Structure
Letters

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Response to The Challenges of Polydisperse SAXS Data Analysis: Two Different SAXS Studies of PICK1 Produce Different Structural Models

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PICK1 is a neuronal scaffolding protein containing a protein-binding PDZ domain and a membrane-binding BAR domain that mediate homo-dimerization (Xu and Xia, 2006-2007). The structure-function relationship of the protein has been worked with for more than a decade (Ammendrup-Johnsen et al., 2012; Madsen et al., 2005, 2008, 2012), and we are very intrigued that this year has offered two solution structures of the protein (Karlsen et al., 2015; Madasu et al., 2015). Both papers use SAXS data to obtain a model of the arrangement of the individual domains within PICK1, but the models differ considerably, in particular with respect to the positioning of the PDZ domains relative to the BAR domain. Madasu et al. (2015) suggest that the PDZ domains are associated with the BAR domain and the linker between the adjacent domain forms a helix, whereas we suggest that the PDZ domain is flexibly attached through an unstructured linker (Karlsen et al., 2015). This controversy is central to the understanding of PICK1 function, because the PDZ domain has been suggested to auto-inhibit the membrane-binding capacity of the BAR domain through steric hindrance (Citri et al., 2010; Lu and Ziff, 2005; Madsen et al., 2008). In a letter in this issue of *Structure*, the Dominguez group now call into question our flexible PICK1 model and deem the two models mutually exclusive (Boczkowska et al., 2015).

PICK1 has higher oligomerization propensity than other BAR proteins we have worked with (endophilin, amphiphysin, arfaptin, SXN1). We demonstrate the cellular relevance of this oligomerization (Karlsen et al., 2015), but the oligomerization complicates structural efforts. The problem was addressed by purifying

PICK1[WT] in Triton X-100 (below the CMC). To further stabilize the protein, three C-terminal residues in PICK1 were substituted to facilitate binding of the C terminus in the PDZ domain (PICK[LKV]), which formed the basis for our modeling of the interdomain arrangement. In addition to SAXS, both PICK1[WT] and PICK1[LKV] were characterized extensively with respect to cellular localization and liposome binding/deformation, as well as to FPLC and AUC. This analysis consistently demonstrated the oligomerization propensity for PICK[WT]; this was reduced for PICK[LKV], which was mainly dimeric and tetrameric (Karlsen et al., 2015).

Madasu et al. (2015) took an alternative approach to stabilize their samples for the SAXS analysis: the WT protein was stabilized in 5% glycerol or the protein was N-terminally fused to MBP (37 kDa). For PICK1-WT, this resulted in SAXS data in a rather limited q-range and with low signal-to-noise. However, oligomerization similar to that observed in our study was reported at the highest concentration. MBP-PICK could be measured in glycerol-free buffer and at much higher concentrations without showing significant signs of oligomerization, which formed the basis for the structural analysis of Madasu et al. (2015).

None of these approaches are ideal for SAXS analysis. For PICK[LKV], the oligomerization had to be taken into account, whereas for MBP-PICK, the two MBP domains are so large, relative to the PDZ-domains, that it is difficult to identify the latter.

For PICK[LKV], we assumed for modeling purposes that only dimers and tetramers were present. The AUC data gave strong support for this approximation, although traces of larger species were visible.

Along with the absolute scaling of the SAXS data, this allowed for decomposition of the SAXS data into the contributions from dimers and tetramers using a particularly simple case of singular value decomposition that consists in solving sets of two equations with two unknowns. The robustness of this approach was clearly demonstrated by virtually identical estimates of the dimer form factor and the corresponding $p(r)$ functions obtained through the different possible combinations of data (Karlsen et al., 2015). This implicitly validated the underlying assumption of dimers and tetramers; indeed, the SAXS data from PICK1[WT] could not be similarly decomposed due to the presence of higher order oligomers. The dimer $p(r)$s from PICK1[LKV] all have a D_{max} close to 200 Å, which is clearly not compatible with a structural model in which the PDZ domains are tightly associated with the BAR-domain as proposed by Madasu et al. (2015). Our SAXS data were first analyzed with a monodisperse, rigid body approach similar to that applied by Madasu et al. (2015). The obtained fits were of comparable quality to the model fit shown by Madasu et al. (2015), but also with similar discrepancies between model and data at low and intermediate q. Furthermore, good fits could only be obtained with the PDZ detached from the BAR-domain, which made it unrealistic that these should be placed in a fixed position. We then generated an ensemble of dimers with different conformations of the PDZ domains and employed EOM to fit our decomposed data. Excellent fits could only be obtained by assuming an ensemble of conformations with the PDZ domains flexibly attached to the BAR domains. Interestingly, the EOM analysis returned conformations with a broad

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D_{max} and R_g distribution, which is generally accepted as indicative of a flexible structure. PDZ-BAR association was never observed in the returned conformations, although it was included in the input ensembles. FRET studies were conducted as an independent control and supported a large distance between the PDZ domains in living cells. All SAXS data (raw data and decomposed data) and models are available to the public for independent verification through SASBDB (SASDAB8).

