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# Molecular Dynamics Simulations of a Membrane Protein−Micelle Complex in Vacuo

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It is estimated that 20−30% of all genes in most genomes encode membrane proteins.[1] However, because of the difficulty in crystallizing membrane proteins, only a fraction of such structures has currently been determined.[2] Simultaneously, 50% of all drugs target membrane proteins of usually unknown structure. The availability of more membrane protein structures would hence be extremely useful for structure-based drug design. The emergence of single-particle imaging techniques based on high brilliance X-ray free-electron lasers (XFELs), such as the Linac Coherent Light Source (LCLS, Stanford, CA) and the European XFEL (Hamburg, Germany), hold the promise of structure determination of biological macromolecules without the need for crystallization.[3] In a single-particle XFEL bioimaging experiment, no biological sample is likely to survive more than one encounter with an X-ray pulse. Consequently, to obtain a three-dimensional (3D) structural reconstitution from several images, the sample needs to be highly reproducible.

Electrospray ionization (ESI) is commonly used to inject biological samples into mass spectrometers. The requirement of sample injection into a container-free sample chamber makes ESI a promising technique for sample delivery.[4] In the ESI process, the sample is transferred from the solution phase to the gas phase, where the relative importance of the electrostatic interactions increases simultaneously with a reduction of the hydrophobic effect.[5] Recent ESI mass spectrometry (MS) experiments have demonstrated that structural interactions in protein−micelle complexes can be preserved in the gas phase.[6]

Lipids play a crucial role in the solubility and stability of membrane proteins in vivo, but they are commonly replaced by detergents in vitro, allowing solubilization of the membrane protein. Hence, lipids and/or detergents need to be present to study the 3D structure of most folded membrane proteins. Micelles, mimicking the milieu of a membrane, could be a suitable vector for the study of membrane proteins in X-ray imaging experiments.

To investigate the stabilizing effect of a thin layer of water covering a protein−micelle complex in vacuo, we report 150 ns molecular dynamics (MD) simulations of the transmembrane N-terminal domain of outer membrane protein A (OmpA171, residues 1 to 171)[7] from *Escherichia coli* embedded in a dodecylphosphocholine (DPC) detergent micelle (80 DPC molecules). MD simulation studies have previously been performed on the stability[8] and self-assembly[9] of DPC micelles around OmpA171. As in previous studies,[10−12] a preparatory water simulation using the GROMOS96 43A1 force field[13] was followed by simulations under vacuum conditions ("vacuum simulations") with various thicknesses of the surrounding water shells (0.6, 0.3, and 0 nm). Drying of droplets in vacuo takes many milliseconds, and the process is accompanied by a temperature drop of more than 50 K within the first millisecond.[14] Our simulations sampled different stages of the process, and since they were started a temperature higher than that of an evaporating droplet, the kinetics were enhanced. Despite this, the protein was very stable, as described below. Simulations were performed using the GROMACS simulation package[15] [see the Supporting Information (SI) for MD simulation details and a movie showing the final structures of the trajectories].

OmpA171 consists of an eight-stranded all-next-neighbor antiparallel $\beta$-barrel with an average strand length of 12.7 amino acid residues. During all of the vacuum simulations, the $\beta$-barrel showed a high degree of stability. With decreasing thickness of the water layer, a few N- and C-terminal $\beta$-strand residues lost their secondary structure, resulting in average strand lengths of 12.5, 12.4, and 10 residues for the 0.6 nm, 0.3, and 0 nm simulations, respectively (Figure S1 and Table 1).

***Table 1.*** Structural Properties of the Protein−Micelle Complex in the MD Simulations[a]

| property | bulk | 0.6 nm | 0.3 nm | 0 nm |
|---|---|---|---|---|
| rmsd $C^\alpha$ | 0.08(1) | 0.219(7) | 0.213(7) | 0.32(1) |
| rmsd $C^\alpha$ ($\beta$-barrel) | 0.061(8) | 0.087(5) | 0.136(5) | 0.14(2) |
| no. $\beta$C | 102(3) | 100(2) | 99(4) | 80(6) |
| $HB_{PP}$ | 142(5) | 143(5) | 147(5) | 154(6) |
| $HB_{BB}$ | 93(4) | 91(3) | 87(4) | 74(4) |
| $HB_{PD}$ | 34(2) | 37(2) | 37(3) | 52(3) |
| $HB_{PS}$ | 179(9) | 188(7) | 121(6) | − |
| $HB_{DS}$ | 336(10) | 324(8) | 195(5) | − |
| $A_P$ | 124(2) | 121(2) | 111(2) | 116(3) |
| $A_D$ | 253(4) | 216(4) | 205(4) | 199(4) |
| $A_{P,hydrophobic}$ | 66% | 65% | 67% | 69% |
| $A_{D,hydrophobic}$ | 47% | 47% | 63% | 76% |
| $R_{G,protein}$ | 1.708(8) | 1.678(6) | 1.652(7) | 1.66(1) |
| $R_{G,detergent}$ | 2.287(9) | 2.169(5) | 2.138(6) | 2.12(1) |
| $R_{G,detergent\text{-}head}$ | 2.55(1) | 2.347(6) | 2.173(6) | 2.03(1) |
| $R_{G,detergent\text{-}tail}$ | 1.962(9) | 1.957(7) | 2.10(1) | 2.21(2) |
| no. $SB_{DD}$ | 145 | 174 | 219 | 235 |

