Spectroscopic Studies to Explore the Impact of Macromolecular Crowding on the Structure and Function of *Escherichia coli* Prolyl-tRNA Synthetase

Lauren Adams, Ryan Andrews, Heidi Schmit,
Scott Bailey-Hartsel, Sanchita Hati*
University of Wisconsin-Eau Claire, Eau Claire, WI, 54702

Acknowledgements:
UWEC-Office of Research and Sponsored Programs
Dr. Elizabeth Glogowski (Spectrofluorometer)
Dr. Bart Dahl and Dr. Jennifer Dahl (Spectrofluorometer)

ABSTRACT

Most computational and experimental studies to understand the molecular mechanism of an enzyme-catalyzed reaction are usually performed in dilute solutions. However, enzymatic activities *in vivo* occur in a crowded environment composed of many macromolecules. We are performing computational, spectroscopic, and kinetic studies to investigate the impact of macromolecular crowding on the structure and enzymatic activity of *Escherichia coli* prolyl-tRNA synthetase. This enzyme is a member of an important family of enzymes, which are essential for the biosynthesis of proteins in all living organisms. The overall goal is to evaluate if there is a need for consideration of the effect of macromolecular crowding for structure-based drug design to inhibit the function of pathogenic prolyl-tRNA synthetases. Preliminary results of the spectroscopic studies are presented herein.

BACKGROUND

❑ **Aminoacyl-tRNA Synthetases (AARSs)**

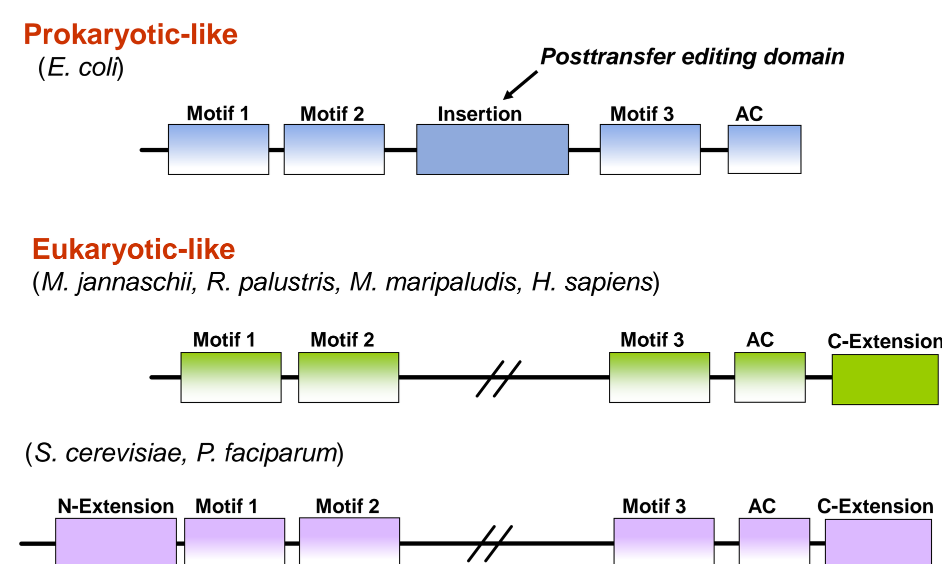
- Multi-domain, allosterically-regulated enzymes [*1*].
- Catalyze the covalent attachment of amino acids to their cognate tRNAs, an important step in protein synthesis.

$$\text{AA} + \text{ATP} + \text{AARS} \leftrightharpoons \text{AARS}\bullet\text{AA-AMP} + \text{PPi} \quad (1)$$

