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Nanoscale

PAPER

View Article Online
View Journal | View Issue

Cite this: *Nanoscale*, 2014, **6**, 6720

The Aβ peptide forms non-amyloid fibrils in the presence of carbon nanotubes†

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Received 16th January 2014
Accepted 11th April 2014

DOI: 10.1039/c4nr00291a

www.rsc.org/nanoscale

Carbon nanotubes have specific properties that make them potentially useful in biomedicine and biotechnology. However, carbon nanotubes may themselves be toxic, making it imperative to understand how carbon nanotubes interact with biomolecules such as proteins. Here, we used NMR, CD, and ThT/fluorescence spectroscopy together with AFM imaging to study pH-dependent molecular interactions between single walled carbon nanotubes (SWNTs) and the amyloid-beta (Aβ) peptide. The aggregation of the Aβ peptide, first into oligomers and later into amyloid fibrils, is considered to be the toxic mechanism behind Alzheimer's disease. We found that SWNTs direct the Aβ peptides to form a new class of β-sheet-rich yet non-amyloid fibrils.

Introduction

Single-walled carbon nanotubes (SWNTs) are considered to hold major promise for biotechnology and biomedicine. Due to their unique electronic and mechanical properties, together with their chemical stability, many possible applications are being explored, ranging from sensors to tissue supports to artificial muscles.[1–6] A potential problem with SWNTs is their poor solubility in water. Proteins and peptides have been used to increase the solubility of SWNTs.[7,8] However, the detailed dynamics and atomic interactions between SWNTs and biomolecules remain largely unknown. Furthermore, the biocompatibility of SWNTs may be an issue, as there is evidence that SWNTs change the secondary structure of certain proteins. SWNTs were reported to accelerate the fibrillation of β2 microglobulin by decreasing the lag phase for fibrillation nucleation.[9] In contrast, SWNTs were reported to prevent the aggregation of human acidic fibroblast growth factor (hFGF-1).[10]

Amyloid fibrillation of many proteins (such as lysozyme, the Aβ peptide, and the tau protein) is associated with neurodegenerative and other diseases, including Alzheimer's disease,[11] Huntington's disease[12] and Parkinson's disease.[13] A common theme is the formation of amyloid fibrils with cross-β-stacking structures through a nucleation-dependent polymerization process, which involves a lag phase followed by elongation into fibrils. For example, aggregation of the Aβ peptide into β-sheet-rich amyloid fibers is considered the key process in Alzheimer's disease. In the lag phase, the protein rearranges its secondary structure/conformation and thus forms a nucleus for elongation.[14,15] Although the mechanism of amyloid fibrillation remains unclear at this level of molecular structure, previous studies indicate that conformational shifts/structural instabilities of proteins induce their aggregation. Both early and late steps in the aggregation may be affected by SWNTs and other nanoparticles, which could either increase or decrease toxicity, depending on how they modulate the aggregation process.

Here, we investigated the effect of hydrophobic SWNTs on the structure, solubility, and aggregation of the Aβ peptide. CD spectroscopy showed that the peptide experiences shifts in the secondary structure and stability in the presence of SWNTs. ThT assays suggested that SWNTs inhibit the nucleation/elongation phase of Aβ peptide fibrillation, while AFM measurements indicated that the Aβ peptide solubilizes SWNTs by adsorbing onto the SWNT surface. NMR measurements indicated that specific Aβ residues interact with the SWNTs, or change conformation in the presence of SWNTs. A cell toxicity assay confirmed the interaction of Aβ and SWNTs. Based on these experimental data, we propose a mechanism for the SWNT–Aβ interaction.

Results and discussion

SWNTs induced a structural transition of the Aβ peptide

During the aggregation, the conformation of the Aβ(1–40) peptide converted from a random coil to a β-sheet structure. Kinetic CD spectroscopy measurements revealed significant, SWNT-dependent alterations of the conformational landscape of 10 μM Aβ(1–40) for 100 minutes in the absence or presence of

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c4nr00291a

0.1 mg ml^{-1} SWNTs (Fig. 1a and b). The initial CD spectrum had a minimum around 197 nm, which is typical for a random coil structure, while the CD spectrum at the end of the structural transitions had a maximum around 195 nm and a minimum around 215 nm, which is considered typical for a β-sheet structure.[16] Monitoring the amplitudes at 195 and 215 nm, it is clear that this structural transition occurred faster in the presence of SWNTs (Fig. 1c). Additionally, in the presence of SWNTs, the amplitude of the Aβ CD spectrum increased significantly for an intermediate spectral form. This suggests a different pathway for Aβ aggregation in the presence of SWNTs. Thus, SWNTs seem to promote the initial conformational change of the Aβ peptide that characterizes amyloid formation. In order to further investigate this effect, the formation of Aβ amyloid fibrils in the presence and absence of SWNTs was studied using a thioflavin T (ThT) binding assay.

