Biological background for bioinformatic studies on the trypanothione synthase

Until now, during our seminar we discussed some elements and approaches of bioinformatics studies, such as search algorithms for recognition between targets and ligands, optimization problems in drug-receptor interactions, the mathematical modeling of activity, affinity, efficacy and selectivity of the drug etc.

The main purpose

to make an attempt to direct some activities of our seminar and some activities of the participants in the project related with bioinformatics studies in SWU, on a concrete object.

The object

an enzyme, related with glutathion and an unique metabolic pathway distrubuted in Unicellular Parasitic protozoa (Eukaryota) species discovered by Alan Fairlamb before 17-18 years.

Unicellular Parasitic protozoa (Eukaryota) species Leishmania and Trypanosoma cause serious diseases.

Trypanosoma brucei cause - African Sleeping sickness in humans or - Nagana in animals.

Trypanosoma cruzi cause - South American Chagas Disease

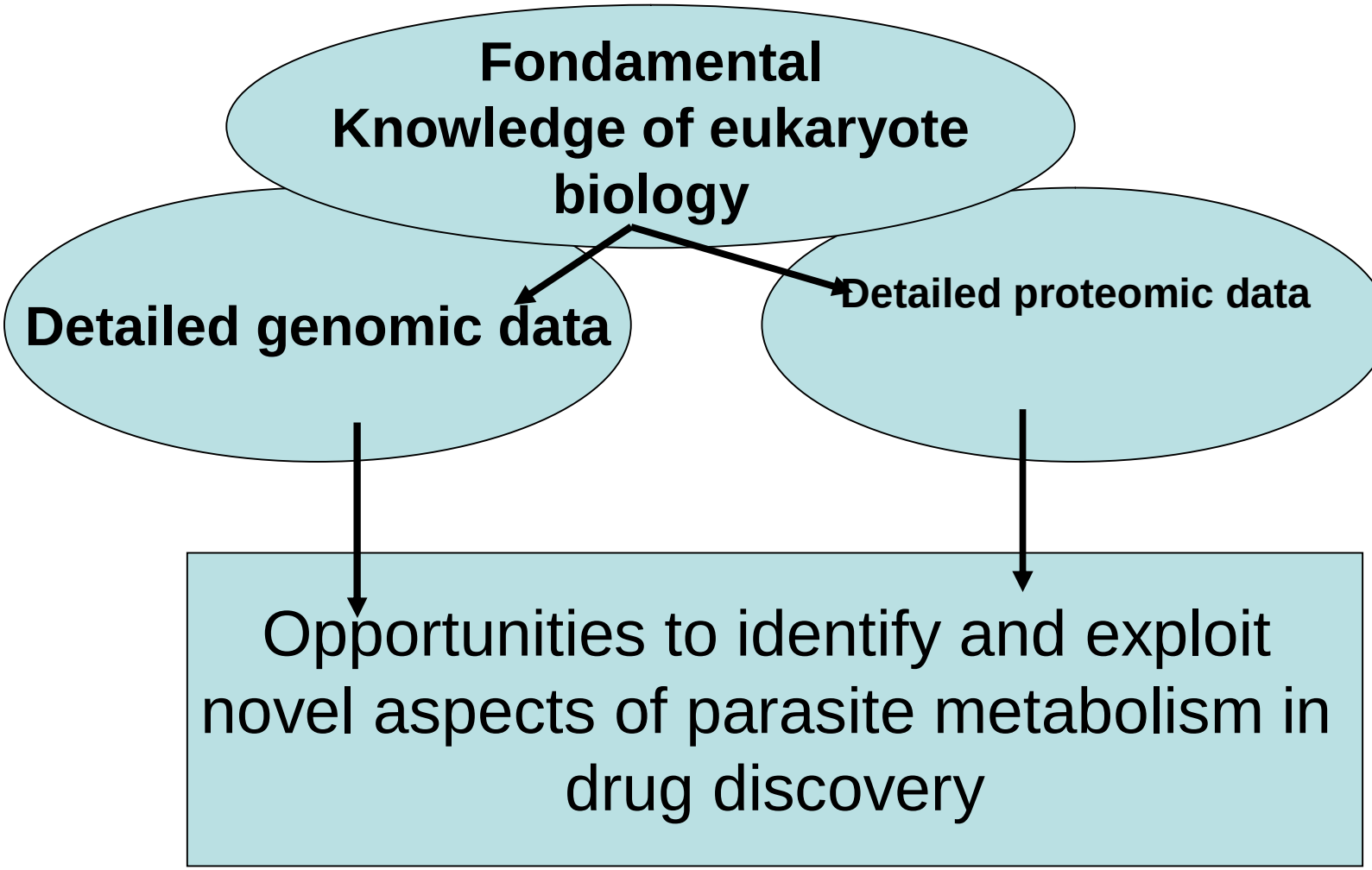
Leishmania major cause – leishmaniasis, which affect and destroy skin;

Leishmania donovani cause – leishmaniasis, which affect the visceral organs.

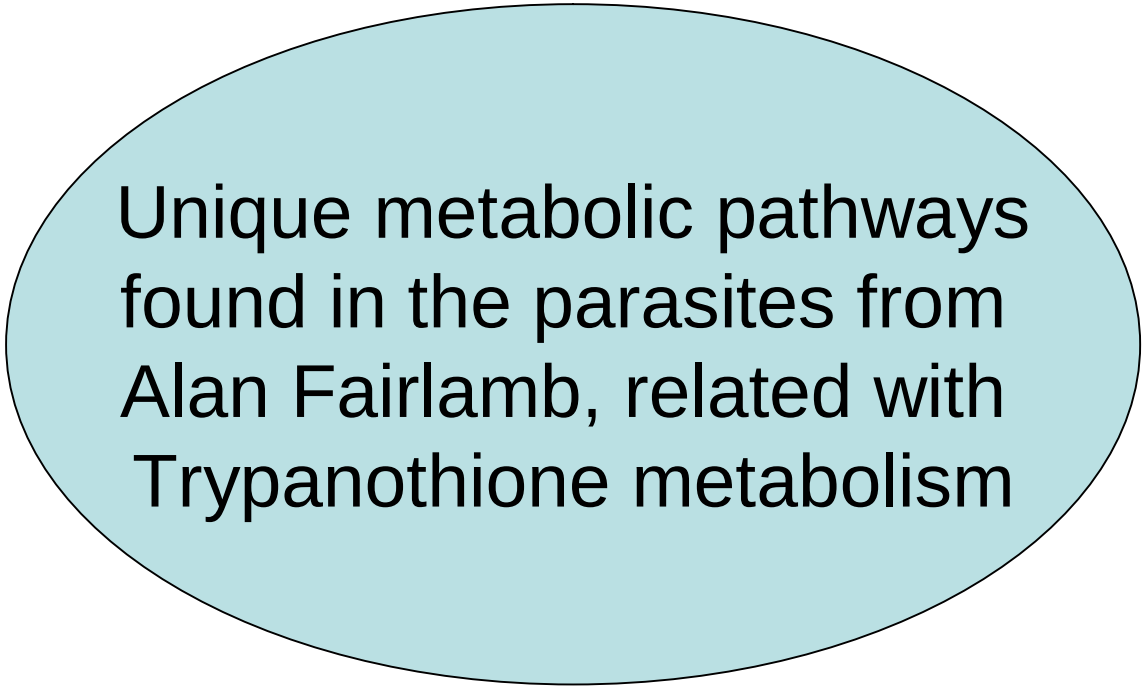
Leishmania currently affects 12 million people in 88 countries, including Bulgaria.

