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TRYPTOPHAN FLUORESCENCE LIFETIMES IN PROTEINS MEASURED BY MULTIFREQUENCY PHASE FLUOROMETRY EMPLOYING MODE-LOCKED LASER EXCITATION

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158. Tryptophan Fluorescence Lifetimes in Proteins Measured by Multifrequency Phase Fluorometry Employing Mode-Locked Laser Excitation. *R. Alcalá*, Franklyn G. Prendergast, and Enrico Gratton. Department of Physics, University of Illinois, Urbana, IL 61801, and Department of Pharmacology, Mayo Foundation, Rochester, MN 55905.

The output of a synchronously pumped and mode-locked argon ion laser was used to pump a dye laser. An acoustooptic modulator was employed to amplitude modulate the dye laser output and to introduce quasi-continuously variable harmonic content (1–416 MHz). UV excitation light was obtained by frequency doubling the acoustooptic modulated dye laser output and was used for a multifrequency phase fluorometry study of indole fluorescence lifetimes in model systems, peptides and proteins. Invariably the fluorescence decays in peptides and proteins were at least biexponential. In contrast, the fluorescence of 5-methylindole complexes in α -cyclodextrin showed a single fluorescence lifetime. Molecular graphics examination of the environs of the tryptophan side chain in these proteins seldom provided an explanation for the observed lifetime. It is unclear whether the two lifetimes observed in single tryptophan proteins represent two protein species; alternative explanations will be discussed.