Experimental report

06/01/2021

Proposal: 8-03-974 **Council:** 4/2019

Title: SANS and USANS of alpha-synuclein protein mutations

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: Thomas MCCOY

Experimental team: Thomas MCCOY
Alexander ROUTH
Alexander ARMSTRONG
Amberley STEPHENS

Local contacts: Anne MARTEL

Samples: Proteins
salts

Instrument	Requested days	Allocated days	From	To
D33	2	0		
S18	3	3	17/01/2020	20/01/2020
D22	0	2	15/01/2020	17/01/2020

Abstract:

In this work, we hope to explore the relationship between surface chemistry and aggregate formation
for a series of alpha;-synuclein mutations, by measuring their structural properties as a function of concentration and carefully chosen salt additives. This study aims to expand on recent work conducted on SANS2D (ISIS) and will involve time and temperature-controlled SANS measurements, to not only determine the internal structure or structures present in each system, but also the key factors governing the aggregation process. In addition, we would like to perform USANS measurements that will serve to characterise large scale aggregation and structures within these systems.

Draft manuscript

Sequence and environment affect monomeric alpha-synuclein structure and aggregation propensity

Amberley D. Stephens, Tom McCoy, Alexander Armstrong, Alex Routh, Gabriele Kaminski Schierle

Chemical Engineering and Biotechnology, University of Cambridge, Philippa Fawcette Drive, Cambridge, CB3 0AS

Previously we observed that the more exposed the N-terminus and the beginning of the NAC region of aSyn are, the more aggregation prone monomeric aSyn conformations become[1]. Here we further investigate this observation by exploring the structure of the six aSyn mutants which lead to early onset Parkinson's Disease and the structure of aSyn in a physiological environment, i.e. with the presence of salt.

The aggregation rate of the six mutants can be defined as 'fast' aggregating and 'slow' aggregating. A30P, G51D and A53E aSyn are defined as 'slow aggregating' and H50Q, A53T and E46K aSyn as 'fast' aggregating. We previously observed A53T and A53E aSyn have differing monomeric structures, where A53E was less exposed to the environment compared to A53T and WT aSyn. We proposed that the initial monomeric structure influences its aggregation propensity, with less exposed structures being less likely to aggregate. We now aim to investigate the structure of the other four mutants and the structure of the monomeric aSyn in differing cellular environments.

SANS

The radius of gyration (R_g) of the the aSyn monomers was calculated from fitting of the SANS data to a gunier-porod model (Supplementary Figure 1). We observe a trend where the 'slow' aggregating mutants increase their R_g when calcium is bound. For the 'fast' mutants A53T, WT and E46K aSyn all have a decrease in R_g, apart from H50Q. The S factor represents shape of the structure where 0 represents spherical shapes and 1 represents more rod-like shapes. The S factor for all aSyn mutants is ~0.7, indicating a more rod-like shape. Upon binding calcium, apart from WT aSyn, the S factor decreases, incdicating the conformational space that aSyn takes becomes more spherical.

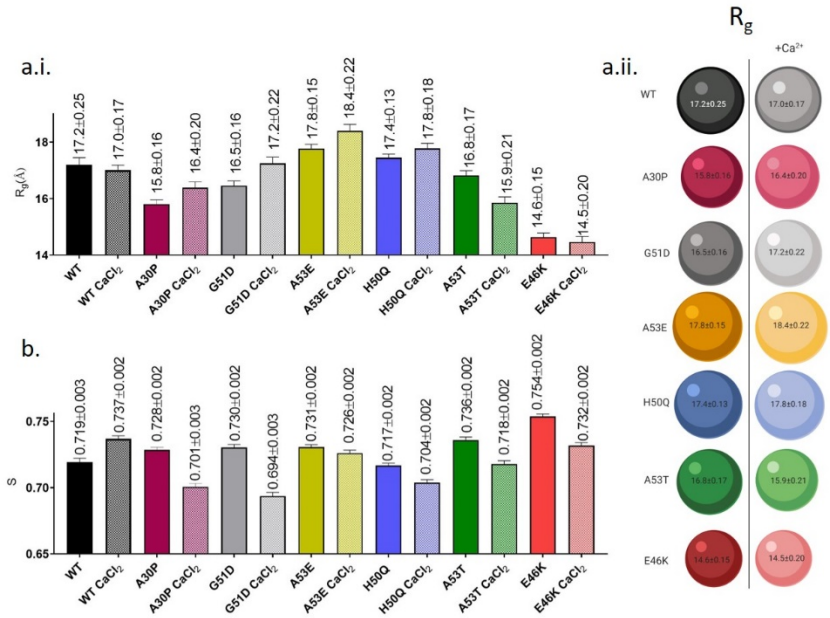
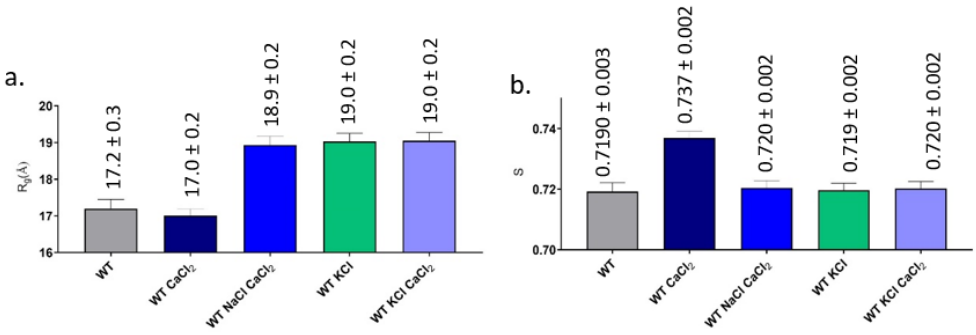