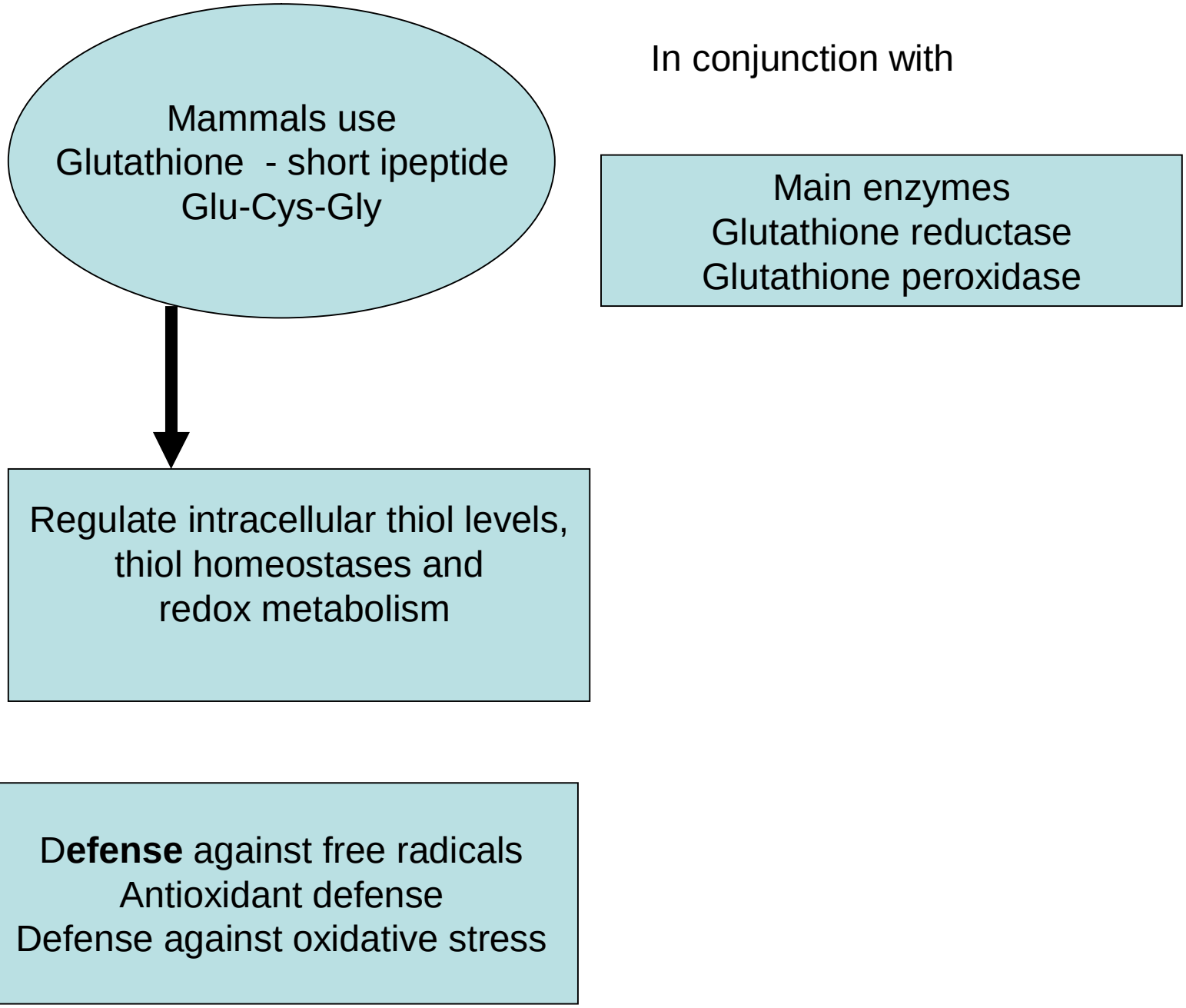
The current few trypanocidal drugs used to combat these infections are unsatisfactory due to:

- toxicity,
- high cost,
- poor efficacy and
- increasing levels of drug resistance.


Fondamental Knowledge of eukaryote biology
Detailed genomic data
Detailed proteomic data
Opportunities to identify and exploit novel aspects of parasite metabolism in drug discovery

Potential trypanocidal drug target




Mammals use
Glutathione - short ipeptide
Glu-Cys-Gly
In conjunction with
Main enzymes
Glutathione reductase
Glutathione peroxidase
Regulate intracellular thiol levels,
thiol homeostases and
redox metabolism
Defense against free radicals
Antioxidant defense
Defense against oxidative stress

Trypanosomatid parasites
exploit the properties of
Trypanothione
contributing defense
against oxidative stress

In conjunction with distinct enzymes

Trypanothyone reductase
Tryparedoxin
Tryparedoxin peroxidase

Trypanothione (Mr = 721.86 g/mol) is
unusual form of glutathione containing
two molecules of glutathione joined by a polyamine spermidine,
which play role of a linker

