

## **Biological membrane as actor in copper biochemistry: chemical models, neuromodulators and a validating bioassay**

Biological compartments are surrounded by membranes - barriers which allow the cells and organisms to control and maintain stable internal environment for complicated networks of chemical reactions which constitute life. These barriers consist of phospholipids, molecules containing hydrocarbon tails and phosphate-based headgroups, which can be further substituted. These molecules, called amphiphilic, have a tendency to self-assemble into large structures – double layers in which the hydrocarbon tails from neighboring molecules align with each other, while phosphate headgroups extend to the outside. The interior of such structure is hydrophobic – does not mix with water molecules, while the exterior is hydrophilic – forms hydrogen bonds with water molecules. Proteins are significant components of biological membranes, providing points of import and export of metabolites, as well as communicating with other cells. For this reason functions of phospholipid membranes are usually considered in conjunction with the corresponding proteins. Phospholipids and related amphiphilic molecules can also form smaller structures, including vesicles (liposomes) and micelles. There is, however, a growing number of functions that can be assigned to membranes apart from their constitutive proteins. One such function is to stabilize alternative structures for membrane proteins/peptides, e.g. to modify their toxicity.

The goal of this project is to elucidate a yet another function of membranes, which has been postulated by us on the basis of preliminary experimental results obtained in our laboratory: the influence of membrane-like environment on chemical properties of copper ions, which might be mediated by neuromodulators, the biological peptides present in the brain and other parts of the nervous systems. The functions of neuromodulators is to modify, strengthen or weaken responses of neurons to stimuli, by which the nervous system acquires ability to act flexibly. Copper ions also participate in these processes on many levels. Nevertheless, all such regulatory effects occur at the level of individual synapses, connections between the nerve cells. The endings of neurons are separated by extremely narrow gaps filled with liquid, called synaptic clefts. Copper ions are released to this gaps in response to the incoming signal, and should be removed and released again for the next signal. The problem is that these signals can arrive very frequently, like every 100 ms, but known chemical reactions of copper ions with molecules present on-site are slower. The hypothesis of this project is that the membrane provides conditions for acceleration of the known reactions or for creation of alternative processes. The postulated role of membrane in synaptic chemistry is strengthened by a particularly high ratio of membrane surface to volume in the synaptic cleft. Working out chemical reaction using real synapses is technically impossible, and even the usage of phospholipid membranes is problematic. Instead, we propose to establish essential chemical properties of amphiphilic environments towards copper using synthetic micelles based on SDS detergent, used in structural studies of proteins. This would guide the follow-up studies using liposomes and living cells. In this way we are going to collect exact chemical data in physically simple systems and validate them in realistic models. Such data can then be extrapolated to conditions of synapses, using additional information from biological studies, such as process times, concentrations of proteins and small molecules etc. The obtained knowledge will contribute to designing innovative metallodrugs and therapies.

We divided the project into nine tasks: (i) chemical synthesis of neuromodulators; (ii) study of the effect of micellar environment on the Cu(II) binding affinity of neuromodulators, with the use of several types of micelles; (iii) determination of three-dimensional structures of selected (most interesting) complexes formed in given types of micelles, with the use of multidimensional nuclear magnetic resonance (NMR), and special research method empowering obtaining structural data despite the interference from Cu(II) ions; (iv) study of susceptibility of selected complex/micelle systems to competition from small hydrophilic ligands: on the basis of our preliminary experiments we can expect that the presence of micelles should weaken some interactions of Cu(II) ions with neuromodulators, which could open the way for new reactions with small molecules abundant in the brain, such as glutamic acid, histidine, carnosine, taurine, as well as dopamine, acetylcholine, noradrenaline and serotonin, and short histidine peptides; (v) study on susceptibility of selected complex/micelle systems to chemical reduction to a Cu(I) complex, which is necessary for its transport out of the synaptic cleft; (vi) research of the effect of micellar environment on the rates of exchange of Cu(II) ions between neuromodulators and other synaptic ligands, in the millisecond or shorter time range; (vii) creation of micelles directly binding the Cu(II) ions, in addition to interactions with neuromodulators. This will provide a new model for Cu(II) binding by phosphatidylserine, a component of natural membranes; (viii) study of interactions of Cu(II) complexes with negatively charged, neutral and Cu(II)-binding liposomes, small membrane-like structures, to validate the micellar models, and (ix) further validation of these models on a basis of a cellular copper intake model developed in our laboratory, which makes possible to study the effects generated directly by individual types of molecules on the HEK293 cell line. Realization of these tasks will provide a robust and comprehensive chemical landscape of membrane role in synaptic copper function.