

Figure 2. A) Aggregation kinetics of the A β 42 wildtype peptide 1 (black) and A β 42 E22e peptide 2 (grey) at 20 μ M, monitored by the Thioflavin T (ThT) fluorescence (λ_{em} = 444 nm, λ_{ex} = 485 nm) at 37 $^{\circ}$ C. B, C) Representative transmission electron microscopy (TEM) images of the fibrillary architectures of the A β 42 wildtype peptide 1 (B) and the A β 42 E22e peptide 2 (C). The samples were incubated in phosphate buffer (20 mM, pH = 7.4) at 222 μ M before being diluted to 200 nM for imaging.

propensity of the peptide 2 for aggregation at the fibrillary endpoint, as well as at prefibrillary stages.

The delayed aggregation kinetics of the E22e peptide 2 led us to investigate whether the fibrillary assemblies of 2 were altered compared to 1. To do this, both A β 42 WT 1 and A β 42 E22e 2 fibrils were grown for 7 days at 37 $^{\circ}$ C following protocols by Tycko et al (see Supporting Information for details).^[9] Transmission electron microscopy (TEM) images of the wildtype A β 42 fibrils (Figure 2B) showed a distinct fibrillary architecture, characterized by the presence of numerous branches extending from the main fibril. This is of particular interest, given recent reports suggesting that the A β 42-fibril formation is a secondary nucleation-dependent process.^[10] In contrast, peptide 2 displayed more elongated, organized fibrillary structures devoid of branches (Figure 2C). Analogous TEM experiments were conducted, following an incubation of the peptides 1 and 2 for 2 h. The results were consistent in terms of branching, which was observable for A β 42 WT, but not for the E22e chiral variant (see Supporting Information for images and further details).

The difference in the fibrillary morphologies between the peptides 1 and 2 led us to further investigate whether alterations in the prefibrillary structural assemblies could account for the striking differences. Photochemically induced crosslinking of unmodified proteins (PICUP) experiments were carried out to gain insight into the distribution of the oligomeric states.^[12] Comparative analyses of the wildtype 1 and the E22e A β 42 peptide 2 were conducted at two time points, either immediately upon reconstitution or following an incubation for 24 h. The oligomerization profiles of the two scaffolds 1 and 2 showed no statistically significant difference in the population states of the oligomers (dimer–heptamer), indicating that any differences in the fibrillary assembly of the two peptides occurred at more advanced stages of the aggregation process (Figure 3A, B, see Supporting Information for details). To investigate these late-stage prefibrillary structures we employed small-angle X-ray scattering (SAXS) analysis. SAXS has been shown to be a powerful technique for monitoring amyloid-re-

lated structural features.^[13] We examined the SAXS curves of both wildtype 1 and E22e A β 42 peptide 2 after initial reconstitution and following 24 h incubation at 37 $^{\circ}$ C (Figure 3C, D). For both time points, SAXS analysis of the peptide 2 demonstrated a Bragg reflection corresponding to a species with a periodicity of 3.7 nm. This value is consistent with the dimensions