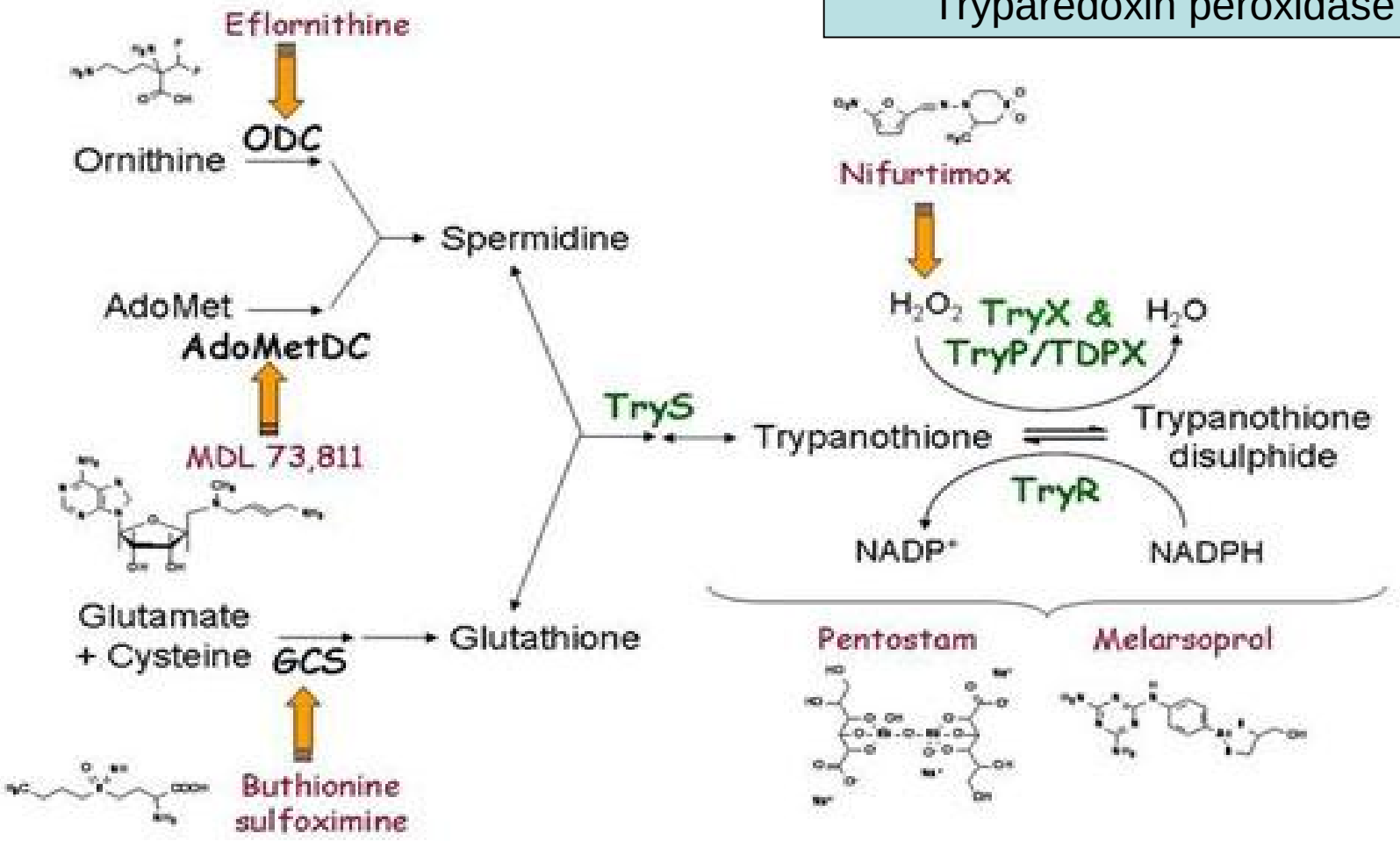
It is found in parasitic protozoa

such as leishmania and trypanosomes.

The differences between host (humans and animals) and parasite present opportunities to target trypanothione metabolism for drug discovery.

Potential drug targets:

Trypanothyone reductase
Tryparedoxin
Tryparedoxin peroxidase



Now we focus on the enzyme responsible for the synthesis and degradation of Trypanothione – **trypanothione synthase**

In enzymology, a **trypanothione synthase** (EC 6.3.1.9.) is an enzyme that catalyzes the folowing chemical reaction:

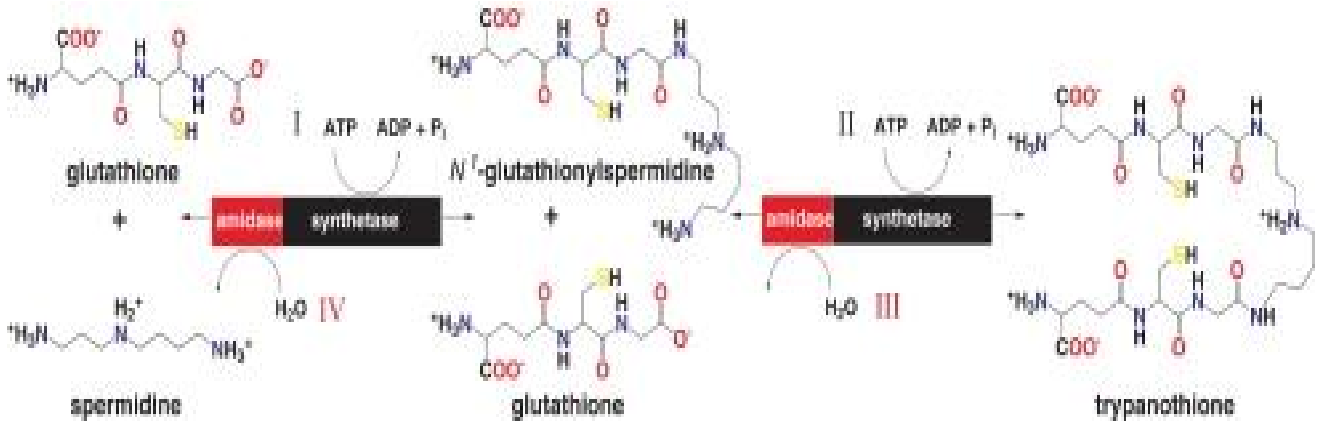
glutathione + glutathionylspermidine + ATP

↓

N1,N8-bis(glutathionyl)spermidine + ADP + phosphate

However, it was established that this enzyme is actually a **Glutationylspermidine synthetase – an** hetrodimer and was identified in the model trypanosomatid *Crithidia fasciculata .*

In the human pathogens Trypanosoma brucei, Trypanosoma cruzi, Leishmania major and Leishmania donovani, a monomeric protein is responsible for the synthesis of thrypanoione and the name of this enzyme is trypanothione synthetase-amidase (TSA).



TSA catalyzes 4 reactions by acting as:

- **Glutationylspermidine synthetase**
- **Trypanothione synthetase**
- **Trypanothione amidase**
- **Glutationylspermidine amidase**

Balance between spermidine and trypanothione maintenance redox metabolism and the level of cell proliferation and cell diffenetiation.

Spermidine, polyamine related with cell proliferation and cell diffenetiation.

So TSA is our target. The target is:

- Protein
- Enzyme, which catalyzes 4 reactions and respectively have at least 3 substrates – spermidine, glutathione, and glutathionylspermidine.
- The eventual drugs or ligands could be **enzyme inhibitors** and eventually analogues of the substrates.

Which kind of questions could be post in order to continue with bioinformatic studies ???

- **Is there data for 3D structure of TSA ???**
- **Which kind of data for 3 D, obtained from biophysical technique such as x-ray crystallography or NMR spectroscopy, are more relevenat for our target?**
- **Which kind of search algorithms have to be used to suggest the biggest pockets?**
- **Since, we have 3 substrates, how many pockets have to be expected ?**

The strength of the non-covalent interaction or binding affinity as a parameter of ligand-target interaction, is predicted by so-called scoring functions. Is it possible to reach these levels of investigations and to initiate drug discovery by virtual screening, lead optimization etc.

