Received: 3 March 2021 | Revised: 18 April 2021 | Accepted: 3 May 2021

DOI: 10.1002/prot.26138

PERSPECTIVE

PROTEINS WILEY

AlphaFold2 predicts the inward-facing conformation of the multidrug transporter LmrP

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Funding information
National Institutes of Health, Grant/Award Number: GM077659

Abstract

As part of the CASP competition, the protein structure prediction algorithm AlphaFold2 generated multiple models of the proton/drug antiporter LmrP. Previous distance restraints from double electron-electron resonance spectroscopy, a technique which reports distance distributions between spin labels attached to proteins, suggest that one of the lower-ranked models may have captured a conformation that has so far eluded experimental structure determination.

Active transporters such as LmrP alternate between outward- and inward-facing conformations (OF and IF, respectively) during their transport cycles.[1-3] Whereas the crystal structure captures LmrP in the former,[4] AlphaFold2 modeled LmrP in the latter[5] (Figure 1A). Because LmrP is a proton/drug antiporter, we carried out DEER distance measurements[6-9] at low and neutral pH to stabilize the IF and OF conformation, respectively (shown in red and blue in Figure 1B,C). To evaluate the IF model's consistency with the low pH DEER data, we modeled the predicted distances in silico using MDDS,[10] a program hosted on the CHARMM-GUI web server.[11] Not only do the predicted distances overlap remarkably well with our experimental data (Figure 1B, C, dashed and solid lines, respectively), but importantly the magnitudes of the experimental distance changes agree with those predicted between the OF crystal structure and AlphaFold2's IF model. These results suggest that the AlphaFold2 model depicts a functionally relevant intermediate of LmrP.

The significance of this breakthrough in modeling transporter conformations is reinforced by comparison of this model to those submitted by other contestants, which overwhelmingly depicted LmrP in an occluded conformation (Figure 1D). Occluded models result from methodological biases that favor compactness.[12] Therefore, the success of AlphaFold2 in modeling IF LmrP suggests that these biases may finally have been overcome. Additionally, it sets the stage for the structural characterization of transporters and their functional intermediates by integrating computational modeling with experimental spectroscopy.

ACKNOWLEDGEMENTS

The work presented here was supported by the National Institutes of Health (GM077659).

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/prot.26138.

DATA AVAILABILITY STATEMENT

Data availability not applicable as no datasets were generated or analyzed during this study.

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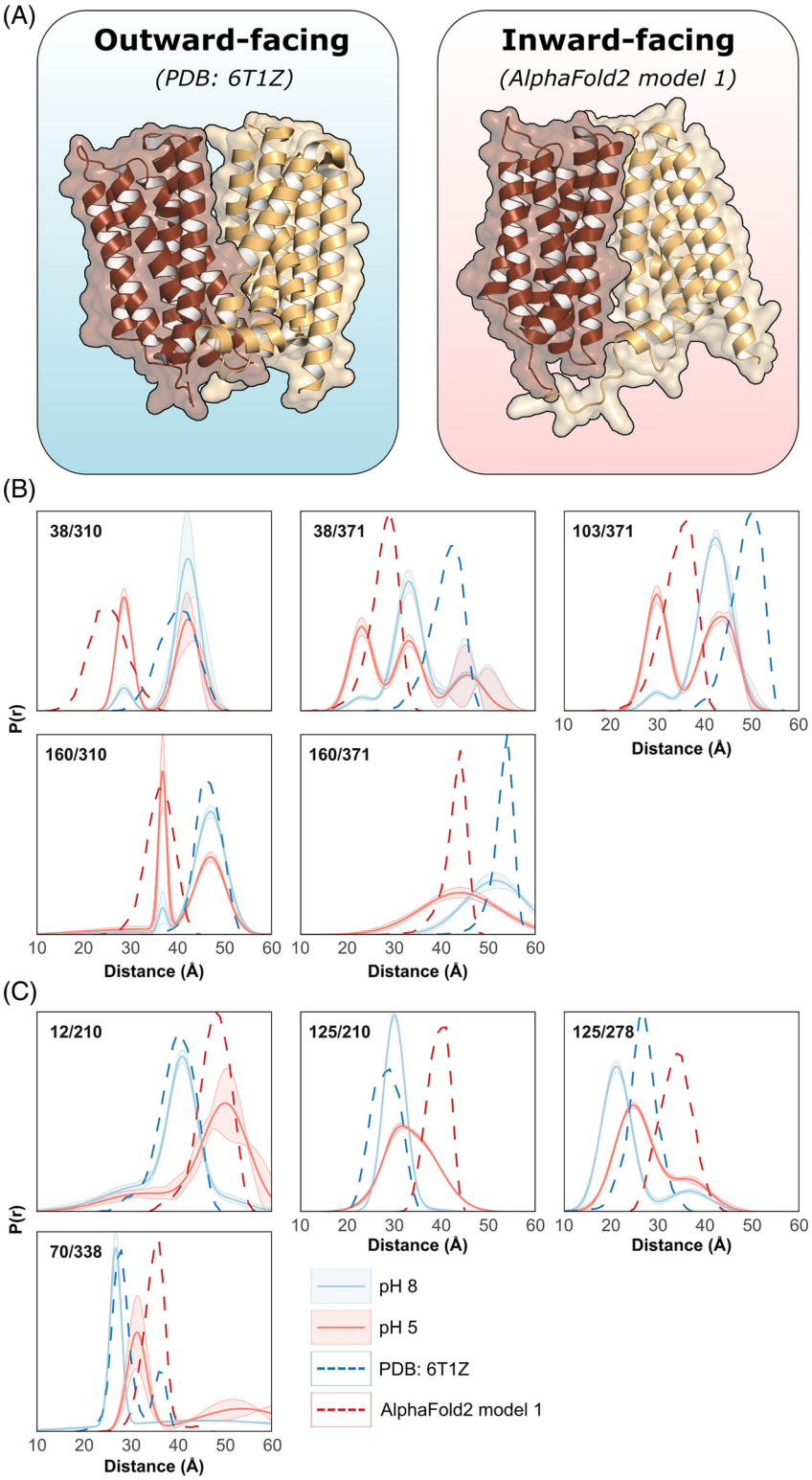

