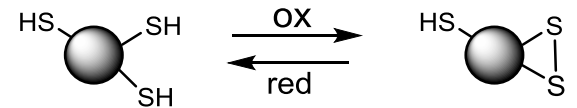


***Cysteine modification of proteins:
Studies of protein oxidation and reduction (redox),
AND modification by catechols and glutathione***



WILLIAM & MARY

founded in 1693



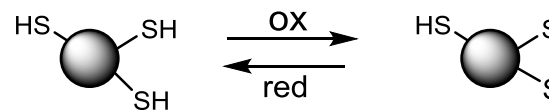
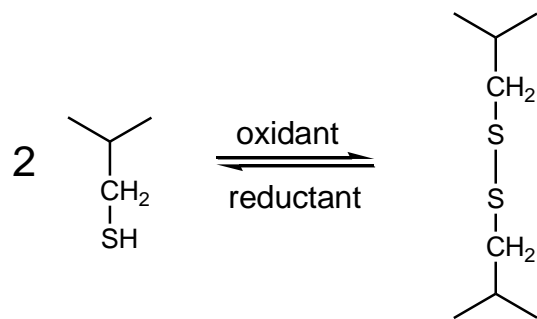
Professor Landino

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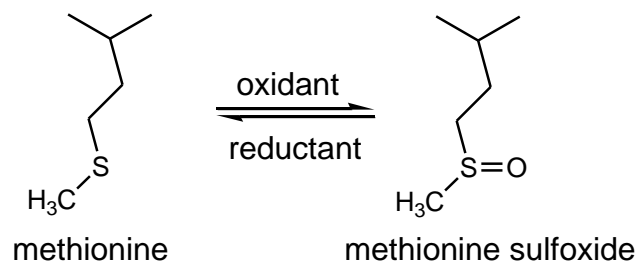
Which amino acids of proteins undergo redox reactions?

Reversible modification of a protein that changes its structure and consequently, its function

cysteine oxidation/reduction



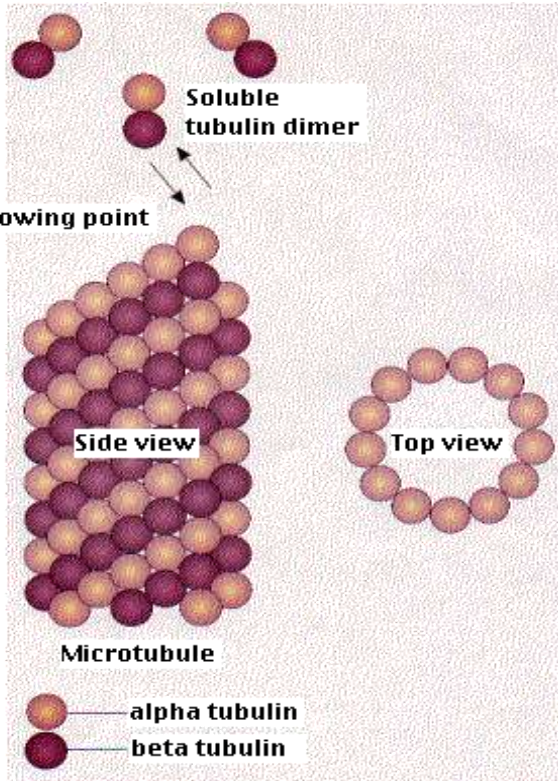
methionine oxidation/reduction



Redox regulation is a delicate balance. Too much oxidation could lead to irreversible damage...

Why were we interested in redox modifications of microtubule proteins?

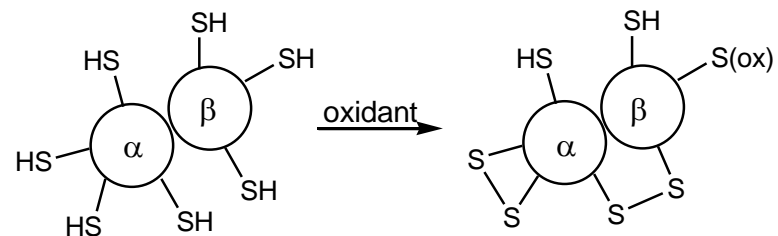
(in the beginning...)



