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Self-assembling DNA–peptide hybrids: morphological consequences of oligonucleotide grafting to a pathogenic amyloid fibrils forming dipeptide†

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For the very first time, highly efficient synthesis of DNA–peptide hybrids to scaffold self-assembled nanostructures is described. Oligonucleotide conjugation to the diphenylalanine dipeptide triggers a morphological transition from fibrillar to vesicular structures which may potentially be used as delivery vehicles, since they exhibit pH triggered release.

Aside from being the universal carrier of the genetic information, DNA and short nucleotide sequences thereof are involved in a plethora of vital biological mechanisms. For instance, suitable nucleotide sequences interfere with various RNA, which may code disease-related proteins, thus acting as therapeutic remedies.[1] However, the main obstacles of using nucleotide sequences as such are their limited plasma half-life as well as cellular penetrability and uptake.[2] There is therefore an increasing interest in designing nucleic acid decorated nanostructures which may help in improving their cell penetrability and potentially be used as carriers.[2] To contrive this aim, nucleotide sequences have been grafted to hydrophobic polymers to induce the formation of self-assembled nanostructures. The synthetic segment might however lack biocompatibility in some instances.[2–4]

Conjugation of nucleotide sequences with biocompatible ligands has thus become a topic of intense research.[5] A peptide sequence may be one of such ligands, since increased cellular uptake has been reported through conjugation with cell penetrating oligopeptides.[6] Moreover peptide sequences possess inherent properties of self-assembling in solution[7] and thus may serve as scaffolds for the formation of oligonucleotide decorated self-assembled structures.

We demonstrate herein that conjugation of the diphenylalanine (FF) dipeptide ligand with a C_6 amino modified CTCTCTCTCTTT nucleotide sequence results in a DNA–peptide hybrid which self-assembles to give rise to oligonucleotide decorated self-assembled structures in aqueous solution. FF is the common structural motif of amyloid fibrils forming peptide sequences known to play a key role in Alzheimer's and related diseases.[8] Bing Xu and co-workers have recently reported that conjugation of nucleobase and nucleoside to FF derivatives leads to a novel series of nucleopeptides which self-assemble in aqueous medium to give rise to nanofibres-based hydrogels.[9a–d] In other reports, nucleobases were conjugated to peptides and their self-assembling properties were studied.[9e] However, to the best of our knowledge, the self-assembling behaviour of DNA–dipeptide conjugates has still not been reported. The present manuscript thus describes for the very first time the highly efficient macromolecular self-assembly of DNA–peptide hybrids to scaffold hollow sphere structures with sizes in the sub-micrometer range. We further demonstrate that conjugation of the nucleotide sequence to the FF dipeptide induces a morphological transition from a fibrillar to a spherical morphology, which is expected to open new avenues to the treatment of amyloid related diseases. The DNA–peptide hybrid (**4**) was synthesised by the procedure given in ESI.† Chemical characterization by HPLC and ESI-MS evidences the synthesis of the novel DNA–peptide hybrid **4** in accordance with its macromolecular structure (Fig. 1).

Self-assembly was induced by direct dissolution in aqueous solution prior to extrusion through 0.45 micron filter membranes to reduce size and size distribution. Initial morphological characterizations were performed through Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM), which both reveal the presence of spherical morphologies of homogeneous size in the range of 200 to 300 nm (Fig. 2b–d). As compared to this, the pristine FF dipeptide forms fibrillar structures[8] (Fig. 2a). Covalent coupling of a nucleotide sequence to the FF dipeptide thus induces a drastic transition from fibrils to spherical structures. However, when the fibrils were co-incubated with the nucleotide sequence without covalent linkage to the dipeptide, a disruption of the fibrillar morphology

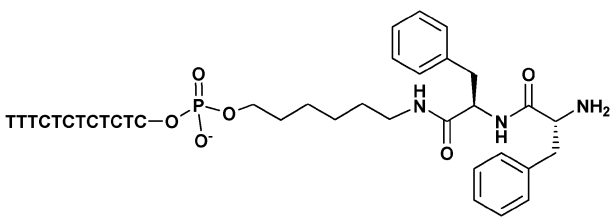

Fig. 1 Macromolecular structure of the DNA–peptide hybrid (**4**).

