Title	Click chemistry for the synthesis of phospholipids to study lipid–protein interactions with EPR and cryo-EM approaches

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# months (min.3)	8

Project description:
Lipids remain one of the most enigmatic classes of biological molecules. While lipids are well known as the basic units of membrane structure and energy storage, they can also act as chemical messengers performing allosteric functions and signaling, and as structural elements for protein stability and folding. Deciphering the exact roles and biological interactions of distinct lipid species has proven elusive. One reason why lipids have been difficult to study is the relative lack of techniques to both interrogate their dynamics and visualize them at structural level. Over the last few decades, lipid-based probes have become more prevalent as powerful tools in chemical and synthetic biology and novel chemical techniques have been described for studying lipids both *in vitro* and *in vivo*.

Applications of the lipidomics include, for instance, understanding the fundamental cell biology of lipid biosynthesis, trafficking and signaling, but also developing cancer drug delivery systems. In the cell, the exact and complex phospholipid composition in the membranes is crucial for mitochondrial functions. Mitochondria are the “powerhouse” of the cell and phospholipids may affect the activity, biogenesis and stability of protein complexes including respiratory chain supercomplexes. In particular, several phospholipid molecules are intertwined with Complex I (NADH:ubiquinone oxidoreductase) which is the entry point of the respiratory chain and the largest membrane-associated enzyme (1 Mda) of our cell. Dysfunctions of Complex I have been implicated in both childhood-related genetic diseases and in adult neurodegenerative syndromes. Lipids can regulate Complex I activity independent of their role in maintaining mitochondrial membrane integrity. Further studies are required for unraveling how lipids regulate CI assembly or function.

The mechanism of lipid-Complex I interactions and their functional implications are still unclear: by synthesizing different bio-mimetic lipids we plan to dissect the interactions of different lipids with Complex I in a multi-technique approach.

In this context, the PhD project “*Click chemistry for the synthesis of phospholipids to study lipid–protein interactions with EPR and cryo-EM approaches*” will focus on studying the molecular recognition mechanisms regulating the interaction between synthetic phospholipids and native Complex I purified from bovine tissues.

The main goals of the project include:

- **Synthesis of bio-mimetic phospholipids.** Copper-catalyzed azide–alkyne cycloaddition (CuAAC) reactions will be used for the synthesis of different biomimetic phospholipids. Long-chain alkyl azide molecules with different insaturation will be used for the reaction with the alkyne scaffold. Alkyl azides will be also used as precursors for the formation of triazole phospholipids when combined with alkyne lysophospholipids. The flexibility of this approach will allow us to label the different lipids with selected probes, either nitroxides, for EPR, or fluorophores, for fluorescence and microscopy.

- **Electron Paramagnetic Resonance (EPR) spectroscopy of spin-labeled lipids.** EPR lipid–protein interactions studies will be tackled using the commercially available nitroxide-labeled lipids and the new biomimetic lipids synthesized using the click-chemistry approach. EPR will allow the evaluation of the dynamics of lipids bound to Complex I and their residency time in the complex. Also, we will estimate both the stoichiometry of lipid–Complex I interaction (i.e., number of lipid sites at the protein perimeter) and the selectivity of the protein for different lipid species (i.e., association constants relative to the background lipid). Different experimental conditions will be evaluated to study the activity of Complex I in the presence of physiologically relevant ligands (e.g., NADH, ubiquinone, and/or rotenone). In parallel, low-temperature EPR will assess the functionality of the protein in terms of electron transport.

- **Cryo-electron microscopy (cryo-EM) on Complex I bound to synthetic lipids**. Nowadays, Cryo-EM allows to obtain structural details of biomolecules at near atomic resolution, including the structure of Complex I bound to lipids molecules. In this project, cryo-EM approaches will be instrumental to unveil the precise location of interacting surfaces between Complex I and the synthetic phospholipids. Additionally, such technique will allow to visualize lipids at structural level, which is still a challenge in chemistry. The structural characterization of such complexes will help to understand the mechanisms by which lipids regulate the assembly and the function of native Complex I.

This multidisciplinary project will be supervised by Dr. Gabriele Giachin for the cryo-EM analysis and lipid synthesis and Prof. Marco Bortolus for the EPR studies. The PhD candidate will spend a secondment period at the Malopolska Centre of Biotechnology (Jagiellonian University, Krakow, Poland) for high-end cryo-EM data collection and analysis using a 300 kV Krios cryo-TEM; here the PhD candidate will acquire highly specialized competences in cryo-EM sample preparation and advance methods for data collection and analysis on membrane proteins.