Response to Comment on “Crystal structures of translocator protein (TSPO) and mutant mimic of a human polymorphism”

Fei Li, Jian Liu, Yi Zheng,* R. Michael Garavito, Shelagh Ferguson-Miller†

Wang comments that the diffraction data for the structure of the A139T mutant of translocator protein TSPO from *Rhodobacter sphaeroides* should be used to 1.65 Å instead of 1.8 Å angstroms and that the density interpreted as porphyrin and monoolein is better fitted as polyethylene glycol. Although different practices of data processing exist, in this case they do not substantially influence the final map. Additional data are presented supporting the fit of a porphyrin and monoolein.

Because no additional information would be gained on the Sa atoms. *Present address: UCSD Skaggs School of Pharmacy and Pharmaceutical Sciences, La Jolla, CA 92039, USA.
†Corresponding author. E-mail: fergus20@msu.edu

Fig. 1. Structure of RsTSPO-A139T obtained in pentaerythritol ethoxylate 15/04 is very similar to that obtained in PEG 400. (A) Structure of RsTSPO-A139T crystallized in PEG 400 (cyan) and pentaerythritol ethoxylate 15/04 (light cyan) show identical features of the porphyrin-like molecules (magenta and light magenta spheres) and most monooleins (orange and yellow sticks). A PEG molecule (green) could be fitted into the 1.8 Å structure but not in the 2.4 Å structure. One asymmetric unit containing three monomers is shown. (B) Structure of pentaerythritol ethoxylate 15/04. (C) Ring-shaped density (2F(obs) − F(calc)) and the fitting of a porphyrin-like molecule in the 2.4 Å structure obtained with pentaerythritol ethoxylate 15/04.
works. Within the lipidic cubic phase, the 60\% (v/v) monoolein roughly corresponds to a concentration of 1.6 to 1.7 M, whereas the PEG 400 can only be as high as 0.35 M. Given that monooleins are critical for the stability of the LCP and the resulting membrane protein crystals, it is highly unlikely that the observed electron densities are correctly interpreted as PEG 400 molecules. Wang further contends that the ligand library density-fitting (LLDF) scores support his argument. However, the LLDF scores are known not to be suitable criteria for surface-bound ligands, such as lipids (7). A quick survey of membrane protein crystal structures determined in LCP with monoolein shows that most designated monooleins have an LLDF higher than 2, demonstrating that this criterion does not work well for lipidic molecules on the surface of the protein. (Structures surveyed include 4QND, 4N6H, 4JKV, 3VW7, 4EIY, 4E1S, 3S8F, 3V5U, 2RH1, 3ZE3, and 4RYR.) On the other hand, we analyzed the redefined structure of 4UC1 modeled with PEG provided by Wang using the PDB validation server. The result shows that most of the PEGs that Wang fitted into the transmembrane region in the place of monoolein have a LLDF score higher than 2 (as high as 206), which undermines his own argument.

However, we agree that raw data instead of merged data deposited in PDB provide more accurate information on data quality. The chosen resolution cut-off of the data at 1.8 Å was supported by the statistics of the raw data. As shown in Table 1, data at 1.65 Å have very high R factors and very low I/σ and CC(1/2)—all indications of high noise—and therefore were excluded in the refinement. Wang has recommended (8) that data should be cut at I/σ around 1, which is ~1.8 Å in this data set, the same as the resolution we reported (2). The decision to limit our data set to 1.8 Å was also supported by the merging statistics for the raw data, which clearly revealed significant anisotropy in the highest shells (1.78 to 1.69 Å). This is quite evident in the analysis of the data by merging and scaling with the program Aimless (9); data within the resolution shell of 1.69 to 1.79 Å, the CC(1/2) for two of the principal directions drops precipitously to under 0.3, and the mean I/σ values have dropped well below 1. In addition, Xtriage from Phenix (10) shows that the data in the highest shells are very weak; less than 23\% of the reflections have an I/σ > 2, and less than 2.6\% have an I/σ > 3. Given that the data in the highest shells display a clear anisotropic distribution, there could be a significant anisotropic contribution to any added “detail” in the resulting electron density maps. This could potentially lead to misleading refinement results and interpretation. Thus, limiting our working data to 1.8 Å is a best-case compromise that adds as much useful data as possible at high resolution but excludes the weak anisotropic data.

In conclusion, we believe that the new structure (5DUO) in pentaerythritol ethoxylate, along with chemical considerations and the statistics of the data, confirm our original conclusion that the best interpretation of the ring-shaped density is a porphyrin-type molecule and that the densities associated with the hydrophobic region of the protein are monooleins.

**REFERENCES**

7. Protein Data Bank, User guide to the wwPDB X-ray validation reports; www.wwpdb.org/validation/ValidationPDFNotes.html.
13. April 2015; accepted 29 September 2015. 10.1126/science.aab2595
Response to Comment on "Crystal structures of translocator protein (TSPO) and mutant mimic of a human polymorphism"

Fei Li, Jian Liu, Yi Zheng, R. Michael Garavito and Shelagh Ferguson-Miller

*Science* 350 (6260), 519.
DOI: 10.1126/science.aab2595