

pole of the dimer, whereas the other pole carries domain swap mutations, which prevent binding to receptor. As HD-VEGF can only bind to monomeric receptors, it does not lead to signal transduction. Thus, HD-VEGF blocks KDR-mediated VEGF activities that are crucial in the angiogenic process and is therefore a promising, multipotent compound in the treatment of angiogenesis-related diseases. The receptor binding domain of wild type VEGF with N-terminal His-tag was expressed as inclusion body in *E. coli* and refolded successfully in our lab. To construct the heterodimeric VEGF variant, the precise KDR binding sites were determined from the crystal structure of VEGF-KDR complex (PDB ID: 3V2A). Considering to the ϕ and ψ dihedral angles, the distance between the first and the last amino acids and the length of these segments, some sequence with equal length and distance compared to those of VEGF were searched in PDB site. The binding sites in VEGF crystal structure were replaced by suitable sequences. The model of designed mutant VEGF was compared to native one after energy minimization process. Based on constructed model, the receptor binding domain of mutant VEGF gene was designed, synthesized and inserted in pET-21a expression vector with additional N-terminal Strep-tag which providing a system that able to purification of heterodimeric from homodimeric variants by two affinity chromatography. The mutant VEGF was expressed in *E. coli* and after purification of heterodimeric variant, its anti angiogenic property will be investigated in future.

Keywords: Angiogenesis, Antagonistic VEGF, Heterodimer, Strep-tag.

Abstract No.187

ZnO and CuO Nanoparticles Increase Extracellular Glutamate Concentration in Synaptosomes of Rat Brain

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In modern life, human beings are exposed to nanoparticles from ambient air and certain work places. There have been increasing reports that inhaled nanoparticles can reach the brain through two main routes: blood circulation and olfactory bulb penetration. More over exposure to nanoparticles in vivo increases the risk of neurodegenerative diseases while in vitro studies show that nanoparticles can kill neurons. However the mechanisms underlying this phenomenon are still unclear. It is believed that the toxicity of

nanoparticles in brain is mediated by induction of oxidative stress in microglia cells and astrocytes. However neuronal presynaptic terminals could be other targets for nanoparticles due to high turn over of synaptic vesicles in these terminals. In this case it has been shown that ferritin, as an iron nanoparticle, inhibits glutamate uptake. Glutamate is the major excitatory neurotransmitter in mammalian central nervous system. Excessive extracellular concentrations of this neurotransmitter could cause hyper activation of glutamate receptors and result in cell death. In the present work the effect of ZnO and CuO, two metal oxide nanoparticles that are widely used in industry, has been studied on the extracellular concentration of glutamate in rat Synaptosomes. We found that ZnO and CuO nanoparticles can cause remarkable increase in extracellular glutamate in Synaptosomes. We suppose that glutamate uptake is inhibited in the presence of these metal oxide nanoparticles.

Keywords: Synaptosomes, Neurodegenerative Diseases, ZnO/CuO Nanoparticles.

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Determination of Microscopic Dissociation Constants of Tryptophan

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The study of the interactions between surfactants and proteins is of significant scientific and technological importance [1]. The macroconstant pKa values are commonly known while the microconstant values (especially tautomerization constant Kz) are often not recognized [2]. The concept of microscopic constants is important and interesting, and it has the advantage of being directly related to the specific functional groups involved, thereby, enabling investigators to interpret such parameters in terms of molecular structure. Determination of microscopic constants and tautomeric ratios has played an important part in understanding the ionic composition of many biologically active molecules, particularly because all proteins fall into this class. Furthermore, the microscopic constants and tautomeric ratio describe the amount of various species as a function of pH [3]. The present work is an attempt to study the effect of anionic surfactant SDS on the dissociation equilibria of the amino acid tryptophan. In this study, we have determined macroconstant Ka1, Ka2 values, tautomerization constant kz and microconstants k11, k12,