# "Invisible" detergents enable a reliable determination of solution structures of native photosystems by small-angle neutron scattering

M. Goluba, J. Gätckeb, S. Subramanianb, A. Kölschb, T. Darwishc, J. K. Howardc, A. Feoktystovd,   
O. Matsarskaiae, A. Martele, L. Porcare, A. Zounib, and J. Piepera\*

a Institute of Physics, University of Tartu, Wilhelm Ostwald str. 1, 50411 Tartu, Estonia

b Humboldt Universität zu Berlin, Philippstr. 13, 10115 Berlin, Germany

c National Deuteration Facility, Australian Nuclear Science and Technology Organization, New Illawarra Road, Lucas Heights NSW 2234, Australia

d Forschungszentrum Jülich GmbH, Jülich Centre for Neutron Science (JCNS) at Heinz Maier-Leibnitz Zentrum (MLZ), Lichtenbergstr. 1, 85748 Garching, Germany

e Institut Laue-Langevin, 71 Avenue des Martyrs CS 20156, 38042 Grenoble Cedex 9, France

\*Author to whom correspondence should be addressed:

Jörg Pieper

Institute of Physics

University of Tartu

W. Ostwald str. 1

50411 Tartu, Estonia

phone.: +(372) 737 4627

email: pieper@ut.ee

**Abstract**

Photosystems I (PSI) and II (PSII) are pigment-protein complexes capable of performing the light-induced charge separation necessary to convert solar energy into a biochemically storable form, an essential step in photosynthesis. Small-angle neutron scattering (SANS) is unique in providing structural information on PSI and PSII in solution under nearly physiological conditions without the need for crystallization or temperature decrease. We show that the reliability of the solution structure critically depends on proper contrast matching of the detergent belt surrounding the protein. Especially, "invisible" specifically deuterated detergents are shown to be properly matched out in SANS experiments by a direct, quantitative comparison with conventional matching strategies. In contrast, protonated detergents necessarily exhibit incomplete matching, so that related SANS results systematically overestimate the size of the membrane protein under study. While the solution structures obtained are close to corresponding high-resolution structures, we show that temperature and solution state lead to individual structural differences compared with high-resolution structures. We attribute these differences to the presence of a manifold of conformational substates accessible by protein dynamics under physiological conditions.

**Introduction**

Photosynthesis is an essential physiological process responsible for producing dioxygen, shaping our atmosphere, and synthesizing energy-rich carbohydrates [1](#_ENREF_1). This process occurs in two membrane-bound pigment-protein supercomplexes denoted as Photosystem I (PSI) and Photosystem II (PSII), present in higher plants, algae, and cyanobacteria. PSII acts as a light-driven water plastoquinone oxidoreductase and catalyzes the electron extraction from water molecules [2-4](#_ENREF_2). PSI is a photoactive enzyme performing the light-induced electron transfer from the reduced plastocyanin or cytochrome *c*6 to ferredoxin. Because of its high stability, PSI is of pivotal importance for biotechnological applications [5-8](#_ENREF_5).

The cryogenic X-ray crystal structure of PSI at 100 K has been determined at nearly atomic resolution for *Thermosynechococcus elongatus* (*T. elongatus)* [9](#_ENREF_9), *Synechocystis* [10](#_ENREF_10), and plant PSI [11](#_ENREF_11). Three monomers constitute the trimeric core complex of PSI from *T. elongatus*. Each monomer comprises 12 protein subunits, binding a total of 127 cofactors responsible for light-harvesting and electron transfer [9](#_ENREF_9). Nine of the subunits are transmembrane, while three subunits are extrinsic and located at the stromal side.

The cryogenic crystal structure of PSII of *T. elongatus* shows the most extended dimensions of the membrane integral part to be 190 Å x 100 Å with a thickness of 40 Å and extends from the stromal side of the membrane by no more than 10 Å. In contrast, the luminal side of each monomer has prominent protrusions of up to 55 Å from the membrane [12-14](#_ENREF_12). Recently, structural data close to 2.0 Å resolution for all four (meta)stable intermediate S-states of the Kok cycle were obtained using femtosecond (fs) serial crystallography at the XFEL at physiological temperatures [15](#_ENREF_15). In comparison to the cryo X-ray structure, an expansion of PSII in all directions was observed[16](#_ENREF_16).

The high-resolution structural studies summarized above required the use of PSI/PSII crystals. However, both functional studies and biotechnological applications of PSI and PSII necessitate the isolation of the proteins in an aqueous solution. As transmembrane proteins, PSI and PSII can only be extracted by solubilization using amphipathic detergent molecules to prevent unspecific protein aggregation [17-19](#_ENREF_17). In most cases, PSI and PSII are isolated, purified, and crystallized using the mild and non-ionic detergent n-dodecyl-ß-D-maltoside (DDM), retaining their functional activity [20](#_ENREF_20), [21](#_ENREF_21). DDM is frequently used to isolate membrane proteins, being an appropriate choice in many solubilization and crystallization studies as well as in reconstitution experiments[22](#_ENREF_22). As a result, the isolated protein is surrounded by a detergent belt in buffer solution, which is not visible in crystal structures because of its significant structural heterogeneity.

Most importantly, however, the structure of a membrane protein solubilized in buffer solution may generally differ from that of a crystal[22-30](#_ENREF_22). This is also indicated, e.g., by a recent cryo-EM study of PSI showing an expansion of the protein in solution, but at cryogenic temperatures [31](#_ENREF_31). Thus, the crystal structure's general relevance for a solubilized membrane protein has to be independently verified. It adds to the complexity that the conformational flexibility of PSII in solution increases drastically above 240 K and depends strongly on temperature and hydration [32-34](#_ENREF_32).

