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### Title

DOES HUMAN SUPEROXIDE-DISMUTASE DISPLAY A MOLTEN GLOBULE STATE - A TIME-RESOLVED FLUORESCENCE STUDY

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Norberto Silva Jr, Enrico Gratton, Giampiero Mei, Nicola Rosato, and Alessandro Finazzi-Agrò. Does human superoxide dismutase display a molten globule state? A time-resolved fluorescence study.

36th Annual Meeting of the Biophysical Society, Houston, Texas, 9-13 February 1992. *Biophys J.* 1992; 61(2 Pt 2): A311, 1799. Abstract

During the past decade an interesting intermediate to the protein folding stage was introduced: the molten globule state. This conformational state is characterized as a compact globule with fluctuating tertiary structure and native secondary structure. Recent timeresolved fluorescence studies of the denaturation of the single tryptophan (Trp) protein human superoxide dismutase (HSOD) has presented some interesting results: 1) The timeresolved fluorescence of native, partially denatured and denatured HSOD is best described by a unimodal distribution of lifetimes. 2) The width of the lifetime distribution of native HSOD is less than that of denatured HSOD. The width of the lifetime distribution characterizes the heterogeneity of the Trp-protein system. 3) The width of the lifetime distribution of HSOD as a function of denaturant displays a maximum not coincident with fully denatured state of HSOD. In contrast, the circular dichroism (CD) of HSOD as a function of denaturant does not seem to support the existence of a molten globule state. However, the width of the lifetime distribution of partially denatured HSOD does increase with decreasing protein concentration while the amide CD signal remains constant. Thus, the possibility may exist that HSOD displays a molten globule state in the monomer stage. Further experimentation including stopped flow experiments using naphthalene dyes are performed on HSOD as a function of protein concentration to explore the question of a molten globule state. This work is supported by NIH grant PHSP41-RR03155.