SWNTs prevented amyloid fibrillation of Aβ peptides

ThT fluorescence is considered a highly sensitive marker for the amyloid state of various aggregating proteins and peptides. ThT in solution displays weak fluorescence in its free form but strong fluorescence when bound to amyloid fibrils.[17] The aggregation kinetics of 10 μM Aβ(1–40) peptides were measured in the presence of 0.0025 mg ml^{-1}, 0.005 mg ml^{-1},

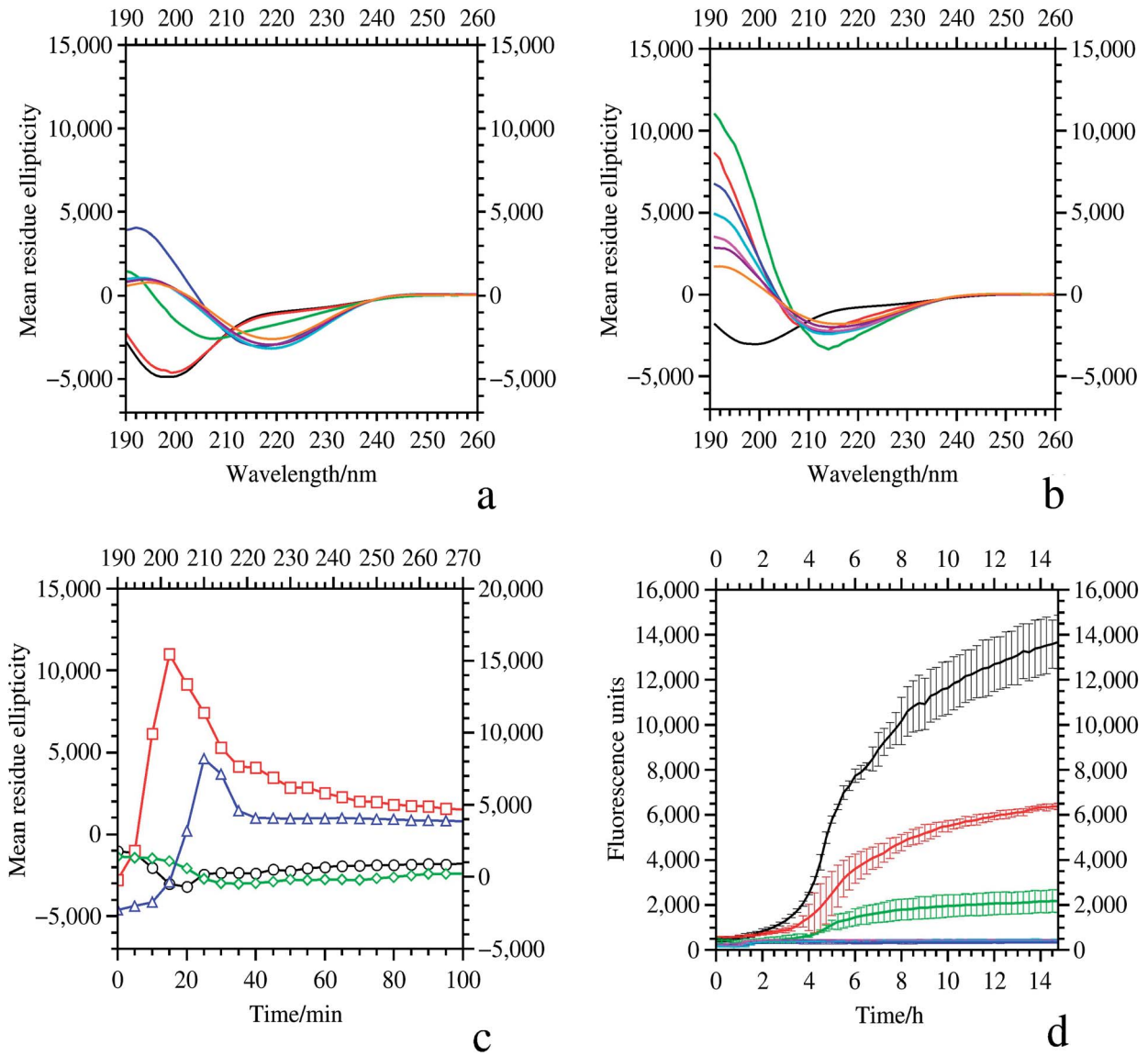

Fig. 1 The effect of SWNTs on the structural transition of Aβ from a random coil to a β-sheet structure. (a and b) CD spectrum of 10 μM Aβ peptide aggregation in the absence (a) and presence (b) of 0.1 mg SWNTs. Black curves: 0 min; red curve: 10 min; green curves: 20 min; blue curves: 30 min; cyan curves: 40 min; magenta curves: 50 min; purple curves: 60 min; orange curves: 100 min. (c) CD intensities at 195 nm (red: Aβ–SWNTs, blue: Aβ alone) and 215 nm (black: Aβ–SWNTs, green: Aβ alone) plotted *versus* time. (d) Aggregation kinetics of the 10 μM Aβ peptide in the absence and presence of SWNTs. (d) ThT assay as a function of SWNT concentration: 0.0025 mg ml^{-1}, 0.005 mg ml^{-1}, 0.025 mg ml^{-1} and 0.1 mg ml^{-1} in pH 7.4. Black curve: 10 μM Aβ; red curve: 10 μM Aβ–0.0025 mg ml^{-1} SWNTs; green curve: 10 μM Aβ–0.005 mg ml^{-1} SWNTs; blue curve: 10 μM Aβ–0.025 mg ml^{-1} SWNTs; 10 μM Aβ–0.1 mg ml^{-1} SWNTs; magenta curve: 0.1 mg ml^{-1} SWNTs.

