

Methods for site-directed introduction of post-translational modifications in peptides or proteins

Technical Journal club

20.05.2014

Tiziana Sonati

Aguzzi's group

Outline

- Background: Post-translational modifications (PTM)
- Methods to introduce site-directed PTMs in proteins or peptides: **SPPS, NCL and EPL**
- Paper 1 (application of SPPS and NCL): One-pot total chemical synthesis of human α -synuclein
- Paper 2 (application of SPPS, NCL and EPL): One-pot semisynthesis of exon 1 of the Huntingtin protein: new tools for elucidating the role of PTM in the pathogenesis of Huntington's disease

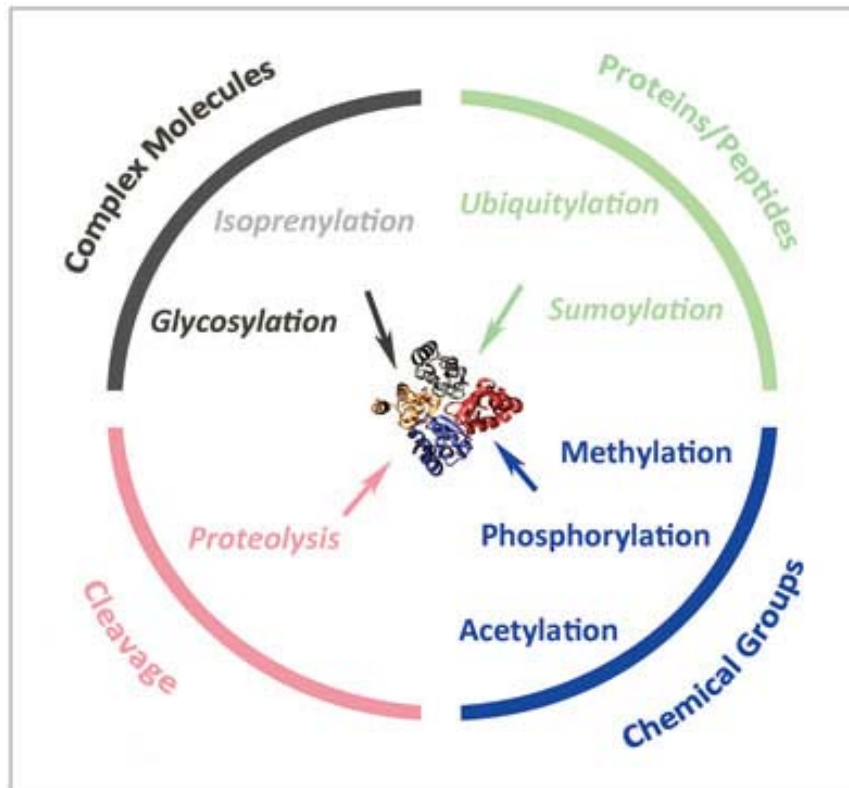
Protein post-translational modifications (PTMs)

- Protein post-translational modifications (PTMs) increase the functional diversity of the proteome by the covalent addition of functional groups or proteins, proteolytic cleavage of regulatory subunits or degradation of entire proteins
- Post-translational modification can occur at any step in the "life cycle" of a protein
 - Shortly after translation is completed to mediate proper protein folding or stability or to direct the nascent protein to distinct cellular compartments (e.g., nucleus, membrane)
 - After folding and localization are completed to activate or inactivate catalytic activity or to otherwise influence the biological activity of the protein
 - Proteins are also covalently linked to tags that target a protein for degradation
- PTMs are most often mediated by enzymes such as, kinases, phosphatases, transferases and ligases, which add or remove functional groups, proteins, lipids or sugars to or from amino acid side chains, and proteases, which cleave peptide bonds to remove specific sequences or regulatory subunits. Many proteins can also modify themselves using autocatalytic domains, such as autokinase and autoprotolytic domains.

PTMs: some examples

Proteins in eukaryotic cells can be edited after translation by a wide variety of reversible and irreversible PTM mechanisms.

The structure, stability and function of proteins in the cells can be dynamically altered by these PTMs.

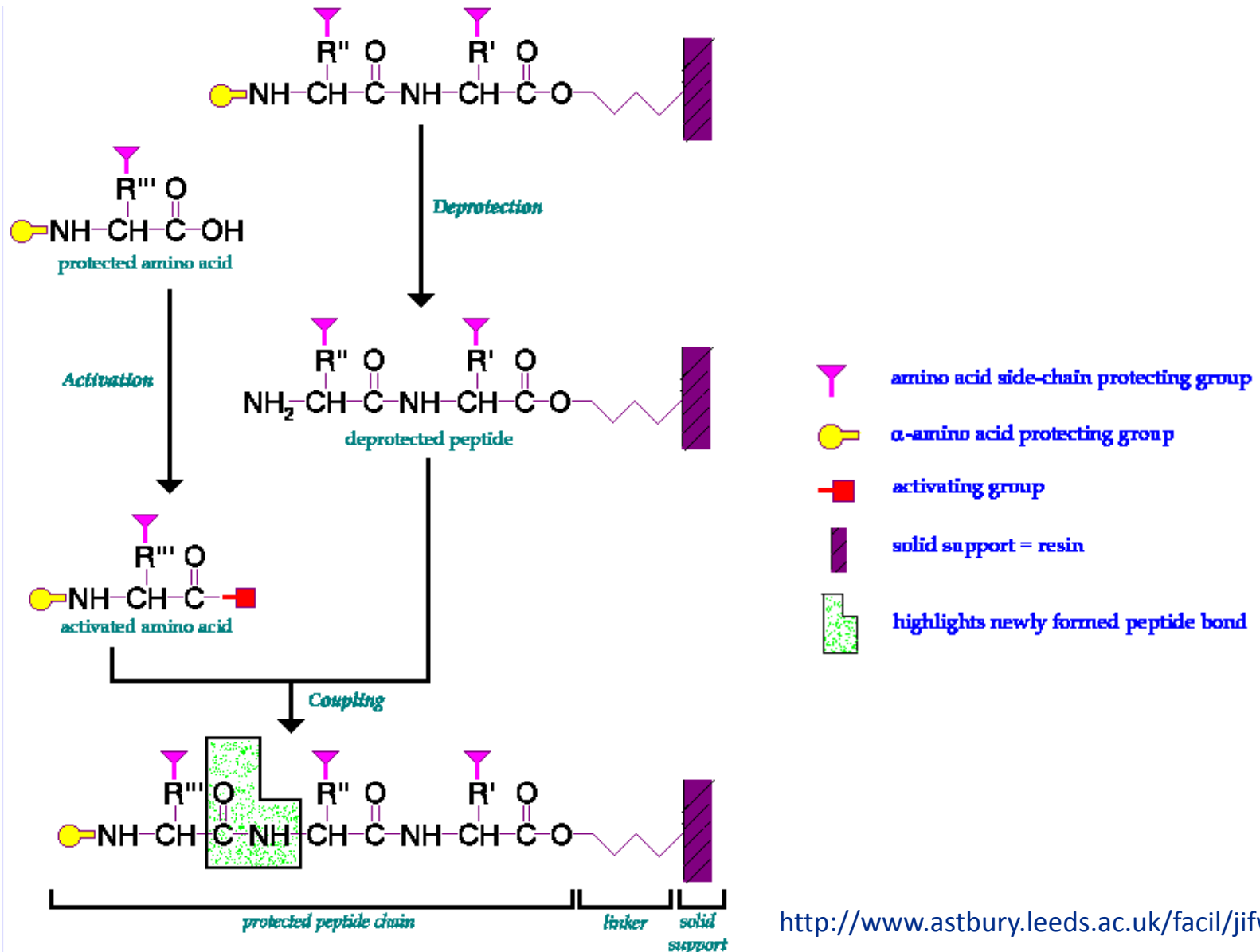


Wang et al. *Cell Research* (2014)

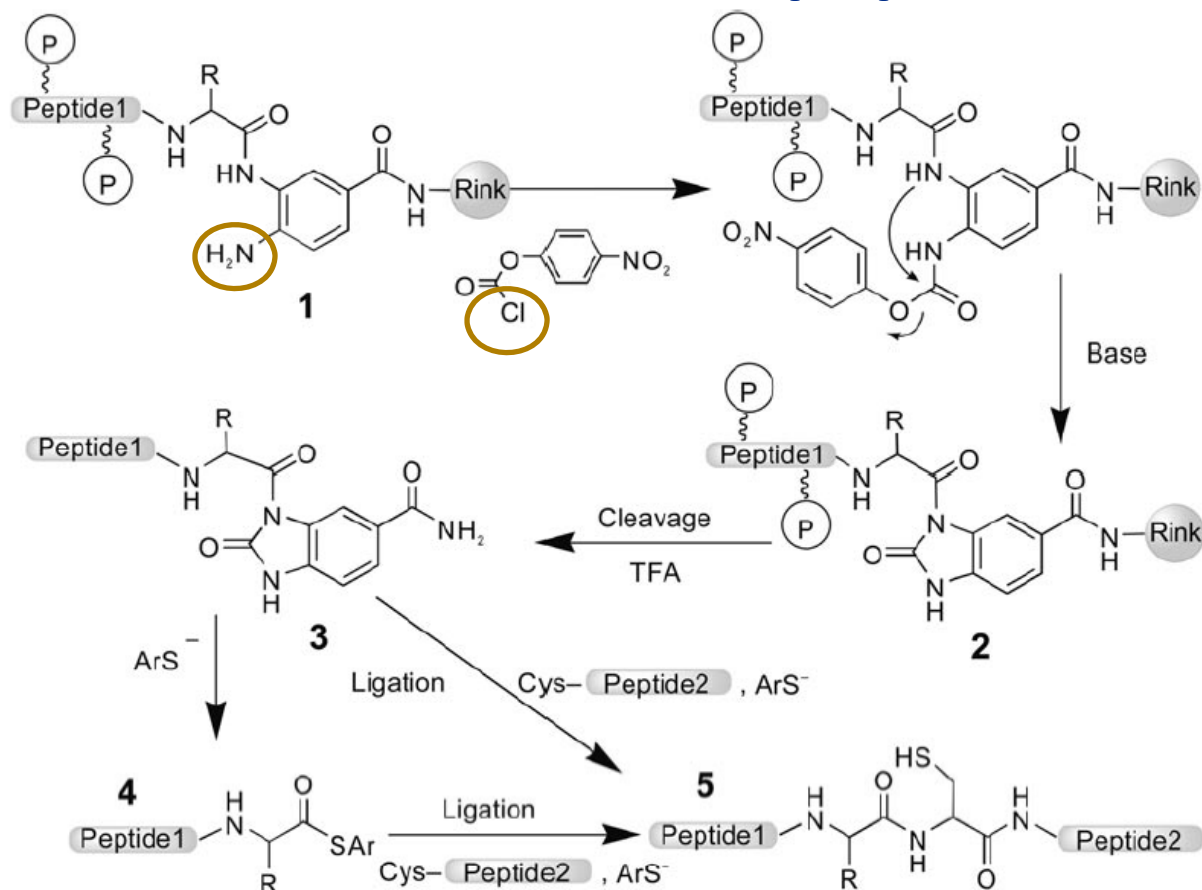
Methods for site-directed introduction of PTMs in peptides or proteins

- Solid-phase peptide synthesis (SPPS)
- Native chemical ligation (NCL)
- Expressed protein ligation (EPL)

Solid-phase peptide synthesis (SPPS)

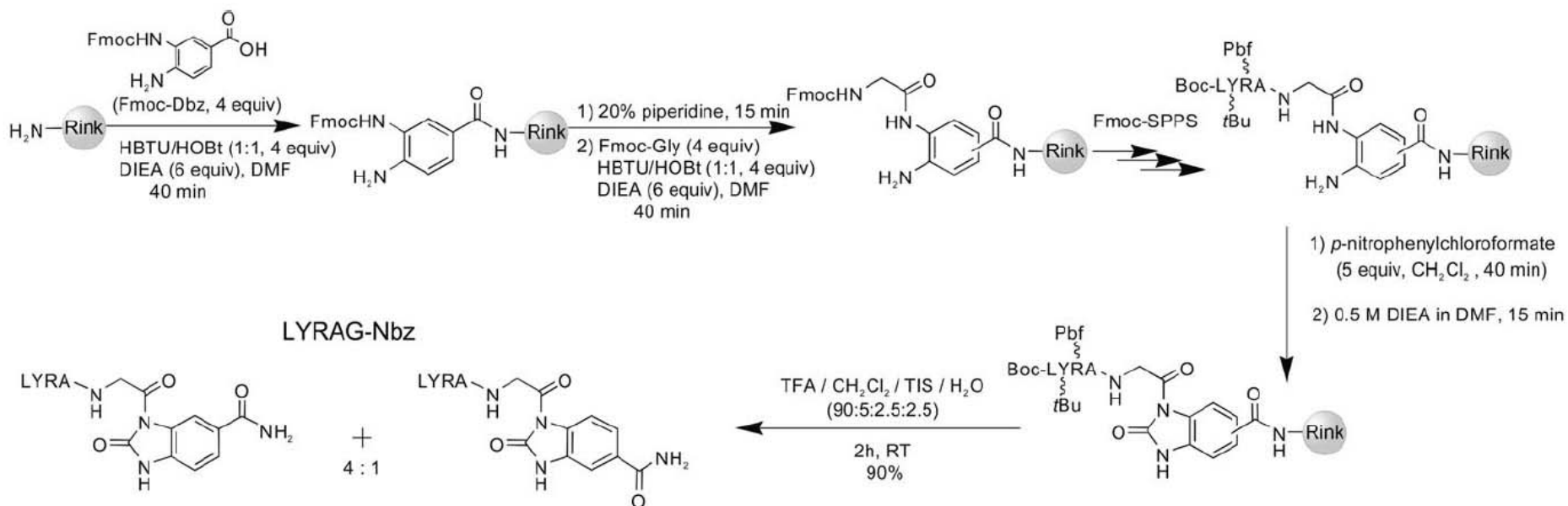


Peptide ligation I: Synthetic strategy for Fmoc-SPPS of thioester peptides



Scheme 1. Synthetic strategy for Fmoc-SPPS of thioester peptides. Following SPPS, aminoanilide 1 undergoes specific acylation and cyclization to yield the resin-bound acylurea peptide 2. Following cleavage and deprotection, peptide-Nbz 3 can undergo thiolysis to yield peptide thioester 4, or direct ligation to yield the native peptide 5.

Peptide ligation II: Synthesis of LYRAG-Nbz

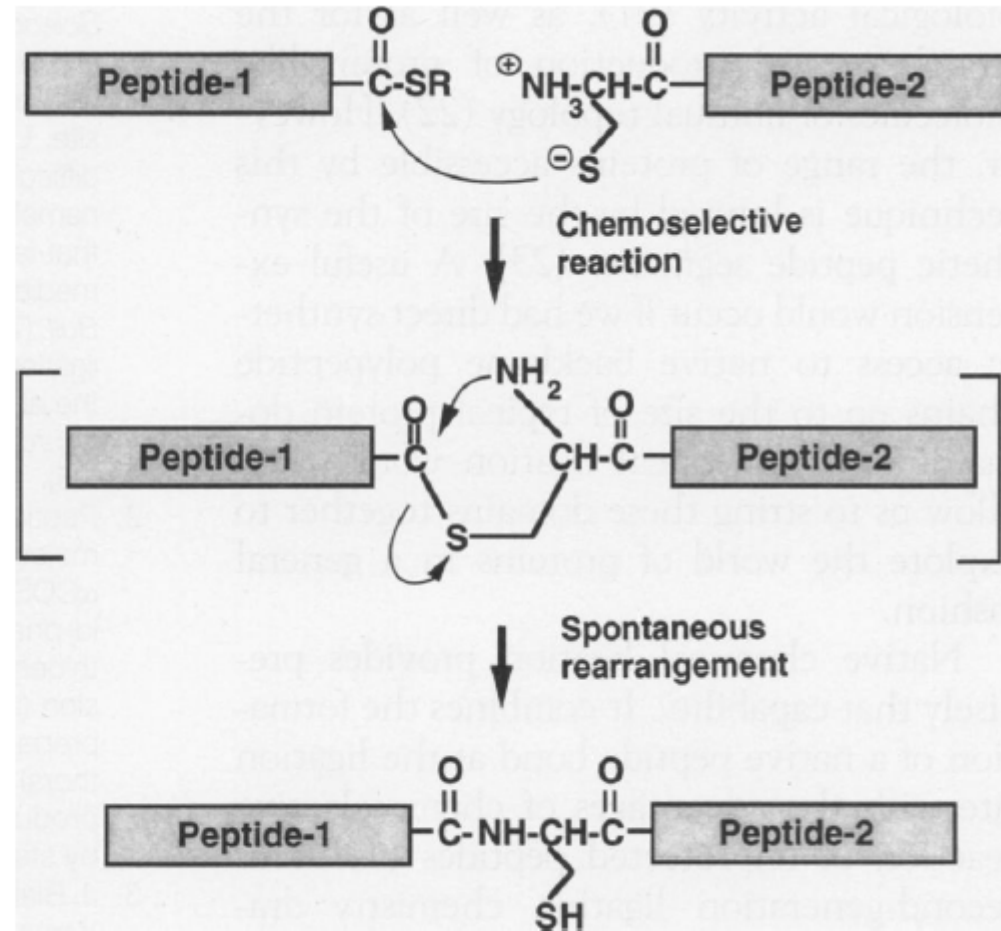


Scheme 2. Synthesis of LYRAG-Nbz. DIEA = *N,N*-diisopropylethylamine, HBTU = *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, HOBt = 1-hydroxybenzotriazole, Pbf = 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl, TIS = triisopropylsilane.

