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Bound Structures of Peripheral Membrane Binding Proteins TIM3 and TIM1 from Molecular Dynamics Informed Analysis of X-ray Reflectivity

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Abstract:

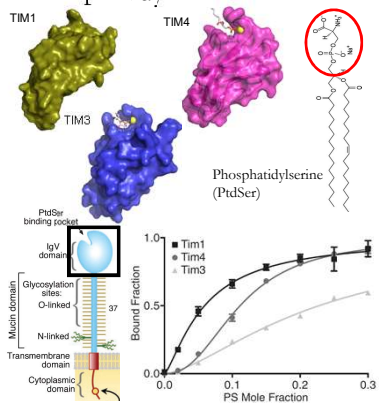
Lipid binding and associating proteins are necessary components of cell signaling pathways historically overlooked for more amenably characterized protein-protein interactions. As peripheral membrane binding proteins attract more attention, reliable structural methods are needed to elucidate the protein-lipid interactions that facilitate their function. Traditional methods such as crystallography or NMR have produced structures of many peripheral membrane binding proteins in isolation, bound to a single lipid, or in a lipid cubic phase but not in complex with full lipid membranes. X-ray reflectivity provides structural characterization of lipid monolayer associated proteins assuming a known structure of the desired protein has already been obtained. Depending on the experimental conditions of the given structure, it is possible this structure is representative of the membrane associated structure. In our studies of three members of the T-cell Immunoglobulin Mucin (TIM) family of proteins, involved in the recognition of the apoptotic cellular signal phosphatidylserine (PtdSer) in lipid membranes, the crystal structure was only representative for TIM4 and not TIM1 or TIM3. TIM1 was crystallized without PtdSer in a closed conformation that cannot represent the PtdSer bound state and TIM3 has much lower affinity resulting in a weak x-ray reflectivity signal. We developed data analysis methods employing molecular dynamics to refine the structures of TIM1 and TIM3 to better represent their membrane bound conformations. The newly obtained structures provide much improved fits of the data and highlight protein-lipid interactions that can explain the differences in binding affinity between the TIM protein family members.

TIM Proteins Bind Phosphatidylserine

The T-cell Immunoglobulin Mucin (TIM) family of proteins interact with lipid membranes through recognition of Phosphatidylserine (PtdSer), a negatively charged lipid that serves as a common cellular signal for apoptosis and several other processes, on the outer leaflet of the cell membrane.

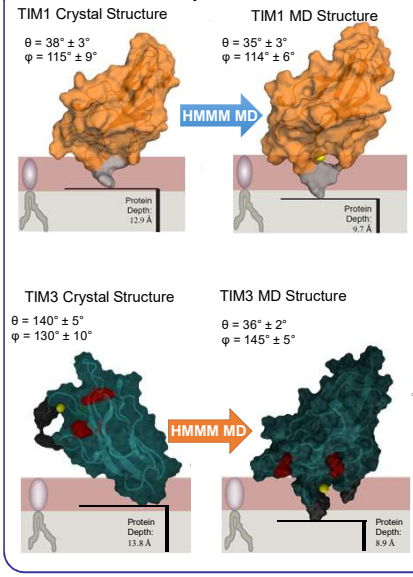
TIM1 anchors motile cells to tissues, regulates function of other immune cells, and is involved in synaptic pruning of neuronal cells.
TIM3 is expressed on T-helper cells and regulates functions of other immune cells, resulting in suppression.
TIM4 is expressed on macrophages and recognizes apoptotic cells for removal.

Each TIM has a conserved, calcium coordinated PtdSer binding pocket. Binding experiments with the immunoglobulin (IgV) domain of the TIMs reveal varying sensitivities to PtdSer containing membranes not explained by their highly similar crystal structures.



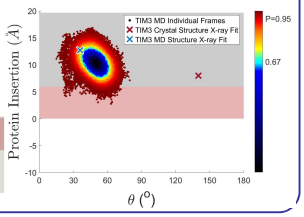
Freeman, G., et al. *Immunol. Rev.* **235**, 172–189 (2010).
Tietjen, G. *et al. Proc. Natl. Acad. Sci. U.S.A.* **111**, E1463–E1472 (2014).

Molecular Dynamics Structures Improve X-ray Fitting

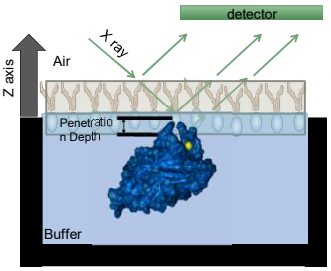


TIM1 was not co-crystalized with PtdSer, and the crystal structure is in a closed pocket conformation. HMMM MD yielded an open pocket and membrane bound conformation which better fit the reflectivity data.

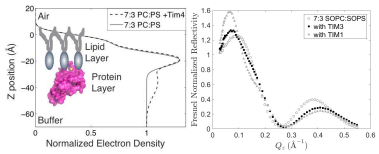
TIM3 was co-crystalized with PtdSer, but not all residues were resolved. Combined with TIM3's low surface coverage of 18%, as compared with TIM1's 50%, the x-ray fit of the crystal structure resulted in an upside down orientation. The resolved HMMM MD structure fit the same data in an upright orientation and was corroborated by MD.



