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Self-aggregation of reverse bis peptide conjugate derived from the unstructured region of the prion protein†

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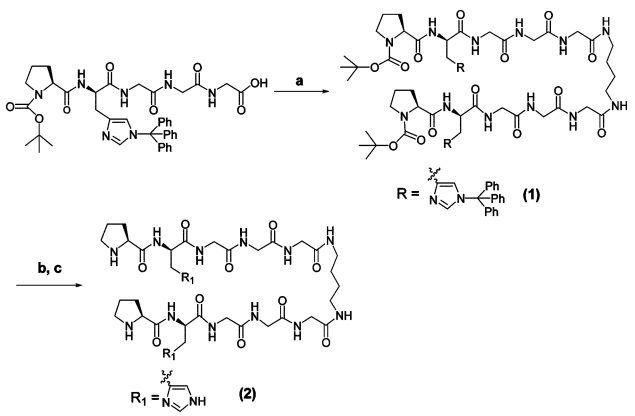
A novel bis scaffold containing a pentapeptide PHGGG, derived from the prion octarepeat, forms fibrils of nanometric dimensions, thus indicating that certain segments in the unstructured region of the prion protein may facilitate an initial fibrillation event.

Misfolding of the normal prion protein (PrP^{c}) converts it to a protease-resistant, pathogenic isoform (PrP^{sc}), which is believed to be the cause of several neurodegenerative diseases.[1] The N-terminal half of the prion protein (amino acids 29–124) contains several high affinity, evolutionary conserved, copper-binding peptide octarepeats of the sequence PHGGGWGQ,[2] which lies in the unstructured region of the protein.[3] Modeling studies of amyloid fibrillar aggregates mostly employ recombinant prion protein rich in α-helical content, which undergoes conformational transition to β-sheets.[4] Synthetic constructs used to mimic fibril formation include: di-, tri-, tetra-, and pentapeptides;[5] artificial peptides such as poly(Leu-Glu)$_n$, poly(Ala), poly(γ-methyl-L-glutamate) grafted-polyallylamine and an oligopeptide (L-Leu-L-Lys)$_8$.[6]

Herein, we report a novel scaffold based on the bis-conjugate of a pentapeptide **PHGGG**, derived from the prion octarepeat, and its propensity to form fibrils of nanometric dimensions. To the best of our knowledge, this is the first report describing macromolecular aggregation of the truncated prion octarepeat sequence present in the unstructured, N-terminus region of prion protein. Solution phase methods were used to prepare the protected pentapeptide *t*-Boc-L-prolyl-N^{im}(trityl)-L-histidyl-glycyl-glycyl-glycine which was then conjugated with 1,4-diaminobutane (DAB), followed by deprotection to afford the bis-pentapeptide, (PHGGG)$_2$DAB (**2**) (Scheme 1). **2** was characterized by standard spectroscopic techniques and the fibril formation was monitored by multiple microscopic techniques *viz.* transmission electron microscopy (TEM)‡, optical microscopy (OM)§, and atomic force microscopy (AFM)¶ (see supporting information).



a). HOBt, DCC, 1,4-diaminobutane, DCM, yield 61%; b). 95% TFA, 3h, RT, 80%; c). Strong anion exchange resin.

Scheme 1 Synthesis of bis-pentapeptide motif.

† Electronic supplementary information (ESI) available: experimental section. See http://www.rsc.org/suppdata/cc/b3/b316793c/

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TEM images of 7 days aged solution of **2** displayed formation of tubular filaments of ~10–12 nm cross-sectional diameter. The existence of tubular fibrils, network and fibrillar tangles was observed in several grid regions (Fig. 1, A–C). This dimension is comparable to the reported cross-sectional diameter of amyloid fibrils.[5d,7] Interestingly, a freshly prepared solution of **2** and a weeklong incubation of pentapeptide methyl ester (data not shown) indicated sparse and thicker fibrils (~200 nm), while extensive aggregation was conspicuous by its absence (Fig. 2A). Circular dichroism (CD) spectral studies with **2** indicate a predominantly random coil structure and a complete lack of β-sheet structural features. In line with the existing nucleation/seeding theory of prion protein, it can be surmised that hydrogen bonding induces intermolecular self-assembly in **2** and upon aging, these assemblies culminate in fibrillar, mesh-like structures bearing a resemblance to prion aggregates.

Congo Red binds to Aβ protein aggregates to provide a diagnostic test for amyloid deposits, as the dye-stained plaques appear green in color under cross-polarized light due to birefringence.[8] Aged fibrils of **2** displayed green birefringence under cross-polarization as evidenced by optical microscopic investigations (Fig. 3). Similar observations have been reported for other synthetic models of amyloid protein.[6ab,7a,9] However, Congo Red in a binding assay is rather promiscuous as it binds to other self-assembled peptide structures as well.[10] Therefore, in the absence of sheet-like morphology, analyzed by CD, this conjugate can be considered to model the aggregational behavior of the prion protein.

