The Role of Nucleic Acids in Protein Folding

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Introduction

A protein's function is strongly dependent on its native conformation, however, protein misfolding and aggregation are a possibility that can lead to serious consequences. Molecular chaperones assist with protein folding, and help the protein achieve its native state. Similarly, it has been indicated that nucleic acids, such as RNA and DNA, can also aid in protein folding, and prevent protein aggregation.

TagRFP675 is a relatively stable red fluorescent protein, and our goal was to optimize the expression and purification of the TagRFP675 in order to further analyze the folding mechanism of the protein and determine how this folding can be affected by nucleic acids in vitro. Even though TagRFP675 is not a disease relevant protein, it is used as a biosensor in *E. coli* to investigate chaperone activity in vivo. Isolating the protein will allow for the verification of the chaperone's action in vivo.

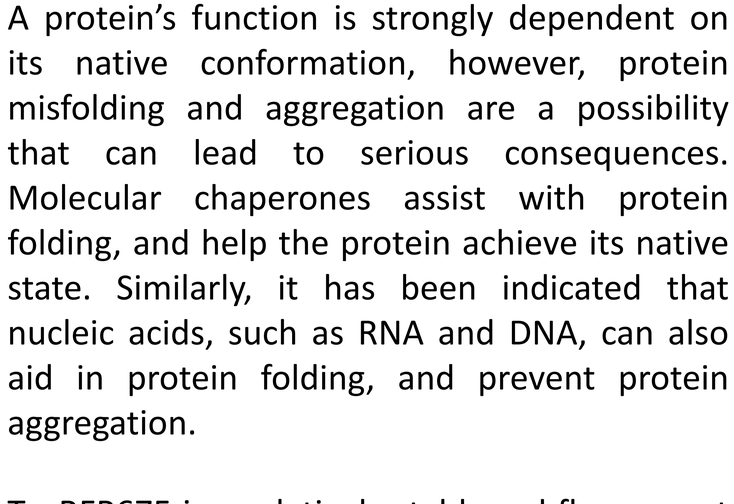

Figure 1—Enhancing fluorescence of biosensor: Previously it has been observed that molecular chaperones and G-quadruplex-containing sequences can enhance the fluorescence of the TagRFP675 protein, indicating that this protein has been folded to its native state in the presence of these chaperones.

TagRFP675 Purification

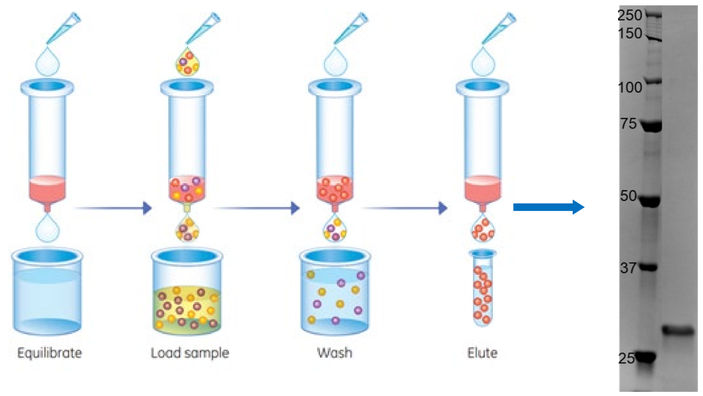

Figure 2—Protein purification: NGC liquid chromatography was used to purify TagRFP675. Upon filtering unwanted debris, TagRFP675 sticks to the column until it is eluted out with imidazole. After TagRFP675 was purified, an SDS-PAGE gel was run, where a pure protein sample is observed.