In contrast to high-resolution techniques, small-angle neutron scattering (SANS) is unique in providing insights into the structure of biomolecules in solution and at physiological temperatures [35-37](#_ENREF_35). SANS bears a great potential for advancing structural biology [38](#_ENREF_38), [39](#_ENREF_39) and drug design [40](#_ENREF_40), [41](#_ENREF_41). However, in the case of membrane proteins, special attention should be paid to the detergent's nontrivial contributions to the scattering data[42](#_ENREF_42). The latter may arise from detergent interacting with the protein itself and from free detergent micelles present in the buffer solution. *In-situ* size exclusion chromatography (SEC) has proven to be an efficient way to remove free micelles from a sample solution [43](#_ENREF_43), [44](#_ENREF_44), whose scattering contribution may otherwise seriously complicate the SANS data analysis [26](#_ENREF_26), [29](#_ENREF_29), [31](#_ENREF_31). In addition, SANS combined with contrast variation is a highly promising approach to match out (and thus "hide") the scattering of detergent molecules in both micelles and protein-detergent complexes [25](#_ENREF_25), [45-47](#_ENREF_45). However, due to the chemically heterogeneous nature of the hydrophilic and hydrophobic parts of protonated detergents, the elimination of their scattering contribution over the entire Q-range remains challenging [27](#_ENREF_27), [42](#_ENREF_42). This may be a reason for the very different solution structures previously reported in the case of PSI, [29](#_ENREF_29), [31](#_ENREF_31), [46](#_ENREF_46), [47](#_ENREF_47) and for their often overestimated sizes compared with the respective crystal structures. More recently, it was shown that matching out detergents can be significantly improved by using adequate deuteration to homogenize the scattering contribution of hydrophilic and hydrophobic parts of a detergent[29](#_ENREF_29).

In the present study, we employ such a specifically deuterated ("invisible") detergent in SANS experiments on native photosystems and directly compare this approach with the previously used strategies for matching out protonated detergents. This permits to quantitatively assess the improvement of the reliability of solution structures obtained by SANS using “invisible" detergents. The combination of SANS with SEC in situ was not used for removing free detergent micelles here, but to exchange protonated detergent with specifically deuterated one, ensuring a detergent-rich environment to the protein as well as its monodispersity. The results reveal that matching protonated detergents remains incomplete, leading to seemingly expanded solution structures of photosystems. In contrast, the use of specifically deuterated detergents leads to proper matching the chemically heterogeneous DDM detergent and, therefore, to the effective elimination of scattering contributions from both free micelles and detergent attached to a solubilized membrane protein. As a result, we obtain reliable solution structures of PSI and PSII at physiological conditions, which correspond very well to the respective high-resolution structures obtained by cryo-EM and XFEL diffraction, respectively.

**Materials and Methods**

**PSI/PSII preparation**

Growth conditions of *T. elongatus* BP-1 and extraction of membrane proteins from thylakoids were performed according to Kern et al. (2005)[20](#_ENREF_20). The PSI isolation was carried out by solubilizing the thylakoid membranes with 2.00 % DDM. PSI preparations were purified from the membrane extract by ionic exchange chromatography in buffers containing 0.02 % DDM, followed by the PSI crystallization. pH values were corrected to pD values using a constant of 0.4. Measurement buffers (20 mM MES, 50 mM MgSO4 and 0.02 % DDM, pH/pD 6.4) were prepared in 99.9 % D2O (Sigma Aldrich) or 100 % H2O and were mixed to final concentrations of 18 % and 5 % D2O. The PSI crystals were washed with MES buffer containing 0.02 % DDM and the corresponding concentration of D2O. The PSI crystals were solubilized to a chlorophyll concentration of 2.5 mM in measurement buffer containing the corresponding concentration of D2O.

The homodimer form of the PSII core complex was isolated from the thermophilic cyanobacterium *T. elongatus*. The isolation procedure implies the usage of the detergent DDM as described by Kern et al., 2005[20](#_ENREF_20). The PSII samples were redissolved with a buffer containing 0.1 M PIPES-NaOH (1,4-piperazinediethanesulfonic acid), pH 7.0, 5 mM CaCl2, and 0.02% DDM. A prerequisite for using SANS/SAXS measurements on the PSII-DDM samples is their monodispersity and homogeneity. Therefore, the monodispersity and uniformity of these samples were increased by a double pre-crystallization procedure. Each pre-crystallization step was done at 0.75 mM chlorophyll (Chl), *a* concentration which is equivalent to 8 mg/ml protein and left overnight at 4 °C[20](#_ENREF_20), [21](#_ENREF_21). The collected crystals were resolubilized, followed by washing in a buffer containing 0.1 M PIPES-NaOH, pH 7.0, 10 mM CaCl2, and 0.02% DDM at a final concentration of 1 mM chlorophyll concentration (equivalent to 10.7 mg/ml protein). For SANS measurements, the PSII samples were rewashed with a buffer containing 0.1 M PIPES-NaOH, pH 7.0, 10 mM CaCl2, and 0.02% DDM with different concentrations of D2O ( 5%, 75%, and 100%).

All prepared samples' activity was assessed at room temperature using steady-state O2 evolution rate under continuous illumination via a Clarke-type electrode (OxyLab, Hansatech instruments). The PSII samples show O2 evolution rates of ~2500 μmol O2/(mg Chl *a* h) as measured in buffer containing 20 mM MES/NaOH, pH 6.5, 20 mM CaCl2, 10 mM MgCl2, and 3 μM DCBQ (2,5-dichloro-p-benzoquinone) as an artificial electron acceptor.

**SANS experiments**

**d-DDM SEC-SANS experiments:** The SANS measurements on PSI and PSII in solution with dDDM were performed at the D22 instrument (ILL, Grenoble, France). The SEC-SANS setup was used to exchange the DDM detergent for its deuterated analog, following the procedure used by Midtgaard et al. [27](#_ENREF_27).

The sample was injected on a Superdex 200 column preequilibrated with the elution buffer: 0.5 mM match-out deuterated DDM, 20 mM Tris/DCl pH 7.5, and 100 mM NaCl in D2O. The elution started at 0.1 mL/min while SANS data were continuously measured in 30 s time frames. This slow elution enables detergent exchange as well as measurement of the buffer scattering signal.

The neutron scattering signal was measured through a 1mm thick quartz cell placed right after the elution column, before the fraction collector. UV absorbance is measured in parallel, on the same sample volume but with a 45° angle (0.141cm pathlength) using a spectrophotomer (OceanOptics 65000 pro) connected by optic fibers. When the sample reached the measurement cell, the elution flow was stopped to accumulate sufficient SANS data statistics at first instrument configuration (2.8 m collimation, 2 m sample-detertor distance), then moved to to collect SANS data in a second instrument configuration (8 m collimation, 8 m sample-detertor distance). The neutron wavelength was 6 Å with 10% wavelength spread. The latter two sample-to-detector distances were covering a Q-range from 0.0075 to 0.04538 Å-1. The data reduction has been performed using the GRASP software: the data were corrected for empty cell and blocked beam signal and normalized by the flux monitored upstream the collimation and the detector sensitivity, and scaled to absolute intensity using direct measurement of the flux at the sample position. The data can be accessed using the DOI: 10.5291/ILL-DATA.TEST-3114.