Membrane Bound Protein Structure from X-Ray Reflectivity



- Lipid monolayer is deposited on an air-water interface.
- Protein is injected into the buffer and equilibrated.
- X-rays probe the system at various small angles and are reflected by the change in electron density of the system's interfacial structure.
- The presence of protein at the membrane is detectable in the reflectivity curve.

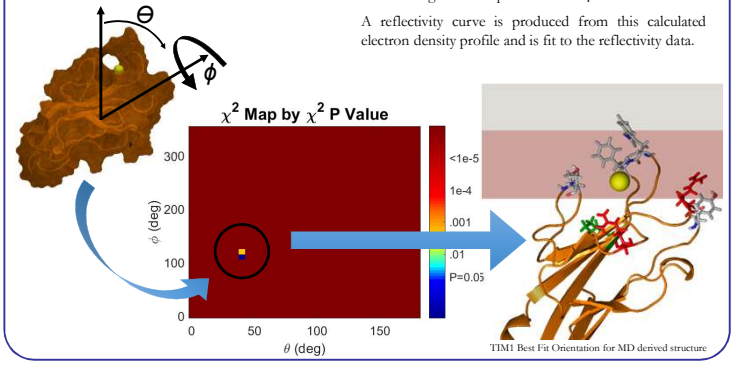


The reflectivity cannot be directly back-solved to yield an electron density profile. The membrane is modeled as two slabs of constant electron density, corresponding to the lipid tails and heads, smeared by a thermodynamic roughness parameter of the film.

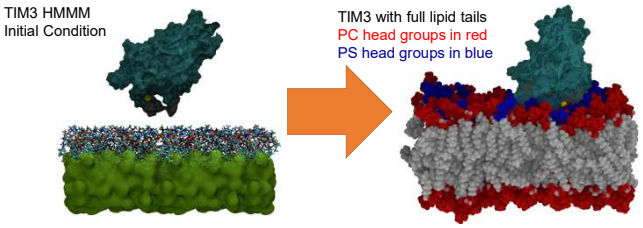
The protein's electron density is calculated from its crystal structure and superimposed on the lipid model. Four parameters determine the contribution of the protein to the electron density:

- Penetration depth
- Coverage: The amount of protein bound to the membrane
- Euler angles of the protein θ and ϕ

A reflectivity curve is produced from this calculated electron density profile and is fit to the reflectivity data.

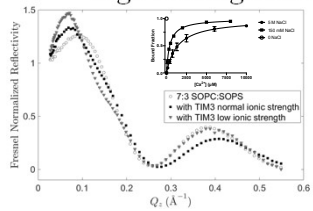


HMMM Molecular Dynamics

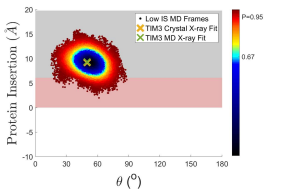


Highly mobile mimetic membrane (HMMM) MD, replaces the lipid tails with a hydrophobic solvent. Lipid dynamics are sped up relative to the protein, raising the likelihood of binding occurring in reasonable simulation time. After the protein binds in this HMMM phase of the simulation, the lipid tails are substituted back in place.

High Coverage Resolves TIM3 Orientation

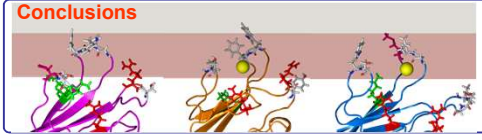


We hypothesized that TIM3's low binding signal resulted in the upside down fit of the crystal structure. To test this claim, we ran x-ray reflectivity experiments under high surface coverage conditions (30-50%), by lowering the NaCl in the buffer, reducing the ionic strength and thereby raising TIM3's affinity.



MD simulations of TIM3 under the same low ionic strength conditions verified that the bound orientation is unchanged from normal ionic strength conditions. The crystal and MD resolved TIM3 structures fit the low ionic strength x-ray data at the same orientation and agree with the normal ionic strength x-ray fit.

Conclusions



X-ray reflectivity analyzed with MD resolved protein structures is a powerful method for characterizing membrane bound orientations of peripheral membrane binding proteins. MD simulations serve to equilibrate the protein structure in a membrane bound context yielding a more representative structure of the protein's actual conformation in x-ray experiments. With the full TIM protein family analyzed, we can implicate the contribution of various residues to their different binding behavior. TIM3 has the weakest affinity for PS in membranes and also contains the least hydrophobic residues as well as requiring the most insertion for its hydrophobic residues to be as inserted as TIM4's and TIM1's hydrophobic residues. TIM4 has several positively charged residues at the interface which can interact with peripheral PS, explaining TIM4's relatively stronger dependence on PS surface density compared with TIM1 and TIM3.

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