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IN SILICO PREDICTION OF ANTIDEPRESSANT-BINDING SITES ON HUMAN GLUTATHIONE REDUCTASE

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Antidepressants

Depression is the oldest and most common psychiatric condition, and is associated with significant disability.

Antidepressants are widely used treatments for major depressive disorder.

They are grouped into various classes of drugs, with slightly different mechanisms of action:

1. selective serotonin reuptake inhibitors or SSRIs (*e.g.* fluoxetine and sertraline)
2. tricyclic antidepressants or TCAs (*e.g.* amitriptyline and clomipramine)
3. alternative/nontraditional antidepressants (*e.g.* hypericin and pseudohypericin)
4. others

Glutathione reductase

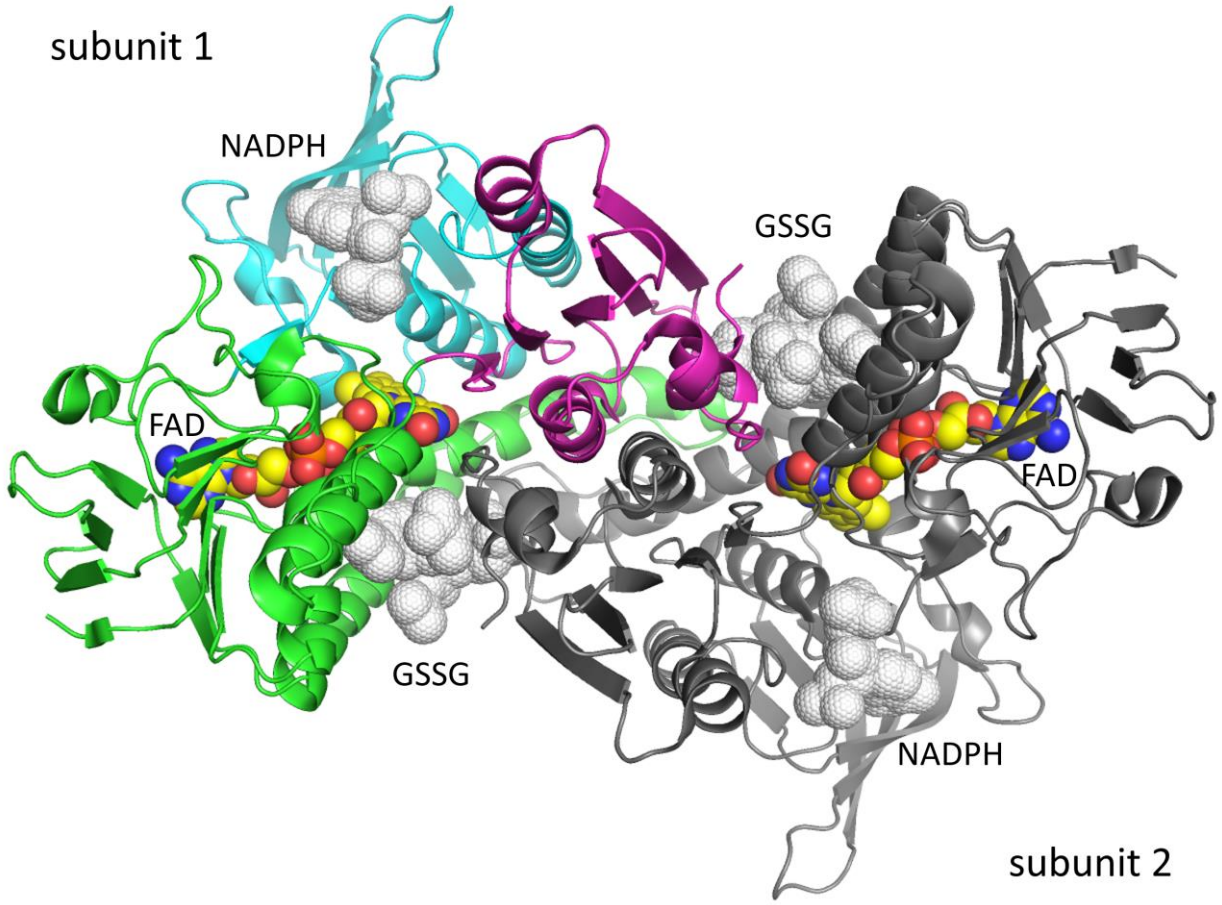
Glutathione reductase (GR; EC 1.6.4.2) is an enzyme belonging to the flavoprotein disulfide oxidoreductase family.

