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# Is the Fluorescence Quantum Yield of Tryptophan Independent of Excitation Wavelength?

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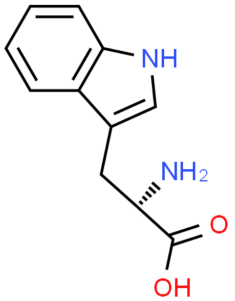
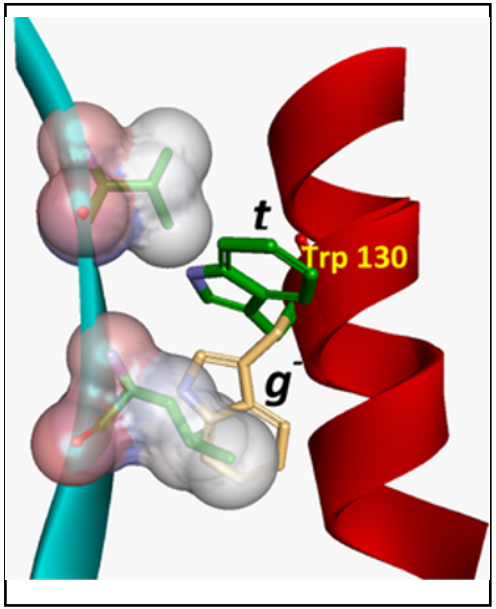
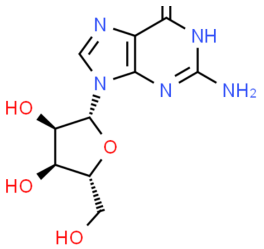
# Background

# Tryptophan (Trp)




- Commonly used as a probe in protein structure-dynamics studies
- Widely used as a convenient standard of fluorescence quantum yields of organic and bioorganic molecules
    - Literature values of the fluorescence quantum yield of Trp range from 0.13-0.15 upon excitation of Trp at 270 nm in aqueous solution



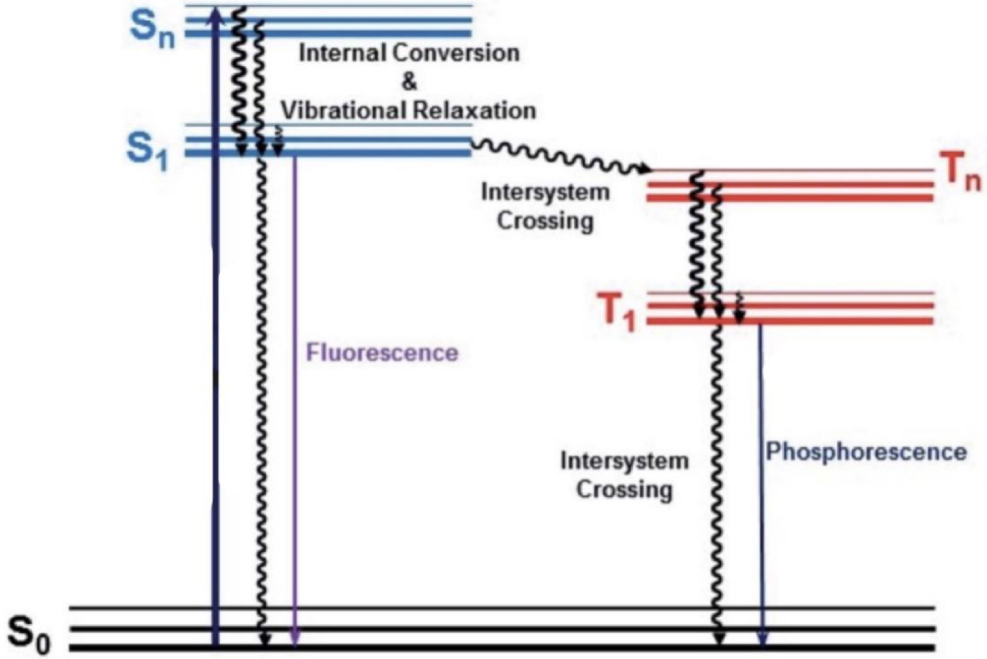

Gasymov OK, Abduragimov AR, Glasgow BJ (2013) Effect of Short- and Long-Range Interactions on Trp Rotamer Populations Determined by Site-Directed Tryptophan Fluorescence of Tear Lipocalin. PLOS ONE 8(10): e78754. https://doi.org/10.1371/journal.pone.0078754

Chen, R. F. (1967). Fluorescence quantum yields of tryptophan and tyrosine. Analytical Letters, 1(1), 35-42.

