How does varying sizes and concentrations of poly(ethylene glycol) affect the conformation and function of the enzyme *Escherichia coli* prolyl-tRNA synthetase?

Jessica Liebau, Bethany Laatsch, Harrison Lowater (PI: Dr. Sanchita Hati)

National Institutes of Health

University *of* Wisconsin Eau Claire

ABSTRACT

Poly(ethylene glycol) (PEG) is a biocompatible, hydrophilic, flexible, non-toxic polyether commonly used in proteomics because it is widely regarded to be a biologically inert molecule. This includes PEG2000 (molecular weight of 2000g/mol), which is a component in the Pfizer-BioNTech and Moderna COVID-19 vaccines. However, recent studies suggest that PEG may impact protein function, depending largely on the molecular weight of the PEG. In this study, we observe the effects of PEG on the function and conformation of enzyme prolyl-tRNA synthetase of *Escherichia coli* (Ec ProRS). ProRS catalyzes the covalent attachment of proline to $tRNA^{Pro}$ and thus is essential for protein biosynthesis. The molecular mechanism of PEG-protein interactions has been probed using enzyme kinetics, fluorescence spectroscopy, and molecular modeling. Herein, we present the results of our study.

BACKGROUND

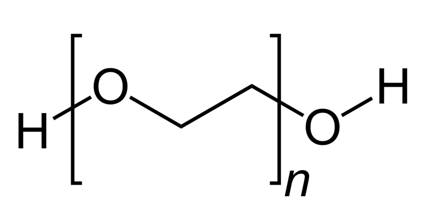
Prolyl-tRNA Synthetase

Prolyl-tRNA synthetase (ProRS) is an enzyme that covalently attaches proline onto its cognate tRNA, $tRNA^{Pro}$, using the following two-step reaction:

(1) ProRS + Pro + ATP $\rightleftharpoons$ ProRS • (Pro-AMP) + PP_i

(2) ProRS • (Pro-AMP) + $tRNA^{Pro} \longrightarrow$ Pro-$tRNA^{Pro}$ + ProRS + AMP

Poly(ethylene glycol)



Synthetic polymer thought to be biologically inert.

Name refers molecular weight (MW) of polymer (PEG600 = MW 600)

Component of the Moderna & Pfizer COVID19 vaccines[1]

Fluorescence Spectroscopy

Molecule absorbs energy; the excited molecules relaxed to ground state by releasing some energy as heat, and the rest is emitted as fluorescence (fig. 1)[2]

Tryptophan (fig. 2) = fluorescent amino acid (dimeric Ec ProRS has 10)

Fluorometer records emission over a variety of wavelengths.