Native chemical ligation (NCL)

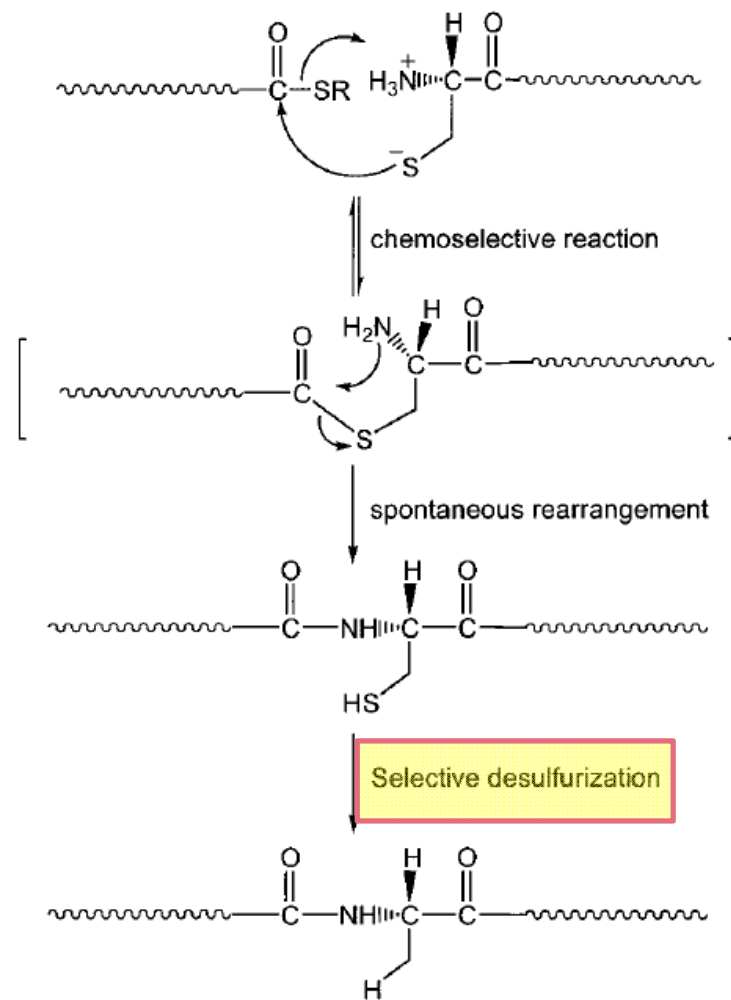
Fig. 1. The principle of native chemical ligation. The synthetic segment, peptide 1, which contains a thioester at the α -carboxyl group, undergoes nucleophilic attack by the side chain of the Cys residue at the amino terminal of peptide 2 (R is an alkyl group).

The initial thioester ligation product undergoes rapid intramolecular reaction because of the favorable geometric arrangement [involving a five-membered ring] of the α -amino group of peptide 2, to yield a product with a native peptide bond at the ligation site. Both reacting peptide segments are in completely unprotected form, and the target peptide is obtained in final form without further manipulation.



NCL for peptides with no Cys

Target protein: ~~~~~ Ala ~~~~~



Protein with its native sequence

Yan et. al, *JACS* (2001)

Mechanism & catalysis of NCL

Mechanism and Catalysis of the Native Chemical Ligation Reaction

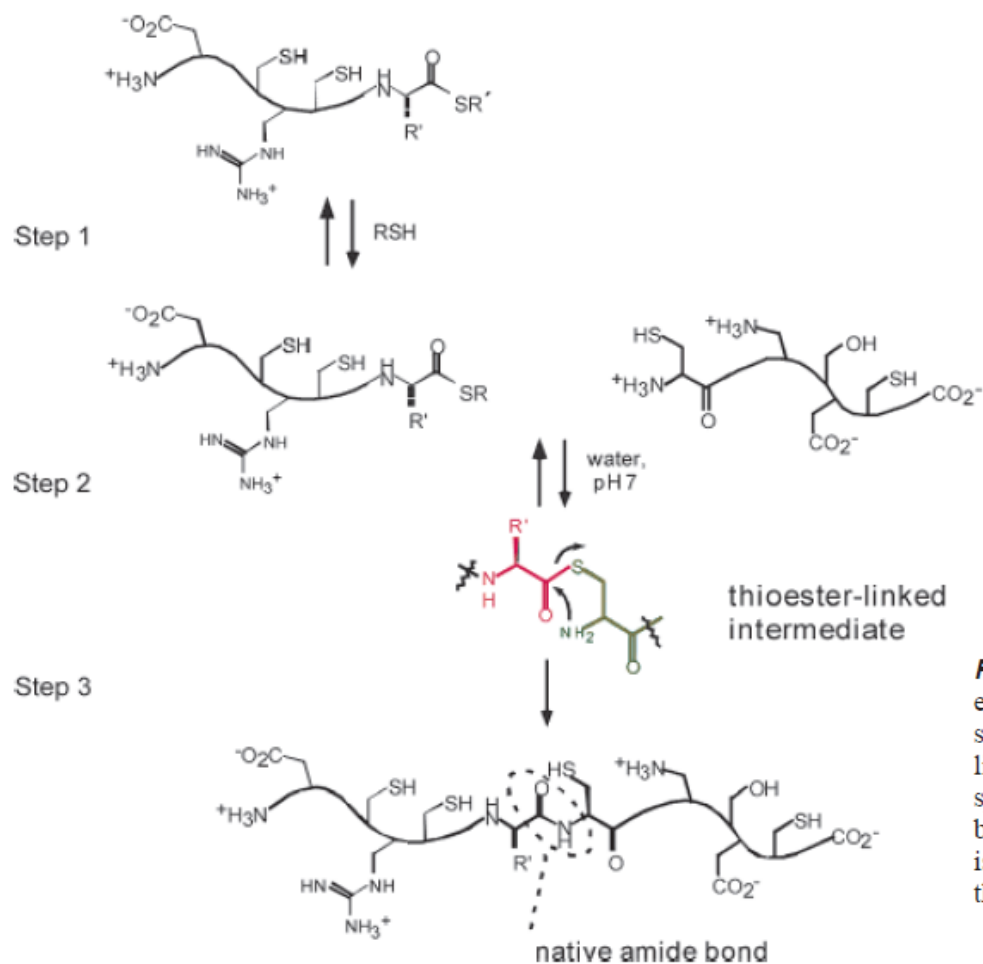
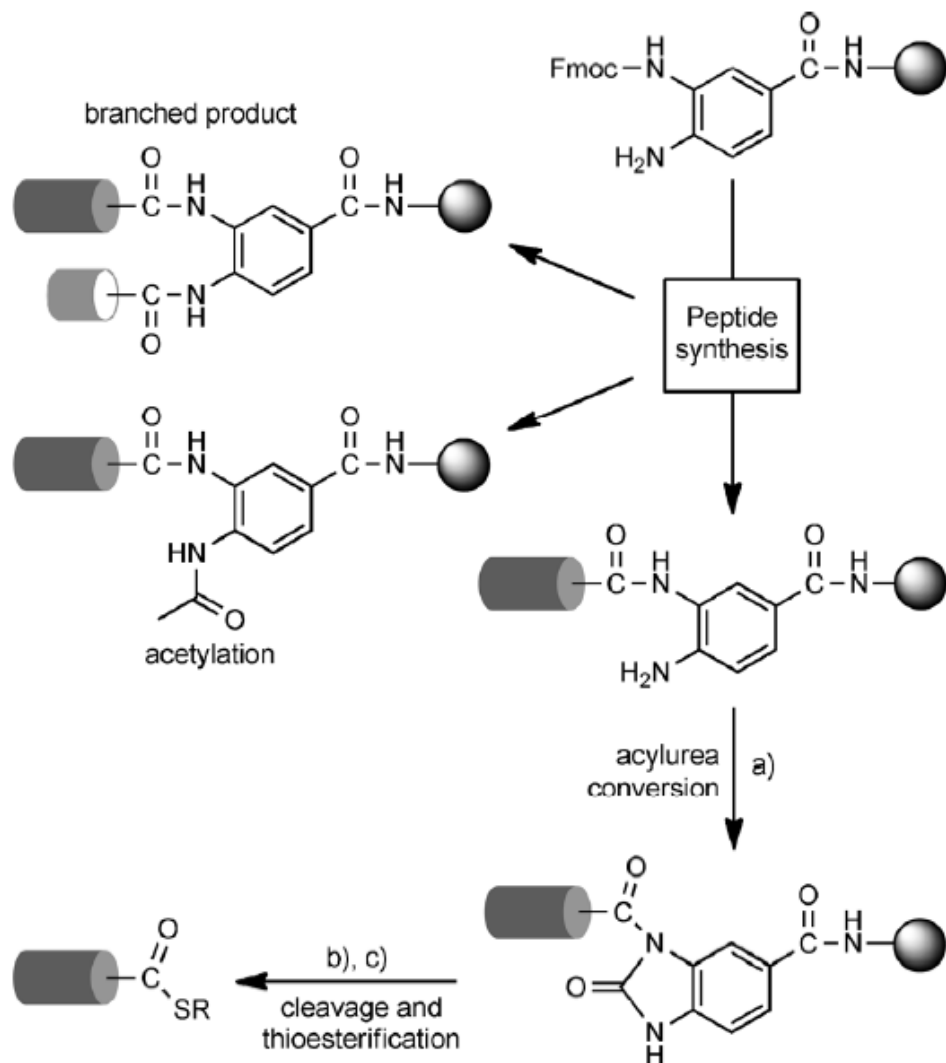


Figure 1. Proposed Mechanism for Native Chemical Ligation. *Step 1*: exogenous thiol-thioester exchange; *Step 2*: transthioesterification with the side chain thiol of N-terminal cysteine creates a thioester-linked intermediate ligation product; *Step 3*: the thioester-linked intermediate undergoes spontaneous intramolecular nucleophilic rearrangement to form an amide bond at the ligation site. Steps 1 & 2 are freely reversible, whereas Step 3 is irreversible, under the conditions used (aqueous solution, pH7, added thiol catalyst).

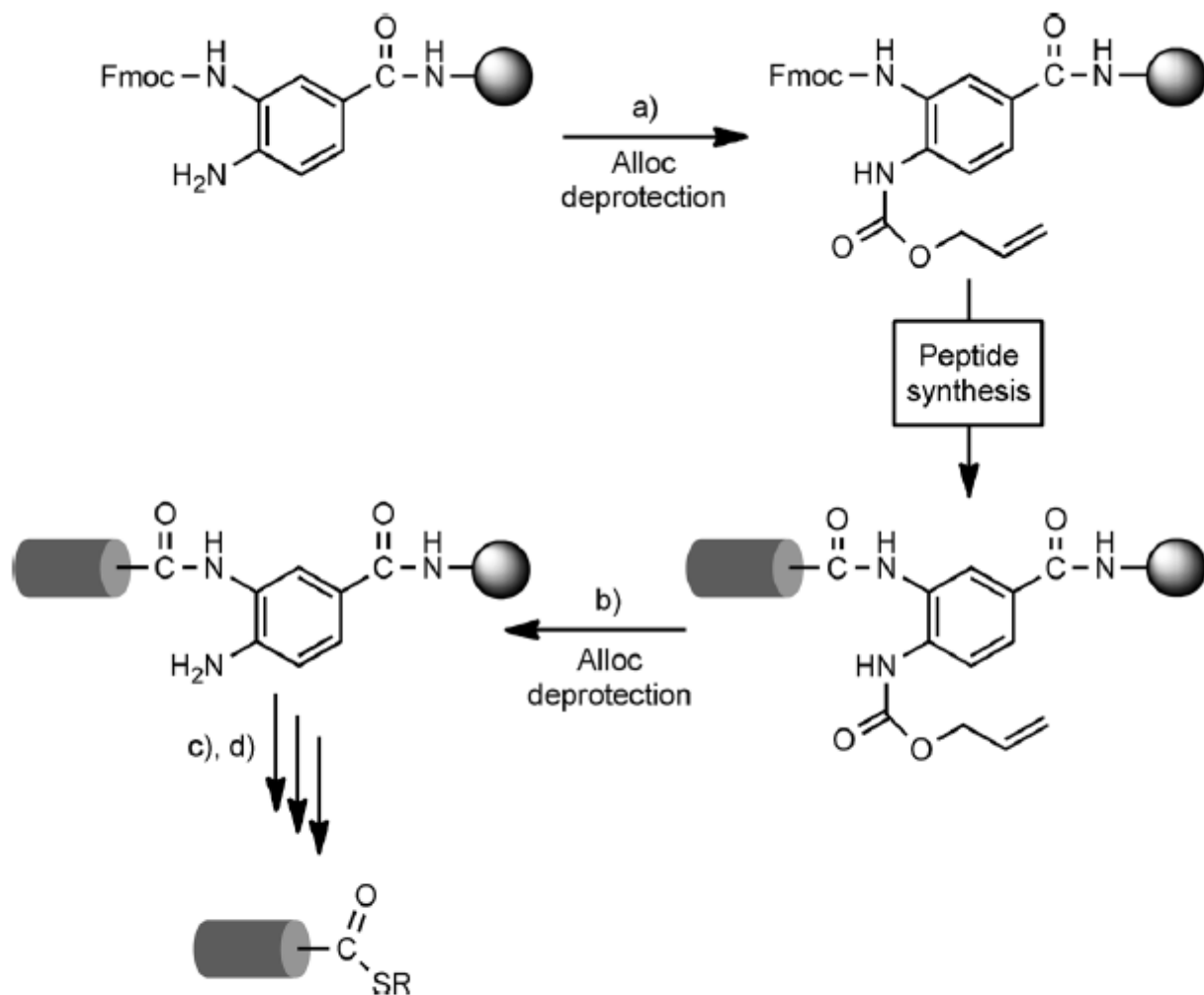
Synthetic scheme for thioester generation by using Dbz as a masked thioester during Fmoc SPPS



Scheme 1. Synthetic scheme for thioester generation by using Dbz as a

Mahto et al. *ChemBiochem* (2011)

Dbz protection strategy



Mahto et al. *ChemBiochem* (2011)

Scheme 2. Dbz protection strategy. a) mono-Fmoc-Dbz is treated with allyl chloroformate to generate the Fmoc-Dbz(Alloc) resin. Peptide synthesis introduces a single peptide chain. b) Treatment with phenylsilane and tetrakis(triphenyl-phosphine) palladium(0) re-generates the peptide-Dbz conjugate on resin, which may be c) converted to Nbz and

One-pot total chemical synthesis of human α -synuclein[†]

Cite this: *Chem. Commun.*, 2013, **49**, 9254

Received 15th July 2013,
Accepted 2nd August 2013

DOI: 10.1039/c3cc45353g

www.rsc.org/chemcomm

Bruno Fauvet,^{‡a} Sara M. Butterfield,^{‡a} Jonas Fuks,^a Ashraf Brik^b and Hilal A. Lashuel^{*a}

Post-translational modifications (PTMs) regulate key aspects of the physiological and pathogenic properties of Parkinson's disease-associated presynaptic protein α -synuclein. We herein describe a one-pot total chemical synthesis that should enable site-specific introduction of single or multiple PTMs or small molecule probes essentially at any site within the protein.