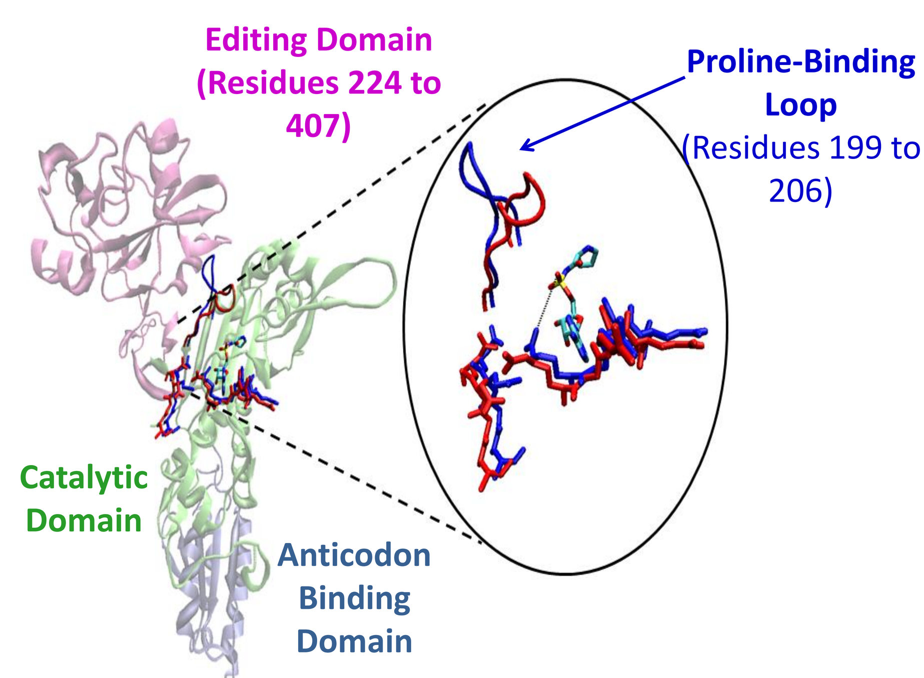
$$\text{AARS}\bullet\text{AA-AMP} + \text{tRNA} \rightarrow \text{AARS} + \text{AA-tRNA} + \text{AMP} \quad (2)$$

❑ **Prolyl-tRNA Synthetases (ProRSs)**

- Catalyze the covalent attachment of proline to $tRNA^{Pro}$.
- Bacterial ProRS occasionally misactivates alanine and cysteine.
- *E. coli* ProRS possesses an editing mechanism and a separate domain to hydrolyze $Ala\text{-}tRNA^{Pro}$ [*2*].
- Prolyl-tRNA Synthetases from different species exhibit variable domain architecture.



- Catalysis involves a substrate-induced conformational change of the catalytically important proline-binding loop (PBL) [*1*].



REFERENCES

(1) Creping et al. (2006) *Structure* 14, 1511-1525.
(2) Beuning et al. (2001) *J. Biol. Chem.* 276, 30779-30785
(3) Ellis, R.J. (2001) *Curr. Opin. Struct. Biol.* 11, 114 -119.
(4) Zimmerman et al (1991), *J. Mol. Bol.* , 222, 599-620.
(5) Batra et al. (2009) *Biophys. J.* 97, 906 – 911.
(6) Schultz et al. (1961) *J. Gen. Physiol.* 44, 1189-1199
(7) Muino et al.(2009) *J. Biophys. B.*, 2572-2577
(8) Hixon, et al. (2009) *MDPI, 2: 1155-1176*
(9) Vivian et al (2001), *Biophys J.*, 80, 2039-2109.

❑ **Macromolecular crowding**

- Approximately 20-30% of interior cellular environments are occupied by macromolecular crowding agents (*in vivo*) [*3, 4*].
- Protein binding affinity as well as catalysis are impacted in the presence of macromolecular crowding agents [5, 6].
- Most *in vitro* studies do not take into account the effects of macromolecular crowding agents on enzymatic rate.

Sucrose (mg/mL)	k_{cat} (s^{-1})	K_M (mM)	k_{cat}/K_M (s^{-1}. mM^{-1})
0	9.12	0.39	23.4
300	2.42	0.13	18.6

*Data collected from a parallel study in our lab, demonstrating increased binding affinity (lower K_M) in the presence of sucrose.

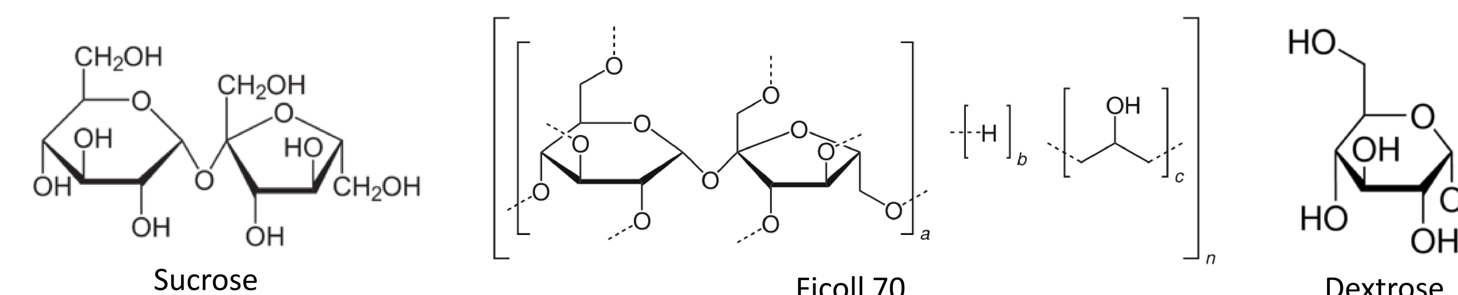
OBJECTIVE

To investigate the impact of macromolecular crowding agents on the structure, dynamics, and function of *E. coli* prolyl-tRNA synthetase.