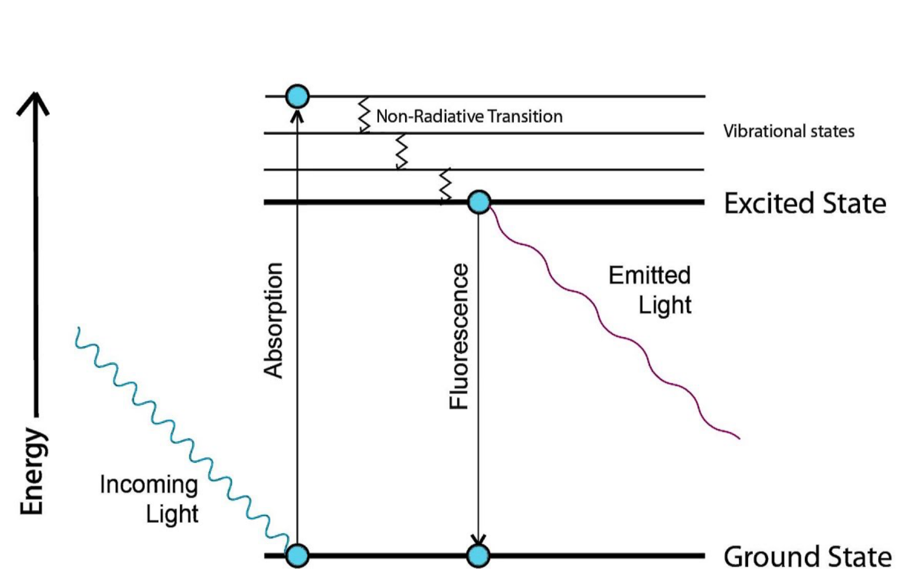


Figure 1. *Jablonski Diagram*

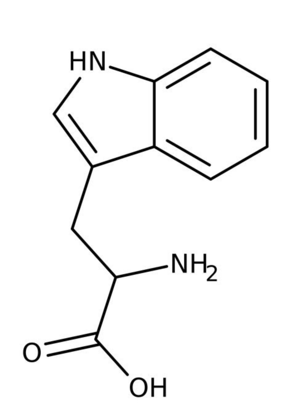


Figure 2. *Tryptophan*

RESULTS

Relative Fluorescence

Comparison of intrinsic fluorescence intensity of Ec ProRS in PEG vs non-PEG solutions

Investigation of the mechanism of *quenching* (transfer of energy from ProRS to surrounding PEG molecules) caused by either hard (steric) or soft (chemical) interactions

Wavelength of Maximum Fluorescence Intensity

Comparison of the wavelength at which the fluorescence emission reaches its peak.

Longer wavelengths indicate the tryptophan is more exposed to polar solvent

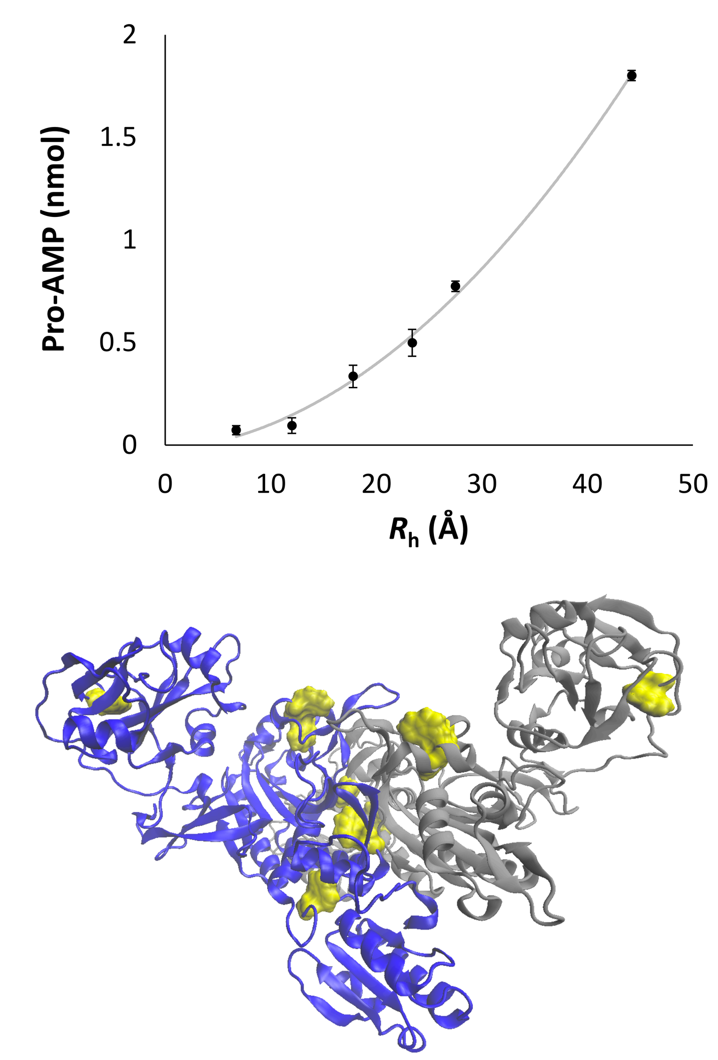


Figure 3. Top: Less product formation in the presence of smaller size PEGs.[3] Bottom: Tryptophan sites in dimeric Ec ProRS.

