

Analysis of small-angle X-ray scattering data of protein–detergent complexes by singular value decomposition

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Small-angle X-ray scattering can be a valuable tool in the structural characterization of membrane protein–detergent complexes (PDCs). However, a major challenge is to separate the PDC scattering signal from that of the ‘empty’ detergent micelle in a protein–detergent mixture. We briefly review an approach that allows approximate determination of the PDC scattering signal at low momentum transfer and present a novel approach that employs a singular value decomposition (SVD) and fitting of scattering data collected at different protein–detergent stoichiometries. The SVD approach allows the scattering profile for the PDC over the entire measured momentum transfer range to be obtained, it is applicable to strongly scattering detergents and can take into account interparticle interference. The two approaches are contrasted and an application to the membrane protein TM0026 from *Thermotoga maritima* is presented.

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1. Introduction

Membrane proteins are located in the cell membrane, where they act as transporters, channels, receptors or enzymes in a range of essential cellular functions. An estimated 30–40% of all genes code for membrane proteins (Wallin & von Heijne, 1998) and they constitute ca 50% of all drug targets (Korepanova *et al.*, 2005). In contrast, less than 1% of the structures currently deposited in the Protein Data Bank (Berman *et al.*, 2000) are of membrane proteins. A major hurdle to structural studies is the necessity to solubilize membrane proteins (Sanders & Sonnichsen, 2006). Micelle forming detergents are routinely used as a mimetic of the cell membrane. The hydrophobic regions of the protein are encapsulated on the inside of the detergent micelle in the resulting protein–detergent complex (PDC).

Insights into the structure of the PDC provide information about the structure of the membrane protein itself, and, furthermore, about protein–detergent interactions. An improved understanding of protein–detergent interactions can be used to choose an optimal detergent for a given protein and application. Finally, knowledge about the interactions of PDCs in solution could help to design better crystallization conditions.

Small-angle X-ray scattering (SAXS) can serve as a powerful probe of the PDC, as it can probe the size, shape and interactions of macromolecular complexes under a variety of solution conditions without the need to crystallize the sample (Doniach, 2001; Svergun & Koch, 2003; Koch *et al.*, 2003). The scattering signal from a mixture of membrane proteins and detergent, however, generally contains contributions both from the PDC and from ‘empty’ detergent micelles. A major challenge is to separate the contributions and to isolate the scattering profile of the PDC.

One apparent possibility is to record a scattering profile of the detergent only, *i.e.* in the absence of protein, for subtraction of the micelle signal. However, as an (*a priori* unknown) fraction of the detergent molecules form PDCs in the presence of protein, the ‘empty’ micelle concentration is different in the absence and presence of the protein and the subtraction will be mismatched (see §3.1). Loll *et al.* (2001) performed light scattering studies after extensive dialysis against a buffer of known detergent concentration to ensure a fixed concentration of detergent micelles. This approach is problematic, however, as for each PDC dialysis the conditions need to be optimized and several days of dialysis are required. An alternative strategy is to match the scattering density of the buffer to that of the detergent, such that the detergent molecules become ‘invisible’ to the scattering experiment. Bu & Engelman (1999) followed this approach and used sucrose solutions of different concentrations for density matching to determine the radius of gyration and molecular weight of a model protein system. However, matching the electron density in this way is only possible for a select few detergents that have a scattering density close to that of water, as the scattering contrast for X-ray scattering (*i.e.* the electron density) is difficult to adjust. This is unlike the case of neutron scattering experiments, where the buffer scattering contrast can be changed over a wide range by adjusting the ratio of D₂O to H₂O (Knoll *et al.*, 1981).

Here, we present two approaches to deconvolving micelle and PDC scattering. First we briefly review a recently developed approximative ‘expansion’ treatment (Columbus *et al.*, 2006) that can be used to obtain an upper and lower limit of the PDC scattering in the low-angle Guinier region from a single measurement of the protein sample. This approximative treatment is well suited to obtaining the radius of gyration and protein oligomerization state of

the PDCs for a large number of protein–detergent combinations in a high-throughput fashion, however, it suffers several important shortcomings.

We then present a novel method to deconvolve the PDC and micelle scattering based on a singular value decomposition (SVD) of scattering data collected at different protein–detergent stoichiometries. Finally, we demonstrate the feasibility of the SVD approach by applying it to scattering data for the integral membrane protein TM0026 from *Thermotoga maritima*. The results are compared with the approximative approach and the relative advantages of the methods are discussed.

