

Molecular Dynamics Protocol – Supporting Information

Starting with the X-ray structure of HEWL (1lee.pdb) solved by Sauter et. al.,^{1S} we prepared 25 protein systems and performed 100ns trajectories using NAMD 2.6^{2S} with the Charmm27 forcefield. Calculations were run across 64 compute-cores on high performance computer clusters.

The first system was created from five HEWL molecules oriented with respect to each other as in the crystal structure with the symmetry group P43212. This step was performed in Discovery Studio v. 2.0.^{3S} Protein molecules were labelled from A to E, starting from the first protein molecule given explicitly in the pdb file (see Fig. 1S). To neutralise the system 5 Na⁺ and 45 Cl⁻ counter ions were added, and the ionic strength was 0.02 M. Such a system was placed in a rectangular box of water molecules (TIP3^{4S}) that extend 2Å from any protein atom. Because of the protein orientations, a few residues were positioned only 2Å away from the edge of the water box, however in the majority of cases the distance was much larger. The total number of atoms was 97 053, including 9 800 protein atoms, 50 ions and 87 203 water atoms. This system was used as a reference for the rest of the trajectories. Other reference data were supplied by our 20ns and 90ns trajectories of single protein adsorption.^{5S-7S}

25 further systems were composed from two, three and five HEWL proteins and a model charge SiO₂ surface (mimicking a mica surface) located in various orientations with respect to proteins' molecules.

In the case of the two – protein systems we used protein molecules denoted as C and D and the surface located on the one side of the protein assembly, in the *x,z* plane (as in the case with five proteins, see Fig. 2 for the relative orientation). Mutation R128G (Arg128 changed to Gly128) on protein C only, on protein D only and on both proteins simultaneously gave 3 additional versions of the two – protein

system. Proteins C and D were used to simulate a dimer because in the crystal structure they were located close to each other ($\sim 2.0\text{\AA}$) with several close contacts between various residues. In the final, fifth two-protein system proteins C and D were pulled out from each other to achieve a distance $\sim 13\text{\AA}$ between them without any additional translations or rotations.

Three – protein systems (were composed from molecules denoted as A, B and C or A, D and E. In the case of the first combination (A, B, C) the surface was located in two positions, above and below the protein system, while in the case of A, D, E the surface was located only below the protein system in the x,y plane. Mutations R128G and R125G separately and simultaneously on all three A,D,E proteins gave 8 different three-protein systems.

The five – protein system was prepared from the original system by adding the surface in the x,z plane on both sides of the ABCDE protein (see Fig. 2). Two simulations were performed for the native protein molecules, and eight with various mutations R128G and R68G. Additional systems were obtained by extending the simulation cell, resulting in a larger protein distance to the image of the surface.

In all cases the protein system together with the surface was placed in a rectangular water box that extended 1\AA from any surface atom and at least 2\AA from any protein atom. Then the protein charge was neutralised by adding the required amount of Na^+ and Cl^- counter ions with ionic strength 0.02M. The amount of water molecules was slightly varying, depending on the system. The final number of atoms was usually 90-95 000, including $\sim 4\ 000$ surface atoms and 1 960 atoms per protein (in the native state). Also the surface dimensions were optimised for each case. The surface was built from a square array of silicon and oxygen atoms located 1.6\AA away from each other with charges $+1.11e$ and $-0.66e$, respectively. Such partial charges

produced a surface charge density $\sigma = -0.0217 \text{ e}/\text{\AA}^2$ which is almost equal to the nominal surface charge density of natural mica at pH=7. For the Si atom, $\epsilon = -0.585 \text{ kcal/mol}$ and $1/2R_{\text{min}}$ was 2.15 \AA , while for oxygen atoms these parameters were -0.152 kcal/mol and 1.77 \AA . A single charged SiO_2 plane surface was used.

All systems were subject to 1000 steps water-only minimization, 100ps water equilibration at constant pressure, 10 000 steps of whole system minimisation, 30ps heating to 300K and 270ps equilibration at this temperature, with a time step 1fs. The equilibration period was enough to obtain a stable total energy level, which subsequently did not increase during the production simulations for the reference protein system without the surface. For this reason we assume that the system is adequately equilibrated to proceed with the adsorption simulations. The production MD simulations were pursued for 100ns at 300K in the NVT ensemble. The integration step was 2fs, the SHAKE algorithm and PBC were used, all four disulphide bridges per protein were kept. For ionizable residues the most probable charge states at pH 7 were chosen. Interaction cutoff distances were all 12\AA , which has already been shown to be efficient yet reliable for single protein adsorption.^{5S}