0.025 mg ml^{-1} and 0.1 mg ml^{-1} SWNTs (Fig. 1d). In the presence of 0.0025 mg ml^{-1} and 0.005 mg ml^{-1} SWNTs, the lag times of the Aβ peptide aggregation decreased from 3.7 h to 2.9 h and 1.0 h, respectively, but the transition times were delayed from 3.3 h to 5.0 h and 8.3 h, respectively. A decreased lag time suggests that the SWNTs promoted nucleation, and the increased transition time suggests that the SWNTs inhibited subsequent growth of these nuclei into amyloids. After addition of 0.1 mg ml^{-1} SWNTs, the amyloid aggregation of the Aβ(1–40) peptide was completely inhibited.

We tested whether our results could also be explained by possible quenching of ThT fluorescence by SWNTs. For this purpose, we generated amyloid from insulin in the presence of ThT, which fixes its conformational state. We then added increasing amounts of SWNTs, observing a queching effect by the nano-material, but the quenching we observed was too low to explain our results (see ESI, Table 1†).

Aβ bound to the SWNTs

Our CD and ThT studies demonstrated that SWNTs promoted the structural transition of Aβ to a β-sheet conformation, but that the amyloid formation of the Aβ peptide was inhibited. This is different from other promoters/inhibitors of amyloid β peptide aggregation. For example, the acceleration of structural transitions of the Aβ peptide caused by, *e.g.*, charged polyamines always promotes aggregation.[18] In order to elucidate this discrepancy, we investigated the structural perturbation of the Aβ peptide in the presence of SWNTs by Atomic Force Microscopy (AFM). Although the method is not able to provide true tertiary/atomic structural information, it did provide the overall structure of the Aβ peptide–SWNT assembly. SWNTs (0.1 mg ml^{-1}) pre-incubated with the 50 μM Aβ peptide at 37 °C, pH 7.4 for 1 h with shaking at 200 rpm, were deposited on freshly cleaved mica and imaged by AFM (Fig. 2). The height of the complex was varied from 16 nm. The heights of early aggregates of the Aβ peptide in the absence of SWNTs, and of SWNTs in the absence of Aβ, were much lower than 10 nm (Fig. S1†). This suggested that the Aβ peptide coated the carbon nanotubes.

Interestingly, the SWNT–Aβ assemblies differed significantly in height at different pH values. Unlike amyloid fibrils, the assembly of SWNT–Aβ displayed a uniform structure (Fig. 2a–c & S2†). At pH 7.4 and 5.5 (Fig. 2b1 and b2 & S2b†), the SWNT–Aβ assembly had a height distribution centered around ±16 nm, whereas at pH 9, the height was significantly lower at ±5 nm. Clearly, the Aβ peptide does not readily interact with the SWNTs at high pH. This indicates that Aβ peptides adsorb onto SWNTs in a pH-dependent process.

The Aβ peptide interacts with the SWNTs mainly through the whole sequence of the Aβ peptide

NMR spectroscopy was used to identify the Aβ residues involved in the SWNT interaction. The ^{1}H-^{15}N-HSQC NMR spectra of the labeled Aβ(1–40) monomer peptide before and after addition of SWNTs demonstrate clear differences in amide cross-peak positions and intensities, proving that SWNTs induce specific conformational changes to the monomeric Aβ(1–40) peptide. These changes persist in fast exchange on the NMR time-scale (Fig. 3). In particular, the amide cross-peak of Aβ residues, Y10–E12, L17–A21 and A30–M35, displayed significant peak shifts in the presence of SWNTs. Furthermore, after the addition of 0.2 mg ml^{-1} SWNTs, most of the amide cross-peaks in the N terminus disappeared and some new amide cross-peaks of Aβ(1–40) appeared, suggesting that the Aβ peptide monomers transit quickly into the new conformation. For instance, the resonances of most of the glycines faded quickly, something which we also observed in other ligand-conjugated Aβ peptide experiments.[18] The signals of these residues are very sensitive to the addition of ligands that promote amyloid formation.[18] Our NMR experiments indicated that not only hydrophobic groups (*i.e.* F20 and Y10) interacted with SWNTs, but also that the chemical environment of some charged residues (*i.e.* E11) changed, as evidenced by obvious NMR shift changes. Thus, addition of SWNTs perturbed both hydrophobic and charged Aβ residues, even though the SWNTs themselves are hydrophobic and do not interact with charged residues. We assume that the interaction between hydrophobic groups of the Aβ peptide and SWNTs could affect the stabilities of the electrostatic network of the Aβ peptide and lead to the conformational changes of the peptide. In previous studies we have reported a similar behavior of the ApoE molecule, which increases the toxicity of Aβ peptides: ApoE also interrupts the electrostatic network of the Aβ peptide.[19]

Aβ reduces the toxicity of SWNTs to SH-SY5Y cells

To probe the toxicity of Aβ–SWNT assembly, we measured the cell viability in the presence of Aβ, SWNTs and Aβ–SWNT complexes. Aβ, SWNTs and their complex were incubated at room temperature for 90 min and added to growing SH-SY5Y cells. As shown in Fig. 3c, 50% cell survived in the presence of Aβ (30 μM) and SWNTs killed most of the cells even at a low concentration of 0.0008 mg ml^{-1}. However, after adding Aβ, SWNTs displayed lower toxicity at concentrations of 0.0008 mg ml^{-1}, 0.0016 mg ml^{-1} and 0.008 mg ml^{-1} SWNTs. The results suggested that Aβ reduced the toxicity of SWNTs. It may be that the reduction of the hydrophobic surface of SWNTs due to the complexation with Aβ reduced the probability of the SWNTs interacting with the cell membrane.

