Structural Changes in the Bacterial Toxin Pneumolysin During Pore Formation

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The bacterial toxin pneumolysin is released as a soluble monomer that kills target cells by assembling into large oligomeric rings that form pores in cholesterol-containing membranes. Using cryo-EM and image processing we have determined the structures of both the prepore and membrane-inserted pore oligomer forms, providing a direct observation of the conformational transition into the pore form of a cholesterol-dependent cytolysin.

To form the pore structure the pneumolysin domains reorganize and double over into an arch, forming a wall that seals the bilayer around the pore. This transformation is accomplished by membrane deformation and the substantial refolding of two of the four protein domains. The pore structure supports the hypothesis that two regions of α-helices refold into β-hairpins that insert into the membrane to form the pore [1, 2]. These hairpins form the largest β-barrels observed; our largest reconstruction of the pore contains 44 subunits forming a 176 strand β-barrel around a 260 Å diameter channel.

[1] Shepard L.A., Heuck A.P., Hamman B.D., Rossjohn J., Parker M.W., Ryan K.R., Johnson, A.E., Tweten R.K., *Biochemistry*, 1998, **37**, 14563. [2] Shatursky O., Heuck A.P., Shepard L.A., Rossjohn J., Parker M.W., Johnson A.E., Tweten R.K., *Cell*, 1999, **99**, 293.

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