2. SAXS measurements

Membrane proteins were expressed and purified as described in Columbus *et al.* (2006). All data were obtained at beamline 12-ID of the Advanced Photon Source at Argonne National Laboratory, USA, using a set-up as described in Lipfert, Millet *et al.* (2006), Beno *et al.* (2001) and Seifert *et al.* (2000). For each data point, a total of ten measurements of 0.1 s integration time each were taken. Data were image corrected and circularly averaged; the ten profiles for each condition were averaged to improve signal quality.

3. Theory

3.1. ‘Expansion’ treatment of the PDC scattering signal

Recently, we have developed a method to obtain the PDC forward scattering intensity $I(0)$ and radius of gyration R_g approximatively (Columbus *et al.*, 2006) from Guinier analysis (Guinier, 1939). The basic idea is to consider two different estimates of the PDC scattering profile: In one limit, referred to as I(complex – buffer), we consider the scattering from the protein–detergent mixture and subtract a suitable buffer (no detergent) profile. This provides an overestimate of the PDC scattering signal, as the contribution of the ‘empty’ detergent micelles is not subtracted. The I(complex – buffer) limit yields an upper bound of the PDC forward scattering intensity and a lower bound of the PDC R_g (Columbus *et al.*, 2006). Denoting the ‘empty’ micelle scattering intensity with I_{mic} , that of the PDC with I_{PDC} , the protein concentration with c_{prot} and that of the ‘empty’ detergent micelles in the presence of the protein with c'_{mic} , we introduce $\epsilon = c'_{mic}I_{mic}(q)/c_{prot}I_{PDC}(q)$. The I(complex – buffer) limit provides a good approximation for small ϵ , *i.e.* whenever the scattering signal of the micelles is weak compared to the PDC scattering and for small concentrations of ‘empty’ detergent micelles. In the other limit, denoted as I(complex – micelle), we subtract the micelle scattering profile recorded at the same detergent concentration from the scattering of the protein–detergent mixture. As the concentration of micelles in the absence of protein c_{mic} is larger than the micelle concentration in the presence of protein c'_{mic} , *i.e.* $c'_{mic} < c_{mic}$, this provides a lower bound for the PDC forward scattering and an upper bound of the PDC R_g . The I(complex – micelle) limit provides a good approximation for small $\hat{\epsilon} = (c_{mic} - c'_{mic})I_{mic}(q)/c_{prot}I_{prot}(q) \geq 0$, *i.e.* for weakly scattering micelles and in the limit that the detergent is in large excess over the protein, such that $c'_{mic} \simeq c_{mic}$.

We have applied this approximative treatment in a screen of eight integral membrane proteins from *Thermotoga maritima* and 11 different detergents (Columbus *et al.*, 2006). For many of the studied protein–detergent pairs the upper and lower bounds for R_g and $I(0)$ from the two different expansions were close and we were able to determine the R_g and oligomerization state of the protein with reasonable accuracy. An advantage of this approximative treatment is

that it only requires a single measurement of the protein sample (and two measurements of the ‘buffer’ and ‘detergent only’ solutions).

However, this approximative approach suffers several important shortcomings. It is inapplicable or provides poor estimates for strongly scattering detergent micelles. Out of the 11 detergents employed in our recent study (Columbus *et al.*, 2006), we found in particular *n*-decyl- β -D-maltoside (DM) and *n*-dodecyl- β -D-maltoside (DoDM) and to a lesser extent 3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate (CHAPS) to be strong scatterers. Even for weakly scattering detergents, a good estimate is only obtained for the PDC scattering at very low momentum transfer q [$q = (4\pi/\lambda)\sin\theta$, where 2θ is the total scattering angle and λ is the X-ray wavelength]. As the PDC is typically much larger and more electron dense than the ‘empty’ micelles, it scatters more strongly at low q . Micelle scattering, however, typically exhibits a strong second peak at intermediate to high q , which results from the interference between the electron dense detergent head groups and the low density aliphatic tail groups in the middle of the micelle. The high- q micelle scattering, therefore, typically exceeds that of the PDC even for weakly scattering detergents. Finally, the approximative treatment neglects interparticle interference effects, and is therefore strictly speaking only applicable to very dilute solutions.

3.2. Analysis by singular value decomposition

We will now present a method that can overcome some of the shortcomings of the approximative ‘expansion’ approach. It requires that scattering data are collected at several different protein–detergent stoichiometries. In return, it allows the scattering profile for the PDC over the entire measured angle range to be obtained, it is applicable to strongly scattering detergents and can take into account interparticle interference.

