The effects of carbamylation on the activity and effector functions of angiotensin I converting enzyme (ACE1)

Posttranslational modifications (PTMs) are biochemical reactions, in which, after synthesis, proteins are further modified by, for example, proteolytic cleavage or the covalent addition of various functional groups. Those changes may affect the chemical character of the whole protein, regulating its stability and biological activity. The occurence of various modifications significantly increases the complexity of the proteome and, moreover, can be an indication of an ongoing pathological process. One of the examples of such PTM is carbamylation, chemical modification of the amino acid lysine to homocitrulline, induced by cyanate generated for example by the decomposition of present in the blood urea. In physiological conditions, this modification occurs at a small scale, however due to pathological changes leading to a loss of glomerular filtration rate, causing the increase in urea concentrations in the blood and, by extension, cyanate, the carbamylation rate is increased. Among disorders characterized by the loss of glomerular function is chronic kidney disease (CKD), with global prevalence thought to be as high as 13%. A variety of factors can increase the risk of occurence of this disorder, like for example diabetes mellitus, glomerulonephritis, hypertension or age. Treatment of the chronic kidney disease is focused mainly on the managment of symptoms and halting further progression of kidney dysfunction, as during the severe stages of this disease the only treatments remain hemodialysis or kidney transplant.

One of the most popular strategies of CKD treatment is the regulation of hypertension by administering angiotensin I convertase (ACE1) inhibitors. ACE1 is one of the key components of renin-angiotensin-aldosterone system due to its ability to convert angiotensin I to the vasocontrictive angiotensin II, and to inactivate several vasodilating peptides (such as bradykinin), further increasing the blood pressure. Moreover, ACE1 plays a role in the regulation of innate and aaptive immunity, and it is even considered as one of the potential mechanisms for the treatment of Alzheimer's disease. Administering ACE1 inhibitors lowers blood pressure, protecting nephrons against further damage, but due to the plethora of functions of ACE1, its inhibition can lead to numerous side effects. As the levels of ACE1 in the blood of patients suffering from chronic kidney disease are heightened, it is highly feasible that it undergoes several types of posttranslational modifications, including carbamylation. That in turn can be another factor responsible for severe complications associated with renal dysfunction.

Therefore, this project's ambition is the elucidation of carbamylation-derived activity changes of ACE1:

1. We will confirm the presence of carbamylated ACE1 in the biological samples from patients undergoing hemodialysis.
2. We will map the likelihood of carbamylation of each lysine residue present in the structure of ACE1.
3. We will evaluate the changes in biological activity of ACE1, including it's affinity to its known biological substrates, such as angiotensin I, bradykinin, substance P and angiotensin 1-7, and the potential of products of ACE1 activity to activate the eukaryotic cells.
4. We will examine the effects of ACE1 carbamylation on the efficacy of inhibitors commonly used in hypertension therapy.

This project will allow for comprehensive evaluation of the role of carbamylation in the regulation of ACE1 activity and biological functions, and the effectiveness of current standard of care CKD therapies. Moreover, it might point towards novel therapeutic strategies, allowing for improvement in CKD patient's quality of life.