes Lennard-Johns potential (switched at 0.9 nm) and the electrostatic potential (shifted from 0 nm to $r_{\text{cut-off}}$) whose parameters are referenced from the MARTINI force field with the extension by the Lorentz-Berthelot rule (Monticelli et al., 2008; Yesylevskyy et al., 2010; Zhang et al., 2011; Jong et al., 2012; Xu et al., 2015)

$$U(r_{ij}) = U_{\text{LJ}}(r_{ij}) + U_{\text{Ele}}(r_{ij}) \\ = 4\epsilon_{ij} \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^6 \right] + \frac{q_i q_j}{4\pi\epsilon_0\epsilon_{\text{rel}}r_{ij}} \quad (1)$$

To construct the initial configuration of the system, the solid layer (thicker than $r_{\text{cut-off}}$) was placed at the bottom of the simulation box. It was set as rigid throughout the simulation. Then, the peptide-chains were inserted at specific distances away from the surface with random configurations. Solvate the peptide-chains with CG water beads whose number was adjusted marginally to ensure that the relative density at locations over 2.00 nm from the surface was kept at 1.00 under the environment of 298 K and 1 atm. Two aggregated water layers were located at 0.375 nm and 0.875 nm with a neutral charge distribution.

Apart from the average distances that were averaged from z coordination of peptide-chains, partitioned force quantification and radius of gyration (R_{gyr}) were used to quantify the movement during the adsorption process. The partitioned force was the summation of LJ force and electrostatic force in the z direction from all neighboring beads of peptide-chains within $r_{\text{cut-off}}$ (Equation 2). It could help to identify dominant forces that lead to the adsorption under different conditions.

$$\overline{F_z} = \sum \left[-\frac{dU(r_{ij})}{dr} \cdot \frac{\overline{r_{ij,z}}}{r_{ij}} \right] \\ = \sum \left[-\frac{d[U_{\text{LJ}}(r_{ij}) + U_{\text{Ele}}(r_{ij})]}{dr} \right] \cdot \frac{\overline{r_{ij,z}}}{r_{ij}} \quad (2)$$

For illustrating the cluster degree of an aggregate which is composed by a group of CG beads, R_{gyr} was used

(Kuszewski et al., 1999) (Equation 3). This value is defined as the average distance from each beads to their centroid. A higher R_{gyr} represents a lower cluster degree.

$$R_{gyr} = \left\{ \sum_{j=1}^N [r_j - (\sum_{i=1}^N r_i / N)]^2 / N \right\}^{1/2} \quad (3)$$

RESULTS AND DISCUSSION

Sixteen linear Ala peptide-chains were used to study the multi-peptide adsorption process. A coarse-grained Ala peptide-chain had no side chains and its LJ parameters were the same as those for the water CG beads (Jong et al., 2012). It is defined that an aggregate has been formed when any part of three peptide-chains are located within $0.5 r_{cut-off}$ of each other. When any part of one peptide-chain is located within $0.5 r_{cut-off}$ of two peptide-chains in one aggregate, this new one should be integrated into the aggregate.

Sixteen peptide-chains were put into the simulation box with random configurations at random locations beyond 2.00 nm above the surface (Fig. 1a). Chain No. 1, 2, 7, 8, 9, 11, 12 and 13 were located closely at the beginning while others were put relatively dispersed.

In the next 75,000 MC steps, Chain No. 10 finished adsorbed on the surface, Chain No. 3, 4 and 7 floated separately and three little aggregates were formed (Fig. 1b):

- Chain No. 5, 6, 8 and 14;
- Chain No. 1, 11 and 13;
- Chain No. 2, 9, 12, 15 and 16.

Followed by the floating away of Chain No. 11, the adsorption of Chain No. 2 and 16, and the joining of Chain No. 3 (Fig. 1c), two small aggregates moved towards each other (Fig. 1d) to form a bigger aggregate. Meanwhile, the last small aggregate finished aggregating at an early time (Fig. 1b) and kept floating in the solution (Fig. 1c-f). Compared with the total amount of peptide-chains in solution, majority of chains aggregated together (i.e., 11 out of 16) rather than adsorbed on the surface (i.e., 4 out of 16) or kept floating in the solution (i.e., 1 out of 16).

