# Research Projects in Biology and Chemistry

# Adam Mickiewicz University

## Chemistry

**1. Sol-gel synthesis of novel inorganic oxide framework materials**
Place: WCAT, Poznan, Poland
Supervisor: Dr. Robert Przekop
The project will include the preparation of ceramic oxide materials based on the sol-gel method. The materials obtained by this method are used as organic molecule matrices, catalyst carriers, ceramic membranes, and coating materials. The project goal is the production of series of SiO2-based materials modified with metal oxides and non-metallic oxides (Sr, Mg, Ca, Ti, P, B, Fe), in a group of 3 to 8 students. In the first step, a SiO2 matrix will be obtained, which will be modified to produce various final forms of the product - a powder, monolithic gel or coating on glass. The resulting materials will be characterized by SEM, SEM/EDS, AFM, optical microscopy, TG/TA, low temperature nitrogen adsorption, XRD, and FTIR spectroscopy. The whole study cycle involves 6 hours work per week for 12 weeks.

2. **Application-driven functionalization of carbon nanotubes.**
Carbon nanotubes (CNTs) have raised much interest during the recent years due to their inherent extraordinary electrical and mechanical properties. For some of the potential applications of this carbon allotrope, highly purified material is necessary, whereas the chemical inertness of the graphitic network presents a major challenge when it comes to composite material fabrication. However, the lack of solubility and the difficult manipulation in any solvents have imposed great limitations to the use of CNT. Indeed, as-produced CNT are insoluble in all organic solvents and aqueous solutions. They can be dispersed in some solvents by sonication, but precipitation immediately occurs when this process is interrupted. On the other hand, it has been demonstrated that CNT can interact with different classes of compounds.
This project aims to utilize wet-chemistry methods in CNTs functionalization, including preparation of well-dispersed CNT solutions in different solvents, preparation of scaffolds for biological applications and hydrophobic surfaces. Following analytic techniques will be used: transmission electron microscopy (TEM), scanning electron microscopy (SEM), atomic force microscopy (AFM), drop shape analysis (DSA) and other.

**3. Two-photon spectroscopy of fluorocrosslink**
Franzen, Skalski and Pedzinski
The project involves laser exctitation of 5-fluoro-4-thuiuridine (FSU) in the presence of thymidine to create the molecules known as fluorocrosslink. This photoreaction to create a fluorescent molecule is possible excited by two-photon excitation. To test this we use infrared light from a Ti:sapphire laser in the range of 720-760 nm to excite the molecule

and then detect the fluorocrosslinking reaction using fluorescence or time-resolved absorption.

# Biology

1. Oxidative stress and antioxidant system activity in plants exposed to biotic or abiotic stress

**A. Generation of reactive oxygen species and the functioning of the enzymatic antioxidant system in abiotic stress**

**B. Generation of nitric oxide in plant cells and antioxidant defense system**

Heavy metals can influence on physical and chemical processes in living organism. One of the effects of heavy metals is increased generation of reactive oxygen species (ROS) such as superoxide anion ($O_2^{-\bullet}$), hydrogen peroxide ($H_2O_2$), and hydroxyl radical ($OH^{\bullet}$) and RNS such as: nitric oxide (NO) which results in oxidative stress. We determine level of ROS and RNS in plant exposed to heavy metals: Cu, Cd, Pb and Cd. At high concentrations ROS and RNS damage major cell components: proteins, lipids and nucleic acids. Plant cells develop endogenous protective mechanisms involving antioxidant molecules (ascorbate, cysteine, glutathione, phytochelatins and α-tocopherol) and enzymes (superoxide dismutases SOD; catalase CAT; ascorbate peroxidase APOX; glutathione reductase GR). We are interested in plant defense mechanisms that allow them to function normally in a polluted environment.

2. Glutathione-phytochelatine system as part of the detoxication mechanism in plants exposed to stress factors

**A. Induction of gene expression of proteins involved in detoxification of heavy metals in plants**.

Plant cells have evolved various mechanisms to store excess metals to prevent their participation in unwanted toxic reactions - generally referred to as a detoxification system. Gene expression patterns change in response to toxic elements. After sensing the heavy metal, the plant activates specific genes to counteract the stress stimuli. We are particularly interested in the expression of enzymes involved in the metabolism of glutathione..

**B. The effect of the presence of growth promoting bacteria on the level of proteins involved in the response to oxidative stress induced by heavy metals.**

Plant growth-promoting bacteria can alleviate the inhibitory effects of various heavy metals on plant growth, via decreasing levels of stress-induced ethylene. However, little has been done to detect any mechanisms specific for heavy metal resistance of this kind of bacteria. We investigate the response of the antioxidative system, especially the level and activity of superoxide dismutase, ascorbate peroxidase and catalase in treated plants.

**C. Ecotoxicological examination of herbicidal ionic liquids by selected tests.**

Ionic liquids (IL), often called *green solvents*, are salts in liquid state, depending on their structure, they can exhibit diverse biological activities, including bacteriostatic, bactericidal and fungicidal properties. We investigate the effect of ionic liquids on the ecophysiological state of pea seedlings after 24 hours of exposition.We examine a range of treatments - 0.1 μM, 25 μM of each ionic liquid. We test for the index of tolerance (for roots, stems, fresh and dry weight), RWC, protein oxidation, chlorophyll a and b content, reactive oxygen species generation and antioxidative enzyme activity.