Consequently, NMR confirmed our CD measurements, and showed that SWNTs trigger a structural transition of Aβ and induce a backbone change of the peptide. This effect of SWNTs on Aβ may also be present for other interactions between proteins and carbon hydrophobic materials, such as graphene and fullerene.

We report that SWNTs promote the structural transition of Aβ(1–40) from a random coil to a β-sheet. This transition is known to precede fibrillation *in vivo*. However, the ThT results show that amyloid formation is inhibited in the presence of SWNTs. Furthermore, Aβ(1–40) was found to coat SWNTs in a pH-dependent fashion. NMR spectroscopy measurements indicated that the hydrophobic residues of the peptides interacted with the SWNTs, and that the chemical environment of some hydrophilic residues changed. Our cell toxicity assay

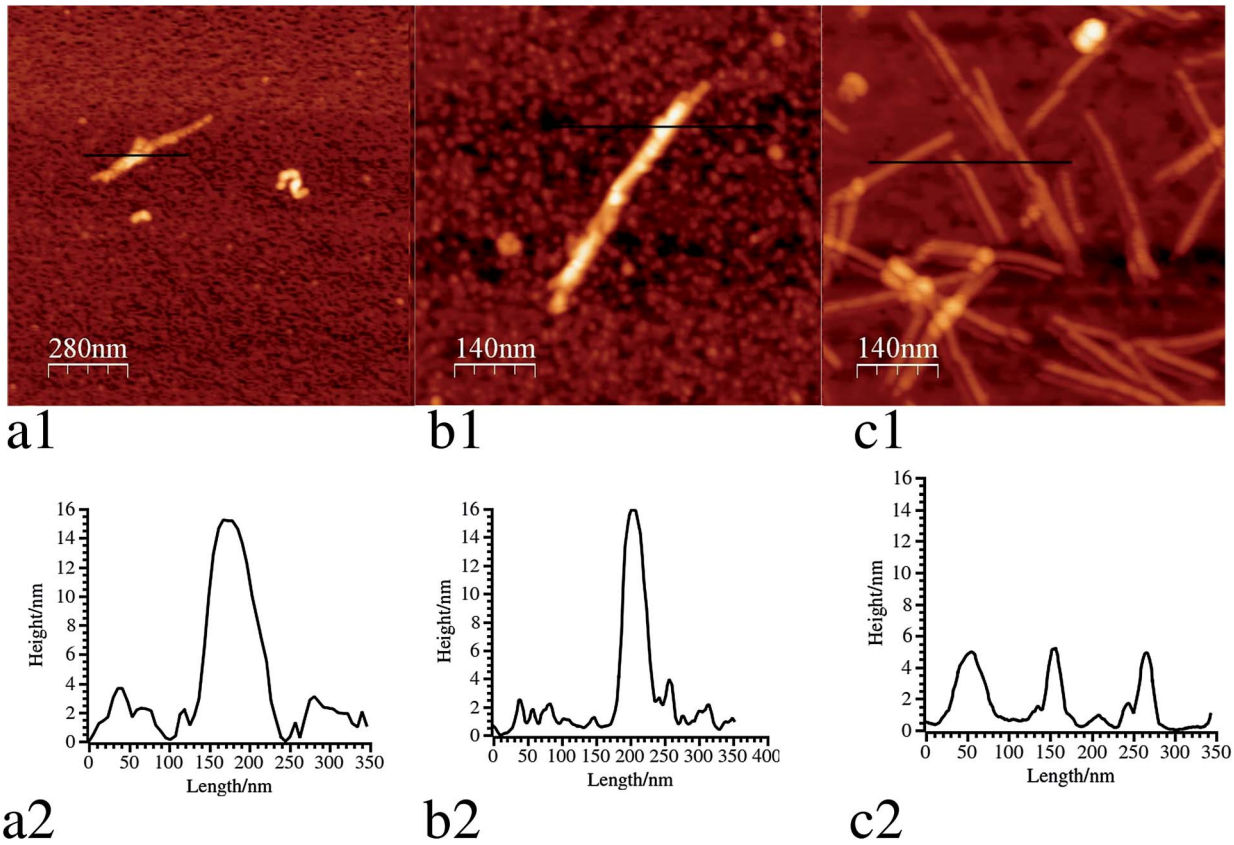

Fig. 2 The morphologies of the incubated Aβ peptide–SWNT complexes at different pHs: (a1) pH 5.5, (b1) pH 7.4 and (c1) pH 9.0. Top panels show the morphologies of the complex of the Aβ peptide and SWNT assembly and bottom panels show the height distribution of the cross-section in link with the top panels.

confirmed that the assemblies with the Aβ peptide are less toxic than SWNTs alone.

