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Effects of metal oxide nanomaterials on cultured human cells: A redox proteomic investigation

David Sheehan, Arjun Prakash Sridharan, Hugh Doyle and Yao Zhu
University College Cork, Ireland

Nanomaterials are an important category of emerging environmental threat given that they are being used in ever-increasing amounts and for an ever-wider range of applications. Nanoparticles, a sub-set of nanomaterials, are particles of average diameter < 100 nm and are readily prepared from metal oxides. Metal oxide nanoparticles are often used in sun creams and other topical preparations. In this study the sensitivity of a panel of human cell lines (HEk, HeLa and A172) to TiO_2, CuO_2 and ZnO_2 nanoparticles was assessed. Cytotoxicity was determined using both neutral red retention and the MTT assays. These suggested that cells had much lower LC_{50} with ZnO_2 and CuO_2 than with TiO_2 and that both assays gave comparable results. A more detailed study was made of CuO_2 effects on HeLa cells using redox proteomics. Thiol-containing proteins were labelled with 5-iodoacetamido-fluorescein (IAF) and fluorescence images were obtained in a Typhoon scanner. Gels were then stained with colloidal coomassie and all images were analysed with SameSpots image analysis software. Differentially expressed and selectively oxidised proteins were identified by peptide mass fingerprinting by LC-tandem MS. Our data suggested that >20 individual proteins are selectively oxidised in response to CuO_2.

d.sheehan@ucc.ie

Phosphorylation of activation loop induces structural changes in c-Src tyrosine kinase leading to a conformational switch to the ATP binding conformation in the presence of the ligand

Encarna Pucheta-Martínez, Nicola D'Amelio and Francesco Luigi Gervasio
University College London, UK

Tyrosine kinases play a crucial role in tumor formation and are the most frequently mutated protein family in cancer. The first viral oncogenic protein discovered, c-Src is involved in metastasis and is mutated in 50% of colon, liver, lung, breast and pancreas tumors. Understanding its conformational dynamics is important to address its activation mechanism and also help the design of more selective inhibitors as in the case of the anti-leukemic drug imatinib (Gleevec). Upon phosphorylation, various conserved structural elements including the activation loop, switch from an inactive to an active form able to bind ATP and phosphorylate a substrate in a cellular signaling process leading to cell replication. Crystal structures of their catalytic domain suggest that phosphorylation restrains the motion of flexible parts, in particular the activation loop which becomes visible in the crystals of the phosphorylated form. In this work, we show how phosphorylation drastically changes the dynamics of the C-lobe in c-Src by NMR analysis, a phenomenon totally invisible by crystallographic data. Flexibility in this region may have the biological role of preparing the structure to harbor the substrate. Besides this effect, signals from other parts of the protein, most likely the activation loop, become visible in agreement with a reduced conformational variability suggested by crystallographic data. NMR also shows that these modifications deeply impact on the mechanism of action through changing the binding affinity for ATP or ADP. In fact, while both the un-phosphorylated and the phosphorylated forms promptly bind ATP, de-phosphorylation greatly reduces the affinity for ADP, as its binding would impede the kinase to work efficiently. We also show that the conformation required for the binding of ATP and ADP to c-Src is already present in the absence of the ligand (independently on the phosphorylation state) and the interaction involves a process of conformational selection rather than induced fit.

encarnapucheta@gmail.com