



DECIPHERING THE MULTI-TARGET MECHANISM OF AURANOFIN IN AN OVARIAN CANCER CELL LINE BY REDOXOMIC AND METALLOMIC PROFILING.

Spectrométrie de Masse Biologique et Protéomique
ESPCI-PSL/CNRS UAR2051

Cancer Cells Cope With High Levels of Reactive Oxygen Species (ROS)

Reactive Oxygen Species (ROS)



Oxygen
(O_2)



Superoxide anion
($O_2^{\bullet-}$)



Peroxide
($O_2^{\bullet-2}$)



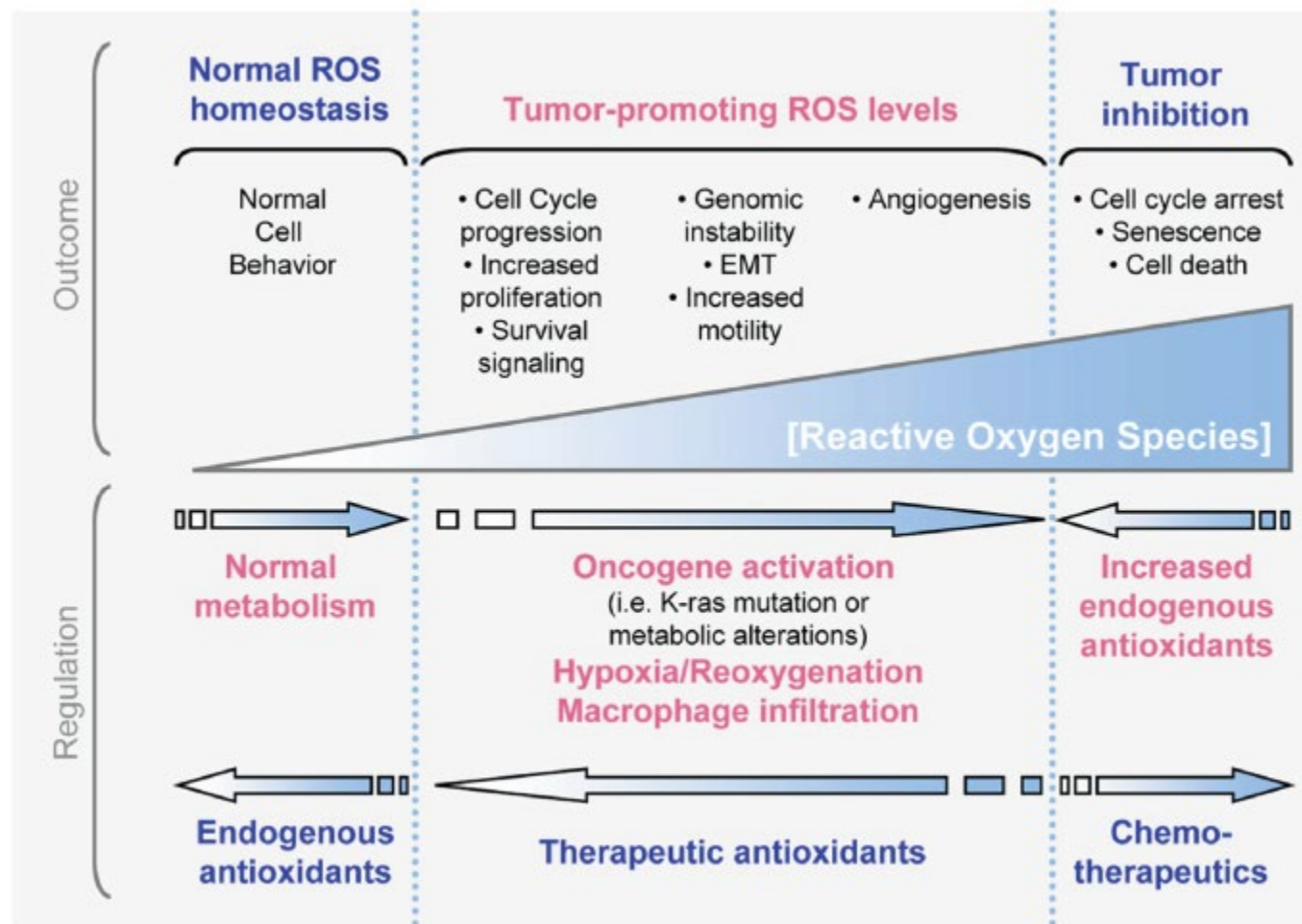
Hydrogen peroxide
(H_2O_2)



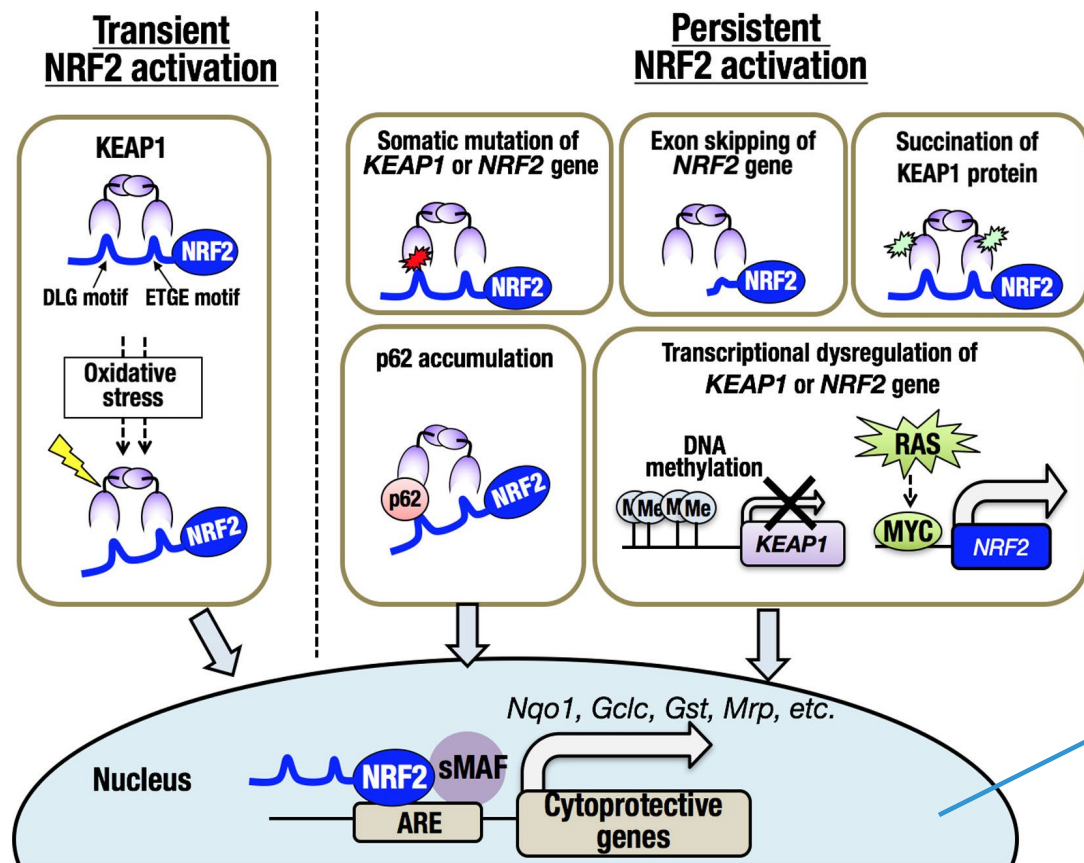
Hydroxyl radical
(OH^*)



Hydroxyl ion
(OH^-)

