Spectrophotometric methods to determine oxidative damage

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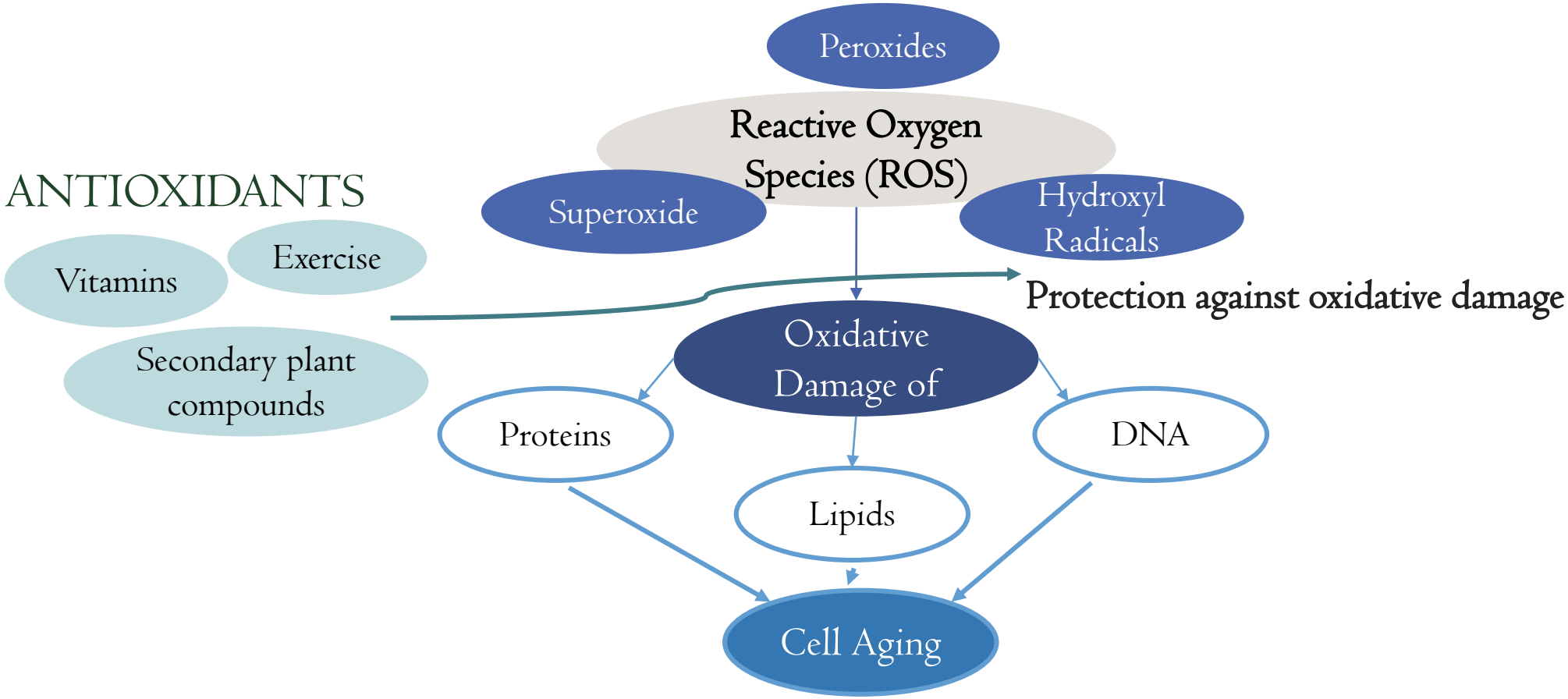
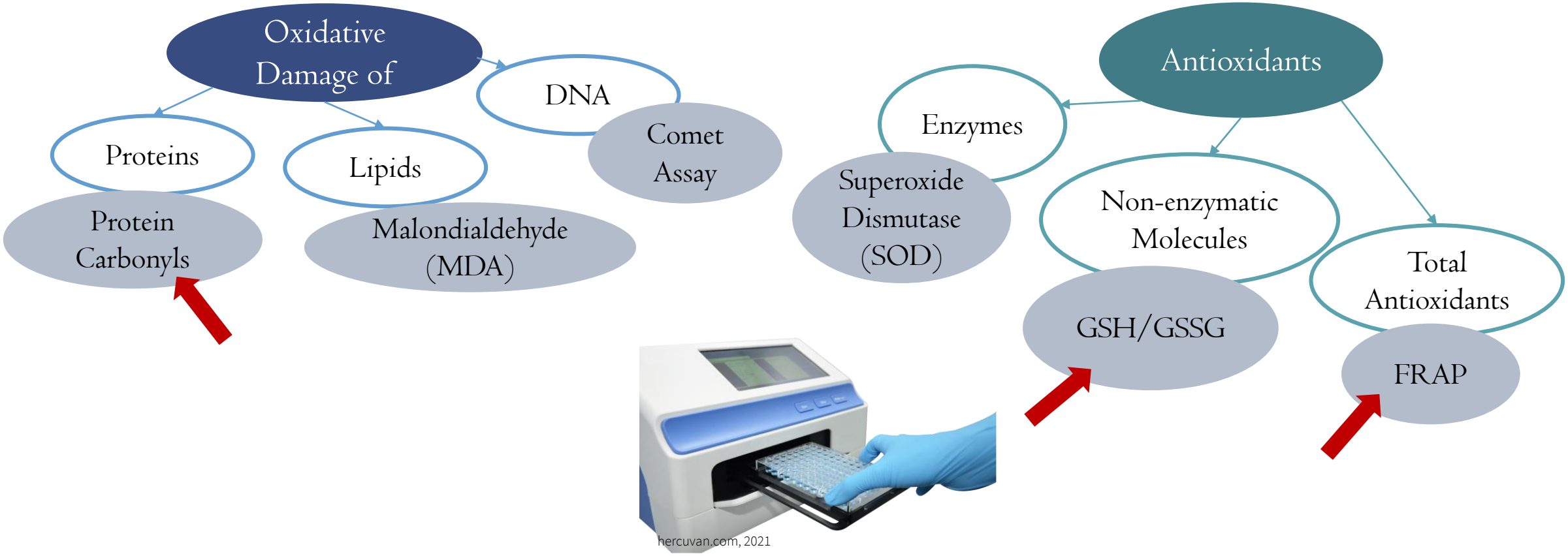
Oxidative Stress Theory of Aging



