Enzymes are proteins synthesized in all living organisms that catalyse many metabolic pathways. Reactions catalyzed by enzymes are crucial in all vital processes of the cell. Each of them is localized at the given place and performs a definite action in the cell, and any malfunction may be fatal. One of the most important enzymes are protein kinases, i.e. enzymes that catalyze reaction of protein phosphorylation (i.e. transfer of the phosphate group). Kinases are involved in numerous crucial functions, also in signal pathways, so the interruption of their expression or activity often causes cancer transformation. Therefore kinases remain one of the main molecular targets purposes in the cancer treatment. Scientists are constantly looking for new compounds that may specifically block the activity of targetted kinase kinase (i.e. inhibitors), many different approaches are used for this purpose. The most commonly used approach exploits compounds with structure close to that of ATP molecule (which is the standard donor of the phosphate group, essential for the phosphorylation). These compounds usually bind at the ATP binding site of a protein kinase, thus blocking the binding of relevant ATP molecule. Next way is to use molecules mimicking a protein substrate, which binds at the substrate binding site, thus blocking the access of a protein to be phosphorylated. It is due to the ability of each kinase to recognize the characteristic sequence of a substrate protein, so the peptides carrying this fragment of the sequence will be also recognized. Another approach is to combine two approaches forming a covalent linking between compounds of the two types mentioned above (so called bi-substrate inhibitor). This way, the inhibitor may block both sites: the ATP- as well as the protein substrate binding site.

Well designed bi-substrate ligand should bind stronger than each of its separate moieties, thus being more specific toward a targetted kinase. However, this approach was neglected and most of known bi-substrate inhibitors of protein kinases were designed by chance, and no further systematic attempts to explore their modifications were performed. Therefore, in this project I intend to test the rationall approach for designing this kind of inhibitors. I will use protein kinase CK2 as the model protein. It is the subject of a high interest because of its key role in controlling almost all cellular functions. This kinase play a crucial role in cell survival and maintain homeostasis. CK2 is constitutively active and distributed ubiquitously in eukaryotic organisms. Protein kinase CK2 has become the therapeutic target for inhibitors in anticancer treatment due to a strong correlation between observed malignancy and an abnormally high level of CK2 found in cancer cells. In order to design the appropriate bi-substrate inhibitor I will be searching for fragments that bind at the ATP binding site and short peptides that will bind at the substrate binding site independently. I will choose only compounds which structure enabling combining them in one molecule by chemical linker in the future. After finding fragments that strongly bind to CK2 I will test if they bind independently i.e. such way that they don't influence of each other binding. It is important for them to form a strong binding particle in the future. Next linker will be test by measuring the binding affinity of ATP mimicking part extended of a part of second inhibitor. The last stage will be to synthesize bi-substrate inhibitor (consisting of two chosen fragments and the appropriate linker) and measure its strength of binding to CK2. That way I will verify the effectiveness of proposed approach. I will be measuring strength of binding of both individual fragment and the entire inhibitor with three various methods. Two of the allow the direct binding affinity measurement and the third method which I will use as a first way to reject poorly binding fragments is indirect method. This method measuring the temperature at which complex of protein-inhibitor changing the structure (is denaturating). Comparing the temperature of denaturation of complex with the temperature of denaturation of protein alone, in our case kinase CK2, giving information about the strength of binding of inhibitor to kinase CK2 (the higher temperature the stronger binding). If presented way of seeking new bi-substrate inhibitors (by searching of every his part individually and joining them after detailed analysis) will turn out to be effective it will be a platform for the fast screening without the need of the troubleshooting synthesis of the final bi-substrate inhibitor at the beginning. The results of this project should help in design of new more specific and potent inhibitors. The proposed inhibitor may be a base for further design of new kind of CKα inhibitor that is much more more specific than as far known ones.