PTMs at the N- or C-terminal regions of α -syn, including N-terminal acetylation, mono-ubiquitination at K6⁵ and K12⁶ and phosphorylation at S129 and Y125⁷. Using these methods, we were able to produce homogeneously modified proteins in milligram quantities and elucidate the role of each modification in regulating α -syn structure,

Fauvet et al. 2013, “One-pot total chemical synthesis of human α -synuclein”

Rationale:

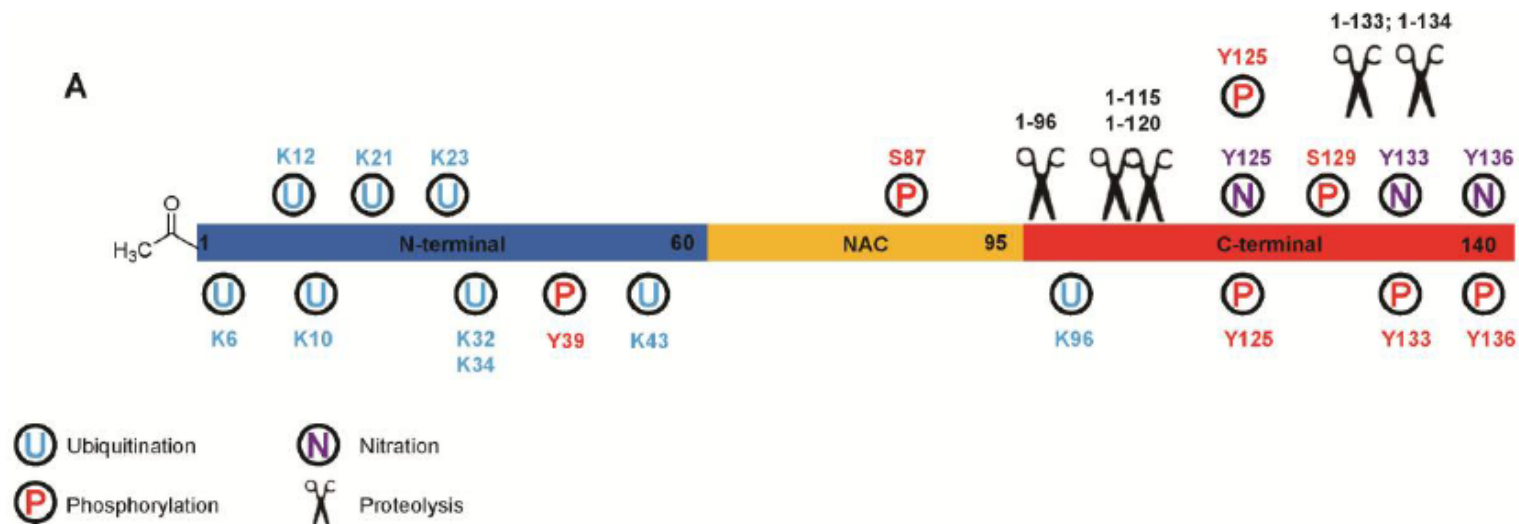
- Several PTMs identified in Lewy bodies (LBs) (i.e. phosphorylation, mono- and poly-ubiquitination at Lys residues, truncations and nitration) suggesting that they can play a role in syn aggregation
- Also, PTMs regulate key aspects of the physiological and pathogenic properties of PD-associated pre-synaptic α -synuclein (i.e. degradation by the proteasome or autophagy, subcellular localization and physiological functions)

Goal:

- Introduction of single or multiple PTMs (or small molecules) at any site of α -synuclein to get homogeneous preparations

PTMs of α -synuclein

PTMs identified in Lewy Bodies

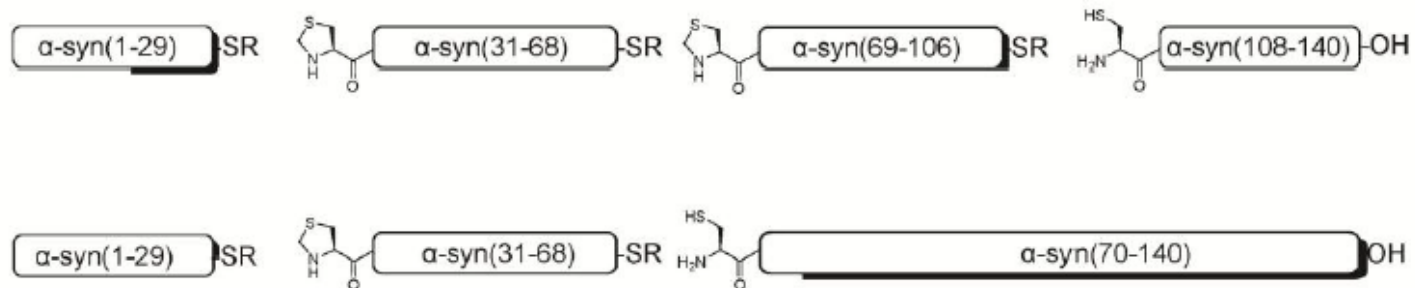


PTMs identified from *in-vitro* studies (below)

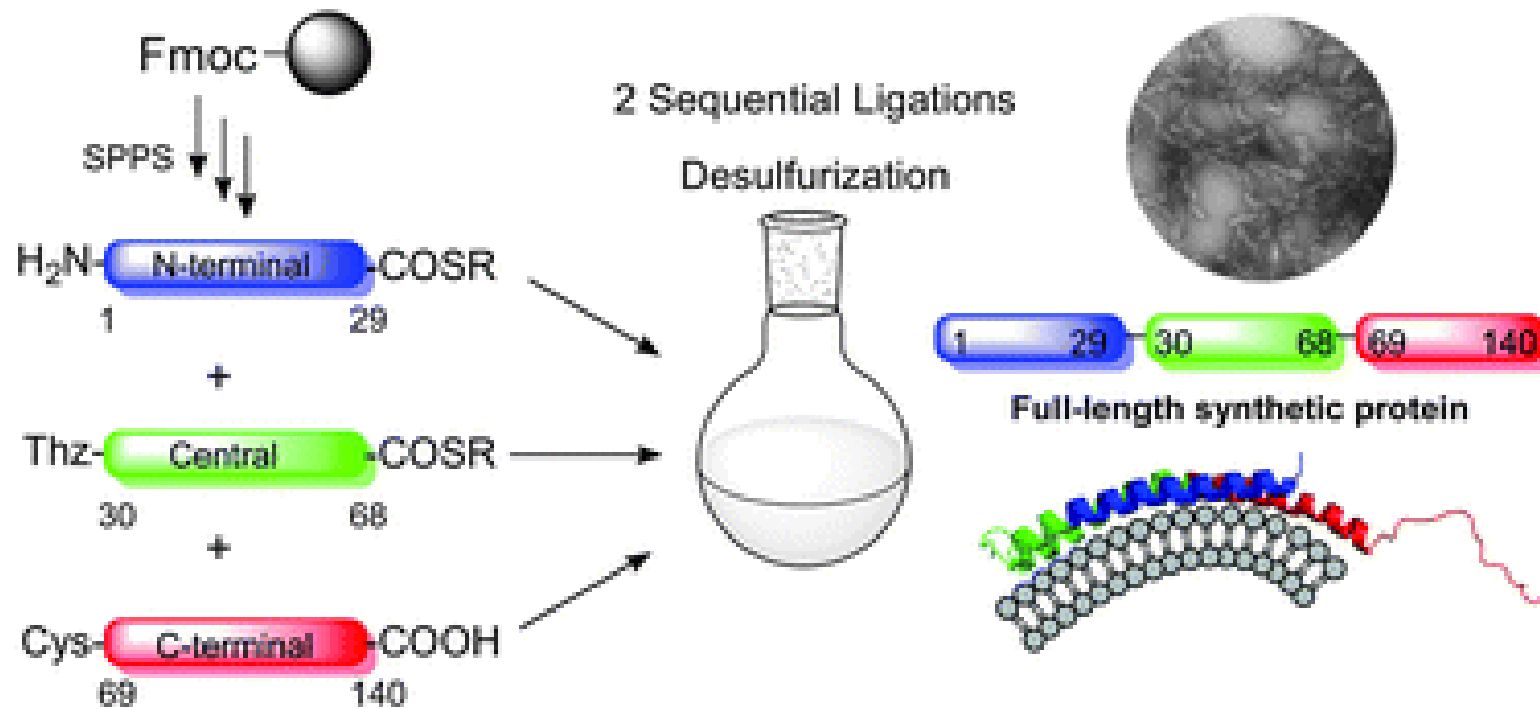
Approach: design of α -synuclein fragments

Four-fragment ligation strategy (top) and
three-fragment scheme (bottom)

B

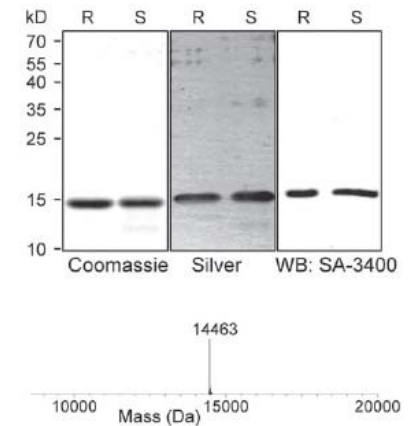
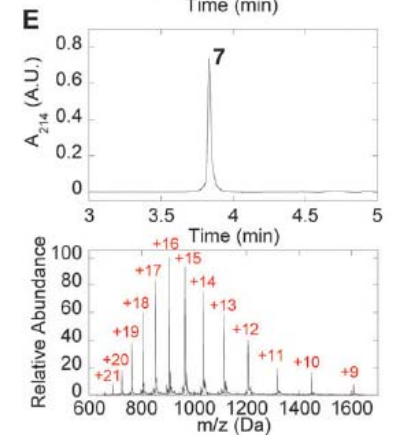
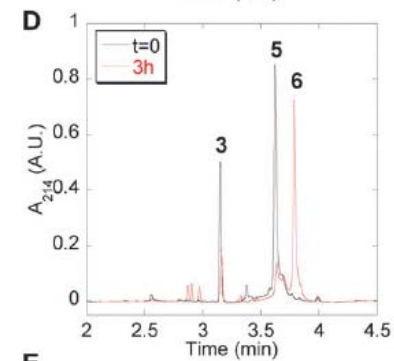
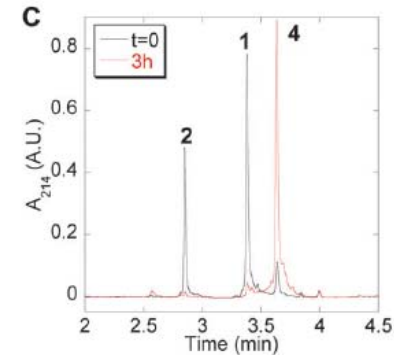
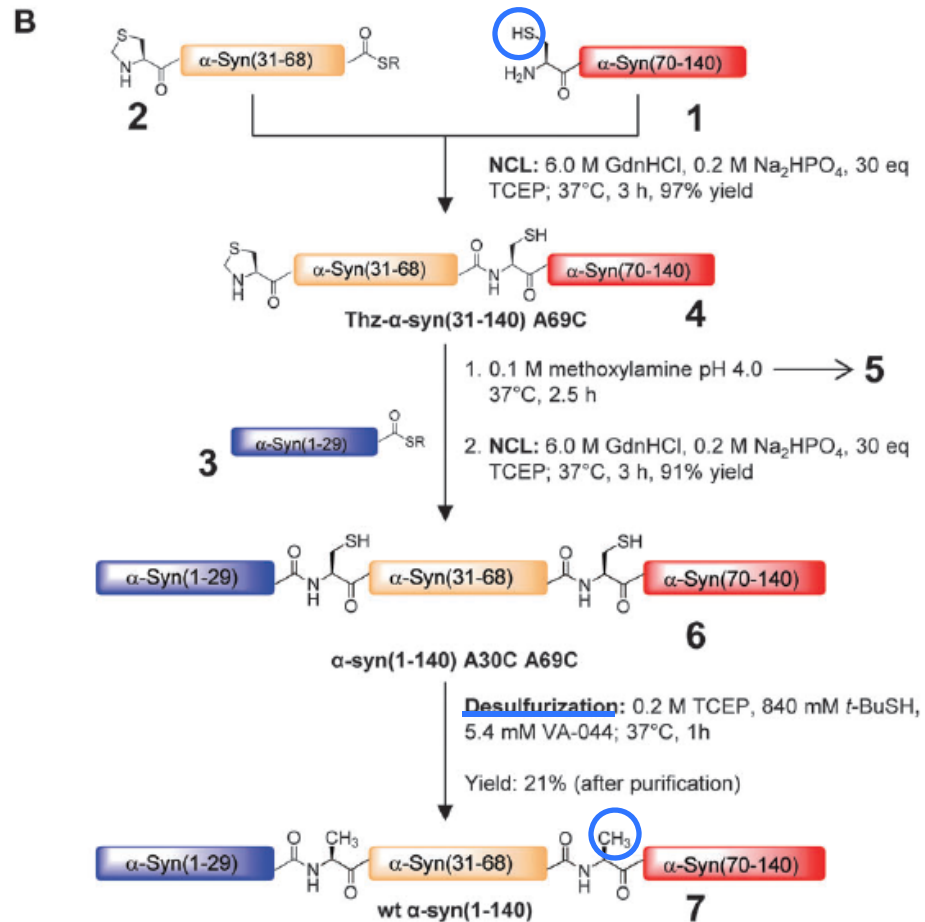
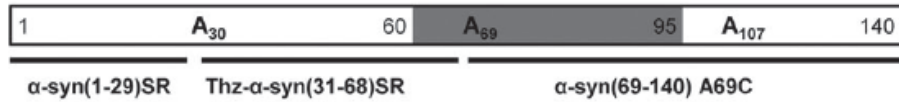


Approach: One-pot total chemical synthesis



One-pot synthesis of α -synuclein

Peptides chosen for 3-fragment strategy



Purity analyses of the peptides used for WT α -syn(1-140) total synthesis

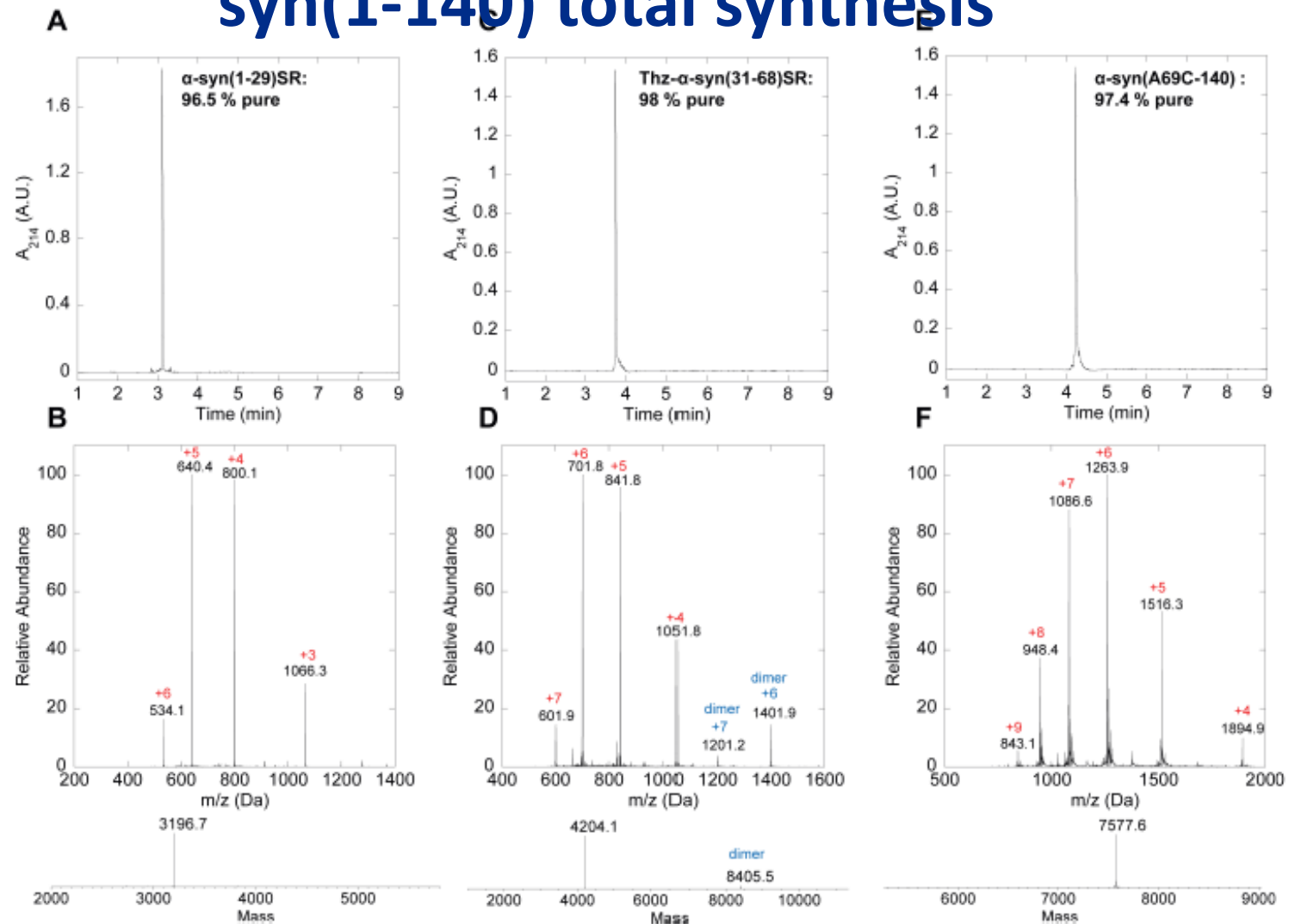


Fig. S3: Purity analyses of the peptides used for WT α -syn(1-140) total synthesis. A-B: analytical RP-UHPLC (A) and ESI-MS (B) of purified α -syn(1-29)SR. C-D: analytical RP-UHPLC (C) and ESI-MS (D) of purified Thz- α -syn(31-68)SR. E-F: analytical RP-UHPLC (E) and ESI-MS (F) of purified α -syn(A69C-140). Panels below ESI-MS spectra correspond to the mass calculated by deconvolution of the ESI-MS spectra (performed with MagTran).

