An Engineered Protein Nanopore using the V-ATPase Rotary Motor

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In this talk, I will discuss the functional reconstitution of the proteolipid ring (*c*-ring) complex of the membrane-bound V_o sector of the H^+-ATPase (V-ATPase) from the yeast *Saccharomyces cerevisiae* using techniques of cryo-electron microscopy, 2D crystallization, and single-molecule electrophysiology. Our work was motivated by, and builds on, a large body of literature spanning more than three decades that suggested that V_o, besides its proton pumping function in holo V-ATPase, has other, so-called "non-canonical" functions in essential processes, such as membrane fusion and neurotransmitter release. Here, I will show that the *c*-ring forms a large-conductance transmembrane pore, which inserted into planar lipid bilayers with a preferred orientation. Moreover, using single-particle cryo-electron microscopy, we demonstrate that the purified *c*-ring forms dimers through an interaction mediated by the cytoplasmic loops of the *c* subunits. Taken together, these findings provide the first direct attestation in support of the proposed non-canonical functions of the V-ATPase V_o sector.[1]

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1. Couoh-Cardel, S.; Hsueh, Y. C.; Wilkens, S.; Movileanu, L., Yeast V-ATPase Proteolipid Ring Acts as a Large-conductance Transmembrane Protein Pore. ***Scientific reports*** **2016, *6*, 24774.**