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Gas-Phase Structure of the *E. coli* OmpA Dimer

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In this issue of *Structure*, Marcoux and colleagues use gas-phase collisional cross section (CCS) measured by ion-mobility mass spectrometry to analyze the CCS of oligomeric states of *E. coli* outer membrane OmpA. CCS of the dimer supports a model of paired periplasmic C-terminal domains projecting away from the transmembrane porins.

The development of ion mobility mass spectrometry (IMS) has enabled the measurement of gas-phase collisional cross section (CCS) (Bohrer et al., 2008; Uetrecht et al., 2010). In this case, during their flight through the mass spectrometer, ions cross a specialized cell of inert drift gas under the influence of an electric field such that their shapes modulate their velocity, achieving a separation similar to the one obtained during electrophoresis. Exit of ions from the ion mobility cell is monitored by fast m/z measurement, allowing calibration with molecules of known shape/CCS and mass. IMS can thus be used to see if gas-phase structures are in agreement with the equivalent aqueous or crystalline species.

A major application for IMS is emerging in the study of native protein complexes largely because of the ability to measure CCS accurately, alongside mass, on time-of-flight instruments with extended m/z capabilities. The extra dimension of separation achieved with an ion-mobility separation prior to MS yields significant benefits in the simplification of spectra that might otherwise be uninterpretable. Importantly, complexes have been observed collapsing to states with smaller CCS or extending to states with larger CCS while maintaining their native oligomeric status (Ruotolo et al., 2005). Robinson's group pioneered the electrospray-ionization MS of native complexes and were the first to apply this technique to integral membrane proteins a decade ago when they demonstrated that detergent molecules retained after spraying micellar solutions of EmrE could be removed by gas-phase dissociation, with the dimeric protein retaining a bound cofactor through to the mass analyzer (Ilag et al., 2004). Considerable progress toward understanding native MS of membrane protein complexes has been made in the subsequent 10 years (Whitelegge, 2013; Laganowsky et al., 2013), and it is now possible to make confident measurements of membrane protein CCS for comparison with structural models.

The porin OmpA is the most abundant *Escherichia coli* outer membrane protein, but its functional significance is controversial because it is difficult to reconcile observed conductance measurements with an eight-stranded β-barrel formed by the N-terminal porin domain alone. Recently, in vivo crosslinking of *E. coli* cells with a specialized tri-functional reagent provided sound evidence for the presence of OmpA dimers in the outer membrane (Zheng et al., 2011). The distance constraints introduced by the reagent and the identity of the crosslinking sites localize the region responsible for dimerization to the "disordered" C-terminal domain. This crosslinking information provided the opportunity to model various potential dimer structures, and, more recently, to test these models via calculation of their respective CCSs for comparison to IMS measurements.

In this issue of *Structure*, Marcoux et al. (2014) found that native IMS of OmpA preparations revealed a mixture

of monomer and dimer in the gas phase that were clearly separated by ion mobility. Protein engineering experiments showed that elimination of the C-terminal domain (residues 188–276) but not the N-terminal domain lead to the loss of dimer formation. Dimerization was never complete, suggesting a monomer/dimer equilibrium in vivo that might underlie previous experimental uncertainties. To model the dimer, they used the crystal structure of the C-terminal domain of OmpA from *Salmonella enterica*, a close homolog of the *E. coli* protein, whose calculated CCS was in good agreement with the measured CCS of the *E. coli* C-terminal domain monomer. SymmDock was used to generate an extensive library of different symmetrical dimer models of the C-terminal domain, which were narrowed down to a handful of structures based upon distance constraints from a previous crosslinking study (Zheng et al., 2011) and the dimer CCS measured in this study. A model that included K192 at the dimer interface but did not involve the last 50 residues of OmpA in dimerization was moved forward based on the native MS data as well as the crosslinking data. The full-length dimer was then modeled, based on symmetry, the length of the flexible linker, and the known structure of the N-terminal domain monomer. Preparations of full-length OmpA were analyzed by IMS and again showed mixtures of monomer and dimer. The measured CCS of dimeric species was in good agreement with the value calculated for the model, while the monomeric species displayed a range of CCSs that corresponded to collapsed species at lower charge states and extended species at higher charge states. The authors conclude that in monomeric OmpA, the C-terminal domain is unstable, while dimerization stabilizes the C-terminal domain in agreement with the model of the full-length species.

The dimeric structural model of OmpA reported by Marcoux et al. (2014) establishes a template for understanding the biological function of this protein. It supports the idea that the C-terminal domains extend away from the periplasmic surface of the outer membrane where they might interact with peptidoglycans. The confinement of the dimer interaction surface to the C-terminal domain is inconsistent with the idea that a pair of transmembrane domains refold into a 16-strand β-barrel to explain temperature-induced conductance changes. Thus, gas-phase CCS measurements are steering structural models for development of testable hypotheses for understanding the structure/function relationships of this fascinating integral membrane protein.

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