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The efficient synthesis and purification of amyloid- β (1–42) using an oligoethylene glycol-containing photocleavable lysine tag†

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A short, monodisperse oligoethylene glycol-containing photocleavable lysine tag was developed to facilitate the efficient purification of hydrophobic and fibril-forming peptides. This new tag was used to prepare a modified A β ₄₂ peptide with increased solubility and decreased propensity to aggregate in aqueous media. The solubilising tag was readily removed by irradiation with UV light and permitted the preparation and isolation of A β ₄₂ in high purity and yield.

Alzheimer's disease is the most common form of neurodegenerative dementia. Hallmarks of the disease include extracellular plaques and intracellular tau-enriched neurofibrillary tangles in the brain.^{1,2} The plaques contain aggregated forms of the amyloid- β (A β) polypeptide ranging in length from 39–43 amino acid residues, formed by proteolytic cleavage of amyloid precursor protein. The two main peptides produced are 40 (A β ₄₀) and 42 (A β ₄₂) amino acid residues in length. The longer A β ₄₂ has a greater propensity for aggregation *in vivo* and is often considered the more toxic.³ Continued efforts to better understand the structure of A β ₄₂ in fibrils and plaques,^{4–6} the toxicity of A β ₄₂ oligomers and assessing potential therapeutic molecules⁷ all require ready access to pure synthetic A β ₄₂.

Solid-phase peptide synthesis (Fmoc SPPS, Fmoc = 9-fluorenylmethoxycarbonyl) can be used to prepare A β ₄₂.⁸ Unfortunately, isolation of A β ₄₂ using conventional chromatographic techniques is extremely challenging. The propensity of A β ₄₂ to aggregate results in broad and asymmetrical peaks when attempting to purify the crude peptide by high performance liquid chromatography (HPLC). This makes fractionation of the target peptide from closely-eluting peptidic impurities difficult.⁹

Several strategies have been employed to improve chromatographic peak resolution and solubility of 'difficult peptides',⁹ including the use of alkaline buffers to alter the net charge of the peptide¹⁰ and high temperature HPLC.¹¹ Temporary chemical modification of hydrophobic peptides with iso-acyl dipeptides¹² and polycationic orthogonally cleavable tags also possess effective solubilising properties in acidic media, which is typically used during RP-HPLC purifications.^{13–17} The addition of PEGylated or OEGylated (PEG = polyethylene glycol, OEG = oligoethylene glycol) functional groups can increase aqueous solubility over a wide pH range without increasing ionic charge and both are simple to incorporate during Fmoc SPPS.¹⁸ It has been shown that *ortho*-nitrobenzyl (*o*Nb) based photocleavable protecting groups are compatible with SPPS.^{19–21} In this manuscript we describe a new amine-reactive, photolabile OEG tag that enhances the solubility of A β ₄₂ and enables its more efficient preparation.

Amine-reactive poly-disperse PEG reagents have been employed to switch on the function of proteins with light, but a small monodisperse OEG-containing substrate was considered more appropriate in this instance since a poly-disperse product would be difficult to isolate.²² It was thought that this approach may also allow some control of fibril formation.²³

An *o*Nb-based substrate containing a *tert*-butyloxycarbonyl (Boc) protected amino-OEG₃ chain (*o*Nb-OEG₃) was synthesised by adapting a method of Rossi and co-workers (Scheme 1).²⁴ Incorporation of an alkyne functional group at the benzylic position of the *o*Nb derivative *via* a Grignard reaction gave **1**.¹⁷ Copper(i)-catalyzed azide-alkyne cyclo-addition (CuAAC) between **1** and **2** gave **3** in a yield of 72%, that was then reacted with *para*-nitrophenyl chloroformate to produce **4** in 45% yield.

