White Rose Research Online

eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk
Universities of Leeds, Sheffield and York

Deposited via The University of Leeds.

White Rose Research Online URL for this paper:
https://eprints.whiterose.ac.uk/id/eprint/155980/

Version: Supplemental Material

Article:
Doherty, CPA, Ulamec, SM, Maya-Martinez, R et al. (2020) A short motif in the N-terminal region of α-synuclein is critical for both aggregation and function. Nature Structural & Molecular Biology, 27. pp. 249-259. ISSN: 1545-9993

https://doi.org/10.1038/s41594-020-0384-x

Reuse
Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown
If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

UNIVERSITY OF LEEDS
University of Sheffield
UNIVERSITY of York

Legends to Supplementary Table 1 and Extended Data figures

Supplementary Table

Supplementary Table S1. Lag times, elongation rates and fibril yield for αSyn variants. The rates of aggregation in each condition were measured in at least triplicate. The errors show the standard deviation of the mean of the replicates. No aggregation after 100 h incubation is indicated by "-". Given errors in estimating fibril yields via SDS PAGE subsequent to centrifugation (see Methods) gels, the values were rounded to the nearest 10%.

Extended data Figures

Extended data Figure 1. Aggregation kinetics of αSyn variants at different pH and salt conditions including TEM images of the aggregates (if any) formed at the end point. ThT fluorescence assays at pH 4.5 or pH 7.5 of a,b) WT αSyn, c,d) ΔP1, e,f) ΔP2 and g,h) ΔΔ at high (200 mM NaCl) or low (20 mM NaCl) ionic strength (n≥3). Negative stain TEM images of representative samples of the aggregates formed at the end point (100 h) are shown alongside each plot using the same colour scheme. The fibril yield under each condition, determined by SDS PAGE subsequent to centrifugation (see Methods) is shown in Supplementary Table S1.

Extended data Figure 2. Cross-seeding αSyn variants using seeds created from WT αSyn. a) ThT fluorescence assays of αSyn variants (100 μM) WT (black), ΔP1 (red), ΔP2 (green), ΔΔ (blue)) seeded with 10 % (v/v) WT αSyn fibril seeds formed at pH 7.5 (n≥3). Seeding assays were performed at pH 7.5, low salt (20 mM added NaCl), 37 °C, quiescent. b) End-point (42 h) TEM images of representative samples of fibrils from the seeding experiments using the same colour codes. Scale bars are 200 nm.

Extended data Figure 3. Aggregation kinetics of ΔC1 and P1P2-GS. a) Schematic of WT, ΔC1, ΔΔ and P1P2-GS αSyn variants including the amino acid sequence of deleted C1 region and substituted P1/P2 region. ThT assays at pH 4.5 or pH 7.5 of b,c) WT αSyn, d,e) ΔC1 (Δ14-20), or f,g) P1P2-GS (dark and light colours depict assays in high (200 mM added NaCl) or low (20 mM added NaCl) conditions, respectively (n≥3). h,i) Negative stain TEM images of representative samples at the endpoint (100 h) of the incubation of h) ΔC1 or i) P1P2-GS, with colour coding consistent with c-f. Scale bars are 200 nm. The fibril yield under each condition, determined by SDS PAGE subsequent to centrifugation (see Methods) is shown in Supplementary Table S1.

Extended data Figure 4. ^{1}H-^{15}N HSQC NMR spectra showing intramolecular PRE NMR experiments on WT αSyn in 20 mM sodium acetate buffer, 20 mM NaCl, pH 4.5, 15 °C. Overlaid are paramagnetic (green) and diamagnetic (orange) spectra for WT αSyn labelled at positions a) A18C, b) A90C or c) A140C. Schematics are shown above each spectrum with the N-terminal (blue), NAC (pink) and C-terminal (red) regions highlighted. The location of spin label is denoted by a yellow circle. Note that small chemical shift changes are observed upon reduction with ascorbic acid, which can be attributed to small changes in pH (see Methods).

As a consequence 2 mM ascorbic acid was used throughout this study, resulting in incomplete reduction of the MTSL-labelled sample. This does not affect the pattern of PREs observed and results in an underestimate of the PRE effect (especially for residues in the NAC region as K80, G84, S87, I88, A89, K96, Q99).

Extended data Figure 5. Intramolecular PRE experiment for P1P2-GS αSyn. a) Intramolecular PRE intensity ratios of amide protons (paramagnetic/diamagnetic) for P1P2-GS αSyn with the MTSL spin label at A90C at low ionic strengths (20 mM NaCl), 15 °C, pH 4.5. Blue, pink and red bars show intensity ratios for residues in the N-terminal, NAC and C-terminal regions, respectively. Dark blue bars highlight residues in the P1/P2 regions that could be assigned and measured. The grey boxes mark the P1/P2 regions. Black arrows show only a small PRE effect is observed in the P1/P2 region for P1P2-GS. Due to the repeating Gly and Ser residues in the P1/P2 sequence not all residues could be assigned (Methods). b) Comparison of a rolling window (over 5 residues for easier comparison) of the PRE effects for WT (blue), ΔΔ (red) and P1P2-GS (orange) αSyn. The black box is zoomed out in c) to highlight residues in the P1/P2-region. The data for WT and ΔΔ are shown in Figures 4d and 5d.

Extended data Figure 6. The role of P1 and P2 in intermolecular interactions. a) Schematic of intermolecular PRE experiments. ^{14}N and ^{15}N αSyn are illustrated as cyan and dark blue chains, respectively. MTSL is shown as a yellow circle. b) H_N-Γ_2 rates for WT αSyn labelled with MTSL at position 40 (A40C) at pH 4.5 in low salt (20 mM added NaCl) (black) or high salt (200 mM added NaCl) (red) conditions, 15 °C. Bars depict residue specific H_N-Γ_2 rates. c) H_N-Γ_2 rates at pH 4.5 under low salt (20 mM added NaCl) for WT (black) or ΔΔ (blue) αSyn labelled at position 129 (S129C). Bars depict residue-specific H_N-Γ_2 rates.

Extended data Figure 7. CD binding assays of αSyn WT, ΔΔ and P1P2-GS to DMPS LUVs. a) Far-UV CD spectra of 25 μM WT αSyn (blue) or ΔΔ (red) incubated in the absence or presence of liposomes (100:1 (M/M) DMPS:αSyn). b) Change of CD signal of WT αSyn (blue), ΔΔ (red) or P1P2-GS (orange) at 220 nm as a function of [DMPS]/[αSyn] ratio. Data were fitted (solid lines) to a single-step binding model, yielding the affinity (K_d) and stoichiometry value (L) (the number of DMPS molecules in the bilayer that are involved in binding to one molecule of αSyn[56]. c) Far-UV CD spectra of 25 μM WT αSyn (blue) or P1P2-GS (orange) incubated in the absence or presence of 100-times molar excess of DMPS LUVs. d) Dynamic light scattering of DMPS liposomes showing they have a hydrodynamic radius (R_H) of on 81 nm.