

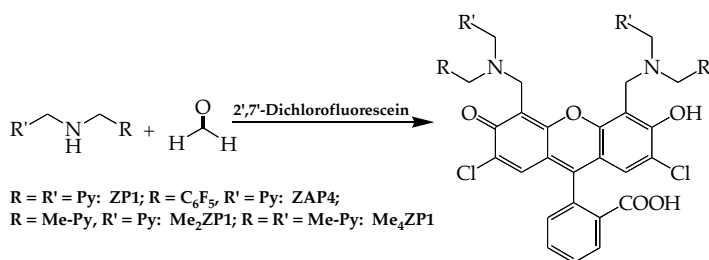
Fluorescent Zinc Sensors with Binding Affinities that Span Four Orders of Magnitude for Quantitating Biologically Relevant Concentrations

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ABSTRACT

Biologically relevant concentrations of mobile zinc are estimated to range from 10 to 500 μM . Current non-peptide zinc sensors having dissociation constants (K_d values) in the nM range cannot accurately measure zinc concentrations near physiologically relevant values. We describe zinc sensors $\text{Me}_2\text{ZP1}$, $\text{Me}_4\text{ZP1}$, and ZAP4 based on the design of Zinpyr-1 (ZP1) (Figure). Methylation of the pyridine rings in ZP1 increases the K_d values of the 1:1 Zn(II) :ligand complexes to 3.3 and 630 nM for $\text{Me}_2\text{ZP1}$ and $\text{Me}_4\text{ZP1}$,



respectively. Substituting pentafluorobenzyl groups for two of the pyridyl moieties of ZP1 has a more dramatic effect. The resultant ZinAlkylPyr-4 (ZAP4) ligand forms a 1:1 Zn(II) :ligand complex with $K_d = 33 \mu\text{M}$. Upon the addition of excess

zinc, a spectroscopically distinct 1:2 Zn(II) :ligand complex with $K_d = 0.5 \text{ mM}$ develops. ZAP4 is unusual in that it displays two large enhancements in fluorescence as the concentration of zinc is increased. The formation of the first species is complete in under 10 s, but the second forms more slowly, requiring 1 hr for full complexation at 295 K. Concomitant with the decreased zinc binding affinities are lowered pK_a values and markedly reduced sensitivities toward other metals for $\text{Me}_4\text{ZP1}$ and ZAP4. The alterations to the ZP1 framework do not affect the ability of the probes to enter cells. All three sensors penetrate cell membranes while retaining their ability to detect zinc fluorimetrically. This work was supported by grant GM 65519 from the National Institute of General Medical Sciences (S. J. L.) and a postdoctoral fellowship from the National Institutes of Health (C. R. G.). We thank Katie R. Barnes for her assistance with cell studies.