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† Electronic supplementary information (ESI) available: Synthesis, ESI-MS, analytical HPLC, details of sample preparation for microscopic visualisation, a proposed hypothetical mechanism of self-assembly and additional microscopic images. See DOI: 10.1039/c2cc31458d

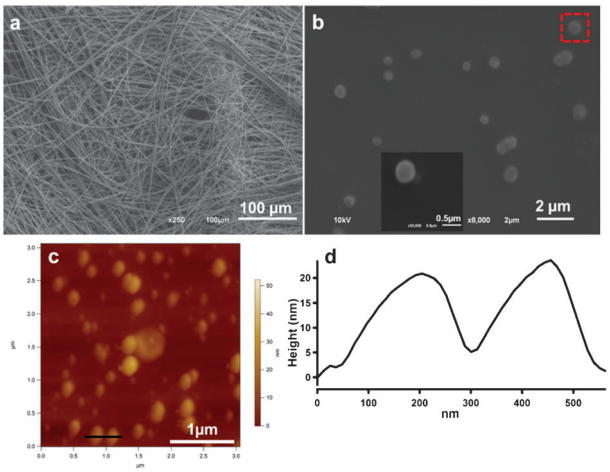

Fig. 2 Microscopic images of **4** (2 mg mL^{-1} passed through 0.45 µm filter). (a) SEM displaying the supramolecular organization of FF into fibrillar structures; (b) SEM of self-assembled peptide–nucleotide hybrids **4** (inset showing the enlarged image of an individual sphere); (c) AFM of **4**; (d) the height profile of two spheres from image (c) (17 nm and 20 nm, respectively).

could be observed but no self-assembly into discrete spherical structures (data not shown).

Specific staining of the nucleotide sequence with acridine orange, which is a nucleic acid selective fluorescent cationic dye,[10] enables to further assess the involvement of the nucleotide sequences in the structure formation of the DNA–peptide hybrid. Binding of acridine orange to the nucleotide sequence composing the DNA–peptide hybrid enables the observation of the self-assembled structures through fluorescence microscopy (Fig. 3a). Hybridization with the FITC-labelled complementary sequence of the one involved in the self-assembly further assesses that the nucleotide sequence retains its functionality after coupling to the dipeptide and subsequent to structure formation (Fig. 3b). These results therefore pave the way for the use of these materials as carriers for targeted delivery by hybridization of the oligonucleotide involved in the self-assembly with its complementary sequence labelled with a ligand of a cell surface receptor for instance.

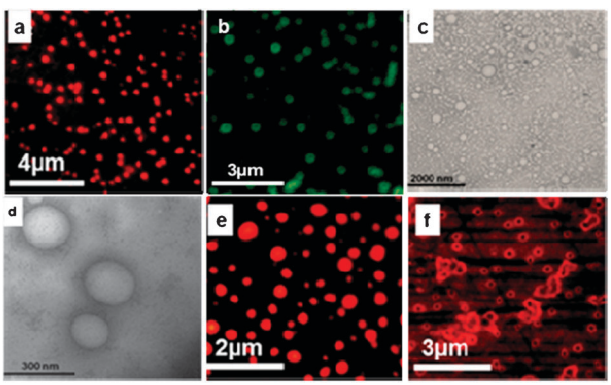

Fig. 3 Microscopic images of **4**. (a) Fluorescence microscopy subsequent to staining with acridine orange; (b) hybridization with the FITC labelled complementary sequence; (c) TEM displaying vesicular structures of **4**; (d) zoomed TEM showing that the hollow spheres have a darker periphery than the inner pool; (e) fluorescence micrograph of **4;** (f) release of the dye at pH 4.5.

From the AFM height profile and the average size of the spherical structures, a vesicular morphology can be assessed. A collapsed height of about 20 nm is in accordance with a bilayer thickness of about 10 nm in agreement with a 4.08 nucleotide long sequence (as assessed by circular dichroism)[4d] linked to the dipeptide. The hollow sphere morphology was further evidenced by Transmission Electron Microscopy (TEM). Due to the electron dense phosphate groups composing the nucleotide sequences, which naturally enhances contrast, imaging could be performed without staining. TEM revealed the presence of spherical structures with a light inner core surrounded by a dark corona (Fig. 3c and d). Encapsulation prior to pH triggered release of sulforhodamine B, a hydrophilic water soluble dye, eventually confirmed the self-assembly into vesicular structures.[11] Upon encapsulation of this dye in the inner aqueous pool of the DNA–peptide hybrid self-assembled structures, fluorescence microscopy enabled the observation of fluorescent spherical aggregates (Fig. 3e), which lose their brightness upon pH reduction from 6.5 to 4.5 (Fig. 3f) due to release of the dye from the inner pool owing to protonation of the cytosine groups composing the nucleotide sequences. Appearance of a fluorescent rim subsequent to pH triggered release of the encapsulated dye, which is due to unspecific sulforhodamine interaction with the DNA–peptide hybrids composing the vesicular wall, enables assessing further the vesicular morphology. This study of the encapsulation of a hydrophilic dye suggests the formation of a vesicular structure composed of a hydrophobic FF membrane surrounded by a nucleotide shell, the sequences pointing both towards the inner aqueous pool and the aqueous surrounding (Fig. 4).

