# POPGST

## Structure-function analysis of proteins involved in stress resistance mechanisms in the model tree *Populus trichocarpa*

*Principle investigator: Nicolas Rouhier*

*Partners: LERMAB (Stéphane Dumarçay, Christine Gérardin)*

*Collaboration : LAE (Frédéric Bourgaud, univ Lorraine) ; CRM2 (Claude Didierjean, univ Lorraine)*

---

**Context** — The 81 poplar glutathione transferases constitute a monophyletic group that can be subdivided in ten classes. These proteins provide a protection against exogenous toxic molecules referred to as xenobiotics and could also participate to the biosynthesis and/or transport of secondary metabolites. They are typically known to catalyze the conjugation of the tripeptide glutathione to compounds containing an electrophilic center to form glutathionylated products. This reaction is an essential step for the detoxification of toxic compounds. Proteomic and transcriptional studies have shown that Phi class GSTs (GSTFs) are strongly induced in response to a broad range of stimuli including stresses. Despite these studies, the substrates and physiological functions of most of these enzymes remain unknown, whereas this class appeared with terrestrial plants before being hugely expanded in some photosynthetic organisms during evolution.

**Objectives** — This project consisted in performing a functional comparative study of all poplar GSTFs using a structure-function approach in order to understand the functional and physiological specificity explaining the expansion of this class in poplar and more generally in plants.

**Approach** — The work program was divided into three main parts combining multidisciplinary approaches:

(i) the study of the enzymatic and structural properties of recombinant proteins after overexpression of the coding sequence in *Escherichia coli* and purification. The idea was to test known substrates, including herbicides/pesticides, secondary metabolites, halogenated pollutant compounds and molecules with aromatic cycles from a chemical library and to try to solve the 3D structures of the proteins alone or in complex with ligands.

(ii) the study of transcript and protein amounts in different poplar organs and in response to various biotic or abiotic stresses.

(iii) the search for physiological substrates (proteins, secondary metabolites, defense compounds) from poplar extracts. This approach was initiated by analyzing protein thermostability with or without ligands and by performing competitive enzymatic experiments with a fluorescent probe that inhibits the GSTF esterase activity. The identification of “active” molecules will then be done by mass spectrometry coupled or not with a separation by liquid chromatography and eventually by NMR.

**Key results** —

- The eight GST Phi isoforms have been purified as well different mutated proteins on catalytic residues.
- The measurement of transcript levels in different poplar organs indicates that most GSTs are not or poorly expressed in roots but display quite comparable expression patterns in aerial organs which do not allow really differentiating them.
- After having tested compounds with aromatic cycles, herbicides/pesticides, secondary metabolites and hormones, it seems that several molecules known to interact with GSTFs

from other organisms do not interact with poplar GSTFs. For other molecules and notably classical model substrates, we have detected differences in the type of catalyzed activities, i.e. (de)glutathionylation or peroxidase activity and for a given activity type, differential affinities and catalytic efficiencies for certain enzyme/substrate couples indicating the existence of a certain enzymatic specificity.

- Owing to the resolution of the 3D structures of 5 GSTFs, we could validate our biochemical and enzymatic observation, notably concerning the versatility of several active site residues present in the loop implicated in the recognition and activation of glutathione.

**Main conclusions including key points of discussion** — The expansion of the GSTF class in poplar does not seem to be linked to specificities in transcript expression territories or in protein subcellular localization (all are predicted as cytosolic) but rather to differences in biochemical and enzymatic properties which are explained by variations at the level of the primary and tertiary sequences and notably by a relatively flexible and permissive active site.

**Future perspectives** — In order to perform a ligand fishing approach, some other tools have been generated such as the purification of protein variants mutated for 1 to 3 active site residues enabling to better accommodate a chemically synthesized modified glutathione. Using this approach, we should be able to isolate glutathionylated metabolites, something which has been rarely achieved so far.

**Valorisation** —

Pégeot H, Koh CS, Petre B, Mathiot S, Duplessis S, Hecker A, Didierjean C, Rouhier N. (2014) The poplar Phi class glutathione transferase: expression, activity and structure of GSTF1. Front Plant Sci. 23;5: 712.

Pégeot H, Mathiot S, Perrot T, Gense F, Hecker A, Didierjean C, Rouhier N. Catalytic versatility in poplar glutathione transferase Phi class: when natural combination of active site loop residues confer dual but opposite biochemical functions. Submitted to Biochem J.

PhD thesis of Henri Pégeot, defense the 11th of December.

Poster presentation at the 9th International Workshop - Sulfur Metabolism in Plants (14-17 april 2014 in Freiburg).