**h-DDM SANS experiments:** The SANS experiments of PSI employing protonated detergent were performed at the KWS-1 small-angle diffractometer (JCNS at MLZ, Garching, Germany)[48](#_ENREF_48), [49](#_ENREF_49). The neutron wavelength used in this experiment was 5 Å with a 10% wavelength spread. The accessible range of the scattering vector Q was extended from 0.006 to 0.45 Å-1 by measuring at two sample-detector distances of 8 and 20 m, respectively. The samples were kept in standard 1 mm thick Hellma cells at a constant temperature of 20 °C. We probed the samples at two different contrast points: 5% and 18% D2O. The data reduction, including the correction according to the detector sensitivity mask, is carried out using the QtiKWS program[50](#_ENREF_50) specifically developed for the KWS-1 instrument.

**SANS Data Analysis**

A model-independent analysis of small-angle scattering data can be performed according to the classical Guinier approximation [51](#_ENREF_51)

****, (1)

where Rg is the radius of gyration, and I(0) is the forward scattering, which is a shape-independent function of the sample's total scattering power. Q is the scalar value of the scattering vector given by the formula:

, (2)

where λ0 is the neutron wavelength and θ is the scattering angle.

The Guinier approximation is valid for dilute solutions of monodisperse particles and at small Q values defined by QRg < 1.3 [51](#_ENREF_51). The data were corrected for the instrument resolution.

In monodisperse macromolecular solutions, the scattering is proportional to the scattering of a single particle averaged over all orientations. The relation between the scattering intensity and the properties of an individual particle is given by

, (3)

where P(R) is the pair distance distribution function, which is non-zero only in the range from 0 to Dmax. Dmax corresponds to the maximum interparticle distance.

The P(R) function and the maximum particle dimension Dmax can be determined using the Inverse Fourier transform (IFT) method employing the software routine GNOM [52](#_ENREF_52). For the IFT analysis, we used the limited Q-range up to 0.07 Å-1.

Further data analysis was carried out using dedicated software packages developed by Dmitri Svergun [53-55](#_ENREF_53). The overall shape of the complexes under study was reconstructed from the P(R) function using the DAMMIF based on a reverse Monte Carlo minimization approach [53](#_ENREF_53), [54](#_ENREF_54). The structural models derived using DAMMIF within this study are averaged over 20 iterations. For each iteration, it is taken into account that both PSI and PSII proteins have oblate shapes. However, the PSI structure requires a P3 symmetry, while the PSII structure shows a P2 symmetry. The normalized spatial discrepancy (NSD) for each of the DAMMIF models ranges from 0.2 to 0.6.

To fit the SANS curves of PSI-dDDM and PSII-dDDM according to the known crystal structures of PSI (pdb 6trd [31](#_ENREF_31)) and PSII (pdb 5kaf [16](#_ENREF_16)), we used the PEPSI routine developed by S. Grudinin [56](#_ENREF_56). The PYMOL program [57](#_ENREF_57) is applied to compare the DAMMIF results with the known pdb structure of a given protein complex.

**Results and Discussion**

**Contrast matching with protonated and specifically deuterated detergent:** SANS data of PSI from *T. elongatus* obtained using specifically deuterated and protonated detergent, respectively, and different contrast match points are shown in Figure 1A. Guinier plots of the same data sets corrected for instrument resolution are shown in Fig. 1B. They clearly exhibit different slopes, which thus seemingly correspond to different radii of gyration for one and the same protein complex. The latter effect is also visible in Figure 1C, where all three data sets are normalized to the same scattering intensity at the smallest values of the scattering vector Q. At first glance, the data set of PSI in specifically deuterated DDM (dDDM) in 100% D2O is shifted towards higher scattering vectors Q compared with the two other SANS curves of PSI in protonated DDM (hDDM) and at 5 and 18% D2O, respectively. The latter effect suggests a seemingly larger size of the same protein complex when protonated detergent is used. This impression is corroborated by the corresponding distance distribution functions P(R) shown in Figure 1D, where the P(R) functions of PSI in hDDM are generally shifted towards larger radii. In addition, the radii of gyration Rg determined from the Guinier plots and from the P(R) functions listed in Table 1 are about 2 to 5 Å larger when using hDDM compared with dDDM. All SANS curves are roughly consistent with trimeric PSI [29](#_ENREF_29), [31](#_ENREF_31), [46](#_ENREF_46), [47](#_ENREF_47), suggesting that the conventional approaches of contrast matching of protonated detergents lead to seemingly larger complex sizes, as indeed previously observed for PSI. [29](#_ENREF_29), [31](#_ENREF_31), [46](#_ENREF_46), [47](#_ENREF_47)

The effects discussed above can be rationalized, taking into account the heterogeneous nature of the detergent as illustrated in Figure 1E, because the contrast match points of detergent tails and head groups differ significantly. In the case of hDDM, the head group's scattering length density (SLD) of about 1.5 10-6 Å-2 is positive, corresponding to a match point of 30% D2O. However, the SLD of the tail group of -0.2 10-6 Å-2 is negative, leading to a match point of 5% D2O.

C:\Users\pcadmin\Desktop\PSI and PSII dDDM paper\figures\Revision Letters\PSI and PSII figures JPhysChemL 6 panels vertical.tif

**Figure 1.** Panel A: SANS data of PSI obtained using specifically deuterated and protonated detergent are shown for the PSI-dDDM complex in 100% D2O (red points), the PSI-hDDM complex in 18% D2O (navy), and the PSI-hDDM complex in 5% D2O (green). The data of PSI at 5% D2O (green) are shown for comparison and taken from Kölsch et al.[31](#_ENREF_31).

Panel B: Guinier plots of the data shown in panel A, the color code is the same as in panel A.

Panel C: SANS data of PSI as shown in panel A, but normalized to the same intensity at the lowest Q-value, the color code is the same as in panel A.

Panel D: Experimental P(R) functions corresponding to the SANS data shown in Panel A.

Panel E: Scheme illustrating the principle of contrast matching of hDDM bound at the surface of PSI, the SLD (right) and corresponding contrast match (left) are shown for the hydrophilic and hydrophobic groups as well as for the average of hDDM.

Panel F: Comparison of the structures obtained for the PSI-dDDM sample in 100% D2O (red spheres) and for the PSI-hDDM sample in 5% D2O (light blue spheres) reconstructed from the SANS data using the DAMMIN routine, see Figure S1 for the corresponding fits.