Sorting all peptide-chains based on their final position and configuration at 270,000th MC step (Fig. 1f), these chains could be divided into three groups as shown below:

- Multiple-peptide aggregates. Two aggregates were formed at the end of the simulation. The bigger one contained seven chains, i.e., Chain No. 1, 3, 7, 9, 12, 13 and 15. The smaller one had 4 chains which were Chain No. 5, 6, 8, and 14;
- Absorbed single chains. Chain No. 2, 4, 10 and 16 finished adsorbed on the surface at the end of simulation with different conformation.
- Floated single chain. Chain No. 11 kept floating in the solution.

In the next section, one aggregate, one floated peptide-chain and one adsorbed chain have been picked out for a detailed study in order to obtain in-depth understanding of the adsorption mechanism.

Single-peptide dynamics

It was observed that most peptide-chains were integrated into aggregates in this case. Single peptide chains could end up with an adsorbed state in the early stage of the process, which implies that they might strongly influence the future

interfacial condition. An adsorbed peptide-chain (Chain No. 10) that was rarely influenced by the aggregates and the only free-floating peptide-chain (Chain No. 11) were used to illustrate the movement in this part.

Initially, Chain No. 10 was surrounded by Chain No. 5 only (Fig. 1a). As shown in Fig. 2, at the first 7,400 MC steps, Chain No. 10 moved away from the surface. But after that, the chain headed for the surface. Although hindered by the second aggregated water layer (0.875 nm away from the surface) for 5,000 MC steps and the first aggregated water layer (0.375 nm away from the surface) for 3,000 MC steps, Chain No. 10 was adsorbed onto the surface successfully. Only a slight larger proportion of negative value of the total force (i.e., overall attractive force between the surface and the peptide) could be observed before peptide-chains adsorption while the negative force became dominant after peptide-chain was adsorbed on the surface at 75,000th MC step (Fig. 2c). In this adsorption process, no clear trend of R_{gyr} can be observed, which is mainly due to the small number of CG beads involved in one peptide-chain (Fig. 2b).

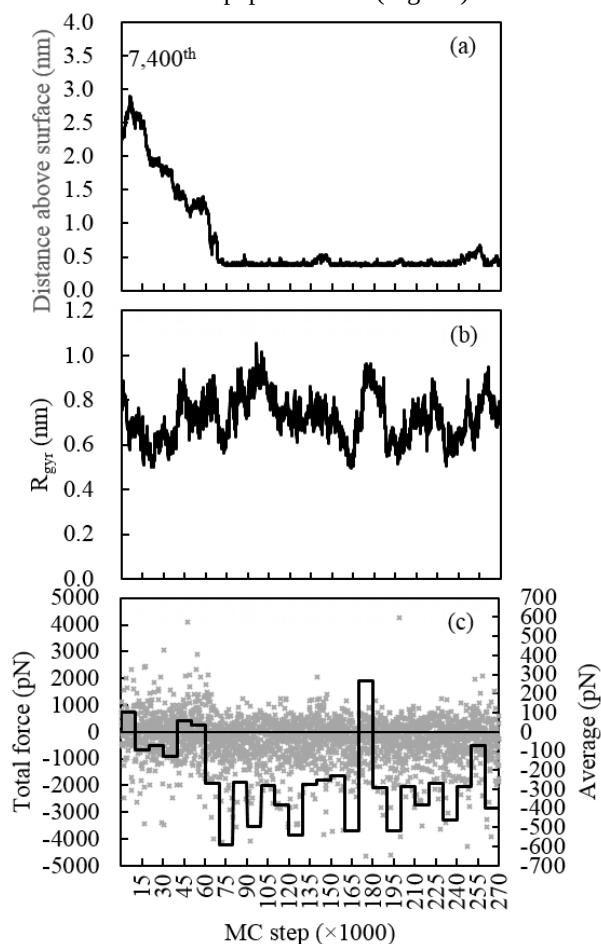


Fig. 2 Chain No. 10's trajectory in terms of the vertical distance from the surface and its radius of gyration, together with the total force exerted by water and surface beads during the adsorption process. In (a), the black and grey horizontal arrows show the locations of the first and the second aggregated water layer, respectively. In (c), the grey points show the in-process data of the total force and the black line represents the average value for every 10,000 MC steps. Negative

values indicate attractive forces between the surface and peptide chains.