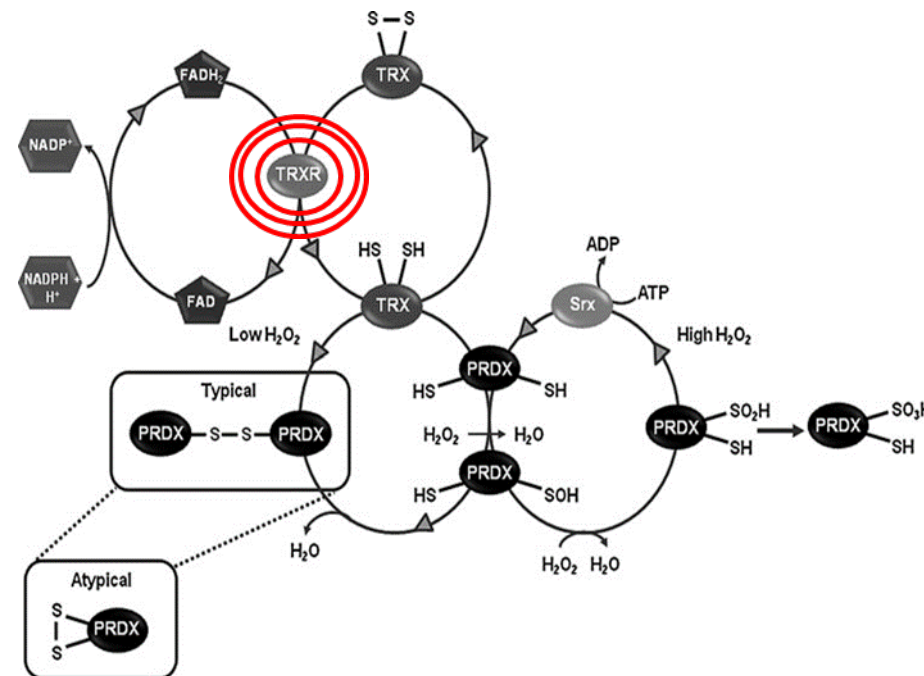


Liou GY, Storz P. *Free Radical Research*. 2010 May;44(5):479-496.



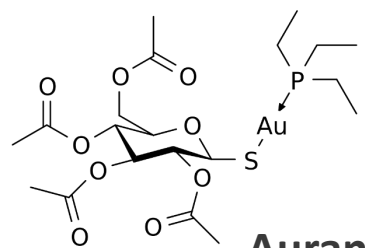
Kitamura H, Motohashi H. *Cancer Sci*. 2018 Apr;109(4):900-911

Thioredoxin (TRX) Pathway



Thioredoxin Reductase (TRXR) is a therapeutic target

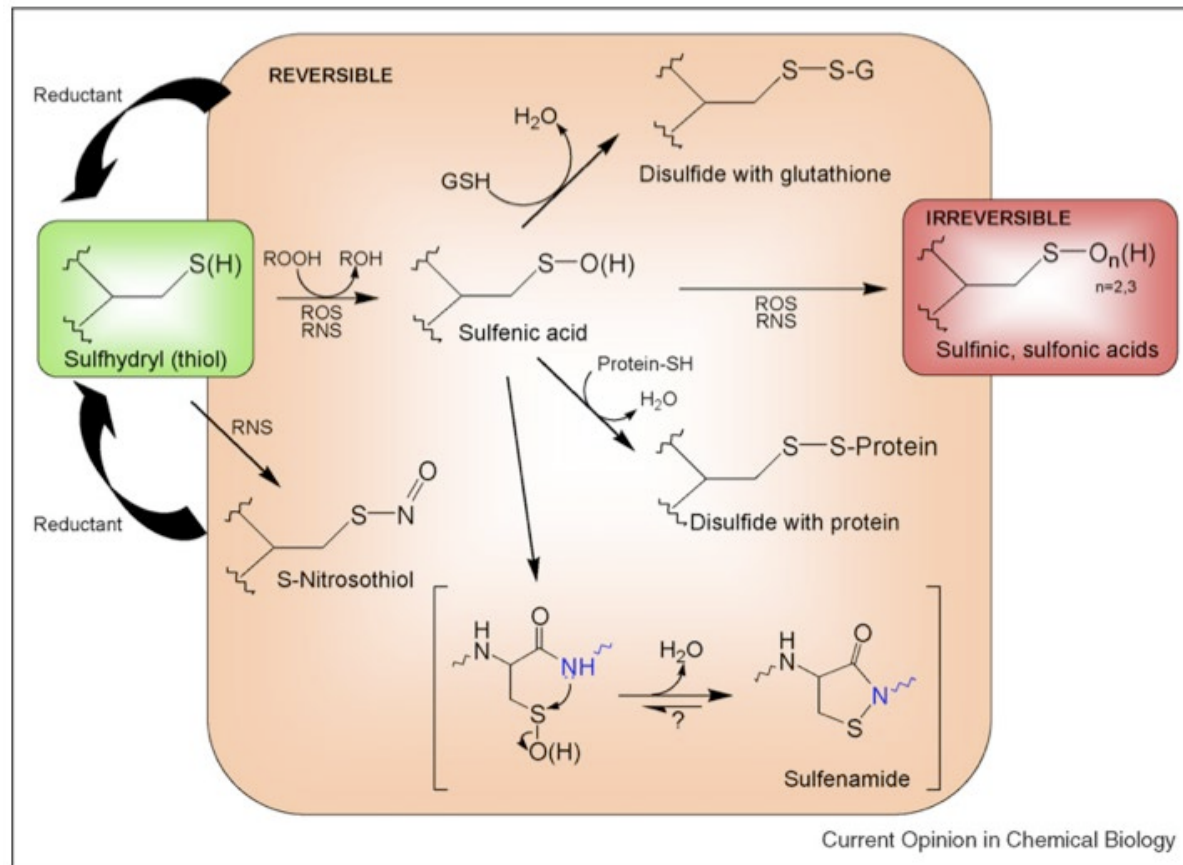
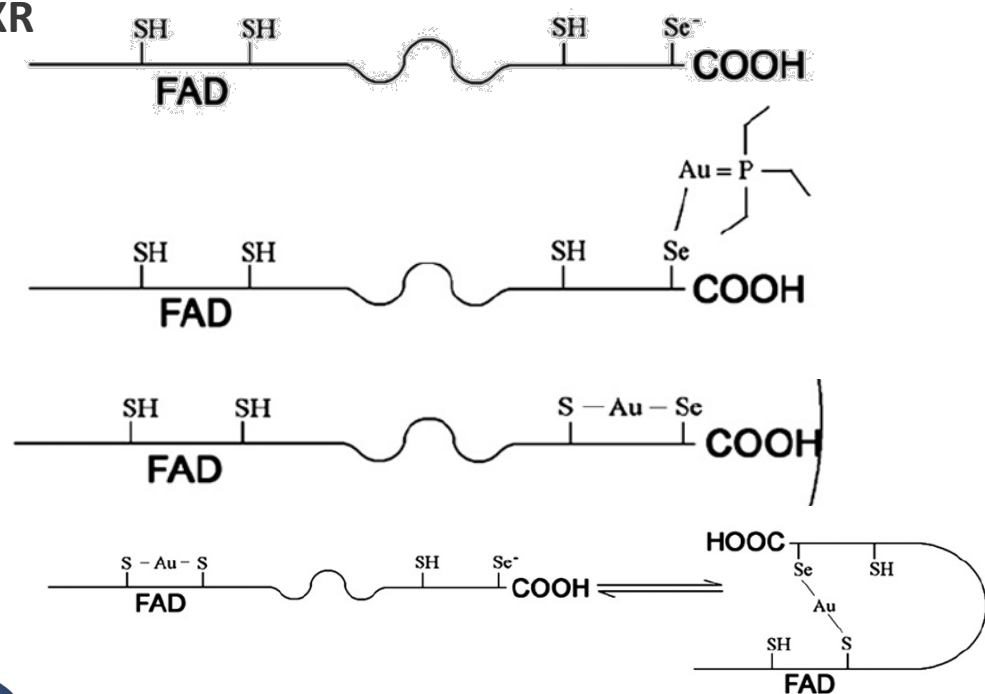
Cysteine Oxidative Post-Translational Modifications