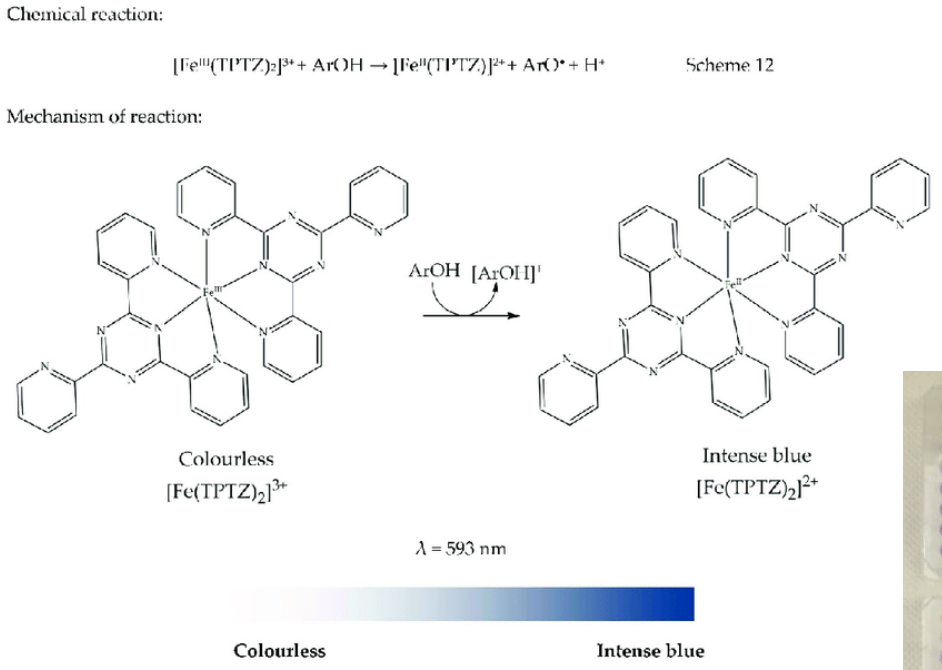
Markers for Oxidative Stress



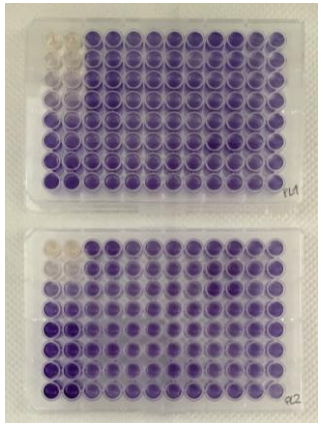
hercuvan.com, 2021

Ferric Reducing Antioxidant Power (FRAP)

- Method established according to Benzie et al., 1996
- Determination of antioxidant capacity of plasma
- Principle: Iron reduction ability of antioxidant substances
- Fe3+-Tripyridyltriazine (Fe3+- TPTZ) complex reduced to Fe2+ → colorless to blue
- Measurement at 593nm



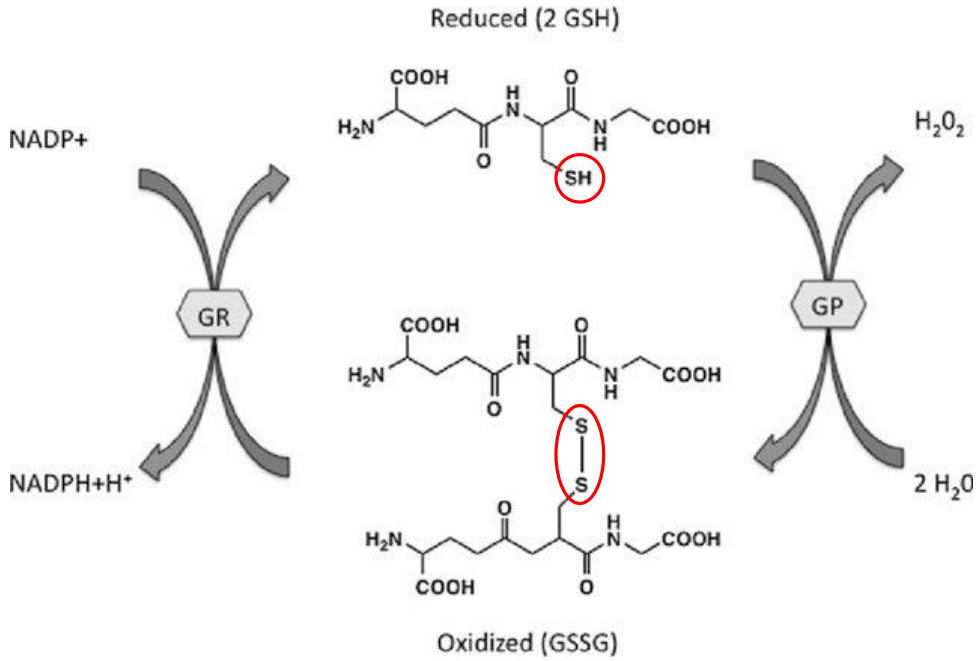
Sadeer, et al., 2020



Measurement of FRAP Assay using microplates

Reduced and Oxidized Glutathione (GSH/GSSG)

