Scientists solve structure of important protein in energy storage of cells

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(Phys.org) -- Scientists at Trinity College Dublin, using a highly specialised crystallography technique have solved a large protein structure that will increase our understanding of energy generation and storage in cells. The findings have been recently published in the online publication of *Nature*.

ATP, the energy currency of the cell, is used to keep the cell alive, growing and replicating. The TCD scientists determined the crystal structure which explains how a particular complex protein machine works at the molecular level in this process.

They created a home-made liquid crystal membrane mimic to crystallise the protein for structure determination by macromolecular X-ray crystallography. The structure reveals the location in 3-dimensional space of all the protein's major constituent atoms. It is the final structure adopted by the protein that determines how the nanometre-sized protein machine works to pull the two oxygen atoms apart in an O2 molecule and to combine each with electrons and protons in a highly regulated process that produces harmless water and at the same time stored energy in the form of a chemical and an electrical (proton) gradient across the membrane that can do work for the cell.

The protein used in this study comes from a bacteria, Thermus thermophilus, that grows optimally at 65 °C and plays a role in composting. It was discovered in a hot spring in Japan. The work shows that the enzyme from Thermus has an extra domain suitably positioned

on the enzyme for fast, efficient and directed tunnelling and channelling of electrons to the active site in the protein's core where all of the intricate oxygen chemistry takes place. The structure reveals a signature chemical motif close to the active site that is poised for proton transfer leading to oxygen reduction and to proton pumping. The work provides insights into how protons and electrons are shuttled in a directed way within proteins. It identifies a novel sugar-coated lipid or fat molecule. It also further demonstrates the general applicability of lipid membrane mimics for the crystallographic structure determination of membrane proteins.

Explaining the structure-function deciphering process, Professor of Membrane Structural and Functional Biology, Martin Caffrey says: "A crystal structure is a static view or a snapshot of the protein, usually in a single state. But the protein is a machine that has many moving parts and myriad states that it cycles through 'robotically' to perform its complex set of functions, over and over again. To capture the entire movie of states that it cycles through from a single crystallographic snapshot requires sophisticated computational work referred to as molecular dynamics simulations (MDS)."

" Dr. Andrei Pisliakov, a collaborator at the RIKEN Advanced Science Institute in Japan, provided MDS support to the project and, in effect, 'breathed life' into the frozen crystal structure. This showed how protons enter the enzyme from the watery interior of the cell and track either into the active site to do oxygen chemistry or fully across the membrane to create the proton gradient. It also illustrated how water formed in the active site exits the protein."

The work was initiated in 2004 when Dr Tewfic Soulimane joined Professor Caffrey's research group, then at the University of Limerick. Orla Slattery, a postgraduate student in Professor Caffrey's laboratory, succeeded in crystallising the purified enzyme provided by Dr

Soulimane. Subsequenctly, another postgraduate student in the Caffrey lab, Joseph Lyons, refined the original crystallisation conditions and together with Dr. David Aragão, a postdoctoral fellow in the group, did the challenging crystallographic work that required numerous trips to synchrotron X-ray sources worldwide in order to solve the structure.

“While the work explains a lot about how these complex protein machines work to harness energy from food in cells, much remains to be understood. The structure provided in this study therefore can be viewed as the embodiment of a set of hypotheses for how the protein does what it does. These hypotheses must now be tested by repeated rounds of experimental laboratory andcomputer work. Some require additional crystal structure determination and all are directed toward understanding how these intricate protein machines, with diverse oxygen affinities, have evolved,” concluded Professor Caffrey

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Provided by Trinity College Dublin