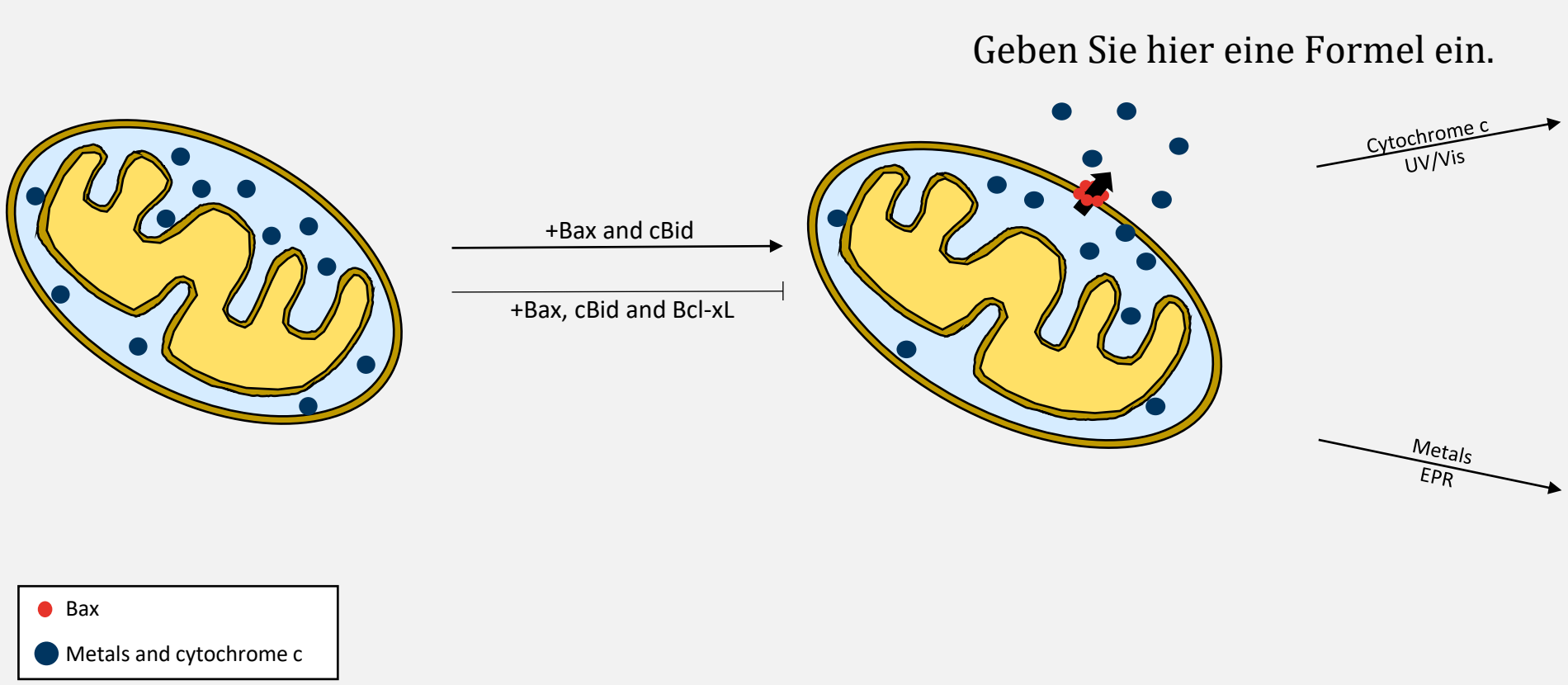
Sensing pore formation in the mitochondrial outer membrane via UV/Vis and EPR spectroscopy

Dominik Gendreizig[1], Christina Elsner[1], Svetlana Kucher[1], Ralf Erdmann[2], Enrica Bordignon[1]