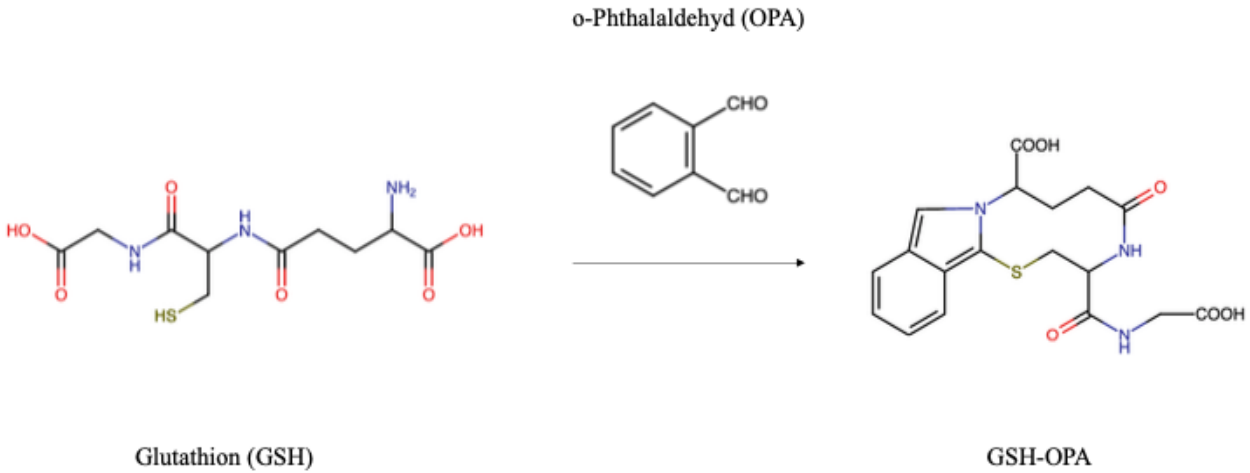
- Thiol proteins: characterized by their free SH group
- Glutathione: can be **oxidized** (GSSG) and **reduced** (GSH)
- Neutralizes ROS
- Ratio between GSH and GSSG commonly used marker for determining antioxidant potential



Glutathione as a biological redox buffer (Xiong et al, 2011)

GSH/GSSG-Assay (Hissin & Hilf 1976)

- Separated measurement of GSH and GSSG
- Fluorescence



Reaction principle of GSH measurement. Binding of the fluorescence reagent OPA to the SH group of GSH.

Reduced Glutathion (GSH) - Method

TCA treated plasma sample

↓

Measurement of GSH

↓

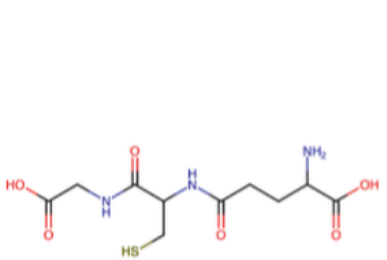
pH 8.0

↓

+ o-Phthaldialdehyd (OPA)
→ 30 min incubation

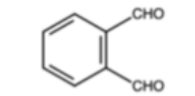
↓

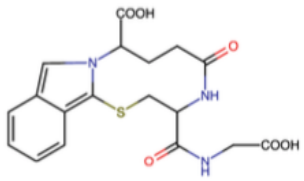
Fluorescence measurement



Glutathion (GSH)

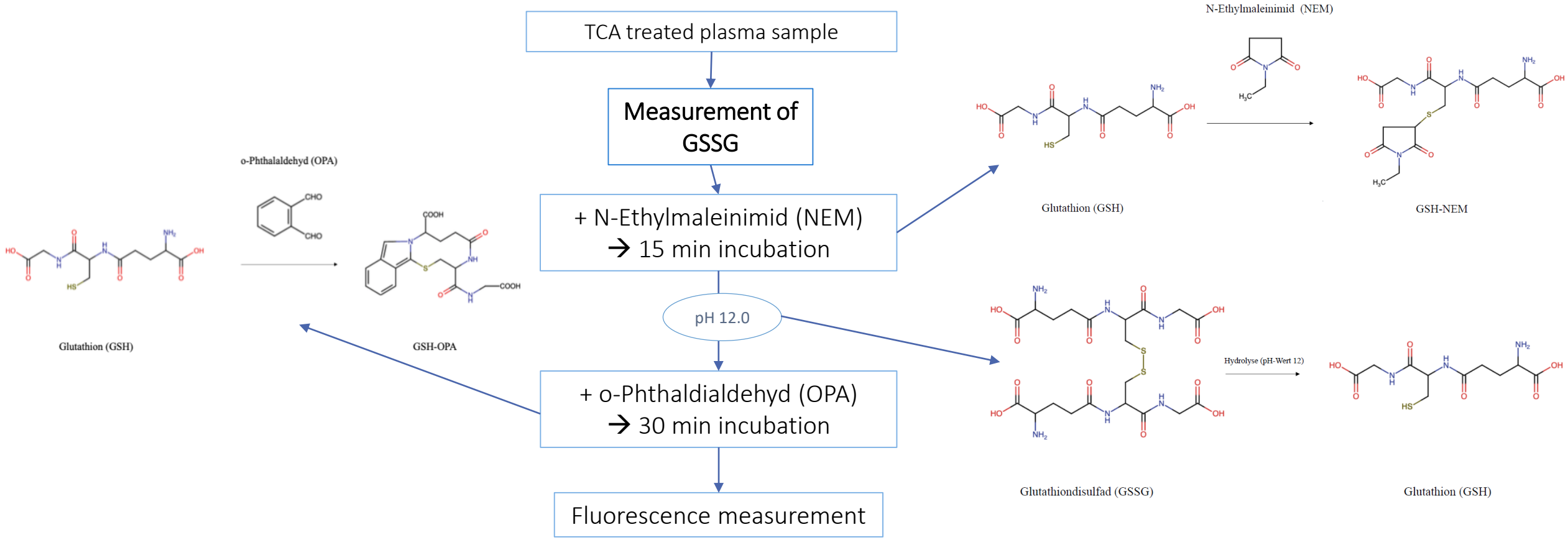
o-Phthalaldehyd (OPA)