One-pot synthesis of α -synuclein

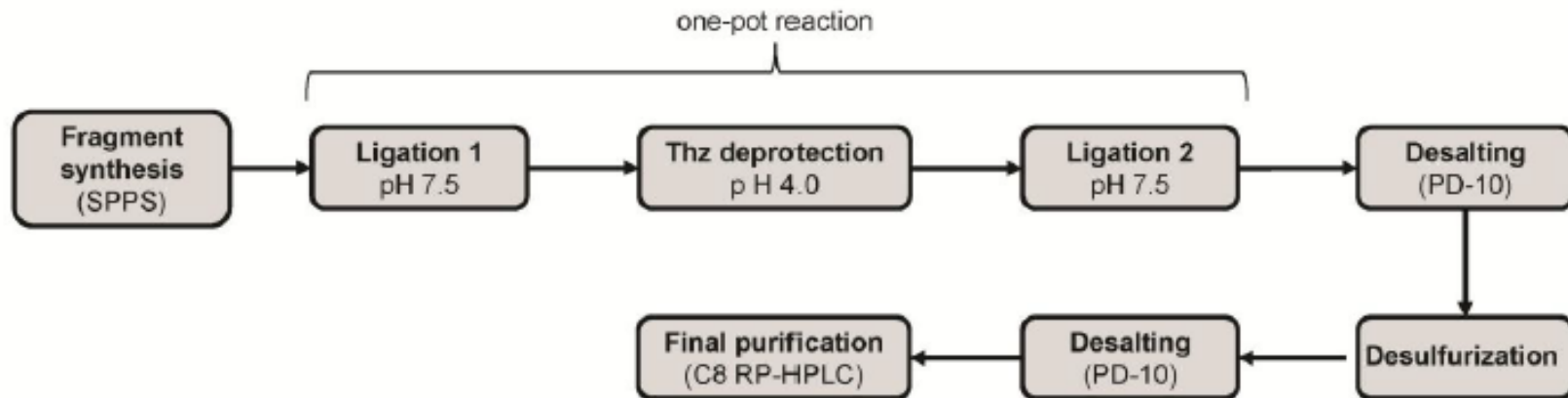


Fig. S2: Workflow diagram describing the synthesis and purification steps for the final (three-fragment) synthesis scheme.

Peptide	Strategy #	Sequence
α -syn(69-140) A69C	2	CVVTGVTAVAQ KT VEGAGSIAAATGFVKKDQLGKNEEGAPQEGILEDMPVDP DNEAYEMPSEEGYQDYEP EA-OH
Thz- α -syn(31-68)SR	1 and 2	Thz-GKTKEGVLYVGS KT KEGVVHGVATVAE KT KEQVTNVGG-SR
α -syn(1-29)SR	1 and 2	MDVFMKGLSKAKEGVVAAAEKTKQGVAEA-SR
Thz- α -syn(70-106)SR	1	Thz-VVTGVTAVAQ KT VEGAGSIAAATGFVKKDQLGKNEEG-SR
α -syn(107-140) A107C	1	CPQEGILEDMPVDPDNEAYEMPSEEGYQDYEP EA-OH

Table S1: Sequences used for the total synthesis of α -synuclein and the structure of (R) thiazolidine-4-carboxylic acid (Thz) which was used as a protecting group for N-terminal Cys. The sites in which structure-disrupting Lys-Thr pseudoproline dipeptides were introduced during the synthesis are shown in bold. The phosphorylation site at S87 is shown in red.

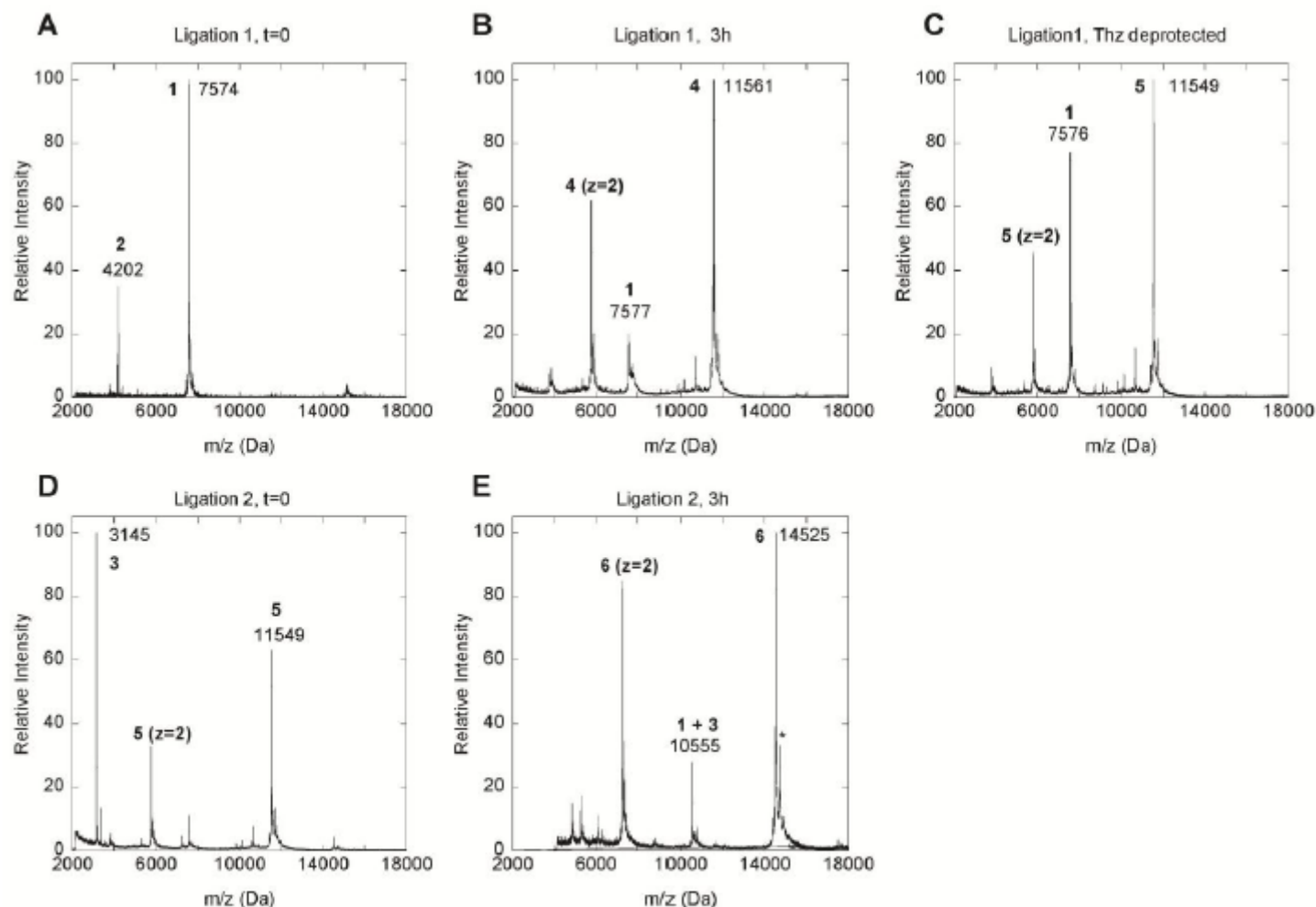
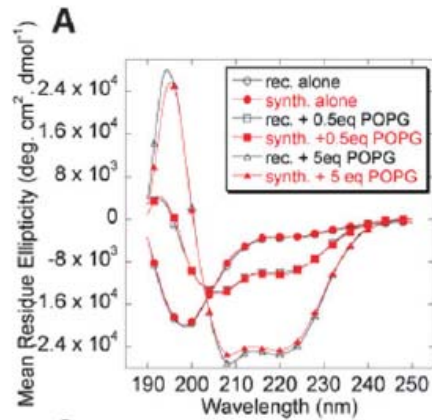


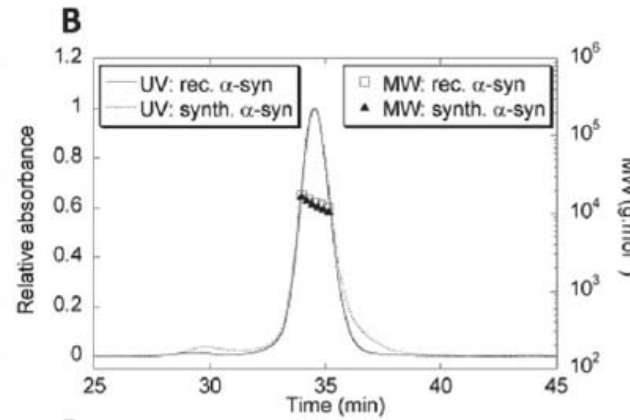
Fig. S4: MALDI-TOF MS analysis of the wt α -syn one-pot synthesis. Samples were diluted 100X in 95:5 water:acetonitrile (each with 0.1% TFA) before being mixed 1:1 with sinapinic acid and spotted on the MALDI plate. **A-B:** Spectra taken at t=0 (**A**) and t=3h (**B**) of the first ligation, between α -syn(A69C-140) and Thz- α -syn(31-140)SR. **C:** Analysis after 1 h of reaction with methoxyamine at pH 4.0, showing the -12 Da mass difference indicative of conversion of the N-terminal Thz in the intermediate 30-140 fragment to a ligation-competent N-terminal cysteine. **D-E:** Spectra taken at t=0 (**D**) and t=3h (**E**) of the first ligation, between α -syn(30-140) A30C A69C and α -syn(1-29)SR.

Biophysical and biochemical characterization of synthetic wt α -syn vs rec α -syn

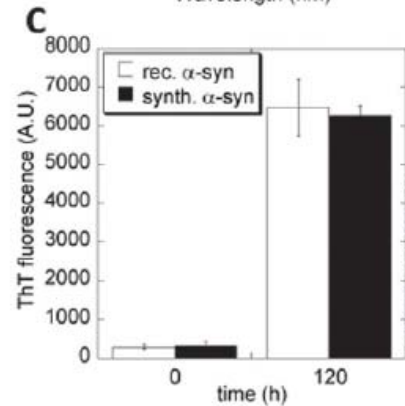
Circular dichroism spectroscopy



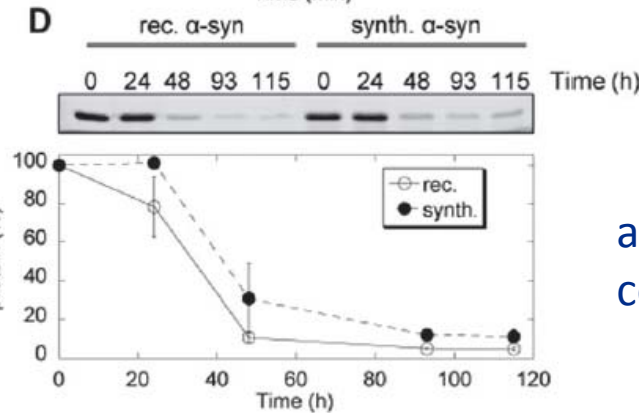
Multi-angle light scattering



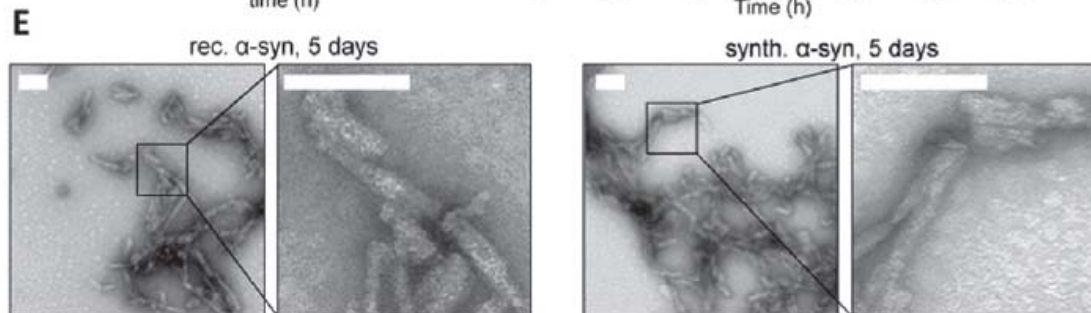
Fibrillization studies



after high-speed centrifugation



TEM



Summary

- Development of a strategy to allow one-pot total chemical synthesis of α -syn using 3 peptide fragments
- Chemical α -syn has comparable biophysical and biochemical properties than rec α -syn
- NCL and SPPS enables synthesis of PTMs in regions inaccessible by semisynthetic methods.

One-Pot Semisynthesis of Exon 1 of the Huntingtin Protein: New Tools for Elucidating the Role of Posttranslational Modifications in the Pathogenesis of Huntington's Disease**

*Annalisa Ansaloni, Zhe-Ming Wang, Jae Sun Jeong, Francesco Simone Ruggeri, Giovanni Dietler, and Hilal A. Lashuel**

Abstract: *The natural enzymes involved in regulating many of the posttranslational modifications (PTMs) within the first 17 residues (Nt17) of Huntingtin exon 1 (Httex1) remain unknown. A semisynthetic strategy that allows the site-specific introduction of PTMs within Nt17 by using expressed protein ligation (EPL) was developed. This strategy was used to produce untagged wild-type (wt) and T3-phosphorylated (pT3) Httex1 containing 23 glutamine residues (Httex1-23Q). Our studies show that pT3 significantly slows the oligomerization and fibrillization of Httex1-23Q and that Httex1 variants containing polyQ repeats below the pathogenic threshold readily aggregate and form fibrils in vitro. These findings suggest that crossing the polyQ pathogenic threshold is not essential for Httex1 aggregation. The ability to produce wt or site-specifically modified tag-free Httex1 should facilitate determining its structure and the role of N-terminal PTMs in regulating the functions of Htt in health and disease.*

Httex1 contains an N-terminal sequence of 17 amino acids (Nt17) that is highly conserved in all vertebrate species.^[4e] Several lines of evidence suggest that Nt17 plays an important role in regulating Httex1 structure,^[5] aggregation,^[6a,b,7] and subcellular localization,^[8] as well as its interactions with organelles^[4c,9] and other proteins. Nt17 also contains several residues that undergo posttranslational modifications (PTMs) including acetylation, phosphorylation, SUMOylation, and ubiquitination.^[6] The high number of PTMs in the N-terminal region of Htt suggest that these modifications might be involved in the regulation of Htt function in health and disease. The close proximity of these different modifications suggests that they might act synergistically, and the cross-talk between different PTMs might constitute an additional molecular switch for regulating the dynamics of Htt function and aggregation. Elucidating the molecular mechanisms that regulate Htt PTMs and the consequences of such modifica-

Ansaloni et al. “One-Pot Semisynthesis of Exon 1 of the Huntingtin Protein: New Tools for Elucidating the Role of Posttranslational Modifications in the Pathogenesis of Huntingtons Disease” (2014)