Consider K scattering profiles collected at different protein and detergent concentrations ($c_{prot,k}$, $c_{det,k}$). The data can be arranged in a matrix A , where the rows correspond to different momentum transfer values q_j and the columns are the intensity profile for the k th condition. Applying a singular value decomposition to the data matrix deconvolves the signal into a set of orthogonal basis functions as follows (Henry & Hofrichter, 1992; Segel *et al.*, 1998; Doniach, 2001)

$$A = UWV^T. \quad (1)$$

For the case of N discrete momentum transfer values, A is an $N \times K$ matrix. The matrix U is also $N \times K$ and has as its columns orthogonal basis functions $U_i(q_j)$ ($= U_{j,i}$). W is a $K \times K$ diagonal matrix containing the *singular values* w_i on the main diagonal. The singular values are ordered, *i.e.* they have the property that $w_1 \geq w_2 \geq w_3 \dots \geq w_K$. Following Henry & Hofrichter (1992), the number of true, independent basis functions $U_i(q)$ corresponds to the number of distinctly scattering species L . For homogeneous populations of micelles and PDCs, we would expect $L = 2$ independently scattering components. However, in practice it is necessary to include more components into the subsequent fitting to account for the effects of interparticle interference.

3.3. Interparticle interference

For approximately spherical particles in solution, the total scattering intensity (\hat{I}) is a product of the concentration-independent *form factor* $I(q)$ [often also denoted $P(q)$], which represents the scattering signal from a single particle at infinite dilution and the *structure factor* $S(q, c)$, which depends in the concentration c and takes into account interparticle effects.

$$\hat{I}(q, c) = I(q)S(q, c). \quad (2)$$

In the limit of infinite dilution $S(q, c) = c$, independent of q , and the scattering intensity is linear in the concentration. We account for interference by expanding $S(q, c)$ in powers of c . Keeping the linear and quadratic term in concentration, the scattering for two molecular species, the PDC and detergent micelle, reads

$$\begin{aligned} \hat{I}(q, c) \simeq & c_{\text{PDC}} I_{\text{PDC}}(q) + (c_{\text{PDC}})^2 I_{\text{int,PDC-PDC}}(q) + c'_{\text{mic}} I_{\text{mic}}(q) \\ & + (c'_{\text{mic}})^2 I_{\text{int,mic-mic}}(q) + c'_{\text{mic}} c_{\text{PDC}} I_{\text{int,mic-PDC}}. \end{aligned} \quad (3)$$

The interference is taken into account by introducing ‘interference components’ $I_{\text{int,PDC-PDC}}$, $I_{\text{int,mic-mic}}$ and $I_{\text{int,mic-PDC}}$ in addition to the particle form factor ‘scattering components’ I_{PDC} and I_{mic} . In principle, it is possible to take into account interference effects to higher order by introducing additional interference components.

3.4. Number of independent components

The number of signal-containing basis functions $U_i(q)$ determines how many ‘scattering’ and ‘interference’ components should be used in the fit. Henry & Hofrichter (1992) suggest the following three criteria to determine the number L of signal-containing components: (1) Inspection of the basis functions: by plotting the basis functions $U_i(q)$ as a function of q , one can estimate which of the $U_i(q)$ contain appreciable levels of signal and which components correspond to noise. (2) Singular values: the size of the singular values gives an estimate of the relative importance of the corresponding basis components. (3) Autocorrelations of the basis functions: by computing the autocorrelation

$$C_i = \sum_{j=1}^{N-1} U_i(q_j) U_i(q_{j+1}) \quad (4)$$

of each of the basis functions, an estimate of the ‘noisiness’ is obtained. Components which contain appreciable signal typically have autocorrelations close to 1.0 (>0.95), whereas components that correspond to noise tend to have $C_i < 0.8$.

The assignment of the independent components to molecular species or ‘interference components’ requires modeling assumptions and must be guided by prior knowledge.

In the following, we treat the case of four independent components: I_{PDC} , I_{mic} , as well as two ‘interference components’ $I_{\text{int,mic-mic}}(q)$ and $I_{\text{int,mic-PDC}}(q)$, *i.e.* we neglect $I_{\text{PDC-PDC}}$ in equation (3) (see below). A generalization of the method to more (or fewer) scattering components is straightforward. Fitting of L independent components requires measurements of at least $K = L$ different protein–detergent mixtures. In practice, it is desirable to have $K > L$ experimental profiles.