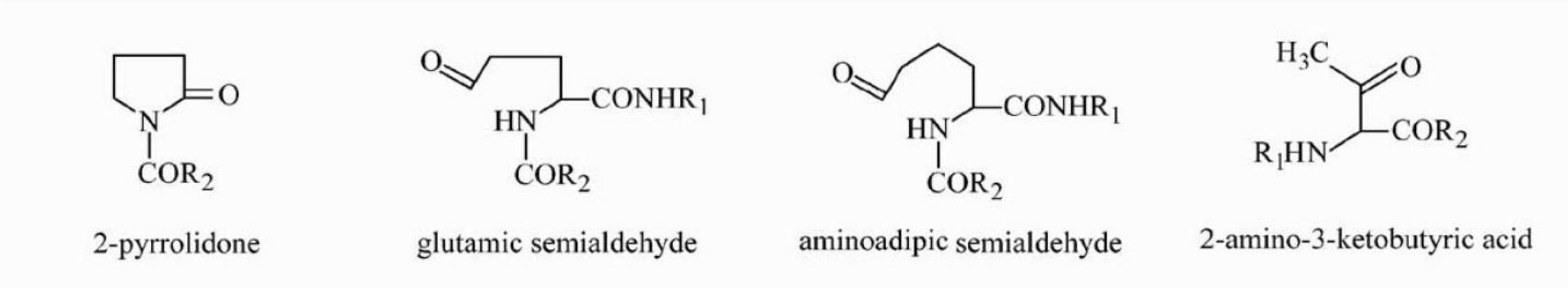
GSH-OPA

Oxidized Glutathion (GSSG) - Method



Protein Carbonyls – Background Information

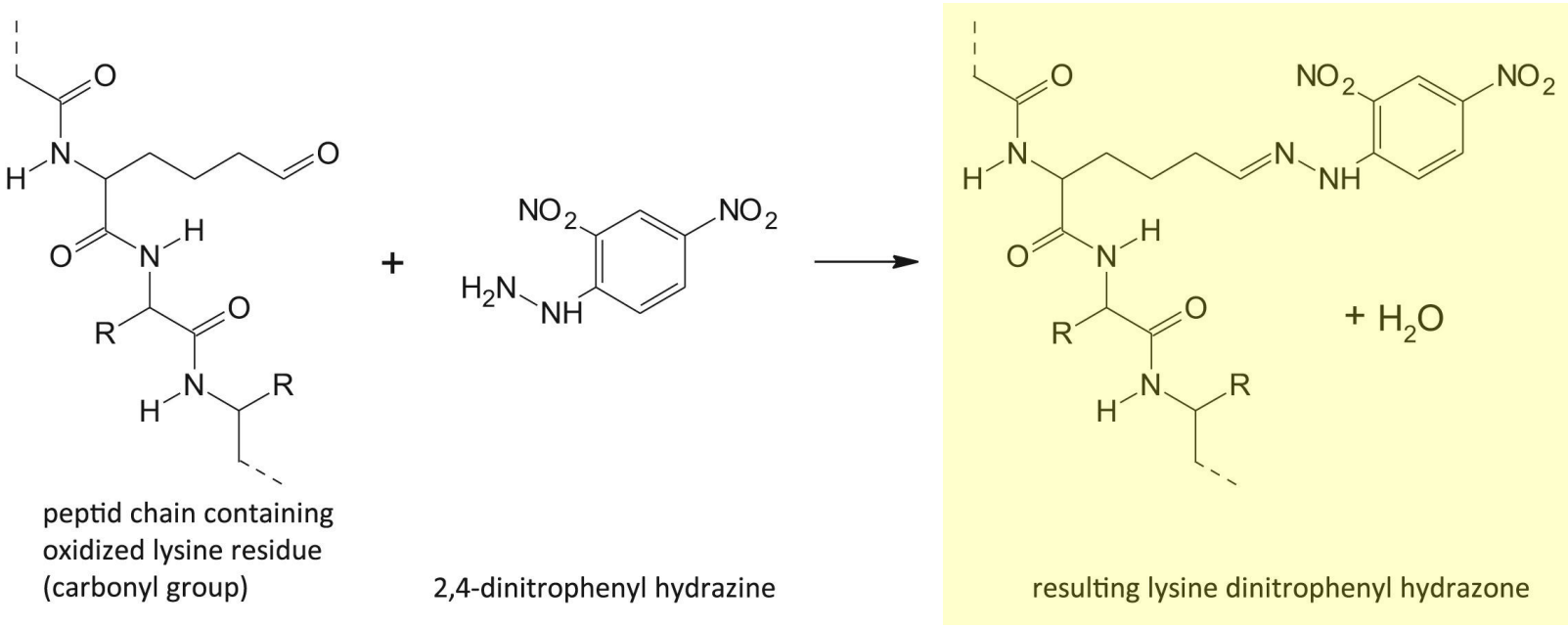
- Protein carbonyl content: commonly used marker for protein oxidation
- Irreversible, chemically stable, oxidative modification



The structure of carbonyl derivatives produced by direct oxidation of amino acid side chains: 2-pyrrolidone from prolyl residue, glutamic semialdehyde from arginyl and prolyl residue, a-aminoadipic semialdehyde from lysyl residue, and 2-amino-3-ketobutyric acid from threonyl residue (Dalle-Donne, 2003)

Protein Carbonyls – Method

