Membrane activity of Adenylate Cyclase Toxin involves formation of tunable size pores with toroidal features

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Adenylate Cyclase Toxin (ACT or CyaA) secreted by the whooping cough bacterium Bordetella pertussis, belongs to the RTX (Repeats in ToXin) family of exoproteins with pore-forming activity. Pore formation by RTX-toxins leads to cell death by dissipation of ionic gradients and membrane potential across the cytoplasmic membrane of target cells. Beyond this, very little is known on the molecular structure or even the nature of the membrane-integrated pores these toxins form. Here we provide the first visualization of ACT molecules in membrane pore structures. We reveal that ACT clusters into heterogeneous oligomeric assemblies presenting a broad distribution of different architectures, including monomers, lines, arcs, and full rings that perforate the membranes. Remarkably we find that ACT pore size is not constant as typically observed in purely proteinaceous channels, but evolves with time, and depends on protein concentration, suggesting a toroidal mechanism. Our data support a new model for the nature of ACT-mediated lesions, in which the toxin delineates pores of different sizes to permeabilize cell membranes. A pore tunable in size adds a new regulatory element in the ACT-mediated cytotoxicity, which may lead to different pore sizes in different physiological scenarios or different cell types.