Chain No. 11 was the only single chain that kept floating in the solution. It was located at the edge of a high-density peptide area (Fig. 1a). It was driven away from the surface by Chain No. 1, 3 and 13 (Fig. 1c and 1d). Because of the loose structure of the cluster formed by these four chains, Chain No. 11 left the cluster and finally floated alone after 270,000th MC step (Fig. 1f). Through the numerical analyses, two attempts of breaking through the aggregated water layers at 62,000th and 243,800th MC step respectively could be observed (Fig. 3a). Stronger influence from other peptide-chains than the one from aggregated water layers caused its first failed attempt at around 62,000th MC step (Fig. 3a). At that point, Chain No. 1 and 13 were adjacent to Chain No. 11 and it was located outside the interaction range of solid surface beads but could interact directly with aggregated water layers (Fig. 1b and 3a). Before the next attempt, Chain No. 11 reached the highest location at around 160,000th MC step. At this time, the average distance of peptide was about 3.0 nm away from the surface which was outside of the interaction range of two aggregated water layers and the total force was about to change from repulsive to attractive (Fig. 3a and 3c). It implies that the main downward attractive force was exerted by other peptide-chains though none of them were located under or above it. It must be pointed out that the top solvent layer in the simulation box was more than 5.00 nm away from the surface, located far away from peptide Chain No. 11. Thus the change of movement direction of Chain 11 at the 160,000th MC step was not due to the restriction of the simulation box size. For the second attempt, it lasted for less than 60,000 MC steps which was too short for a peptide-chain to obtain a configuration/morphology that could secure an adsorbed state. The movement of Chain No. 11 was mainly influenced by its neighboring peptide-chains, dominant attraction force from the surface cannot be identified in Fig. 3 (i.e., the overall attractive force is slightly higher than the overall repulsive force).

Due to the small size of a single 12-Ala peptide-chain, no specific trend of R_{gyr} could be observed in both Fig. 2b and 3b. But these results can be used for the comparison with the R_{gyr} dynamics of aggregates in the next section.

In this case, the leaving of Chain No. 11 from the loose-structure cluster implies a hypothesis that there might be a maximum number of peptide-chains that an aggregate can contain. Similar hypothesis has been reported by Eby *et al.*, that the hydrophobic residues might relax the peptide into small aggregates (Eby, 2010). The interaction in an aggregate not only needs to integrate all chains together but also offer a certain level of flexibility so that the aggregate can respond to the environment and demonstrate mobility.

Multi-peptide aggregate dynamics

In this work, when any part of a peptide-chain is located within $0.5 r_{\text{cut-off}}$ of other two chains, these three peptide-chains are regarded as a multi-peptide aggregate. As shown in Fig. 1f, at the end of simulation (i.e., the 270,000th MC step), two aggregates (with four and seven peptide-chains, respectively) were formed. In this part, the dynamics of the

bigger multi-peptide aggregate would be studied in detail, while the dynamics of the smaller one were affected by the periodic boundary condition.

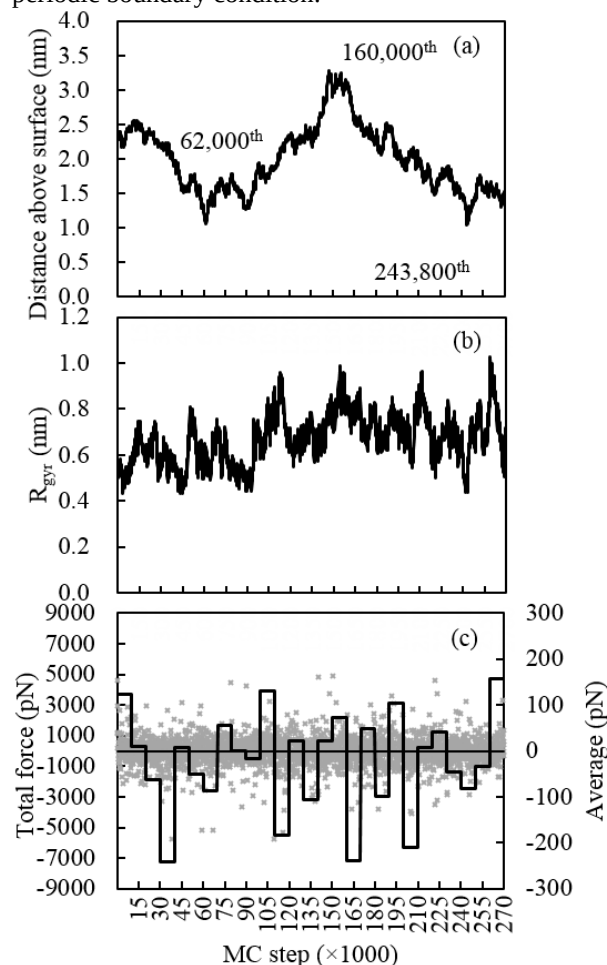


Fig. 3 Chain No. 11's trajectory in terms of the vertical distance from the surface and its radius of gyration, together with the total force exerted by water and surface beads during the adsorption process. In (c), the grey points show the in-process data of the total force and the black line represents the average value for every 10,000 MC steps. Negative values indicate attractive forces between the surface and peptide chains.

