Oral presentation

The elusive "*slack* state" of mitochondrial Complex I is stabilized by an assembly factor

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Respiratory Complex I (NADH: ubiquinone oxidoreductase) stands as the largest multi-subunit mitochondrial enzyme, pivotal for energy transduction within cells, essential for oxidative phosphorylation, and crucial in regulating NAD+/NADH pools as entry point of the electron respiratory chain. Recent advancements in electron cryogenic microscopy (cryo-EM) have unravelled the conserved architecture of Complex I. Moreover, due to its ability to separate molecules in a mixed population into distinct classes, cryo-EM single-particle analysis (SPA) has enabled the identification and characterisation of various physiologically relevant conformations [1-2]. In the Complex I context, due to variations between species, preparations, and cryo-EM SPA strategies, different numbers of states have been observed. However, unravelling their catalytic and regulatory properties to underpin mechanistic hypotheses about Complex I redox catalysis has posed a challenge. Notably, within this structurally diverse pool, an enigmatic conformation termed the "slack state" has emerged for the bovine Complex I enzyme, marked by disorder in specific membrane domain elements [3]. The functional significance of this "slack state" remains elusive, posing a compelling question in mitochondrial biology. Further exploration of this state within a defined biochemical state and a homogeneous population is necessary to establish the relevance of this intriguing enzyme state. In this study, we have reconstituted the bovine Complex I in LMNG detergent, bound to a known Complex I assembly factor [4] (acyl-CoA dehydrogenase family member 9, ACAD9), and elucidated a novel structure of Complex I at 2.9 Å resolution, in a uniformly de-active conformation. Comparative analysis with previously reported cryo-EM structures has unveiled structural similarities with the elusive "slack state" albeit in a more extended conformation, hence termed by us as "slack β-state". We propose that ACAD9 stabilises this distinctive conformation, likely modulating Complex I functions.

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*[2] Kampjut, D., & Sazanov L., A. (2020) Science, **370**: eabc4209.*

*[3] Chung, I., Wright, J.J., Bridges, H.R., et al. (2022). Nature Communications, **13**: 2758.*

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