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The Challenges of Polydisperse SAXS Data Analysis - Two Different SAXS Studies of PICK1 Produce Structural Models

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PICK1 is a BAR domain protein, featuring additionally an N-terminal PDZ domain and an acidic C-terminal tail (ACT). PICK1 has been implicated in trafficking of several neuronal proteins, including the AMPA receptor. A recent article (Karlsen et al., 2015) reports a structural study of PICK1 using small angle x-ray scattering (SAXS). This work reaches different conclusions than a prior study from our laboratory that used a similar approach (Madasu et al., 2015). However, the new study was apparently unaware of our work, and did not address important contrary evidence. Here, we discuss how different approaches to overcome sample polydispersity (aggregation) in SAXS data analysis may explain the different structural and functional conclusions, and encourage scientists in the field to test the two diverging models.

SAXS is a low-resolution method that in favorable cases can yield information about the overall dimensions and shape of macromolecules in solution (Trewhella et al., 2013). However, given the limited information embedded in a SAXS scattering curve, the method can easily lead to erroneous interpretations. PICK1 is particularly challenging for SAXS since, like many BAR domain proteins that oligomerize on cellular membranes, it is prone to aggregation. Because the scattering intensity is directly proportional to the mass of the scattering particle, higher order aggregates tend to dominate the scattering, and produce unrealistically large particle dimensions.

The two studies on PICK1 took different approaches to circumvent protein aggregation. In our study, the recognition that the scattering intensity of full-length PICK1 was plagued by aggregation, as revealed by a non-linear dependence of the scattering with protein concentration, led us to design an MBP-PICK1 fusion protein that was monodisperse and produced reliable scattering intensities up to a concentration of 7.5 mg/ml (Figure 1 in Madasu et al., 2015). To further reduce the chances of aggregation and concentration effects, subsequent analysis was based on the data collected at 3.75 mg/ml, which was well within the linear region of the Intensity *vs.* Concentration plot. We note, however, that even this apparently clean sample could in principle contain residual aggregates, such that a slight overestimation of particle dimensions cannot be completely ruled out. Nevertheless, parameters such as the radius of gyration (*Rg*), molecular mass (regarded by the SAXS

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SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure and can be found with this Letter online

community as an essential diagnosis for aggregation) and the maximum particle dimensions (*Dmax*) are all consistent with the expected values for MBP-PICK1 and PICK1 (estimated from the monodisperse portion of the data collected at lower concentration), also suggesting that MBP does not affect the overall structure.

The use of MBP-PICK1 had one additional advantage. Like all BAR domain proteins, PICK1 is an antiparallel dimer. MBP (371 aa) is only marginally smaller than PICK1 (415 aa), and is easily recognizable at both ends of the *ab initio* SAXS envelope. Because we used a short 3-alanine linker between MBP and the PDZ domain of PICK1, this also defines quite precisely the location of the PDZ domain (albeit not its orientation) with respect to the BAR domain. Indeed, in our SAXS envelope, MBP-PDZ-BAR-ACT form a contiguous elongated shape, with two-fold symmetry and bent in the middle as expected for a BAR domain protein, indicating that the PDZ domain must lie adjacent to the BAR domain. An atomic model based on known structures of MBP, the PDZ domain and a related BAR domain structure fits well the *ab initio* SAXS envelope (Figure 2 in Madasu et al., 2015). The atomic model and scattering data of MBP-PICK1 were deposited with www.sasbdb.org (accession code: SASDBL2)

In contrast, Karlsen et al. used a decomposition method to render the scattering data from the aggregated PICK1 sample interpretable, and assumed that the sample consisted solely of dimers and tetramers. They applied this method to a mutant ($PICK1^{LKV}$) in which the last three amino acids of PICK1 ($^{413}CDS^{415}$) where replaced by the sequence LKV that binds in the pocket of the PDZ domain, marginally alleviating aggregation, but possibly altering the overall structure. They then focused their analysis on the dimeric portion of the decomposed data. However, even this portion of the data did not fit a single monodisperse species. Therefore, they resorted to a combination of rigid body modeling and ensemble optimization method (EOM) to conclude that the BAR and PDZ domains were well separated from each other and connected by a flexible linker, thus satisfying their rather large particle dimensions. They finally extended the EOM analysis to the tetrameric species in an attempt to provide a model of BAR-BAR interactions in the higher oligomeric states, which they consider physiologically relevant.

