

Neurospora crassa (N. crassa)

Non-pathogenic filamentous fungus; Widely used as a model multicellular eukaryote.

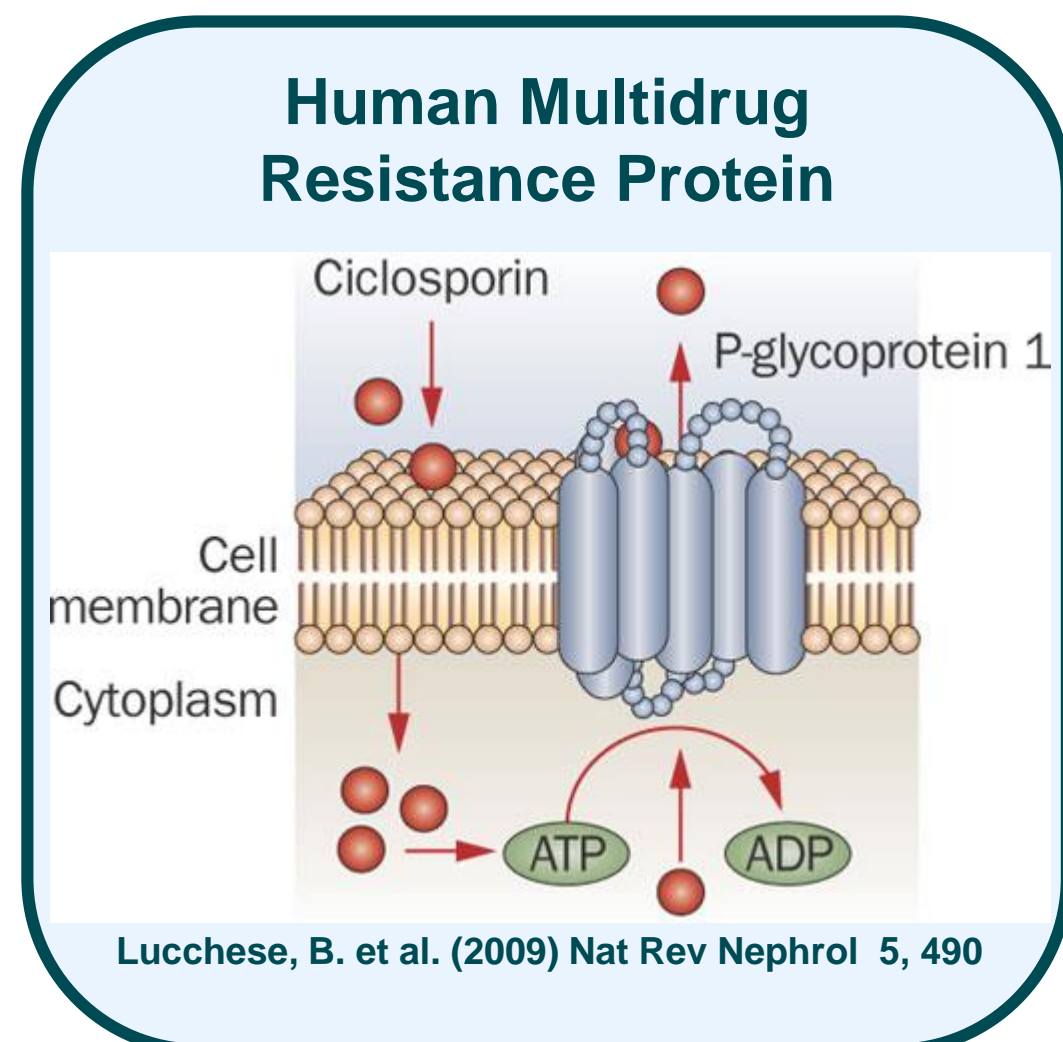


Wild type

VS. *abc3*
N. crassa strain without ABC3 protein

Neurospora ABC3 protein is a STS exporter

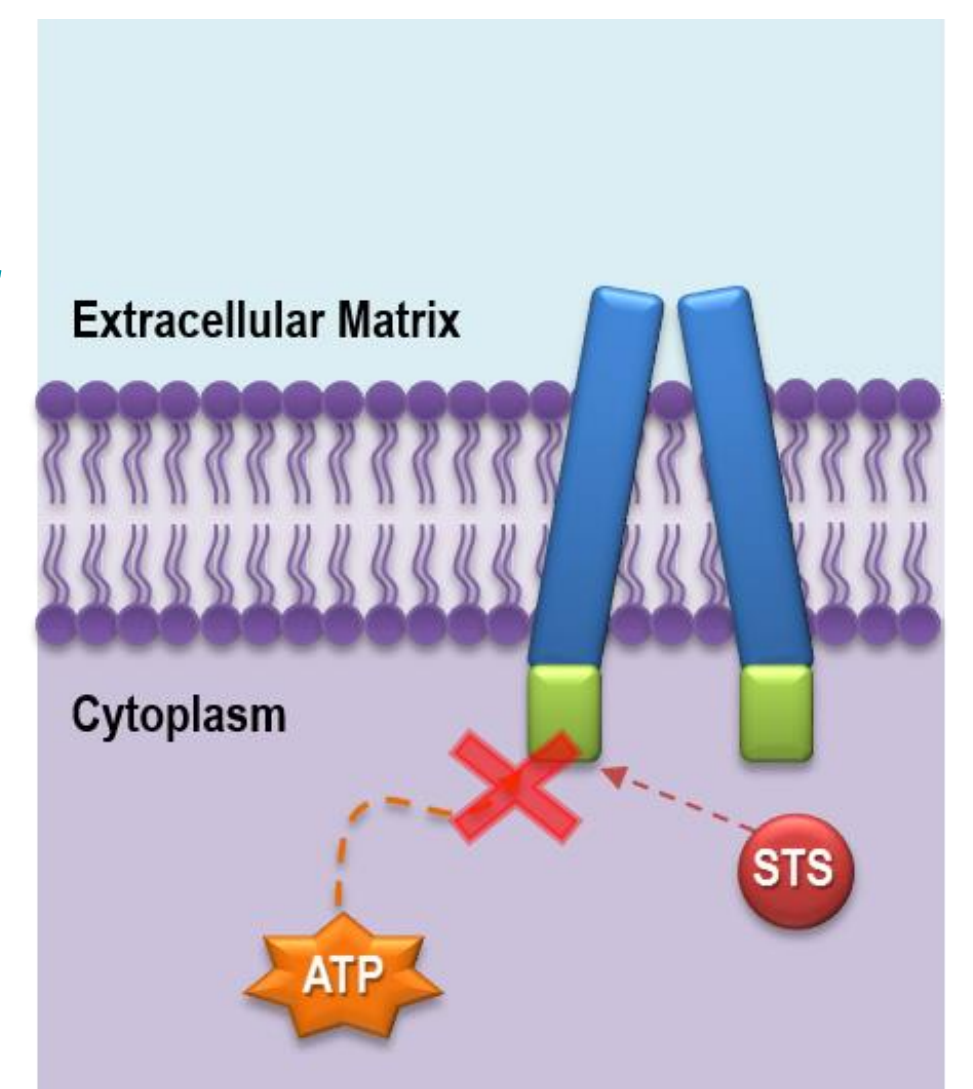
Castro, A. et al. (2010) Eukaryot Cell 9, 906-914



