

Job offer: postdoctoral Fellow

Project Title: Enzymatic Controlled glycosylation of natural flavonoid mixtures

Research Fields: antioxidants, flavonoids, enzymatic engineering, agricultural co-products, phytochemical analysis

Work Place: Nantes & Angers

Research Laboratories: UFIP (Unité Fonctionnalité et Ingénierie des Protéines) – UMR CNRS 6286, University of Nantes & SONAS (Substances d'Origine Naturelle et Analogues Structuraux) - UPRES EA 921, University of Angers

Head(s) of the Scientific Project: Dr Corinne Miral (UFIP) & Dr Séverine Boisard (SONAS)

Offer type: postdoctoral researcher: short term contract 18 months

Hiring Institution: University of Nantes

Application deadline: 01/06/2019

Job Starting Date: 15/06/2019

Environment

Hiring institution:

Fundamental, applied or technological, research is the bedrock of the University of Nantes. Multidisciplinary and oriented towards interdisciplinary, the University of Nantes is part of a dynamic of excellence of its research with the will to accompany the emergence of new projects and to explore ever more new fields of research to answer the challenges of tomorrow. For this, the University of Nantes can now count on 43 research laboratories, covering all scientific fields, and rely on the activity of nearly 2,600 dedicated staff.

Research Laboratories:

The partner laboratories of this project develop complementary skills:

- UFIP has significant expertise in the field of enzyme engineering using sugars as substrates but also in structural bioinformatics. Some of the methods developed by UFIP will, in the framework of this project, be applied to these new enzymatic systems to synthesize functionalized flavonoids.
- SONAS is specialized in the valorisation of secondary metabolism of plants through phytochemical analysis, from the resolution of complex mixtures to the structural characterization of purified compounds, and biological tests. This laboratory is also expert on the analytical approach envisaged *via* the use of classical structural characterization and elucidation techniques but also using LDI (Laser Desorption Ionization) mass spectrometry.

Context of the project

Flavonoids are secondary metabolites naturally present in abundance in many plants. These polyphenols, co-products of the agricultural industry, can be obtained by fractionation of fruits and vegetables and are of major interest because they are powerful antioxidants with many applications in the field of "health foods", cosmetics or pharmacy. Many of their biological effects appear to be related to their ability to modulate certain cell signalling cascades. Flavonoids, through various mechanisms of *in vitro* action validated on different animal models, have thus demonstrated anti-inflammatory, anti-thrombogenic, antidiabetic, anti-cancer and neuroprotective properties. They are generally functionalised (predominantly glycosylation) that strongly modifies their properties, in particular in terms of solubility, stability, bioavailability and bioactivity. The possibility of access to functionalized flavonoids and their fine characterization is of major interest for researchers and the industrial world.

The extraction and purification of glycosylated flavonoids from plants is however particularly difficult and therefore expensive. The controlled functionalization by total chemical synthesis of these phenolic units also has multiple locks. Regarding the enzymatic pathways, the enzymes that selectively functionalize flavonoids are rare, whereas the requirements for antioxidants functionalized both in research and in industry are considerable. Thus, because they use sucrose, a cheap and highly reactive donor substrate and because of their promiscuity in terms of co-substrates (flavonoid acceptors) and the availability of thermostable homologs, sucrose phosphorylases are very good candidates for the enzymatic functionalization of flavonoids.

This project aims, through the use of enzymes derived from bacterial biodiversity, the controlled functionalization of natural flavonoids with applications in the fields of health, nutraceuticals and cosmetics.

The project's objectives

The main objective of this project is to develop an original approach for the screening of bacterial biodiversity in order to identify enzymatic actors able to glycosylate complex mixtures of flavonoids and to characterize their specificities and selectivity. In this context, this project also aims to develop an innovative methodology for the analytical characterization of flavonoids thus functionalized. Their antioxidant properties and their biological potential will also be studied during this project.

Mission & methodology

The applied methodology will be divided into 4 main steps:

1. The choice of a panel of candidate enzymes for experimental studies will be rationalized by molecular phylogeny and structural bioinformatics approaches. To this end, bacterial biodiversity will be explored to identify candidate sucrose phosphorylases ("In silico Protein Design" team, UFIP).
2. The enzymes identified in step 1 will be produced and characterized experimentally by classical approaches in molecular biology, biochemistry and enzymology. A library of thirty enzymes will be produced. The study of their ability to functionalize flavonoids will be performed *in vitro* ("Molecular Engineering and Glycobiology" team, UFIP) on a library of flavonoid mixtures provided by the SONAS laboratory.
3. The characterization of glycosylated flavonoids obtained enzymatically will be carried out *via* innovative analytical chemistry approaches using two complementary approaches: capillary electrophoresis (team "Molecular Engineering and Glycobiology", UFIP) and mass spectrometry, coupled or not: LDI-MS and UPLC-MSⁿ and ¹³C-NMR-dereplicative analysis (SONAS Laboratory).
4. The evaluation of the antioxidant potential of the functionalized flavonoids will be carried out (DPPH, ORAC and anti-AGEs tests) within the SONAS laboratory, and a screening of the biological activities on cell models (in particular on the glioblastoma) of the glycosylated flavonoids obtained will be realized (UFIP collaboration with an external partner).