METHODS

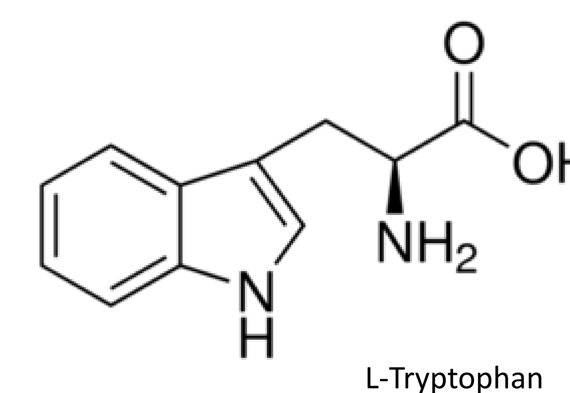
❑ **General Procedure and Materials**

- With a constant concentration of protein of 2 µM, we add varying concentrations of sucrose (hydrodynamic radius 5.9Å), ficoll 70 (hydrodynamic radius 40Å), and dextrose simulating the crowded cell environment [6].



❑ **Intrinsic Fluorescence**

- The aromatic amino acids of proteins are able to absorb and subsequently emit light. This phenomenon can be induced and recorded by a spectrofluorometer [7].
- The aromatic amino acid, tryptophan, is highly sensitive to the surrounding environment as a result of the redistribution of electron density after photon induced excitation due to its asymmetric indole ring [8].



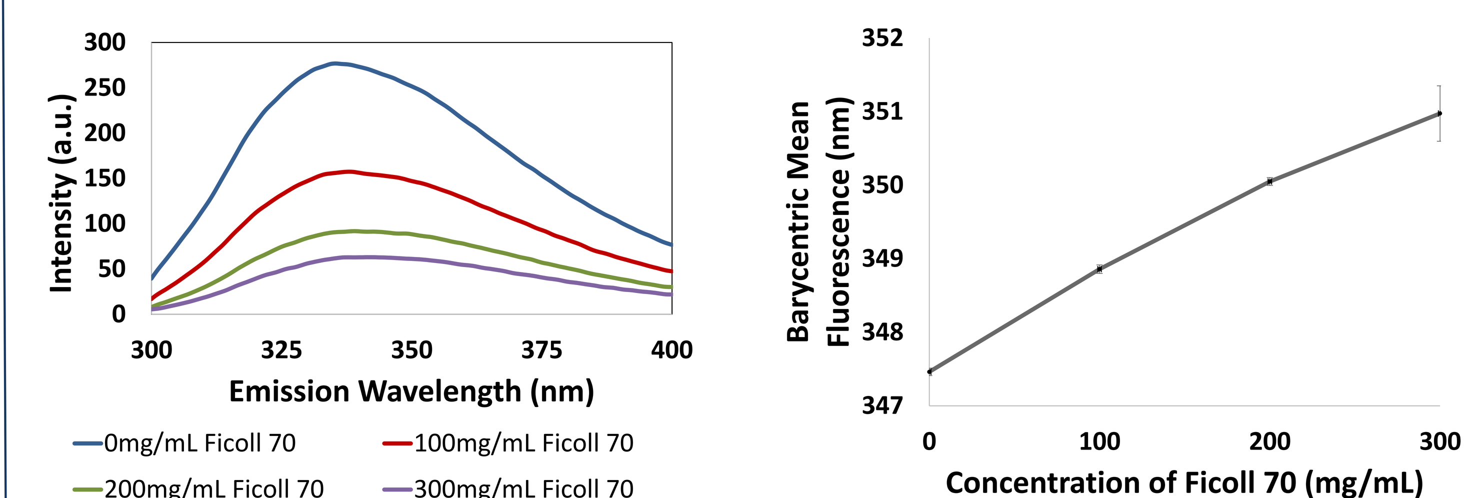
- The fluorescence emission of aromatic amino acids is solvent dependent, any change in the emission spectra (wavelength or intensity) provides information on the structural alteration [9].