It reduces GSSG to GSH at the expense of NADPH, providing cells with a high intracellular GSH/GSSG ratio:

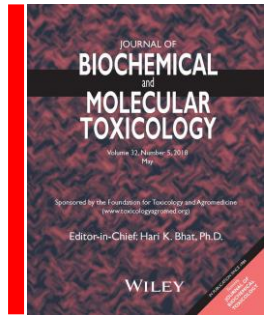
$GSSG + H^+ + NADPH \rightarrow 2GSH + NADP^+$

Human erythrocyte GR is composed of two 50-kDa monomers, each with an FAD-binding domain, an NADPH-binding domain, and a dimerization domain.

Glutathione is bound to the FAD-binding domain of one subunit and the dimerization domain of the other subunit.



Kinetic research on GR from baker's yeast

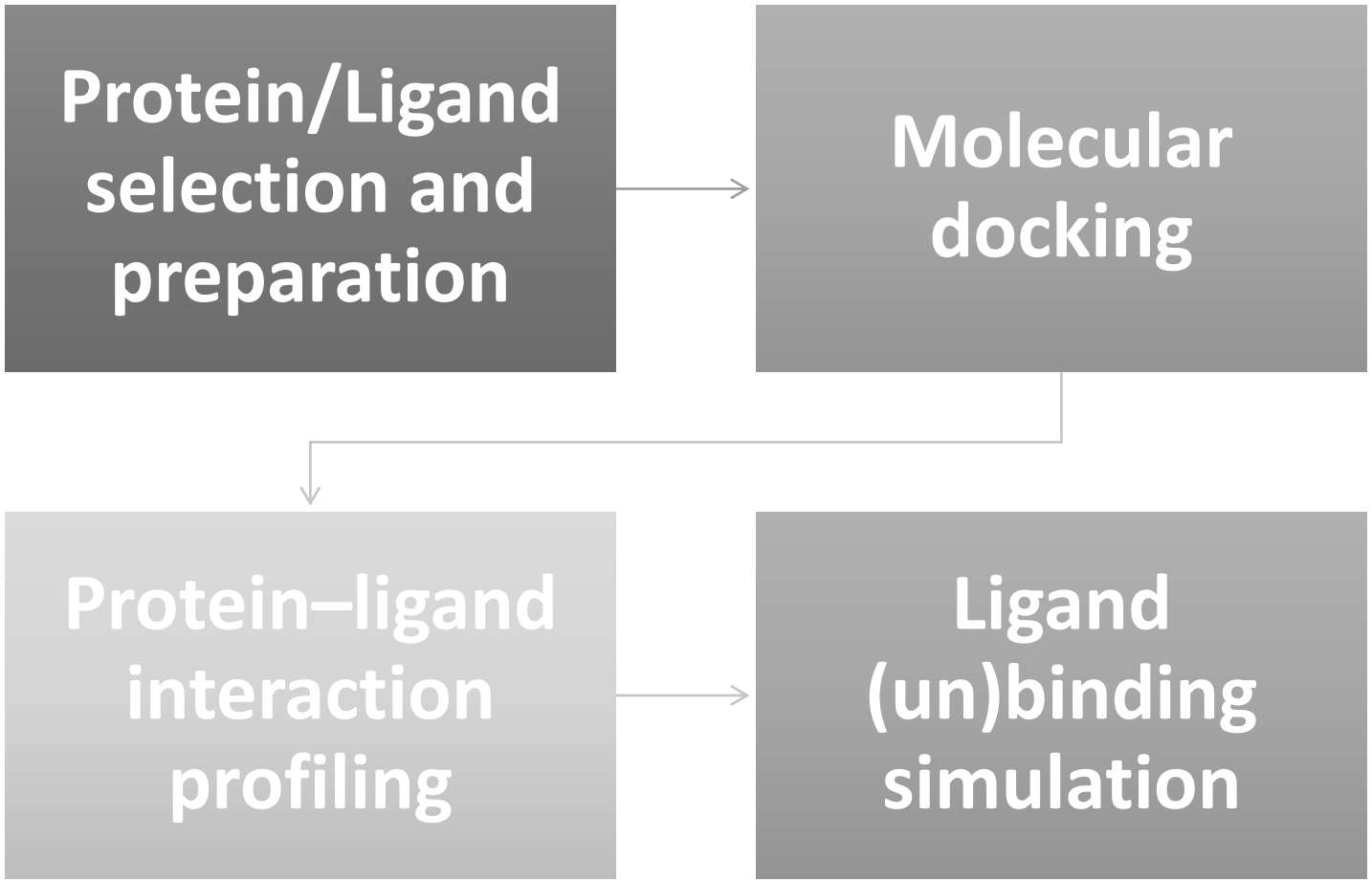


Antidepressant	Variable substrate	Type of inhibition	Potency
Hypericin	i. GSSG ii. NADPH	i. Competitive ii. Linear mixed-type competitive	i. K_i = 2.92 ± 0.73 µM ii. K_i = 2.63 ± 0.50 µM; α = 3.48 ± 1.31
Fluoxetine	i. GSSG ii. NADPH	i. Linear mixed-type competitive ii. Non-competitive	i. K_i = 279 ± 32 µM; α = 5.48 ± 1.29 ii. K_i = 879 ± 82 µM

Dalmizrak, O., Teralı, K., Abdullah, R.K. & Ozer, N. 2018, "Mechanistic and structural insights into the *in vitro* inhibitory action of hypericin on glutathione reductase purified from baker's yeast", *Journal of Biochemical and Molecular Toxicology,* vol. 32, no. 5, p. e22051.

Dalmizrak, O., Teralı, K., Asuquo, E.B., Ogus, I.H. & Ozer, N. 2019, "The relevance of glutathione reductase inhibition by fluoxetine to human health and disease: insights derived from a combined kinetic and docking study", *The Protein Journal,* vol. 38, no. 5, pp. 515–524.

