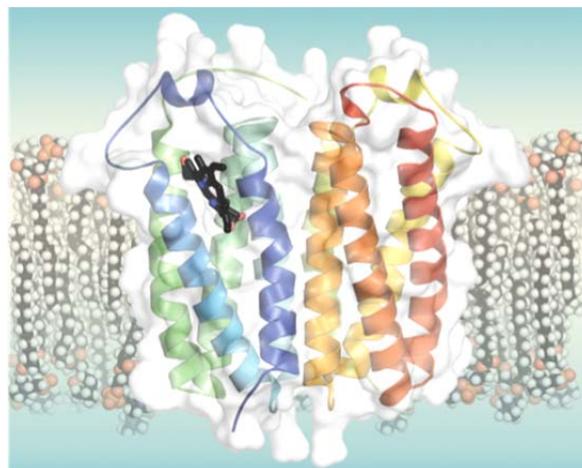


## Translocator Protein Structure and Function

Translocator protein (TSPO) is an ancient conserved protein whose functions in bacteria and higher eukaryotes are yet to be clearly defined in spite of more than 30 years of study. In mitochondria, it was first recognized as an outer membrane protein that binds benzodiazepine drugs, but distinct from the central nervous system site, the GABA<sub>A</sub> receptor (1). Originally called the *peripheral benzodiazepine receptor* (PBR), it was renamed the translocator protein or TSPO, in recognition of its far-reaching phylogeny and mounting evidence of involvement in a number of complex cellular processes, including cholesterol transport, porphyrin transport, inflammation, tumor progression, and Parkinson's and Alzheimer's disease (1, 2).

The variety of conditions in which TSPO appears to be involved is remarkable, likely accounting for the difficulty in defining its precise function. Of particular recent interest is the identification of a human single polymorphism in TSPO associated with anxiety-related diseases, making it a potential target for anxiolytic drugs. There is also widespread use of TSPO ligands for imaging of brain damage, due to the consistently high expression of TSPO in regions of neuroinflammation (3). Independent of its recognition in animal systems, TSPO was discovered in photosynthetic bacteria as a regulator of the transition between respiration and photosynthesis (4), an activity that appears related to its ability to bind and translocate porphyrins. Importantly, the bacterial homolog in *Rhodobacter sphaeroides* can be functionally replaced by rat TSPO, indicating a highly conserved function for TSPO beyond its ability to bind drug ligands.

To gain insights into the TSPO function, the Ferguson-Miller lab determined the high-resolution crystal structures of the *Rhodobacter* homolog of mitochondrial translocator protein 18 kDa (TSPO), as well as a mutant form mimicking a human disease-related polymorphism, using lipidic cubic phase technology. This was a very demanding endeavor because of the small fragile crystals, requiring access and expert support at the best synchrotron facilities in the country, including time on Beam Line 12-2 at SSRL. The resulting crystal structures of *Rs*TSPO at 1.8 to 2.5 Å resolutions show a 5 transmembrane helical bundle, unlike most outer membrane proteins which are beta-barrels (Fig. 1). The structures also show significant differences between the wild-type and the mutant mimic of the human polymorphism. The mutant reveals a more closed conformation and reduced affinity for cholesterol and porphyrin, consistent with the human phenotype. A closely-apposed dimer (Fig.1) was found in three different crystal forms, implying functional significance, but the tight dimer interface does not suggest a role in transport, as often proposed. An endogenous ligand, porphyrin, was also resolved in the x-ray structures, but no drug or cholesterol molecules were observed, leaving many unanswered questions regarding the function of this ancient multifaceted protein.



**Figure 1.** Crystal structure of *Rs*TSPO (4UC1) with an endogenous ligand, a porphyrin (black), in the central cavity of one monomer of the dimeric structure, represented in a simulated membrane.

### Primary Citation

F. Li, J. Liu, Y. Zheng, R. M. Garavito and S. Ferguson-Miller, "Crystal Structures of Translocator Protein (TSPO) and Mutant Mimic of a Human Polymorphism", *Science* **347**, 555 (2015), DOI: 10.1126/science.1260590.

### References

1. J. Fan, P. Lindemann, M. G. Feuilloy and V. Papadopoulos, "Structural and Functional Evolution of the Translocator Protein (18 kDa)", *Curr. Mol. Med.* **12**, 369 (2012)
2. J. Gatliff and M. Campanella, "The 18 kDa Translocator Protein (TSPO): A New Perspective in Mitochondrial Biology", *Curr. Mol. Med.* **12**, 356 (2012).
3. V. Papadopoulos and L. Lecanu, "Translocator Protein (18 kDa) TSPO: An Emerging Therapeutic Target in Neurotrauma", *Exp. Neurol.* **219**, 53 (2009)
4. A. A. Yeliseev and S. Kaplan, "A Sensory Transducer Homologous to the Mammalian Peripheral-type Benzodiazepine Receptor Regulates Photosynthetic Membrane Complex formation in *Rhodobacter sphaeroides* 2.4.1", *J. Biol. Chem.* **270**, 21167 (1995)

### Contact

*Shelagh Ferguson-Miller, Michigan State University*

SSRL is primarily supported by the DOE Offices of Basic Energy Sciences and Biological and Environmental Research, with additional support from the National Institutes of Health, National Institute of General Medical Sciences.