**Table 1:** Radii of gyration of PSI and PSII obtained in this study via Guinier and IFT analysis

|  |  |  |
| --- | --- | --- |
| Sample | Rg (Å)  from Guinier analysis | Rg (Å)  from IFT analysis (Gnom) |
| High-resolution structures | | |
| PSI crystal structure 1JBO[9](#_ENREF_9) | 68.2 | 68.28 |
| PSI cryo-EM structure 6TRD[31](#_ENREF_31) | 68.15 | 68.51 |
| SANS data, this study | | |
| PSI-dDDM 100% D2O | 69.7±1 | 70.5±1 |
| PSI-hDDM 5% D2O | 75.2 ± 3 | 75.8 ± 3 |
| PSI-hDDM 18% D2O | 72.4 ± 3 | 72.5 ± 3 |
| PSII-dDDM 100% D2O | 57.23±1 | 57.25±1 |

The general approach to reducing the detergent signal has so far been to use a contrast match point with an average SLD of the entire detergent molecule, which corresponds to about 18% D2O in the case of hDDM. Figures 1A,C show the scattering profile of the PSI-hDDM complex measured at 18% D2O (blue symbols), permitting an approximate estimation of the PSI solution structure. However, the protein signal-to-noise ratio is relatively low at this contrast point, and the background becomes dominant at Q values higher than 0.08 Å-1. Another approach is to match the hydrophobic part of the hDDM detergent selectively at 5% D2O (green symbols in Figures 1A,C). The normalized SANS curves measured at 5% and 18% D2O (see Figure 1C) are relatively similar for Q-values smaller than 0.07 Å-1, while the P(R) function indicates a larger maximum particle dimension. The latter effect may be attributed to the detergent head groups, which are not matched at 5% D2O. In addition, it has to be kept in mind that incomplete matching does not only concern hDDM molecules within the detergent belt surrounding the protein, but also in free detergent micelles that may be present in the buffer solution, see below.

In contrast, a novel approach to match out the detergent is to deuterate its head and tail groups specifically so that their resulting SLDs become equal and can be properly matched using a single contrast level of the solvent. The deuteration levels in the hydrophobic and the hydrophilic groups of the dDDM used here are 89% and 57%, respectively, corresponding to an SLD of 6.36 10-6 Å-2, which can be almost perfectly matched out at 100% D2O [27](#_ENREF_27). In the present study, we measured a SANS curve of PSI in a solution with dDDM at 100% D2O at the D22 instrument (ILL, Grenoble, France). Because of the individual hydrogen/deuterium levels of the dDDM head and tail sections, all detergent is matched out in heavy water, and only the signal arising from the membrane protein remains visible in the SANS scattering profile that differs from those measured using hDDM (see red symbols in Figures 1A-D). More details about the experiment are given in the supporting information.

Comparing the experimental P(R) functions for all three SANS curves shown in Figure 1D, the improvement of the detergent matching is clearly visible. The maximum particle dimension (Dmax) obtained from the PSI-dDDM SANS curve is 206.5 Å, while PSI-hDDM curves at 5% and 18% D2O yield Dmax-values of 230 Å and 215 Å, respectively. As mentioned above, the radii of gyration Rg (see Table 1) are also about 2 to 5 Å larger when using hDDM compared with dDDM. Figure 1F provides a comparison of the solution structures of the PSI-dDDM complex in 100% D2O (red dots) and the PSI-hDDM complex in 5% D2O (blue spheres) obtained from their P(R) functions by *ab-initio* reconstruction using the DAMMIN program . One detergent molecule has a length of about 23 Å, which is roughly equal to the size of two spheres of the DAMMIN bead models shown in Figure 1F. The difference between the two DAMMIN structures is mostly in the order of 1-2 spheres, which indicates that it is most probably be due to incomplete matching of the DDM belt surrounding the protein in the case of hDDM. Furthermore, the difference between the reconstructed structures suggests that the solution structure of the PSI-hDDM complex appears artificially enlarged and, thus, yields only a rough estimate of the native solution structure due to the incomplete matching of the DDM belt surrounding the protein. In contrast, a proper detergent match is achieved only for the PSI-dDDM sample resulting in a realistic solution structure.



**Figure 2:** PSI SANS data (green points) measured at 5% D2O: The fit function (black line) is composed of (i) the PSI trimer contribution (dashed grey line) and (ii) a feature stemming from free βDM micelles (dotted grey line).

While the latter analysis of the PSI-dDDM data by DAMMIN is straightforward due to the complete detergent matching, the presence of free (incompletely matched) detergent micelles has to be considered as additional contribution in the case of the PSI-hDDM complex. Free detergent micelles were visible as a separate peak at about 0.17 Å−1 in previous PSI SAXS data[27](#_ENREF_27). Taking the latter lineshape into account, the SANS data of the PSI–hDDM complex shown in Figure 2 have to be fitted by two components[29](#_ENREF_29): a) the DAMMIN model of the PSI-hDDM complex and b) a spherical core shell representing free hDDM micelles. A closer inspection of Figure 2 indicates that the contribution of the PSI-hDDM complex is shifted further towards low Q-values compared with the measured SANS curve of the PSI-hDDM complex at 5% D2O. This means that the difference between the SANS curves of the PSI-dDDM and of the PSI-hDDM complexes visible in Figures 1A and 1C is even larger, when the presence of free detergent micelles is taken into account.

**Solution structures of PSI and PSII:** The SANS data set of the PSI-dDDM complex from *T. elongatus* (red points in Figure 3A) can be well described over the entire Q-range by a simulation obtained using the PEPSI program[56](#_ENREF_56) based on a high-resolution (Cryo-EM) structure of PSI (pdb code 6trd) [31](#_ENREF_31) reflecting the solution state (black line in Figure 3A). The experimentally obtained P(R) function of the PSI-dDDM complex is also very similar to the theoretical P(R) function of the PSI cryo-EM structure (see red and black lines in Figure 3B, respectively). The theoretical value of Dmax for the PSI crystal structure is about 200 Å, which is only 5 Å smaller than the experimental Dmax for the PSI-dDDM complex. The *ab initio* structure reconstituted from the P(R) function of the PSI-dDDM complex and the PSI high-resolution structure (see Figures 3C and D) are very similar, corroborating the superior matching efficiency observed for dDDM in contrast to previous approaches.

