**Use of Short Amyloidogenic Peptides for Nanotechnology**

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**Abstract**

Amyloid fibers, often associated with several human degenerative diseases (such as Alzheimer's and Parkinson's diseases) may also have physiological roles, having even been suggested as potential novel biomaterials [1]. In general, amyloid fibers share a common β-sheet rich architecture that is behind their exceptional stability, mechanical strength and resistance to degradation, rendering them excellent nanomaterial candidates [1,2]. The potential to form amyloids (and other protein/peptide aggregates) can be predicted from the peptide sequence [1, 3].

Here, we used atomic force microscopy (AFM), circular dichroism (CD) and Fourier transform infrared spectroscopy (FTIR) to evaluate which among three possible amyloidosis models (peptides with sequence QVQIIE, ISFLIF and GNNQQNY) would form characteristic amyloid fibrils at physiologic pH conditions. FTIR and CD were used to determine the structural conformation of the amyloid fibrils formed. The CD spectra of QVQIIE are presented in **Figure 1A.** These spectra demonstrate that this peptide sequence can acquire a β-sheet conformation characteristic of amyloid fibrils. However, at the pH and temperature conditions tested, the GNNQQNY peptide forms structures with a random coil conformation (**Figure 1A**), inconsistent with classical amyloid fibril morphology. Due to lower solubility, ISFLIF was prepared at a lower concentration than the other peptide sequences. As at those concentrations the structural characterization of the peptide by CD is not feasible, FTIR was used instead (**Figure 1B**). It is clear that the ISFLIF peptide acquires β-sheet conformation at physiologic pH, with a characteristic band around 1635 $cm^{-1}$ (classical β-sheet peaks are found in the 1623-1640 $cm^{-1}$ region [4]). From these results it is clear the peptide GNNQQNY, in the conditions tested, does not acquire a β-sheet conformation typical of amyloid fibrils. The peptides were further investigated via their abilities to bind Congo Red (a dye commonly used to detect amyloid fibrils in solution [5]). In line with the studies of secondary structure, GNNQQNY does not bind Congo Red. Congo Red binding assays of ISFLIF and QVQIIE are presented in F**igure 1C**. In the presence of amyloid fibrils, Congo Red absorbance spectrum changes, resulting in a maximal spectral difference at 540 nm [5]. QVQIIE spectra did not suffer these characteristic maximal spectral differences, indicating that amyloid fibrils are not formed at the pH and temperature incubation conditions. ISFLIF, however, suffers a clear shift, indicative of amyloid structure. Having established the peptides secondary structure content, AFM was employed in the subsequent studies, as it is a microscopic technique very useful for the study of amyloid fibrils because it allows the imaging of surfaces with high resolution and sensibility (**Figure 1D**). Negative and positive controls behaved as expected. GNNQQNY does not form amyloid-like structures, while QVQIIE shows structures that are not consistent with classic well-structured mature amyloid fibrils. Regarding ISFLIF, this peptide clearly forms an amyloid fibril structure, similar to the positive control. AFM-based morphological characterization of the ISFLIF amyloid fibrils (**Figure 1E-F**) shows that these fibrils have and average diameter of 159.6 ± 3.2 nm and 10.0 ± 0.1 nm of average height. ISFLIF amyloid fibrils formed under these physiological conditions of pH and temperature seem to be similar to standard amyloid fibrils, constituting promising biomaterials [1, 2].

AFM, CD, FTIR and Congo Red data, taken together, indicate the peptide ISFLIF as the most reproducible and amenable peptide for developing amyloid-based nanotechnology approaches, in line with previous work [2, 3], where short amyloidogenic peptides are sought for nanotechnology applications.

