Copper Sensing Based on the Far-Red Fluorescent Protein, HcRed, from *Heteractis crispa*

Eric Hunt

Abstract:

HcRed was initially isolated as a chromoprotein from the reef coral *Heteractis crispa* and converted to its current far-red fluorescent form through random mutagenesis by PCR. We report, for the first time, on the natural affinity of HcRed to bind ionic copper(II) and on its application in the development of a reagentless copper-sensing system. Metal binding studies indicate that HcRed binds copper(II) with high specificity over other divalent cations, resulting in the quenching of its fluorescence. Stern-Volmer relationships developed from temperature-dependent fluorescent studies, as well as evidence from UV-Visible and CD spectroscopy, indicate that this quenching proceeds by some static mechanism. Chemical modification of histidine and cysteine residues resulted in a substantial decrease in copper binding, providing evidence for the formation of a metal-protein complex. These findings served as the basis for the development of a reagentless copper-sensing system, using HcRed as the biosensor, with a nanomolar detection limit. The far-red properties of HcRed (ex. 598 nm, em. 645 nm) make it viable for *in vivo* applications, as animal tissues are nearly translucent in this spectral region. Copper is involved in several metabolic pathways, the degradation of which often leads to some form of neurological disease, and is also a common element in heavy metal pollution, thus making it an important target for simple, inexpensive detection.