Auranofin



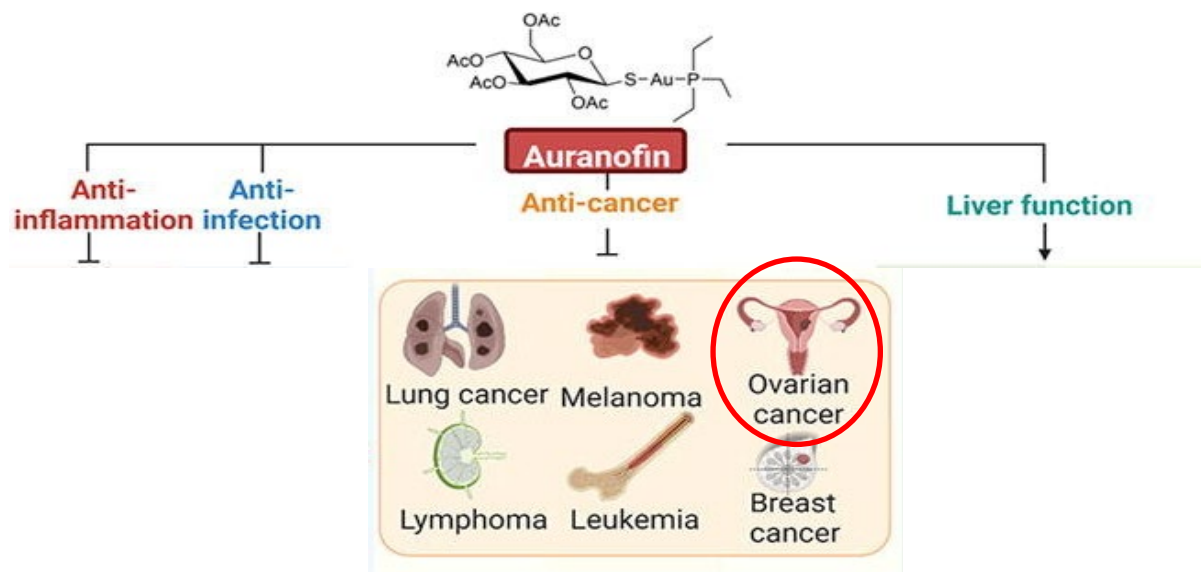
TRXR





Redox Proteomic Analysis of Auranofin-Treated Ovarian Cancer Cells

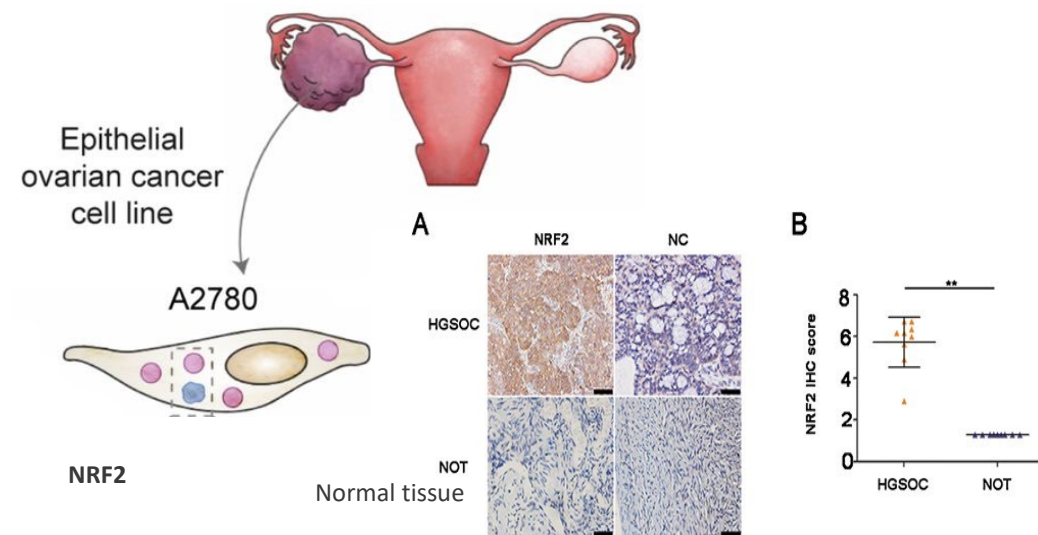
Redox Proteomics Analysis of Auranofin Treated Ovarian Cancer Cells (A2780)



Shuying S, et al. *Coord.Chem.Rev.* 2023, 493, 15, 215323

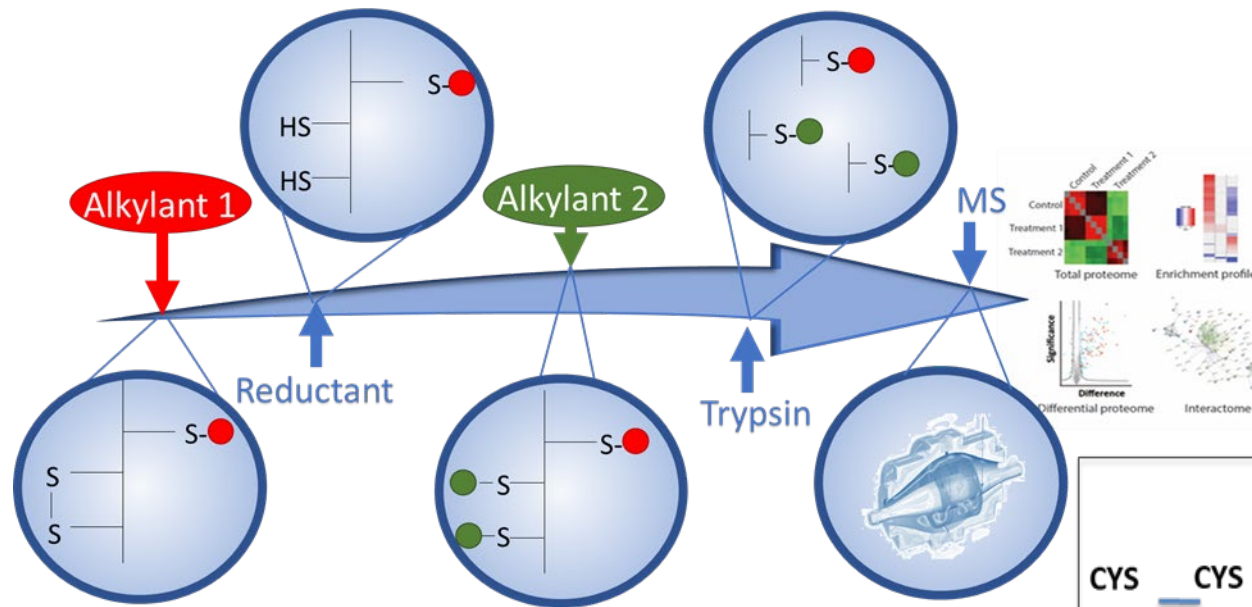
Auranofin is highly cytotoxic in NRF2 addicted cells

NRF2 is aberrantly activated in High-Grade Serous Ovarian Cancer (HGSOC)

