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Title

Binding studies of ANS to TUNA apomyoglobin using fluorescence correlation spectroscopy.

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Journal

BIOPHYSICAL JOURNAL, 78(1)

ISSN

0006-3495

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Publication Date

2000

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Peer reviewed

Don C Lamb, Ettore Bismuto, G Ulrich Nienhaus, and Enrico Gratton.

Binding studies of ans to tuna apomyoglobin using fluorescence correlation spectroscopy.

44th Annual Meeting of the Biophysical Society, New Orleans, Louisiana, 2000.

Biophys J. 2000; 78(1 Pt 2), 2600-Pos.

Abstract

ANS (1-anilino-8-naphthalene sulfonate) is often used as a hydrophobic probe because of its high sensitivity to its environment. For example, the quantum yield of ANS increases by over 200 fold upon binding to the heme pocket of apomyoglobin. The study of ANS binding to apomyoglobin has been used to investigate protein denaturation and refolding. Here, we have used the binding of ANS to apomyoglobin to probe protein dynamics using Fluctuation Correlation Spectroscopy (FCS). FCS measures the changes in fluorescence intensity arising from equilibrium thermodynamic fluctuations in the sample. Assuming a bimolecular reaction, it is possible to determine both the on and off rates of ANS binding from the FCS data and have been analyzed as a function of temperature and pH. At pH 7 the on rate is diffusion limited. At pH 3, the on rate is too fast to be attributed to diffusion, suggesting that the ligand is fluctuating between multiple binding sites within the protein. The temperature dependence of the off rate is non Arrhenius, suggesting that protein fluctuations limit the rate of ANS escape from the protein. Supported by grants from the National Institutes of Health (RR03155).