RESULTS

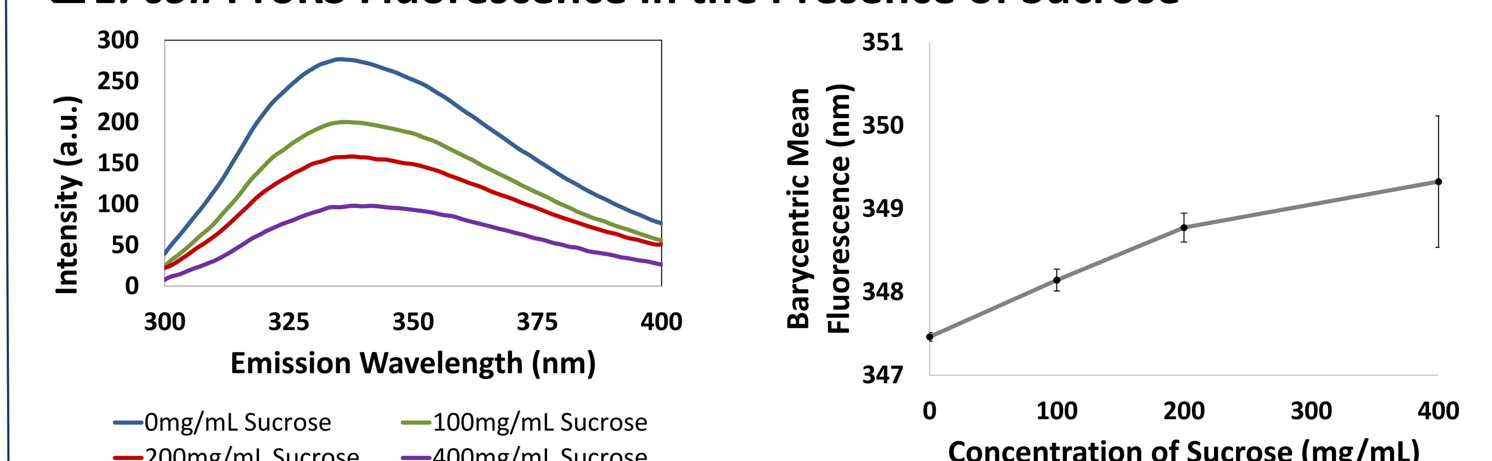
[Macromolecules] (mg/mL)	Sucrose	Ficoll 70	Dextrose
	Barycentric Mean Fluorescence Wavelength (nm)		
0	347.46±0.05	347.46±0.05	348.19±0.014
100	348.14±0.13	348.86±0.06	348.06±0.03
200	348.77±0.17	350.05±0.05	347.97±0.02
300	N/A	350.97±0.38	347.92±0.05
400	349.33±0.79	N/A	N/A

Concentration of Ficoll 70 & Sucrose (mg/mL)	Barycentric Mean Fluorescence Wavelength (nm)
0 Ficoll 70 & 0 Sucrose	347.99±0.33
50 Ficoll 70 & 100 Sucrose	349.20±0.39
100 Ficoll 70 & 200 Sucrose	350.37±0.65

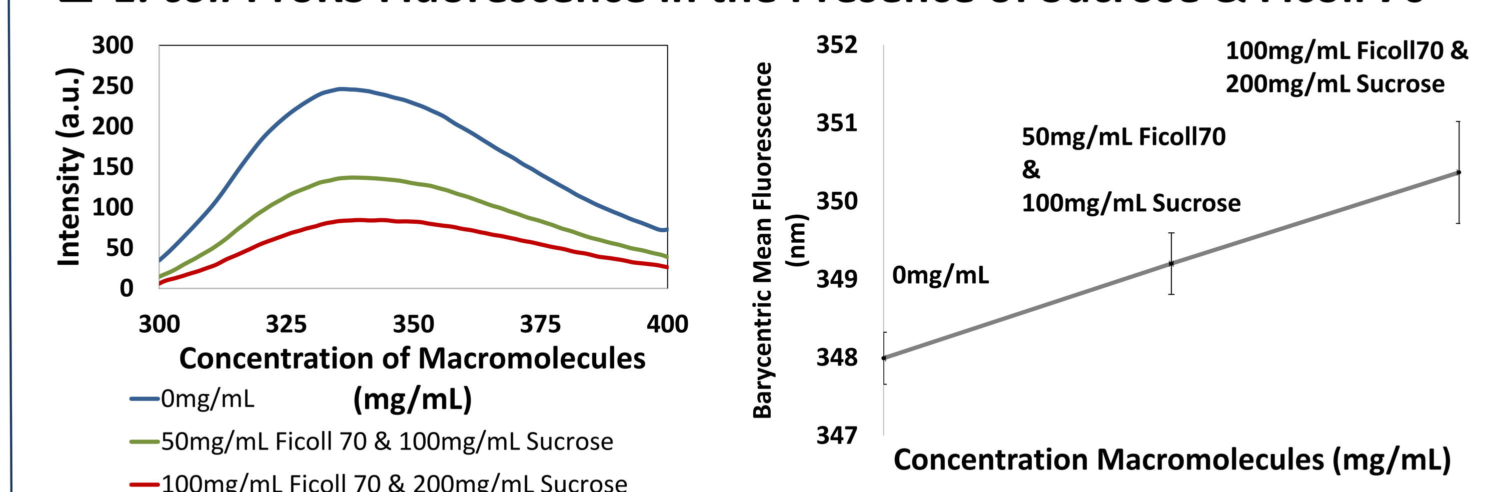
❑ ***E. coli* ProRS Fluorescence in the Presence of Ficoll 70**