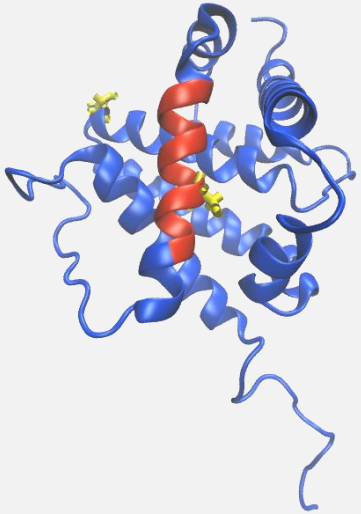
[1]*Faculty of Chemistry and Biochemistry, Ruhr University of Bochum, Bochum, Germany,* [2]*Faculty of Medicine, Ruhr University of Bochum, Bochum, Germany.*

Dominik.Gendreizig@rub.de

Geben Sie hier eine Formel ein.

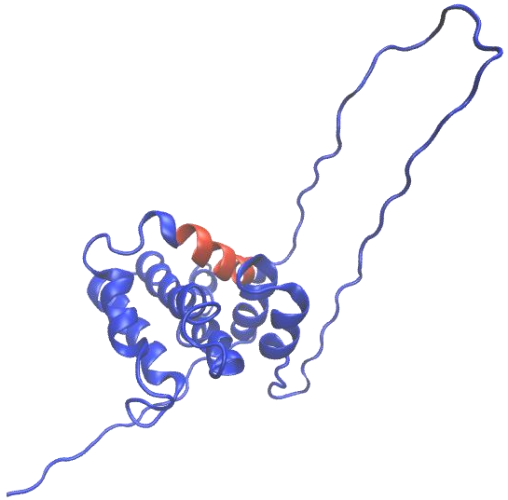


A minimal Bcl-2 interaction network



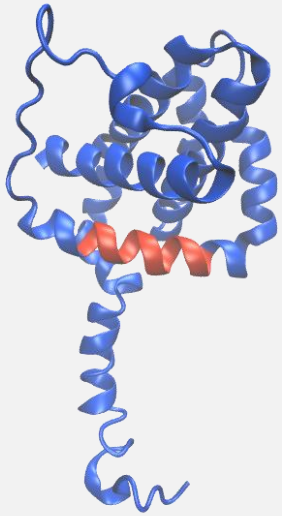
Bax

- Pro apoptotic, pore forming protein
- It has some auto activity
- Oligomerization and membrane insertion facilitated by cBid



Bid

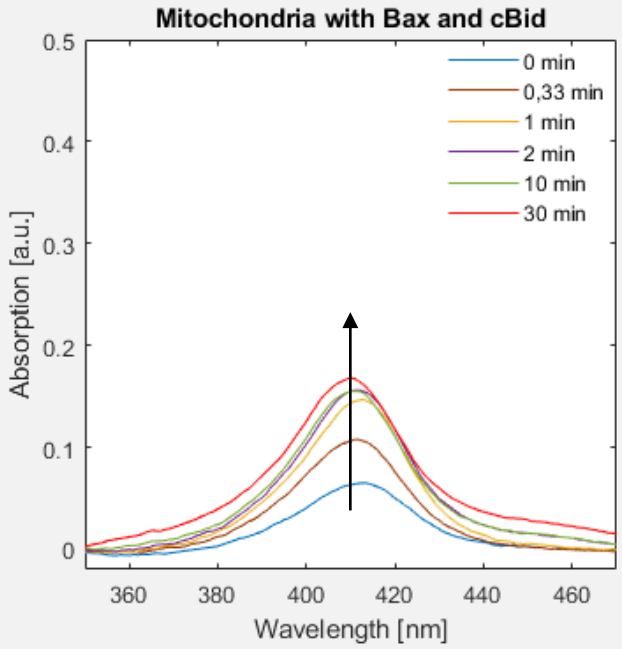
- Pro apoptotic
- Cleaved by caspase 9 into active form
- Facilitates Bax insertion in a catalytic manner

