Integrative structural biology in molecular parasitology: new strategies for old diseases

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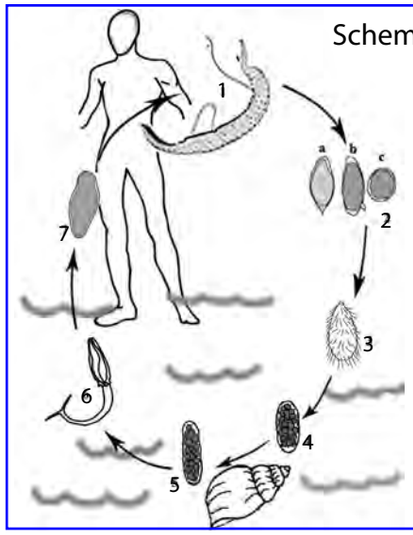
Why studying parasitic diseases?
- They are very ancient eucaryotes (at least as their host)
- They helped shaping the immune system -> host-pathogen interactions
- Many are vector borne -> adapted to different hosts/ecological niches
- They affect >1/5 of the world population -> global health problem-> we need beter diagnosis and better cures
- Learn from your enemy -> understand the host immune system by the way it is hijacked by parasites

Which one in this poster? Schistosomiasis, endemic in 78 countries

Objetives? Focused structural genomics for chemotherapy and diagnosis, by selecting pathways and macromolecules with established keyroles in the human host infecting stages:

- Thiol-mediated peroxides detoxification metabolism ***- Excreted/secreted proteins***

How? Integration of bioinformatics, structural & functional analysis (MX, SAXS, TEM, fast kinetics, SPR, BLI), medicinal chemistry and animal model validation -> multidisciplinary approach



Schematic Life cycle of *Schistosoma* parasites

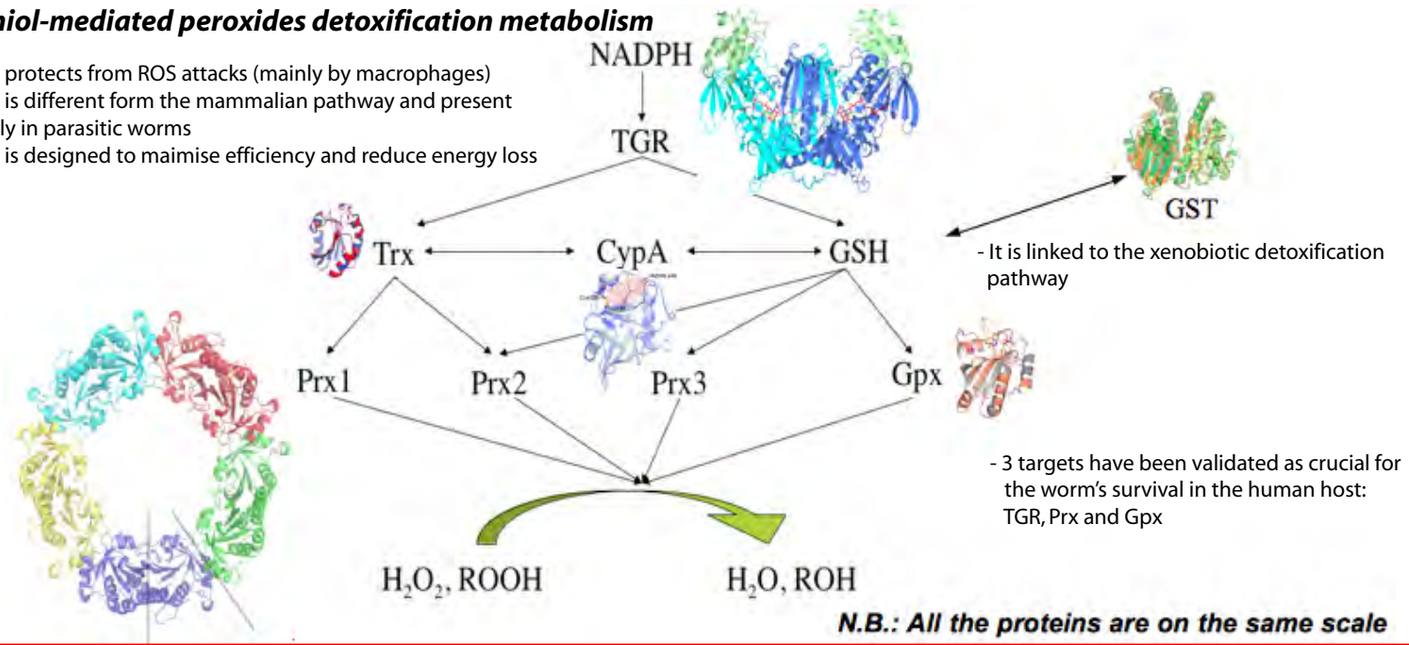
1. **Adult pair** -> the dwellers. They do not induce humoral response, but enhance the T_{H2} and T_{reg} cellular response, to evade the immune system
2. **Eggs** (a *S. haematobium*, b *S. mansoni*, c *S. japonicum*) -> the perpetuators. They induce proinflammatory response to facilitate excretion
3. **Myracidium** -> mollusk host sensor & invader. Free living in freshwater

