Structure of the complete human PINK1-blocked TOM Complex

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Mitochondrial DNA codes for only 13 of the ~1200 proteins required for healthy mitochondrial function. The remainder of these proteins are imported from the cytosol, through the translocase of the outer mitochondrial membrane (TOM) complex. Acting as a mitochondrial health marker, PINK1 is constitutively expressed in the cytosol, imported through the TOM complex, cleaved by PARL at the inner mitochondrial membrane and then released back into the cytosol to be degraded. When the mitochondrial import gradient fails, PINK1 accumulates at the TOM complex, auto-phosphorylates and then activates Parkin, an E3 ligase that ubiquitinates a variety of proteins as well as the mitochondria itself, promoting mitophagy of the damaged mitochondrion to prevent further damage from mitochondrial leakage. PINK1/Parkin mutations are linked to familial Parkinson's disease and other mutations in this pathway are linked to a variety of neurodegenerative diseases.

Here we reveal a cryoEM structure of the entire PINK1-blocked TOM complex in an unexpected array with VDAC, pulled directly from human cells. This structure (1) resolves human PINK1 for the first time, illustrating the importance of a multitude of cysteine residues; (2) demonstrates an unexpected role for VDAC in seeding this complex; (3) demonstrates clearly the structure and role of TOM20 in guiding proteins to TOM40 and capturing PINK1 specifically; (4) reveals the first high resolution capture of a human TOM40 substrate within the TOM lumen and (5) reveals unexpected roles for small TOMs in the stabilisation of this complex. We also reveal the sites of several disease-linked mutations, improve upon the mechanism of PINK1 activation and reveal tantalising clues as to the structure of the human TOM/TIM supercomplex.

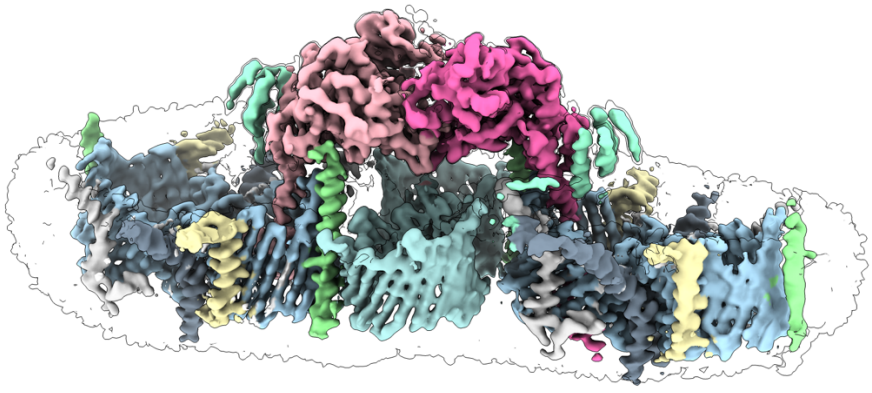

Figure 1. CryoEM density of the PINK1-TOM-VDAC array