The changed behavior of the Aβ peptide in the presence of SWNTs is most likely due to the adsorption of the peptide onto the hydrophobic surface of the SWNTs. With the knowledge of the fine fibril structure obtained by cryo-EM,[20] we realized that the SWNT–Aβ complex has a similar diameter to the fibrils. Miller *et al.* reported 10 different fibril models at both acidic and physiological pHs.[20] Considering the diameter of SWNTs and the hollow core of fibril models, we tried to dock a hydrophobic SWNT into the hollow hydrophobic core structure of the Aβ fibrils (M21 in Miller's paper[20]), and it fitted very well because the diameter of the carbon nanotube was around 1–2 nm and the size of the hollow core was from 2 nm to 3 nm (Fig. 4). In this mode, residues I31, M35, V39 and I41 in the C terminus of the Aβ peptide are proposed to interact with SWNTs. However, although a SWNT would fit inside the amyloid fibril, we have no experimental evidence for such a structure. We did observe using AFM that the diameter of the Aβ–SWNT assembly was 14–15 nm, which is very close to that of the Aβ fibrils. Furthermore, our NMR experiments indicated that the hydrophobic groups of the Aβ peptide adsorbed onto the SWNTs. However, it is unlikely that the conformation of the peptides in the SWNT–Aβ assembly is identical to that of the peptides in the Aβ fibrils. The self-assembly of the SWNTs furthermore suggests that the core of the Aβ–SWNT assembly may consist of SWNT bundles.

Our experimental data are in agreement with a mechanism in which SWNTs quickly bind Aβ peptides that have changed from a random coil into a parallel β-sheet conformation. After this binding, the interactions between hydrophobic residues of the peptide and the SWNT surface will stabilize the conformation of the Aβ peptide. This would inhibit the subsequent structural rearrangement of the peptide into the cross-β-sheet conformation that is typical for amyloid formation. Thus, the SWNTs would scavenge peptides that have undergone the first structural transition that is required for the formation of a stable nucleus for fibrillation, but, by binding, slow down the subsequent structural rearrangement into the cross-β-strand conformation (Fig. S3†). In addition, cross amyloid interaction of Aβ and tau protein results in polymorphism of the amyloid and induces the aggregation of their oligomeric complexes.[21,22] In theory, the new structural shapes induced by SWNTs could therefore also induce polymorphism. The techniques that we used here are inconclusive if this is the case. The detailed mechanism of polymorphism of Aβ peptide oligomers induced by SWNTs remains to be clarified. Our data do suggest, however, that SWNTs would slow down or even reduce amyloid formation.

