A Hybrid Approach to Map the Misfolded State of a Pathogenic Rhodopsin Variant

Victoria Most,[1]* Jacklyn M. Gallagher,[2]* Hope Woods,[3,4] Andrew G. McKee,[2] Wesley D. Penn,[2] Charles P. Kuntz,[2] Jonathan P. Schlebach,[2] and Jens Meiler[1,3]

[1]*Institute for Drug Development, Leipzig University, Leipzig, SAG, Germany*
[2]*Department of Chemistry, Indiana University, Bloomington, IN, USA*
[3]*Department of Chemistry, Vanderbilt University, Nashville, TN, USA*
[4]*Chemical and Physical Biology Program, Vanderbilt University, Nashville, TN, USA*

*Authors contributed equally

Mutations that disrupt the folding of integral membrane protein folding are associated with numerous genetic diseases. Despite significant advances in the experimental characterization of native membrane protein structures, the structural properties of the misfolded conformations that promote their premature cellular degradation remain poorly understood. We previously demonstrated that the cotranslational misfolding of rhodopsin is stimulated by polar mutations within TMD7 that stabilize non-native topology such as the retinitis pigmentosa mutation S297R. To identify non-native tertiary contacts that stabilize this topology, we utilized Rosetta modeling to generate an ensemble of low energy non-native structures that are consistent with the topological effects of the S297R mutation. We identified putative non-native conformations in which the mutated side chain forms tertiary contacts with TMD2. *In silico* mutational scanning suggests the native conformation can be re-stabilized by a variety of secondary mutations within TMD2. To test this prediction, we utilized deep mutational scanning to assess the effects of 501 secondary mutations on the plasma membrane expression of S297R rhodopsin in HEK293T cells. Our experimental results confirm that numerous mutations within TMD2 restore folding to the S297R variant and suggest that distinct tertiary contacts are formed in the context of the misfolded state. We are currently developing new approaches to search the non-native ensembles generated by Rosetta to identify misfolded structures that are most consistent with our experimental observations. Together, our results provide novel insights into the structural basis of membrane protein misfolding in the cell.