3.5. Thermodynamic model

From the known concentrations of protein and detergent for the K stoichiometries ($c_{\text{prot},k}$, $c_{\text{det},k}$), we estimate the concentration of micelles in the presence of protein as

$$c'_{\text{mic},k} \simeq (c_{\text{det},k} - m_{\text{PDC}} c_{\text{PDC},k}) / m_{\text{mic}}. \quad (5)$$

Out of the total number of detergent molecules c_{det} , $m_{\text{PDC}} c_{\text{PDC}}$ participate in PDCs, *i.e.* each PDC contains one protein and m_{PDC} detergent monomers. The remaining detergent molecules form ‘empty’ micelles of aggregation number m_{mic} . This assumes that the protein is monomeric inside the PDC, *i.e.* that $c_{\text{PDC}} = c_{\text{prot}}$. The oligomerization state of the protein inside the PDC can be obtained from the approximative ‘expansion’ treatment or from *e.g.* chemical

cross-linking experiments (Columbus *et al.*, 2006). For higher protein oligomers c_{prot} is to be divided by the appropriate factor.

We neglect free detergent monomers (*i.e.* monomers that neither participate in micelles nor in PDCs). The concentration of free detergent is of the order of the critical micelle concentration, which is typically much lower than the detergent concentrations used in our experiments. Furthermore, we neglect the weak dependence of micelle size and aggregation number on detergent concentration (empirically $m_{\text{mic}} \propto c_{\text{det}}^{1/4}$; Quina *et al.*, 1995). We also found in a recent study that the dominant effect on the scattering profile for DM with increasing detergent concentration is interparticle interference, and not micelle growth (Lipfert, Columbus *et al.*, 2006). Values for m_{mic} are available for many detergents from the literature or can be determined from Guinier analysis of the detergent forward scattering intensity (Lipfert, Columbus *et al.*, 2006). m_{mic} needs to be only approximately known, see below. The parameter m_{PDC} is determined from the fit.

3.6. Fitting to the SVD data

The data matrix $A_{j,k}$ with columns $\hat{I}(q_j, c_k)$ can be approximated by the first L components $U_i(q)$, with the weights given by the SVD as

$$\hat{I}(q, c_k) = \sum_{i=1}^L w_i V_{k,i} U_i(q). \quad (6)$$

It is a general property of the SVD that equation (6) is the best approximation of the data in the least-squares sense for any set of L vectors (Golub & Van Loan, 1996). As the $U_i(q)$ form a linear independent basis set, the (yet to be determined) scattering profiles $I_{\text{mic}}(q)$ and $I_{\text{PDC}}(q)$ as well as $I_{\text{int,mic-mic}}(q)$ and $I_{\text{int,mic-PDC}}(q)$ can be written as linear combinations

$$I_{\text{PDC}}(q) = \sum_{i=1}^L w_i b_i^{\text{PDC}} U_i(q), \quad (7)$$

$$I_{\text{mic}}(q) = \sum_{i=1}^L w_i b_i^{\text{mic}} U_i(q), \quad (8)$$

$$I_{\text{int,mic-mic}}(q) = \sum_{i=1}^L w_i b_i^{\text{int,mic-mic}} U_i(q), \quad (9)$$

$$I_{\text{int,prot}}(q) = \sum_{i=1}^L w_i b_i^{\text{int,mic-PDC}} U_i(q). \quad (10)$$

The coefficients b_i^{PDC} , b_i^{mic} , $b_i^{\text{int,mic-mic}}$ and $b_i^{\text{int,mic-PDC}}$ are to be determined by the fitting procedure.

Combining equations (7–10) with (3) and comparing coefficients component by component (as the U_i are linearly independent) with equation (6) we find that

$$\begin{aligned} V_{k,j} = & c_{\text{PDC},k} b_j^{\text{PDC}} + c'_{\text{mic},k} b_j^{\text{mic}} \\ & + c_{\text{PDC},k} c'_{\text{mic},k} b_j^{\text{int,mic-PDC}} + (c'_{\text{mic},k})^2 b_j^{\text{int,mic-mic}} \end{aligned} \quad (11)$$

for $j = 1, \dots, 4$. We can employ a nonlinear fitting routine in order to determine the coefficients b_i as well as the aggregation number m_{PDC} to obtain an optimal fit to the data by minimizing the function

$$\chi^2 = \sum_{i=1}^L \sum_{k=1}^K \frac{w_i^2 (V_{k,i}^{\text{obs}} - V_{k,i}^{\text{calc}})^2}{\sigma_{k,i}^2}. \quad (12)$$