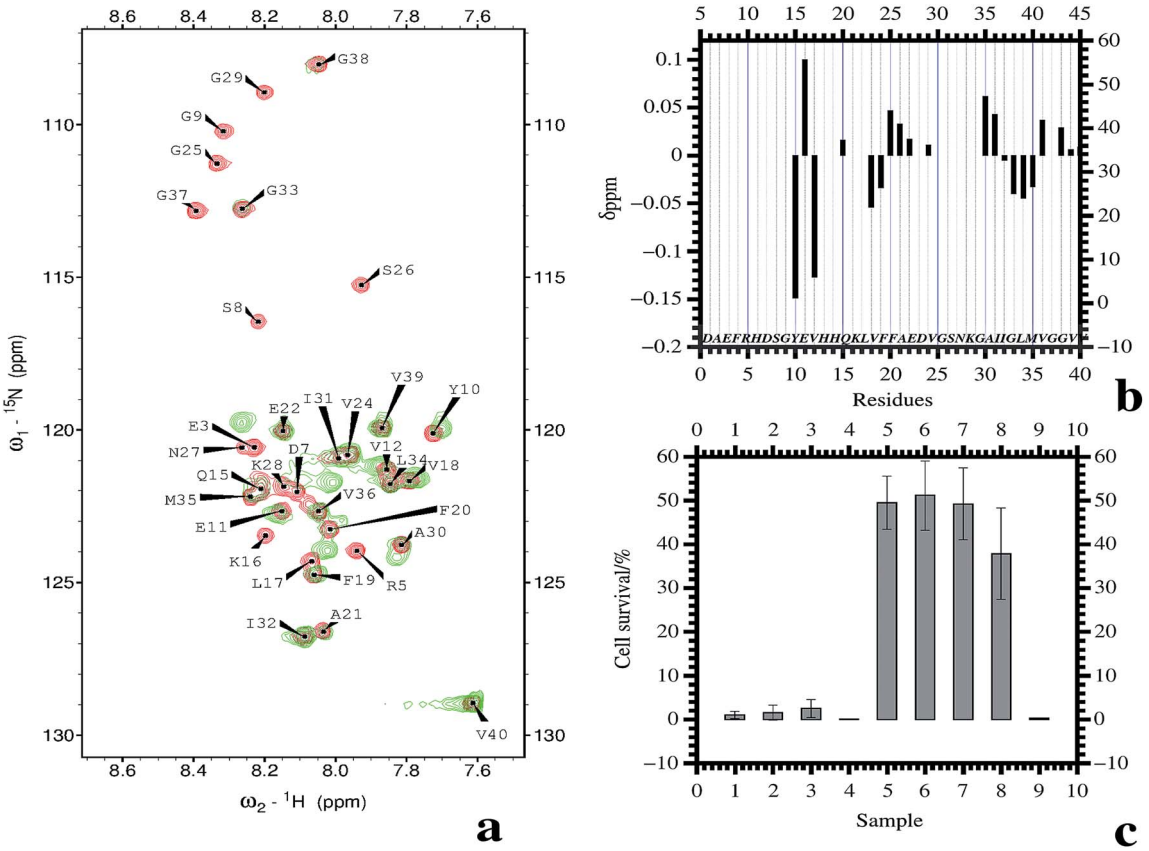

Fig. 3 (a) ^{1}H-^{15}N-HSQC amide region spectra at 5 °C of the 100 μM ^{15}N-labeled Aβ(1–40) peptide in 20 mM sodium phosphate buffer at pH 7.3, in the absence (red) and presence (green) of 0.2 mg ml^{-1} SWNTs. (b) ^{15}N chemical shift differences in ^{1}H-^{15}N-HSQC spectra at 5 °C of 100 μM ^{15}N-labeled Aβ(1–40) peptide in the absence (red) and presence (green) of 0.2 mg ml^{-1} SWNTs. (c) Neuroblastoma cell (SH-SY5Y) viability affected in the presence and absence of SWNTs or/and ^{15}N-labeled Aβ(1–40). Samples 1–9 are 0.0008 mg ml^{-1} SWNTs, 0.0016 mg ml^{-1} SWNTs, 0.008 mg ml^{-1} SWNTs, 0.03 mg ml^{-1} SWNTs, 30 μM Aβ, 0.0008 mg ml^{-1} SWNT–30 μM Aβ, 0.0016 mg ml^{-1} SWNT–30 μM Aβ, 0.008 mg ml^{-1} SWNT–30 μM Aβ, and 0.03 mg ml^{-1} SWNT–30 μM Aβ, respectively.

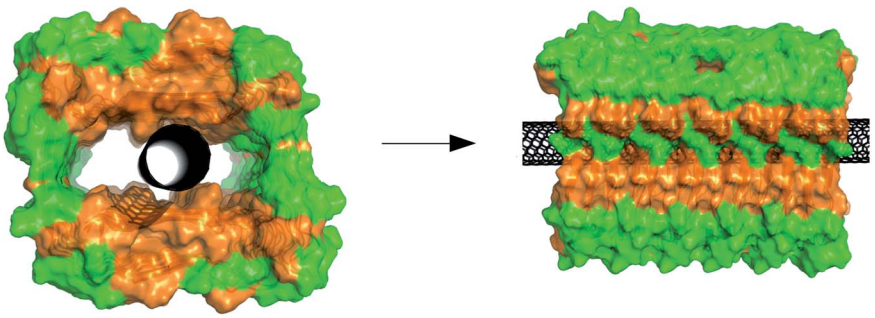

Fig. 4 Illustration of a SWNT located in the hollow core of an amyloid beta peptide fibril. Orange and green represent hydrophobic and hydrophilic residues and the black one is the carbon nanotube. Although this structure is theoretically possible, it is not backed by experimental evidence.

The proposed mechanism has wider implications, as it may be extended to other hydrophobic nano-surfaces. It would explain the action of fullerene and modified fullerene analogues that have been developed to prevent the cytotoxicity of the aggregates of the amyloid β peptide.[23,24] It even raises questions about Aβ peptide aggregation on or around cell membrane surfaces! It is worth pointing out that existing antibodies and small detection molecules such as ThT have been developed to identify amyloid fibrils. We here show that Aβ can also form non-amyloid fibrils in suitable environments, but whether such structures exist *in vivo* we presently cannot say since we lack the tools to detect them. Both SWNTs and Aβ oligomer are toxic to mammalian cells. We found that SWNTs do not have an effect on the toxicity of the Aβ oligomer, but that the Aβ peptide

reduces the toxicity of SWNTs. Therefore, we hope that our findings will stimulate further studies on the possible toxicities and protective effects of proteins on nanomaterials. Our studies also open new directions for biologically functionalizing carbon nanotubes (and perhaps other hydrophobic nano-materials), in view of the straightforward, high yield coating of SWNTs with Aβ peptides and the reduced cytotoxicity of coated SWNTs we report. Modified versions of such peptides that carry functional groups could yield a host of novel nanostructured materials if drug spontaneously coats such materials like Aβ does with SWNTs.

Materials and methods

Sample preparation

The Aβ(1–40) peptide was bought either unlabeled or ^{15}N-labeled from AlexoTech AB (Umeå, Sweden) and prepared according to previously described protocols.[18] The peptide was dissolved in 10 mM NaOH to a peptide concentration of 1 mg ml^{-1} and sonicated in an ice bath for 1 min. Then, this stock solution was diluted for every assay.

Single-walled carbon nanotubes (SWNTs) produced by high-pressure decomposition of carbon monoxide (the HiPco process) were purchased from Unidym and used without modification. SWNT dispersions were prepared immediately prior to use for each assay by sonicating in Eppendorf tubes a 1 ml solution containing SWNTs in DMSO. A VCX 750 tip sonicator (Sonics and Materials Inc.) was used for 10 min with a 24% amplitude and a 10 seconds on-and-off pulse. The tip of the sonicator was immersed to appropriately a half depth of solution in the Eppendorf tube and the supernatant was collected for ThT assay, AFM and NMR measurements. For the CD measurements, addition of DMSO increases the noise of the CD signal. SWNTs without dissolving in DMSO were mixed with the Aβ peptide and mechanical rotary stirring was used to mix the sample of Aβ and SWNTs.