Suzuki, K., Kobayashi, A., Kaneko, S., Takehira, K., Yoshihara, T., Ishida, H., ... & Tobita, S. (2009). Reevaluation of absolute luminescence quantum yields of standard solutions using a spectrometer with an integrating sphere and a back-thinned CCD detector. Physical Chemistry Chemical Physics, 11(42), 9850-9860.

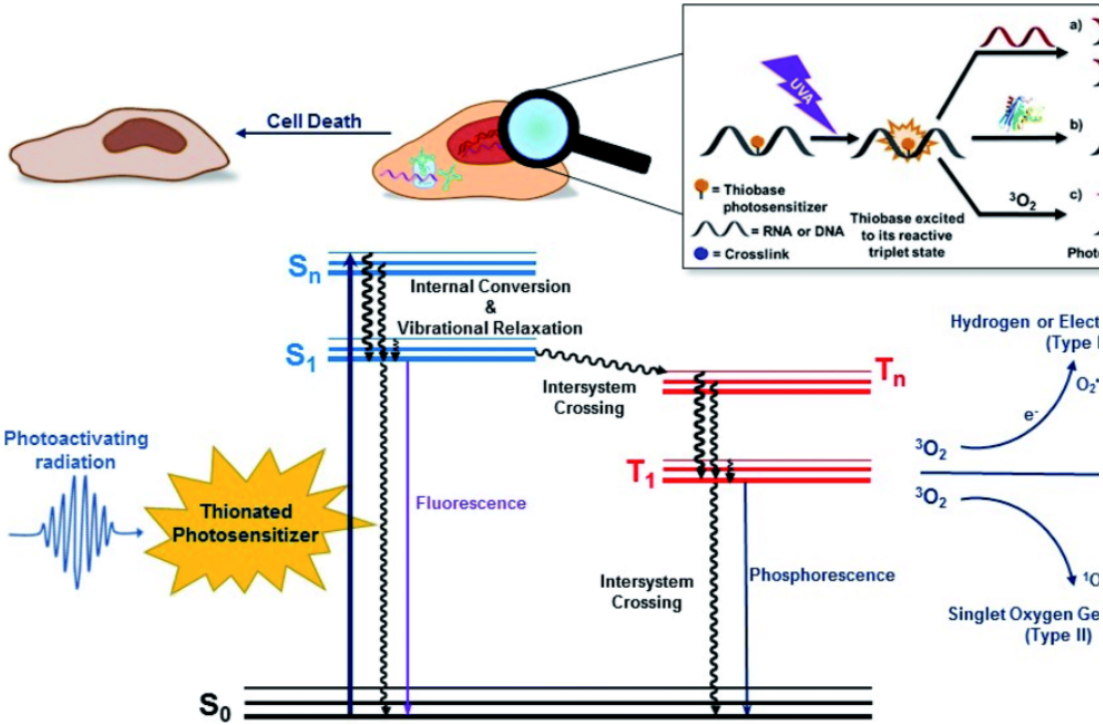
# Fluorescence Quantum Yield

**Fluorescence Quantum Yield($\Phi_F$)** - ratio of photons emitted to photons absorbed by the system.
$\Phi_F$= Photons Emitted/Photons Absorbed




**Scheme 1.** Jablonski Diagram

# Jablonski Diagram




**Scheme 2.** Jablonski Diagram demonstrating possible deactivation pathways of a photosensitizer including photosensitized reactions.

Ortiz-Rodríguez, L. A., & Crespo-Hernández, C. E. (2020). Thionated organic compounds as emerging heavy-atom-free photodynamic therapy agents. *Chemical Science*.