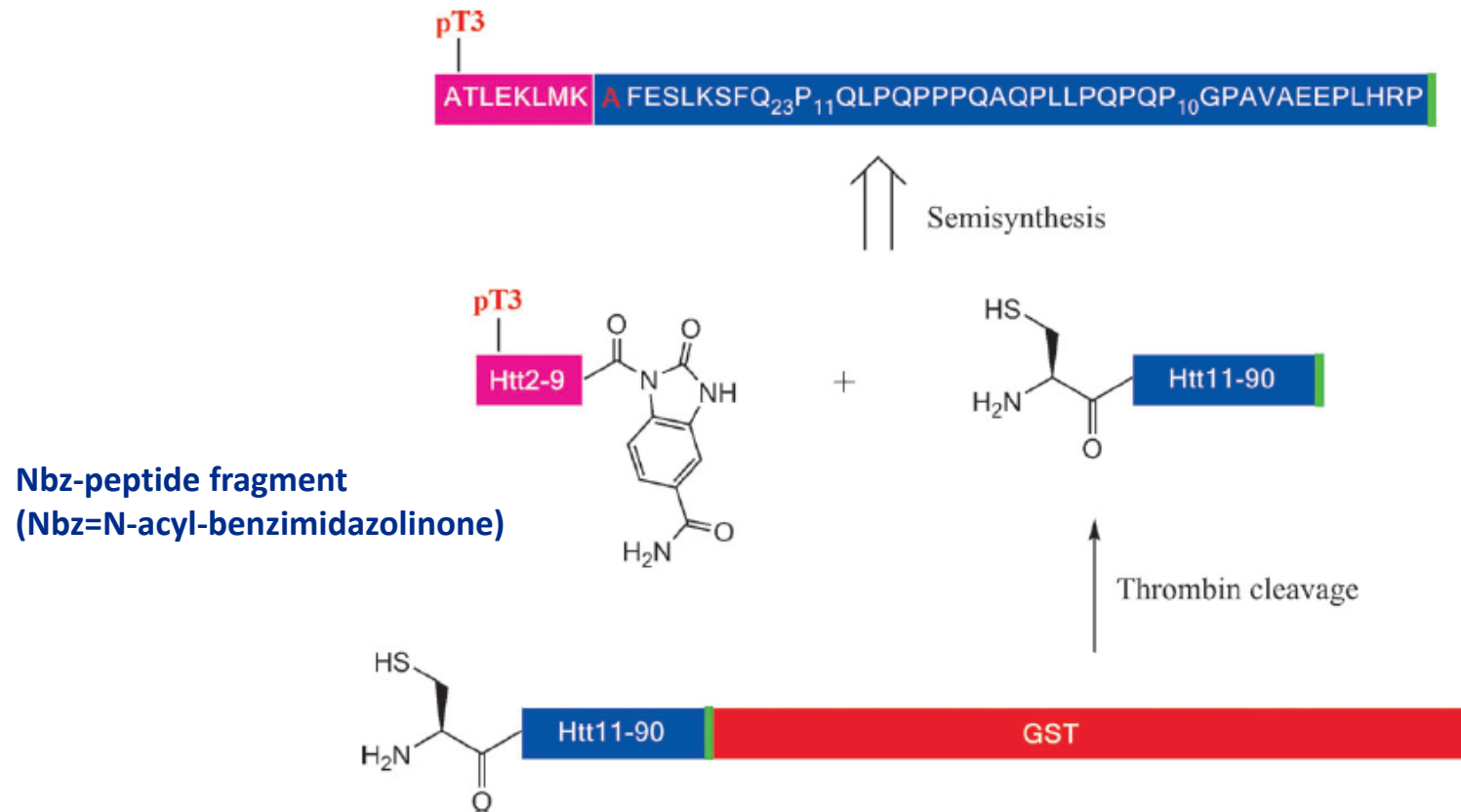
Rationale

- **Huntingtons disease (HD)** is an autosomal dominant neurodegenerative disorder caused by polyglutamine repeat expansion (**polyQ repeat>37Q**) within the first exon (ex1) of the Huntingtin (Htt) gene → enhanced Htt aggregation, increased toxicity in animal models of HD, and early disease onset in humans
- High number of PTMs in the Nt17 (highly conserved among species) of Htt (i.e. acetylation, phosphorylation, SUMOylation, and Ubiquitination) → regulation of Htt function in health and Disease

Goal

- Site-specific introduction of **pT3 in the Nt17 domain of the Httex1** (in mg amounts) and to determine the effects of such PTM on structure and aggregation

Approach: semisynthetic strategy by EPL



Scheme 1. Schematic depiction showing the amino acid sequence of exon 1 of the Huntingtin protein (Httex1) and our semisynthetic strategy for preparing wt and T3-phosphorylated Httex1-23Q. The green line represents the additional four amino acids (LVPR) remaining after thrombin cleavage and removal of the GST fusion tag at the C terminus of the recombinant fragment Htt10-90(A10C)-23Q.

Semisynthesis of wt and pT3 Httex-23Q

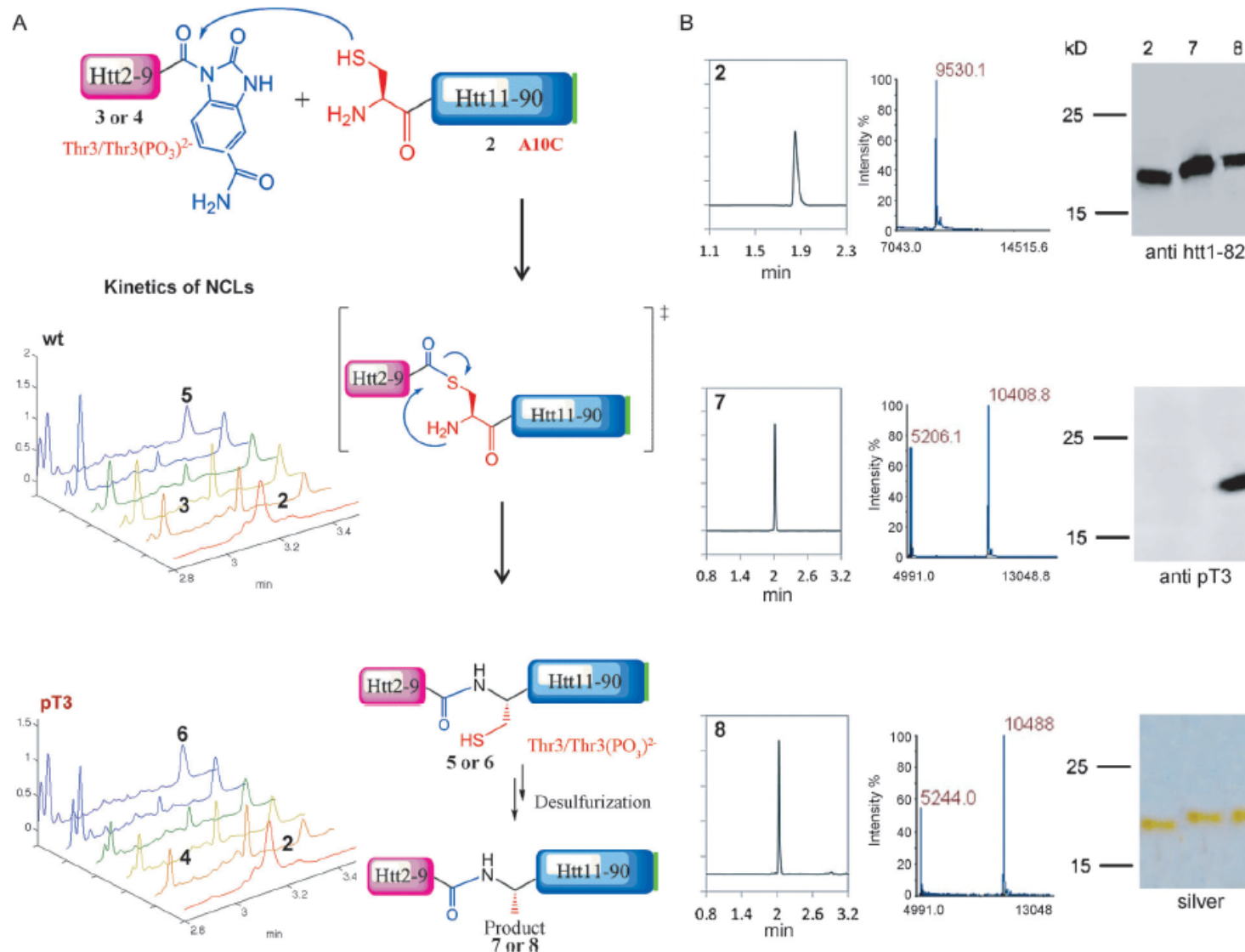
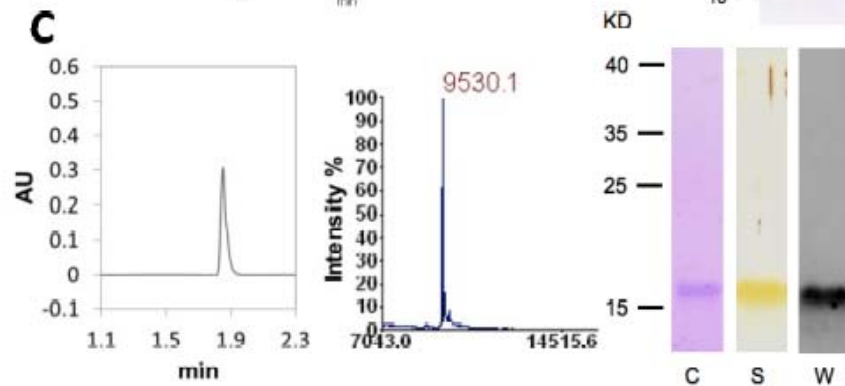
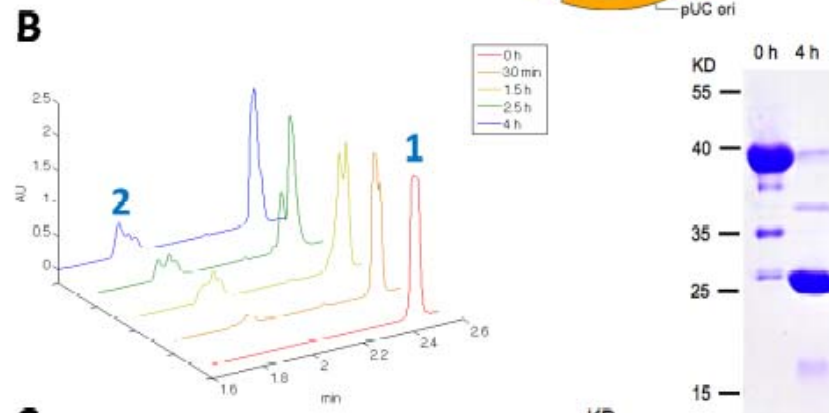
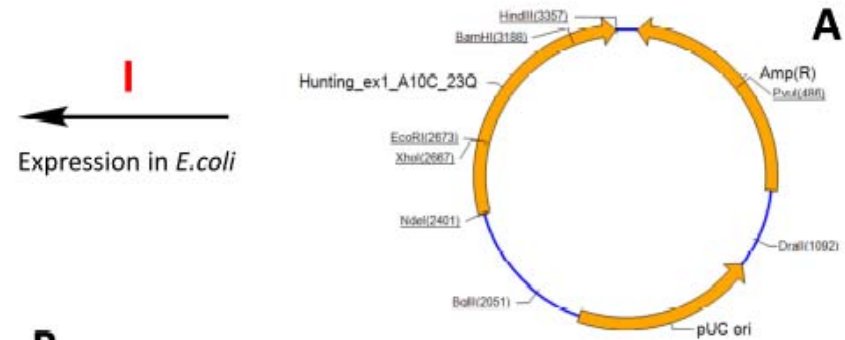
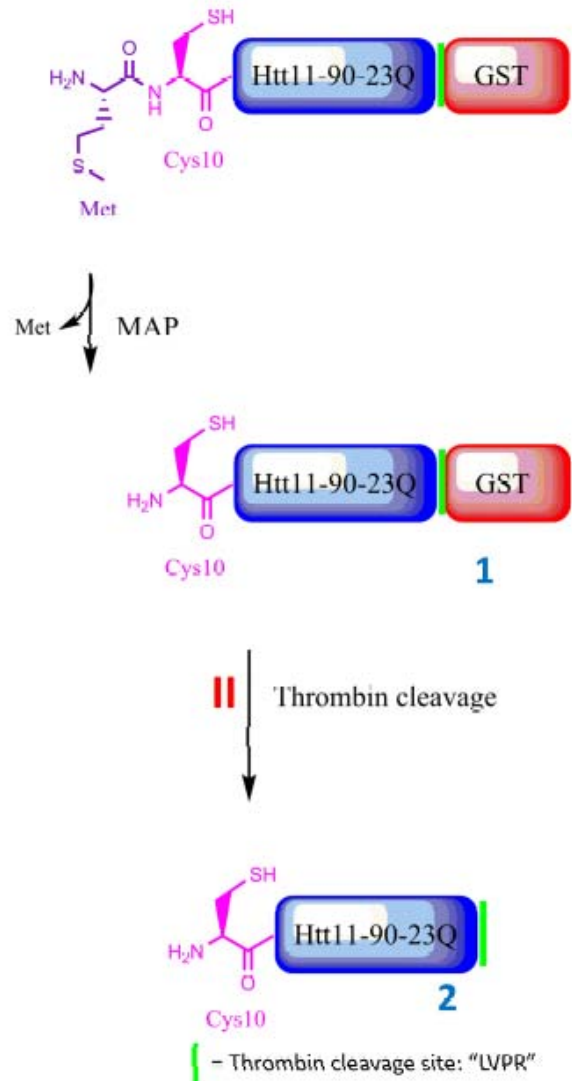


Figure 1. A) Schematic depiction of the semisynthesis of wt (7) and pT3 (8) Httex1-23Q. The native chemical ligation reactions were monitored by UPLC. B) The purity of fragment 2 and the final products 7 and 8 was assessed by UPLC, MALDI-TOF, silver staining, and western blotting with antibodies against the polyQ repeat region (anti htt1-82 MAB5492, Millipore) and a phospho-T3-specific antibody.

Preparation of recombinant Htt10-90(A10C)-23Q



Purification and analysis of Htt10-90(A10C)GST-23Q

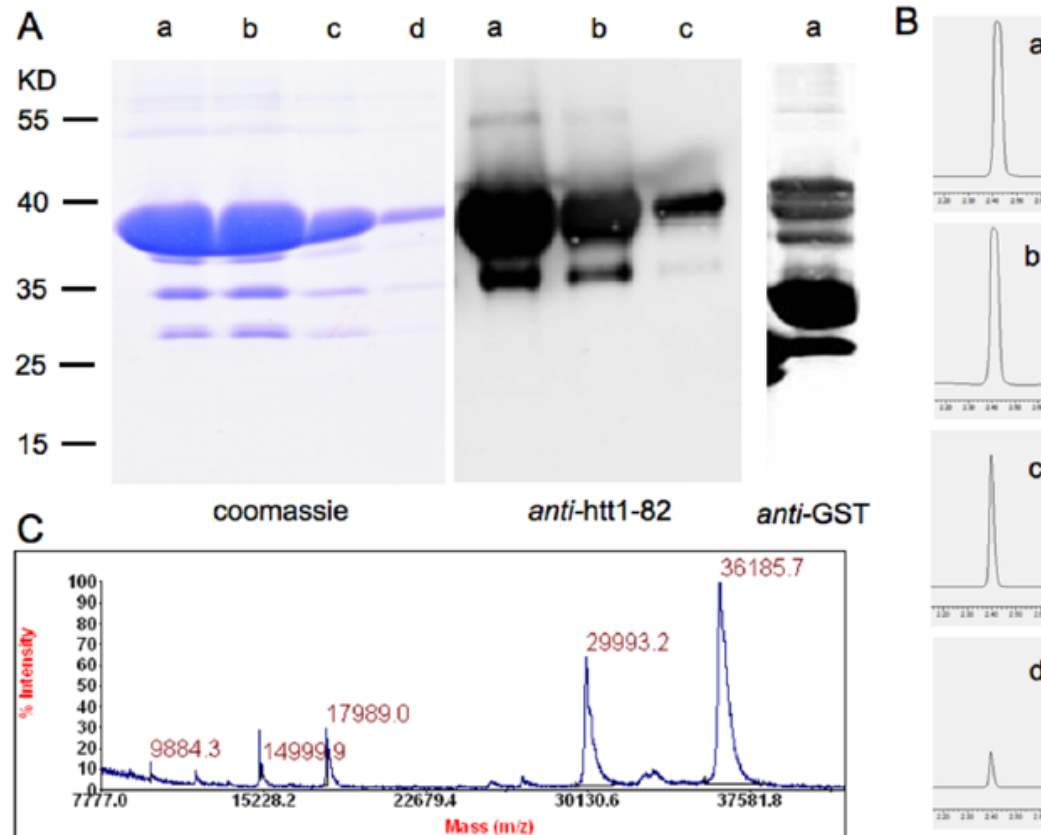


Figure S2: Glutathione affinity purification and characterization of Htt10-90(A10C)GST-23Q: A) analyses of FPLC elution fractions from the affinity column using SDS-PAGE / Coomassie staining and Western blot analysis. Our analysis of the lower bands (35 and 27 kDa) revealed that these fragments correspond to partial cleavages of the fusion protein within GST, as they were recognized by an anti-GST antibody, but not by the anti-Htt antibody. B) RP-UHPLC analysis of the FPLC elution fractions. C) Molecular mass of the GST fusion protein measured by MALDI-TOF MS (calculated: 36176.3 Da, measured 36185.7 Da). The molecular mass of Da. 29993 Da represents an impurity which is formed after partial cleavage of Htt-GST within GST.

