Description for the general public

Glucose is the main energy source used by brain cells. The tight regulation of glucose metabolism is crucial for brain physiology affecting both the brain itself, as well as the entire organism. By studying rats, a research team from Japan in 2006 identified a short peptide called nesfatin-1, which reduced appetite and the production of body fat when injected into the brain of the animals. As a metabolism-regulated peptide, nesfatin-1 has been widely studied in the field of glucose and fatty acid metabolism. It was found to be cleaved from the precursor protein Nucleobindin-2 (Nucb2). This small macromolecule shortly attracted the attention of several independent groups and up to date together with Nucb2 is found not only in the brain but also in peripheral tissues including the stomach, where it is expressed at the highest level in the body, pancreas, adipocytes and blood. Initially ascribed as a satiety molecule which inhibits food intake and body weight and regulates glucose homeostasis, both Nucb2 and nesfatin-1 have subsequently been shown to influence other homeostasis systems encompassing water intake, gastric emptying, stress-related endocrine and anxiogenic responses and reproduction. Moreover, recent studies in humans discussed a potential use of Nucb2 and nesfatin-1 as biomarkers in specific diseases.

For a number of key problems in molecular biochemistry, such as protein folding or protein-ligand interactions, direct analyses of higher order protein structure provide significant insight into it molecular features, often critical for the biological function. Nucb2 is a calcium ion-binding, multi-domain protein. It undergoes proteolytic processing not only to the nesfatin-1, but also to two more peptide products called nesfatin-2 and nesfatin-3. Surprisingly, of these three peptides, only nesfatin-1 exhibits some biological activity. The roles, if any, of nesfatin-2 and -3 still remain unknown. The putative complex structure of Nucb2 may explain diverse functions of this protein. Proteins with multiple domains, in general, have been shown to be central to numerous signal pathways acting as hubs facilitating signal integration and transduction. It is of interest to note that although the role of Nub2 is nowadays broadly studied, nothing is known about its molecular structure and the molecular details of its action. It has been long believed that a defined three-dimensional structure is indispensable for proteins and their biological functions. However, recent analyses have demonstrated that many proteins are unable to fold spontaneously into stable, globular structures but are rather dynamically disordered and adopt in solution multiple conformations. Based on our preliminary research data, we hypothesize that the multiple and astonishingly diverse functions that Nucb2 has been found to be involved in, might be explained by the fact that this protein very likely belongs to the family of intrinsically disordered proteins. The ability of proteins to exhibit multiple functions that are additionally not due to gene fusions or splice variants, is known as protein moonlighting. According to some authors, there might be a relationship between protein conformational fluctuations and the diversity of its functions. Therefore, such structural adaptability of an intrinsically disordered protein confers the possibility that many of them might also moonlight. To better understand the biological functions of Nucb2 and nesfatins we propose research project focused on unresolved issue of Nucb2 structure in solution. The project is aimed at gaining an understanding of the molecular structure characteristics of Nucb2 and shed some light on how this structure is modulated by the interactions between this protein and its potential ligands. In order to provide this missing but important knowledge we will test our hypothesis that both, conformational flexibility and intrinsically disordered regions are crucial for structure–function relationships in the Nucb2 molecules. Our biophysical characterisation will include the analysis of a secondary structure of studied proteins – i.e. providing information on secondary structure features with circular dichroism spectroscopy and mapping of the position of intrinsically disordered regions using hydrogen/deuterium exchange mass spectrometry as well as characterization of the tertiary structure of Nucb2s and nesfatins with dynamic light scattering, size-exclusion chromatography with multi-angle laser light scattering, analytical ultracentrifugation and small-angle scattering of X-rays. The project includes also investigation of the molecular role of divalent metal cations on Nucb2/nesfatins conformation studied using isothermal titration calorimetry and thermal shift assay.

A novel aspect of our study will be to integrate structural and functional relationships of Nucb2 and nesfatins in the presence and absence of their ligands on the molecular level. Our research and analysis techniques will aid future scientific efforts and may even provide novel opportunities to develop new promising clinical treatments. Unravelling the molecular mechanisms of Nucb2/nesfatins action will promote the discovery of new strategies that might help to fight obesity, obesity-associated insulin resistance, thermogenesis, diabetes, aging, many other metabolic diseases and cancer. It seems that Nucb2 might be a novel target for biomedical research or therapeutic intervention. Therefore, as a result of this project, experimental determination of details regarding Nucb2 structure might build connections between basic and applied science and integrate explored recently functional research knowledge into one conceptual framework.