(https://www.sigmaaldrich.com/technicaldocuments/protocols/biology/affinity-chromatography-tagged-proteins/manual-purification-using-his-gravitrap-talon.html)

TagRFP675 Folding

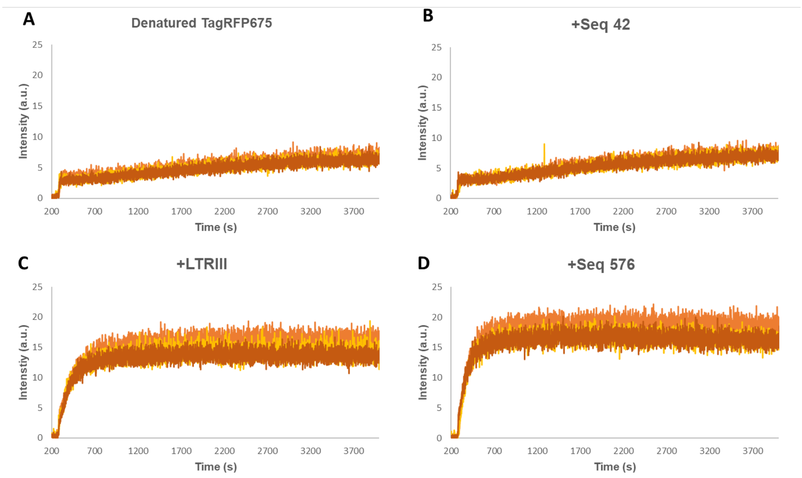

Figure 3—Effect of nucleic acids containing G-quadruplex structures on the refolding of TagRFP675 in vitro. TagRFP675 was denatured in 6M guanidine-hydrochloride (Gu-HCl). Denatured protein was diluted in potassium phosphate and the increase in fluorescent intensity was measured over time in the absence of nucleic acids (**A**) and in the presence of 1μM of sequence 42 (**B**), LTRIII (**C**), and sequence 576 (**D**). LTRIII and sequence 576 contain G-quadruplex structures.

TagRFP675 Folding - Temperature

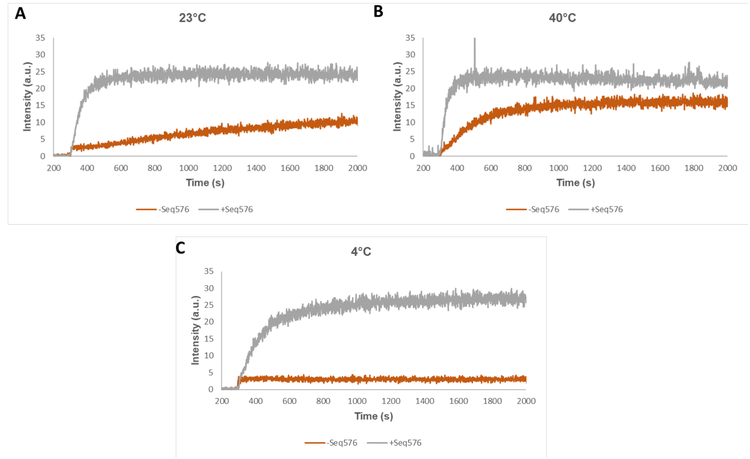

Figure 4—Effect of temperature on the fluorescence recovery of TagRFP675. The fluorescence recovery of denatured TagRFP675 was measured under different temperatures including room temperature (23°C) **(A),** 40°C (**B**) and 4°C **(C).** A higher temperature seems to catalyze the protein folding compared to room temperature and lower temperature.

Next Steps

Fluorescence recovery will be tested with different ratio concentrations of protein to nucleic acids to help uncover the mechanism's stoichiometry. The different conditions being tested: the presence of nucleic acids, temperature, and concentration ratio will give us an insight into the interactions between a misfolded protein and nucleic acids in vitro. Circular dichroism (CD) will also be used to investigate further the structure of the denatured and nondenatured protein to the structure of the protein in the presence and absence of nucleic acids.

References

Nelson, D. L., Cox, M. M., & Lehninger, A. L. (2017). Lehninger principles of biochemistry. New York, NY: W.H. Freeman and Company.

Protein Production. (n.d.). UNSW http://2018.igem.org/Team:UNSW_Australia/ Lab/Protein

Manual Purification of Histidine-Tagged Proteins using His GraviTrap TALON. (n.d.). Sigma Aldrich. https://www.sigmaaldrich.com/technical-documents/protocols/biology/affinity-chromatography-tagged-proteins/manual-purification-using-his-gravitrap-talon.html