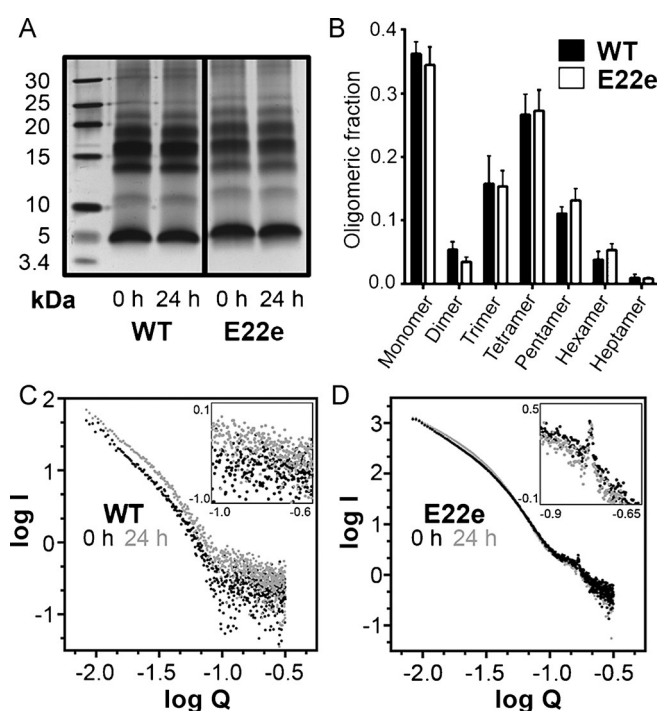


Figure 3. All experiments were carried out in phosphate buffer (20 mM, pH = 7.4) A) Representative PICUP (photochemically induced crosslinking of unmodified proteins) gels at both $t = 0$ h and $t = 24$ h. All PICUP experiments were carried out in phosphate buffer at 50 μ M, either directly after reconstitution, or following incubation for 24 h. Corresponding experiments were also performed at 20 μ M (see the Supporting Information). B) Densitometric analysis of oligomeric band intensity at $t = 0$ h, (see Supporting Information for $t = 24$ h). C) Small-angle X-ray scattering (SAXS) measurements of the A β 42 wildtype peptide 1 at $t = 0$ h (black) and $t = 24$ h (grey). D) SAXS measurements of the A β 42 E22e peptide 2 at $t = 0$ h (black) and $t = 24$ h (grey).

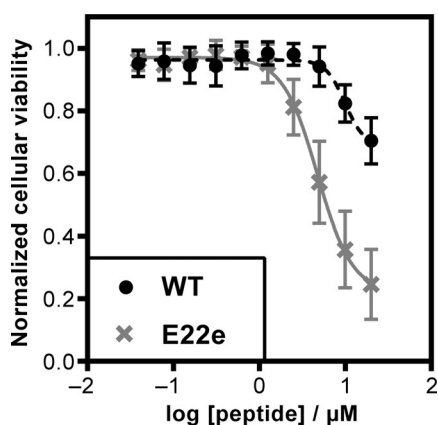


Figure 4. Dose-response curves of both wildtype A β 42 **1** (black) and A β 42 E22e **2** (grey) peptides against the rat pheochromocytoma PC12 adhesive cell line. Cells were plated at 5000 cells per well and allowed to adhere for 24 h prior to peptide addition, followed by incubation for additional 72 h. The cellular viability was determined at the endpoint of the assay using the cell proliferation reagent WST-1.

of a single unit of the wildtype A β 42 sequence found within a fibril using NMR and in silico structural models.^[14] No Bragg reflection and an increase in the heterogeneity of the sample was observed for peptide **1**, reflected by the large variance at high Q values.

The mechanism underlying the toxicity of A β 42 remains a subject of active research. Diverse modes of cytotoxicity have been proposed, including membrane disruption, induction of tau hyperphosphorylation, oxidative stress mediated through copper complexation, brain insulin resistance/signaling, and mitochondrial toxicity.^[15] The original (fibril-centric) amyloid-cascade hypothesis was reformulated when diffusible A β 42 oligomers emerged as the more toxic species.^[4c] To test whether the prefibrillary stabilized structure of peptide **2** exhibited an increase in cytotoxicity, we monitored the effects of varying concentrations of the wildtype peptide **1** and the E22e peptide **2** on rat pheochromocytoma PC12 cells (Figure 4). Addition of either peptide resulted in a reduction in the cellular viability, determined by the cell proliferation reagent WST-1. At 20 μ M, a 30% reduction in the cellular viability was observed when dosing with the wildtype peptide **1** (Figure 4, WT). However, addition of the same concentration of the E22e peptide **2** resulted in an 80% reduction in the viability (Figure 4, E22e). The cellular viability of the PC12 cells was also found to be lower when peptide **2** was dosed at 10 μ M, with a reduction in the cellular viability close to 65%, compared with a 20% reduction when dosing with the same concentration of peptide **1** (for detailed graphical analysis see Supporting Information). Preincubation (2 h or 4 h) of the peptides prior to administration did not affect their cytotoxicity (see Supporting Information). Our E22e variant offers a unique way of trapping an advanced aggregation intermediate of A β 42 with enhanced toxicity, and highlights how a subtle structural change—a single chiral substitution—can have profound effects on aggregation and neurotoxicity.

In conclusion, incorporation of D-glutamate at position 22 of A β 42 resulted in a peptide with attenuated propensity for mis-

folding and aggregation. Transmission electron microscopy showed a striking difference in the fibril morphology. The E22e peptide **2** exhibited elongated, ordered amyloid-beta fibrils. This is in stark contrast to the A β 42 WT peptide **1**, which displayed a fibrillary architecture, characterized by the presence of a large number of sidechains protruding from the main fibril. No difference in the population density of the oligomers (dimer–heptamer) between the two peptides was observed. However, SAXS analysis of the E22e peptide **2** showed the presence of a unique Bragg reflection corresponding to a soluble species with a periodicity of 3.7 nm. Cell culture studies established a three- to fourfold increase in the cytotoxicity in response to the E22e substitution in A β 42. This subtle molecular edit therewith offers a tool to improve our understanding of the A β 42 neurotoxicity.

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