1. Aim of the research: Hydrolysis of peptide bond by proteases, is irreversible, so it must be tightly controlled to prevent damage to the cell or whole organism. One way of controlling proteolytic activity, especially in multicellular organisms, is synthesis of proteinaceous protease inhibitors. Almost all inhibitors are specific for particular proteases that share similar substrate specificity and interact directly with the active site cleft, blocking access to it for all substrates. In stark contrast, members of the α_2-macroglobulin (A2M) inhibitor family inhibit a broad spectrum of proteases without disturbing their active sites. The inhibition is achieved by irreversible trap mechanisms resulting from large conformational rearrangements upon a prey protease's cleavage in the bait region resulting in encaging of the protease. The mechanism of action of A2Ms could be compared to carnivorous plants such as sundew and *Venus flytrap* hunting on insects. In stark contrast to these plants, trapped proteases are intact and active against small substrates. Outside metazoans, putative A2M genes are generally absent from archaea, protozoa, fungi and plants. Strikingly, they are found in Gram-negative bacteria colonising mammals but not in free-living environmental microorganisms. In this context, it is fascinating that two bacterial species express A2Ms: *Porphyromonas gingivalis (Pg)* and *Tannerella forsythia (Tf)*, which are major etiologic factors of periodontal diseases, affecting in its severe forms even 15% of adults worldwide. If untreated, the condition leads to the destruction of teeth supporting tissues resulting finally in tooth loss and contributes to the development and/or progression of systemic diseases such as diabetes, atherosclerosis, rheumatoid arthritis, and neurodegenerative diseases. *Therefore, the main aim of the proposed project is not only a detailed biochemical and structural description of the inhibitory mechanism of periodontal A2Ms, but also their role in periodontopathogens virulence.*

2. Description of research: TfA2M and PgA2M will be obtained as recombinant proteins employing a bacterial expression system. First, we will identify proteases, both human and endogenous, inhibited by investigated A2Ms and biochemically characterise the inhibition process in detail. To achieve it, we will determine the stoichiometry of inhibition (SI), the number of A2M molecules required for inhibition of one molecule of active protease, and association rate constant (k_{ass}), a parameter describing how fast protease activity is scavenged, identify the molecular mass of smallest proteinaceous substrate for which inhibition occurred, and analyse the formation and chemical nature of inhibitory complexes. In this step, we will also analyse the inhibitory flexibility of A2Ms by introducing different changes to A2M, aiming to improve the inhibitory properties of A2Ms. Secondly, we will solve the three-dimensional structure of A2Ms: native and in complex with target proteases employing X-ray crystallography and cryogenic electron microscopy (cryo-EM). Finally, we will describe the biological role of A2Ms. To do it, we will check using *P. gingivalis* and *T. forsythia* cells: wild-type and deletion mutants lacking A2M if A2Ms protects the bacteria against killing by human phagocytic (neutrophils, macrophages) and epithelial cells. We will also check if A2Ms, through inhibition of a protease, dentilisin, limit virulence of another periodontopathogen, *Treponema denticola*.

3. Reason for doing research: In stark contrast to mammals, A2Ms are rarely found in bacteria and surprisingly predominantly in microorganisms colonising mammals. Moreover, bacterial, opposite to mammalian, A2Ms are very inefficient inhibitors, which even have not allowed their detailed biochemical characterisation. In such a context, it is fascinating that two A2Ms produced by human periodontopathogens are very effective inhibitors. Due to low similarity to other characterised A2Ms structural studies of TfA2M and PgA2M may reveal a novel mechanism of inhibition for this family of inhibitors. Finally, due to the sporadic distribution of A2Ms among bacteria, it is tempting to speculate that A2Ms play an essential role in the virulence of *P. gingivalis* and *T. forsythia*. Due to involvement in numerous physiological processes and aetiology of diseases, proteases are considered an attractive target for drug development. Example of such conditions includes periodontal diseases, Crohn disease, irritated bowel syndrome and ulcerative colitis. In such a context, A2Ms seems like a good candidate for drug development, because on contrary to the majority of known inhibitors, they could inhibit many completely different proteases.

4. Outcome of research: The realisation of the project will lead not only to the detailed description of the inhibitory mechanism of periodontal A2Ms but also of the role of A2Ms in periodontopathogens virulence. Altogether, compared with results obtained for other A2Ms, this data will also shed more light on the evolution of bacterial A2Ms. Thus, these results improved our knowledge in such fields as biochemistry, structural biology and microbiology. Despite the pure scientific outcome, we may also identify a novel inhibitory molecule based on the A2Ms scaffold, which could be the first step of developing a drug, which may find application in the treatment of disease, in which aetiology numerous proteases play a crucial role.