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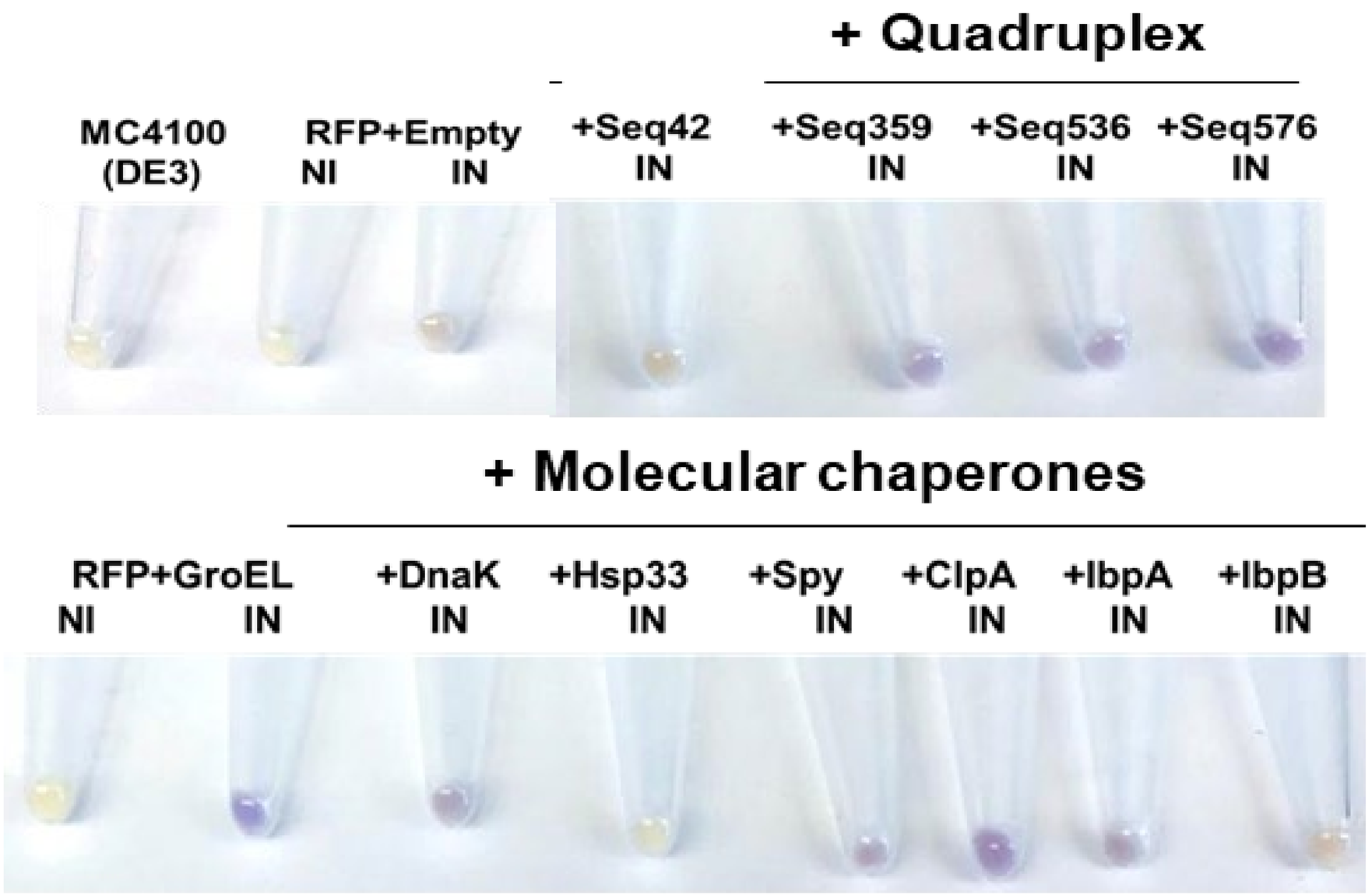
Enhancing Fluorescence of Biosensor



