Invited Lecture

Helicobacter pylori drug discovery using cryo EM:

Targeting the evil duo of a pH-gated urea channel and a cytoplasmic urease

Hartmut Luecke

Nova University, Lisbon, Portugal

Helicobacter pylori's proton-gated plasma membrane urea channel and cytoplasmic urease are essential for the survival of this carcinogen the human stomach. The channel is closed at neutral pH and opens at acidic pH to allow the rapid access of urea to cytoplasmic urease. Urease hydrolyzes urea into 2 NH_3 and CO_2, neutralizing protons and thus buffering its cytoplasm even in gastric juice when the pH is below 2.0. We determined the crystal structure of the channel, revealing six protomers assembled in a hexameric ring surrounding a central bilayer plug of ordered lipids. Each protomer encloses a channel formed by a twisted bundle of six transmembrane helices. The bundle defines a previously unobserved fold comprising a two-helix hairpin motif repeated three times around the central axis of the channel, without the inverted repeat of mammalian-type urea transporters. Both the channel and the protomer interface contain residues conserved in the AmiS/UreI superfamily, suggesting the preservation of channel architecture and oligomeric state in this superfamily. Predominantly aromatic or aliphatic side chains line the entire channel and define two consecutive constriction sites in the middle of the channel. Mutation of Trp153 in the cytoplasmic constriction site to Ala or Phe decreases the selectivity for urea in comparison with thiourea, suggesting that solute interaction with Trp153 contributes specificity. The structure suggests a novel mechanism for the permeation of urea and other small amide solutes in prokaryotes and archaea. Follow-up microsecond-scale unrestrained molecular dynamics studies provide a detailed mechanism of urea and water transport by the channel. More recently, we have determined the structure of the 1.1 MDa urease with various bound inhibitors to resolutions up to 1.5 Å using cryo electron microscopy. Structure-based inhibitor discovery against both targets is in progress.

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[2]Luecke, H. & Sachs, G. "*Helicobacter pylori's* Achilles' Heel" (2013) *Immuno-Gastroenterology* **2**, 76.

[3]McNulty, R., Ulmschneider, J.P., Luecke, H., Ulmschneider, M.B. "Mechanisms of molecular transport through the urea channel of Helicobacter pylori" (2013) *Nature Communications* **4**, 2900.

[4]Cunha, E.S., Chen, X., Sanz Gaitero, M., Mills, D., Luecke, H. "Cryo EM Structure of *Helicobacter pylori* inhibitor-bound urease at 2.0 Å resolution." (2021) Nature Communications **12**, 230. https://rdcu.be/cdnuc