Lucchese, B. et al. (2009) Nat Rev Nephrol 5, 490

Staurosporine (STS)

Protein kinases inhibition through the prevention of ATP binding to the kinase.



N. crassa challenged with STS

Studying *N. crassa* strains with and without a STS stimulus

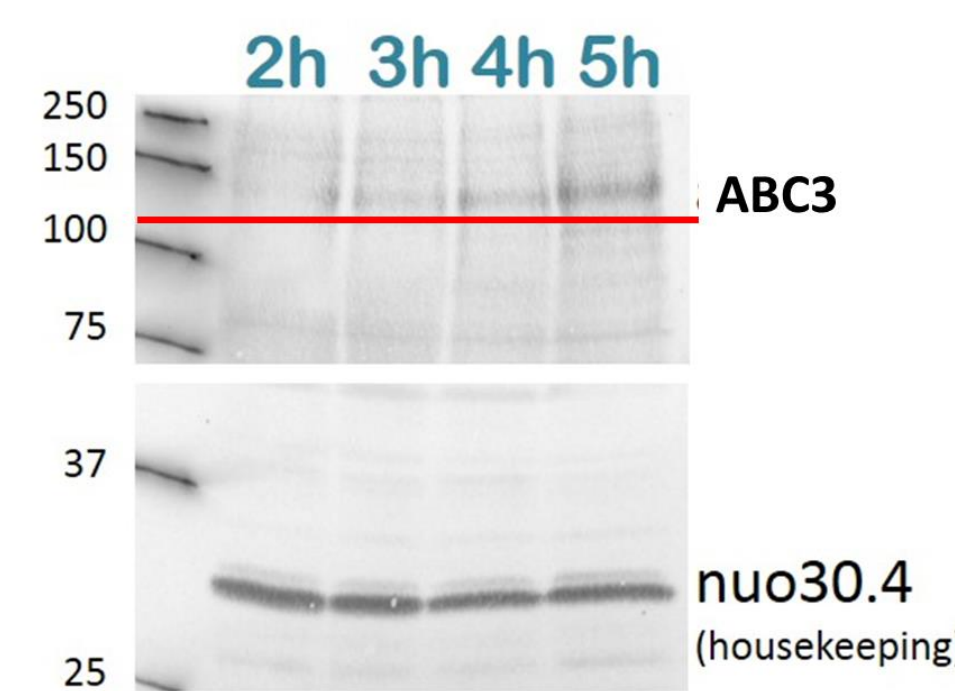
Resistance model

Main goal:

