

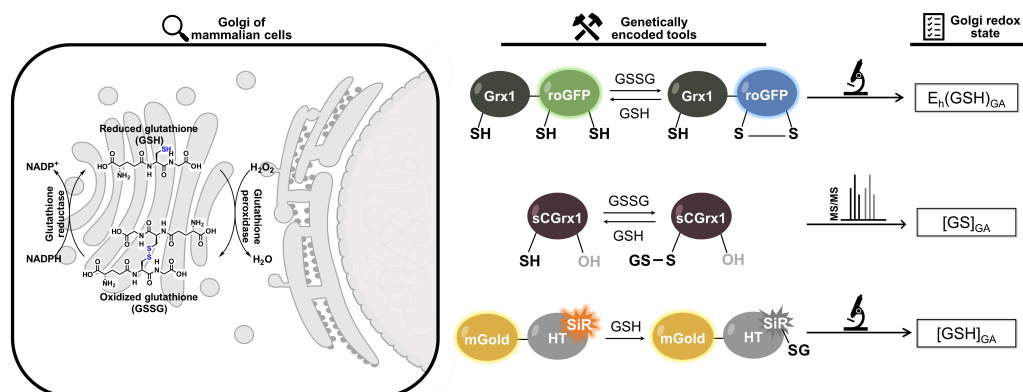
Characterization of the Glutathione Redox State of the Golgi Apparatus

C. Miró-Vinyals¹, S. Emmert^{1,2}, G. Grammbitter², P. Rivera-Fuentes^{1*}

¹Chemistry Department, University of Zürich, CH-8057, Zürich, Switzerland, ²Institute of Chemical Sciences and Engineering, Ecole Polytechnique Fédéral de Lausanne, CH-1015, Lausanne, Switzerland.

Redox homeostasis is essential for cell functioning and its study in a compartmentalized manner is key due to the differentiated redox pairs ratios in each organelle.¹ The redox state of an organelle can be determined by quantifying the redox potential (E_h) and the concentration of a given redox pair. In our case, we chose to study glutathione (GSH) and its oxidized counterpart (GSSG) because of their high cellular concentration (mM).¹ The Golgi apparatus (GA) is a central organelle responsible for protein glycosylation and protein sorting and its dysfunction is linked to cancer and neurodegenerative diseases. It is considered one of the most oxidizing organelles in the cell, yet there are no reports of redox potential nor values for absolute GSH+GSSG concentrations in the Golgi.²

In this work, we determined the redox state in the Golgi by using different and independent genetically encoded tools. A redox-sensitive green-fluorescent protein (roGFP1-iE)³ allowed us to calculate the $E_h(\text{GSH})_{\text{GA}}$. Together with a single-cysteine glutaredoxin (sCGrx1p)⁴ we calculated the total GSH+GSSG concentration in the organelle ($[\text{GS}]_{\text{GA}}$). Finally, using a GSH sensor (TRaQ-G),⁵ consisting of a fusion protein of mGold and HaloTag (HT) conjugated to a GSH-reactive silicon rhodamine (SiR), we were able to calculate the absolute GSH concentration ($[\text{GSH}]_{\text{GA}}$). These results allowed us to present for the first time a quantitative redox profile in the Golgi apparatus and allow for further enlightenment on how the redox state is maintained in the specified organelle.



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