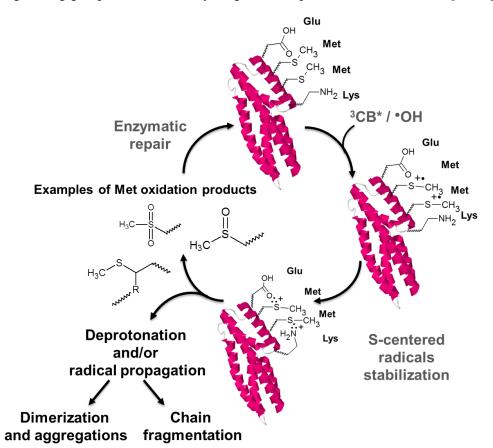
## Can stabilization of sulfur-centered radical cation influence protein functionality? From model compounds to real bio-systems.

The aim of this project is to analyze and understand the influence of radicals stabilization on protein structure and stability. Due to the fact, that proteins are built from hundreds different amino acids, their analysis, especially for fast reactions, is complicated.

Free radicals and oxidizing species are considered to cause many diseases states (e.g. cardiovascular) but also aging. One electron oxidants (such as hydroxyl radicals \*OH or excited triplet states of aromatic molecules) oxidize quickly methionine-containing proteins yielding **sulfur-centered radical cation MetS\***. This species is short lived and can undergo either deprotonation from adjacent carbon atom to form carbon centered radicals or can be stabilized by formation of exotic, unusual **two-centered three-electron** (**2c-3e**) bond with electron rich atom (S, N or O). These species have been investigated by many groups including project leader for almost 20 years, however still new controversies arise in scientific publications considering way of MetS\*+ stabilization. Thus it is important from scientific (and further protein analysis) point of view, that these processes are well characterized.

In this project, we are going to first investigate model compounds and make **final settlement** for primary and secondary processes occurring after oxidation using advanced techniques. Short lived species will be analyzed by time-resolved pulse radiolysis (PR) and laser flash photolysis (LFP) with optical (UV-Vis, Raman) and conductivity detection in order to follow reactions step by step. Model compounds have been chosen to form only S:S, S:O or S:N (2c-3e) species. Stable products identification by chromatography and mass spectrometry will give the answer, if there is any specific product of given stabilization. Results obtained from model compounds will be applied for proteins containing from one to six Met residues in their sequence with specific and different neighboring groups in close vicinity. A good example is *Histidine-containing Phosphotransfer Protein MtHPt1* 



(PDB 3US6) from Medicago truncatula. It contains two (from total 6) Met on the surface in position 6 and 19 in close vicinity with Lys15 (model for S:N) and Glu2 (model for S:Oformation). We are going analyze radical processes occurring in proteins via time resolved and LFP. Stable products analysis will be done by analyzing structural changes (mass spectra, X-ray, electrophoresis, ELYSA), sequence changes (amino acids composition) and functionality.