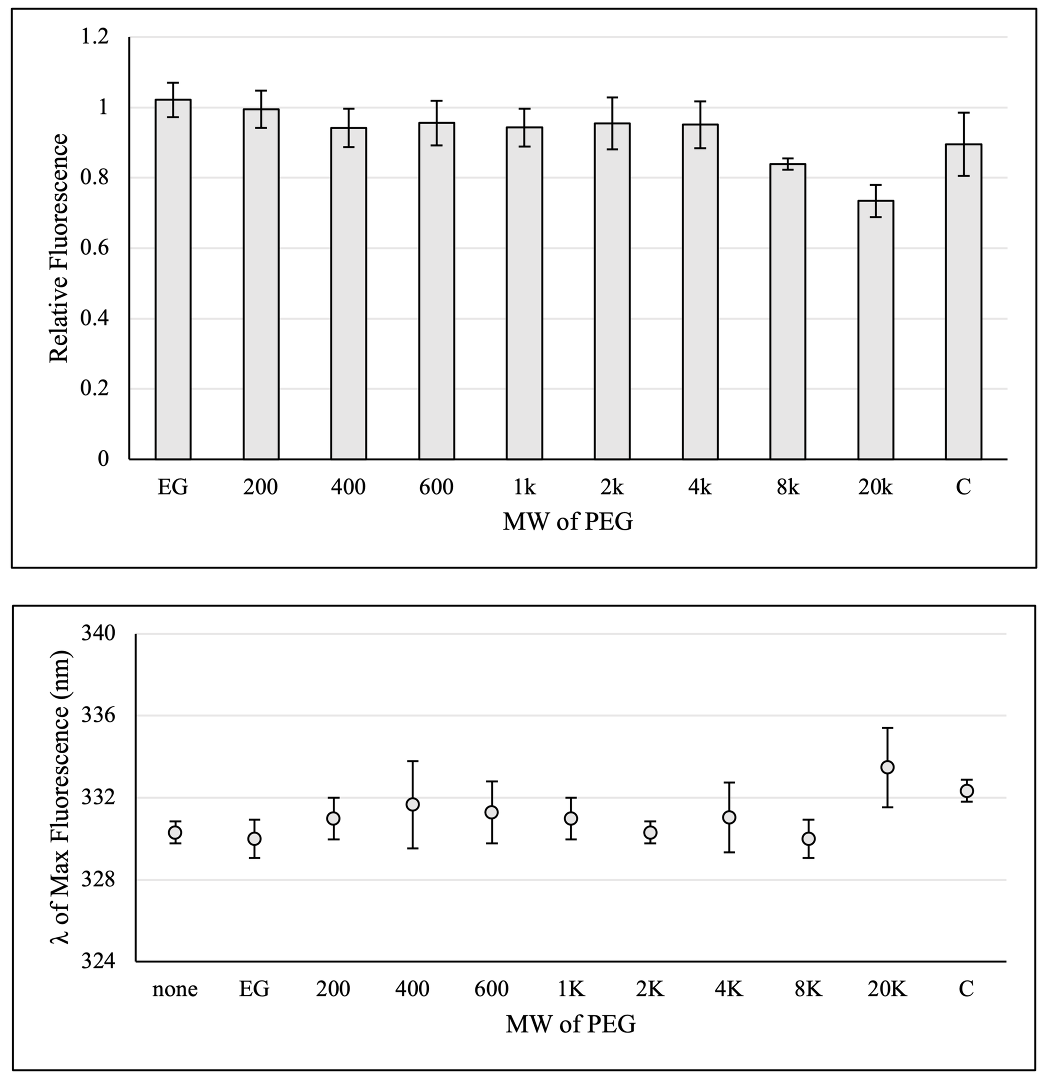


Figure 4. *MW variation.* Ten different molecular weights were examined along with a cocktail of all PEG molecules (C). 50g/L PEG and 1 µM ProRS were used for all trials. Experiments were performed in triplicate. Error bars represent standard deviation.