In comparison, the Dominguez group used ab initio modeling of a molecular envelope through the program DAMMIF to fit their data (Madasu et al., 2015). Although it is an unbiased approach to extraction of a low-resolution protein structure from SAXS data, a routine such a DAMMIF suffers from the intrinsic limitation that it fits a single structure to the data; hence, it implicitly assumes monodispersity and structural rigidity. For Madasu et al. (2015), this was furthermore constrained by imposing P2 symmetry. An atomic model for the construct was fitted into the direct space envelope obtained through 20 DAMMIF runs and finally refined against one of the SAXS datasets. Good agreement between model and data was obtained, but with significant systematic deviations both at low q and, interestingly, also at intermediate q (around $q = 0.12$ 1/Å). These systematic deviations indicate the same type of flexibility as observed in our study, and it would have been interesting to see whether a better model fit could be obtained by assuming a flexible association of the PDZ and MBP domains. It would also have been interesting to see whether the isolated PICK1 model of Madasu et al. (2015) is compatible with their PICK1^{WT} data.

In their letter, Boczkowska et al., 2015 raise several points that they view as favoring their compact model over our flexible one. Here, we provide brief replies to these: regarding the first and third points in Boczkowska et al. (2015), it is indeed challenging to reconcile the role of the PDZ domain with respect to membrane binding capacity, which has been described to be both auto-inhibitory and facilitating in nature (Citri et al., 2010; Lu and Ziff, 2005; Madsen et al., 2008; Pan et al., 2007). Importantly, neither auto-

inhibition nor coincidence detection, to our knowledge, necessitates physical interaction. Indeed, several crystal structures show physical interaction between BAR and accessory domians; however, crystallization is completely biased toward non-flexible conditions and proteins. This makes in-solution structure determinations all the more relevant, and their unbiased interpretation crucial. We note that PICK1 has not been crystalized despite strong efforts, which consistent with our flexible model.

We speculate that functional duality of the PDZ domain may imply different functional states of the protein, perhaps reflected in the alternative conformational states proposed by the two studies (Karlsen et al., 2015; Madasu et al., 2015). How these conformations relate to the different functional states of the protein, however, requires further study.

Regarding the second point in Boczkowska et al. (2015), we note that the constructs used in Lu and Ziff (2005) (1–135 and 121–416) are highly prone to aggregation which render them inappropriate for protein-protein interaction studies, without proper negative controls.

In terms of the fourth point in Boczkowska et al. (2015), the flexible C terminus can easily reach the flexible PDZ domains in our model (Karlsen et al., 2015). In contrast, Madasu et al. (2015) model a helix (based entirely on secondary structure predictions) in the C terminus, which constricts the length/flexibility in their model. We presented NMR data of the N and C termini that showed no such helical structure (Karlsen et al., 2015). We now demonstrate by NMR that these regions are unstructured, also in the context of full-length PICK1 (Figure S1). By overlaying the full-length PICK1 spectrum with the spectra originating from the truncated constructs, we could unambiguously assign several residues showing no or little chemical shift perturbation (indicated with their assignments). This experiment likewise invalidates the H1 prediction that Madasu et al. (2015) model in the N terminus of the protein.

Finally, with respect to fifth point of Boczkowska et al. (2015), we do indeed agree that a helix can form between the PDZ and the BAR domain (118–130), and we have just submitted a manuscript

(shared with the editor for review) describing its role in membrane binding and function of PICK1. NMR, circular dicroism, and molecular dynamics, however, show that this region only adopts its helical conformation in the presence of membrane, similar to other N-BAR proteins.

Our goal throughout the analysis has been to stay true to the data and derive the simplest possible structural model that could describe the data. We have prioritized transparency in the biochemical characterization of the constructs, as well as in the data treatment and modeling, and deposited everything online (SASDAB8). We hope this will allow others to build on our research and promote future studies of PICK1.

SUPPLEMENTAL INFORMATION

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