- **Which kind of popular docking approaches, such as shape complementarity, simulation etc. are better and who will master them ???**

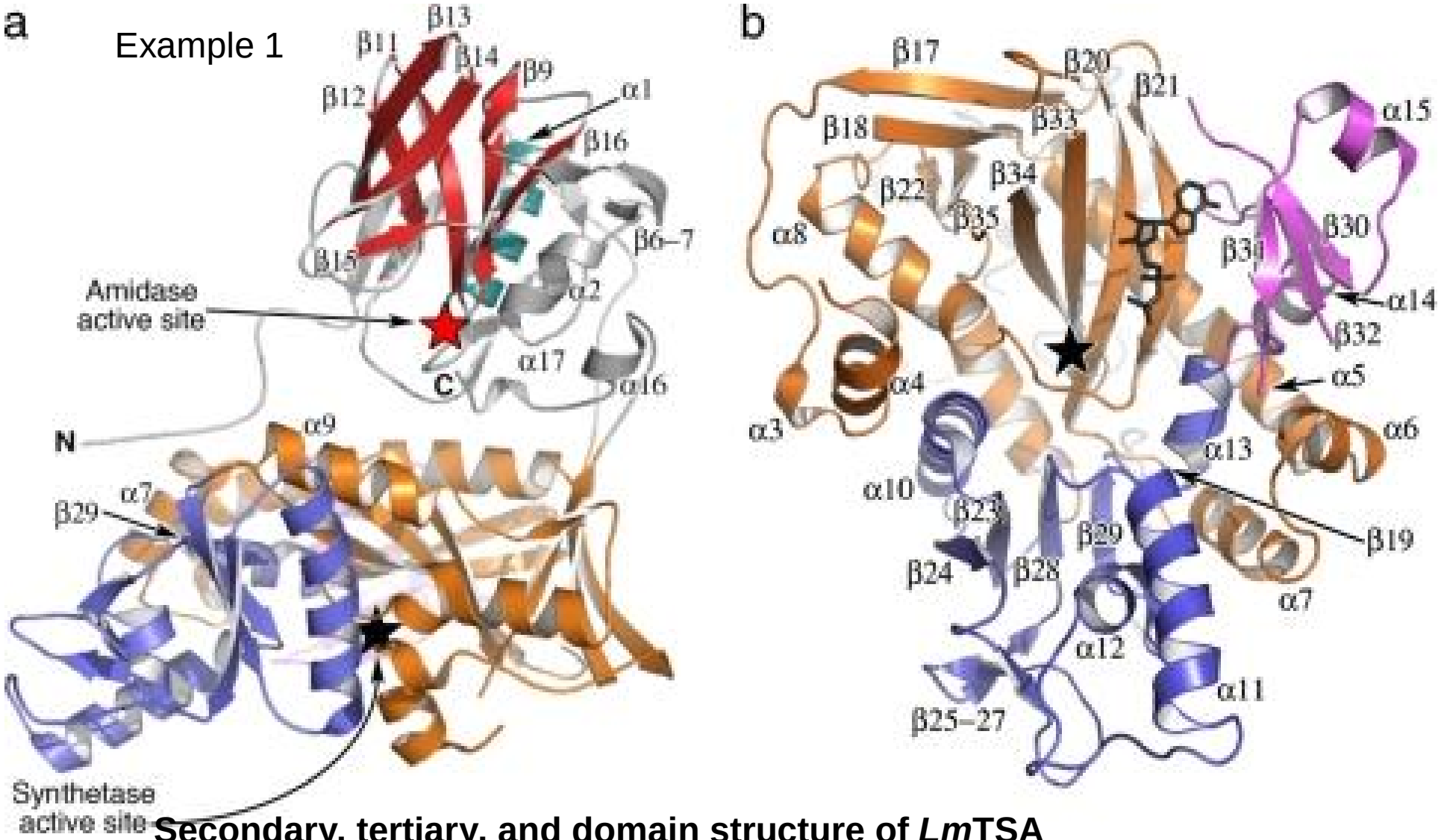
Two examples from the

Fyfe P.K., Oza S.L., Fairlamb A.H., Hunter W.N.

Leishmania Trypanothione Synthetase-Amidase Structure Reveals a Basis for Regulation of Conflicting Synthetic and Hydrolytic Activities

THE JOURNAL OF BIOLOGICAL CHEMISTRY 2008, 283 (25), 17672-17680.

Example 1



Secondary, tertiary, and domain structure of *Lm*TSA

N-terminal domain – responsible for the amidase activity (protease) red star

C-terminal domain – responsible for the synthetase activity – black star

Selected elements of the secondary structure (a or b-barrel) are caloured

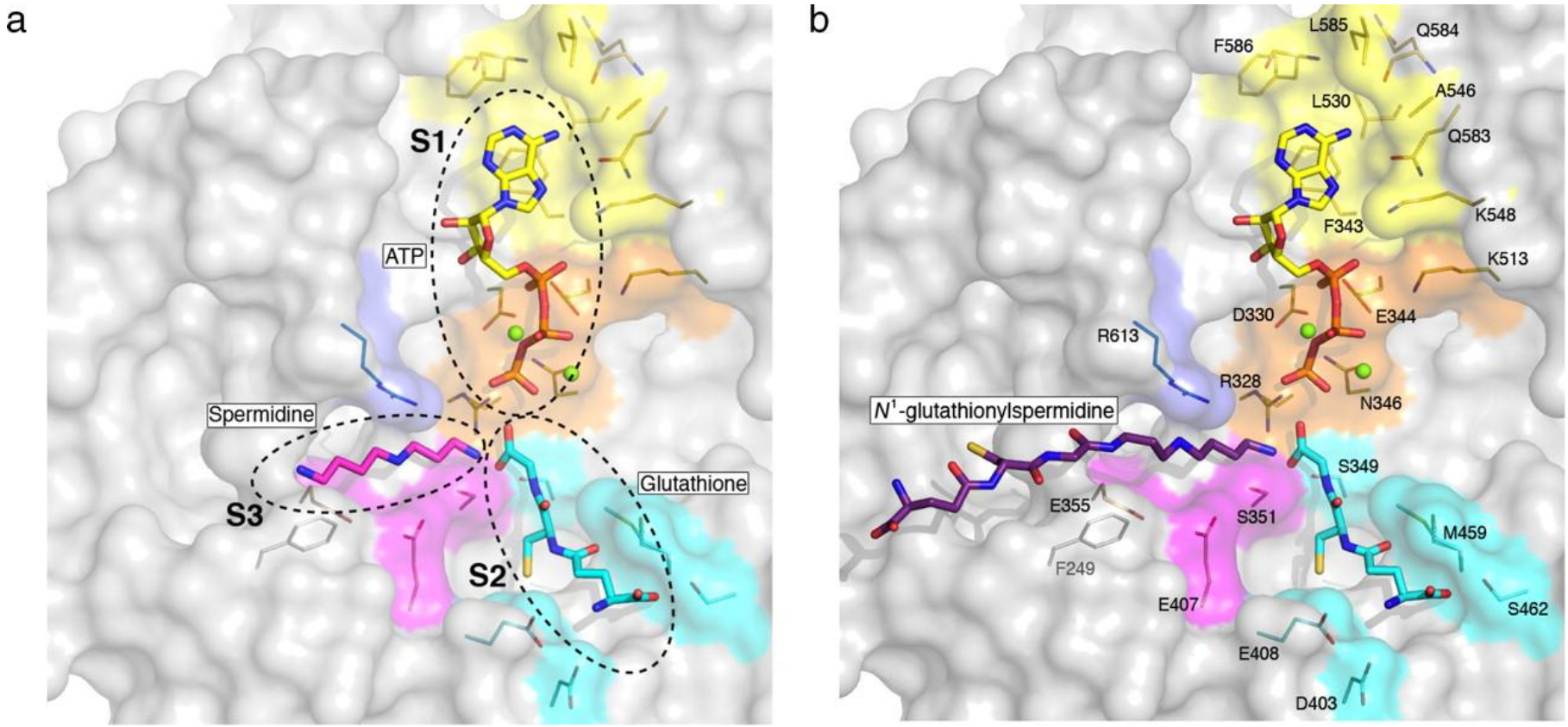
On right – subdomain strucutre of ATP-grasp synthetase domain, which binds nucleotide.