The screen shots shown in Fig. 1 clearly demonstrate the formation of this aggregate. At the beginning, all chains were located at about 2.2 nm away from the surface and none of them within 2.00 nm (Fig. 1a and 4a). The initial positions of Chain No. 1, 7, 9, 12, 13, 15 were close to each other and Chain No. 2, 8, 11, 16 were located adjacent to them. Chain No. 3 was far away from them (Fig. 1a). After some time, three groups were formed. One group contained Chain No. 7 only. Another group contained Chain No. 1, 3, 8, 11, 13. Due to its loose structure, Chain No. 8 left and joined into a new aggregate while Chain No. 11 started to leave and become a stand-alone one (Fig. 1b and 1c). The last biggest group contained Chain No. 2, 9, 12, 15, 16 and could be labelled as an aggregate (Fig. 1b and 1c). As time went by, the first two groups moved towards the biggest group and were integrated into it (Fig. 1d). During the integration, Chain No. 2 and 16 attempted to lock down on the surface. Although Chain No.

2 broke through the aggregated water layers (between the 75,000th and the 113,000th MC step) later than Chain No. 16 did (before the 75,000th MC step), it was adsorbed on the surface earlier (at the 215,000th MC step) than Chain No. 16 who finished at the 270,000th MC step. The two adsorbed chains finally left the aggregate, thus did not contribute to the adsorption of the aggregate. Chain No. 9 was influenced by them at around the 175,000th MC step but then moved back towards the aggregate (Fig. 1e and 1f).

The movement of this aggregate was analyzed in detail. Before the 113,000th MC step, R_{gyr} was decreased slowly from 2.3 nm to 1.7 nm (Fig. 4c). Two abrupt increases at around the 48,900th and 50,000th MC step were caused by the periodic boundary condition of Chain No. 3 when it moved across the simulation boundary (Fig. 1a-c). Between the 113,000th and 130,900th MC step, a dramatic drop of R_{gyr} indicates the formation of a big aggregate from three small groups (Fig. 1c to 1d). After that, the whole aggregate slightly moved away from the surface with a stable R_{gyr} at around 1.5 nm. Because of the large amount of CG beads in this aggregate, this backward movement could barely be observed in Fig. 4a. But this trend could be forecasted since the overall repulsive force was higher than the overall attractive force between the surface and the aggregate (see Fig. 4b).

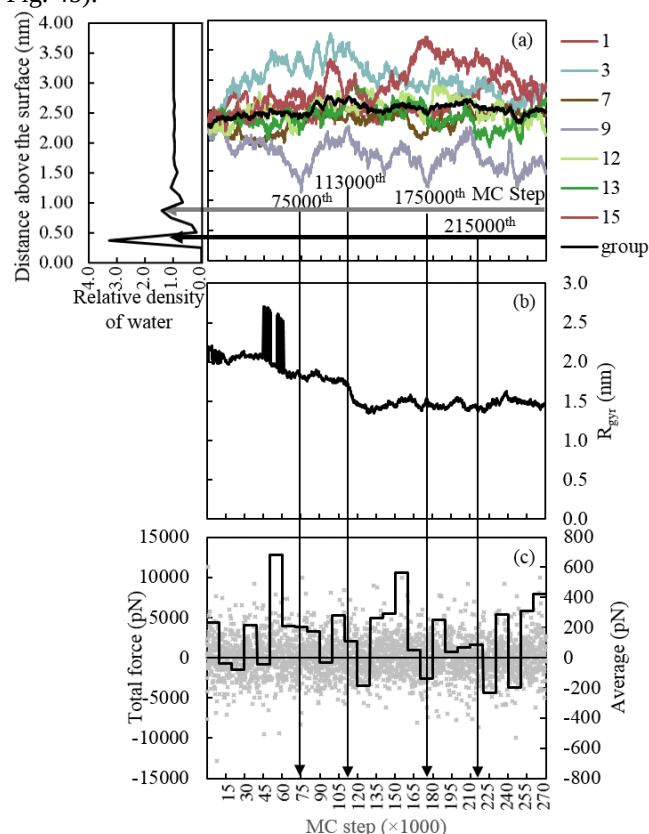


Fig. 4 The evolution of average distances above surface, total force and the radius of gyration of the aggregate. In (a), the central positions for all members are shown. The black and grey horizontal arrows point out the locations of the first and the second aggregated water layers, respectively. In (b), the grey points show the in-process average value for every 10,000 MC steps. The negative value indicates a force pointing towards the surface,

while a positive one indicates a force pointing away from the surface. In (c), remarkable increases of R_{gyr} between the 45,000th and 60,000th MC steps were caused by PBC when Chain No. 3 moved across the boundary of the simulation box.

