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A general model of dynamic quenching revisited.

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Abstract

Gratton et al. (1984) outlined a model for dynamic quenching of a fluorophore in the protein interior. This model reconciled the apparent contradiction resulting when the classical Stern-Volmer analysis, which can be applied to a free fluorophore in solution, i used for the analysis of the quenching behavior of internally buried fluorophores of proteins (Vaughn & Weber 1970 Lackowicz & Weber 1973). Gratton et al. proposed that, for internally buried fluorophores with single exponential decays, the presence of quencher molecules leads to doubly exponential decay times. These decay times or rates are eigenvalues that encompass the acquisition rate of the quencher by protein, the migration rate of the quencher in the protein interior, and the exit rate from the protein. Longer lifetime probes necessitate less quencher concentration and will respond to the acquisition rate of the quencher by the protein. Shorter lifetime probes, due to the greater quencher concentration needed to cause quenching, will respond to the migration rate of the quencher within the protein. These rates, having different orders of magnitude, result in different quenching behavior. In this study, the quenching by 02 of Zinc Protoporphyrin IX reconstituted Horse Skeletal myoglobin (ZNPPkGLOBIN) is monitored using frequency domain lifetime acquisition in the MHz frequency range at two emission wavelengths as a function of temperature. The advantage of the ZNPPIXGLOBIN system is the presence of two distinct lifetimes that are present at zero quencher concentration. Selection of emission wavelengths allows the study of each lifetime individually on the same sample. According ... [truncated at 250 words]