However, minor differences between the high-resolution and solution structures of PSI remain, for example, in the form of a slight deviation indicated by a blue arrow in Figure 3A at Q > 0.1 Å‑1. While the cryo-EM structure[31](#_ENREF_31) predicts a small and broad peak at this position, no such structure is visible in the SANS data of PSI. In this regard, quasielastic neutron scattering data of PSII show that internal motions of protein sidechains on the picosecond timescale are largely suppressed below 240 K and, thus, not reflected in cryo-EM structures but activated at physiological temperatures and in solution [32-34](#_ENREF_32). Therefore, it is reasonable to assume that accessibility of additional conformational substates due to the presence of internal flexibility may smear out the observed peak under physiological (non-cryogenic) conditions.

It is also instructive to compare the experimentally obtained P(R) function of the PSI-dDDM complex with P(R) functions calculated based on different high-resolution structures (see Figure 3B). The P(R) function deviating from that of the solution structure the most is the low-temperature PSI crystal structure (pdb 1jb0 [9](#_ENREF_9), green line). In contrast, both the PSI cryo-EM structure (pdb 6trd [31](#_ENREF_31), black line) and the room temperature PSI crystal structure obtained by XFEL diffraction (pdb 6pgk [58](#_ENREF_58), blue line) appear to be shifted towards the P(R) function reflecting the solution structure of PSI at physiological temperatures because of expansions in the order of 5 Å in all directions compared with the low temperature crystal structure. This can be rationalized by recalling that temperature decrease as well as crystal formation required to obtain high-resolution structures may restrict the motional freedom of a protein and thus the available set of conformational substates as already mentioned above [32-34](#_ENREF_32). As an example, it was shown that electron transfer in PSI and PSII has multiple initial conformations and requires protein dynamics to accommodate subsequent conformational changes (see ref. 33and references therein). Thus, the solution structure has to encompass all the latter conformational substates, while a crystal structure can naturally reflect only a single conformation. The latter results highlight the importance of obtaining solution structures of photosynthetic protein complexes and of membrane proteins in general using SANS under physiological conditions.

C:\Users\pcadmin\Desktop\PSI and PSII dDDM paper\figures\Revision Letters\Figure 2.tif

**Figure 3.** Panel A: SANS data of trimeric PSI obtained using dDDM and at 100% D2O (red points). The black line represents the fit curve obtained using PEPSI [56](#_ENREF_56) based on the PSI cryo-EM structure (pdb 6trd [31](#_ENREF_31)). The blue arrow indicates a slight deviation of the theoretical fit from the experimental data, see also inset for a magnification.

Panel B: P(R) function obtained from PSI-dDDM SANS data (red line). P(R) functions calculated based on different high-resolution structures of PSI are shown for comparison: a) the low-temperature PSI crystal structure (pdb 1jb0 [9](#_ENREF_9), green line), the PSI cryo-EM structure (pdb 6trd [31](#_ENREF_31), black line), and the room temperature PSI crystal structure obtained by XFEL diffraction (pdb 6pgk[58](#_ENREF_58), blue line).

Panels C and D: Top and side views of the PSI-dDDM complex structure reconstructed from the SANS data using the DAMMIN routine (gray spheres, see Figure S1 for the corresponding fits) superimposed to the PSI cryo-EM structure (pdb 6trd [31](#_ENREF_31)) shown in red, yellow, and green.

**C:\Users\pcadmin\Desktop\PSI and PSII dDDM paper\figures\Revision Letters\Figure 3.tifFigure 4.** Panel A: SANS data of dimeric PSII obtained using dDDM at 100% D2O (cyan points). The black line represents the fit curve obtained using PEPSI [56](#_ENREF_56) based on the PSII crystal structure (pdb 5kaf [16](#_ENREF_16))

Panel B: P(R) function obtained from PSI-dDDM SANS data (cyan line). For comparison, the P(R) function calculated from the PSII crystal structure (pdb 5kaf [16](#_ENREF_16)) is shown as a black line.

Panels C and D: Comparison of the PSII-dDDM complex structure reconstructed from the SANS data using the DAMMIN routine (gray spheres, see Figure S2 for the corresponding fits) with the PSII crystal structure (pdb 5kaf [16](#_ENREF_16)) shown in cyan and violet.

The second protein considered is the dimeric PSII core complex from *T. elongatus*. Analogous to the approach used for PSI above, we performed SANS measurements of PSII in a solution with dDDM at 100% D2O. The analysis using PEPSI [56](#_ENREF_56) shows that the theoretical SANS curve of the room temperature PSII crystal structure (pdb code 5kaf [16](#_ENREF_16)) fits well to the measured SANS curve of the PSII-dDDM complex (see Figure 4A). We also find that the experimental P(R) function of the PSII-dDDM complex and the theoretical P(R) function of the PSII crystal structure are very similar, taking into account the experimental uncertainty (see Figure 4B). The Dmax values determined from the experimental and theoretical P(R) functions are 187.7 Å and 195.5 Å, respectively, indicating a slight expansion on PSII in solution similar to PSI above. The *ab initio* structure obtained by the DAMMIN program based on the P(R) function of the PSII-dDDM complex and the room temperature PSII crystal structure are compared in Figure 4C and D. The reconstituted structure mimics dimeric PSII very well, so that we find an excellent agreement between the crystal and the solution structure of dimeric PSII core complexes, which demonstrates again the remarkable matching efficiency achieved for dDDM.

**Conclusions:** In summary, we have investigated the solution structures of PSI and PSII from *T. elongatus* using SANS with contrast variation. Primarily, we have compared SANS data of samples solubilized in specifically deuterated detergent with conventional contrast matching strategies employed when using protonated detergents. We find that SANS data of PSI solubilized in hDDM yielded only a rough estimate of the complex structure, while its size appeared to be systematically overestimated. The latter effect can be attributed to the incomplete matching of protonated detergent molecules bound at the surface of the protein and in free micelles in the buffer solution. In contrast, specifically deuterated detergent can be matched out entirely so that SANS data of the PSI-dDDM complex can be solely attributed to the membrane protein. The absence of a detergent contribution simplifies the data analysis and reduces the number of modeling approximations needed to account for the detergent contribution.

As a result, we determine reliable solution structures of two native photosystems, PSI and PSII, which cannot be achieved by conventional contrast variation attempts. Both solution structures are more similar to the corresponding high-resolution structures than previously published. Residual differences can most probably be attributed to molecular flexibility, which is present under physiological conditions, but suppressed in crystals or at low temperature – i.e. under conditions commonly applied to determine high-resolution structures. We anticipate that similar effects can be expected in the case of other membrane proteins.

