**Controlling life and death with proteins: A protein-protein interaction investigated with neutron reflectometry.**

Sophie Ayscough\*1,4, Luke Clifton2, Jörgen Ådén3, Tamás Nagy3, Sebastian Köhler5, Gerhard Groebner3 and Hanna Wacklin-Knecht1,4

1 Division of Physical Chemistry, Lund University, Lund, Sweden

2 ISIS neutron and muon source, Didcot, United Kingdom

3Department of Chemistry,Umeå University, Umeå, Sweden

4European Spallation Source, Lund, Sweden

5LINXS-Institute of advanced Neutron and X-ray science, Lund, Sweden

Mitochondria are well known as the ‘powerhouse of the cell’. Mitochondria also play a crucial role in apoptosis, a programmed mechanism of cell death. This process is tightly regulated by proteins at the mitochondrial outer membrane (MOM).

In this mechanism of cell death, the pro-apoptotic Bax protein is attracted to the outer mitichondrial membrane (MOM), where it induces membrane leakage. [1] In healthy cells, Bax is neutralized by the anti-apoptotic Bcl-2 residing in the MOM.[2]

The complexity of the MOM system makes it difficult to obtain an overall picture of its organisation. However, preparation of planar lipid bilayers and analysis with neutron reflectivity (NR) and attenuated total reflection- fourier transform infrared spectroscopy (ATR-FTIR) can be used to identify the position of the two crucial proteins in respect to the membrane and to identify the kinetics of the interaction. We can characterise this biological effect by employing hydrogen/deuterium labeling to create a stong contrast between the lipid and protein components.

Our results provide the first structural evidence of Bcl-2 preventing membrane perforation by Bax as part of its anti-apoptotic mechanism. In the absence of Bcl-2, the Bax protein both inserts into the lipid bilayer and removes lipids, forming a lipid-protein complex on top of the original bilayer structure. However, when Bcl-2 is reconstituted into POPC bilayers, Bax associates to the bilayer but does not insert into it or remove lipids. Separately when Bcl-2 is reconstituted into 9:1 POPC: cardiolipin bilayers (a higher cardiolipin content than the average MOM), Bax still perforates the bilayer with slower kinetics than in the absence of Bcl-2.[3] These results indicate that membrane lipids play an important role in apoptotic pore formation, and life hangs in the balance of membrane protein content and lipid composition.

[1] M. Lidman, *BBA-Biomembranes* **1858**, 1288-1297 (2016).

[2] A. Mushtaq, *Commun Biol* **4**, 507 (2021).

[3] L. A. Clifton, L.A., *Science Advances*, **9**,22, (2023)

\* Correspondence: [sophie.ayscough@fkem1.lu.se](mailto:sophie.ayscough@fkem1.lu.se)