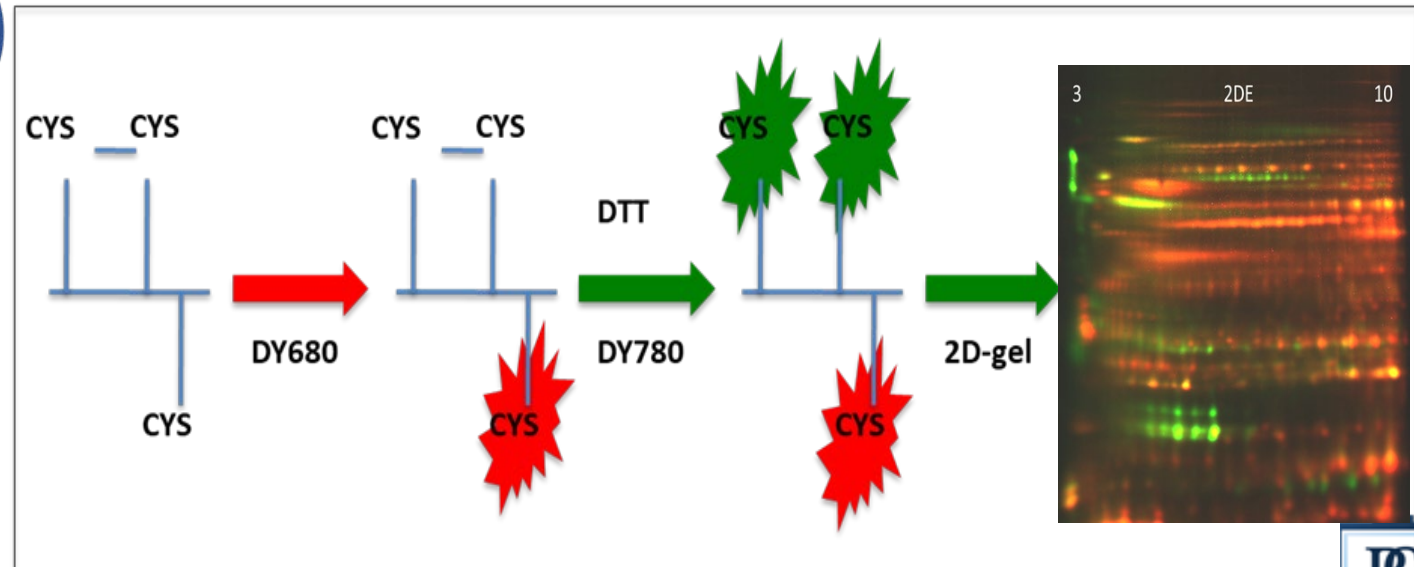


Lisio MA, et al. *Int J Mol Sci.* 2019 Feb 22;20(4):952

Thiol Redox Proteomics

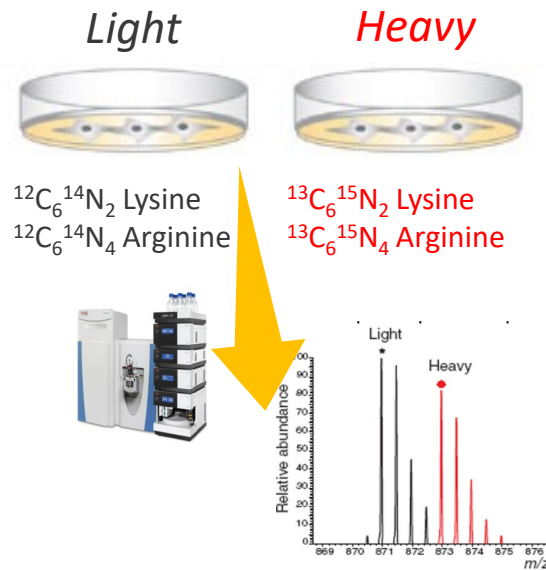


Chiappetta G, et al. *Methods Enzymol.* 2010;473:199-216

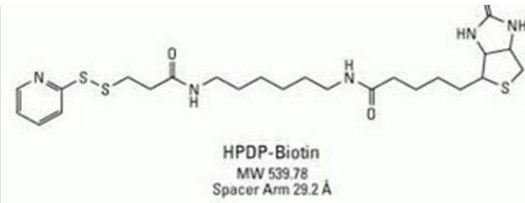


Thiol Redox Proteomics: OcSILAC

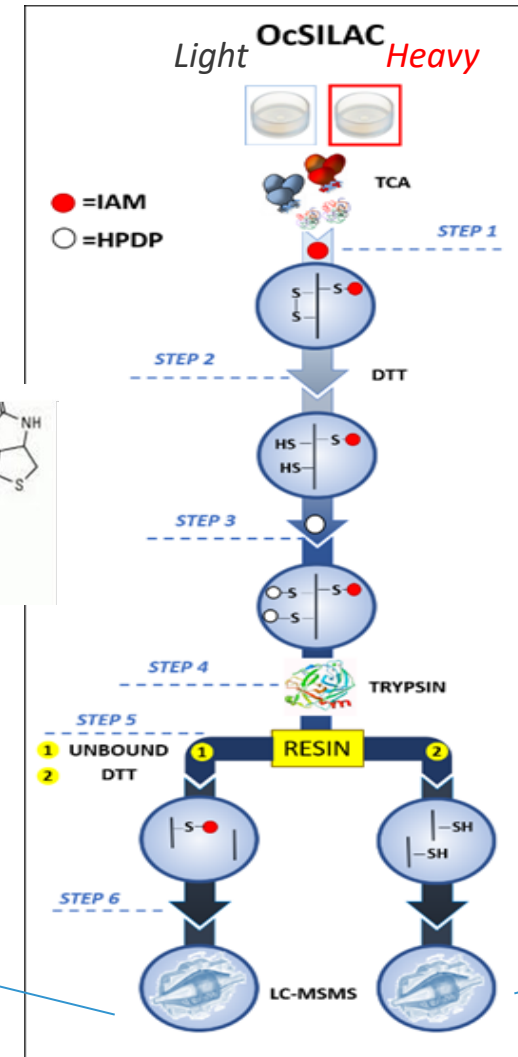
Stable Isotope Labeling in Cell culture (SILAC)



Biotin Switch



- Reduced Cys
- Protein levels

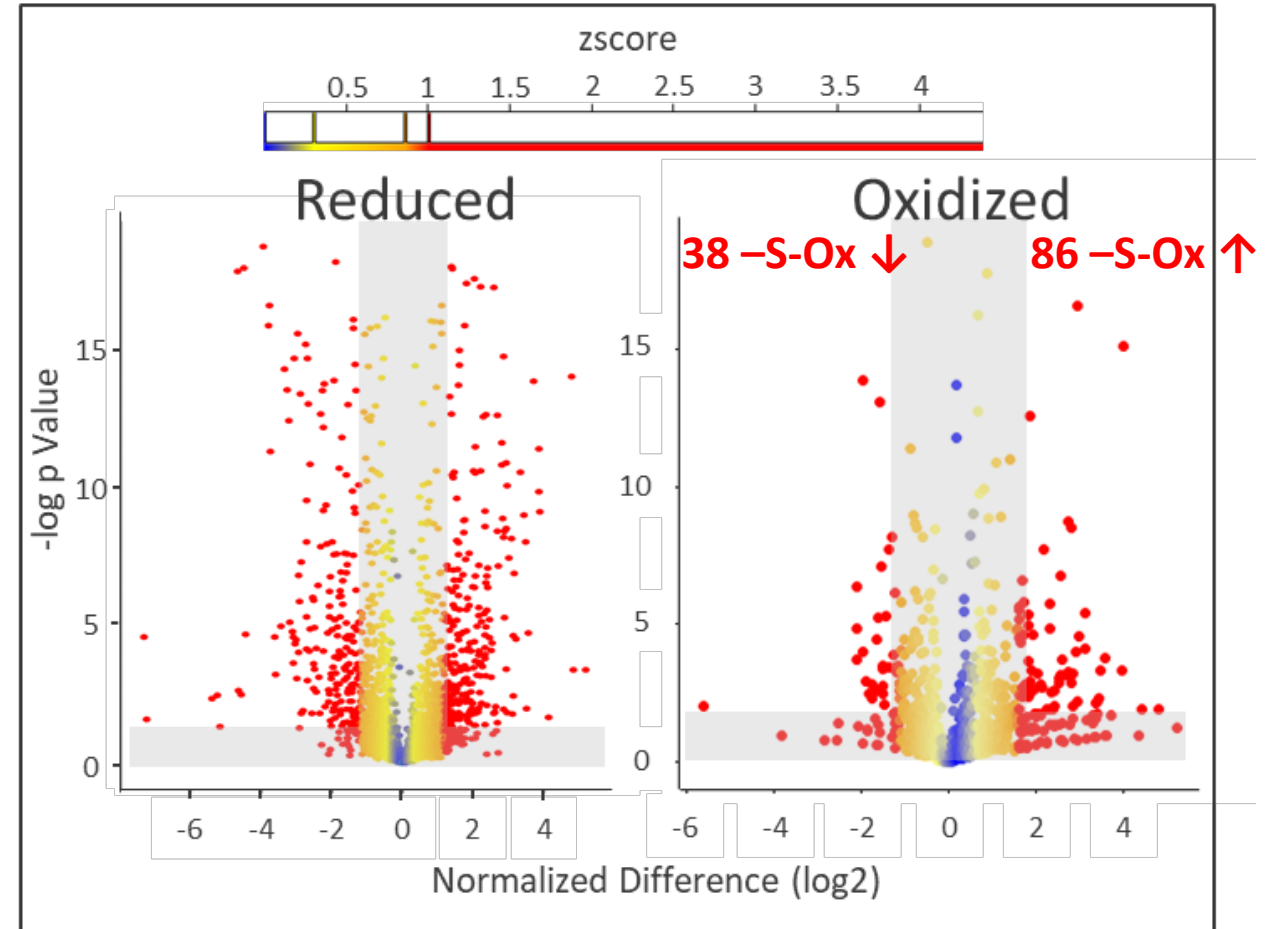
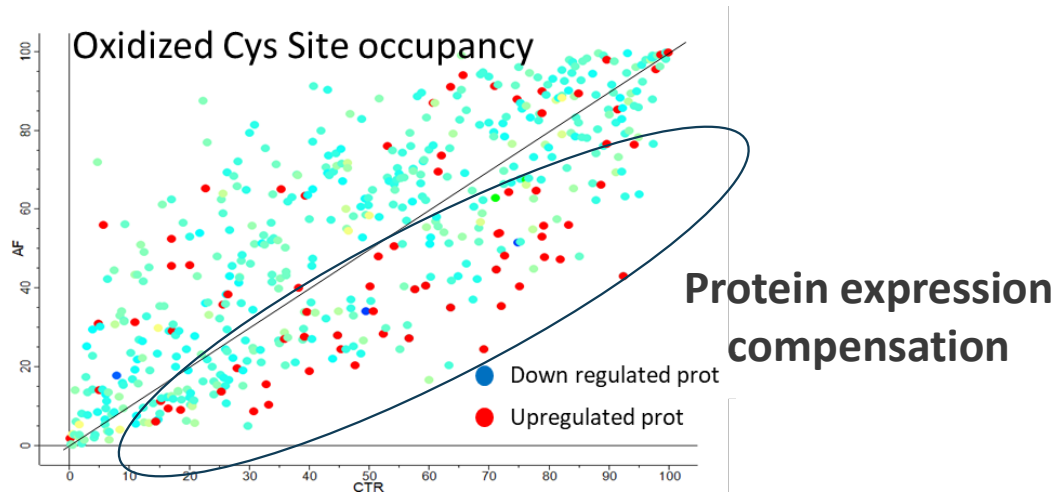
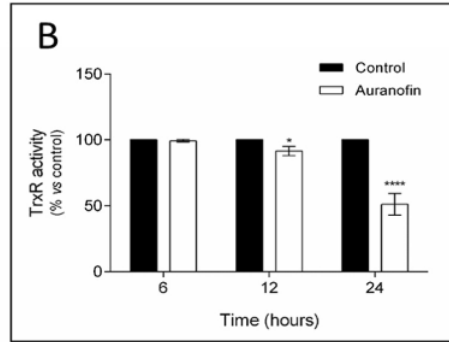
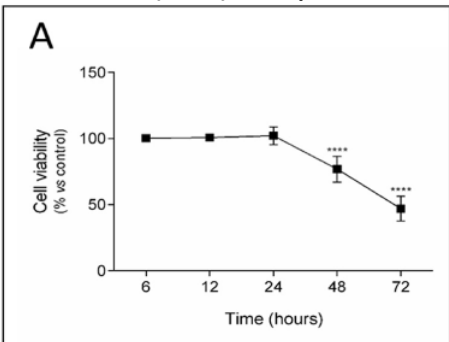