In addition, the size of the detergent belt surrounding a membrane protein plays a crucial role in the crystallization process and in the formation of high-quality crystals for X-ray structural analysis. As a prerequisite for investigations of the detergent belt structure, the true size of the complex should be determined using SANS measurements and deuterated detergent as demonstrated in the present study. This could lead to improved reproducibility and quality of the crystals as well as to improved X-ray or even XFEL data analysis of membrane proteins like PSI and PSII.

**Supporting Information:**

Dammif fits of SANS data of the PSI-dDDM complex in 100% D2O, the PSI-hDDM complex in 18% D2O, and the PSI-hDDM complex in 5% D2O (Figure S1); Dammif fit of SANS data of the PSII-dDDM in 100% D2O (Figure S2); details of data collection and structure reconstitution, fitting parameters for SANS curves of PSII/PSI-dDDM in 100% D2O contrast (Table S1).

**Acknowledgement**

Financial support by the Estonian Research Council (Grants PRG 539 and SLOKT 12026 T) is gratefully acknowledged. This work was also partly financed by the Baltic-German University Liaison Office supported by the German Academic Exchange Service (DAAD) with funds from the Foreign Office of the Federal Republic Germany. A.Z. is grateful for financial support by Germany`s Excellence Strategy (Project EXC 2008/1-390540038 (A.Z.) coordinated by T.U. Berlin and by the German Research Foundaton (DFG) via the Collaborative Research Center SFB1078 (Humboldt Universität zu Berlin), TP A5 (A.Z., J.G.) and for the support by the Bundesministerium für Bildung und Forschung, Germany, 2020, projects 031B0557 AþB (A.K., A.Z. and S.S.).

We also thank the ILL (Grenoble, France) for the allocation of beamtime at the SANS instrument D22 and the Jülich Centre for Neutron Science (JCNS) at the Heinz Maier-Leibnitz Zentrum MLZ (Garching, Germany) for beamtime at the KWS-1 SANS instrument. The ILL SANS data are permanently curated by the ILL and accessible via the DOI 10.5291/ILL-DATA.TEST-3114. Finally, we are very grateful to the National Deuteration Facility, Australian Nuclear Science and Technology Organization ANSTO (Lucas Heights, Australia) for providing the deuterated detergent used in our experiments. The National Deuteration Facility is partly supported by the National Collaborative Research Infrastructure Strategy – an initiative of the Australian Government.

**References:**

1. Nelson, N.; Yocum, C. F. Structure and Function of Photosystems I and II. *Annu. Rev. Plant. Biol.* **2006,** *57*, 521-565.

2. Satoh, K.; Wydrzynski, T. J., Introduction to Photosystem II. In *Photosystem II*, Springer, Dordrecht: 2005; Vol. 22, pp 11-22.

3. Müh, F.; Zouni, A. Light-Induced Water Oxidation in Photosystem II. *Front. Biosci.* **2011,** *16*, 3072-3132.

4. Lambreva, M. D.; Russo, D.; Polticelli, F.; Scognamiglio, V.; Antonacci, A.; Zobnina, V.; Campi, G.; Rea, G. Structure/Function/Dynamics of Photosystem II Plastoquinone Binding Sites. *Curr. Protein Pept. Sci.* **2014,** *15*, 285-295.

5. Gorka, M.; Schartner, J.; van der Est, A.; Rogner, M.; Golbeck, J. H. Light-Mediated Hydrogen Generation in Photosystem I: Attachment of a Naphthoquinone-Molecular Wire-Pt Nanoparticle to the A1a and A1b Sites. *Biochemistry* **2014,** *53*, 2295-2306.

6. Saboe, P. O.; Lubner, C. E.; McCool, N. S.; Vargas-Barbosa, N. M.; Yan, H.; Chan, S.; Ferlez, B.; Bazan, G. C.; Golbeck, J. H.; Kumar, M. Two-Dimensional Protein Crystals for Solar Energy Conversion. *Adv. Mater.* **2014,** *26*, 7064-7069.

7. Feifel, S. C.; Lokstein, H.; Hejazi, M.; Zouni, A.; Lisdat, F. Unidirectional Photocurrent of Photosystem I on Pi-System-Modified Graphene Electrodes: Nanobionic Approaches for the Construction of Photobiohybrid Systems. *Langmuir* **2015,** *31*, 10590-10598.

8. Stieger, K. R.; CiornII, D.; Kolsch, A.; Hejazi, M.; Lokstein, H.; Feifel, S. C.; Zouni, A.; Lisdat, F. Engineering of Supramolecular Photoactive Protein Architectures: The Defined Co-Assembly of Photosystem I and Cytochrome C Using a Nanoscaled DNA-Matrix. *Nanoscale* **2016,** *8*, 10695-10705.

9. Jordan, P.; Fromme, P.; Witt, H. T.; Klukas, O.; Saenger, W.; Krauss, N. Three-Dimensional Structure of Cyanobacterial Photosystem I at 2.5 a Resolution. *Nature* **2001,** *411*, 909-917.

10. Mazor, Y.; Nataf, D.; Toporik, H.; Nelson, N. Crystal Structures of Virus-Like Photosystem I Complexes from the Mesophilic Cyanobacterium Synochocystic Pcc 6803. *Elife* **2014,** *3*, 1-17.

11. Mazor, Y.; Borovikova, A.; Caspy, I.; Nelson, N. Structure of the Plant Photosystem I Supercomplex at 2.6 a Resolution. *Nat. Plants* **2017,** *3*, 1-9.

12. Loll, B.; Kern, J.; Saenger, W.; Zouni, A.; Biesiadka, J. Towards Complete Cofactor Arrangement in the 3.0 Å Resolution Structure of Photosystem II. *Nature* **2005,** *438*, 1040-1044.

13. Umena, Y.; Kawakami, K.; Shen, J. R.; Kamiya, N. Crystal Structure of Oxygen-Evolving Photosystem II at a Resolution of 1.9 A. *Nature* **2011,** *473*, 55-60.

14. Shen, J. R. The Structure of Photosystem II and the Mechanism of Water Oxidation in Photosynthesis. *Annu. Rev. Plant Biol.* **2015,** *66*, 23-48.

15. Kern, J.; Chatterjee, R.; Young, I. D.; Fuller, F. D.; Lassalle, L.; Ibrahim, M.; Gul, S.; Fransson, T.; Brewster, A. S.; Alonso-Mori, R., et al. Structures of the Intermediates of Kok's Photosynthetic Water Oxidation Clock. *Nature* **2018,** *563*, 421-425.

