# Atomistic Characterization of Collective Protein–Water–Membrane Dynamics

Christopher Päslack,<sup>†</sup> Lars V. Schäfer,<sup>\*,†</sup> and Matthias Heyden<sup>\*,‡</sup>

<sup>†</sup>Center for Theoretical Chemistry, Ruhr University Bochum, D-44780 Bochum, Germany <sup>‡</sup>School of Molecular Sciences, Arizona State University, Tempe, AZ 85287-1604, USA

E-mail: lars.schaefer@ruhr-universitaet-bochum.de; heyden@asu.edu

## Protein position & stability

Figure S1 shows the mass density profiles with respect to the z-axis computed from the NVE simulations. The simulation box is large enough, since even at a separation distance of R = 30 Å there is enough water between the protein and the lipid bilayer in the periodic image cell to avoid artificial couplings.



Figure S1: Mass densities along the z-coordinate for selected protein–membrane distances R.

Since the production simulations in the NVE ensemble were entirely unbiased, we checked whether the orientation of the protein remained stable over the course of the 100 ps simulations. Figure S2 shows the angles of the anchor residue pairs Ser35/Val195 and Glu138/His254 (see above) with respect to the z-axis. The data was taken from single, representative NVE simulations. Figure S2 demonstrates that during the 100 ps simulations, the orientation of Anx did not change significantly.

Furthermore, we assessed the stability of the protein distance R to the membrane surface. Figure S3 shows that the protein-membrane distance does not change significantly over the



Figure S2: Angles of the anchor residue pairs Ser35/Val195 and Glu138/His254 with respect to the z-axis as a function of simulation time. The data shown were taken from single, representative NVE simulations for the shown protein-membrane distances.

duration of the NVE simulations.

For the data shown in Figure 3 in the main text, control NVE simulations of Anx in water (i.e., no membrane present) and of a solvated DOPC/DOPS bilayer (i.e., without protein) were carried out. We used equilibrated starting structures (298 K, 1 bar) from our previous work<sup>1</sup> to start these simulations.

The distributions of the protein and membrane atoms selected for cross-correlation analysis obtained from the NVE simulations are shown in Figure S4. The distributions are cut off on the right shoulders because only protein atoms with a position  $z < r_{com}$  were considered for analysis.

#### Analysis of correlated vibrations

To account for the mobility of water molecules over the sampling time of the correlation functions (tens of ps), localized densities of mass-weighted velocities were employed for water oxygen atoms for the calculation of protein–water and membrane–water cross-correlation spectra. The width of the Gaussian velocity density kernels was set to  $\sigma = 0.4$  Å in accordance with Heyden & Tobias.<sup>2</sup> Figure S5 illustrates the sampling point positions for two selected distances to the protein surface. All spectra were calculated using the longitudi-



Figure S3: Protein-membrane distance R as a function of simulation time. The data were taken from single, representative NVE simulations for the indicated protein-membrane distances. Over the short time course of the NVE simulations (100 ps), there is very little change of the Anx position relative to the membrane.



Figure S4: Distributions of z-coordinates of the atoms selected for cross-correlation analysis. The data were obtained from representative NVE simulations for the indicated proteinmembrane distances.

nal components of the velocity vectors, *i.e.*, components parallel to the protein/membrane surface normal.

For each surface type (protein and membrane), approximately 1500 sampling points were randomly chosen for each distance to the surface. The sampling point distances to the protein and membrane surfaces range from 2.5 to 10 Å, in steps of 0.5 Å. Velocity densities and atomic velocities were recorded for each density sampling point and its closest protein or membrane heavy atom, respectively. Then, time cross-correlation functions were computed and Fourier-transformed to obtain the spectra shown in Fig. 3 of the main text.

For the protein–membrane cross-correlation spectra shown in Fig. 2 of the main text, only explicit velocities of protein and membrane heavy atoms were used. The selection of protein and membrane surface atoms that was used for this analysis is shown in Figure 1 C,D of the main text.

Figure S6 shows the protein–water and membrane–water cross-correlation spectra of mass-weighted atomic velocities and localized velocity densities for each of the protein–membrane distances.



Figure S5: Illustration of the sampling point positions for the calculation of localized, massweighted velocity densities according to Eq. 2 in the main text. The sampling points are randomly distributed around the bottom surface of the protein, which is defined by all nonhydrogen protein atoms that are located at least 10 Å below the center of mass of the protein and within 3.5 Å of any water atom.



Figure S6: Protein–water and membrane–water cross-correlation spectra of mass-weighted atomic velocities and localized velocity densities for each of the protein–membrane distances (10–30 Å). The spectra show correlated vibrations of non-hydrogen protein or membrane surface atoms with hydration water oxygen atoms as a function of reciprocal distance  $k = 2\pi/r_{S-OW}$ . The left column shows the Anx–water spectra and the right column shows the membrane–water spectra.

## Analysis of hydrogen bond lifetimes

The reorientation dynamics of water molecules can be characterized by hydrogen bond autocorrelation functions, which quantify the lifetime of hydrogen bonds:

$$C_{HB}(t) = \left\langle \frac{h(\tau)h(\tau+t)}{h(\tau)h(\tau)} \right\rangle_{\tau}$$
(1)

The operator h(t) yields the value 1 if a hydrogen bond is intact at time t and 0 if it is broken. We defined H-bonds using a geometric criterion with a maximum donor-acceptor distance of 3.5 Å and a minimum donor-hydrogen-acceptor angle of 150°.

The correlation function  $C_{HB}(t)$  generally describes the dynamics of several coupled processes hat can lead to a multi- or stretched-exponential decay. Here, instead of assuming a certain functional form of  $C_{HB}(t)$ , the net relaxation times  $\tau_{HB}$  were directly obtained from the correlation functions as  $C(\tau_{HB}) = 1/e$ . A detailed description of hydrogen bond dynamics in neat water and in various environments can be found elsewhere.<sup>3-9</sup>

We analyzed water-water hydrogen bond dynamics involving the water molecules between the protein and the membrane (see Fig. 1B, main text). At first, the average HB lifetimes of water molecules as a function of protein-membrane distance R were evaluated (Fig. 5A, main text). Then, the function  $C_{HB}(t)$  was computed for water molecules within several distance ranges (0–5 Å, 5–8 Å, 8–11 Å, ..., 17–20 Å) from the membrane and the membrane-facing side of Anx, respectively. The first range involving the first and second hydration shells was wider since the reorientation dynamics within 3 Å from the membrane or protein surfaces can be dominated by contributions from immobilized water molecules directly bound to the respective surface. Usually, bulk dynamics are restored at distances of 8–10 Å from a biomolecular surface, but here we extended the analysis up to distances of 20 Å from the membrane and Anx. Therefore, it was possible to study the effects of hydration shell overlap for small enough protein-membrane distances R. The average HB lifetimes involving water molecules selected as a function of distance from either the protein or membrane surface are shown in Fig. 5B, top and bottom panels, respectively.

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