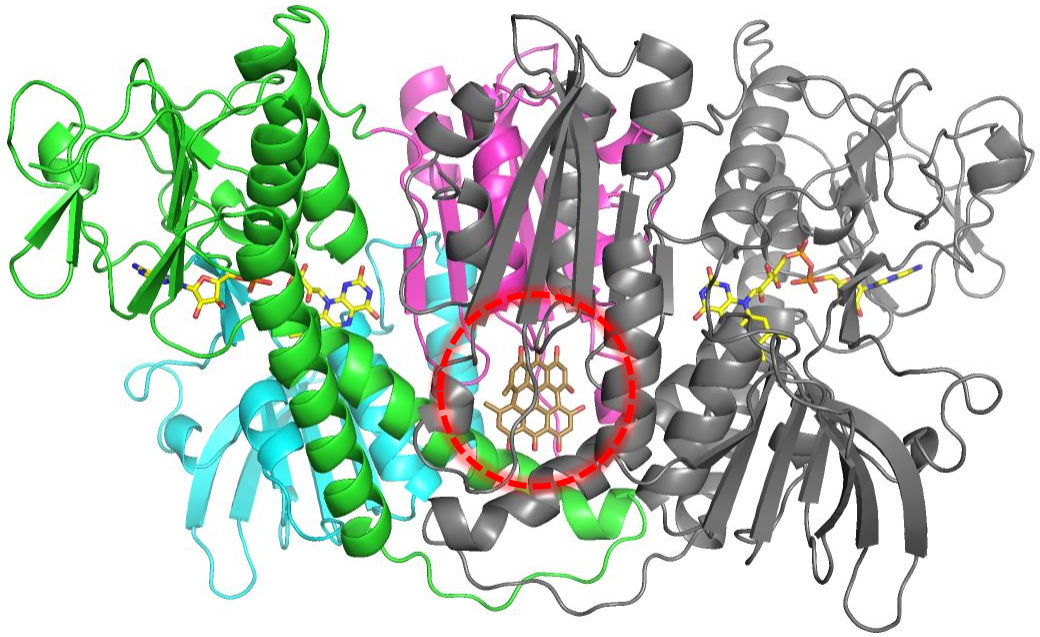
Methodology

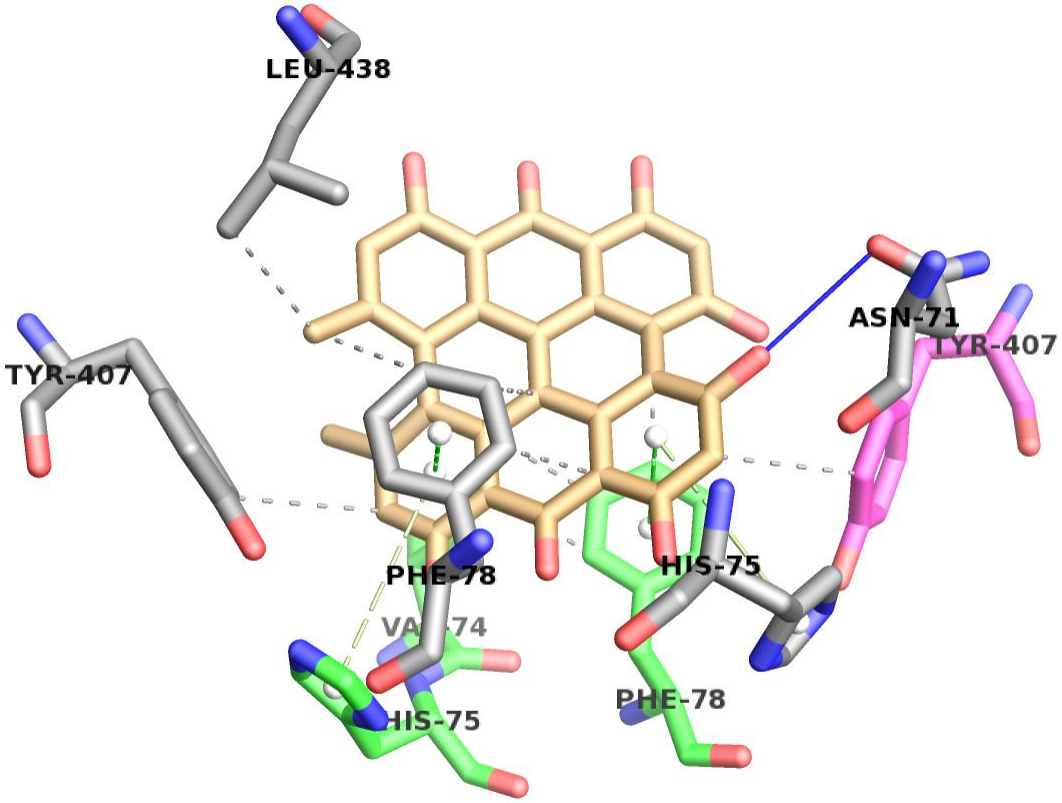


Redocking and cross-docking results

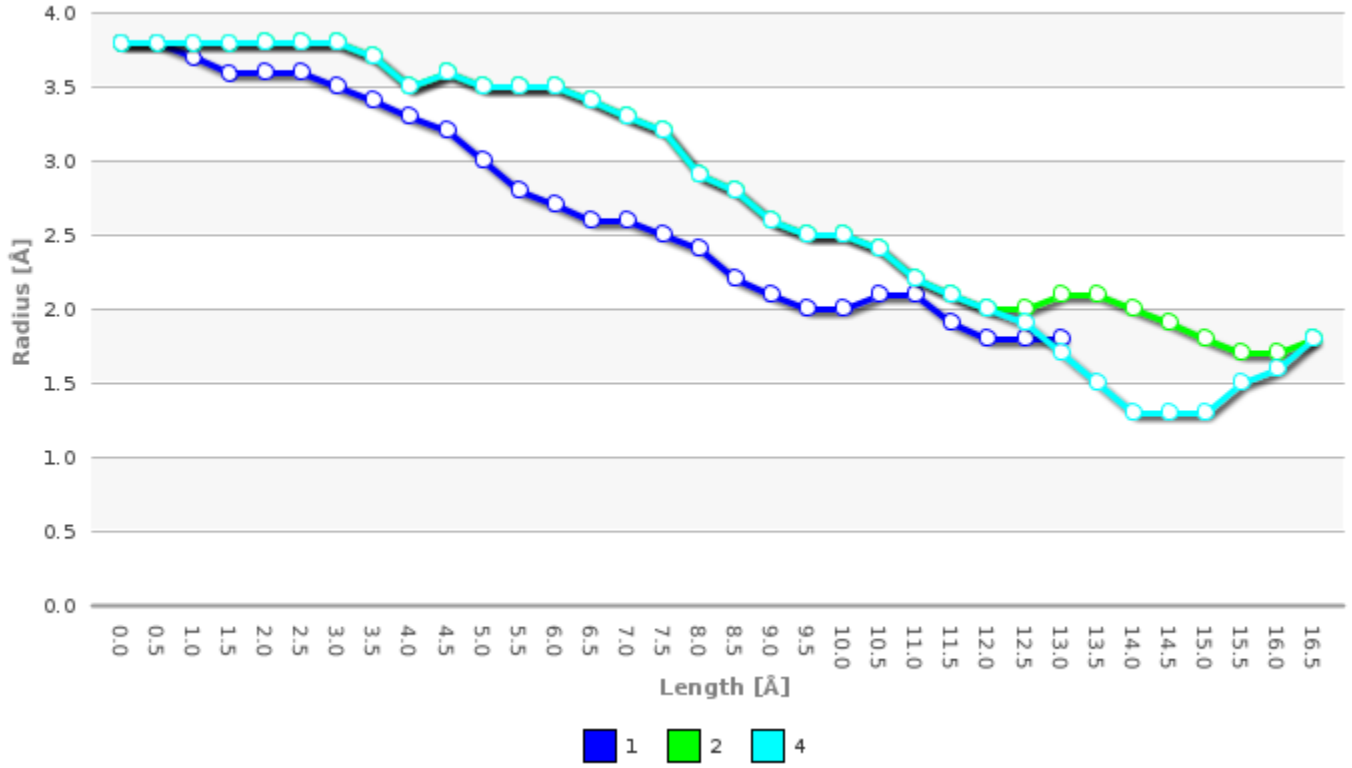
Ligand	Binding energy (in kcal mol^{-1})	Poses in cluster
Hypericin	**–14.10**	**84**
Pseudohypericin	**–14.00**	**81**
Amitriptyline	**–9.30**	**151**
Fluoxetine	**–8.70**	**153**
Sertraline	**–8.70**	**143**
Clomipramine	**–8.50**	**146**
Pyocyanin	**–8.10**	**160**