- PC determination according to Levine et al., 1990
- Derivatization of the carbonyl groups using Dinitrophenyl hydrazine (DNPH)



Reaction of protein carbonyl group with 2,4-dinitrophenylhydrazine (Weber, 2015)

Protein Carbonyls - Protocol

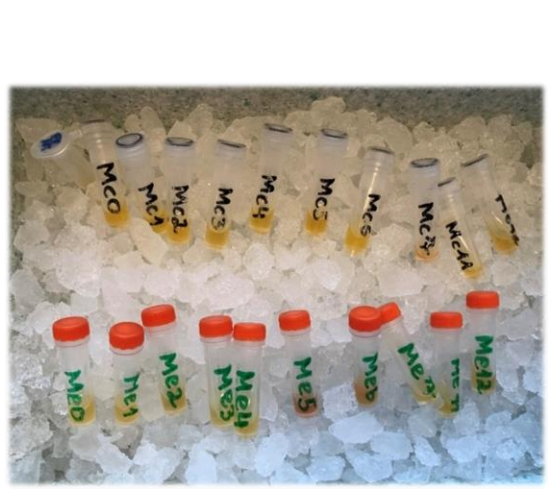
Albumin standard

2 Tubes per sample:

→ Protein measurement at 276nm (UV)

→ Carbonyl measurement at 350nm

1. Derivatization with DNPH
2. Precipitation with trichloroacetic acid
3. Washing steps (3) with ethanol-ethyl acetate
4. Dissolve in Guanidin buffer
5. Spectrophotometric measurement



Multi-Mode-Mikroplate-Reader SpectraMax M3

Thank you for your attention!