During the complete process, these chains' z-coordinates were relatively stable except for Chain No. 3, 9 and 15. Chain No. 3 was the newest member in this aggregate. It was fully integrated into the aggregate after the 175,000th MC step when a downward movement trend showed up (Fig. 4a) and the aggregate's integrity level was stable (Fig. 4c). Chain No. 15 located at the edge of the aggregate had a higher mobility than other members. Restricted by the slow movement of the complete aggregate, it was pushed away from the surface after the 215,000th MC step. Chain No. 9 fluctuated at the bottom of the aggregate (Fig. 4a) and was finally pulled away from the surface by its neighboring chains after three failed adsorption attempts. Compared with two adsorbed peptide Chain No. 2 and 16, the failed attempts of Chain No. 9 indicate the influence from the surrounding environment. In the initial configuration, all Chain No. 2, 9, 16 were located at the bottom of aggregate (Fig. 1b). Both Chain No. 9 and 16 were attached to the surface with one terminal in a relatively vertical posture. This posture was not preferable for final adsorption because the peptide's center of mass was too high to move downward in a reasonable short time period. For Chain No. 2, though its anchoring attempt started later than Chain No. 9 and 16, it was attached successfully to the surface with both terminals at the 113,000th MC step due to its' lower center of mass (Fig. 1d). Two major factors contributed the final adsorption of Chain No. 16: (1) the weak attraction from the aggregate since it was located at the edge of the aggregate, (2) the strong downward force exerted by the aggregated water layers, and (3) negligible hindrance caused by other adsorbed chains.

CONCLUSIONS

This work introduced a mesoscale coarse-grained modeling method to study the adsorption of multiple peptides. The following conclusions can be drawn:

1. A small proportion (5 out of 16) of peptide-chains would keep separated while most of them would tend to aggregate together (11 out of 16).
2. For a single peptide, its movement is much faster than an aggregate. It tends to be hindered by the aggregated water layers when it attempts to lockdown on the surface (Fig. 3).
3. When the gravity effect can be neglected, the single peptide tends to be adsorbed on the solid surface earlier than an aggregate does. This result is supported by Jimenez *et al.*, in experiment (Jimenez, et al., 2013).

Molecular-level understanding of adsorption mechanism from this study can be supported by the experimental results by Jimenez *et al.*, (Jimenez, et al., 2013). It helps further produce a hypothesis of protein fouling on solid surface: As time proceeds, the adsorbed stand-alone peptide would change the interfacial characteristics of the surface and weaken the influence of the solid surface on the adsorption of either peptides or aggregates. The interaction contributing

to the adsorption process would no longer involve metal atoms. This understanding will in future help us pursue effective anti-fouling strategies.

NOMENCLATURE

CG	Coarse-Grained
F	Force, pN
N	The total number
q_i	Charge, C
R_{gyr}	Radius of gyration, nm
$r_{\text{cut-off}}$	Cut-off radius, nm
r_{ij}	Distance between CG beads i and j, nm
$U(r_{ij})$	Total potential, kJ/mol
$U_{\text{LJ}}(r_{ij})$	Lennard-Jones potential, kJ/mol
$U_{\text{Ele}}(r_{ij})$	Electrostatic potential, kJ/mol
ϵ_0	Vacuum permeability, F/m
ϵ_{ij}	Well depth, kJ/mol
ϵ_{rel}	Relative dielectric constant
σ_{ij}	Lennard-Jones parameter, nm

Subscript

Ele	Electrostatic potential
i	CG bead i
j	CG bead j
LJ	Lennard-Jones potential
z	The z partial differential