FIGURE 1 An inward-facing model of LmrP generated by AlphaFold2 is consistent with experimental data. A, Outward- and inward-facing conformations determined using X-ray crystallography and modeled by AlphaFold2, respectively. B and C, Experimental double electron-electron resonance (DEER) distance distributions on the extracellular and intracellular sides of the protein, respectively, overlap with distances predicted by AlphaFold2 model 1. Dashed lines are distance distributions predicted by either the crystal structure (blue) or the model (red). These data have been previously published. D, Average predicted DEER distance of all CASP14 LmrP models on the intracellular side (X-axis) and extracellular side (Y-axis). Solid blue and red circles represent components from the experimental DEER data corresponding to outward- and inward-facing conformations, respectively. The inward-facing AlphaFold2 model shown in panel A is located on the bottom-right

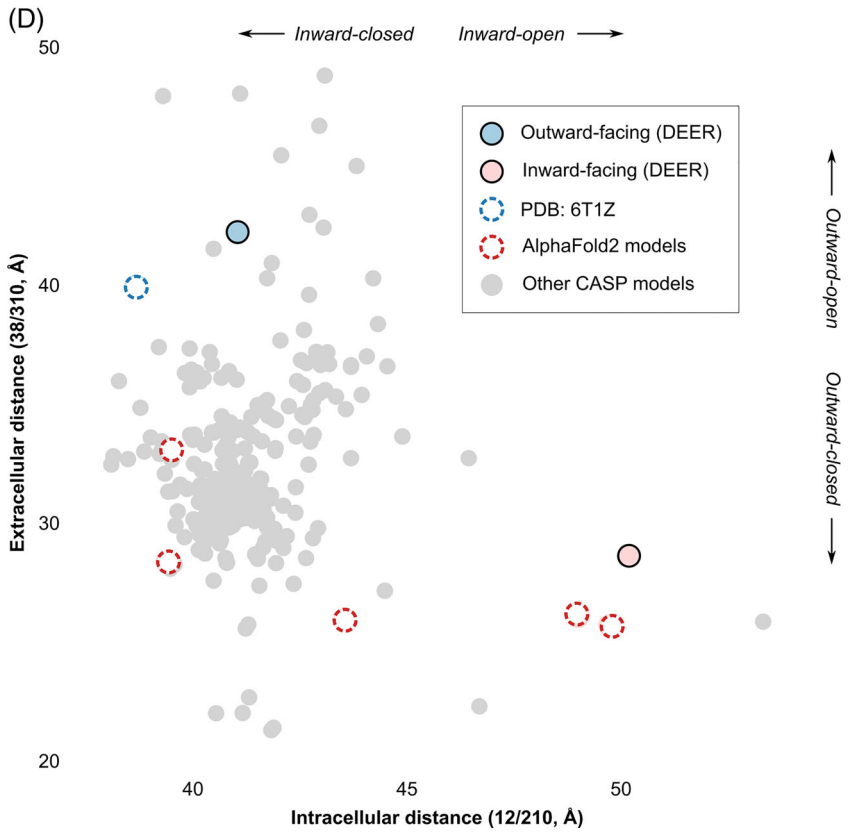

FIGURE 1 (Continued)

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How to cite this article: del Alamo D, Govaerts C, Mchaourab HS. AlphaFold2 predicts the inward-facing conformation of the multidrug transporter LmrP. *Proteins*. 2021;89(9):1226–1228. https://doi.org/10.1002/prot.26138