Example 2

The synthetase active site

a – Model of substrates (ATP, GSH and spermidine) at the onset of Ist reaction. The protein surface is depicted as a semi-transparent van der Waals surface

b – Model for the onset of the second reaction.



Findings of Noel Dognin

About trypanothione reductase and synthetase structures in the Protein Data Bank:

The reductase cav be found in both – bound and unbound state, and thanks to the bounded structures, we have the definition of the domaines and their SCOP classification.
The structures come from brucei, cruzy and leishmania.

The synthetase can only be found in unbound state, and structures only come from leishmania.

Thank you !

Docking glossary

•**Receptor** or **host** – The "receiving" molecule, most commonly a protein or other biopolymer.

• **Ligand** or **guest** – The complementary partner molecule which binds to the receptor. Ligands are most often small molecules but could also be another biopolymer.

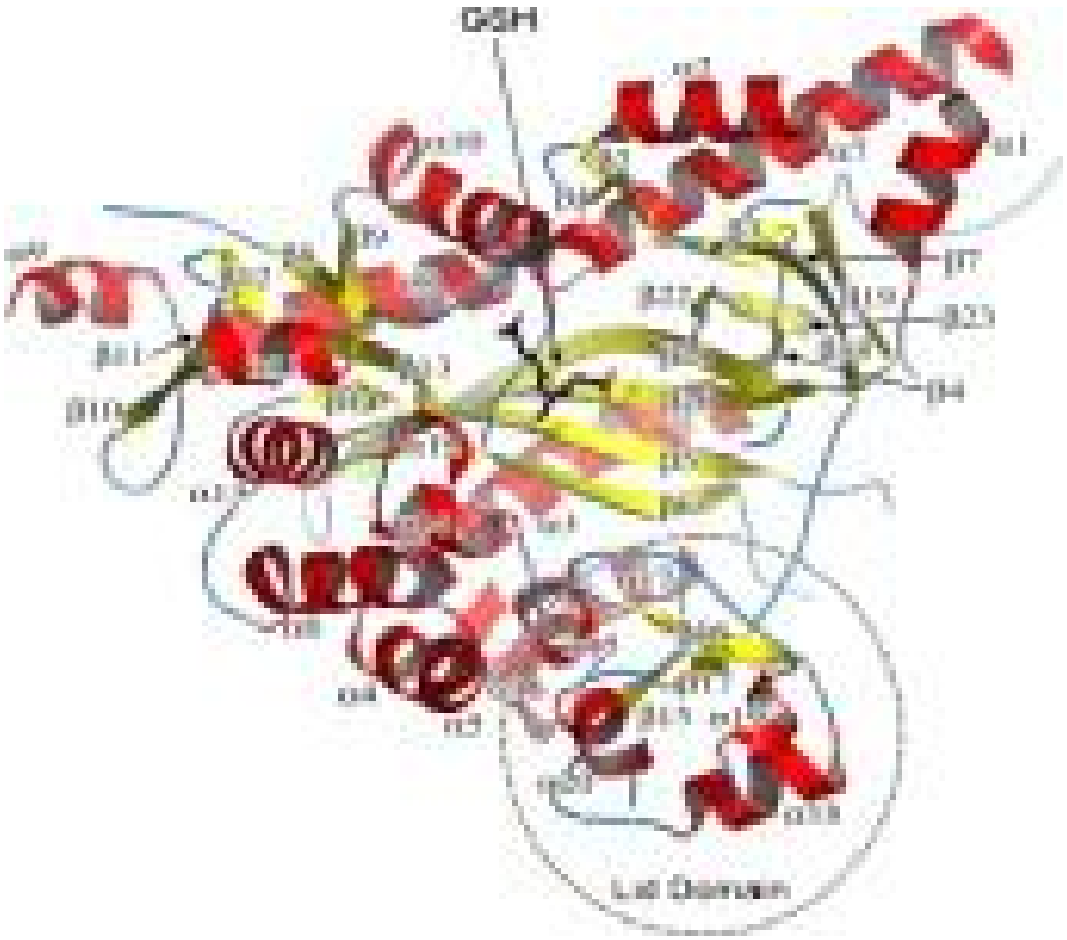
• **Docking** – Computational simulation of a candidate ligand binding to a receptor.

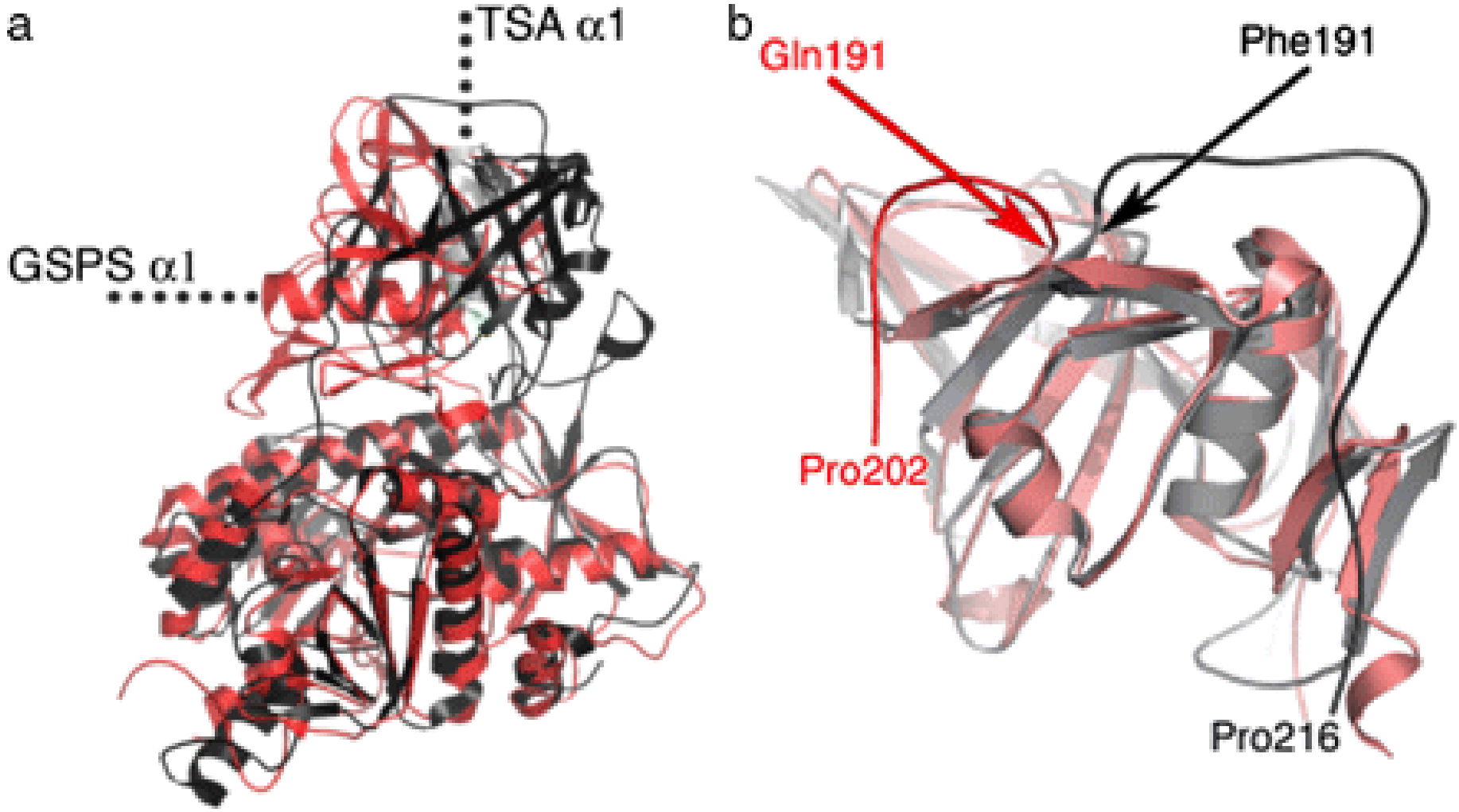
• **Binding mode** – The orientation of the ligand relative to the receptor as well as the conformation of the ligand and receptor when bound to each other.