This was further studied by using AFM, a technique routinely used to study ultrastructural details of biomolecular architectures.[11] AFM characterization of freshly prepared solution of **2** revealed uniformly distributed, punctated structures with an average thick-

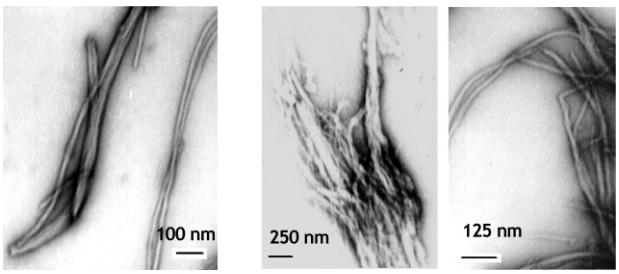

Fig. 1 TEM images for seven days aged peptide solution (1 mM), (A) individual filaments; (B) bundles; (C) mesh.

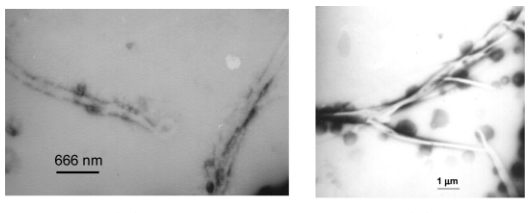

Fig. 2 TEM images of freshly prepared **2** (A) and seven days aged bis (HGGG)$_2$DAB.

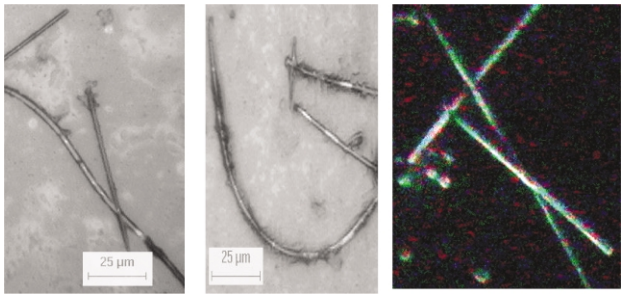

Fig. 3 Congo Red staining of aged **2** solution: fibrils and green birefringence.

ness of 6–7 nm (Fig. 4A), while aging of this solution resulted in a highly clustered morphology and the aggregates so formed displayed a mean thickness of ~250 nm (Fig. 4B). This indicates rapid self-assembly of peptide conjugate and a time-dependent aggregation to yield distinctive plaque-like structures.

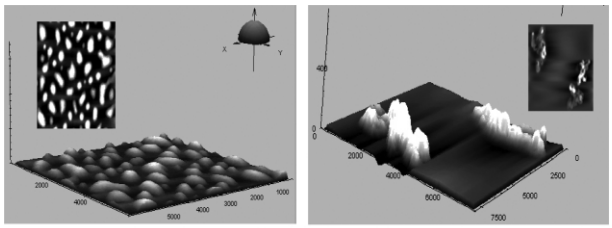

Fig. 4 AFM images of (A) freshly prepared, and (B) seven days aged solution of **2** (1 mM).

The N-terminal proline residue was deleted in **2** resulting in a new tetrapeptide conjugate, $(HGGG)_2DAB$ (see supporting information). Interestingly, this tetrapeptide did not exhibit aggregation and even a weeklong incubation only revealed fibers (~150–200 nm) similar to the freshly prepared solution of **2** (Fig. 2B). This suggests the crucial presence of terminal prolines contributing to the overall stability and aggregation of **2** *via* interchain, non-bonded contacts. Expectedly, the lack of side chains in glycine and net positive charge on histidine imidazole rings, prevent peptide aggregation. In a different context, proline-aided interactions leading to self-assemblies are shown to be critical for triple helical collagen stability.[12]

The fact that tethered peptide sequences from prion octarepeat can self-assemble strongly points towards a possible scenario where certain segments in the unstructured region of the prion protein may independently facilitate an initial fibrillation event, which is otherwise attributed to the C-terminus of the protein.[13] It is curious to note that mammalian octarepeats undergo pH-dependent folding by adopting loop and β-turn like secondary structures, embedded within octarepeat sequences.[14]

In summary, we have synthesized **2** derived from the cellular prion octarepeat sequence and studied the morphology of the aggregates formed through non-covalent self-assembly of **2**. It is expected that our results will provide an impetus for a closer scrutiny of conserved sequences within the prion protein and Aβ peptide acting as mediators/initiators for the formation of protofilaments, fibrillogenesis and aggregation.

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Notes and references

‡ Transmission electron microscopy: 7 days aged solution of **2** (8 μL, 1 mM) was transferred onto Formvar coated TEM grids and dried. Grids were stained with 2% uranyl acetate, dried and examined under a JEOL 2000FX-II electron microscope, at an operating voltage of 100 kV.
§ Optical microscopy: Congo Red solution (2 μL, 150 μM) was added to 7 days aged solution of **2** (98 μL, 1 mM) and the mixture was left for 6 h at room temperature. 50 μL of this solution were transferred on to a glass slide, dried and then viewed under optical microscopy (AX10 Lab, Zeiss) with cross-polarized light (500×).
¶ Atomic force microscopy: 7 days aged solution of **2** (10 μL, 1 mM) was transferred to a freshly cleaved mica piece, followed by uniform spreading of the sample with the aid of a spin-coater. The mica piece was dried for 30 min followed by AFM imaging (Molecular Imaging, USA).

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