Several observations appear to support our “compact” model of the PICK1 structure:

1. Full-length PICK1 adopts an autoinhibited conformation, characterized by its uniform cytoplasmic localization. However, several laboratories have observed that PICK1 clusters on vesicle-like structures either when the PDZ domain is removed or when it binds to a ligand at the membrane, which appears to expose the membrane-binding surface of the BAR domain (Lu and Ziff, 2005; Madasu et al., 2015; Madsen et al., 2008; Perez et al., 2001). These results suggest that the PDZ domain participates in autoinhibitory interactions with the other domains (BAR and ACT). How could this be achieved if the PDZ domain is free and detached from the other domains?
2. The PDZ and BAR domains have been shown to directly interact with each other (Lu and Ziff, 2005).

3. It has been reported that the PDZ domain contributes along with the BAR domain to membrane binding (Jin et al., 2006; Pan et al., 2007). More generally, the contribution of so-called accessory domains to the membrane binding capacity of the BAR domain has been documented for several BAR domain proteins, a mechanism known as coincidence detection (Moravcevic et al., 2012). Importantly, in all the BAR domain proteins analyzed to date coincidence detection is achieved through tight domain-domain or protein-protein association, presumably because this allows for more efficient communication between lipid-binding folds than when these domains are disconnected. Thus, all the high-resolution structures of BAR domain proteins featuring accessory domains show these domains interacting extensively with the BAR domain (Supplementary Information Figure 1), including the BAR-PH of APPL1 (PDB code: 4H8S), the PX-BAR of SNX9 (PDB code: 2RAJ) and the F-BAR-SH3 domain of syndapin-1 (PDB code: 2X3W). A tight interaction is also observed in the structure of a complex of Arfaptin2, a BAR domain protein, with the GTPase Arl1 (PDB code 4DCN).

4. Karlsen et al.'s own observation that the PICK1^{LKV} mutant is more stable, presumably because the C-terminal residues LKV bind in the pocket of the N-terminal PDZ domain, supports our model in which these two elements are within interacting distance of each other, and opposes their model where the PDZ domain would be too far apart to interact with the C-terminus. If anything, their model predicts that this mutation should result in the formation of higher order interactions, as the PDZ domain would be more likely to interact inter-molecularly when fully exposed.

5. The linker between the PDZ and BAR domains (~129-146), which Karlsen et al. assume is unstructured, is strongly predicted to contain a large helical segment. In all the BAR-accessory domain structures listed above such inter-domain sequences fold at the interface between domains and help glue them together.

To summarize, in our study we treated aggregation as an unfortunate property observed with many BAR domain proteins, and designed a monodisperse MBP-PICK1 fusion protein that could be studied by SAXS without pre-assumptions or compromises. We find that the BAR and PDZ domains lie adjacent to each other. This result agrees with several observations from other laboratories. Our analysis does not negate, however, the existence of flexibility within the PICK1 molecule; we believe flexibility does exist, but it most likely concerns the ACT, which is heavily charged and predicted unstructured. By applying data decomposition to a polydisperse SAXS sample, Karlsen et al. have produced a model in which the PDZ domain is separated and moves freely with respect to the BAR domain. They treated aggregation as an intermediate step toward physiological BAR domain oligomerization, which normally occurs on membranes. These two models are radically different, and mutually exclusive, and should inspire scientists in the field, our laboratory included, to use alternative approaches to test their legitimacy. Lastly, we hope that as a result of this debate aggregation issues in SAXS data analyses receive the close scrutiny they deserve. The recent

availability of in-line SEC-SAXS at an increasing number of beamlines worldwide should help mitigate aggregation problems, although this is not a universal solution.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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