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When Macromolecules Mishbehave -how Poly(Amino) PPAs Help Us to Understand Disease Patterns

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Project Title: **When macromolecules misbehave – how poly(amino acid)s, PAAs help us to understand disease patterns**

Project Summary:

There are 25,000 to 30,000 people in the US who have been diagnosed with Huntington's disease, HD, with another 150,000 to 250,000 persons being at risk.[1] HD is a neurodegenerative, genetic disorder that manifests itself with chorea (abnormal involuntary movements), cognitive dysfunction and noticeable behavioral problems and eventually leads to a total physical and mental deterioration.[2]

At the center of the disease is poly(*L*-Glutamine), p(*L*-Gln)[3], a poly(amino acid), PAA, which forms deposits in the brain, known as plaques. These expanded p(*L*-Gln) sequences form at the end of the N-terminus of the huntingtin protein due to a genetic malfunction of the gene that encodes for the huntingtin protein, htt, which has an expanded trinucleotide section. If the length of the repeated trinucleotide section exceeds the normal range the huntingtin protein forms with excessive p(*L*-Gln) segments, which then form these 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 is the disease 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. There are many medical implications that cannot be considered here, however, the disease seems to present an interesting connection to the polymer physics of self-assembly and folding of PAA chains. It needs to be understood what is so special about the polymer chain lengths and why are comparatively small changes in chain length so significant for the course of HD. The framework of the RCEU program provides an ideal setting for the synthesis of p(*L*-Gln) polymers with distinct, HD related chain lengths. The research effort proposed here will be a **polymer synthesis project.** The student involved in this project will have the opportunity to become proficient in state-of-the-art synthesis techniques, specifically in using a Schlenk line, a technique that cannot be taught in regular teaching labs, as it is too challenging and demanding. At the end of the project the student will have acquired unique polymer synthetic skills: Synthesis of p(*L*-Gln) N-carboxyanhydride, NCA, monomers, ring-opening polymerization, ROP, of trityl-protected *L*-Gln NCAs, Deprotection of terminal polymer side chains by hydrolysis. Several p(*L*-Gln) polymers

will be synthesized with distinct chain lengths: 25, 36, 40 and 45 p(*L*-Gln) units, and analyzed spectroscopically. The polymer physical analysis of folding patterns of the polymers produced by the student is outside the current RCEU effort and will be conducted later in collaboration with our colleagues at Martin Luther University in Halle.

Student Duties:

The student will be responsible for all monomer and polymer syntheses. First, the student will synthesize the monomer trt-*L*-Gln NCA, preliminary data have been established by my group. Learning objectives: (i) handling triphosgene (special safety instruction), (ii) isolating the NCA monomer from the reaction, (iii) purifying by repeated recrystallizations and (iv) solvent/non-solvent techniques. The structure and purity monomer will be monitored and characterized by ^{1}H NMR. Learning objective: recording and interpreting ^{1}H NMR spectra.

When satisfactorily results have been achieved for the monomer synthesis, the trt-*L*-Gln NCA will be polymerized by ROP using hexylamine as initiator. Learning objectives: (i) purification of solvents by distillation, (ii) stoichiometric calculations for polymerizations (monomer:initiator ratios), (iii) anhydrous polymerization using a Schlenk line and (iv) isolation and purification of the polymer by dialysis. The molecular architecture and chain length will be determined by ^{1}H NMR. Anhydrous polymerizations using a Schlenk line is a challenging task and the student will initially be supervised and supported by either my graduate students or myself. ***The Schlenk technique used here is a difficult chemical method; being proficient in using a Schlenk line will provide the student with a rather unique chemical skill and a distinct advantage***.

Finally, the student will investigate the deprotection of p(*L*-Gln) polymers. Learning Objective: (i) hydrolysis reactions applied to polymers and (ii) determination of complete deprotection reactions by ^{1}H NMR.

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 teach the student the use of all the equipment and instrumentation (especially Schlenk technique) that is needed to fulfill this project. I will be available continuously throughout the day if questions or problems arise. Additional supervision will be provided by my graduate students David Ulkoski and Samuel Nkrumah-Agyeefi. 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 2015.
Expected workload: 10 weeks at 40 hrs/week

1 Harper, P.S. in: Huntington's disease Oxford Monographs on Medical Genetics, 3rd ed. **2002;** 2 Frank, S. *Neurotherapeutics*, **2014**, 11, 153; 3 Assarsson, A. et al. *Langmuir*, **2014**, 30, 8812