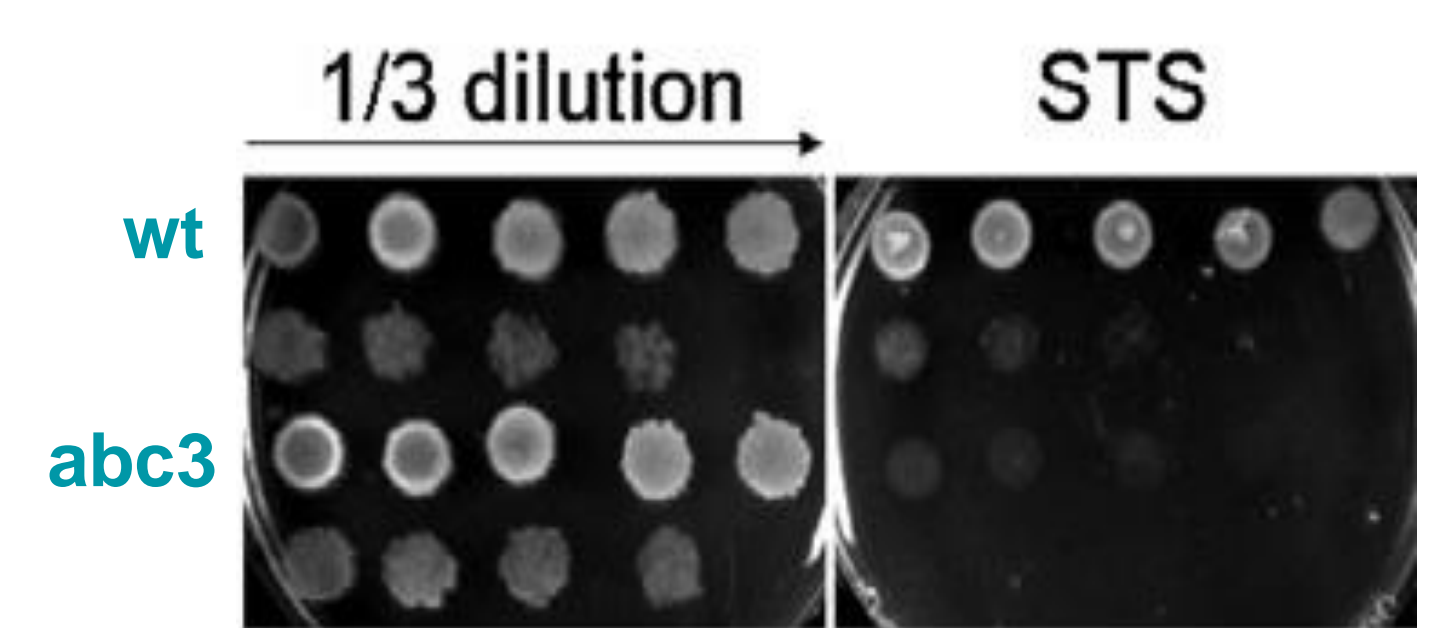
Understand if plasma membrane (PM) biophysical properties are involved.