Tubulin is abundant in neurons (10-15% of total protein); therefore, a likely candidate for modification by oxidants.

Easy to purify from brain tissue in good yield and functional form.

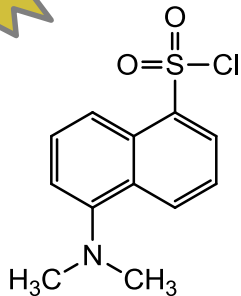
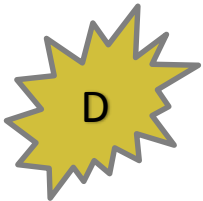
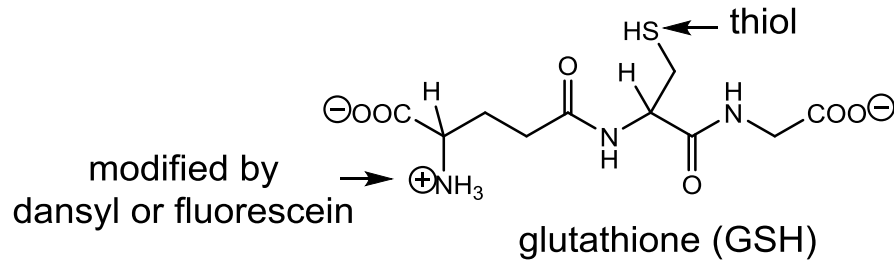
Porcine brain tubulin contains 20 free cysteines – the most easily oxidized amino acid in proteins.



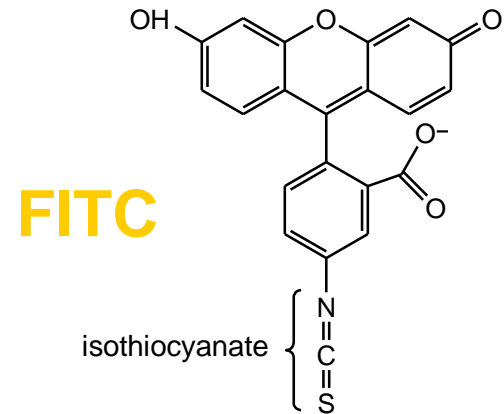
Tau, a neuron-specific MAP, is the major component of paired helical filaments found in neurofibrillary tangles in Alzheimer's disease brain. Furthermore, oxidative damage to proteins may contribute to neurodegeneration.

What is cysteine modification by catechols and glutathione?

Glutathione



dansyl chloride

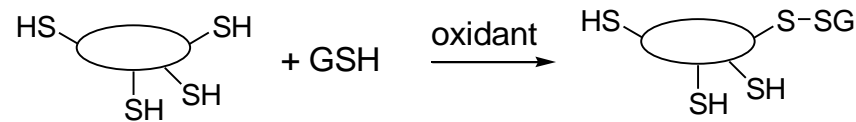


FITC = fluorescein isothiocyanate

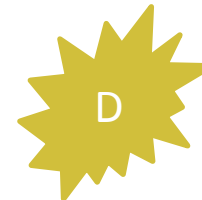
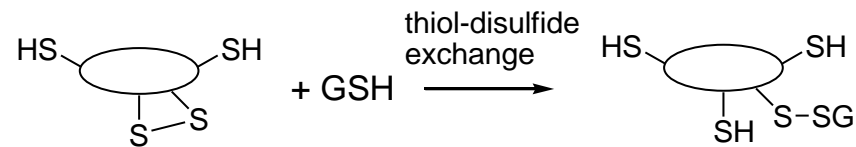
This is a synthesis and purification project.

How do we attach GSH to proteins via their cysteine thiols?

