## MS04-01 | Mechanisms Of C-Ring Protonation And Flexible F1-Fo Coupling In A Mitochondrial ATP Synthase

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$F_1F_o$-ATP synthases play a central role in cellular metabolism across all domains of life. Although much is known about the structure and mechanism of these complexes, open questions remain. The stoichiometric mismatch between processes at $F_1$ (3-fold symmetry) and $F_o$ (8- to 17-fold symmetry) is a challenge to efficient energy conversion within the complex. Flexible coupling between the two nanomotors is proposed to mitigate this challenge, but the structural basis is not established. We have determined the single-particle cryo-EM structure of active dimeric ATP synthase from mitochondria of Polytomella sp. at 2.7- 2.8 Å resolution, and have used 3D classification to separate 13 well-defined rotary sub states, providing a detailed picture of the molecular motions that accompany c-ring rotation. We show that the $F_1$ head rotates along with the central stalk and c-ring rotor for the first ~30° of each 120° primary rotary step, which would result in flexible coupling of $F_1$ and $F_o$. Rotation of $F_1$ is mediated primarily by the interdomain hinge of the conserved OSCP subunit, a well-established target of physiologically important inhibitors. The membrane-bound $F_o$ subunit, where proton translocation drives c-ring rotation, is resolved at 2.7Å, allowing us to model ordered water molecules in the aqueous half-channels and at the conserved gating Arg-239. A histidine residue essential to proton translocation binds a metal ion in the proton access channel, and the coordination of the metal changes with c-ring position, suggesting that this may play a role in synchronising c-ring protonation with rotation.