Thiol Redox Levels at 24h

Cell survival
IC₅₀(72h)=0.7μM

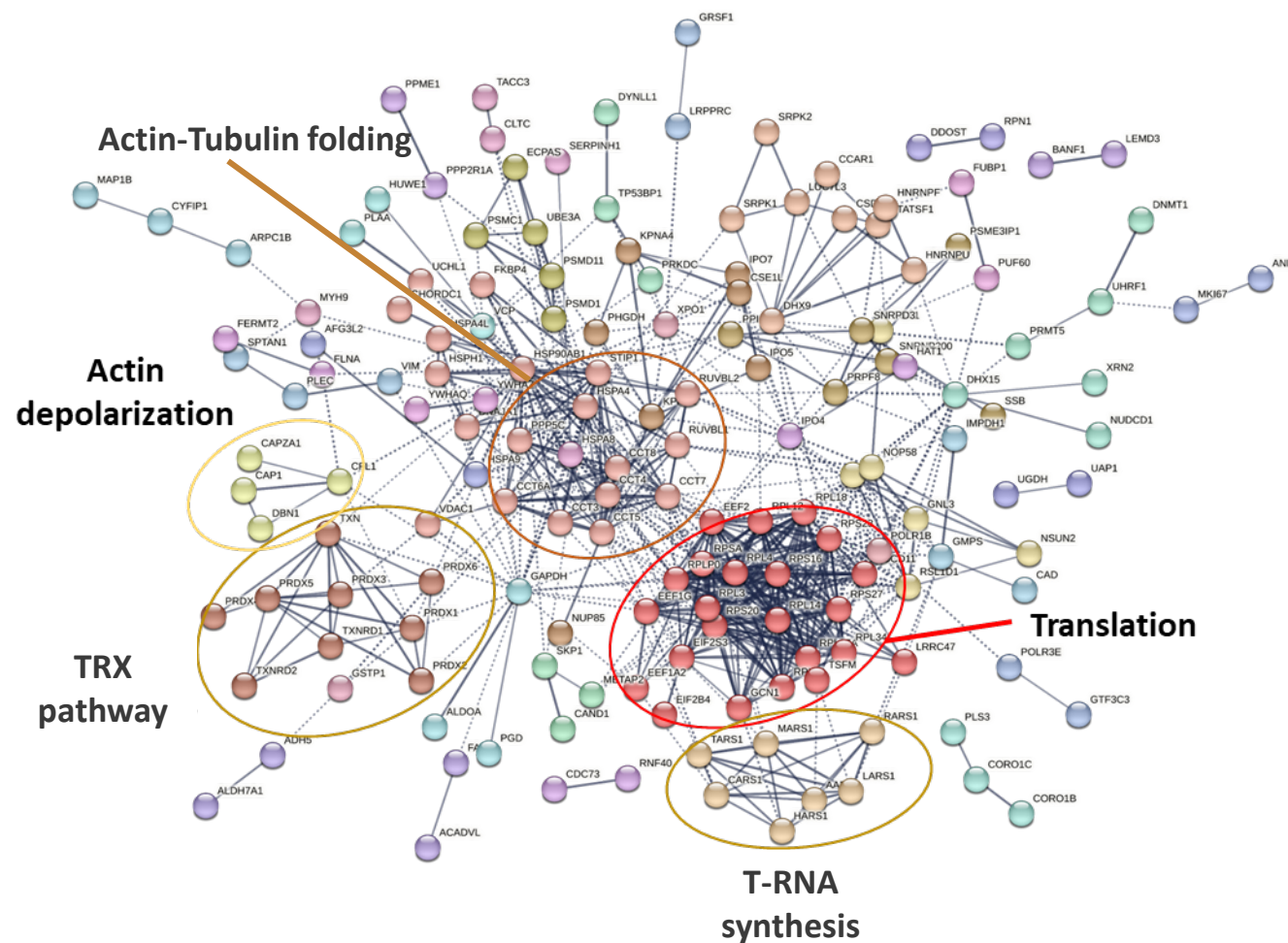
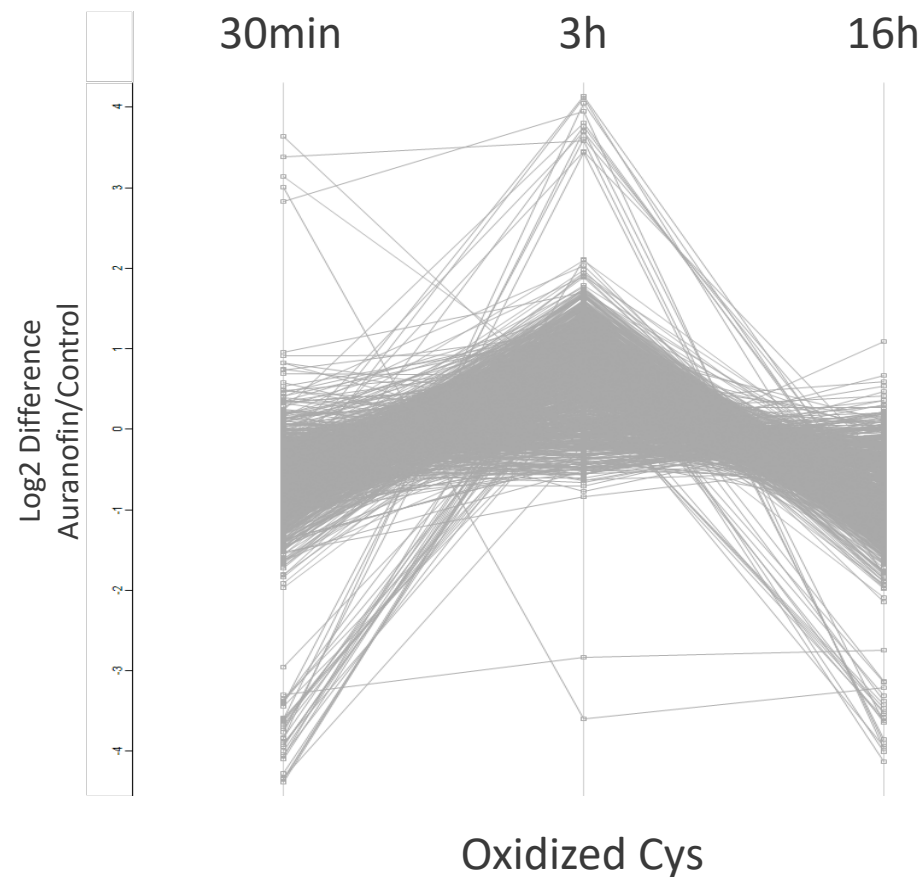
TrxR activity