16. Young, I. D.; Ibrahim, M.; Chatterjee, R.; Gul, S.; Fuller, F.; Koroidov, S.; Brewster, A. S.; Tran, R.; Alonso-Mori, R.; Kroll, T., et al. Structure of Photosystem II and Substrate Binding at Room Temperature. *Nature* **2016,** *540*, 453-457.

17. Vasilev, S.; Irrgang, K. D.; Schrotter, T.; Bergmann, A.; Eichler, H. J.; Renger, G. Quenching of Chlorophyll Alpha Fluorescence in the Aggregates of LHCII: Steady State Fluorescence and Picosecond Relaxation Kinetics. *Biochemistry* **1997,** *36*, 7503-7512.

18. Pieper, J.; Irrgang, K.-D.; Rätsep, M.; Jankowiak, R.; Schrötter, T.; Voigt, J.; Small, G.; Renger, G. Effects of Aggregation on Trimeric Light-Harvesting Complex II of Green Plants: A Hole-Burning Study. *J. Phys. Chem. A* **1999,** *103*, 2422-2428.

19. Voigt, B.; Krikunova, M.; Lokstein, H. Influence of Detergent Concentration on Aggregation and Spectroscopic Properties of Light-Harvesting Complex II. *Photosynth. Res.* **2008,** *95*, 317-325.

20. Kern, J.; Loll, B.; Luneberg, C.; DiFiore, D.; Biesiadka, J.; Irrgang, K. D.; Zouni, A. Purification, Characterisation and Crystallisation of Photosystem II from Thermosynechococcus Elongatus Cultivated in a New Type of Photobioreactor. *Biochim. Biophys. Acta* **2005,** *1706*, 147-157.

21. Hussein, R.; Ibrahim, M.; Chatterjee, R.; Coates, L.; Müh, F.; Yachandra, V. K.; Yano, J.; Kern, J.; Dobbek, H.; Zouni, A. Optimizing Crystal Size of Photosystem II by Macroseeding: Toward Neutron Protein Crystallography. *Cryst. Growth. Des.* **2018,** *18*, 85-94.

22. le Maire, M.; Champeil, P.; Möller, J. V. Interaction of Membrane Proteins and Lipids with Solubilizing Detergents. *Biochim. Biophys. Acta* **2000,** *1508*, 86-111.

23. Seddon, A. M.; Curnow, P.; Booth, P. J. Membrane Proteins, Lipids and Detergents: Not Just a Soap Opera. *Biochim. Biophys. Acta* **2004,** *1666*, 105-117.

24. Mo, Y.; Lee, B. K.; Ankner, J. F.; Becker, J. M.; Heller, W. T. Detergent-Associated Solution Conformations of Helical and Beta-Barrel Membrane Proteins. *J. Phys. Chem. B* **2008,** *112*, 13349-13354.

25. Cardoso, M. B.; Smolensky, D.; Heller, W. T.; O'Neill, H. Insight into the Structure of Light-Harvesting Complex II and Its Stabilization in Detergent Solution. *J. Phys. Chem. B* **2009,** *113*, 16377-16383.

26. Golub, M.; Hejazi, M.; Kölsch, A.; Lokstein, H.; Wieland, D. C. F.; Zouni, A.; Pieper, J. Solution Structure of Monomeric and Trimeric Photosystem I of Thermosynechococcus Elongatus Investigated by Small-Angle X-Ray Scattering. *Photosynth. Res.* **2017,** *133*, 163-173.

27. Midtgaard, S. R.; Darwish, T. A.; Pedersen, M. C.; Huda, P.; Larsen, A. H.; Jensen, G. V.; Kynde, S. A. R.; Skar-Gislinge, N.; Nielsen, A. J. Z.; Olesen, C., et al. Invisible Detergents for Structure Determination of Membrane Proteins by Small-Angle Neutron Scattering. *FEBS J.* **2018,** *285*, 357-371.

28. Golub, M.; Moldenhauer, M.; Schmitt, F. J.; Feoktystov, A.; Mandar, H.; Maksimov, E.; Friedrich, T.; Pieper, J. Solution Structure and Conformational Flexibility in the Active State of the Orange Carotenoid Protein: Part I. Small-Angle Scattering. *J. Phys. Chem. B* **2019,** *123*, 9525-9535.

29. Golub, M.; Kölsch, A.; Feoktystov, A.; Zouni, A.; Pieper, J. Insights into Solution Structures of Photosynthetic Protein Complexes from Small-Angle Scattering Methods. *Crystals* **2021,** *11*, 1-16.

30. Golub, M.; Lokstein, H.; Soloviov, D.; Kuklin, A.; Wieland, D. C. F.; Pieper, J. Light-Harvesting Complex II Adopts Different Quaternary Structures in Solution as Observed Using Small-Angle Scattering. *J. Phys. Chem. Lett.* **2022,** *13*, 1258-1265.

31. Kölsch, A.; Radon, C.; Golub, M.; Baumert, A.; Bürger, J.; Mielke, T.; Lisdat, F.; Feoktystov, A.; Pieper, J.; Zouni, A., et al. Current Limits of Structural Biology: The Transient Interaction between Cytochrome C6 and Photosystem I. *Curr. Res. Struct. Biol.* **2020,** *2*, 171-179.

32. Kühn, P.; Pieper, J.; Kaminskaya, O.; Eckert, H. J.; Lechner, R. E.; Shuvalov, V.; Renger, G. Reaction Pattern of Photosystem II: Oxidative Water Cleavage and Protein Flexibility. *Photosynth. Res.* **2005,** *84*, 317-23.

33. Pieper, J.; Hauss, T.; Buchsteiner, A.; Renger, G. The Effect of Hydration on Protein Flexibility in Photosystem II of Green Plants Studied by Quasielastic Neutron Scattering. *Eur. Biophys. J.* **2008,** *37*, 657-663.

34. Pieper, J.; Trapp, M.; Skomorokhov, A.; Natkaniec, I.; Peters, J.; Renger, G. Temperature-Dependent Vibrational and Conformational Dynamics of Photosystem II Membrane Fragments from Spinach Investigated by Elastic and Inelastic Neutron Scattering. *Biochem. Biophys. Acta* **2012,** *1817*, 1213-1219.

35. Jacques, D. A.; Trewhella, J. Small-Angle Scattering for Structural Biology--Expanding the Frontier While Avoiding the Pitfalls. *Protein Sci.* **2010,** *19*, 642-657.