By applying this approach, it will be possible to explain retrospectively the rules underlying the activity and selectivity of sucrose phosphorylases on the target flavonoids.

Expected results

This work will enable the development of new efficient enzyme systems adapted to the controlled glycosylation of flavonoid pools derived from vegetable raw extracts, obtained from local and renewable sources (plants grown in the Pays de la Loire zone, co-products of agro-food processing...) and to study its selectivity. Innovative methodologies for the fine characterization of these complex mixtures will be developed. Thus, the LDI mass spectrometry, recently developed by SONAS in the case of (poly) phenols, should provide a powerful and innovative tool for the identification of flavonoid-enzyme-carbohydrate combinations and for the fine structural characterization of synthesized glycosylated flavonoids.

Required Profile

Doctor (PhD) in biochemistry/molecular biology, maximum 3 years of experience after thesis defense¹. An international experience in research is required (during or after Doctorate). Candidates must not have supported their thesis in the hiring institution and not previously worked in the host research unit.

The candidate must have solid experience in the fields of chemistry-biology to be able to implement approaches in enzymology, molecular biology and biochemistry. Complementary skills in the field of analytical characterization of natural substances are also required.

Usefull References

Website:

University of Nantes: <https://www.univ-nantes.fr/version-francaise/page-accueil-site-institutionnel-universite-de-nantes-2037832.kjsp>

UFIP (Unité Fonctionnalité et Ingénierie des Protéines) – UMR CNRS 6286, University of Nantes : <http://ufip.univ-nantes.fr/>

SONAS (Substances d'Origine Naturelle et Analogues Structuraux) - UPRES EA 921, University of Angers : <http://sonas.univ-angers.fr/fr/index.html/>

Références:

1. Aerts D, Verhaeghe TF, Roman BI, Stevens CV, Desmet T, Soetaert W (2011) Transglucosylation potential of six sucrose phosphorylases toward different classes of acceptors. *Carbohydr. Res.* 346:1860–1867.
2. Dirks-Hofmeister ME, Verhaeghe T, De Winter K, Desmet T (2015) Creating Space for Large Acceptors: Rational Biocatalyst Design for Resveratrol Glycosylation in an Aqueous System. *Angewandte Chemie* 127:9421–9424.
3. Kraus M, Grimm C, Seibel J (2016) Redesign of the Active Site of Sucrose Phosphorylase through a Clash-Induced Cascade of Loop Shifts. *Chembiochem* 17:33–36.
4. Kraus M, Grimm C, Seibel J (2018) Reversibility of a point mutation induced domain shift : expanding the conformational space of a Sucrose Phosphorylase *Sci Rep.* 8(1):10490
5. **Kraus** M, Grimm C, **Seibel** J. (2017) Switching enzyme specificity from phosphate to resveratrol glycosylation *Chem Commun (Camb)* 53(90):12181-12184
6. **Kraus** M, Görl J, Timm M, **Seibel** J. (2016) Synthesis of the rare disaccharide nigerose by structure-based design of a phosphorylase mutant with altered regioselectivity. *Chem Commun (Camb)* 28;52(25):4625-7
7. Verhaeghe T, De Winter K, Berland M, De Vreese R, Dhooghe M, Offmann B, Desmet T (2016) Converting bulk sugars into prebiotics: semi-rational design of a transglucosylase with controlled selectivity. *Chem. Commun. (Camb.)* 52:3687–3689.

¹ The thesis defense must have taken place after 31/08/2014, except in rare exceptions. Periods of sickness, maternity or parental leave shall not be counted in this 3 years period.

How to apply ?

Please send the following documents by email to:

Corinne MIRAL (corinne.miral@univ-nantes.fr) with copy to recherche@u-bretagne Loire.fr

- Short Curriculum Vitae and a covering letter showing your interest and especially addressing your professional project
- A list of your major works (2 pages max.) : scientific publications, patents and others scientific productions
- Letters of recommendation (not required)
- A copy of your PhD diploma²

The general selection process is described here: <https://u-bretagne Loire.fr/dossiers/postdoc/candidatures>

Selection process

The candidates whose profile will be selected will be auditioned at the beginning of June by a selection panel made up of representatives of the two research laboratories carrying the project and a representative from each of the concerned departments.

Further information

Annual Gross Salary: 44500 Euros

This Fellowship is co-funded by Université Bretagne Loire and Région Pays de la Loire

² For doctors graduated from a French establishment, a link to the thesis notice in the [SUDOC Catalogue](#) or the French official portal [Theses.fr](#) is sufficient.