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REFERENCES

- Bansal, B., and Chen, X. D., 2006, A critical review of milk fouling in heat exchangers: *Comprehensive Reviews in Food Science & Food Safety*, Vol. 5, pp. 27-33.
- Blanpain-Avet, P., Hédoux, A., Guinet, Y., Paccou, L., Petit, J., Six, T., and Delaplace, G., 2012, Analysis by Raman spectroscopy of the conformational structure of whey proteins constituting fouling deposits during the processing in a heat exchanger: *Journal of Food Engineering*, Vol. 110, pp. 86-94.
- Bouvier, L., Moreau, A., Ronse, G., Six, T., Petit, J., and Delaplace, G., 2014, A CFD model as a tool to simulate β -lactoglobulin heat-induced denaturation and aggregation in a plate heat exchanger: *Journal of Food Engineering*, Vol. 136, pp. 56-63.
- Carmesin, I., and Kremer, K., 1988, The bond fluctuation method: A new effective algorithm for the dynamics of polymers in all spatial dimensions: *Macromolecules*, Vol. 21, pp. 2819-2823.
- Christian, G. K., Changani, S. D., and Fryer, P. J., 2003, The effect of adding minerals on fouling from whey protein concentrate: Development of a model fouling fluid for a plate heat exchanger: *Food & Bioproducts Processing*, Vol. 80, pp. 231-239.
- Eby, D. M., Johnson, G. R., Farmer, B. L., and Pandey, R. B., 2010, Supramolecular assembly of a biomineralizing antimicrobial peptide in coarse-grained Monte Carlo simulations: *Physical Chemistry Chemical Physics Pccp*, Vol. 13, pp. 1123.
- Fickak, A., Al-Raisi, A., and Xiao, D. C., 2011, Effect of whey protein concentration on the fouling and cleaning of a heat transfer surface: *Journal of Food Engineering*, Vol. 104, pp. 323-331.
- Foo, G. M., and Pandey, R. B., 1997, Electro-deposition of polymer chains on an adsorbing wall: Density profiles and wall coverage: *Journal of Chemical Physics*, Vol. 107, pp. 10260-10267.
- Foo, G. M., and Pandey, R. B., 1998, Conformation and dynamics of polymer chains on dirty surfaces: A discrete-to-continuum approach: *Journal of Chemical Physics*, Vol. 109, pp. 1162-1169.
- Georgiadis, M. C., Rotstein, G. E., and Sro, M., 1998, Optimal design and operation of heat exchangers under milk fouling: *Aiche Journal*, Vol. 44, pp. 2099-2111.
- Hagiwara, T., Sakiyama, T., and Watanabe, H., 2009, Molecular simulation of bovine β -lactoglobulin adsorbed onto a positively charged solid surface: *Langmuir the Acs Journal of Surfaces & Colloids*, Vol. 25, pp. 226.
- Hanke, A. T., and Ottens, M., 2014, Purifying biopharmaceuticals: Knowledge-based chromatographic process development: *Trends in Biotechnology*, Vol. 32, pp. 210-220.
- Heinz, H., Vaia, R. A., Farmer, B. L., and Naik, R. R., 2008, Accurate simulation of surfaces and interfaces of face-centered cubic metals using 12-6 and 9-6 Lennard-Jones potentials: *Journal of Physical Chemistry C*, Vol. 112, pp. 17281-17290.
- Jimenez, M., Delaplace, G., Nuns, N., Bellayer, S., Deresmes, D., Ronse, G., Alogaili, G., Collinet-Fressancourt, M., and Traisnel, M., 2013, Toward the understanding of the interfacial dairy fouling deposition and growth mechanisms at a stainless steel surface: A multiscale approach: *Journal of Colloid & Interface Science*, Vol. 404, pp. 192-200.
- Jong, D. H. D., Singh, G., Bennett, W. F. W., Arnarez, C., Wassenaar, T. A., Schäfer, L. V., Periole, X., Tieleman, D. P., and Marrink, S. J., 2012, Improved parameters for the Martini coarse-grained protein force field: *Journal of Chemical Theory and Computation*, Vol. 9, pp. 687-697.
- Jun, S., and Puri, V. M., 2005, 3D milk-fouling model of plate heat exchangers using computational fluid dynamics: *International Journal of Dairy Technology*, Vol. 58, pp. 214-224.
- Kröner, F., Hanke, A., Nfor, B., Pinkse, M., Verhaert, P., Ottens, M., and Hubbuch, J., 2013, Analytical