Up-regulates ABC transporter ABC-3

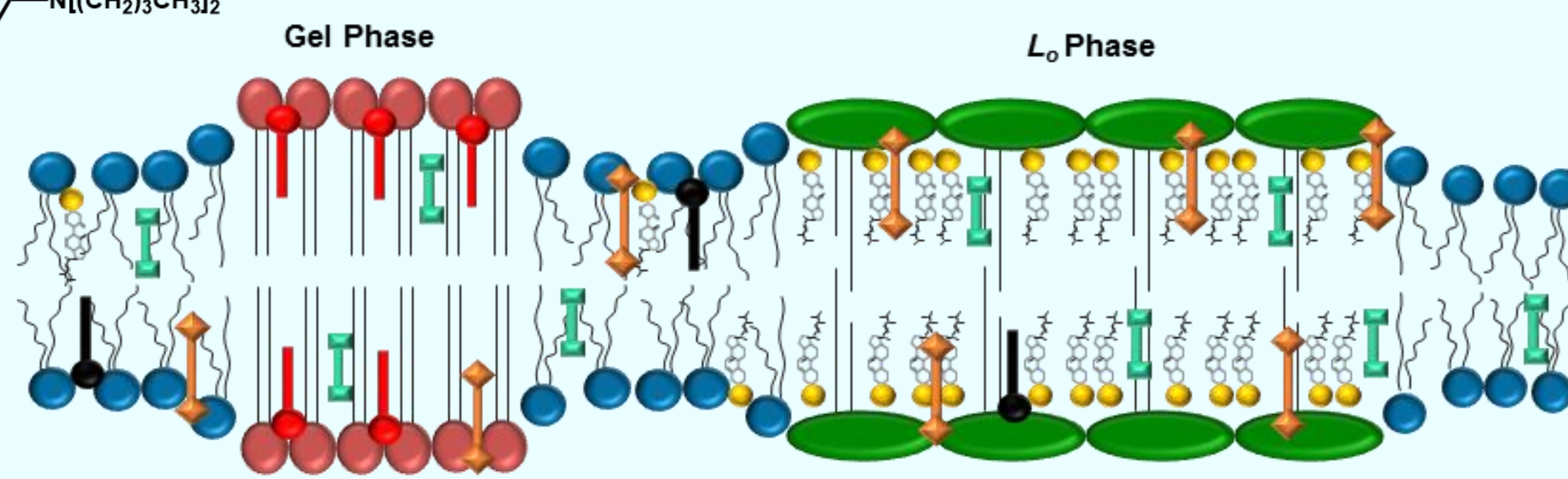
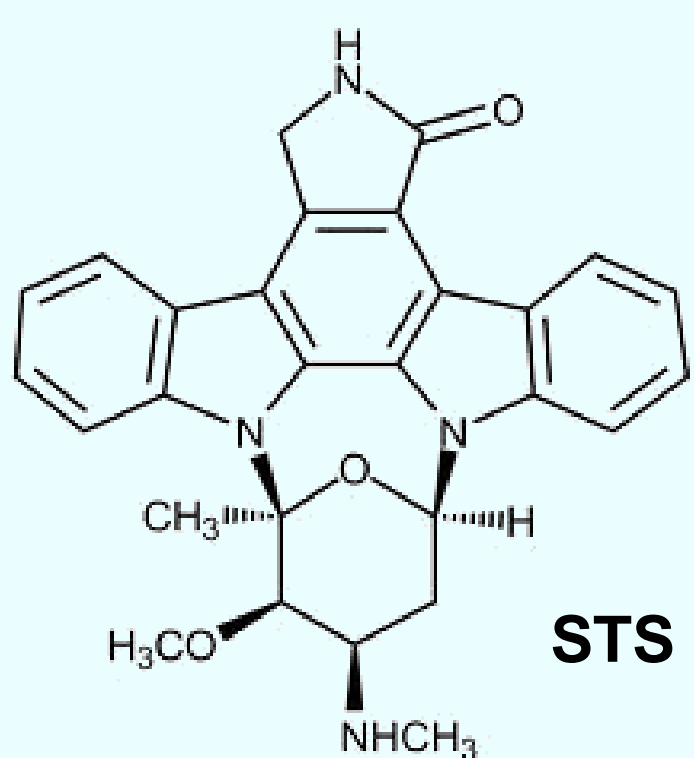
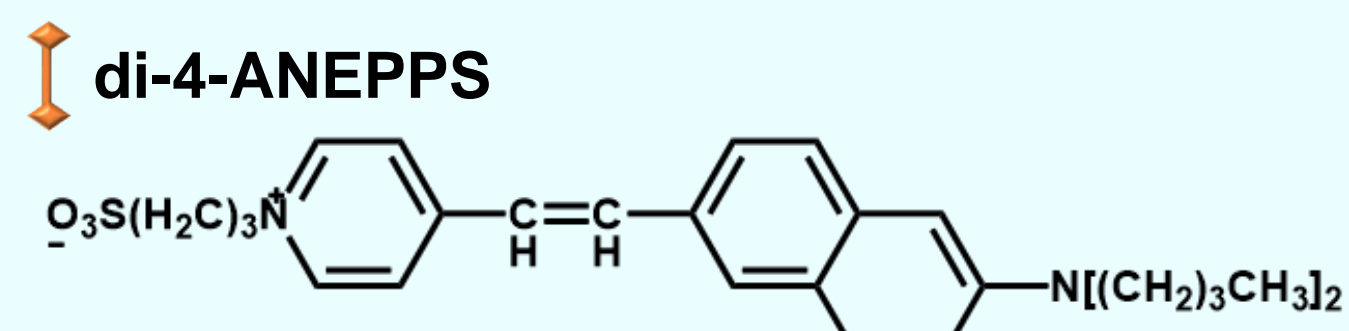
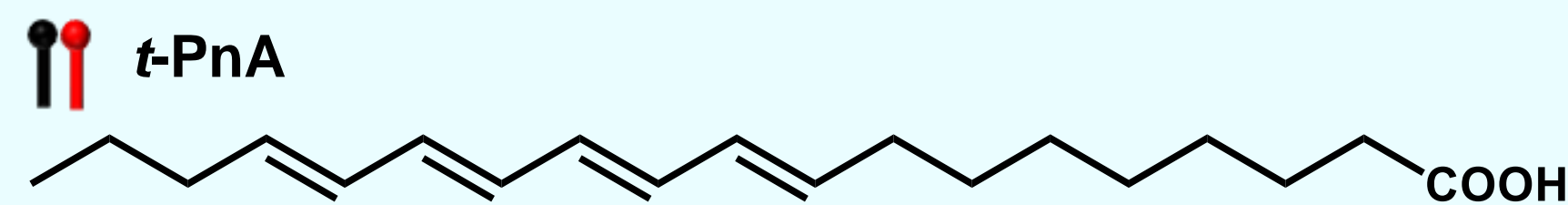
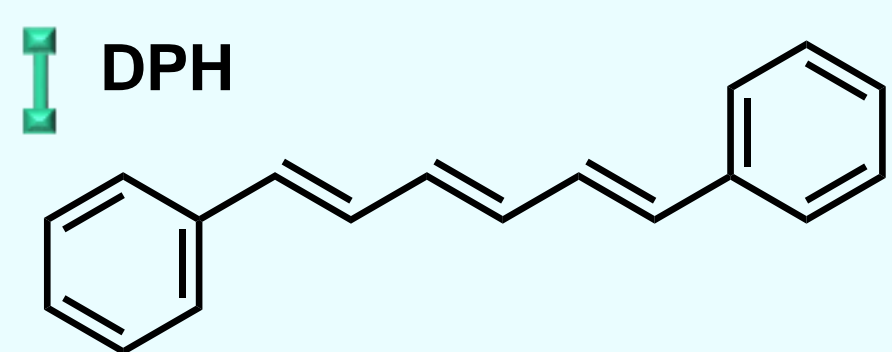