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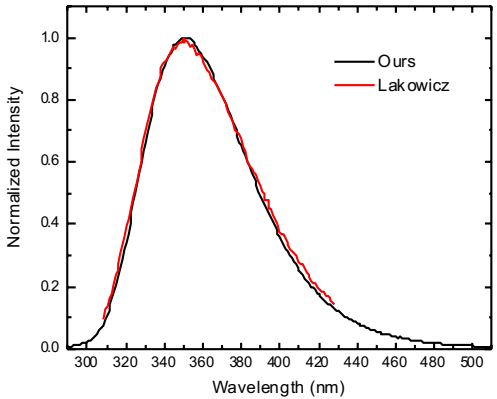
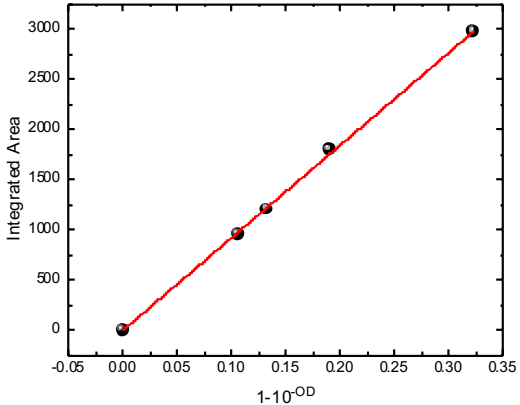
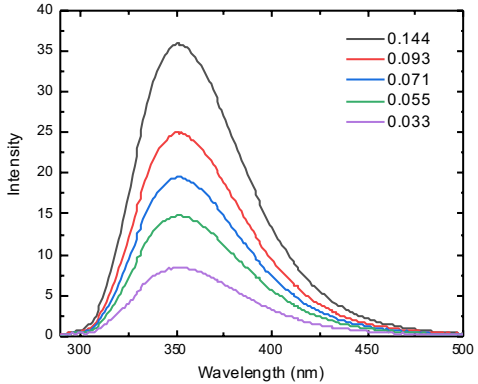
# Research Design and Methodology



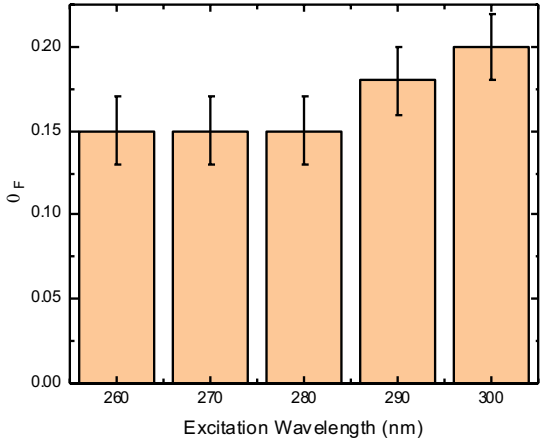

$$\Phi_{F\,sample} = \Phi_{F\,standard}\left(\frac{m_{sample}}{m_{standard}}\right)\left(\frac{\eta_{sample}}{\eta_{standard}}\right)^2$$

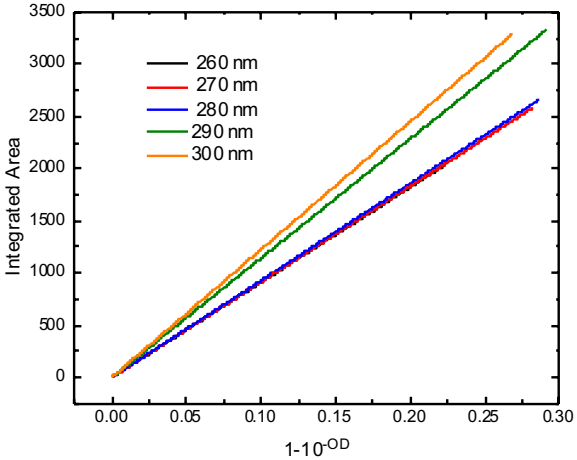
- Excitation wavelength dependence?
  - Range: 260-300 nm
- Dependence on ionic strength of the phosphate buffer?
  - 5-100 mM


Lakowicz, J. R. (Ed.). (2013). Principles of fluorescence spectroscopy. Springer science & business media.

Suzuki, K., Kobayashi, A., Kaneko, S., Takehira, K., Yoshihara, T., Ishida, H., ... & Tobita, S. (2009). Reevaluation of absolute luminescence quantum yields of standard solutions using a spectrometer with an integrating sphere and a back-thinned CCD detector. Physical Chemistry Chemical Physics, 11(42), 9850-9860.