- characterization of complex, biotechnological feedstocks by pH gradient ion exchange chromatography for purification process development: *Journal of Chromatography A*, Vol. 1311, pp. 55-64.
- Kuszewski, J., Gronenborn, A. M., and Clore, G. M., 1999, Improving the packing and accuracy of NMR structures with a pseudopotential for the radius of gyration: *Journal of the American Chemical Society*, Vol. 121, pp. 225-236.
- Michalski, C. B., Brackett, R. E., Hung, Y. C., and Ezeike, G. O., 1999, Use of capillary tubes and plate heat exchanger to validate U.S. Department of Agriculture pasteurization protocols for elimination of *Salmonella enteritidis* from liquid egg products: *J Food Prot*, Vol. 62, pp. 112-117.
- Miller, F. P., Vandome, A. F., and Mcbrewster, J., 2010, GROMACS, *Alphascript Publishing*.
- Monticelli, L., Kandasamy, S. K., Periole, X., Larson, R. G., Tieleman, D. P., and Marrink, S.-J., 2008, The MARTINI coarse-grained force field: Extension to proteins: *Journal of Chemical Theory and Computation*, Vol. 4, pp. 819-834.
- Pandey, R. B., and Farmer, B. L., 2010, Globular structure of a human immunodeficiency virus-1 protease (1DIFA dimer) in an effective solvent medium by a Monte Carlo simulation: *Journal of Chemical Physics*, Vol. 132, pp. 125101.
- Pandey, R. B., Heinz, H., Feng, J., Farmer, B. L., Slocik, J. M., Drummy, L. F., and Naik, R. R., 2009, Adsorption of peptides (A3, Flg, Pd2, Pd4) on gold and palladium surfaces by a coarse-grained Monte Carlo simulation: *Physical Chemistry Chemical Physics*, Vol. 11, pp. 1989-2001.
- Pandey, R. B., Kuang, Z., Farmer, B. L., Kim, S. S., and Naik, R. R., 2012, Stability of peptide (P1 and P2) binding to a graphene sheet via an all-atom to all-residue coarse-grained approach: *Soft Matter*, Vol. 8, pp. 9101-9109.
- Pelegrine, D. H. G., and Gomes, M. T. M. S., 2012, Analysis of whey proteins solubility at high temperatures: *International Journal of Food Engineering*, Vol. 8.
- Penna, M. J., Milan, M., and Biggs, M. J., 2014, Molecular-level understanding of protein adsorption at the interface between water and a strongly interacting uncharged solid surface: *Journal of the American Chemical Society*, Vol. 136, pp. 5323-5331.
- Punidades, P., and Mckellar, R. C., 1999, Selected physical properties of liquid egg products at pasteurization temperatures: *Journal of Food Processing & Preservation*, Vol. 23, pp. 153-169.
- Qiu, R., Xiao, J., and Chen, X. D., 2017, Further understanding of the biased diffusion for peptide adsorption on uncharged solid surfaces that strongly interact with water molecules: *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, Vol. 518, pp. 197-207.
- Rouault, Y., and Milchev, A., 1995, Monte Carlo study of living polymers with the bond-fluctuation method: *Physical Review E Statistical Physics Plasmas Fluids & Related Interdisciplinary Topics*, Vol. 51, pp. 5905-5910.
- Sakiyama, T., Aya, A., Embutsu, M., Imamura, K., and Nakanishi, K., 2006, Protease susceptibility of β -lactoglobulin adsorbed on stainless steel surface as evidence of contribution of its specific segment to adsorption: *Journal of Bioscience & Bioengineering*, Vol. 101.
- Xiao, J., and Huang, Y., 2009, Microstructure-property-quality-correlated paint design: An LMC-based approach: *Aiche Journal*, Vol. 55, pp. 132-149.
- Xu, W., Lan, Z., Peng, B. L., Wen, R. F., and Ma, X. H., 2015, Effect of surface free energies on the heterogeneous nucleation of water droplet: A molecular dynamics simulation approach: *Journal of Chemical Physics*, Vol. 142.
- Yesylevskyy, S. O., Schäfer, L. V., Sengupta, D., and Marrink, S. J., 2010, Polarizable water model for the coarse-grained MARTINI force field: *Plos Computational Biology*, Vol. 6, pp. 364-364.
- Yu, J., Becker, M. L., and Carri, G. A., 2012, The influence of amino acid sequence and functionality on the binding process of peptides onto gold surfaces: *Langmuir*, Vol. 28, pp. 1408-1417.
- Zhang, L., Bai, S., and Sun, Y., 2011, Modification of Martini force field for molecular dynamics simulation of hydrophobic charge induction chromatography of lysozyme: *Journal of Molecular Graphics & Modelling*, Vol. 29, pp. 906.

