Séminaire

CONFÉRENCIER INVITÉ

Vendredi 22 Mars 2019 à 11h

Salle des séminaires

Institut de biologie structurale - 71 avenue des Martyrs CS 10090 38044 Grenoble Cedex 9 - T.+33 (0)4 57 42 85 00

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The cytosolic NADPH oxidase complex: from stoichiometry to topology in living cells

Protein complexes composed of proteins containing well-structured domains connected by intrinsically disordered protein segments are difficult to investigate. We developed an analytical strategy based on the use of the best fluorescent proteins combined with FRET–fluorescence lifetime imaging (FRET-FLIM) and fluorescence cross-correlation spectroscopy (FCCS) to characterize protein-protein interactions in such protein complexes in living cells.

The strategy was applied to the phagocyte NADPH oxidase, an enzyme that produces superoxide anions, a precursor of reactive oxygen species (ROS) critical for host responses to microbial infections. It consists of two membranous (Nox2 and p22) and three cytosolic subunits (p40, p47, and p67) that undergo structural changes during enzyme activation.

I will show how we characterized the inter- and intramolecular interactions of the cytosolic subunits and elucidated their conformation, stoichiometry, interacting fraction, and affinities in live cells. Furthermore, combining FRET data with small-angle X-ray scattering (SAXS) models and published crystal structures of isolated domains and subunits, we built a 3D model of the entire cytosolic complex.

Hôte : Franck Fieschi (IBS/groupe Membrane & Pathogens)