Although it would be simplest to incorporate the hydrophilic tag at the N-terminus, we chose to functionalise Lys₂₈ due to its close proximity to the hydrophobic C-terminus that is essential for aggregation. Residues 28–42 of A β ₄₂ were assembled on the solid support by microwave-assisted Fmoc SPPS, with monomethoxytrityl (Mmt) protection at Lys₂₈ (Scheme 2). The ϵ -amino group was liberated after multiple treatments with dilute

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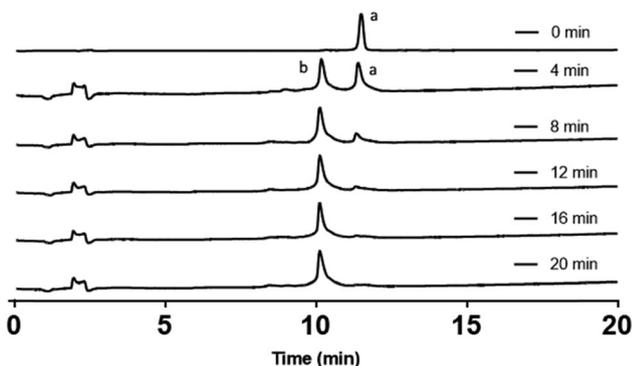


Fig. 2 Analytical RP-HPLC traces of a time course for the photolysis reaction. Peak (a) α Nb-OEG-A β_{42} ; peak (b) A β_{42} obtained after photolysis of α Nb-OEG-A β_{42} .

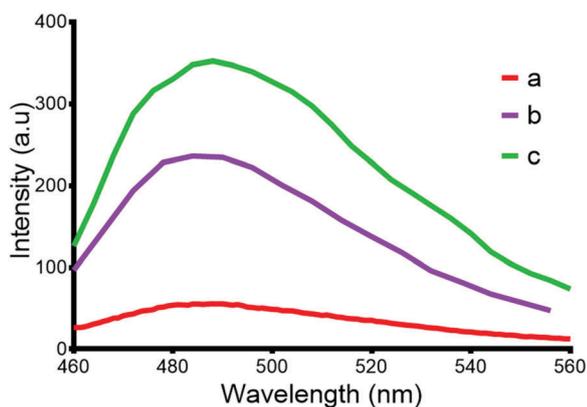


Fig. 3 ThT fluorescence curves after incubation for 48 hours at 37 °C in PBS ($\lambda_{\text{ex}} = 444 \text{ nm}$). (a) ThT only in PBS; (b) α Nb-OEG-A β_{42} (20 μM); (c) A β_{42} peptide (20 μM) obtained by photolysis of α Nb-OEG-A β_{42} .

photolysis of α Nb-OEG-A β_{42} (Fig. 3). This is presumably due to the tag hindering the formation of amyloid fibrils. The structural morphology of the aggregates after photolysis of α Nb-OEG-A β_{42} was investigated by transmission electron microscopy (TEM), and confirmed the formation of A β_{42} fibrils (Fig. 4b). In contrast, the TEM image of α Nb-OEG-A β_{42} does not show well defined fibril structures characteristic of native peptide aggregates (Fig. 4a). Given these results, it appears that

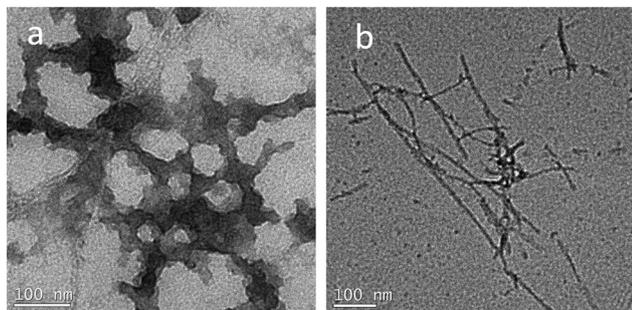


Fig. 4 TEM images after incubation for 48 hours at 37 °C in PBS, negatively stained with 2% uranyl acetate (a) α Nb-OEG-A β_{42} (10 μM); and (b) A β_{42} (10 μM) prepared by photolysis of α Nb-OEG-A β_{42} .

strategic placement of the α Nb-OEG₃ tag is effective in suppressing amyloid fibril formation. This could explain why α Nb-OEG-A β_{42} was obtained at such a high purity.

In conclusion, a photocleavable hydrophilic tag, α Nb-OEG, has been prepared and incorporated into A β_{42} at Lys₂₈ using Fmoc SPPS. The presence of the tag improved the solubility of this hydrophobic peptide in aqueous media and suppressed the formation of aggregates and fibrils. The increased solubility enabled the synthesis and isolation of α Nb-OEG-A β_{42} in good yield and high purity. The tag could be readily removed by photolysis. It is likely that this strategy could be used for the synthesis of other hydrophobic and fibril-forming “difficult” peptides.⁹

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