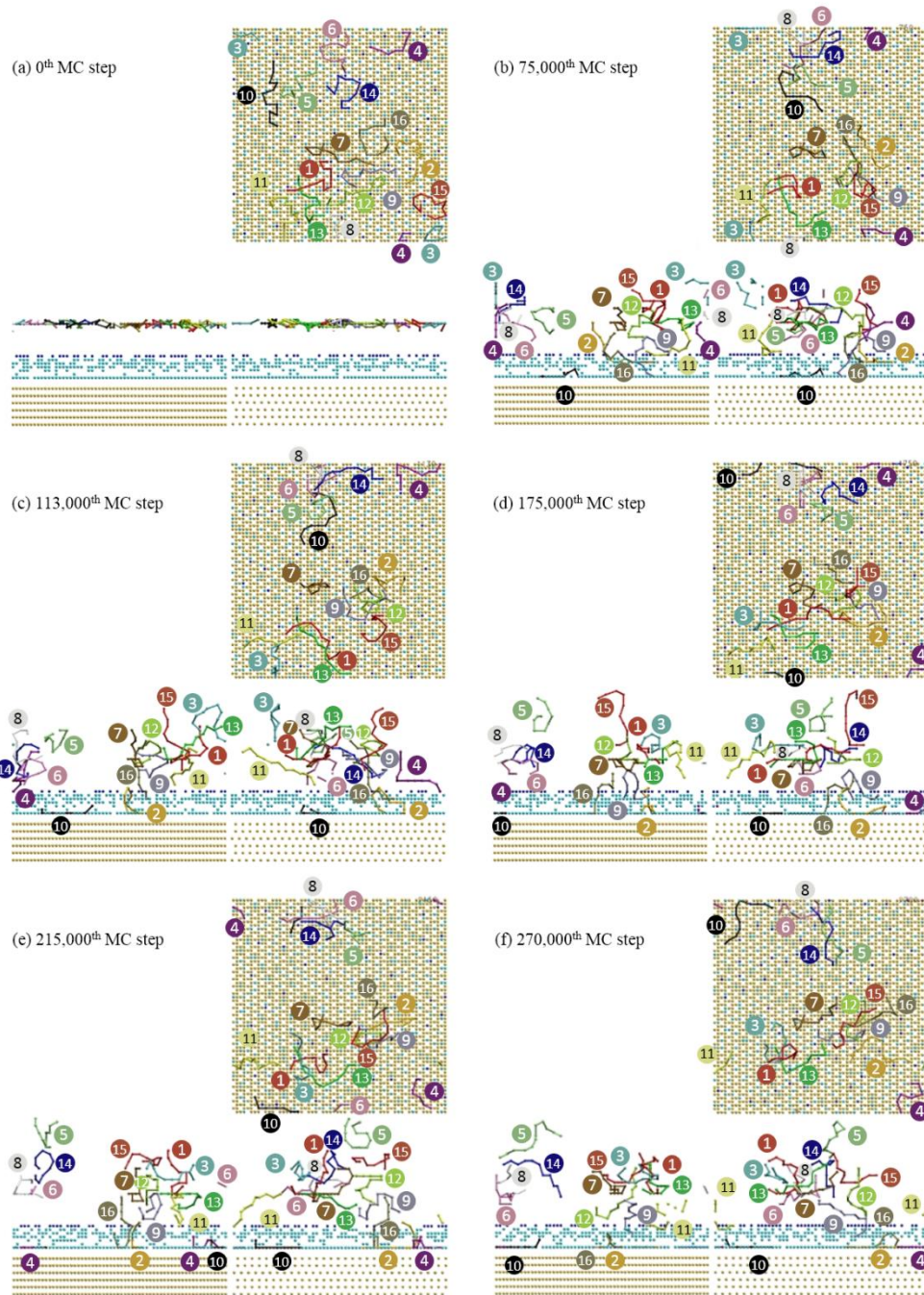


Fig. 1 The representative screenshots in an adsorption process. The numbered lines represent Ala peptide-chains with twelve amino acid residues in each chain. The ordered yellow beads at the bottom of the simulation domain are Au atoms; the dark blue beads indicate the interaction cut-off range from surface beads while the light blue ones represent the water beads that could interact with the surface beads. All water beads beyond 1.20 nm above the surface are hidden in these figures. In each subplot a specific MC step, the top right, bottom left and bottom right ones show respectively the top view, left view and front view of the simulation box.