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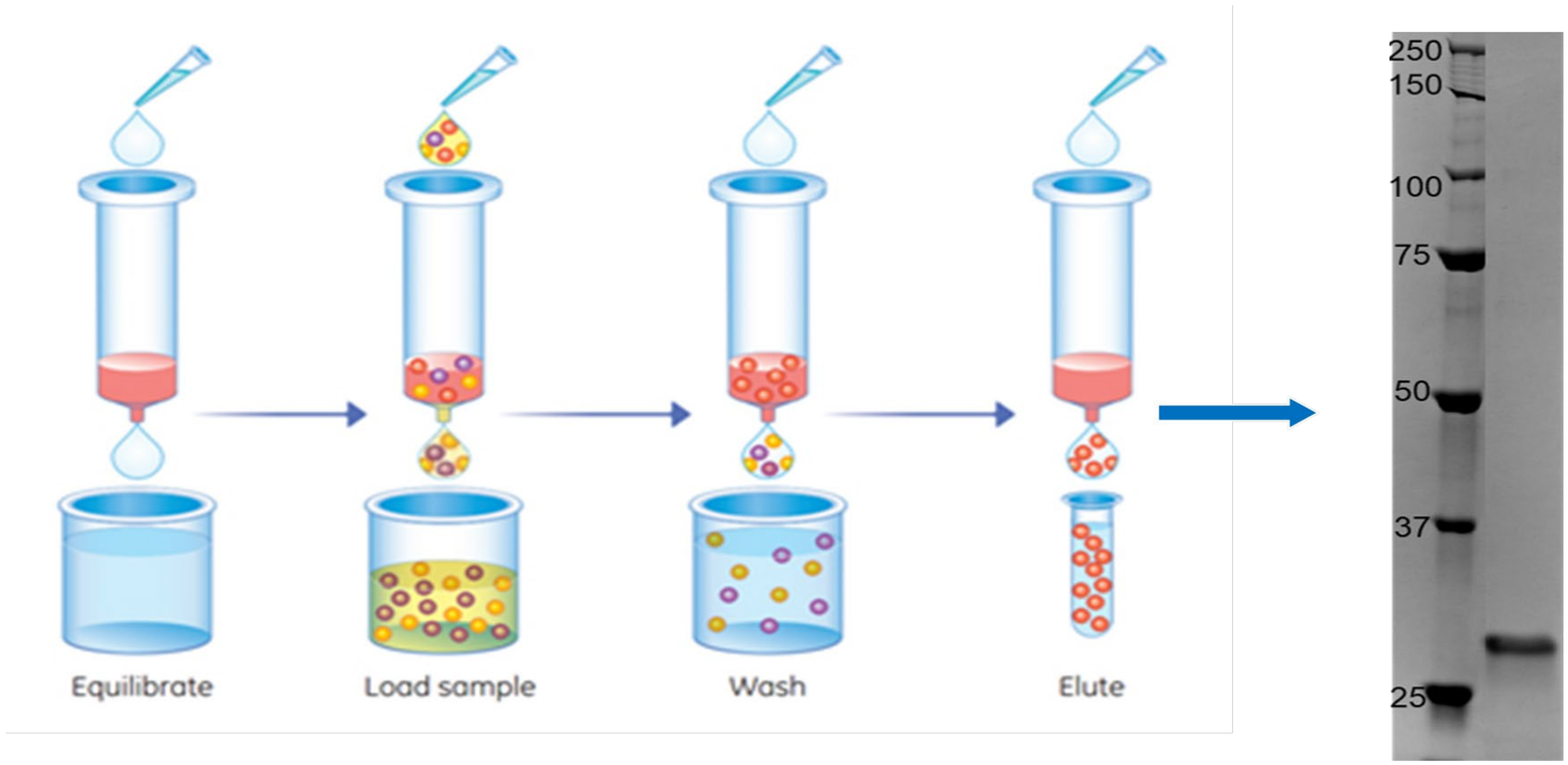
TagRFP675 Purification