❑ ***E. coli* ProRS Fluorescence in the Presence of Sucrose**

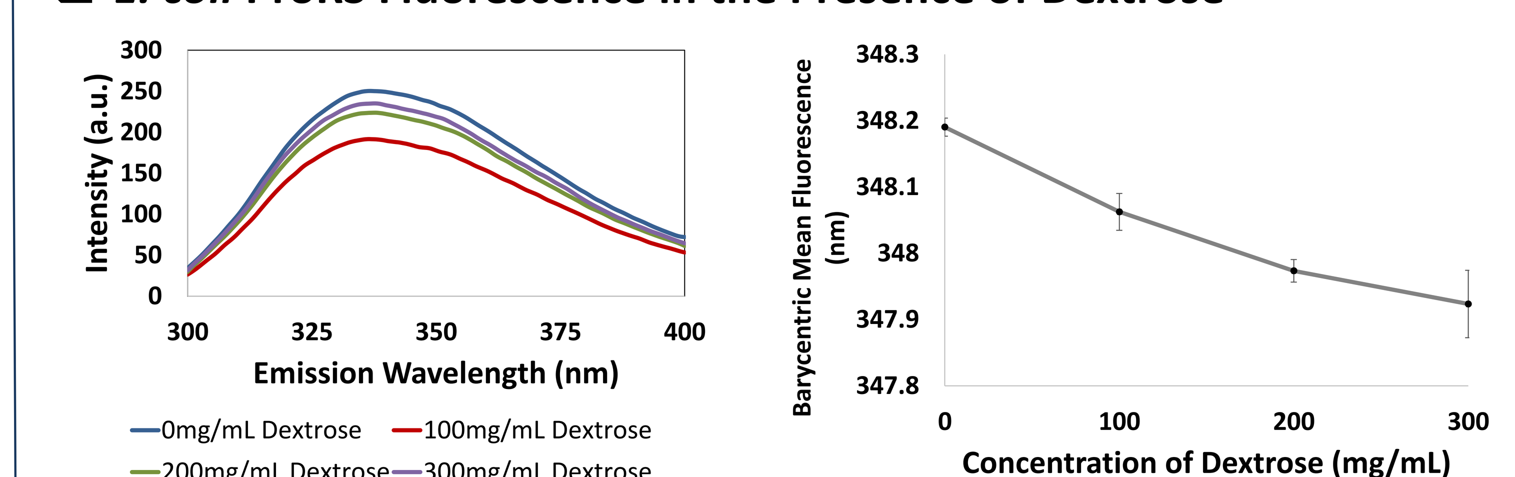


❑ ***E. coli* ProRS Fluorescence in the Presence of Sucrose & Ficoll 70**



- A combination of sucrose and ficoll 70 displayed an additive effect on both fluorescence intensity and Barycentric mean fluorescence wavelength (i.e. 50mg/mL ficoll 70 & 100mg/ mL sucrose resembled the effect of 100mg/mL ficoll 70 or 200mg/mL sucrose).

❑ ***E. coli* ProRS Fluorescence in the Presence of Dextrose**



CONCLUSIONS

- As the concentration of sucrose and ficoll 70 increases, a conformational change is occurring that shifts the maximum emission wavelength toward a higher wavelength (red-shift), indicating that one or more tryptophans are becoming more exposed to solution.
- Dextrose displays the opposite trend of sucrose and ficoll 70, suggesting a more hydrophobic environment surrounding the tryptophans [7].

FUTURE DIRECTIONS

- Examine the impact of a combination of macromolecules.
- Perform kinetic studies in the presence of different macromolecules to investigate the impact of macromolecular crowding on catalytic efficiency.
- Site directed mutagenesis in order to alter the number and location of tryptophans to pinpoint the exact site of structural and dynamical changes, which lead to shifts in the maximum emission wavelength.
- Performing long-timescale computational simulations in the presence of macromolecules.