Protein–ligand interaction profiling results

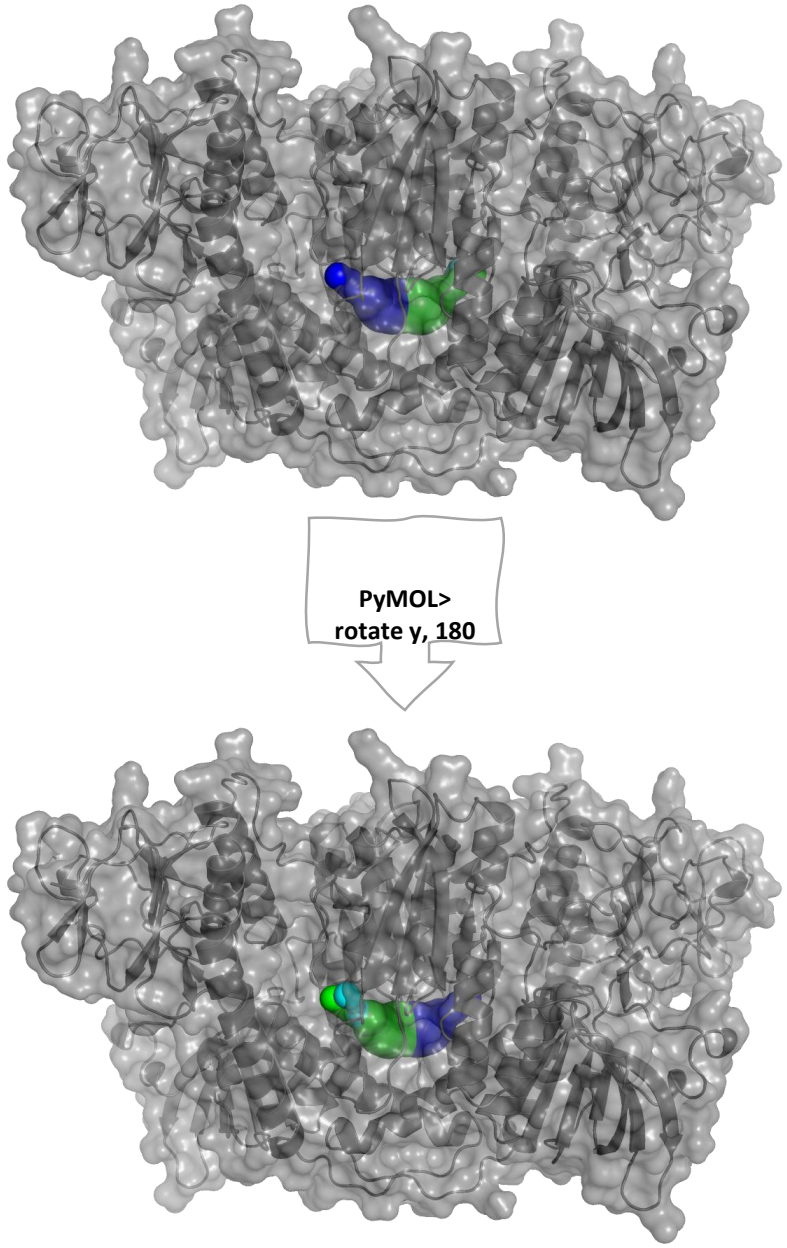




Ligand (un)binding simulation results



Tunnel	Bottleneck radius (in Å)	Length (in Å)	Curvature	Throughput
1	1.8	12.4	1.1	0.8
2	1.7	15.8	1.2	0.8
4	1.3	16.0	1.2	0.7



An eccentric and medicinally attractive site?

Human GR is a good antimalarial target as the parasite *Plasmodium falciparum* is very sensitive to oxidative stress; it needs proper functioning of GR in its host erythrocytes for survival.

Therefore, antimalarial agents (*e.g.* methylene blue), designed to inactivate erythrocyte GR, have been used as therapy.

To date, several antimalarial dyes, including **safranin O** (not deposited in the Protein Data Bank), **xanthene** (PDB entry: 1XAN) and **pyocyanin** (PDB entry: 3SQP), have been shown to bind in the large intermonomer cavity of human GR.

It should prove interesting to test the antimalarial activity of hypericin in an *ex vivo* or *in vivo* setting.