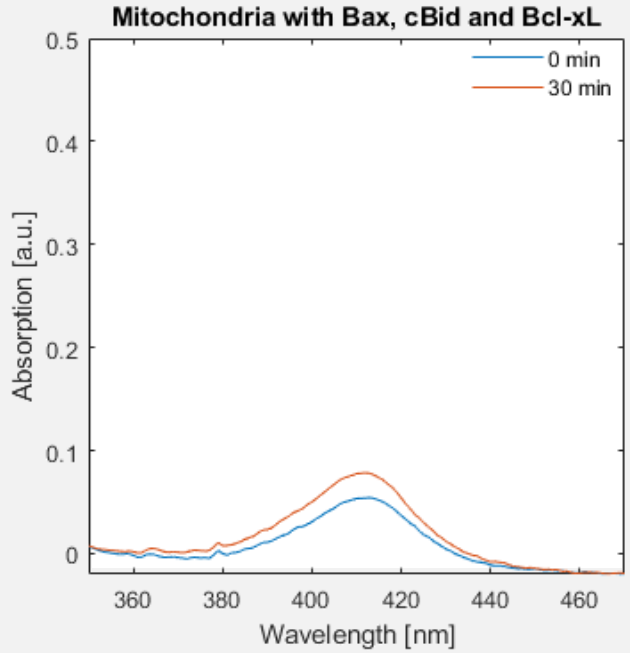


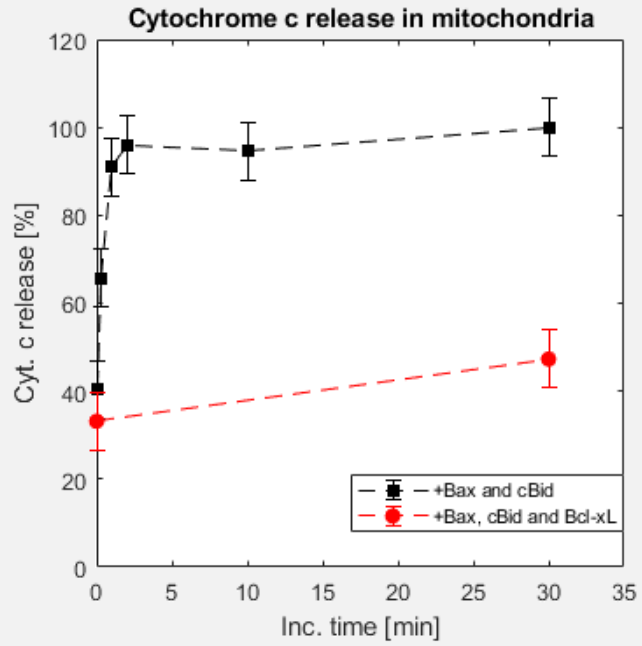
Bcl-xL

- Anti apoptotic protein
- Inhibits mitochondrial outer membrane permeabilization (MOMP)
- Mechanism of apoptosis prevention yet unknown