abc3 null mutant is extremely sensitive to STS



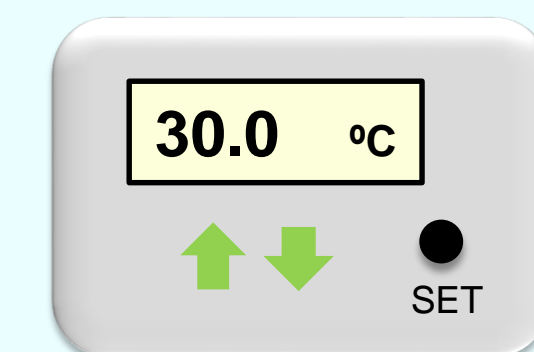
Fernandes, A.S. et al. (2011) Fungal Genet Biol, 48, 1130-1138.

Biophysical Studies in Living Cells: Fluorescence Spectroscopy



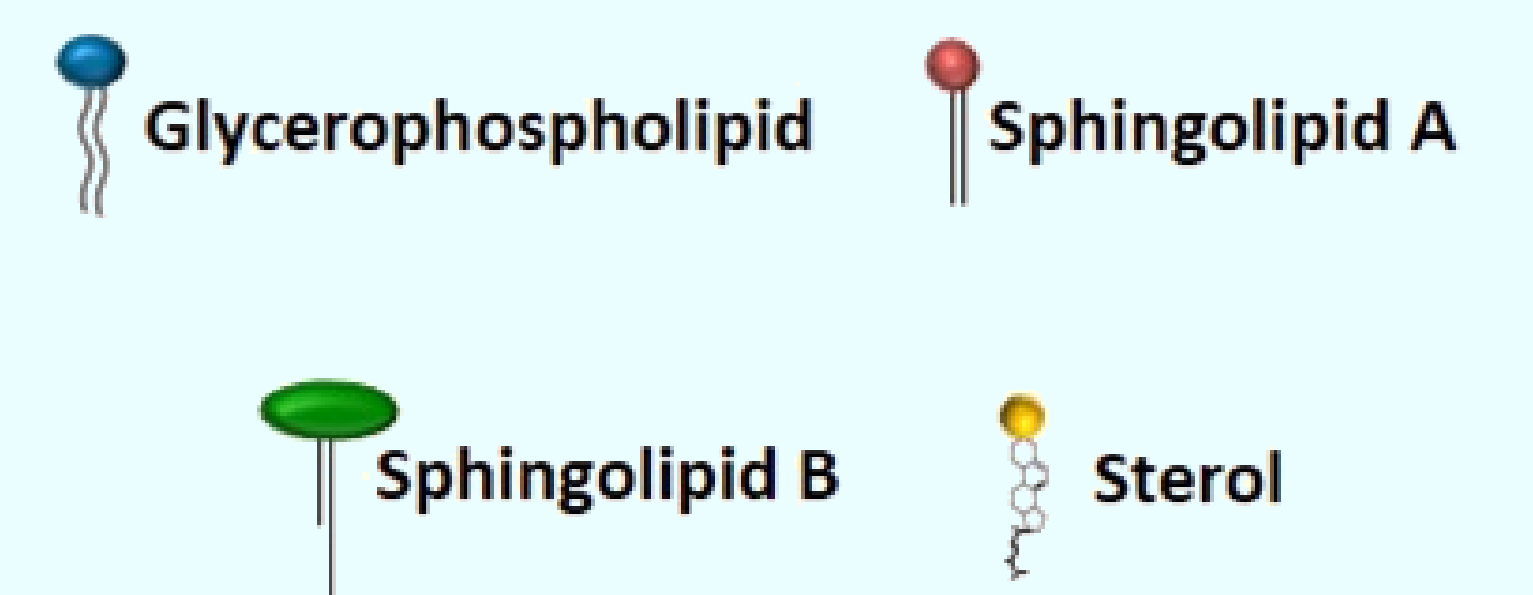
Fluorescence Lifetime