**References**

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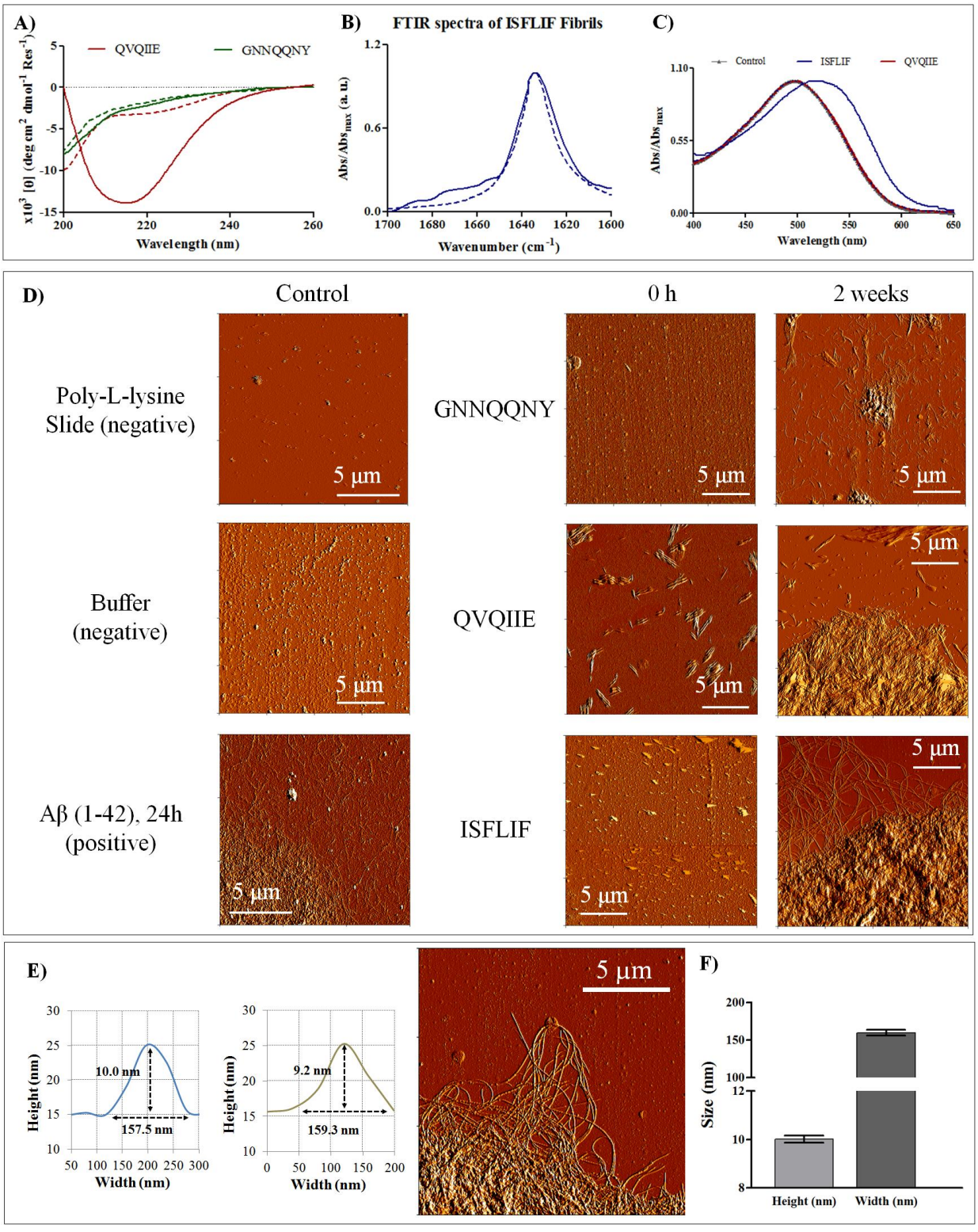


**Fig. 1. Amyloidogenic properties of three short peptide sequences, GNNQQNY, QVQIIE and ISFLIF, tested at physiological conditions of pH and temperature.** (**A**) Far UV CD spectra of GNNQQNY and QVQIIE (dotted line: 0h of incubation; continuous line: 2 weeks of incubation). (**B**) FTIR spectra of ISFLIF in the amide I region (dotted line: 0h of incubation; continuous line: 2 weeks of incubation). (**C**) Congo Red binding assay of QVQIIE and ISFLIF. GNNQQNY shows spectra with no binding, similar to QVQIIE (data no shown) (dotted line: 0h of incubation; continuous line: 2 weeks of incubation). (**D**) AFM study of the three peptides morphology (center and right columns), displaying also positive and negative controls (left column). (**E**) Study of ISFLIF amyloid structure, imaged by AFM. (**F**) Height and width of ISFLIF amyloid fibrils, determined by AFM cross-sections (N = 120).