36. Kikhney, A. G.; Svergun, D. I. A Practical Guide to Small Angle X-Ray Scattering (Saxs) of Flexible and Intrinsically Disordered Proteins. *FEBS Lett.* **2015,** *589*, 2570-2577.

37. Franke, D.; Petoukhov, M. V.; Konarev, P. V.; Panjkovich, A.; Tuukkanen, A.; Mertens, H. D. T.; Kikhney, A. G.; Hajizadeh, N. R.; Franklin, J. M.; Jeffries, C. M., et al. Atsas 2.8: A Comprehensive Data Analysis Suite for Small-Angle Scattering from Macromolecular Solutions. *J. Appl. Crystallogr.* **2017,** *50*, 1212-1225.

38. Gabel, F., Applications of Sans to Study Membrane Protein Systems. In *Biological Small Angle Scattering: Techniques, Strategies and Tips*, Chaudhuri, B.; Muñoz, I. G.; Qian, S.; Urban, V. S., Eds. Springer Singapore: Singapore, 2017; pp 201-214.

39. Conn, C. E.; de Campo, L.; Whitten, A. E.; Garvey, C. J.; Krause-Heuer, A. M.; van 't Hag, L. Membrane Protein Structures in Lipid Bilayers; Small-Angle Neutron Scattering with Contrast-Matched Bicontinuous Cubic Phases. *Front. Chem.* **2020,** *8*, 619470.

40. Di Cola, E.; Grillo, I.; Ristori, S. Small Angle X-Ray and Neutron Scattering: Powerful Tools for Studying the Structure of Drug-Loaded Liposomes. *Pharmaceutics* **2016,** *8*, 1-16.

41. Amaro, R. E.; Mulholland, A. J. Multiscale Methods in Drug Design Bridge Chemical and Biological Complexity in the Search for Cures. *Nat. Rev. Chem.* **2018,** *2*, 1-12.

42. Koutsioubas, A. Low-Resolution Structure of Detergent-Solubilized Membrane Proteins from Small-Angle Scattering Data. *Biophys. J.* **2017,** *113*, 2373-2382.

43. Berthaud, A.; Manzi, J.; Perez, J.; Mangenot, S. Modeling Detergent Organization around Aquaporin-0 Using Small-Angle X-Ray Scattering. *J. Am. Chem. Soc.* **2012,** *134*, 10080-8.

44. Chen, P. C.; Hub, J. S. Structural Properties of Protein-Detergent Complexes from Saxs and Md Simulations. *J. Phys. Chem. Lett.* **2015,** *6*, 5116-21.

45. Breyton, C.; Gabel, F.; Lethier, M.; Flayhan, A.; Durand, G.; Jault, J. M.; Juillan-Binard, C.; Imbert, L.; Moulin, M.; Ravaud, S., et al. Small-Angle Neutron Scattering for the Study of Solubilised Membrane Proteins. *Eur. Phys. J. E* **2013,** *36*, 1-16.

46. Le, R. K.; Harris, B. J.; Iwuchukwu, I. J.; Bruce, B. D.; Cheng, X.; Qian, S.; Heller, W. T.; O'Neill, H.; Frymier, P. D. Analysis of the Solution Structure of Thermosynechococcus Elongatus Photosystem I in N-Dodecyl-Beta-D-Maltoside Using Small-Angle Neutron Scattering and Molecular Dynamics Simulation. *Arch. Biochem. Biophys.* **2014,** *550-551*, 50-57.

47. Golub, M.; Hussein, R.; Ibrahim, M.; Hecht, M.; Wieland, D. C. F.; Martel, A.; Machado, B.; Zouni, A.; Pieper, J. Solution Structure of the Detergent-Photosystem II Core Complex Investigated by Small Angle Scattering Techniques. *J. Phys. Chem. B* **2020,** *124*, 8583-8592.

48. Feoktystov, A. V.; Frielinghaus, H.; Di, Z. Y.; Jaksch, S.; Pipich, V.; Appavou, M. S.; Babcock, E.; Hanslik, R.; Engels, R.; Kemmerling, G., et al. Kws-1 High-Resolution Small-Angle Neutron Scattering Instrument at Jcns: Current State. *J. Appl. Crystallogr.* **2015,** *48*, 61-70.

49. Frielinghaus, H.; Feoktystov, A.; Berts, I.; Mangiapia, G. Kws-1: Small-Angle Scattering Di Ractometer. *J. Large-Scale Res. Facil.* **2015,** *1*, 1-4.

50. Pipich, V. Qtikws. [www.qtikws.de](http://www.qtikws.de) (accessed Oct 3, 2017).

51. Guinier, A.; Fournet, G. *Small-Angle Scattering of X-Rays*. John Wiley and Sons: New York, 1955.

52. Svergun, D. I. Determination of the Regularization Parameter in Indirect-Transform Methods Using Perceptual Criteria. *J. Appl. Crystallogr.* **1992,** *25*, 495-503.

53. Konarev, P. V.; Volkov, V. V.; Sokolova, A. V.; Koch, M. H. J.; Svergun, D. I. Primus: A Windows Pc-Based System for Small-Angle Scattering Data Analysis. *J. Appl. Crystallogr.* **2003,** *36*, 1277-1282.

54. Franke, D.; Svergun, D. I. Dammif, a Program for Rapid Ab-Initio Shape Determination in Small-Angle Scattering. *J. Appl. Crystallogr.* **2009,** *42*, 342-346.

55. Petoukhov, M. V.; Franke, D.; Shkumatov, A. V.; Tria, G.; Kikhney, A. G.; Gajda, M.; Gorba, C.; Mertens, H. D.; Konarev, P. V.; Svergun, D. I. New Developments in the Atsas Program Package for Small-Angle Scattering Data Analysis. *J. Appl. Crystallogr.* **2012,** *45*, 342-350.

56. Grudinin, S.; Garkavenko, M.; Kazennov, A. Pepsi-Saxs: An Adaptive Method for Rapid and Accurate Computation of Small-Angle X-Ray Scattering Profiles. *Acta Crystallogr., Sect. D: Struct. Biol.* **2017,** *73*, 449-464.

57. DeLano, W. L. *Pymol Molecular Graphics System* 0.99; California U.S.A., 2006.

58. Gisriel, C.; Coe, J.; Letrun, R.; Yefanov, O. M.; Luna-Chavez, C.; Stander, N. E.; Lisova, S.; Mariani, V.; Kuhn, M.; Aplin, S., et al. Membrane Protein Megahertz Crystallography at the European Xfel. *Nat. Commun.* **2019,** *10*, 5021.

TOC graphics