Here the $V_{k,i}^{\text{obs}}$ are the coefficients obtained from the SVD of the data matrix [equation (6)] and the $V_{k,i}^{\text{calc}}$ are the modeled coefficients from equation (11). The errors $\sigma_{k,i}^2$ are the variances of the coefficients from the SVD and are simple linear combinations of the experimental errors (Henry & Hofrichter, 1992). We used a nonlinear fitting

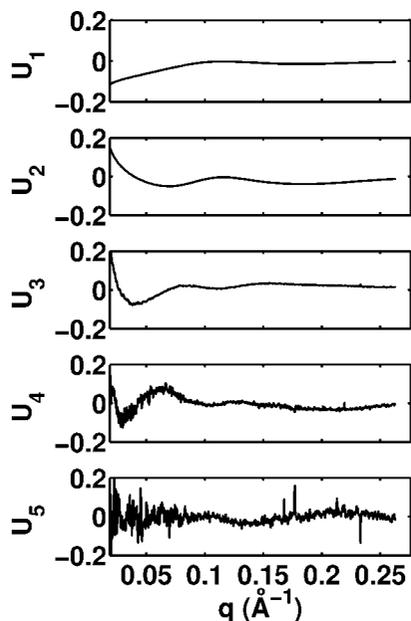


Figure 1
The first five basis functions $U_i(q)$ from an SVD of SAXS data of the membrane protein TM0026 in DM at six different stoichiometries (see text). The momentum transfer q is equal to $(4\pi/\lambda)\sin(\theta)$, where 2θ is the total scattering angle and λ is the X-ray wavelength.

routine implemented in *Matlab* (Mathworks) to obtain fits to the SVD data from our model. The aggregation number of the free micelle m_{mic} in equation (5) is not a free parameter, as it simply sets the scale of the b_i^{mic} and $b_i^{int,mic-mic}$. However, we need to ensure that the numerical value of m_{mic} is such that the concentrations c'_{mic} are smaller than unity.

4. Results

Scattering data for the membrane protein TM0026 in *n*-decyl- β -D-maltoside (DM) were obtained as described in §2. TM0026 is an α -helical protein with two predicted transmembrane helices and a molecular weight of 9.6 kDa. Judging from one- and two-dimensional

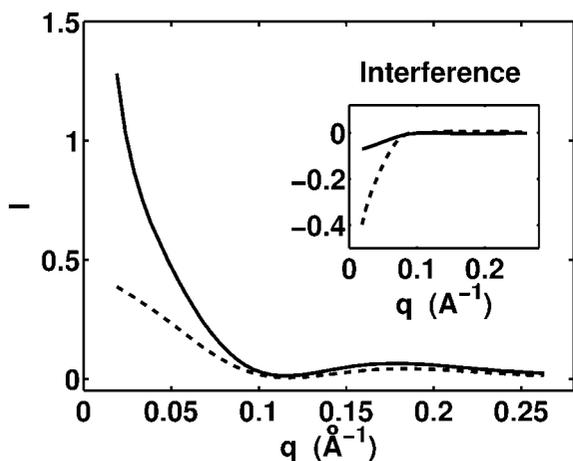


Figure 2
Scattering profiles and interference profiles for TM0026 in DM obtained from fitting to the SVD data. Main graph: $I_{PDC}(q)$ (solid line) and $I_{mic}(q)$ (dashed line). Inset: Interference components $I_{int,mic-PDC}(q)$ (solid line) and $I_{int,mic-mic}(q)$ (dashed line).

nuclear magnetic resonance and circular dichroism spectroscopy, TM0026 is well folded and does not aggregate when solubilized in DM micelles (Columbus *et al.*, 2006). Using the ‘expansion’ approach, it was determined to be monomeric in PDCs formed by five different detergents, including DM (Columbus *et al.*, 2006).

For this work, a total of six scattering profiles were collected, three at a protein concentration of 0.18 mM and detergent concentrations of 88, 150 and 300 mM, another three at identical detergent concentrations and a protein concentration of 0.36 mM. All measurements were performed in 20 mM phosphate buffer, pH 7.0, with 150 mM NaCl added. Scattering profiles of this buffer were subtracted for background correction.

We determine the number of signal-containing components by applying the criteria of Henry & Hofrichter (1992). Fig. 1 shows the first five basis components $U_i(q)$ obtained from an SVD of the scattering data matrix. The plots of the basis functions suggest that the first four components contain significant signal, whereas the fifth (and sixth, not shown) are representative of noise. This finding is corroborated by the autocorrelations computed from equation (4), which are found to be 0.99, 0.99, 0.97, 0.94, 0.63 and 0.49 for $i = 1, \dots, 6$. The first four components have autocorrelations of 94% and higher, while the last two components exhibit much lower values.