CD spectroscopy

A Chirascan CD unit from Applied Photophysics was used to monitor the kinetics of a 10 μM solution of the Aβ(1–40) peptide dissolved in 5 mM sodium phosphate buffer at pH 7.0, either with or without 0.1 mg ml^{-1} SWNTs. At 5 min intervals CD spectra were recorded between 190–270 nm using a step size of 2 nm and a slit size providing 1 nm resolution. The sample was held at 37 °C in a 10 mm path length quartz cuvette (lot: 119.004-QS, Hellma Analytics), and mechanical rotary stirring with a magnet was used to speed up the aggregation process.

ThT assay

A 10 mM ThT stock solution was prepared in 50 mM Tris buffer (pH 7.4) and filtered to remove ThT particles. This solution was combined with the Aβ(1–40) sample and SWNTs to yield a mixture containing 5 μM ThT, 10 μM Aβ(1–40), 50 mM Tris buffer, and either 0.0025 mg ml^{-1}, 0.005 mg ml^{-1}, 0.025 mg ml^{-1}, or 0.1 mg ml^{-1} SWNTs. In addition, a sample of pure Aβ(1–40) was prepared at different pH values (5.5, 7.4 and 9.0). The various mixtures were pipetted into a plate with 384 wells holding 45 μl each, set to a temperature of 37 °C. Fluorescence measurements were recorded with a Tecan Safire2 plate reader every 15 minutes, with excitation and emission wavelengths of 446 nm and 490 nm, respectively. The plate was set to automatically shake the wells 30 seconds before each measurement. Each sample was prepared in duplicate, and average fluorescence signals were calculated after the baseline fluorescence of control samples (*i.e.* samples without the Aβ peptide) had been subtracted from each measurement. The experiments were performed in triplicate. The data were fitted using previously described methods for calculating the transition and lag times of the amyloid formation process.[18,25,26]

AFM imaging

Samples containing 50 mM Tris buffer (pH 7.4) and either 0.1 mg ml^{-1} SWNTs, 50 μM Aβ(1–40) peptide, or a mixture of 0.1 mg ml^{-1} SWNTs and 50 μM Aβ peptide after 1 hour of 200 rpm shaking incubation at 37 °C were diluted 1 : 1 with 50 mM Tris buffer (pH 7.4) and deposited on freshly cleaved mica for 5 min. Then the excess liquid was shaken off, the substrate rinsed once with 50 mM Tris buffer (pH 7.4), and dried in a stream of dry nitrogen. The specimens were then mounted on a multi-mode atomic force microscope, and imaging was carried out in air using silicon cantilevers. The images were collected in tapping mode at a frequency of 300 kHz.

NMR spectroscopy

A Bruker Avance 500 MHz spectrometer was used to record ^{1}H-^{15}N-HSQC spectra at 5 °C of the 100 μM ^{15}N-labeled Aβ(1–40) peptide in 20 mM sodium phosphate at pH 7.3 (90/10 H_2O–D_2O), both in the absence and presence of 0.2 mg ml^{-1} SWNTs. The spectrometer was equipped with a triple-resonance cryogenically cooled probe-head, and the spectra were referenced to the water signal.

Cell viability assay

Neuroblastoma SH-SY5Y cells (in a maximum passage number of 15) were cultured in Dulbecco's modified Eagle medium, a 1 : 1 mixture of DMEM and Ham's F12 medium, and 10% supplemental fetal bovine serum, containing 1% (v/v) penicillin–streptomycin at 37 °C, 5% CO_2 to a confluency of 85% in a 75 cm^2 flask (Greiner Bio-one, cat. 658170). Cells were detached by 5 mM EDTA–PBS for 3 min at 37 °C. Then cells were resuspended at a concentration of 2 000 000 cells per ml in DMEM/F12, containing 1% (v/v) penicillin–streptomycin. The resuspended cells were plated at a volume of 50 μl and a cell density of 20 000 cells per well in a 96-well plate. The plated cells were incubated for 48 h at 37 °C at 5% CO_2. SWNTs, Aβ40 or SWNT–Aβ40 complexes were incubated for 1.5 h at concentrations of 0.0025 mg ml^{-1} SWNTs, 0.005 mg ml^{-1} SWNTs, 0.025 mg ml^{-1} SWNTs, 0.1 mg ml^{-1} SWNTs, 90 μM Aβ, 0.0025 mg ml^{-1} SWNT–90 μM Aβ, 0.005 mg ml^{-1} SWNT–90 μM Aβ, 0.025 mg ml^{-1} SWNT–90 μM Aβ, and 0.1 mg ml^{-1} SWNT–90 μM Aβ, added to growing cells. The concentration of the incubated Aβ aggregates/SWNTs/assemblies was diluted 3 times in experimental

wells. The phosphate buffer as a control was added at a volume of 50 μl in the medium to each well and left to incubate for 48 h. After 48 h, the plate was equilibrated at room temperature for approximately 30 minutes. 50 μl of CellTiter-Glo® Luminescent Cell Viability Assay (Promega, cat. G7571) compound was added to each well and then the contents in the plate were mixed on an orbital shaker for 2 minutes to induce cell lysis.[27] Luminescence intensity was measured on a 384-well plate reader (Infinite M1000 PRO microplate reader) at 1000 ms integration time. Measurements were performed in three independent experiments and statistical analysis was performed to calculate average values and standard deviations.

