Copper is an essential trace element in the human body. The main role of copper is to provide mitochondrial enzymes with chemical competences to harness energy produced by interaction of oxygen with glucose and other nutrients in the process called oxidative phosphorylation. This process releases trace amounts of highly toxic reactive oxygen species (ROS) Thus another function of copper is to defend the body from ROS, turning them into less toxic substances, also in specific enzymes. Other copper enzymes help maintain neurotransmission or provide skin pigmentation in controlled oxidation reactions. In all these reactions the role of copper is to handle electrons by using its ability to change its state of oxidation between Cu(II) and Cu(I). But copper is a toxic element itself, because its compounds can generate ROS catalytically on their own, by the same Cu(II)/Cu(I) redox couple as in the enzymes. In order to prevent such toxicity, the organisms evolved ways of dealing with copper in a safe, nonreactive form. Copper ions are provided to cells and transported inside them by a set of dedicated proteins, channels and chaperones, as relatively unreactive Cu(I) ions, until they are handed over to the respective enzymes. Such mechanisms are not possible, however, in blood and other body fluids through which copper must travel from food in the intestine to its targets inside cells. The amount of oxygen in blood is high and Cu(I) complexes get easily damaged by oxidation. Therefore, a different system of transport is needed, based on Cu(II) which is stable in oxygenated media. This system is much less understood in comparison with the intracellular one.

Serum albumin (HSA), a universal carrier protein of human blood is considered to be the major participant of copper transport in this fluid. The way it might deliver copper to hCtr1, the protein responsible for acquisition of this element by the cells remain elusive, however. hCtr1 can channel only Cu(I) ions across the cell membrane, thus the extracellular Cu(II) need to be reduced prior to transfer. The direct reactions of copper exchange from HSA and to hCtr1 studied so far appear to be much too slow for the physiological requirements, and the copper reduction sites and mechanisms remain unknown.

We want to understand these processes because there is growing evidence that they are related to pathological processes in cancer, diabetes and neurodegenerative diseases, such as Alzheimer's disease. In order to achieve this, we ought to study the chemical principles underlying copper physiology, because only the detailed molecular level knowledge empowers one to design remedies, such as new drugs or lifestyle changes.

On the basis of a series of recently published and unpublished preliminary studies performed in our research group we propose that the actual mechanisms of exchange and reduction require the participation of further molecules in addition to HSA and other copper carriers in blood and the recipient hCtr1 protein. One important aim of this project is to identify such molecules on the basis of their chemical properties. We are going to start our research with accurate investigations of rates of association and dissociation of Cu(II) complexes with peptides being simpler models of copper managing proteins in order to derive the reaction mechanisms. Next, we will screen several types of molecules that we expect to be able to assist in Cu(II) exchange by interacting with the Cu(II) releasing complexes. After building up sufficient expertise, we will perform experiments with HSA to identify what molecules present physiologically in human blood may participate in copper transport. Another objective of the project is to identify the molecules that participate in Cu(II) reduction to Cu(I) without producing ROS. In the final stage of the project we will use a special instrument in which molecules of hCtr1 will be placed in a minute stretch of biological membrane and we will measure the electric current flowing through them in the form of Cu^{+} ions. In this system we will perform the ultimate tests of results of all preceding experiments, to deliver to the public a comprehensive proposal of the molecular mechanism of copper transport in blood, ready for further biological tests.

Most of the work will be performed in our laboratories at IBB PAS, but some key elements require Polish and international cooperation. We will synthesize most of the peptides we will use in our studies and both proteins, HSA and hCtr1, but peptide models of a domain of hCtr1 required for some tests will be provided by the collaborating groups in the USA and France. The dissociation reactions will be studied at IBB PAS by a number of spectroscopic techniques, but the fastest reactions require the specially constructed equipment which is not available comercially. Such equipment will be made available to us by a collaborating group in the Netherlands. Electrochemical studies of copper oxidation and reduction will be performed at Warsaw Technology University, and the functional studies of isolated hCtr1 described above will be initially performed in Scotland. Then, the similar apparatus will be installed in Warsaw so that the experiments will be done in parallel in both laboratories.