Figure 1. Radius of gyration and shape factor of WT aSyn and aSyn mutants alter upon calcium binding. (a.i.) The R_g was calculated from SANS data using a gunier-porod model. (a.ii.) A schematic represents the

conformational space taken by the average of the Rg of each aSyn mutant with and without calcium. (b) The Shape factor (S) was calculated from the gunier-porod model, 0 represents spherical shapes and 1 represents rod-like shapes. Upon binding calcium, apart from WT aSyn, the S factor decreases indicating aSyn becomes more spherical.

We then proceeded to investigate the size of aSyn and its conformational space in more physiological conditions, by the addition of salt to mimic the intracellular (140 mM KCl) and extracellular environments (140 mM KCl, 2 mM $CaCl_2$). For WT aSyn we observe the addition of salt increases the R_g due to charge shielding occuring at the positively charrged N-terminis and negatively charged C-terminus of the monomeric protein by the salt ions (Figure 2a). The S factor does not greatly differ for WT aSyn in salt buffers compared to WT aSyn in a no salt buffer (Figure 2b) . The greatest difference in S factor is observed when aSyn is bound



to calcium only.

Figure 2. Radius of gyration of WT aSyn increases when salt is present in the buffer, however shape factor only differs when calcium only is present. (a.) The Rg was calculated from SANS data using a gunier-porod model. The Rg of WT aSyn in 20 mM Tris (WT) or 20 mM Tris + 2 mM CaCl2 is ~ 17 Å. The Rg increases upon addition of salt, where we mimic the extracellular space (140 mM KCl, 2 mM CaCl2), the intracellular space (140 mM KCl) or the intracellular space near calcium channels (140 mM KCl, 200 µM CaCl2). (b) The Shape factor (S) was calculated from the gunier-porod model, 0 represents spherical shapes and 1 represents rod-like shames. The S factor remains similar in conditions with the addition of salt vs Tris only (WT), yet upon additoin of calcium the WT aSyn becomes slightly more rod-like.

We then compared the R_g and S factor of the WT aSyn to a 'fast' aggregating mutant A53T aSyn and a 'slow' aggregating mutant A53E aSyn, where the mutation occurs at the same residue but leads to different aggregation propensity. We observe when mimicking intracellular and extracellular conditions that the R_g for both A53T and A53E aSyn is smaller than for WT aSyn, yet does not change significantly between the two buffer conditions (Figure 3a). The S factor for A53T and A53E aSyn is smaller than for WT aSyn, indicating they occupy a more spherical space. For both the A53T and A53E aSyn, the presence of calcium in the 'extracellular' condition leads to a slight increase in S factor, and therefore a more rod-like shape, which is not observed for the WT aSyn (Figure 3b).