General scheme of the cleavage and purification of Htt10-90(A10C)-23Q

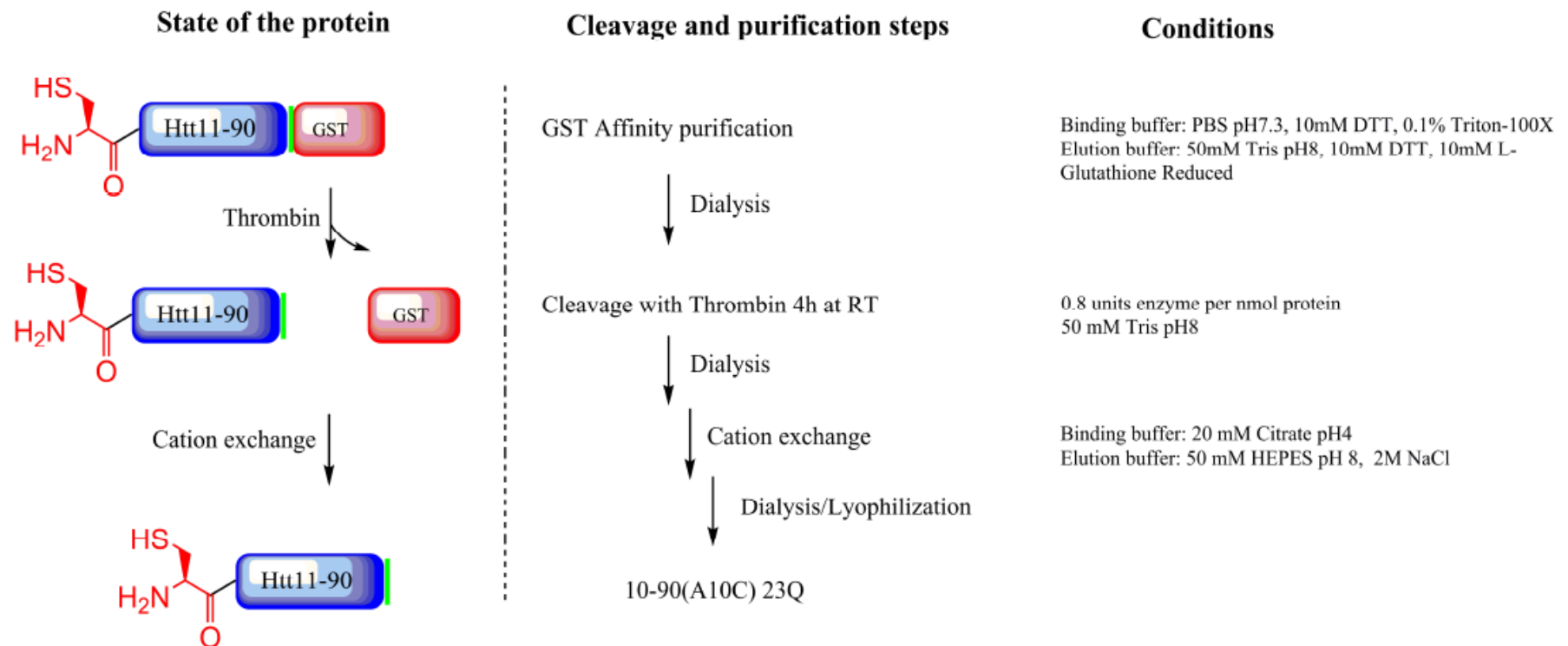


Figure S3: Schematic depiction of the primary steps involved in thrombin cleavage and protein purification to generate pure Htt10-90(A10C)-23Q.

MALDI-TOF analyses of the miscleaved products

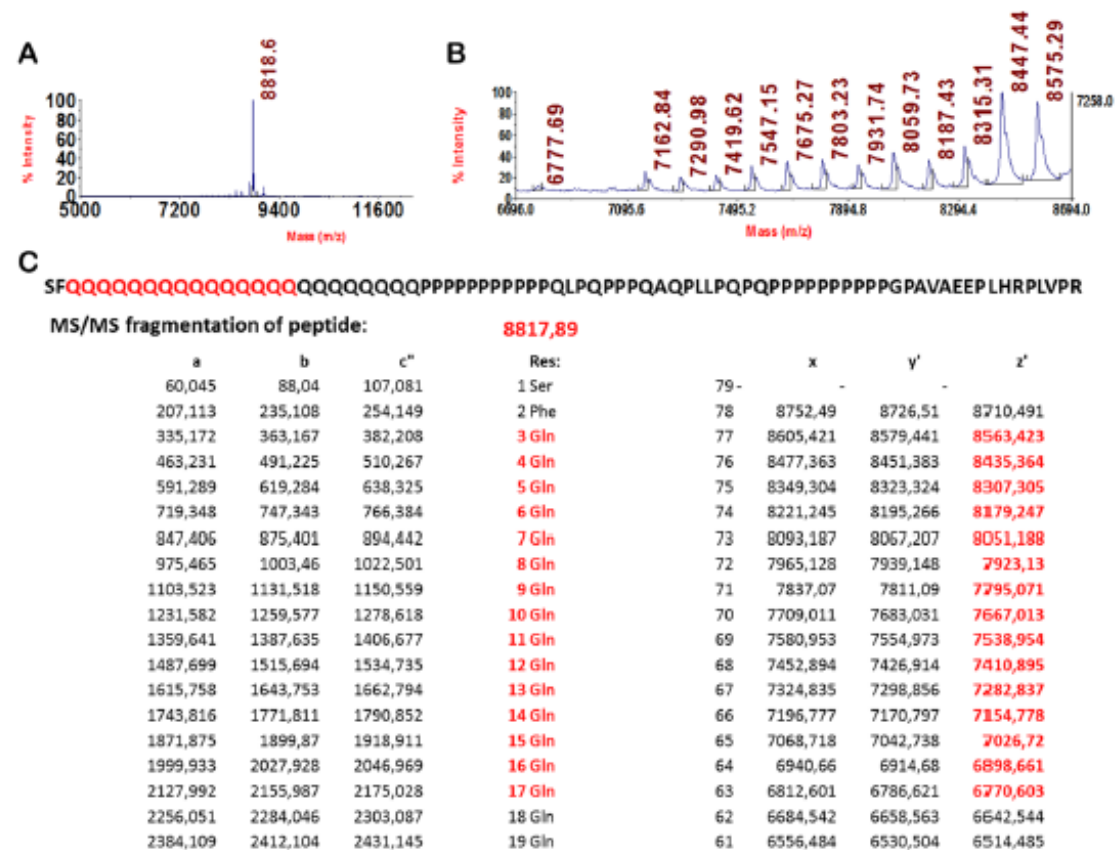
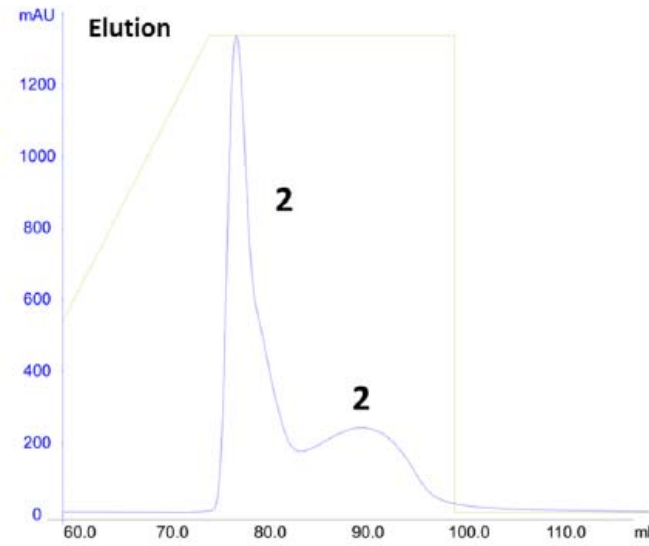
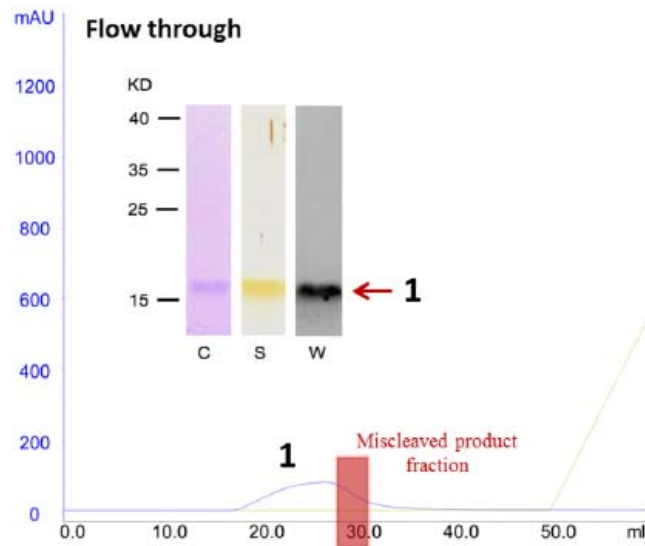
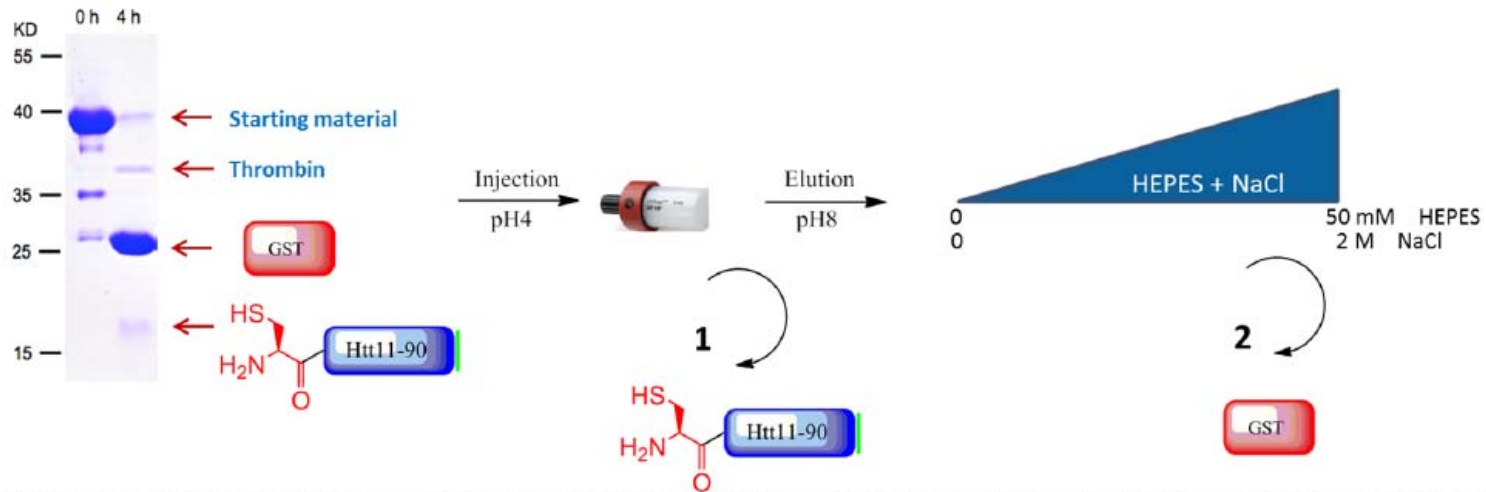


Figure S5: MS analysis of the miscleaved side-products observed during the expression of Htt10-90(A10C)GST-23Q: A) intact molecular mass, B) fragmentation of miscleaved side product under MALDI-TOF MS condition (in-source decay), C) theoretical fragmentation table of the miscleaved product, with the amino acids (central column) and their respective z^1 ions (right column) underlined in red. The z^1 ion values correspond to the masses observed with the fragmentation of the side product using MALDI-TOF MS B). These values are slightly shifted compared with intact mass values, reflecting error in the linear operating mode. Our results suggest that thrombin cleaves between K15 and S16 within the Nt17, which is consistent with the resulting 8817 Da mass corresponding to the expected N-terminal truncated Httex1-23Q at S16. Interestingly, a previous study by *Vu et al.* demonstrated that thrombin cleaves between arginine (R41) and serine (S42) residues within the N-terminus of the thrombin receptor.^[1]

Cation-exchange purification of Htt10-90(A10C)-23Q



Circular dichroism spectroscopy

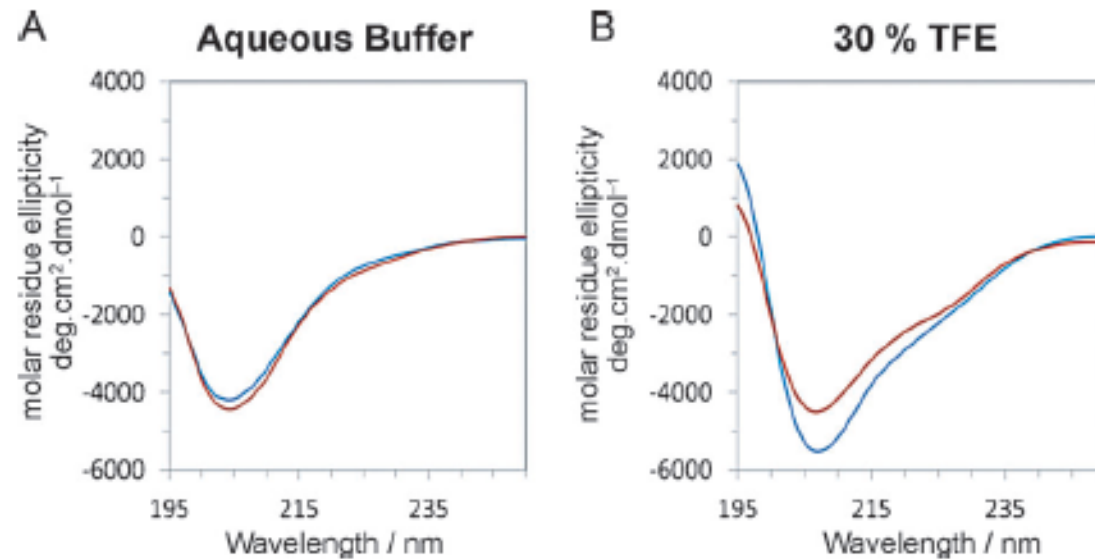


Figure 2. Far-UV CD of wt (blue) and pT3 (red) Httex1-23Q in aqueous buffer (A) and in the presence of 30% of TFE (B). Samples in aqueous buffer were used for aggregation studies to a final concentration of 50 μM , while a concentration of 30 μM was used for the TFE treatments.

TFE titration studies

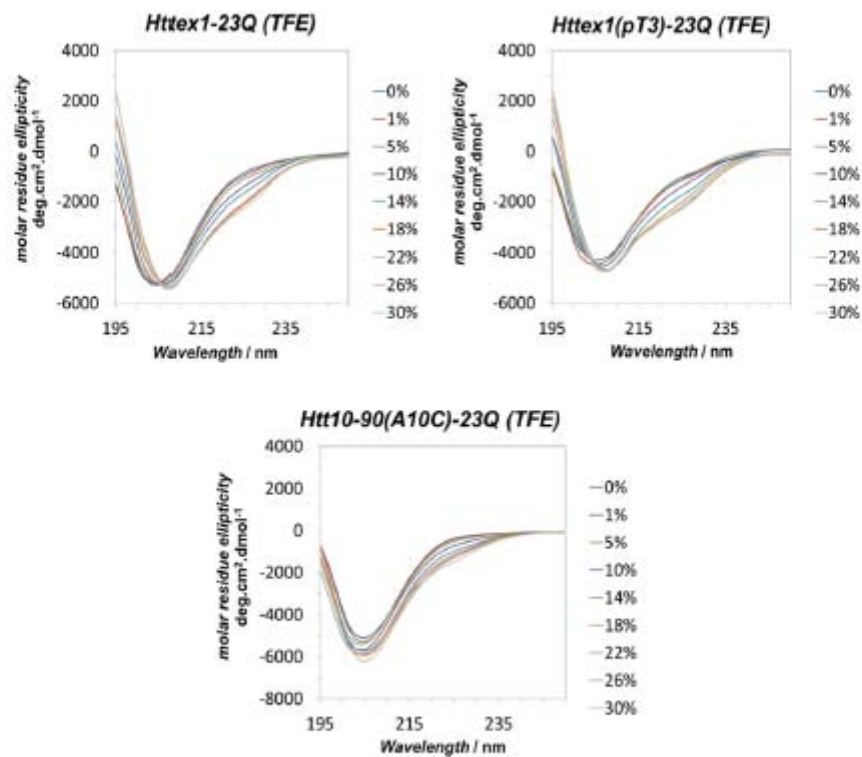


Figure S6: Far-UV CD analysis of Httex1-23Q and its T3-phosphorylated (Httex1(pT3)-23Q) variant in the presence of increasing amounts of TFE (top panels). A TFE titration of recombinant fragment lacking Nt17, Htt10-90(A10C)-23Q, is also shown in the bottom panel. 30 μ M protein samples were prepared in 10 mM Tris, 75 mM NaCl, pH 7.4.