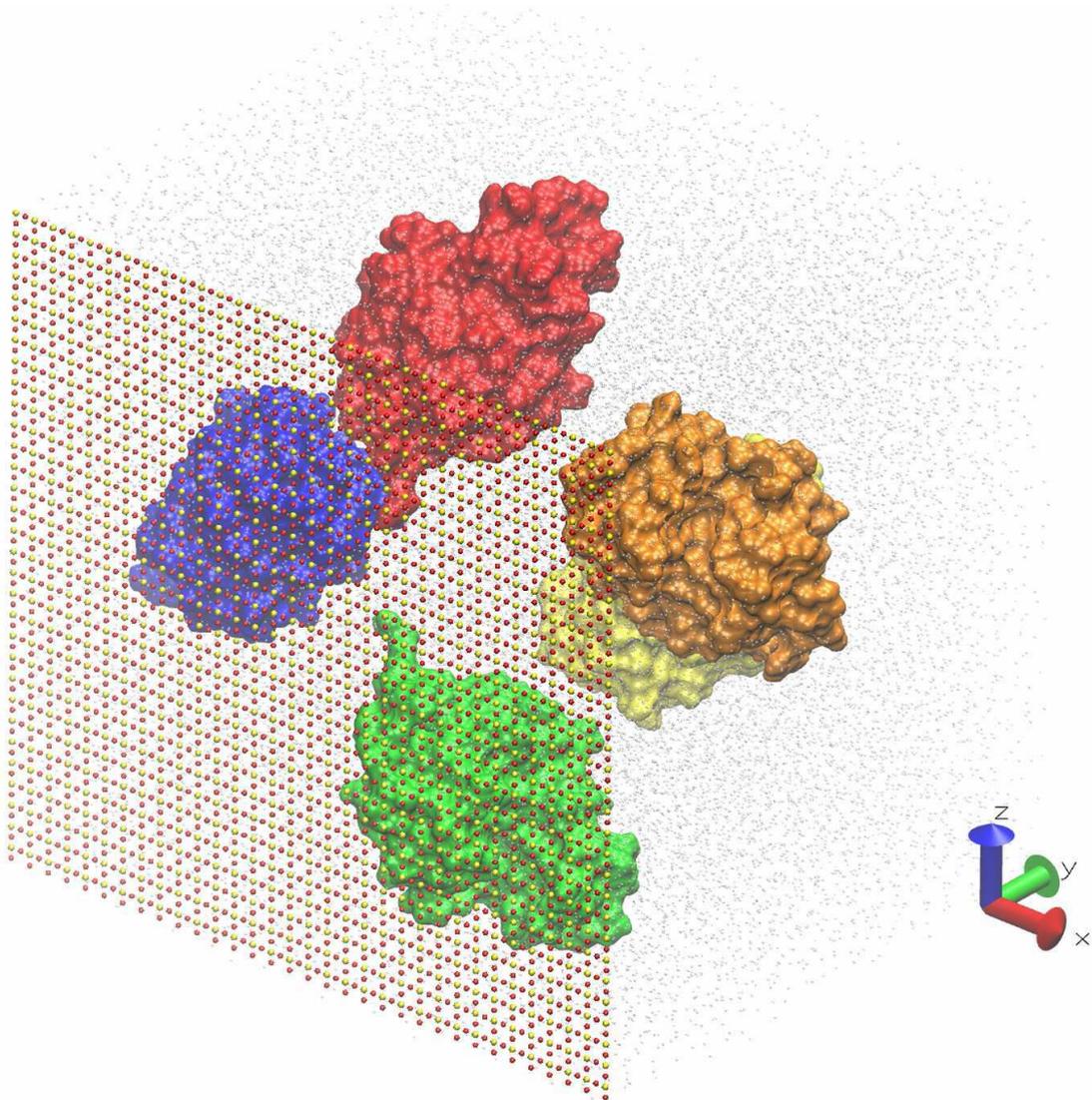
Movies

The following movies are available. Due to file size constraints, the trajectories are sampled at finite rates which have been left apparent to the viewer rather than smoothed.

1. “dimer.avi” shows the trajectory of the dimer discussed in the *Two Protein System* section of the main paper.
2. “dimer_trans.avi” shows the dimer trajectory where one protein has been translated by 20\AA so that their initial separation is 13\AA .

3. “cluster3.avi” shows the trajectory discussed in the *Three Protein System* section of the main paper.
4. “cluster3_opp.avi” shows the continued trajectory discussed in the *Three Protein System* section of the main paper.
5. “cluster5_1.avi” shows one view of the trajectory discussed in the *Five Protein System* section of the main paper.
6. “cluster5_2.avi” shows another view of the trajectory discussed in the *Five Protein System* section of the main paper.
7. “cluster5_3.avi” shows the situation after 100ns as discussed in the *Five Protein System* section of the main paper.
8. “binding_sites.avi” shows the binding sites identified in the simulations as discussed in the *Cluster Formation Mechanisms* section of the main paper.

Figure 1S. Initial HEWL orientations with respect to each other. The proteins are shown as a surface, with molecule A is shown in blue, molecule B in red, molecule C in orange, molecule D in yellow and molecule E in green. Water molecules are indicated by grey dots. The surface is located as in the simulations with five proteins. The surface atoms are shown as red (oxygen atoms) and yellow (silicon) spheres.



References

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