UPTAKE OF CU(II) VISUALIZED BY FLIM

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Heavy-metal ions play an ambiguous role in the life sciences. At the one hand, these ions often constitute the active centers of proteins and are, therefore, essential. Redox enzymes or oxygen transporters are prominent examples. At the other hand, excess concentrations can be poisonous as some metal ions have a strong affinity to several cellular compounds. Several human diseases are related to a malfunctioning metal-ion metabolism. The most relevant heavy-metal ions are the late transition metal ions like iron, nickel, cobalt, copper and zinc. Copper, for example, is integral part of some human ferri-oxidoreductases, but it is also associated with the occurrence of Alzheimer’s disease. It is therefore highly desirable to detect, to quantify copper-ions in its higher oxidation state, i.e. Cu$^{2+}$, and to distinguish it from the lower oxidation state, i.e. Cu$^{2+}$. Both ion states might be present in cellular systems, the discussed concentration ranges in cellular environments are between $10^{-18}$ M and $10^{-4}$ M.

Fluorescent proteins (FPs) are nowadays integral part of the molecular biology toolbox. They can be easily expressed in pro- and eukaryotic cells at arbitrary cellular locations. In order to study the above mentioned pathologies in model organisms, FPs are obvious targets for establishing sensors for the above-mentioned metal ions. Some sensors on the framework of FPs exist and exploit the static quenching. As the fluorescence intensity is not self-referencing, i.e. the intensity depends on the sensor concentration, such an approach might only qualitatively indicate Cu$^{2+}$. Photoprocesses in FPs might impose further challenges [1], [2].

The problems associated with fluctuating intensities are overcome by exploiting the fluorescence lifetime of FPs. We will present a titration experiment with a His$_6$-tag as the Cu binding site including the determination of the underlying dissociation constant $K_D \sim 10^{-5}$ M. The proposed approach is only sensitive to the concentration changes of Cu$^{2+}$, but unaffected by the other relevant metal ions. We were able to visualize the uptake of Cu$^{2+}$ into E.coli and plant root cells. Exterior concentrations of $10^{-4}$ M are applied, and the decrease of the fluorescence lifetime is reversed by the addition of strongly chelating agents. Finally, we will discuss how lower concentration ranges of Cu$^{2+}$ can be accessed.