$$I(t) = \sum_{i=1}^n \alpha_i \cdot \exp\left(-\frac{t}{\tau_i}\right)$$

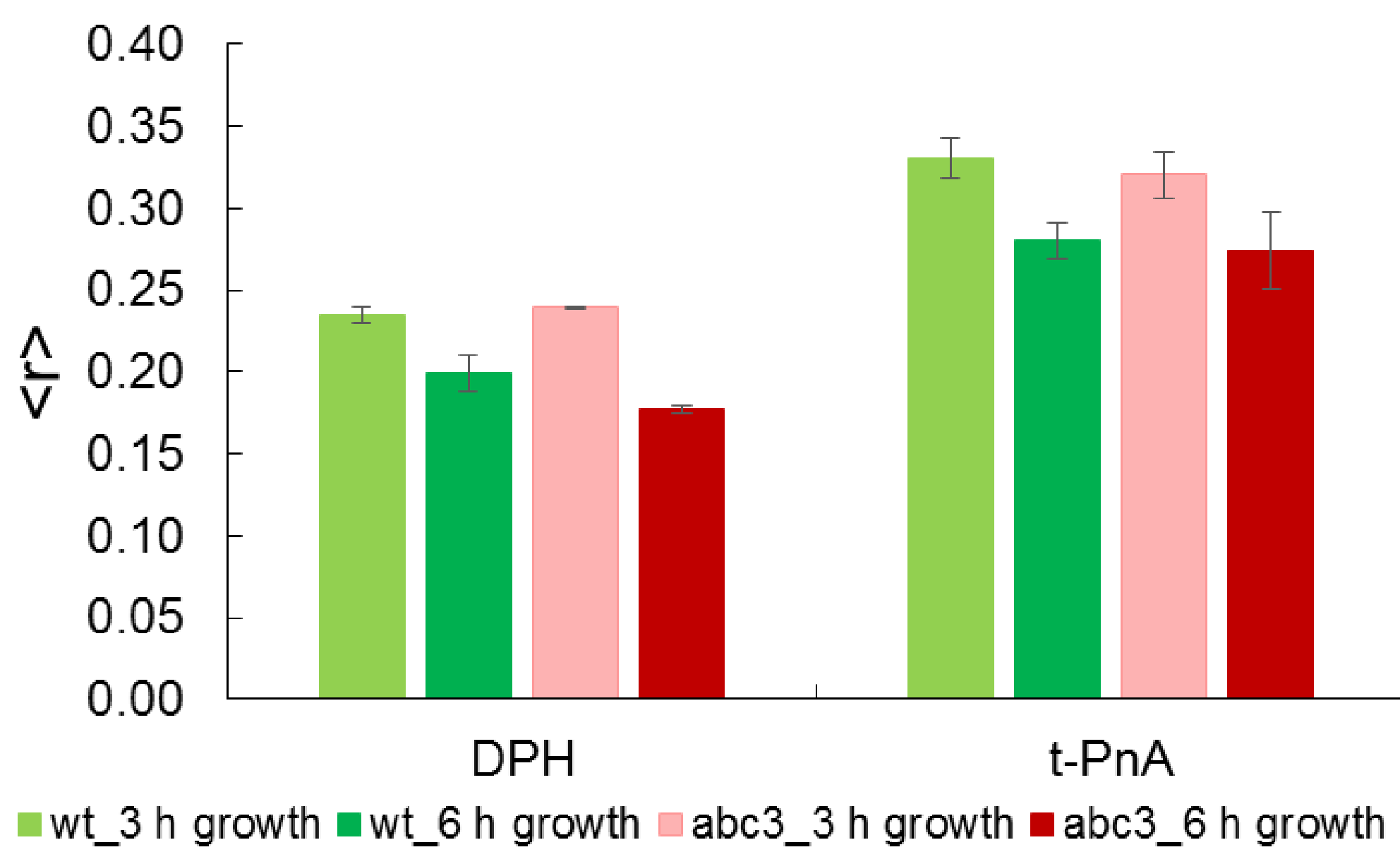


Steady-State anisotropy

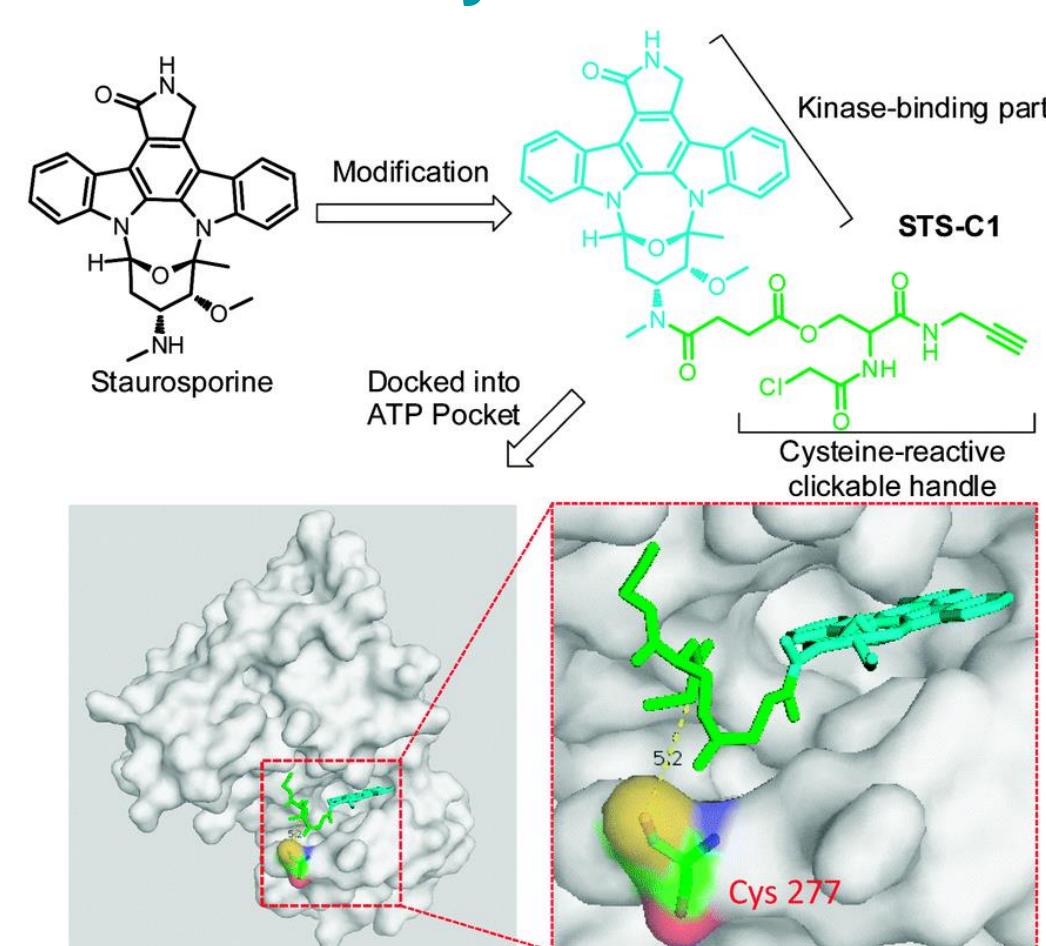
$$\langle r \rangle = \frac{I_{VV} - G \cdot I_{VH}}{I_{VV} + 2 \cdot G \cdot I_{VH}}$$



