

Emission Ratiometric Intracellular Zinc Probes

Eric M. Kawamoto,¹ Janet L. Wolford,¹ and Thomas V. O'Halloran^{1,2}

¹*Department of Chemistry and* ²*Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University*

The Zinbo series, a group of new ratiometric fluorescent sensors for zinc, has been synthesized and characterized. These probes are built around a highly fluorescent 2-(2'-Hydroxyphenyl) benzoxazole core. The probes exhibit large metal-induced changes in emission, shifting from 400-420 nm for the apo- forms to 440-490 nm for the zinc bound forms. The ratiometric properties are only seen with Zn²⁺ and Cd²⁺. The other physiologically relevant metals show quenching (Mn²⁺, Co²⁺, Ni²⁺, Fe²⁺, and Cu²⁺) while a few show no response (Na⁺, K⁺, and Ca²⁺). The ratiometric properties show no significant difference over a wide range of physiological pH values. The dissociation constants from these probes range from nM to pM to even tighter binding than TPEN (fM), which will allow for greater flexibility in the applications for these probes. Cell permeable and cell impermeable probes allow for intracellular and extracellular applications. Zinbo probes have proved valuable in experiments that examine zinc distribution in tissues such as the hippocampus, where discrete packets of free zinc are stored and released. By using the shift in emission rather than excitation wavelengths, these probes have the potential to determine zinc concentration in tissues using two-photon microscopy.