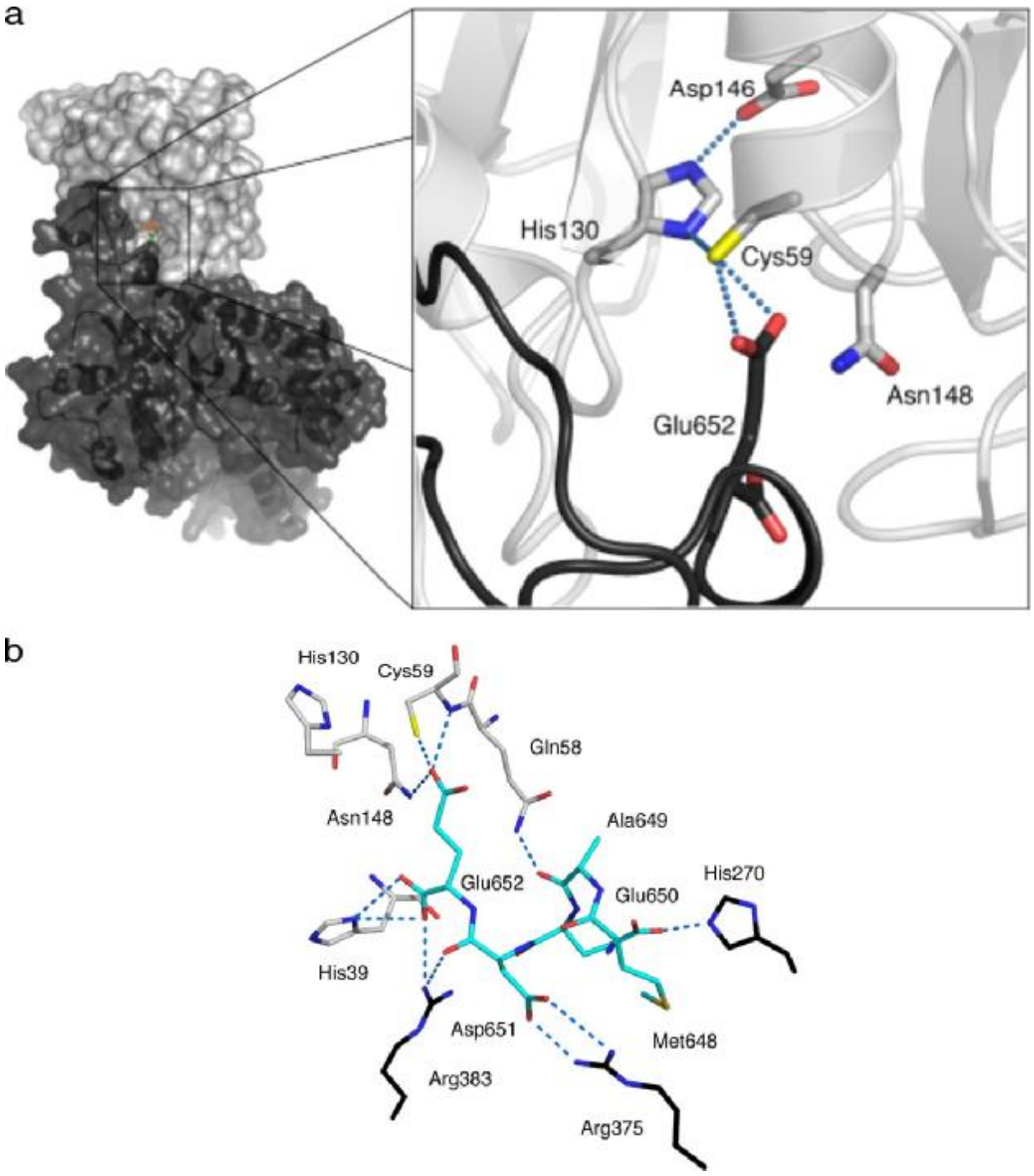
• **Pose** – A candidate binding mode.