4-5. **Sporocysts** -> asexual reproductors

6. **Cercaria** -> human host sensor and invader. Free living
7. **Schistosomulum** -> the traveller

Thiol-mediated peroxides detoxification metabolism

- It protects from ROS attacks (mainly by macrophages)
- It is different form the mammalian pathway and present only in parasitic worms
- It is designed to maimise efficiency and reduce energy loss



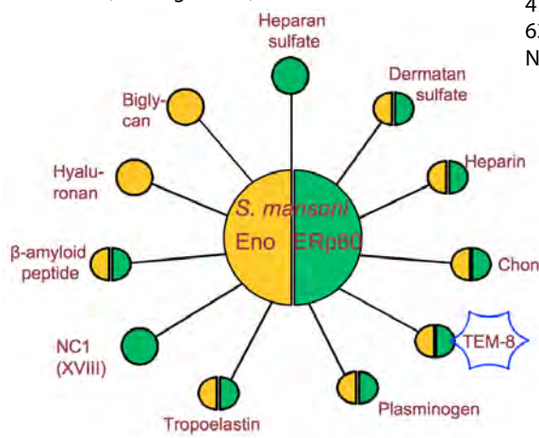
- It is linked to the xenobiotic detoxification pathway
- 3 targets have been validated as crucial for the worm's survival in the human host: TGR, Prx and Gpx

N.B.: All the proteins are on the same scale

Excreted/secreted proteins

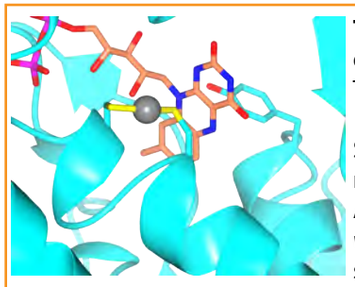
Proteomic analysis of adult worms and eggs secretomes has highlighted the presence of:
- surface antigens (such as the high variable Venom allergen-like proteins, micro-exons genes)
- moonlighting proteins (enolase, protein disulfide isomerase ERp60, Prx)
- glycoproteins (circulating catodic and anodic antigens, omega-1)
- immune modulators (IL6, IL10, PD-1)
- hormones (oestrogen-like)

47% of E/S proteins do not have a signal peptide
63% have a secretion signal -> 17% a canonical N-ter and 46% an internal signal

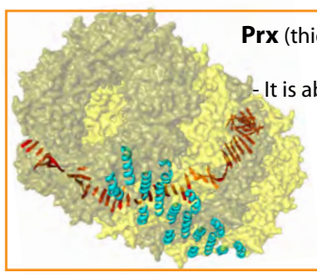


Which roles for these molecules?
- They modulate the host cellular response (anti- *vs* pro-inflammatory)
- increase the vasculature permeability
- induce modification of the host extracellular matrix (ECM)
- induce host Glc production and secretion
- induce host lipolysis

-> **Host-parasite interactions**



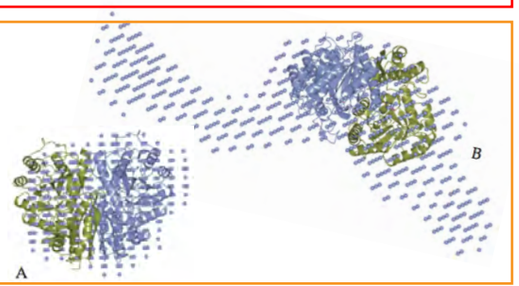
TGR (thioredoxin-glutathione reductase) is a druggable bottleneck
The last 4 aa on a flexible arm are:
Gly-Cys-Sec-Gly -> a mimick of GSH
Selenocysteine is a more versatile entry and exit redox group than Cys
All the inhibitors able to reduce worm burden *in vivo* are mechanism-based -> the worst case scenario for rational drug design
Auranofin, Au(I)-based orphan drug is the most potent inhibitor so far validated in animal models



Prx (thioredoxin peroxidase or peroxiredoxin) is a moonlighting protein
- It is able to polymerize from donuts to nanotubes when pH changes from 7.0 to 6.5
- Only donuts are redox active
- The tubes are holdases. ATP- independent chaperons

Eno (enolase) is a glycolytic enzyme and a moonlighting protein by counter-ion:
in ↑K^+ -> active enzyme (2PG↔ PEP) **(panel A)**
in ↑Na^+ -> inactive -> it binds plasminogen & a few other ECM components, out of 80 tested -> **CS in B**

ERp60 is a flexible protein disulfide isomerase and redox-regulated chaperone by increasing the local concentration



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