3. Use of biodegradable chelators and microorganisms for assisted phyto-assisted extraction

**A. Study of the transport route of root-leaf zinc ions in crop plants**

Plants are able to minimize the adverse effects of excess HMs by regulating the distribution and translocation of HMs within their organs or cells. The most common forms of this are the much higher amounts of HMs found in plant roots than in shoots, and reduced translocation of HM to the shoots. Although phytoremediation techniques favor plant species capable of transporting metals to the aboveground parts. Through the use of confocal microscopy and laser ablation, we investigate the pathway of zinc ion translocation in individual tissues and organs.

**B. Increasing metal mobility by nitrilotriacetic acid and bacterial surfactant supplementation**

Phytoextraction is an interesting alternative to conventional remediation technologies. However, calcareous soils with relatively high total metal contents are difficult to phytoremediate due to low soluble metal concentrations. Soil amendments and microorganisms are suggested to increase heavy metal bioavailability and uptake in aboveground plant parts. We are researching the possibilities of using NTA, ramnolipids and probiotic bacteria to increase both the level of metal removal from soil and transport to the aboveground parts of treated plants.

**4. Comparative proteomic analysis of Chelidonium majus plant latex and extracts after virus infection using 2-D electrophoresis**

**Introduction**

Greater Celandine (*Chelidonium majus* L.) is a perennial herbaceous plant from Papaveraceae family growing wild both in Europe and America. *C. majus* stems are usually 50 cm long, light green and hairy with pinnate leaves, small yellow flowers and yellow milky sap, which exudes after injury of any part of the plant. Fruits are linear, with two valved capsule and many seeds. Seeds are small, black and possess an elaiosome which attracts ants to disperse them (Arora and Sharma 2013). *C. majus* latex is a cytoplasm of an internal, articulated, and non-anastomosing laticifer system localized throughout the whole plant (Hagel et al. 2008). *C. majus* was known in traditional herbal medicine for its extensive medicinal properties. The fresh latex was used for skin

complaints such as *Tinea* infections, corns, eczema and tumors of the skin. Fresh plant extracts were often used for liver and biliary disorders and as a chalogogue (Barnes et al. 2007; Etxenagusia et al. 2000). One of the most important medicinal and traditional use of the fresh milky sap is its external application to treat warts, papillae and condylomas, which are caused by human papilloma virus (HPV) infections (Etxenagusia et al. 2000). The most pronounced medicinal activity is connected with the period of plant and latex collection, which is the strongest at the beginning of May during flowering and also fruit ripening (Nawrot et al. 2007, 2016, 2017; Arora and Sharma 2013; Etxenagusia et al. 2000; Monavari et al. 2012).

However, the literature information about antiviral activity of *C. majus* latex against plant virus is scarce, probably because the plant seems not to be affected by diseases, especially of viral origin (Pospieszny et al. 2004). To my best knowledge, the only plant virus which was shown to infect *C. majus* in its natural habitats is cucumber mosaic virus (CMV), which produce only very mild mottling on infected plants (Brcak 1979) or do not produce any visible disease symptoms (Pospieszny et al 2004). Symptomless *C. majus* plants were found to be natural hosts of CMV encapsidating satRNA (Hrzenjak et al. 1999).

## Project goal

The aim of the study is the proteomic comparison of *Chelidonium majus* milky sap and extracts collected from plants before and after virus infection using 2-D electrophoresis and MS/MS analysis of differentiated proteins to screen for proteins with upregulated expression during infection.

## Materials and methods

Samples of *C. majus* milky sap will be collected at AMU Morasko campus in September 2017. 4 healthy plants and 4 infected plants will be studied. In case of infected plants, they will be mechanically wounded using carborundum (SiC - sillicon carbide) and subsequently inoculated with potyvirus (PVY Ny and PVY LW) in controlled mini-greenhouse conditions.

The milky sap samples will be collected from both healthy and infected *C. majus* plants of similar developmental stage (height of the plant ca. 50 cm). The stems of adult *C. majus* plants will be cut and the exuding orange milky sap will be collected. The samples will be directly dissolved in 0.1 M Tris-Cl buffer, pH 8.0, containing 10% glycerol (sap : buffer ratio 1:2).

Both extract and milky sap samples will be separated into a supernatant, referred to as a protein extract, and a pellet fraction by centrifugation at 12,000 rpm for 20 min at 4ºC as described in Nawrot et al. (2007), with modifications. Supernatants will be stored at -20ºC for further analysis.

For protein extraction from *Chelidonium majus* TCA/Aceton method after Damerval 1986 with modifications (Rabilloud, 1999) will be used. Proteins will be isolated, dialysed, and 50 µg will be applied to 7 cm IPG strip (pH 4-7). The first dimension of two-dimensional (2-D) electrophoresis will be carried out using PROTEAN i12 IEF (BIO-RAD). This system is designed for isoelectric focusing (IEF) proteins in immobilized pH gradient (IPG) strips. GE Immobiline DryStrip pH 3-10, 7 cm (Product code: 17-6001-11) will be loaded with 50 µg of protein in two replicates.

After IEF, the second dimension of 2-DE will be carried out in a slab mini-gel apparatus using 13% polyacrylamide as the separating gel and 1.5% agarose as the stacking gel and stained with cCBB. 2-D gels will be scanned and analysed using Phoretix 2D software (Non-linear Dynamics). Selected protein spots will be excised from the gel and analyzed by liquid chromatography coupled to mass spectrometer in the Laboratory of Mass Spectrometry, Institute of Biochemistry and Biophysics, PAS, Warsaw, Poland.