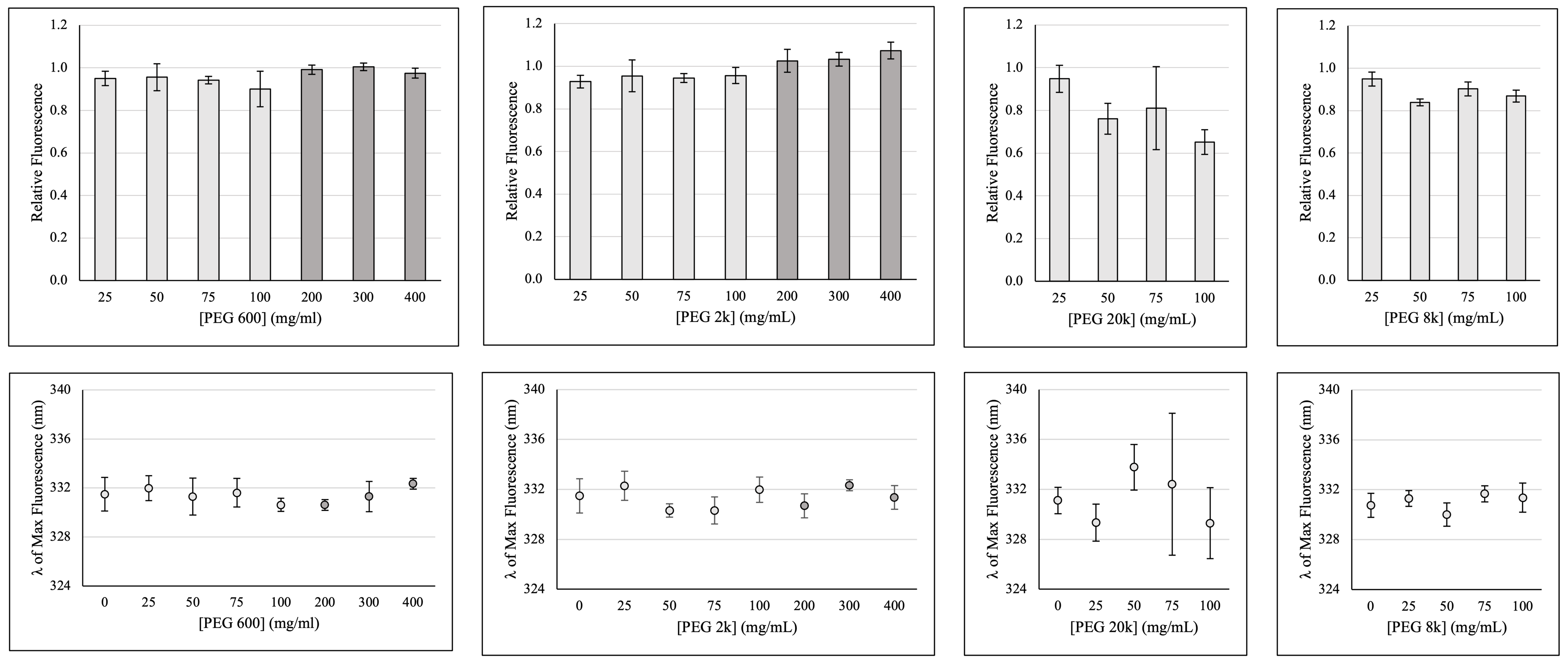


Figure 5. *Concentration variation.* A range of sizes of PEG were selected from the MW variation study to examine effects of concentration. Low MW PEG 600 and 2k concentration ranged from 25-400 mg/mL while high MW PEG 8k and 20k ranged from 25-100 mg/mL. A concentration of 1 µM ProRS was used. Experiments were performed in triplicate. Error bars represent the standard deviation.

CONCLUSIONS

- Larger PEG molecules cause quenching and red-shift of emission wavelengths, indicating a shift to a more open conformation of ProRS.
- Higher concentrations of larger PEG molecules cause more fluorescence quenching.
- Small PEG molecules do not have a significant impact on fluorescence intensity or wavelength, even at higher concentrations.

FUTURE DIRECTIONS

- Fluorescence spectroscopy to observe how PEG affects the **thermal stability** of ProRS.
- UV spectroscopy study to observe how PEG affects the **rate of growth** of *E. coli* cells.
- Kinetics assay to observe how PEG affects the **catalytic rate** of ProRS.
- Atomic force microscopy to **image** the effects of PEG on ProRS.
- Computational chemistry to **simulate the interactions** between PEG and ProRS.

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