Notes and references

1 M. Swierczewska, K. Y. Choi, E. L. Mertz, X. Huang, F. Zhang, L. Zhu, H. Y. Yoon, J. H. Park, A. Bhirde, S. Lee and X. Chen, *Nano Lett.*, 2012, **12**, 3613–3620.
2 A. Bianco, K. Kostarelos and M. Prato, *Curr. Opin. Chem. Biol.*, 2005, **9**, 674–679.
3 Y. Weizmann, D. M. Chenoweth and T. M. Swager, *J. Am. Chem. Soc.*, 2010, **132**, 14009–14011.
4 F. Patolsky, G. Zheng and C. M. Lieber, *Nanomedicine*, 2006, **1**, 51–65.
5 S. Penn, *Curr. Opin. Chem. Biol.*, 2003, **7**, 609–615.
6 C. R. Martin and P. Kohli, *Nat. Rev. Drug Discovery*, 2003, **2**, 29–37.
7 M. J. Pender, L. A. Sowards, J. D. Hartgerink, M. O. Stone and R. R. Naik, *Nano Lett.*, 2006, **6**, 40–44.
8 Y. Choi, T. J. Olsen, P. C. Sims, I. S. Moody, B. L. Corso, M. N. Dang, G. Weiss and P. G. Collins, *Nano Lett.*, 2013, **13**, 625–631.
9 S. Linse, C. Cabaleiro-Lago, W.-F. Xue, I. Lynch, S. Lindman, E. Thulin, S. E. Radford and K. a. Dawson, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 8691–8696.
10 A. V. Ghule, K. M. Kathir, T. K. Suresh Kumar, S.-H. Tzing, J.-Y. Chang, C. Yu and Y.-C. Ling, *Carbon*, 2007, **45**, 1586–1589.
11 T. Wisniewski, J. Ghiso and B. Frangione, *Neurobiol. Dis.*, 1997, **4**, 313–328.
12 C. A. Ross and S. J. Tabrizi, *Lancet Neurol.*, **10**, 83–98.
13 G. B. Irvine, O. M. El-Agnaf, G. M. Shankar and D. M. Walsh, *Mol. Med.*, 2008, **14**, 451–464.
14 J. T. Jarrett and P. T. Lansbury, *Biochemistry*, 1992, **31**, 12345–12352.
15 A. Lomakin, D. B. Teplow, D. A. Kirschner and G. B. Benedek, *Proc. Natl. Acad. Sci. U. S. A.*, 1997, **94**, 7942–7947.
16 N. J. Greenfield, *Nat. Protoc.*, 2006, **1**, 2876–2890.
17 M. Biancalana and S. Koide, *Biochim. Biophys. Acta*, 2010, **1804**, 1405–1412.
18 J. Luo, C.-H. Yu, H. Yu, R. Borstnar, S. C. L. Kamerlin, A. Gräslund, J. P. Abrahams and S. K. T. S. Wärmländer, *ACS Chem. Neurosci.*, 2013, **4**, 454–462.
19 J. Luo, J.-D. Maréchal, S. Wärmländer, A. Gräslund and A. Perálvarez-Marín, *PLoS Comput. Biol.*, 2010, **6**, e1000663.
20 Y. Miller, B. Ma, C.-J. Tsai and R. Nussinov, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 14128–14133.
21 Y. Raz and Y. Miller, *PLoS One*, 2013, **8**, e73303.
22 Y. Miller, B. Ma and R. Nussinov, *Biochemistry*, 2011, **50**, 5172–5181.
23 C.-M. Lee, S.-T. Huang, S.-H. Huang, H.-W. Lin, H.-P. Tsai, J.-Y. Wu, C.-M. Lin and C.-T. Chen, *Nanomedicine*, 2011, **7**, 107–114.
24 G. Bobylev, B. Kornev, L. G. Bobyleva, M. D. Shpagina, I. S. Fadeeva, R. S. Fadeev, D. G. Deryabin, J. Balzarini, P. Troshin and Z. Podlubnaya, *Org. Biomol. Chem.*, 2011, **9**, 5714–5719.
25 J. Luo, S. K. T. S. Wärmländer, A. Gräslund and J. P. Abrahams, *Chem. Commun.*, 2013, **49**, 6507–6509.
26 J. Luo, J. M. Otero, C. H. Yu, S. K. T. S. Wärmländer, A. Gräslund, M. Overhand and J. P. Abrahams, *Chem. – Eur. J.*, 2013, **19**, 17338–17348.
27 J. Luo, I. Mohammed, S. K. T. S. Wärmländer, Y. Hiruma, A. Gräslund and J. P. Abrahams, *Biomacromolecules*, 2014, DOI: 10.1021/bm401874j.