Cysteine Oxidation



Chiappetta G. et al., *Redox Biol.* 2022; 52: 102294

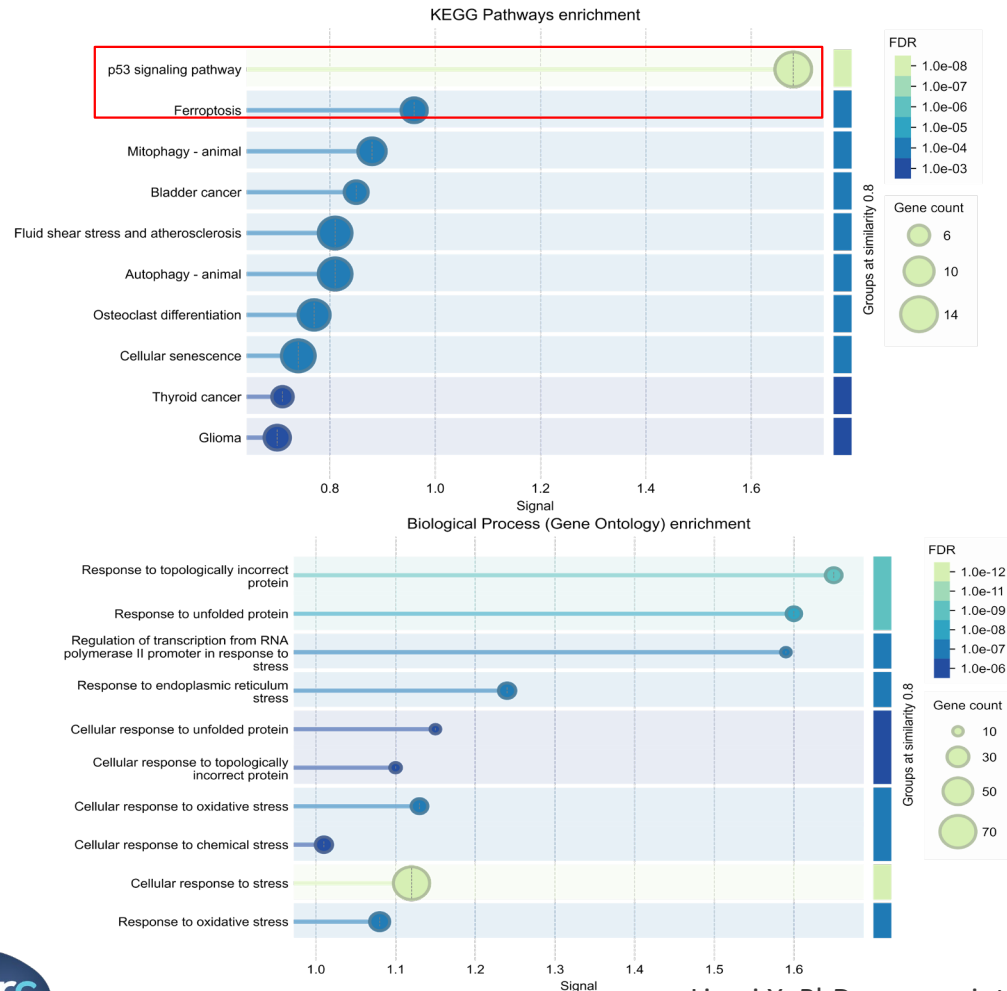
Auranofin Induces a Fast Oxidative Burst



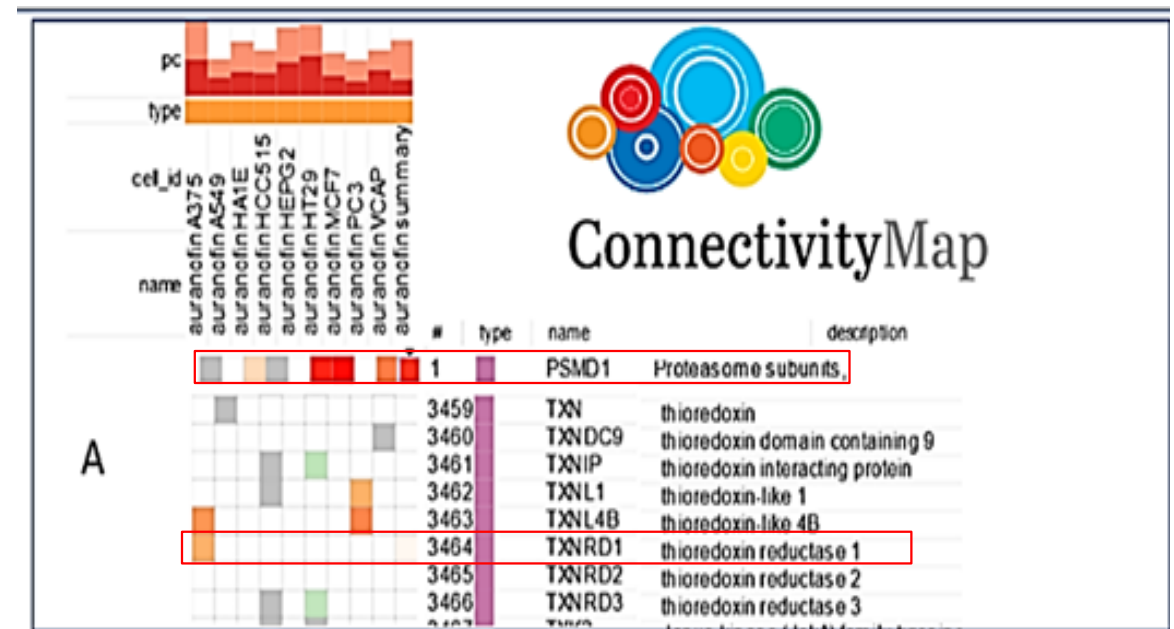


Auranofin Induces ER stress-UPR and Ferroptosis

Protein expression at 24h



Auranofin correlates with Proteasome inhibitors



Gamberi T., et al. *Med Res Rev.* 2022; 42(3): 1111–1146



Metallomics Detection of Auranofin Protein Targets

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Analytical Chemistry

A Metallomics Strategy to Reveal Protein Targets of Auranofin through Direct and Indirect Detection of Gold–Protein Adducts

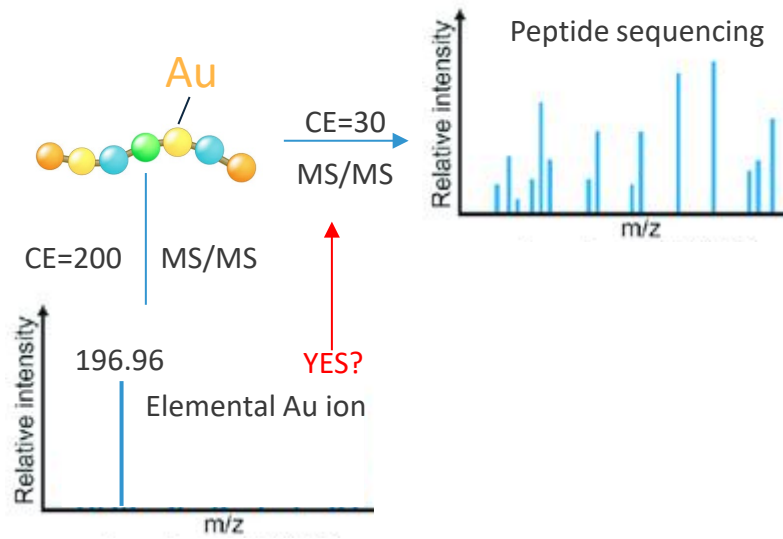
06 August 2025, Version 1

Working Paper

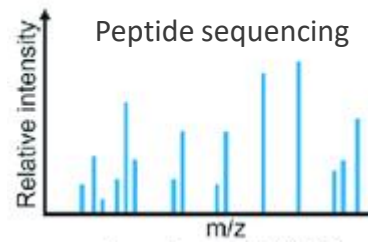
[Giovanni Chiappetta](#) ^{id}, [Xhesika Limaj](#), [Madeleine Frelon](#), [Joelle Vinh](#) [Show author details](#) [▼]

