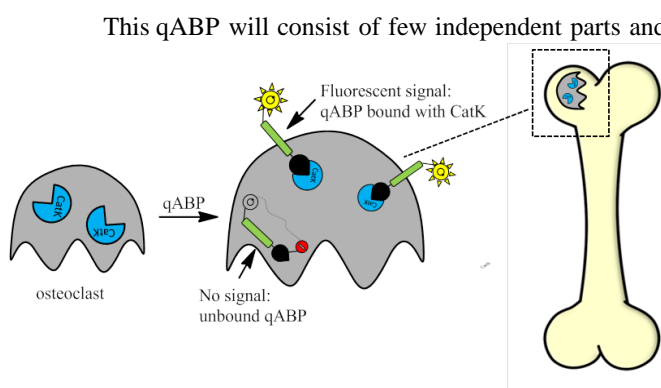


Proteases is one of the most common group of enzymes, which are involved in almost each physiological process in organism, starting from digestion through blood coagulation cascade and programmed cell death. Proteases are divided for smaller groups, among other is one of the most abundant group, called cysteine proteases. This group is named from the presence of reactive cysteine group in the enzyme active site what guarantees hydrolysis.

Cathepsin K is one of cysteine proteases which participates in a correct bone growth, bone remodeling and bone repair after injury. This enzyme was found in osteoclasts (bone loss cells) and, along with HNE (human neutrophil elastase), belongs to one of the most potent proteases thus its ability to extracellular matrix degradation. However, there are many factors, which prevent abnormal CatK activity and therefore allow to host protection. From time to time this natural prevention is broken, what leads to abnormal bone resorption. This kind of activity was found in osteoporosis and bone cancer. Osteoporosis progress based on bone resorption, and therefore leads to bone density changes, making it susceptible for breaking.

This enzyme-inhibitor imbalance is caused by many factors like enzyme overexpression or inhibitor insufficiency, however this process is not well investigated. To date there is no effective method of bone diseases treatment due to lack of accurate knowledge about particular factors participate in bone resorption. One of those factors is CatK, which is called osteoclast's biological marker, therefore aim of this proposal is design and synthesis of specific chemical marker as a tool to osteoclast's CatK visualization. It is believed that this probe will provide more basic information about CatK presence and function for further researches.



This qABP will consist of few independent parts and each part will have defined function. First part is specific peptide fragment which guarantee specific binding with CatK. This peptide sequence will be design based on results obtained with combinatorial substrate library screening. This library will consist of naturally occurring and synthetic amino acids, increases structural diversification and substrate-enzyme matching. Second part is warhead, which is responsible for covalent binding of qABP with enzyme. This feature is necessary during *in vitro* enzyme investigations. Third fragment is fluorescent dye which will give a signal form qABP. However, instead of specific sequence, one of the most important part of qABP is quencher which function is quenching fluorescence signal from unbind qABP. While

probe will bind with CatK, quencher will be released and fluorescence signal will be released. This feature lead to omit false fluorescence signal from unbind probe and is significant in *in vitro* CatK assays and can provide information about presence, amount, secretion and storage of enzyme in osteoclasts, and thereby increase our knowledge in this field.