Reaction 1

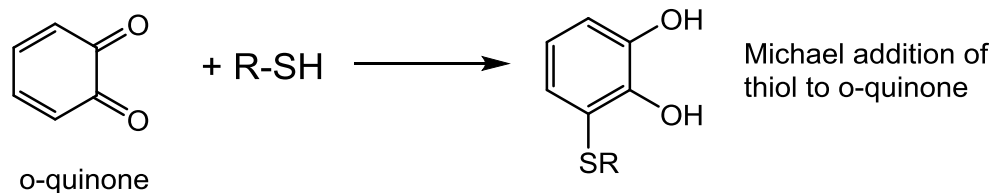
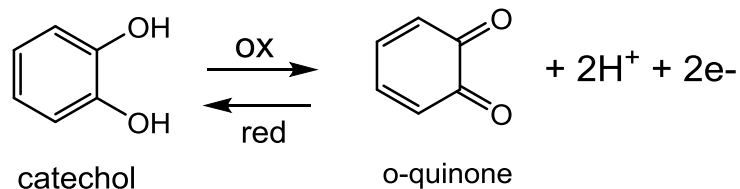


Reaction 2

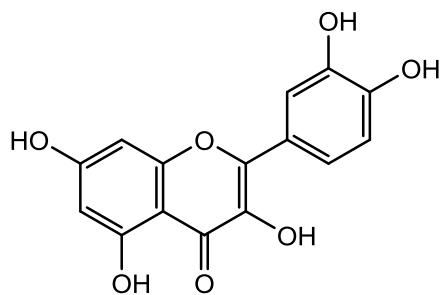


If using D-GSH or F-GSH, protein becomes fluorescent!

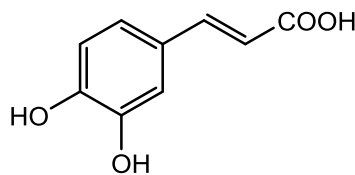
What is cysteine modification by catechols?



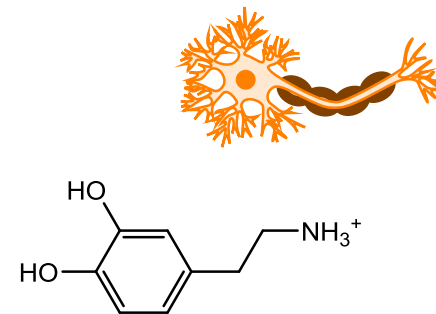
many more molecules to explore!



quercetin
abundant dietary flavinoid

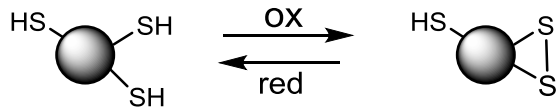


caffeic acid
present in all plants

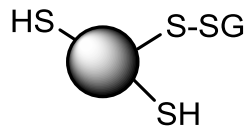


dopamine
neurotransmitter

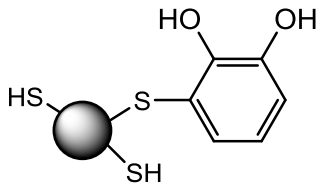
Is modification of protein cysteines by GSH or catechols GOOD or BAD?



Generally, oxidation is BAD and reduction is GOOD



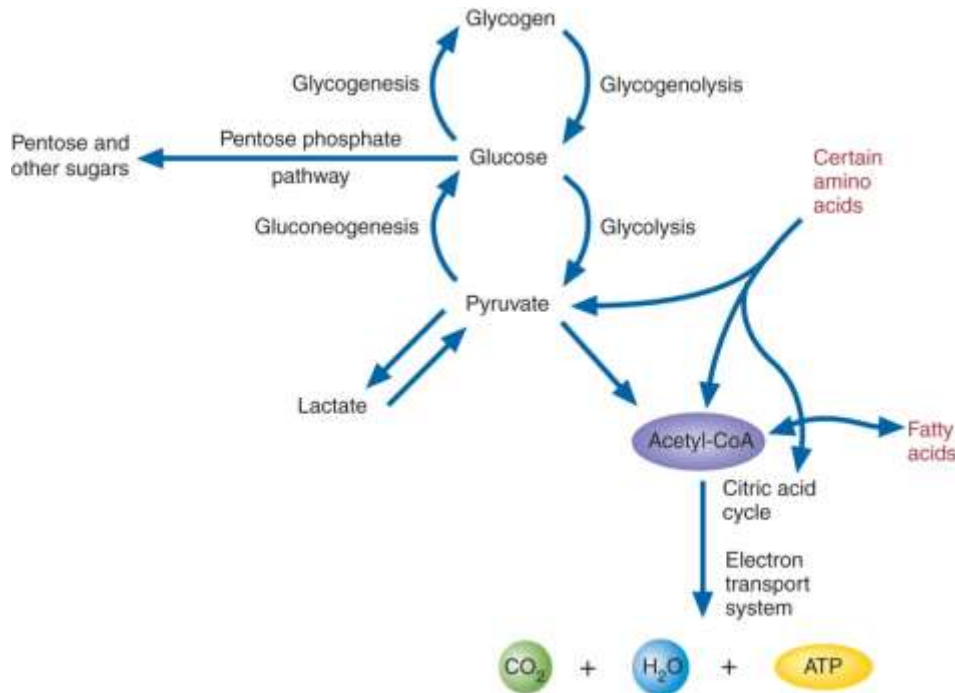
Modification by GSH can protect cysteines from oxidation, so it's GOOD? Even if it's BAD, it can be removed by a reducing agent