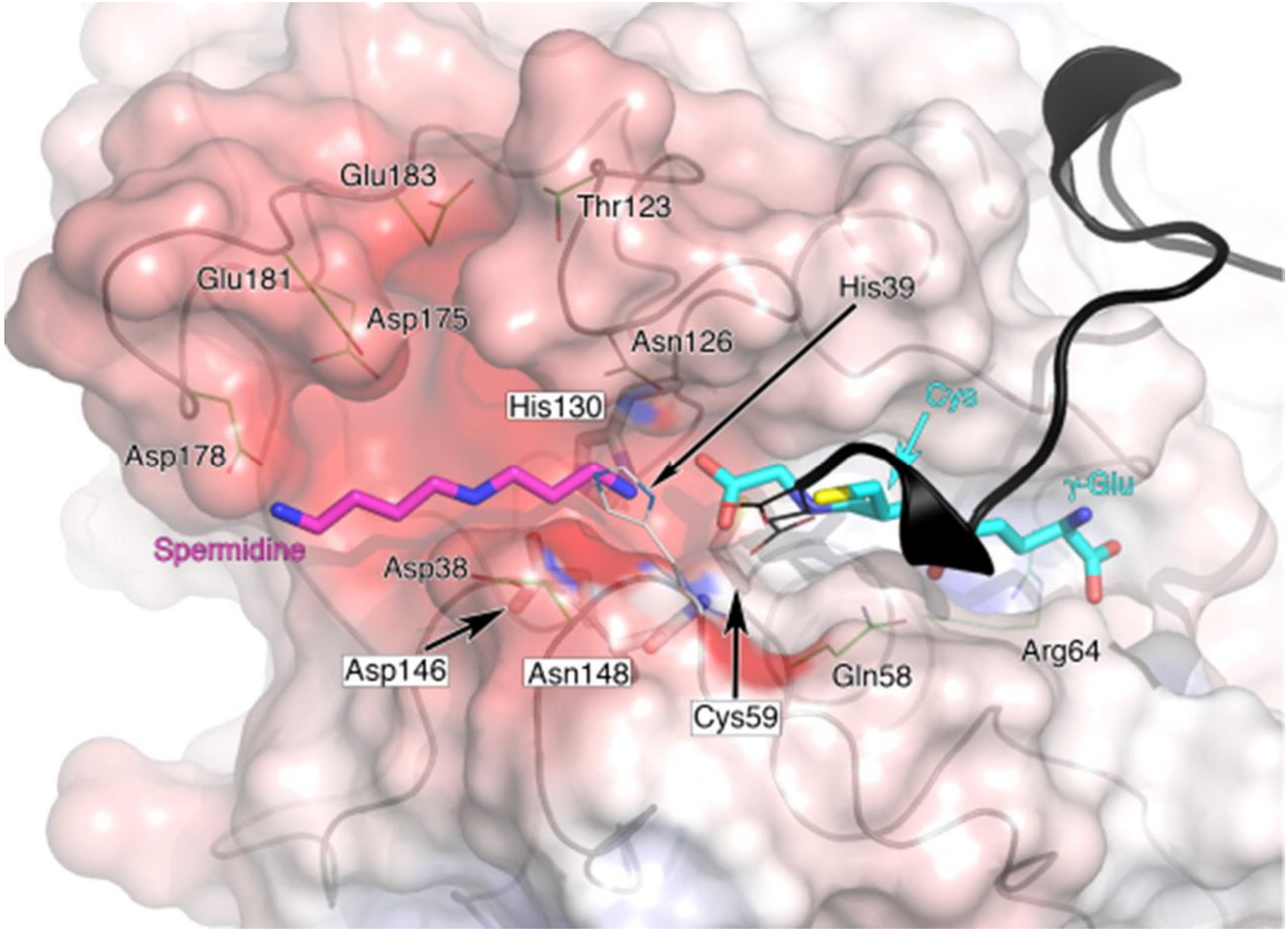
• **Scoring** – The process of evaluating a particular pose by counting the number of favorable intermolecular interactions such as hydrogen bonds and hydrophobic contacts.

• **Ranking** – The process of classifying which ligands are most likely to interact favorably to a particular receptor based on the predicted free-energy of binding.


GSH
Lid Domain

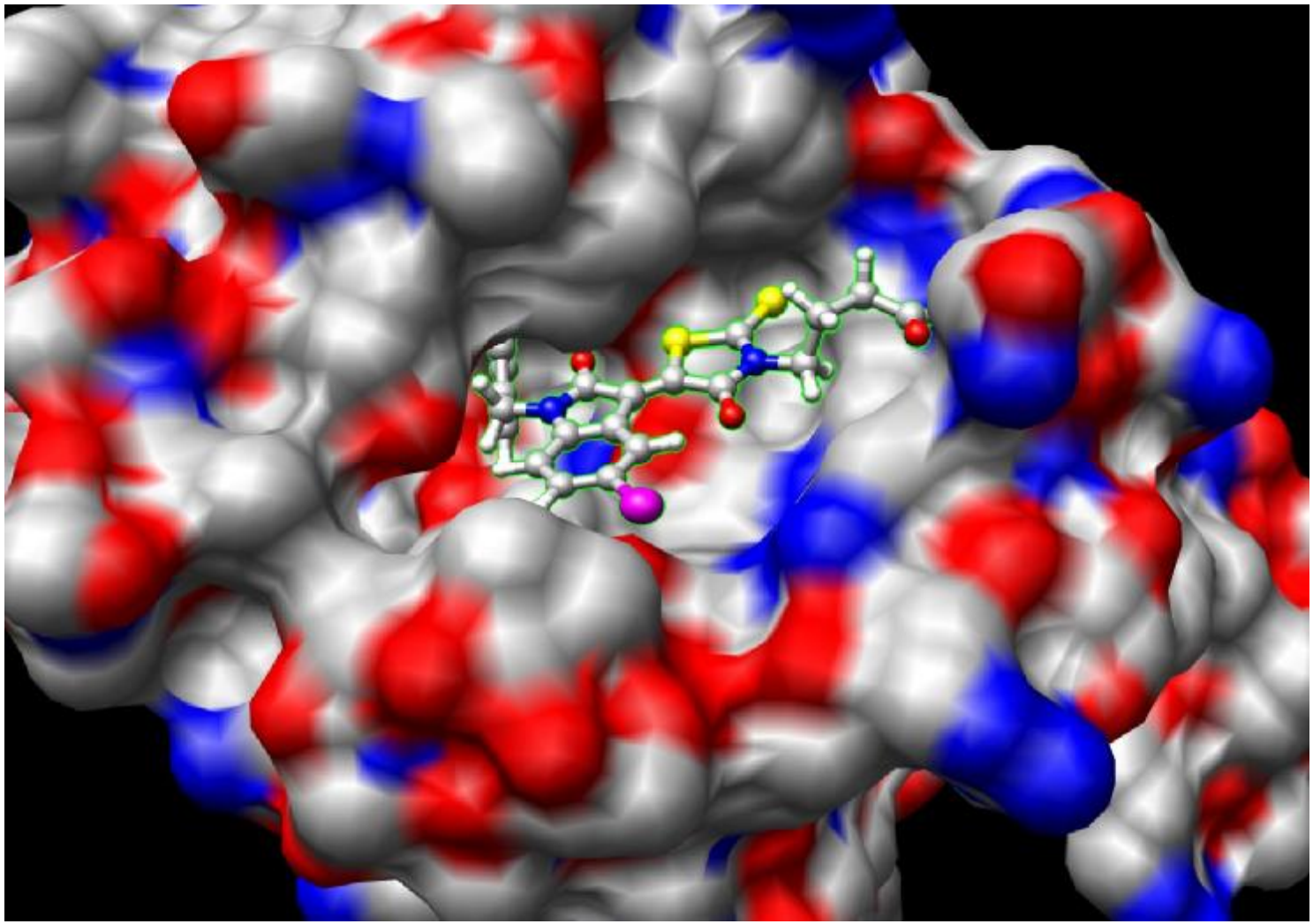

a
TSA α1
GSPS α1
b
Gln191
Phe191
Pro202
Pro216


a
Asp146
His130
Cys59
Asn148
Glu652
b
His130
Cys59
Gln58
Asn148
Ala649
Glu652
Glu650
His270
His39
Asp651
Met648
Arg383
Arg375


Glu183
Thr123
Glu181
Asp175
His39
Asn126
His130
Cys
Asp178
γ-Glu
Spermidine
Asp38
Asp146
Asn148
Gln58
Arg64
Cys59

Trypanosomes are a group of kinetoplastid protozoa distinguished by having only a single flagellum. All members are exclusively parasitic, found primarily in insects.[1] A few genera have life-cycles involving a secondary host, which may be a vertebrate or a plant. These include several species that cause major diseases in humans.[2]
The most important trypanosomal diseases are trypanosomiasis (African Sleeping Sickness and South American Chagas Disease); these are caused by species of *Trypanosoma*. The Leishmaniases are a set of trypanosomal diseases caused by various species of *Leishmania*.