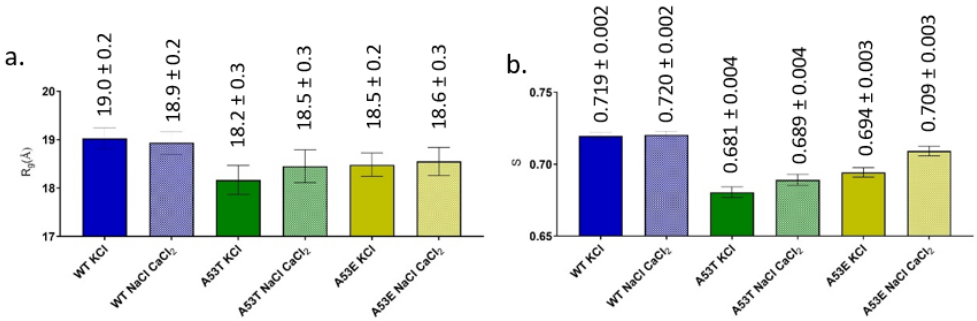
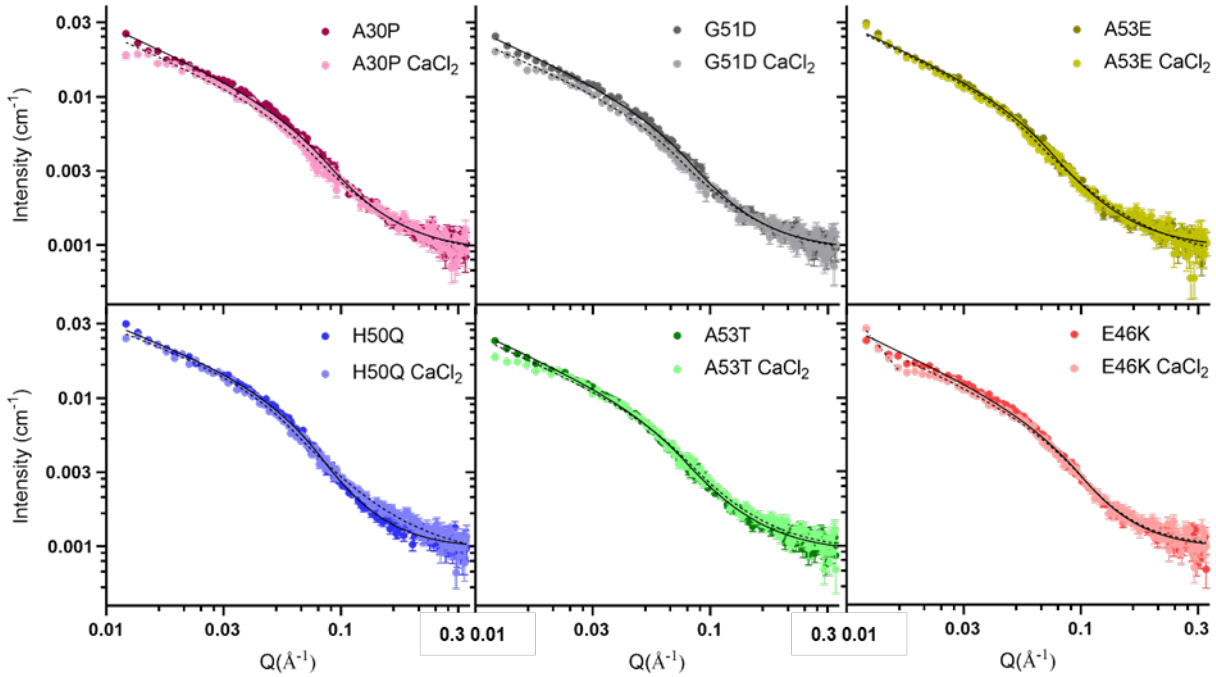


Figure 3. Radius of gyration of A53T and A53E aSyn in salt buffers is smaller than WT aSyn and their S factor more spherical than WT aSyn. (a.) The Rg was calculated from SANS data using a gunier-porod model. The Rg of A53T and A53E aSyn is smaller than WT aSyn buffers mimmicing the intracellular space (140 mM KCl) or the extracellular space (140 mM KCl, 2 mM CaCl2). (b) The Shape factor (S) was smaller for A53T and A53E indicating they are more spherical than WT aSyn in these conditions.

Supplementary Figures



Supplementary Figure 1. Raw data and gunnier-porod fitting of SANS data from aSyn mutants.

SANS data was collected for monomeric aSyn mutants, A30P (pink), G51D (grey), A53E (yellow), H50Q (blue), A53T (green) and E46K (red) aSyn in 20 mM Tris (dark spots) and with 2 mM CaCl2 (light spots). Data was fitted using a gunier-porod model (filled line for 20 mM Tris, dashed lined for with 2 mM CaCl2). For E46K aSyn + CaCl2, a power law was fitted between 0.01212 and 0.0175 Q(Å^{-1}) due to the presence of small aggregates (dash and spotted line).