Irreversible and therefore BAD if cysteine is required for function

Ignore "scale" – protein is way bigger than catechol

Possible proteins to study: glycolysis pathway



Which ones? How? Who cares?

We are interested in:
pyruvate kinase (#10)
GAPDH (#5)
lactate dehydrogenase (#11)

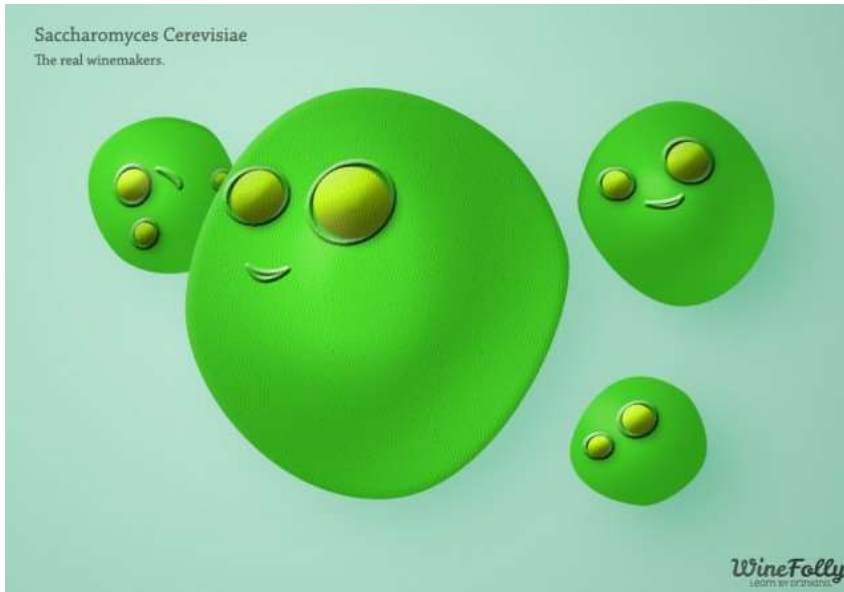
All 3 are positively charged and therefore will bind to negative tubulin.

All have reactive cysteines.

All have been identified as targets for oxidation in proteomic studies.

If neurons can't get energy from glucose metabolism, they may die!

New area: what about studying protein redox reactions, and cysteine modification reactions in yeast?



- Yeast have many of the same proteins that we do!
- Yeast proteins undergo the same types of cysteine modification reactions.
- Perhaps a useful cell model?

- *Saccharomyces cerevisiae* (sugar fungus)
- eukaroyte (has a nucleus)
- “life with 6000 genes”

Skills/techniques used in the Landino lab:

- *Purification of modified GSH/GSSG by column chromatography (C8/C18)*
- *UV/Vis spectroscopy to quantitate and study molecules*
- *Fluorescence spectroscopy to study protein labeling at cysteines & amines*
- *Purification of proteins by chromatography (ion exchange and desalting)*
- *Enzyme kinetics (usually by monitoring absorbance changes)*
- *Growing yeast and preparing cell extracts*
- *Studying protein modification by catechols*