Leishmania is a genus of trypanosome protozoa, and is the parasite responsible for the disease leishmaniasis.[1][2] It is spread through sandflies of the genus *Phlebotomus* in the Old World, and of the genus *Lutzomyia* in the New World. Their primary hosts are vertebrates; *Leishmania* commonly infects hyraxes, canids, rodents, and humans. *Leishmania* currently affects 12 million people in 88 countries.
The parasite was named in 1903 after the Scottish pathologist William Boog Leishman.



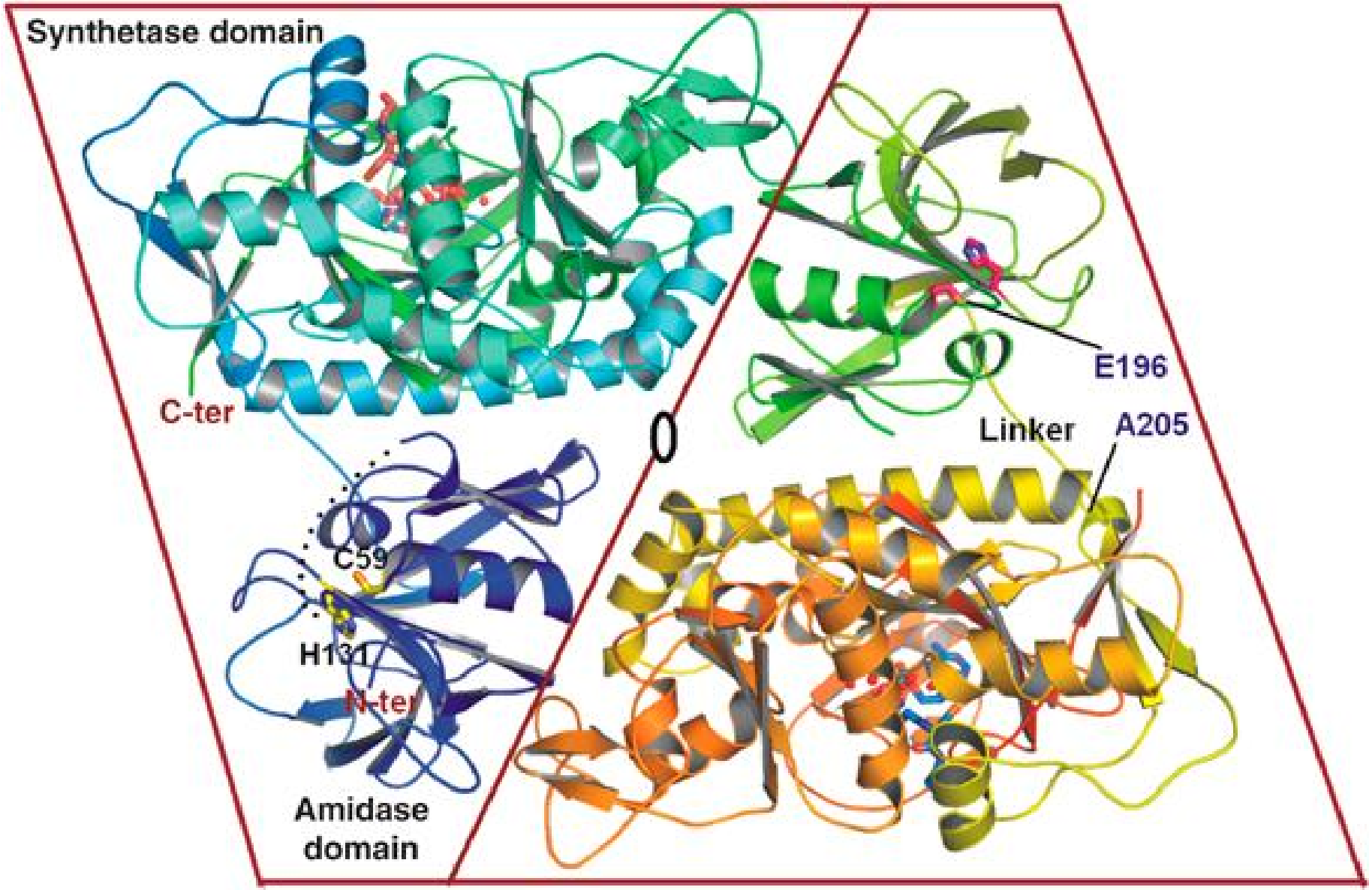
Docking

Chemical Compound docked to Protein structure (acetyltransferase)

Trypanothione (Mr = 721.86 g/mol) is an unusual form of glutathione containing two molecules of glutathione joined by a spermidine (polyamine) linker. It is found in parasitic protozoa such as leishmania and trypanosomes.[1] These protozoal parasites are the cause of leishmaniasis, sleeping sickness and Chagas' disease. Trypanothione was discovered by Alan Fairlamb. Its structure was proven by chemical synthesis.[2] It is unique to the Kinetoplastida and not found in other parasitic protozoa such as *Entamoeba histolytica*.[3] Since this thiol is absent from humans and is essential for the survival of the parasites, the enzymes that make and use this molecule are targets for the development of new drugs to treat these diseases.[4]

Trypanothione-dependent enzymes include reductases, peroxidases, glyoxalases and transferases. Trypanothione-disulfide reductase (TryR) was the first trypanothione-dependent enzyme to be discovered (EC 1.8.1.12). It is an NADPH-dependent flavoenzyme that reduces trypanothione disulfide. TryR is essential for survival of these parasites both *in vitro* and in the human host.[5][6]

A major function of trypanothione is in the defence against oxidative stress.[7] Here, trypanothione-dependent enzymes such as tryparedoxin peroxidase (TryP) reduce peroxides using electrons donated either directly from trypanothione, or via the redox intermediate tryparedoxin (TryX). Trypanothione-dependent hydrogen peroxide metabolism is particularly important in these organisms because they lack catalase. Since the trypanosomatids also lack an equivalent of thioredoxin reductase, trypanothione reductase is the sole path that electrons can take from NADPH to these antioxidant enzymes


Synthetase domain
C-ter
E196
Linker
A205
C59
H131
N-ter
Amidase
domain