Optochemical control of Cu(I) homeostasis in mammalian cells

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The redox-active copper atom is a crucial co-factor for the essential cellular processes in all forms of life. Electron exchange by cuproenzymes helps in metabolism, synthesizes hormones, and acts as neurotransmitters. Cellular homeostasis is often required and in the case of copper, it is inevitable. The increase in copper levels evidently enhances the chances of cancer, and Wilson's disease, even lead to neurodegenerative diseases like Alzheimer's.[1] Since copper is an essential element, the complete depletion of copper by the chelators could have adverse effect in many cellular functions. To overcome this issue, an additional layer of control/stimuli over the chelator would be beneficial.[2] In the last several years, light has been used as an external stimulus to control various biological functions due to its non-invasive nature. In this regard, we have designed a photocaged copper chelator that can be activated by light on demand. The efficiency of the copper chelation was confirmed by absorption and fluorescence studies. We have further investigated the copper chelation in HeLa cells in the presence of a turn-on copper specific fluorophore.[3] The copper dependent trafficking of ATPases was studied by immunostaining after incubation with Photocaged chelator for both in presence and absence of light.

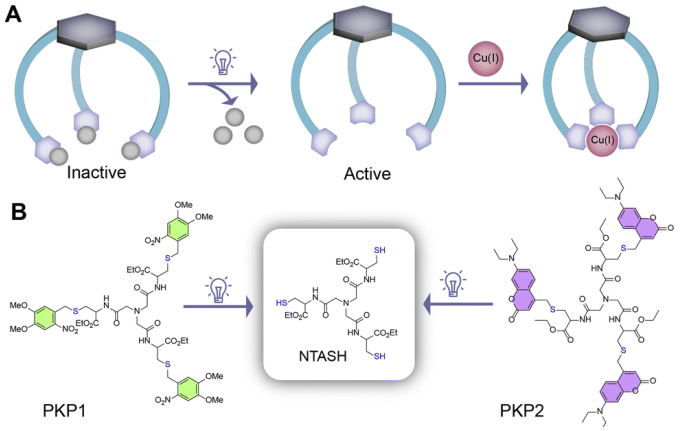

Figure 1 (A) Schematic representation of the photocaged Cu(I) chelator. (B) Cu(I) chelators PKP1 and PKP2 photocaged with NVOC and the coumarin group.

In finding another path to maintain copper homeostasis, we have designed a chelator which can be turned-off in presence of light. A small molecule based tripodal chelator which shows high affinity towards Cu(I), both biochemically and inside mammalian cells. Upon irradiation with light, with copper-specific fluorophore; we have observed increase in the fluorescence leading us to believe that no copper chelation after irradiation is occurring. Mitochondrial activity in cells and copper abundance are intertwined, we are further investigating the changes in mitochondria and cellular activities before and after chelation.[4]

[1] S. Lutsenko, *Curr. Opin. Chem. Biol.,* **2010**, *14,* 211-217.
[2] A. M. Pujol, M. Cuillel, A. S. Jullien, C. Lebrun, D. Cassio, E. Mintz, C. Gateau, P. Delangle, *Angew. Chemie. Int. Ed.,* **2012**, *51*, 7445-7448.
[3] P. K. Pain, D. Palit, M. Shegane, R. P. Singh, D. Manna, *Chem. Commun*., **2023**, *59*, 2315-2318.
[4] P. K. Pain, D. Manna, *Manuscript under preparation*.