Membrane binding studies

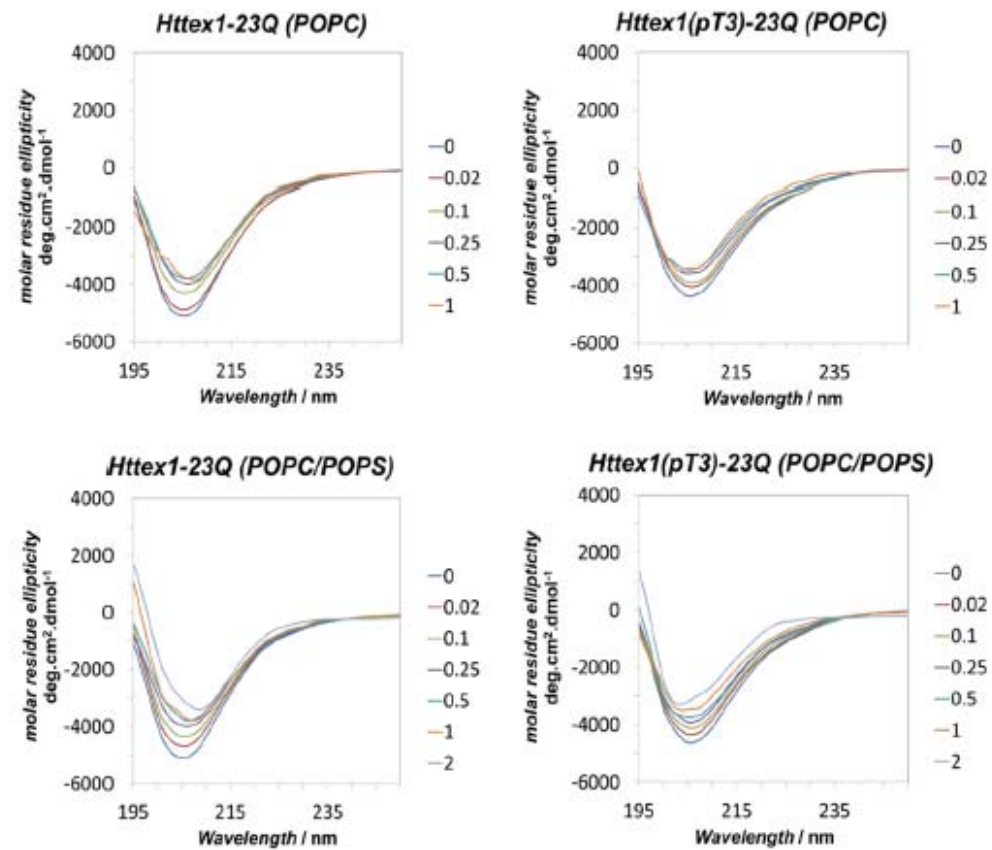
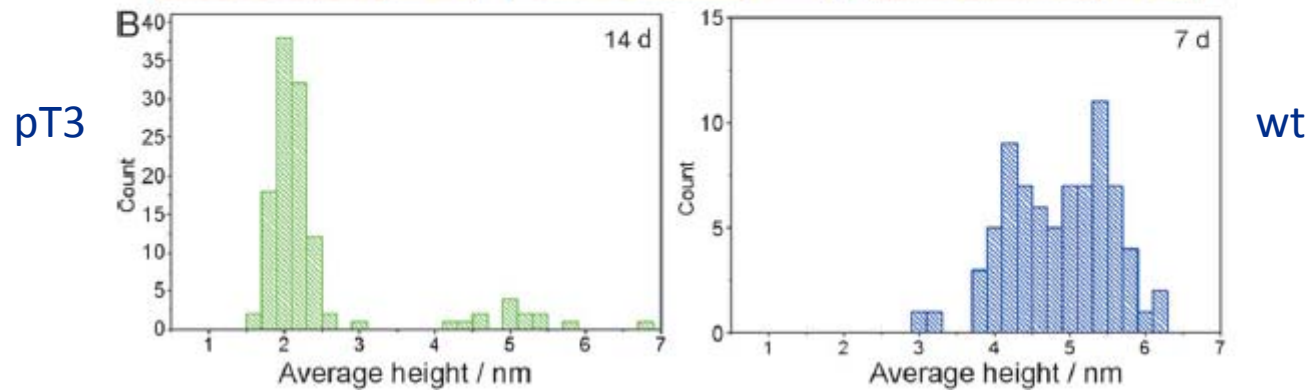
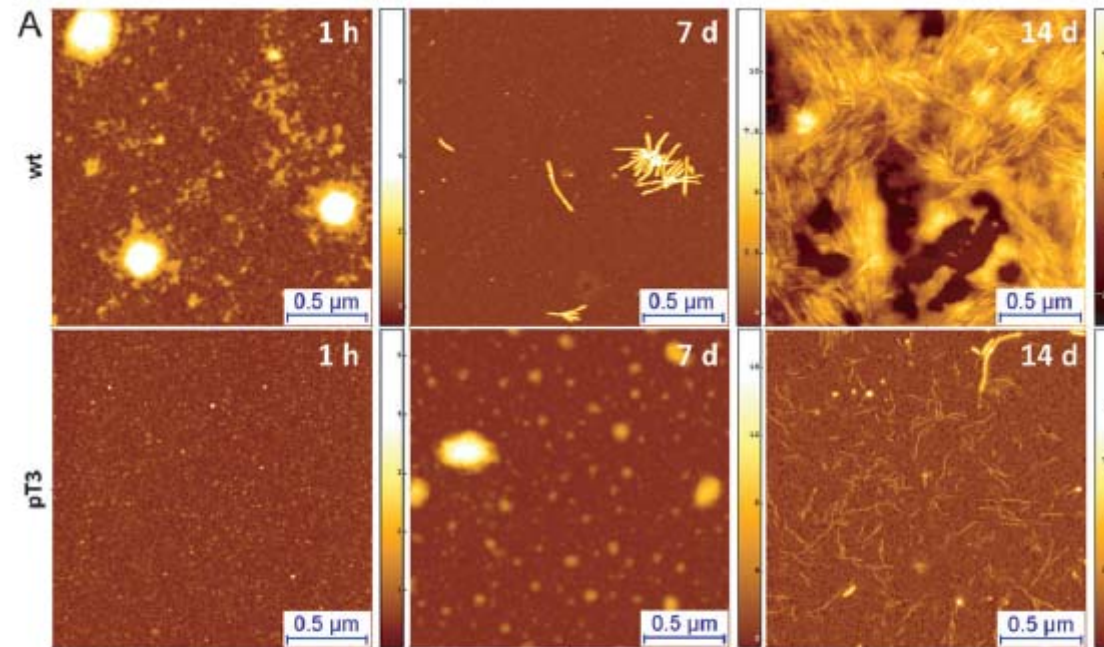


Figure S7: CD spectroscopy of WT and pT3 Httex1-23Q incubated with POPC (top panels) and POPC/POPS lipids (3:1 ratio) (bottom panels). 30 μ M protein samples were prepared in 10 mM Tris, 75 mM NaCl, pH 7.4.

AFM analyses



AFM analyses

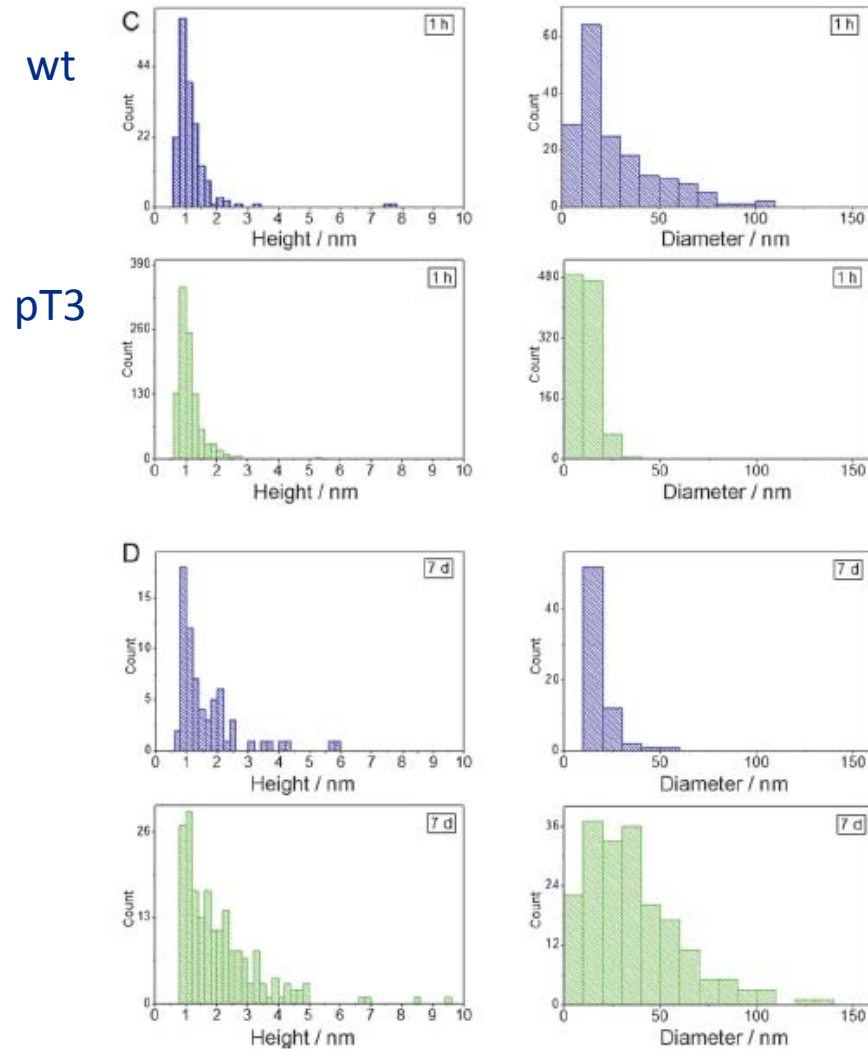


Figure 3. A) AFM images of wt and pT3 Httex1-23Q aggregates at 1 h, 7 days, and 14 days. Samples were prepared at a concentration of 50 μM in 10 mM Tris, 75 mM NaCl, pH 7.4 and statically incubated at 37°C. B) Average height distribution of wt (blue) and pT3 (green) Httex1-23Q aggregates after 7 and 14 days, respectively. The plotted values correspond to the average height along the length of each fibril measured. Height and diameter distribution of wt (blue) and pT3 (green) Httex1-23Q oligomers formed after 1 h (C) and 7 days (D).

Aggregation of WT and pT3 Httex1-23Q monitored by TEM

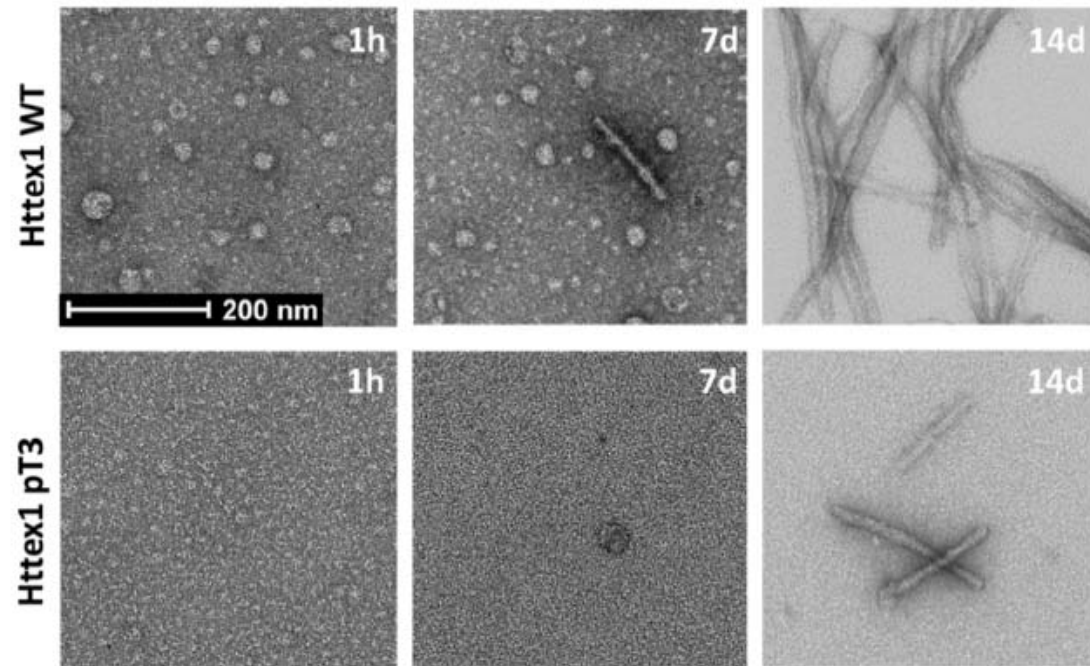


Figure S9: TEM images of WT and pT3 Httex1-23Q aggregates at 1h, 7 and 14 days. The samples were prepared at a concentration of 50 μ M in 10 mM Tris, 75 mM NaCl pH 7.4, filtered through a 100 kDa cutoff filters to remove any pre-formed aggregates and statically incubated at 37 $^{\circ}$ C.

Aggregation of WT and pT3 Httex1-23Q monitored by Analytical SEC

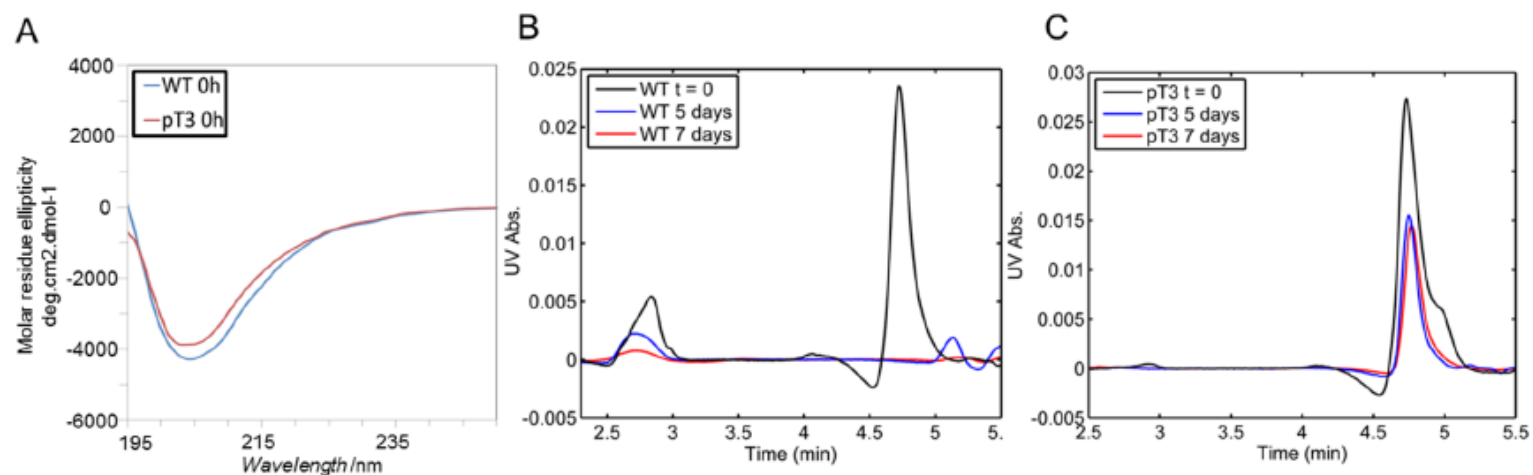


Figure S10: The extent of aggregation by WT and pT3 Httex1-23Q was also assessed by size-exclusion chromatography (SEC). A) CD spectra of WT and pT3 Httex1-23Q at t=0. The loss of protein and appearance of an oligomer peak was assessed as a function of time. Analytical SEC was used to follow Httex1-23Q WT B) and pT3 C) aggregation at time 0, 5 and 7 days (samples concentration: 40 μ M). Before each SEC injection, the samples were spun down at 14000 rpm for 10 min at 4 $^{\circ}$ C in order to remove any insoluble aggregates. Under the conditions used in this experiment, WT Httex1-23Q formed stable oligomers almost immediately as shown by the oligomer peak at 2.7 min. After 5 days, the peak corresponding to monomeric Httex1-23Q disappears. The area of the oligomer peak also decreased with time, consistently with the rapid conversion of the protein into amyloid fibrils. In the case of pT3 Httex1-23Q, only a minor oligomer peak was observed at t = 0 and significant amount of monomeric protein was still present event after 7 days of incubation, consistently with the slow aggregation of pT3 Httex1-23Q observed by AFM and TEM.

