Since GCPII is a potential pharmacological target, it is being extensively studied in many labs all around the world and these studies comprise many topics. This is mirrored also by this PhD thesis. The papers included here concern two major issues: 1. analysis of GCPII structure and interactions with ligands; 2. study of GCPII distribution in tissues of human and two other species considered as potential animal models and kinetic characterization of the corresponding GCPII orthologs.

Structural studies of GCPII active site and substrate binding are driven by the attempt to broaden the information that could help the rational design of novel small GCPII ligands, functioning either as inhibitors in neuronal damage or as imaging agents in cancer diagnosis. Two papers included in this thesis describe ligand binding in the GCPII active site in detail, with particular emphasis on the S1' pocket in one case and on the S1 pocket in the other. Based on our findings, we can describe a set of interactions governing GCPII affinity to a substrate, accommodations that GCPII active site is capable of during ligand binding, the limits imposed on the ligand and tolerance of the enzyme to varying ligand nature. All these pieces of information are useful for the design of novel compounds with high affinity to GCPII, sufficient bioavailability, convenient pharmacokinetic and pharmacodynamic parameters, and in case of inhibitors also with high inhibition potency. This could be further assisted by the new paper of Bařinka, Plechanovová *et al.* that analyzes the reaction mechanism, particularly the role of Glu424, the predicted proton shuttle [manuscript in preparation].

Unfortunately, there is a relevant obstacle that could limit the benefit of some of the therapeutic approaches targeting GCPII: the widespread distribution of GCPII in human body. In the beginning of studying GCPII, the enzyme was thought to be prostate-specific, which, together with its upregulated expression in prostate carcinoma, made it an excellent candidate target for prostate cancer treatment. However, as the time went by, more and more results have been showing that the GCPII expression is not restricted to the prostate. The third paper included in the thesis involves a systematic study of GCPII expression in twenty-one human tissues and confirms more or less pronounced GCPII expression in the majority of them. This finding must certainly be taken into account in planning new anticancer strategies exploiting GCPII as a target (for example, antibodies recognizing GCPII conjugated to toxins or radiolabeled), especially with respect to a potential harm that could be made to healthy tissues

(particularly nervous system, spleen, liver, kidney, and small intestine that also display high GCPII levels).

This paper provides one more warning yet: GCPII expression in human, rat, and pig differ significantly, which is relevant especially with respect to the fact that rat is one of the most common animal models that are currently used. One should still keep in mind that GCPII is absent in several rat tissues that are GCPII-positive in human and vice versa, when using rat as an animal model for testing new GCPII-based diagnostic/therapeutic strategies *in vivo*.

The good news given by this paper is that rat and pig GCPII display very similar kinetic parameters (K_m and k_{cat}) as human enzyme and, moreover, are susceptible to its selective inhibitor 2-PMPA. It can thus be inferred that these orthologs could serve as a suitable approximation of human GCPII in kinetic studies, for example inhibitor testing.

However, it should be noted that the activity as well as immuno(histo)chemistry results could represent superposition of signals of human GCPII and its homologs, especially the closest of them, GCPIII. Human GCPIII is capable of NAAG hydrolysis with a comparable catalytic efficiency as GCPII and is also susceptible to 2-PMPA (measured at our laboratory; [43]). Not surprisingly, the structure of human GCPIII, determined by Hlouchová and Bařinka [manuscript in preparation], is very similar to that of GCPII. Given a very similar structure of these enzymes, it seems that it would be difficult to synthesize a specific ligand of GCPII that is not recognized by GCPIII.

For the therapeutic exploitation of GCPII, it would be very useful to know the precise physiological role of GCPII outside the nervous system and small intestine. Based on its structural similarity to the transferrin receptor and capability to internalize, we hypothesize that GCPII acts not only as a hydrolase, but could also serve as a receptor for a yet unknown ligand. The author of this thesis spent several years searching for the GCPII ligand, unfortunately with no result. Answer to this question could help to establish new GCPII-based therapeutic interventions, which is also the aim of all the papers included in the thesis.