

Rational Design of Fluorescence Sensors for the Biological Chemistry of Copper

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Copper is an essential micronutrient that plays a central role for a broad range of biological processes. While there is compelling evidence that the cytoplasm does not contain any free copper ions, the rapid kinetics of copper uptake and release suggests the presence of a labile intracellular copper pool. To elucidate the subcellular localization of this pool we designed a series of fluorescent probes that either respond with a wavelength shift or bright emission enhancement upon binding of monovalent copper. The sensors exhibit excellent selectivity towards Cu(I), and their emission response is not compromised by the presence of millimolar concentrations of Ca(II) or Mg(II) ions. Variable temperature NMR studies revealed a rapid Cu(I) self-exchange equilibrium with a low activation barrier at room temperature ($\Delta G^\ddagger = 44$ kJ/mol). Mouse fibroblast cells incubated with the sensor produced a perinuclear staining pattern that is responsive to the extracellular availability of copper. The intracellular staining pattern agrees qualitatively well with the subcellular topography of copper as determined by synchrotron-based micro x-ray fluorescence microscopy (micro-XRF). The data provide a coherent picture with strong evidence for a kinetically labile copper pool, which is predominantly localized in the mitochondria and the Golgi apparatus.