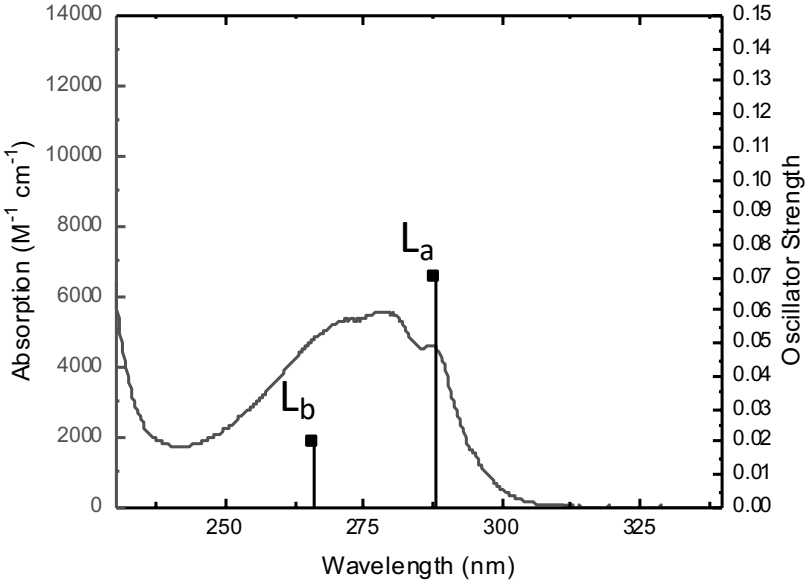
# Results

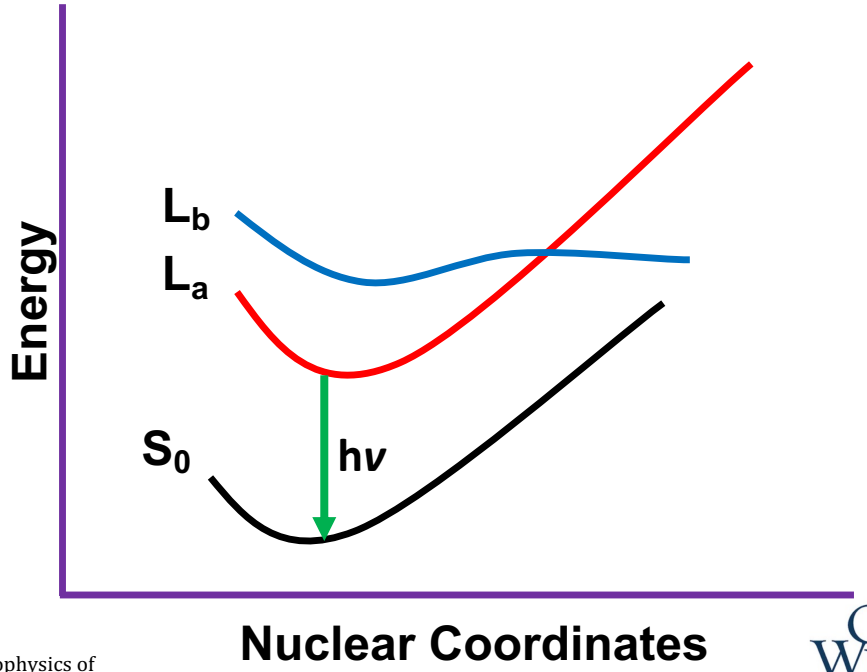



| Buffer Concentration (mM) | 260 nm | | | | |
|---|---|---|---|---|---|
| 5 | 0.15 ± 0.02 | 0.15 ± 0.02 | 0.15 ± 0.02 | 0.18 ± 0.02 | 0.20 ± 0.02 |
| 16 | 0.15 ± 0.02 | 0.15 ± 0.02 | 0.15 ± 0.02 | 0.18 ± 0.02 | 0.20 ± 0.02 |
| 25 | 0.15 ± 0.02 | 0.15 ± 0.02 | 0.15 ± 0.02 | 0.18 ± 0.02 | 0.20 ± 0.02 |
| 50 | 0.15 ± 0.02 | 0.15 ± 0.02 | 0.15 ± 0.02 | 0.18 ± 0.03 | 0.20 ± 0.03 |
| 100 | 0.15 ± 0.03 | 0.15 ± 0.02 | 0.15 ± 0.03 | 0.18 ± 0.03 | 0.20 ± 0.03 |

# Proposed Mechanism for the Wavelength Dependence of the $\Phi_F$




Sobolewski, A. L., Shemesh, D., & Domcke, W. (2009). Computational studies of the photophysics of neutral and zwitterionic amino acids in an aqueous environment: tyrosine–(H2O) 2 and tryptophan–(H2O) 2 clusters. The Journal of Physical Chemistry A, 113(3), 542-550.

# Conclusion

- We demonstrated that the $\Phi_F$ of Trp is excitation wavelength dependent. However, it is independent of the ionic strength of the buffered solution in a salt concentration range of 5-100 mM.

# Acknowledgements