Preparation and purification of recombinant Htt18-90(Q18C)-42Q

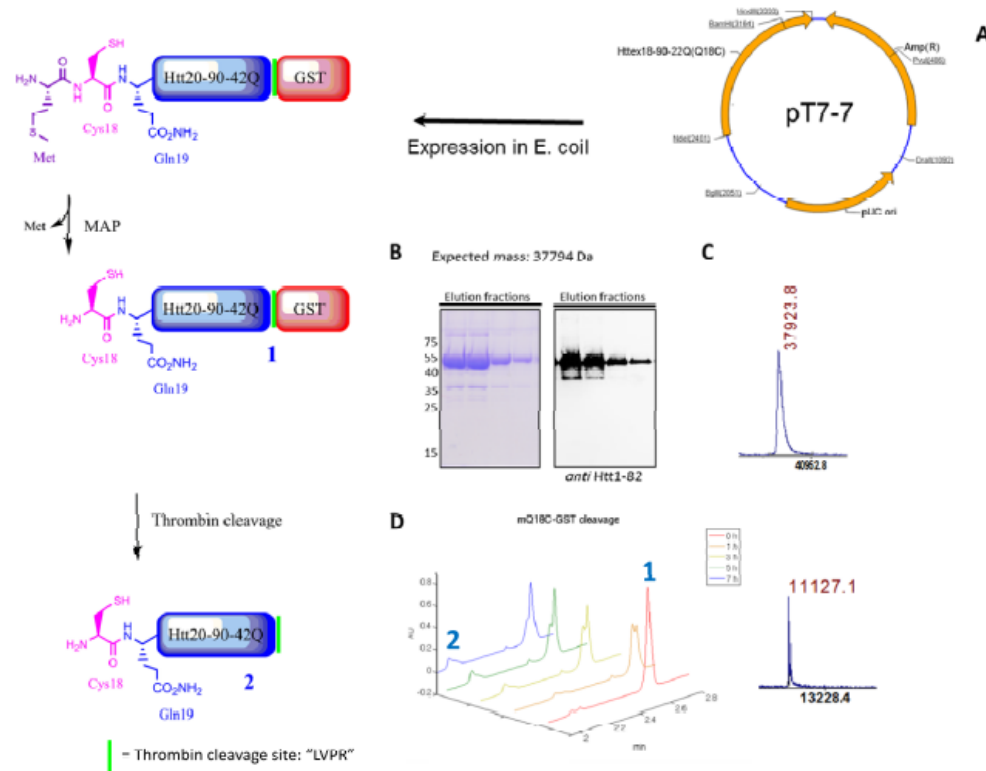


Figure S11: Preparation, purification and characterization of recombinant Htt18-90(Q18C)-42Q **2** from recombinantly expressed Htt18-90(Q18C)GST-42Q. **A**) The Htt18-90(Q18C)GST 42Q protein expresses very well in *E. coli* and showed significantly less impurities compared to Htt10-90(A10C)GST-23Q. **B-C**) SDS-PAGE and MALDI-TOF MS analyses of Htt18-90(Q18C)GST-42Q after affinity purification (calculated mass: 37794 Da; measured mass: 37923.8 Da); **D**) The kinetic of thrombin cleavage was monitored by RP-UHPLC and SDS-PAGE. After 1 h the cleavage reached almost 50%; the major product now is represented by two closely-eluting peaks which correspond to cleaved GST (on the right) and to full length protein (on the left). A small peak starts to appear at the left of the graph corresponding to the cleaved product **2**. The cleavage arrived almost at completion (90%) after 7 h of incubation at room temperature. Since the cleaved protein couldn't be detected by SDS-PAGE, the identity and purity of the protein were assessed by MALDI-TOF MS (calculated: 11121 Da, measured: 11127.1 Da).

Semisynthesis of Httex1(Q18C)-42Q

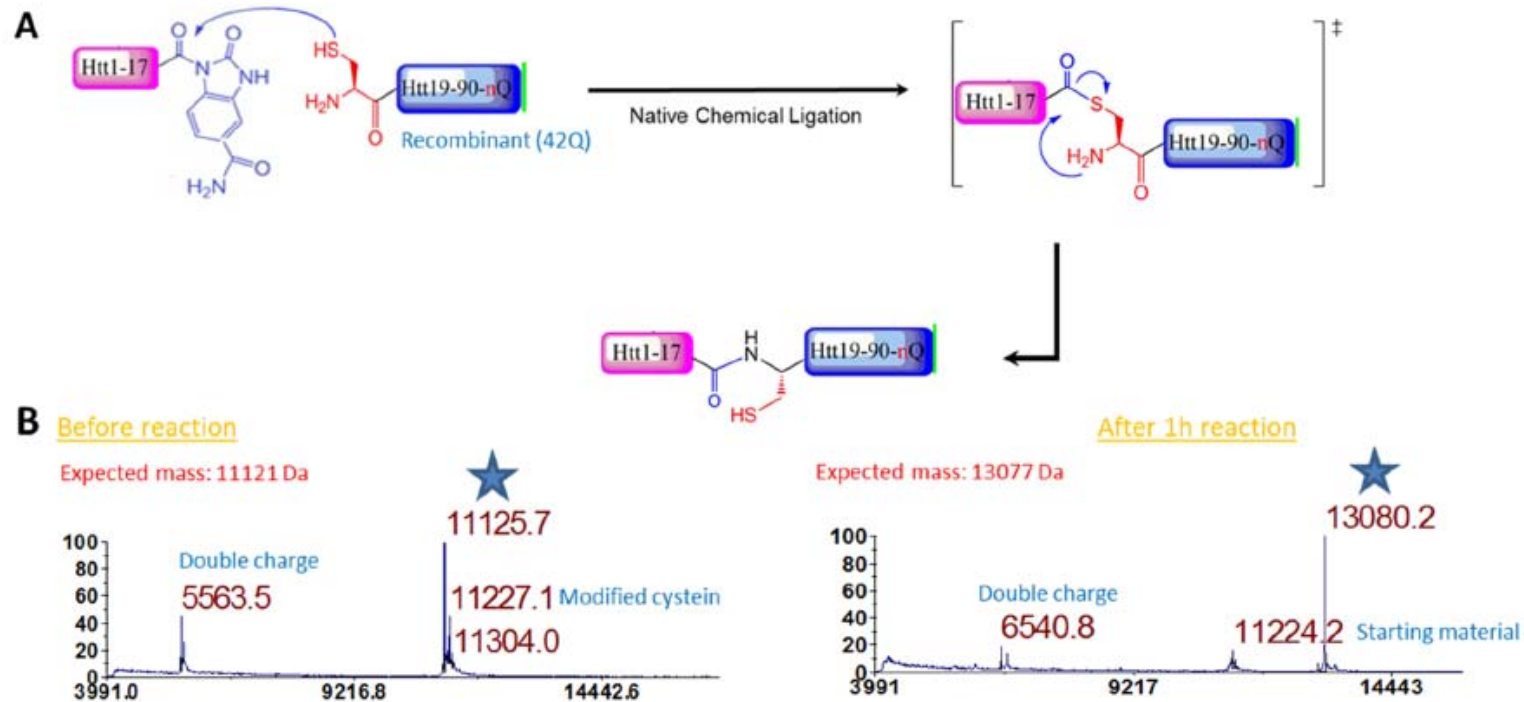


Figure S12: A) Schematic depiction of the semisynthesis of Httex1(Q18C)-42Q via native chemical ligation. B) MALDI-TOF MS analysis showed the formation of the desired product (calculated: 13077 Da, measured: 13080.2 Da). Unfortunately the yield of the final product was very low and did not allow us to proceed with desulfurization and purification of the final product by RP-HPLC.

Analysis of Htt18-90(Q18C)GST-42Q during GST purification and thrombin cleavage

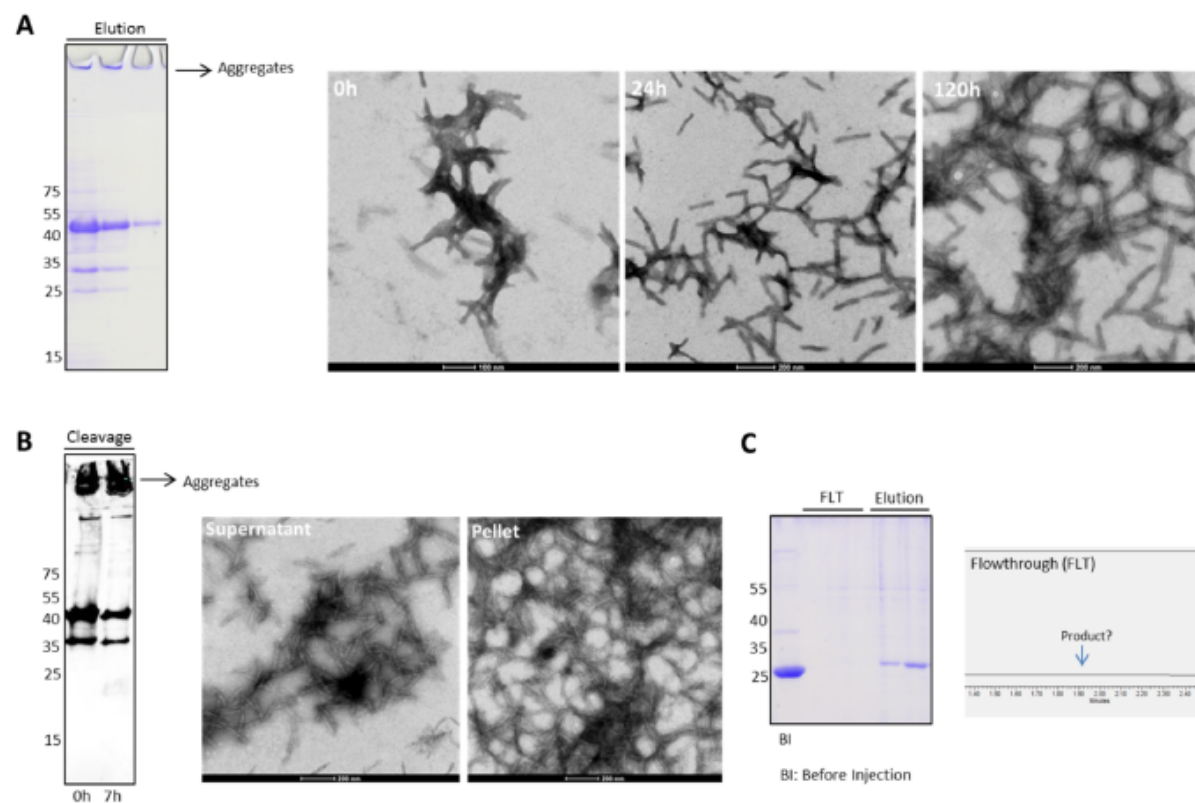
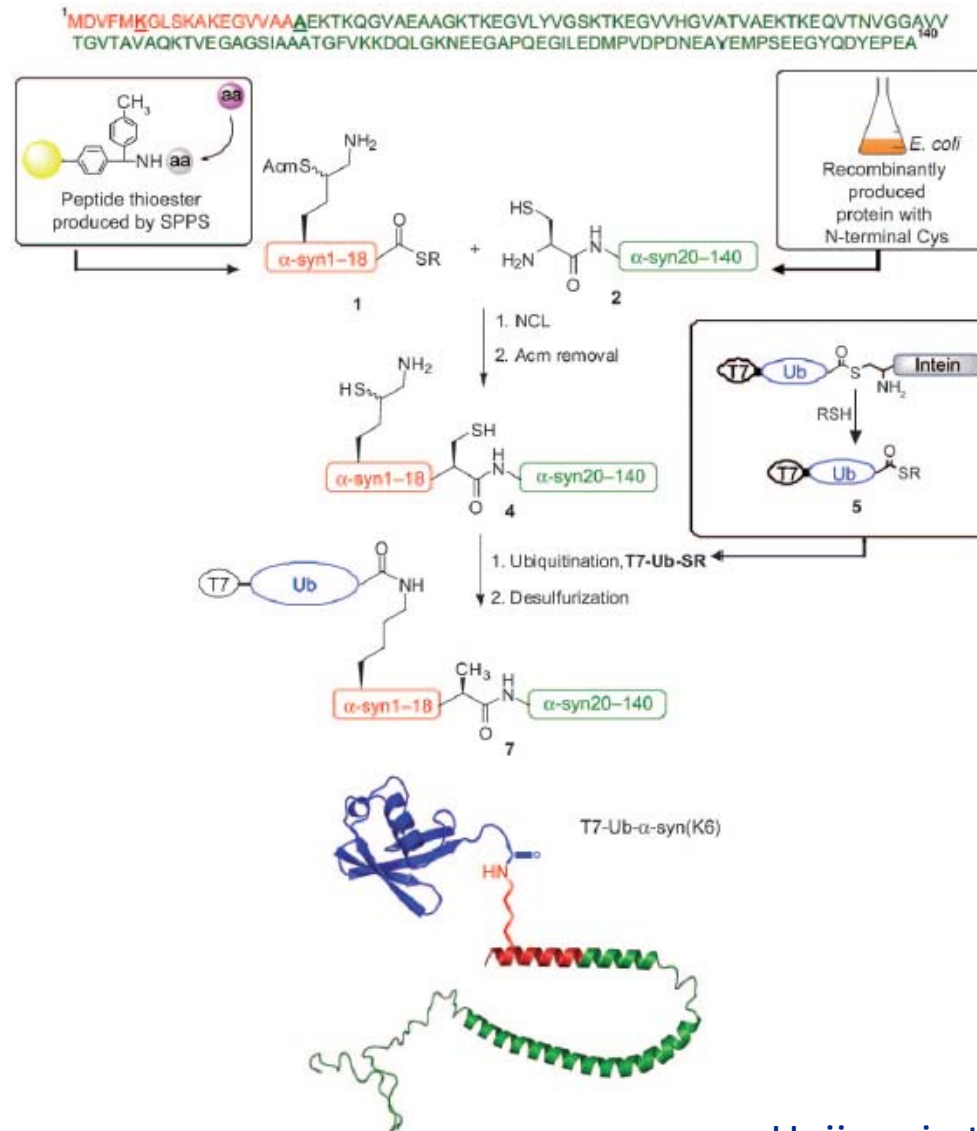


Figure S13: The main reason for the low yield of semisynthetic Httex1(Q18C)-42Q is the high aggregation propensity of the C-terminal recombinant fragment Htt18-90(Q18C)GST-42Q prior to, during and after removal of GST. In addition, further aggregation was observed during the purifications steps, thus resulting in dramatic reduction in the amount of Htt18-90(Q18C)-42Q protein that can be obtained and used in the native ligation reaction. A) SDS-PAGE gel (Coomassie staining) after Htt18-90(Q18C)GST-42Q GST purification. The stacking gel already shows the accumulation of insoluble material which was validated by TEM analyses at different time points. B) GST cleavage monitored by SDS-PAGE / WB also reveals the formation of more aggregates (in the stacking gel) during the cleavage reaction. No band corresponding to the cleaved product Htt18-90(Q18C)-42Q is detectable under these conditions. TEM pictures of supernatant and pellet of the cleavage solution (after 7h reaction) are shown. C) SDS-PAGE gel and RP-UHPLC analyses of anion exchange purification of Htt18-90(Q18C)-42Q.

Protein ubiquitination example



Scheme 1. Semisynthetic strategy for the ubiquitination of $\alpha\text{-syn}$ at K6.

Hejjaoui et al. *Angewandte* (2011)

Summary

- Httex1 with polyQ repeats below the pathogenic threshold (23Q) leads to aggregates and spherical and short fibril-like structures in contrast to previous findings (in fusion proteins in which peptides or proteins were fused to the N and/or C termini of Httex1, the effect of the cleaved protein tags on the aggregation process was not assessed or accounted for).
- The general semisynthetic strategy described here can be easily extended to generate homogeneously phosphorylated, acetylated, or ubiquitylated forms of Httex1-23Q in mg quantities as for α -syn (add refs)

PTMs intro

- Chemical modifications that playing a key role in functional proteomics, because they regulate activity, localization and interaction with other cellular molecules such as proteins, nucleic acids, lipids, and cofactors → normal cell biology and pathogenesis
- Post-translational modification can occur at any step in the "life cycle" of a protein. For example, many proteins are modified shortly after translation is completed to mediate proper protein folding or stability or to direct the nascent protein to distinct cellular compartments (e.g., nucleus, membrane). Other modifications occur after folding and localization are completed to activate or inactivate catalytic activity or to otherwise influence the biological activity of the protein. Proteins are also covalently linked to tags that target a protein for degradation. Besides single modifications, proteins are often modified through a combination of post-translational cleavage and the addition of functional groups through a step-wise mechanism of protein maturation or activation.
- Protein PTMs can also be reversible depending on the nature of the modification. For example, kinases phosphorylate proteins at specific amino acid side chains, which is a common method of catalytic activation or inactivation. Conversely, phosphatases hydrolyze the phosphate group to remove it from the protein and reverse the biological activity. Proteolytic cleavage of peptide bonds is a thermodynamically favorable reaction and therefore permanently removes peptide sequences or regulatory domains
- Protein post-translational modifications (PTMs) increases the functional diversity of the proteome by the covalent addition of functional groups or proteins, proteolytic cleavage of regulatory subunits or degradation of entire proteins.
- **Regulate cellular activity**

PTMs occur at distinct amino acid side chains or peptide linkages and are most often mediated by enzymatic activity. Indeed, it is estimated that 5% of the proteome comprises enzymes that perform more than 200 types of post-translational modifications (4). These enzymes include kinases, phosphatases, transferases and ligases, which add or remove functional groups, proteins, lipids or sugars to or from amino acid side chains, and proteases, which cleave peptide bonds to remove specific sequences or regulatory subunits. Many proteins can also modify themselves using autocatalytic domains, such as autokinase and autoprotolytic domains.