Cytochrome c measured via UV/Vis spectroscopy

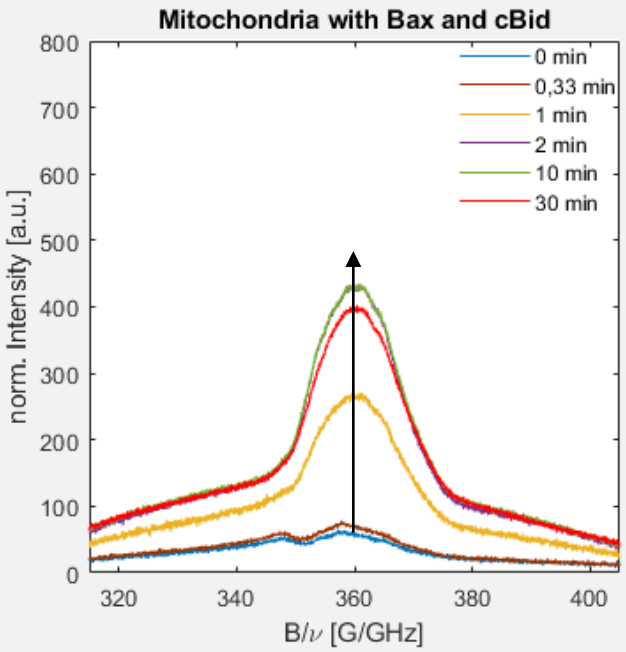


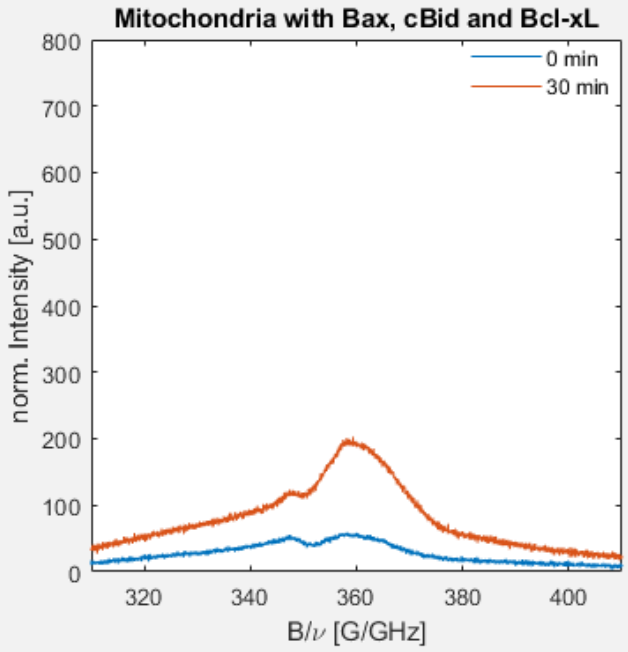


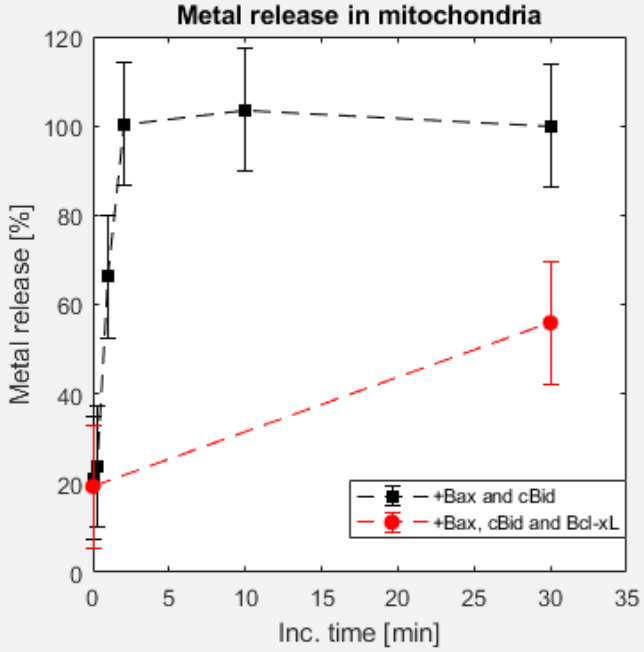


- Upon MOMP cytochrome c is released from the intermembrane space
- Permeabilization in presence of cBid is fast (approx. 2 minutes)
- In presence of Bcl-xL MOMP is inhibited

Metal release measured via EPR spectroscopy







- After MOMP, the release of metals can be detected via EDFS in the mitochondrial supernatant
- Metal and cytochrome c release display similar kinetics
- Metals are yet to be identified

Conclusion

- Dynamic interaction of a minimal Bcl-2 protein interactome *in organelle*
- An extension of the interactome is easily possible by including drugs and anti-cancer peptides
- Future studies of spin-labeled Bcl-2 proteins *in organelle*

Thank you for your attention

References

P. E. Czabotar, G. Lessene, A. Strasser, J. M. Adams, *Nat Rev Mol Cell Biol* **2014**, *15*, 49.

J. Kale, E. J. Osterlund, D. W. Andrews, *Cell Death Differ* **2018**, *25*, 65.