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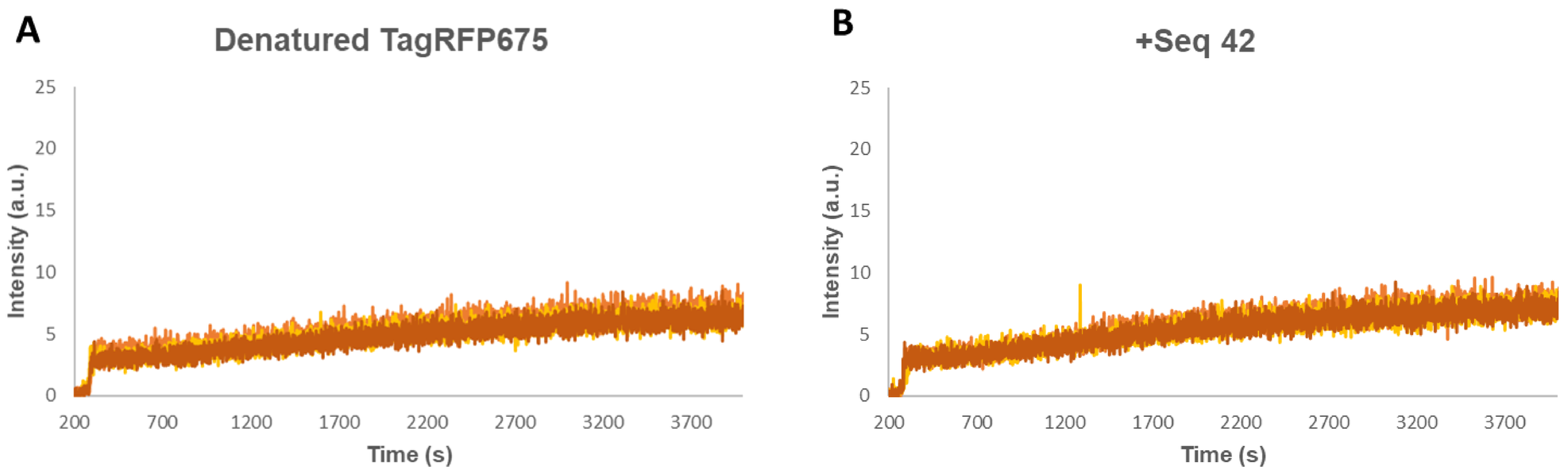

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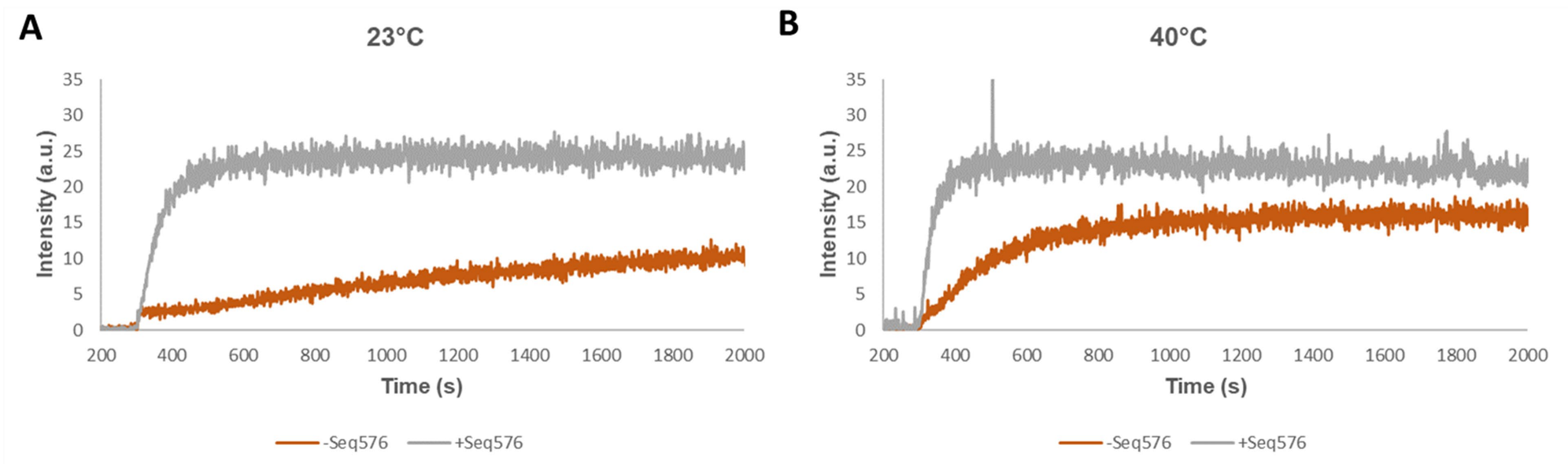
TagRFP675 Folding - Temperature



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