Reches and Gazit assessed that FF aromatic interactions provide energy and directionality to form the dipeptide pleated sheet.[12] In their seminal work, they showed that FF fibres change to spherical structures when conjugated to cysteine, which has been attributed to the formation of disulphide bridges that causes aromatic stacking along the two directions to result in a more compact spherical structure.[13] Conjugation of the dipeptide to the nucleotide sequence might lead to such a structural transition from tubular to spherical structures owing to hydrogen bonding and amphiphilicity.[12,13] To examine the effect of hydrogen bonding, we co-incubated both the DNA–peptide hybrid spheres as well as the FF fibres with urea. While there was no significant change in the morphology of the FF fibres, a dramatic shift in the morphology of the spheres self-assembled from the DNA–peptide hybrid upon incubation with this denaturing agent could be observed (Fig. 5). Since urea plays

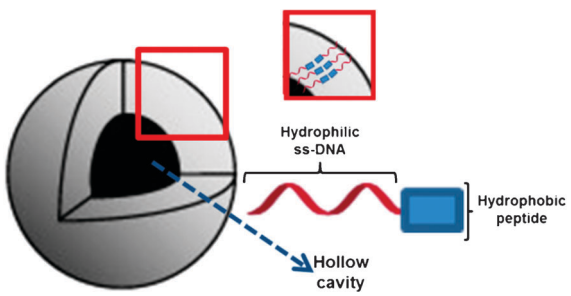

Fig. 4 Proposed structure of a vesicle self-assembled by **4** based on AFM, TEM and encapsulation studies.

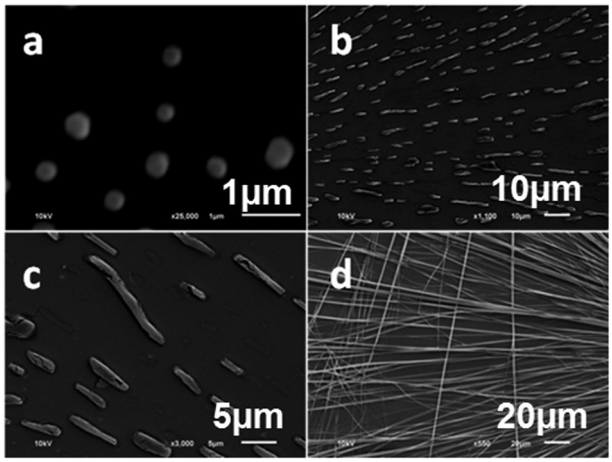

Fig. 5 (a) SEM displaying spherical structures self-assembled from the peptide–nucleotide hybrid; (b) SEM subsequent to co-incubation with urea, (c) zoomed SEM of (b), (d) SEM of FF fibrils incubated with urea.

a crucial role in inhibiting hydrogen bonding between the nucleotide sequences composing the self-assembled structures, the morphology of the spheres appeared elongated through recovering of the stacking interactions along one axis solely, which is attributed to be the cause of fibril organization by FF. Hydrogen bonds favour the formation of a bilayer-like structure of the DNA–peptide hybrid which closes then into a hollow sphere.

We thus get from these investigations an indirect evidence for the role of hydrogen bonding in inducing the morphological transition from fibrillar to spherical structures. Based on these studies we have proposed a hypothetical mechanism which is illustrated in ESI.†[14] However with these preliminary results we cannot come to any such conclusion with exact certainty. Investigations are currently in progress to ascertain the exact modes of structure formation and interaction of this novel DNA–peptide hybrid, based on these hypotheses.

In conclusion, we demonstrated that conjugation of nucleotide sequences to the FF dipeptide leads to a dramatic shift of the pristine morphology, *i.e.* a morphological transition from fibrillar to hollow spherical structures is induced. To the best of our knowledge this is the first report in which self-assembly of DNA–peptide hybrids is described, driven by both amphiphilicity and intermolecular interactions between the nucleotide sequences involved in the self-assembly. Although self-assembly of bioinspired amphiphiles is well documented in the literature,[15] the self-assembling behaviour of the hybrids is unique since the hydrophobic to hydrophilic number of residues is not balanced like for conventional amphiphiles. The spherical self-assemblies are formed spontaneously in aqueous solution. Vesicular structures efficiently encapsulate a hydrophilic dye and pH triggered release reveals their potential for application as carriers for drug delivery for instance. Future endeavours thus include further assessing their biocompatibility and their potential as delivery cargo. Morphological consequences of the grafting of oligonucleotides to the diphenylalanine dipeptide, a common structural motif of amyloid, also motivate the study of the therapeutic potential of these novel conjugates and open in the future new avenues for the treatment of amyloid related diseases.

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