Direct Excitation of Tryptophan Phosphorescence: A New Method for Triplet States Investigation

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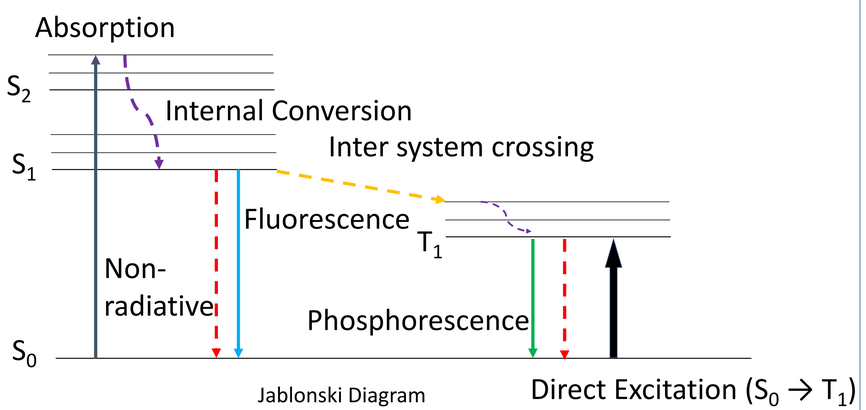
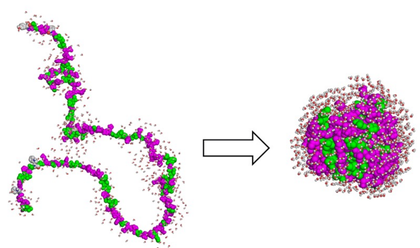
Motivation

We studied room temperature phosphorescence of tryptophan (TRP) embedded in poly (vinyl alcohol) [PVA] films. With UV (285 nm) excitation, the phosphorescence spectrum of TRP appears at about 460 nm. We also observed the TRP phosphorescence with blue light excitation at 410 nm, well outside of the $S_0 \rightarrow S_1$ absorption. This excitation reaches the triplet state of TRP directly without the involvement of the singlet excited state. The phosphorescence lifetime of TRP is in the sub-millisecond range. The long-wavelength direct excitation to the triplet state results in high phosphorescence anisotropy which can be useful in macromolecule dynamics study via time-resolved phosphorescence.



Jablonski Diagram

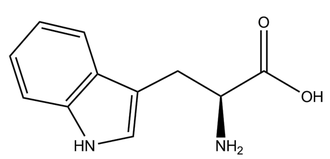
Direct Excitation ($S_0 \rightarrow T_1$)



Unfolded

Folded

Protein Folding

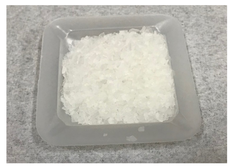


Tryptophan structure

Methods & Results

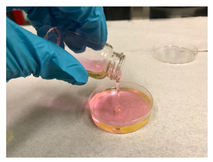
PVA was polymerized in a span of three days until it was clear to introduce the fluorophore, TRP, into the PVA matrix. After a week of drying, the film was removed and ready for spectroscopic measurements.

Raw PVA



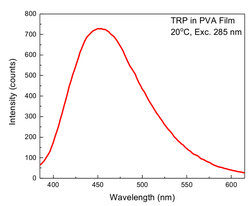
Dissolve PVA

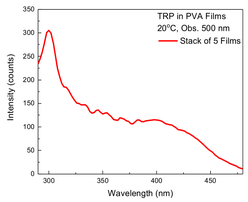
Introduce dye in dissolved PVA

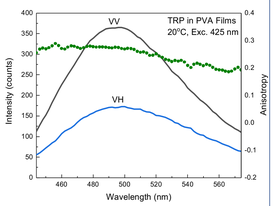


Dye is doped in PVA matrix

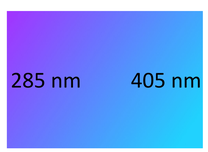
Traditionally phosphorescence emission was conducted with an ultraviolet (UV) excitation. Consequently, in biology with a UV excitation, a huge emission of background emission (autofluorescence) is prevalent. However, in PVA TRP had a new (longer) wavelength of excitation of phosphorescence.

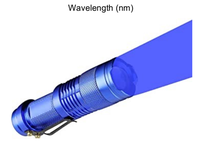




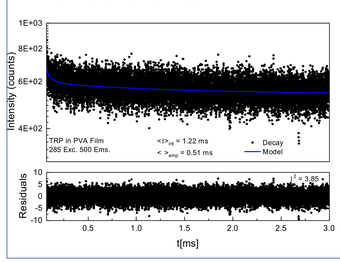


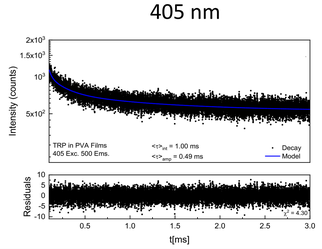
UV excitation 285 nm





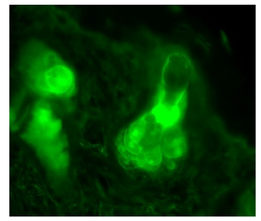
Longer wavelength excitation 405 nm





Conclusions

It has been shown that it is possible to excite TRP directly to the triplet state. This excitation results in phosphorescence emission, which we believe to be an important finding. PVA and most solvents and buffers do not absorb the blue light used for the direct TRP excitation. Powerful laser light sources are readily available in this spectral range. Also, long-wavelength excitation results in lower scattering, which might be important in macromolecules (proteins) studies. Biological background noise will be reduced as compared to typical UV excitation.



Tissue autofluorescence

405 nm laser

1. "On the Possibility of Direct Triplet State Excitation of Indole." **Jose Chavez**, Luca Ceresa, Emma Kitchner, Joseph Kimball, Tanya Shtoyko, Rafal Fudala, Julian Borejdo, Zygmunt Gryczynski, Ignacy Gryczynski. Journal of Photochemistry and Photobiology B: Biology. Volume 208. May 16, (2020) .
2. "Luminescence Properties of 5-Bromoindole in PVA Films at Room Temperature. Direct Triplet State Excitation." **Jose Chavez**, Joseph Kimball, Luca Ceresa, Emma Kitchner, Tanya Shtoyko, Rafal Fudala, Julian Borejdo, Zygmunt Gryczynski, Ignacy Gryczynski. Journal of Luminescence. Volume 230. November 6, (2020).
3. "Photophysical Properties of 2-Phenylindole in Poly (vinyl alcohol) Film at Room Temperature. Enhanced Phosphorescence Anisotropy with Direct Triplet State Excitation." Z. Gryczynski, J. Kimball, R. Fudala, **J. Chavez**, L. Ceresa, M. Szabelski, J. Borejdo, I. Gryczynski, Methods and Applications in Fluorescence. Volume 8. February 3, (2020).
4. "Direct Excitation of Tryptophan Phosphorescence. A New Method for Triplet States Investigation." **Jose Chavez**, Luca Ceresa, John M. Reeks, Yuri M. Strzhemechny, Joseph Kimball, Emma Kitchner, Zygmunt Gryczynski, and Ignacy Gryczynski. Methods and Applications in Fluorescence. Volume 10. Article 025001. January 18, (2022).
5. Emily Casanova, "Autofluorescence: When Tissues Light Up Under Your Microscope For No Apparent Reason."