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# On the Track of a Misbehaving Polymer: Poly(Glutamine) and Huntington's Disease

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# Research and Creative Experience for Undergraduates (RCEU) Program, Summer 2019


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Project Title: **On the track of a misbehaving polymer: Poly(Glutamine) and Huntington's disease**


Project Summary:

Poly(*L*-Glutamine), p(*L*-Gln), a homopolymer consisting of *L*-Glutamine repeat units, is at the center of Huntington's disease[1]. Expanded p(*L*-Gln) sequences form at the end of the N-terminus of the huntingtin protein due to a genetic malfunction. If the length of the repeated trinucleotide section exceeds the normal range the huntingtin protein forms excessive p(*L*-Gln) segments, which then form plaques. The magic cut-off number is 36 p(*L*-Gln) repeat units. Why 36 repeat units – nobody knows. Fact is, the more p(*L*-Gln) units are present, the more severe the disease, which affects 25,000 to 30,000 people in the US, and the more likely it is that the disease is past-on to an offspring. Less than 26 p(*L*-Gln) on the htt protein is normal, the person is not at risk, 26 – 35 units, the person may not develop the disease, but there is a risk for the offspring, 36 and more p(*L*-Gln) units, the person will develop the disease, and there is 50% chance that the offspring will have the disease as well. The longer the p(*L*-Gln) chain the more severe and the more accelerated is the progress of the disease.

This proposal does not claim to find a cure for Huntington's disease, but we need to understand why 36 repeat units are the crucial point in the development of this neurodegenerative disease. The answer to this question lies in the physical properties of this polymer, its three-dimensional structure and interaction with its environment, all of which could be easily studied if the polymer was readily available. The synthesis of this polymer has proven challenging, however, with the help of two former RCEU students, Corban Swain and Allana Schafer, significant progress has been made (proof that a direct synthesis is not possible and development of a viable method for the polymer-analogous conversion of p(*L*-glutamate), p(*L*-Glu)) into the desired p(*L*-Gln).
Now, that the basic synthetic approach has been established, polymers need to be synthesized with pre-determined numbers of repeat units that cover the entire relevant spectrum from 20 to 40 repeat units, so that physical analyses can be performed on the material.



1 Harper, P.S. in: Huntington's disease Oxford Monographs on Medical Genetics, 3rd ed. **2002**


Student Duties:

The student will learn about and perform the syntheses of the monomers and polymers. Benzyl-*L*-Glutamate will be converted into its reactive, cyclic form: Benzyl-*L*-Glu *N*-carboxyanhydride, NCA through the reaction with triphosgene. The polymer will be produced by ring-opening polymerization of the Bz-*L*-Glu NCA, initiated with a

primary amine compound yielding p(Bz-*L*-Glu)$_x$; x indicates the number of repeat units and is set to match the spectrum relevant to Huntington's disease, x= 20, 25, 30, 36, 40. The resulting p(Bz-*L*-Glu)x will be deprotected using trimethyl iodide, yielding the p*(L*-Glu)$_x$. Thereafter, the side chains will be activated for the polymer-analogous reaction that will yield the desired p(*L*-Gln). It has been determined that the formation of an *N*-hydroxysuccinimide ester catalyzed by 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide is the most effective activation of the p(*L*-Glu). This intermediate will then undergo aminolysis using ammonia dissolved in tetrahydrofuran, which was proven to be the most efficient reagent that converts the side chains but does not break the polymer backbone. Learning objectives for monomer synthesis: (i) handling triphosgene (special safety instruction), (ii) isolating the NCA monomer, (iii) purifying the NCA by repeated recrystallizations. Learning objectives for polymerization: (i) purification of solvents by distillation, (ii) stoichiometric calculations for polymerizations (monomer:initiator ratios to control molecular weight), (iii) anhydrous polymerization using a Schlenk line and (iv) isolation and purification of the polymer by dialysis.
Upon synthesizing the polymers the student will assist in the structure verification of the produced polymers using $^1$H NMR. Learning objective: recording or assisting in recording. i.e. preparing polymer solutions, and interpreting $^1$H NMR spectra.
The polymer-physical analysis discussed above is outside the scope of this RCEU project.

Student Pre-requisites:

The RCEU student for this project should be majoring in Chemistry or a related field or have an interest in biomedical research. The student must also have a keen interest in organic polymer synthesis. Successful completion of CH 331 (and CH 332) is preferred.

Mentor Supervision and Interaction

I will meet with the student every morning for 15 to 20 minutes and discuss results of the previous day, and plan experiments for that day, thus he/she will have the benefit of daily supervision by me. I will be available continuously throughout the day if questions or problems arise. The RCEU student will participate in my bi-weekly group meetings and will be expected to provide research updates using power point presentations, as it is standard in my group. This will be an excellent opportunity for him/her to learn how to present research data and how to interpret and defend them in a group of fellow researchers. The student's progress on the project will be evaluated at these group meetings. He/she is also expected to summarize the entire research work in a formal setting by presenting the results to his/her peers and prepare a poster to be presented at the RCEU poster session in September 2019.

Expected workload: 10 weeks at 40 hrs/week