With the number of signal-containing components determined to be $L = 4$, we fit $I_{PDC}(q)$, $I_{mic}(q)$ and two ‘interference components’ to the data. As the aggregation number for DM is ~ 70 (Sigma Aldrich, 2004), the micelle concentration is higher than the PDC concentration for all experimental stoichiometries. Therefore, we neglect the $(c_{PDC})^2$ term in equation (3) and fit $I_{int,mic-mic}$ and $I_{int,mic-PDC}$ as interference components. This approach is further corroborated by the fact that scattering data collected on DM detergent micelles for DM concentrations ranging from 5 to 200 mM yield two signal-containing components (data not shown).

The $I_{PDC}(q)$, $I_{mic}(q)$ and two ‘interference profiles’ obtained from the best fit to the data are shown in Fig. 2. The number of detergent monomers in the PDC was fitted to be ~ 100 –120. Interestingly, this value is larger than the aggregation number of the empty micelle, which suggests that the detergent packing is significantly perturbed in the PDC as compared to the micelle. By Guinier analysis of the fitted $I_{PDC}(q)$ and $I_{mic}(q)$, the radius of gyration of the PDC was determined to be 40 Å, that of the micelle to be 27 Å. Using the ‘expansion’ approach, we had previously only been able to bracket the R_g of the TM0026–DM PDC coarsely as $24 < R_g^{PDC} < 47$ Å, as DM is a strongly scattering detergent (Columbus *et al.*, 2006). The value of 27 Å for the micelle R_g is in excellent agreement with the value of 27 ± 0.5 Å determined from direct measurements of detergent scattering (Lipfert, Columbus *et al.*, 2006). The fitted scattering profile $I_{mic}(q)$ agrees well with the measured scattering profiles for ‘empty’ DM micelles (not shown). Overall, the fit to the data is excellent, as shown in Fig. 3.

The fitted interference components (inset of Fig. 2) quickly go to zero for high q , as is to be expected as generally $\lim_{q \rightarrow \infty} S(q, c) = 1.0$. For low q values they are negative, characteristic of interparticle repulsion. As DM is a non-ionic detergent, this repulsion is likely to be due to excluded volume effects.

5. Conclusion

We have shown that the scattering profile of the PDC can be separated from the micelle scattering by using SVD analysis and fitting to data of protein–detergent mixtures at different stoichiometries. This approach, in contrast to the approximative ‘expansion’ treatment,

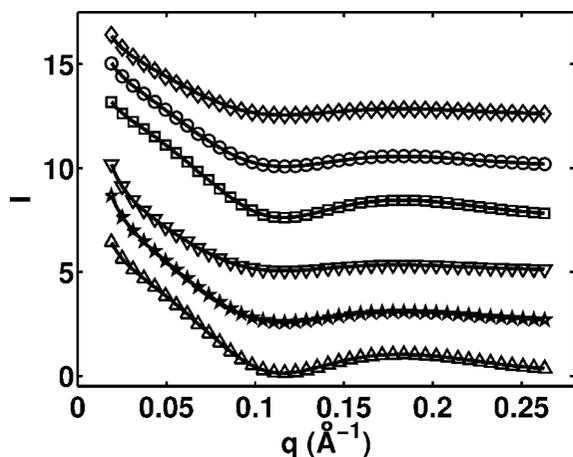


Figure 3

Experimental SAXS profiles for TM0026 in DM (symbols) and fitted profiles from the SVD (solid lines). The protein detergent and concentrations are, from top to bottom, ($c_{\text{prot}} =$) 0.18 mM, ($c_{\text{det}} =$) 88 mM (diamonds), 0.18, 150 mM (circles), 0.18, 300 mM (squares), 0.36, 88 mM (downwards pointing triangles), 0.36, 150 mM (stars), 0.36, 300 mM (upwards pointing triangles). For clarity, the number of experimental momentum transfer values was reduced and the profiles were vertically offset.

requires measurements of several protein samples, which makes it less well suited to high-throughput data collection. In return, it allows the reconstruction of the scattering profile for the PDC over the entire measured q range, which is advantageous for subsequent modeling of the PDC. The SVD approach is applicable even to strongly scattering detergents; furthermore, interparticle interference can be taken into account.

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