Results: Comparison of 2 strains with different STS sensitivity

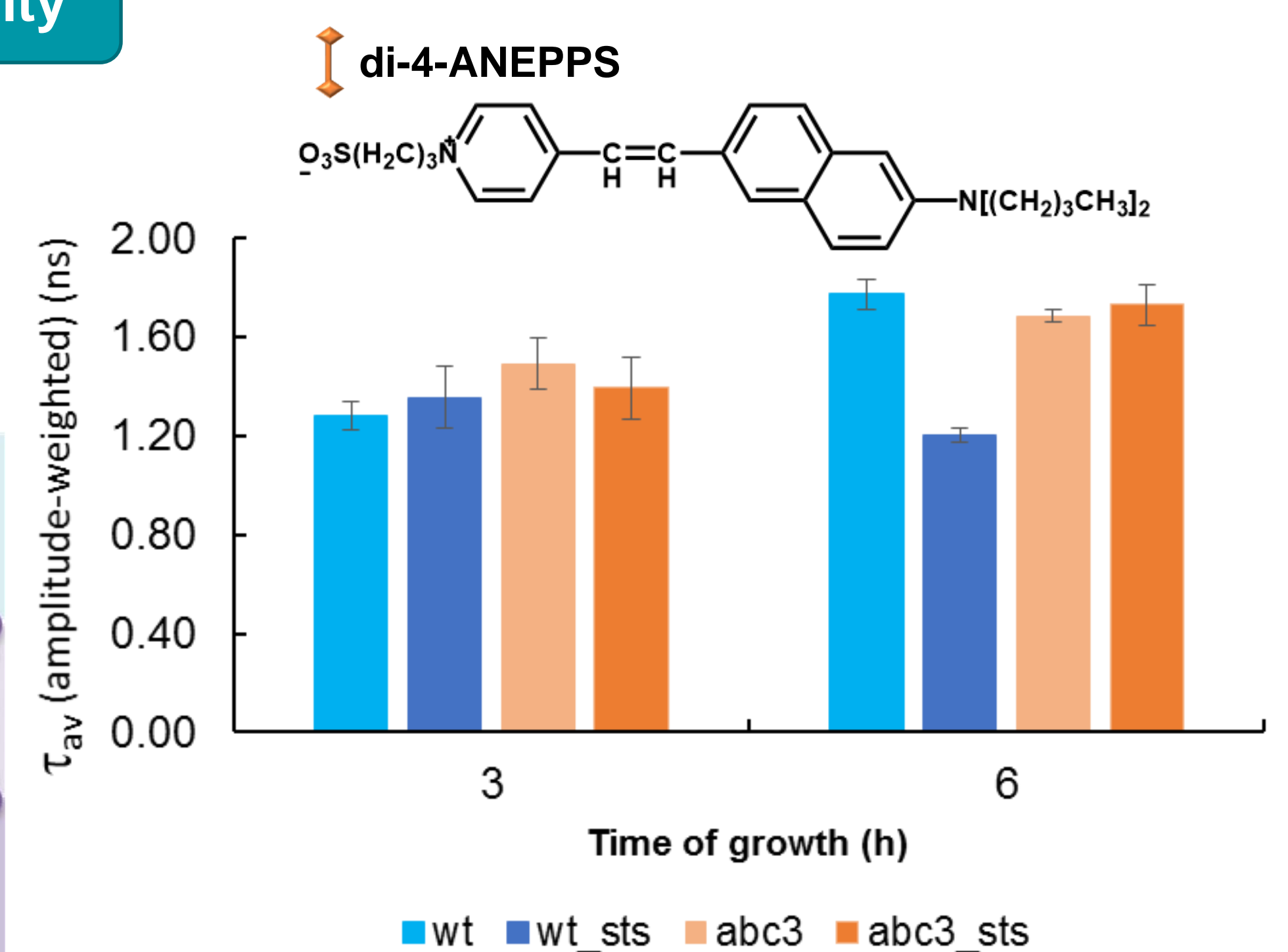
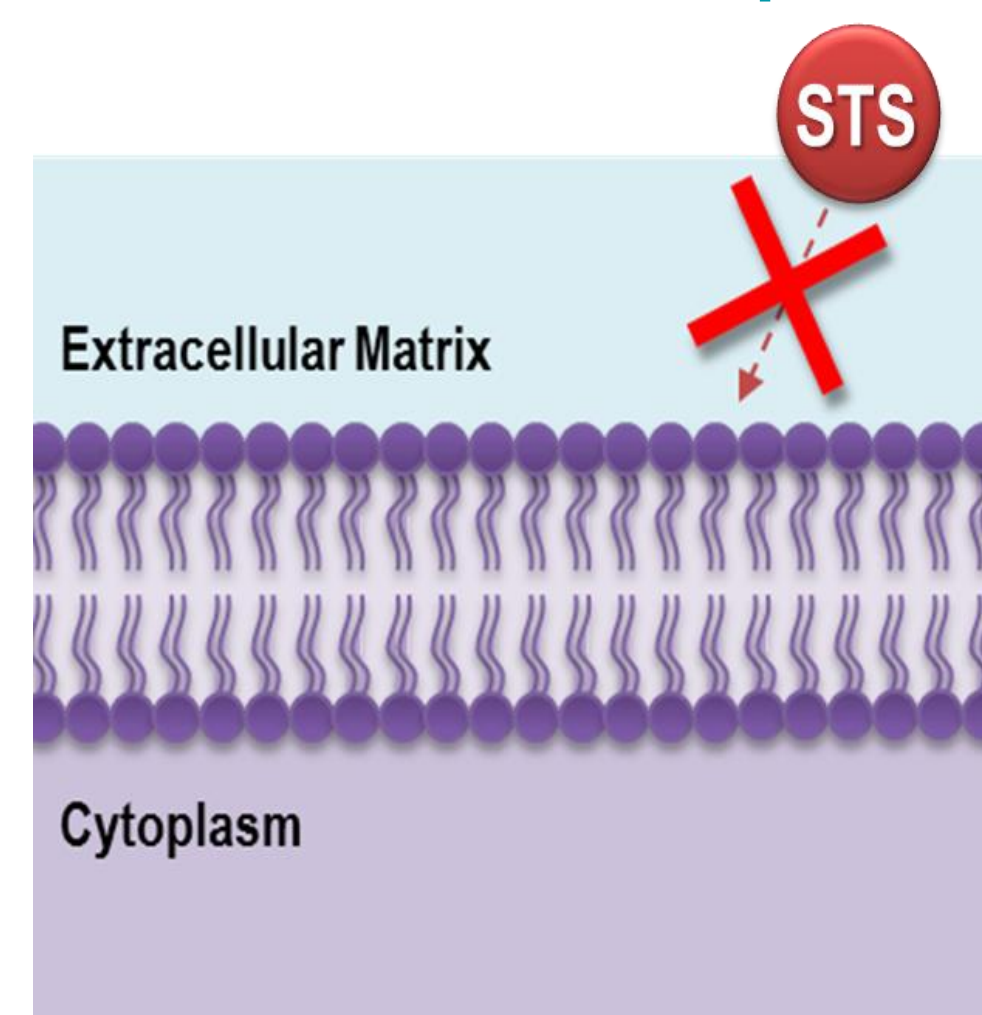


Protein kinase inhibited by STS



Cheng, X. et al. (2014) Chem. Commun., 50, 2851-2853.

STS does not interact with membrane lipids



Concluding Remarks

- Global membrane order (DPH) increases for *abc3*.
- Related to ordered domains detected by *t-PnA*.
- In the absence of STS *wt* and *abc3* have identical behavior.
- Upon a STS challenge the response of membrane biophysical properties is distinct for 6 h growth.
- N. crassa* possesses a highly ordered PM.
- Important biological roles of PM biophysical properties in *N. crassa*.

Acknowledgments

This work was supported by Fundação para a Ciência e a Tecnologia (FCT), Portugal (Projects UID/Multi/00612/2013, IF2012 initiative (POPH, Fundo Social Europeu)). F.C.S. acknowledges Ph.D. scholarship SFRH/BD/108031/2015, also from FCT.