Identification of AF target of an *in vitro* labeled protein cell extract

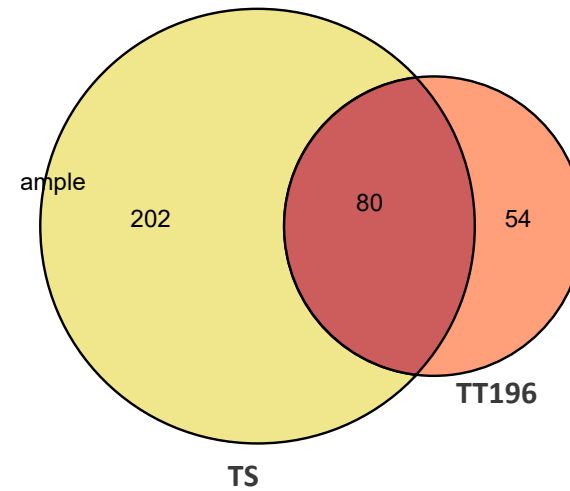
- **TS:** Top speed (≈ 40 precursors/sec)
- **TT196:** Tandem Trigger



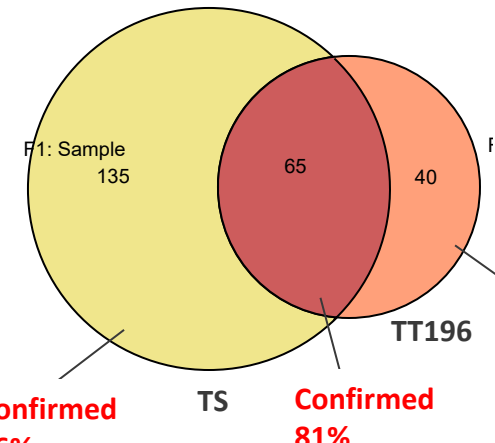
Tandem Mass Trigger Method



Detected Gold-peptides



**Higher Quality MS/MS spectra
Xcorr (SEQUEST) > 3**



**Confirmed
66%**

**Confirmed
81%**

**Confirmed
74%**

- does not improve the identification rates
- produces more robust identifications
- provides complementary identifications

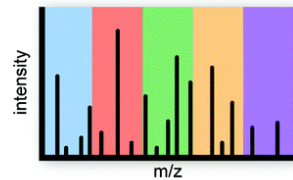
Identification of AF target *in cellulo*

NO CONFIDENTLY IDENTIFIED GOLD-PEPTIDES

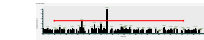
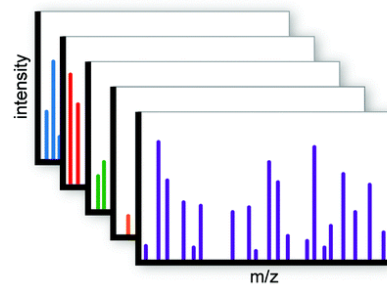
High Energy DIA

DIA-MS

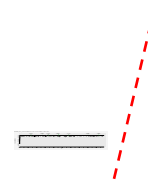
survey scan
across all isolation windows



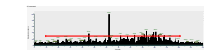
fragmentation of
all precursors in each window



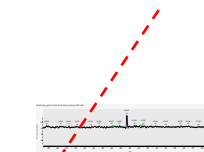
XIC 196.96



Untreated cells

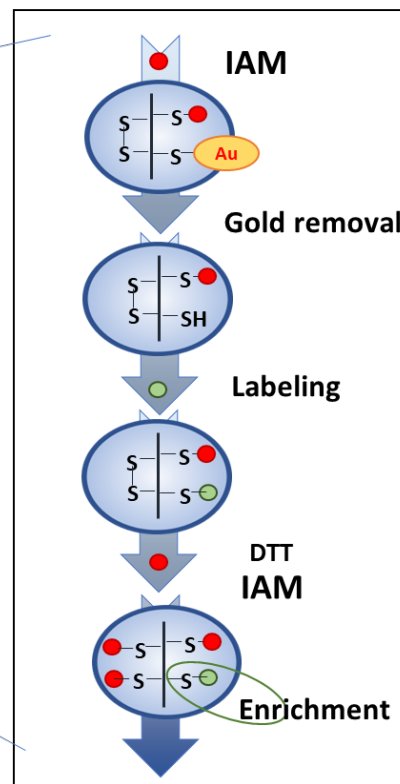


XIC 196.96



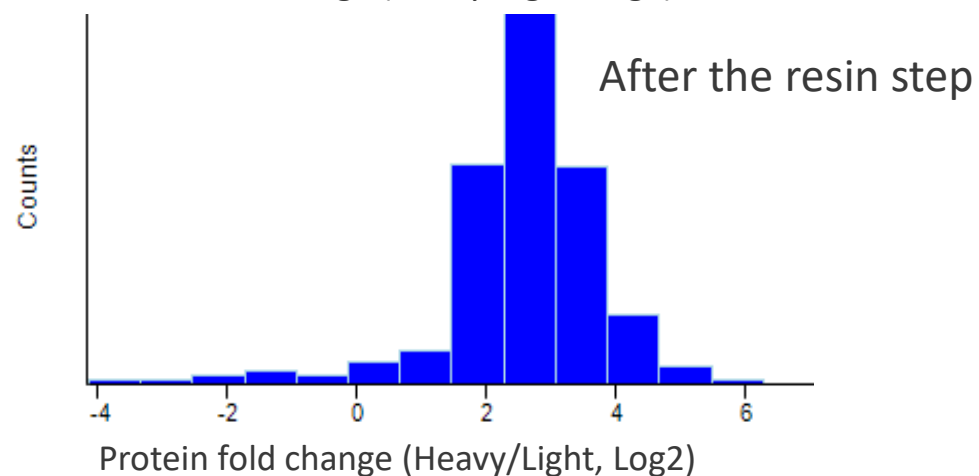
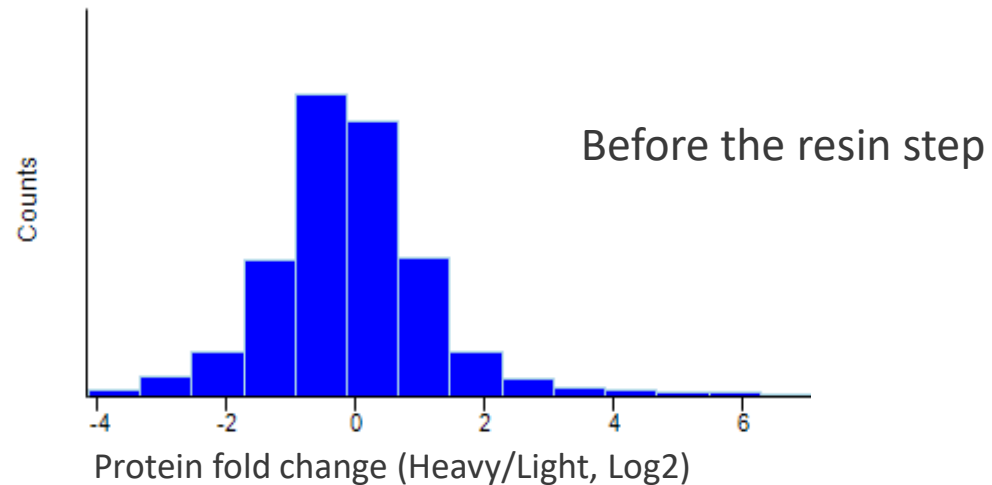
S/N=2...LOD

AF cells



Gold Speciation of Auranofin Treated Ovarian Cancer Cells (A2780)

Auranofin *in cellulo* (Heavy cells + Auranofin vs Light cells)