[a] The properties are abbreviated as follows: rmsd's of $C^\alpha$ (in nm) compared to a bulk-water reference (see the SI) for both the whole protein and the membrane spanning $\beta$-barrel (rmsd $C^\alpha$); the number of amino acid residues in the $\beta$-sheet conformation (no. $\beta$C); the numbers of intramolecular H-bonds within the protein ($HB_{PP}$), within the $\beta$-barrel ($HB_{BB}$), between the protein and the detergent molecules ($HB_{PD}$), between the protein and the water molecules ($HB_{PS}$), and between the detergent and water molecules ($HB_{DS}$); the solvent-accessible surface areas (in $nm^2$) of the protein omitting the micelle ($A_P$) and the detergent molecules ($A_D$) as well as the corresponding percentages that are hydrophobic ($A_{P,hydrophobic}$, $A_{D,hydrophobic}$); the radii of gyration ($R_G$) of the protein, the detergent molecules, and the head and tail groups of the latter; and the number of detergent−detergent salt bridges (no. $SB_{DD}$). Values are time averages over the final half of the simulations, and the numbers in parentheses are standard deviations in the last digit.

The root-mean-square deviations (rmsd's, Table 1) of the vacuum simulations with respect to the bulk-water equilibrated structure (see the SI) show that the structures change with lessening of the surrounding water layer. The inherently flexible loops that extend beyond the membrane contribute to a larger degree to the overall deviation, and the membrane-spanning barrel is much better preserved (Table 1). However, the structural drift of the membrane



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protein in the protein− micelle vacuum simulations is significantly less than what was observed previously for soluble proteins,[10,11] despite the fact that the simulations were an order of magnitude longer.

Figure 1 shows the protein−micelle complex after the initial bulk-water simulation as well as the final structures (as defined in the SI) from 150 ns simulations in vacuo with different thicknesses of proximate water layers around the complex. The water layer around the protein−micelle complex has a strong influence on the encasing detergents. In bulk water, the detergent molecules form a normal-phase micelle in which the DPC molecules are stacked in an ordered manner with the charged head groups facing outward toward the water interface, thereby sequestering the aliphatic detergent tails in the interior. Because of the water evaporation, the favorable hydrogen bonds between the DPC surfactant and solvent decrease. Furthermore, the electrostatic contacts between the choline nitrogens of the zwitterionic DPC detergent molecules and the phosphate groups of neighboring DPC molecules increase (Table 1). As a consequence, the alignment of the DPC molecules along the radial direction vanishes, and the surfactants start to flank around the protein (Figure 1 and Figure S3), leading to a smaller, more hydrophobic micellar surface area with an increase in the number of electrostatic interactions between detergent head groups and protein (Table 1). The total solvent-accessible surface area of the protein remains constant or decreases slightly. However, the accessible protein surface areas with hydrophobic and hydrophilic properties are stable over all of the vacuum simulations.

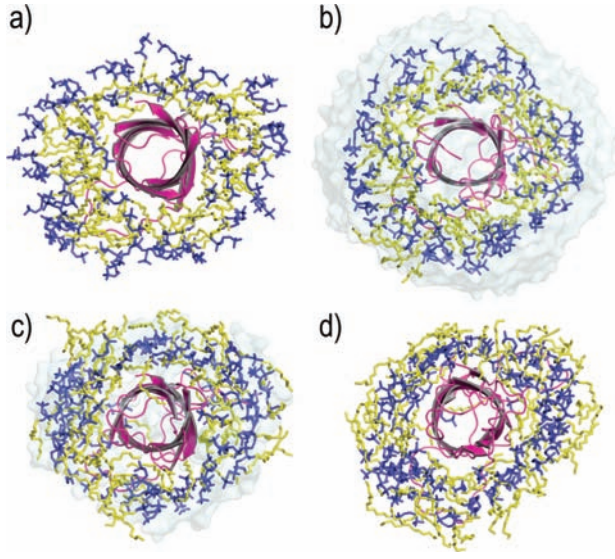


***Figure 1.*** (a) Final bulk-water simulation structure and (b−d) final simulation products under vacuum conditions with various thicknesses of the surrounding water shell: (b) 0.6, (c) 0.3, and (d) 0 nm. Water molecules are shown in the semitransparent surface representation. OmpA171 is represented in pink, and the ionic/polar and nonpolar parts of DPC are shown in blue and yellow, respectively.

Unlike the vacuum simulations of soluble proteins,[10,11] the number of intramolecular protein hydrogen bonds of OmpA171 did not increase drastically with evaporation of water molecules (Table 1). This was also concluded in the vacuum simulation of the soluble protein myoglobin encapsulated in a cetyl trimethylammonium bromide reversed-phase micelle.[11]

The results presented here demonstrate that structures of membrane protein−detergent complexes are not very sensitive to the vacuum environment. It is assuring for MS and future single-particle XFEL bioimaging measurements that micellar structures seem to act as a shelter, protecting both soluble and membrane proteins from vacuum-induced structural changes. In order to make reproducible protein−detergent/lipid complexes, extensive testing of different surfactants (in silico and in vitro) is needed. The ability to encapsulate membrane proteins with a well-defined number of surfactant molecules will have a tremendous impact on single-molecule bioimaging as well as ligand binding studies by MS, which is important for drug design. In summary, we have shown that the transfer from water to vacuum would to some extent affect the structure of the OmpA171−DPC micelle complex. However, the major structural change occurs in the surrounding micelle, whereas the protein is less influenced. The water and surfactant molecules act as a safeguard, protecting the membrane protein from dehydration-related conformational changes.

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**Supporting Information Available:** A movie (QT), methodological details, supplementary analysis, and complete ref 4. This material is available free of charge via the Internet at http://pubs.acs.org.

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