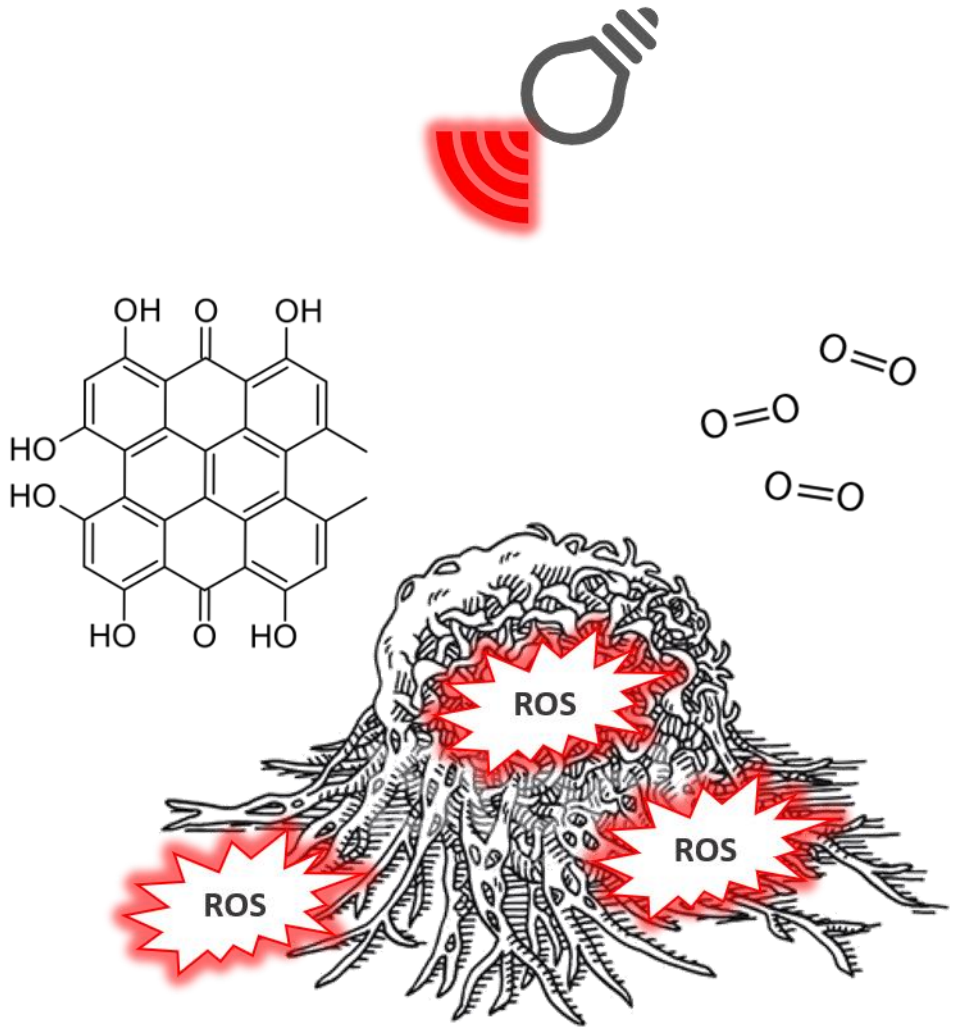
GR and the cancer connection

Increased GSH levels are interrelated with cell cycle progression and cell proliferation, making GR an attractive target for anticancer therapy.

Photodynamic therapy is a therapeutic approach in which optical illumination selectively activates light-sensitive drugs (photosensitizers) and destroys cancer cells.

Hypericin is a natural photosensitizer and generates reactive oxygen species (ROS) in the presence of light (at $\lambda \approx 600$ nm) and oxygen.

Therefore, besides its contributory role in weakening cancer cells through inhibiting GR, hypericin can also promote oxidative stress via the formation of ROS in photodynamic therapy.



Thank you for listening!

Any questions?