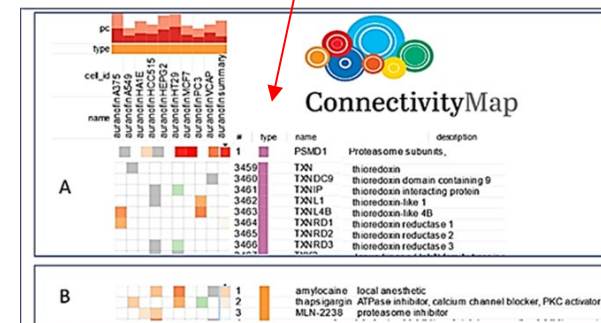


Auranofin targets

Fasta headers	MEDIANE
sp P51784 UBP11_HUMAN Ubiquitin carboxyl-terminal hydrolase 11 OS=H	12.9701212
sp Q16881 TRXR1_HUMAN Thioredoxin reductase 1, cytoplasmic OS=Hom	11.6212008
sp Q5JTH9 RRP12_HUMAN RRP12-like protein OS=Homo sapiens OX=9606	10.6248799
sp O60884 DNJA2_HUMAN DnaJ homolog subfamily A member 2 OS=Hom	9.6211857

...about 100 candidate targets

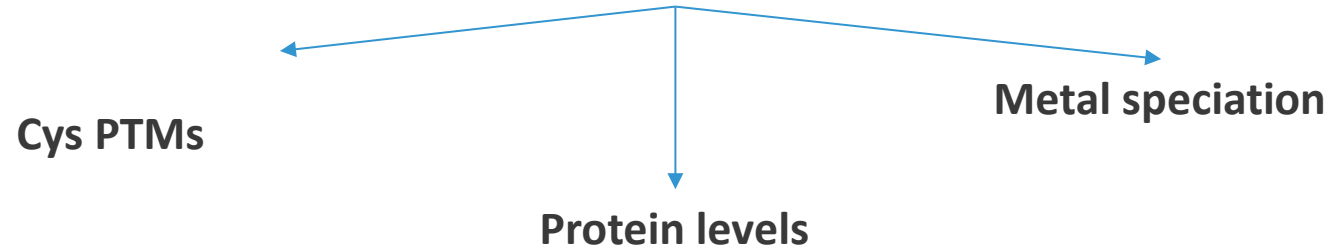
Enrichment FDR	nGenes	Pathway Genes	Fold Enrichment	Pathways (click for details)
1.4E-06	6	46	30.7	Proteasome
3.6E-04	4	44	21.4	Aminoacyl-tRNA biosynthesis
2.6E-02	2	22	21.4	Histidine metabolism
1.0E-06	8	108	17.4	Nucleocytoplasmic transport



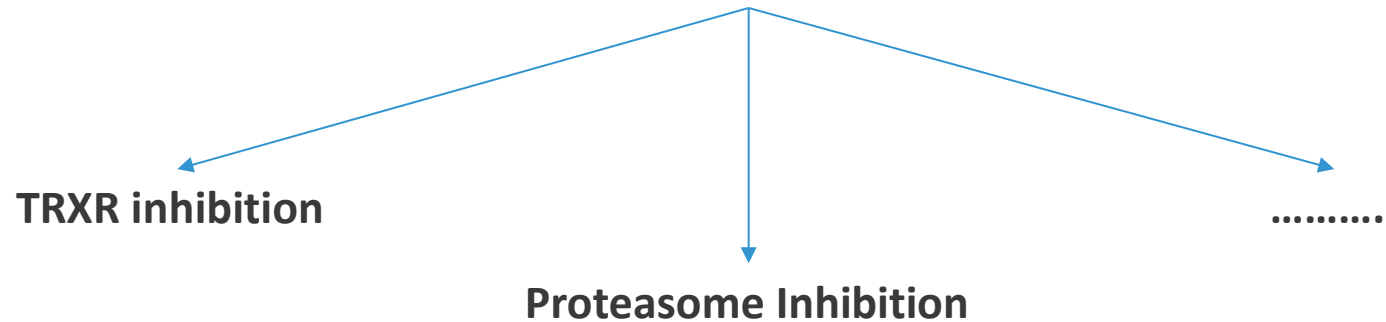


Conclusions

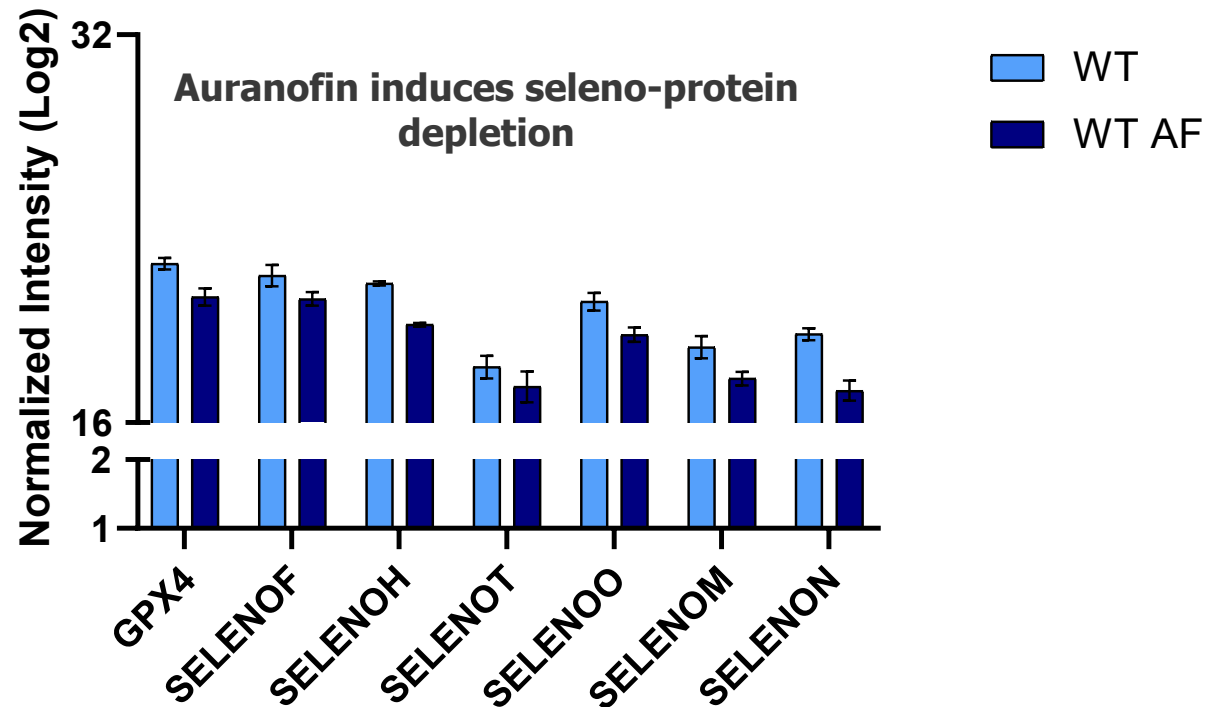
Proteomics is a flexible tool



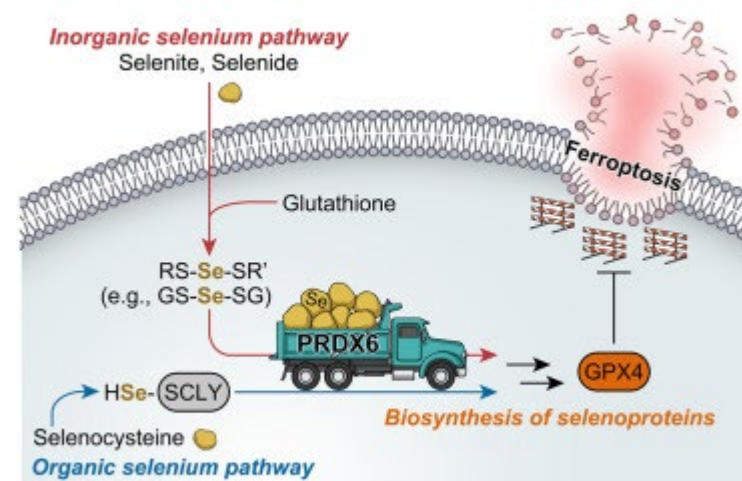
Auranofin has a multitarget mechanism



PRDX6 is an Auranofin Target: Implications for Ferroptosis



Limaj X. PhD manuscript, Dec. 2024



Ito J., et al., *Mol Cell*. 2024 Dec 5;84(23):4629-4644.e9.

IS PRDX6 INHIBITION BY AURANOFIN THE TRIGGER OF FERROPTOSIS ?



Thank you for the attention!



Joelle Vinh
Yann Verdier
Iman Haddad
Emmanuelle Demey
Xhesika Limaj
Sai Nagendra
Madeleine Frelon