Modulating the antigen density on the surface of peptide nanofibrils by molecular co-assembly

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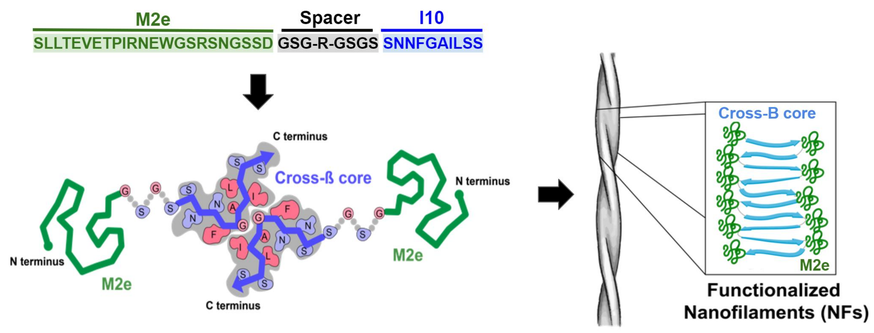
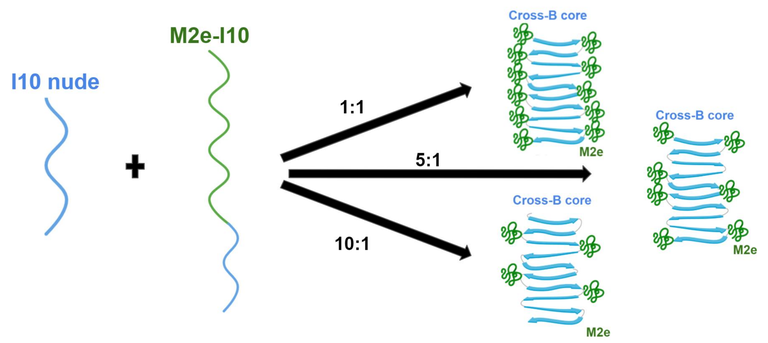
Introduction

- Peptides with the ability to self-assemble into defined nanoparticles have gained increase interest for the design of antigen delivery platform for subunit vaccines [1].
- By modulating the primary sequences and the self-assembly conditions, the shape, size and surface chemistry of the supramolecular structures can be precisely modulated, opening to a diversity of immunological functionalities [2].
- We recently reported that nanofilaments assembled from a short 10-mer amyloidogenic sequence (I10) derived from the islet amyloid polypeptide (IAPP) constitute promising assemblies suitable for anchoring antigenic determinants and increasing their immunogenicity [1].
- In the present study, we took advantage of non-covalent molecular self-assembly to integrate different densities of antigens on the fibril surface in a controlled manner by adjusting the stoichiometry of the different building blocks.



Methodology

- The M2e epitope derived from the matrix 2 protein of the influenza virus was conjugated to I10 by a flexible short linker on solid support to obtain M2e-I10.
- Chimeric M2e-I10 peptides were assembled in presence of different molar ratio of I10 under continuous rotary agitation in Tris buffer, pH 7.5.
- Structural conversion of the soluble peptides into cross-β-sheet filaments was followed by thioflavin T (ThT) and anilinonaphthalene-8-sulfonic acid (ANS) fluorescence, circular dichroism spectroscopy and atomic force microscopy (AFM).
- The density of the M2e epitope accessible on the fibril surface was evaluated by the enzyme-linked immunosorbent assay (ELISA).
- The capacity of the cross-β-sheet assemblies to activate the Toll-like receptor 2 (TLR2) was evaluated using HEK-Blue-hTLR6/TLR2 cells that have a NF-κB-inducible reporter gene SEAP (secreted embryonic alkaline phosphatase).



Results

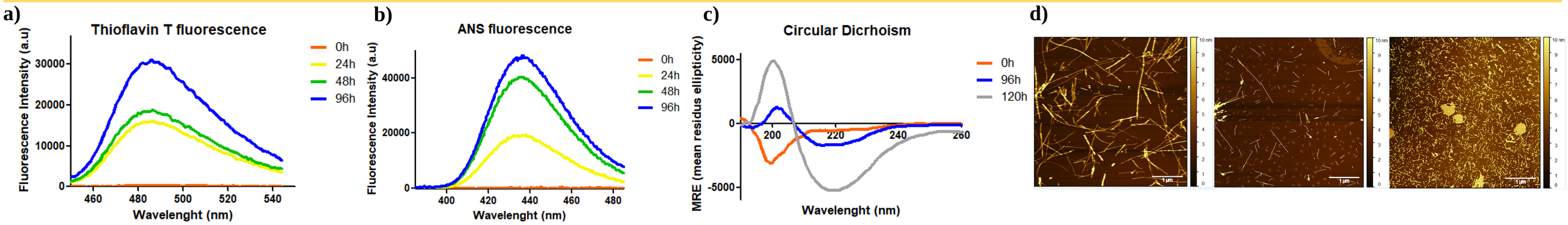


Figure 1: Biophysical characterization of the co-assembly of I10 and M2e-I10 (10:1) into nanofilaments. Kinetics of the self-assembly was evaluated by (a) ThT fluorescence, (b) ANS fluorescence, (c) and circular dichroism spectroscopy. (d) Representative AFM images of the nanofilaments assembled from I10 (left), M2e-I10 (right) and at the molar ratio of 10:1 (I10:M2e-I10), assembled for 96 h under continuous rotation.

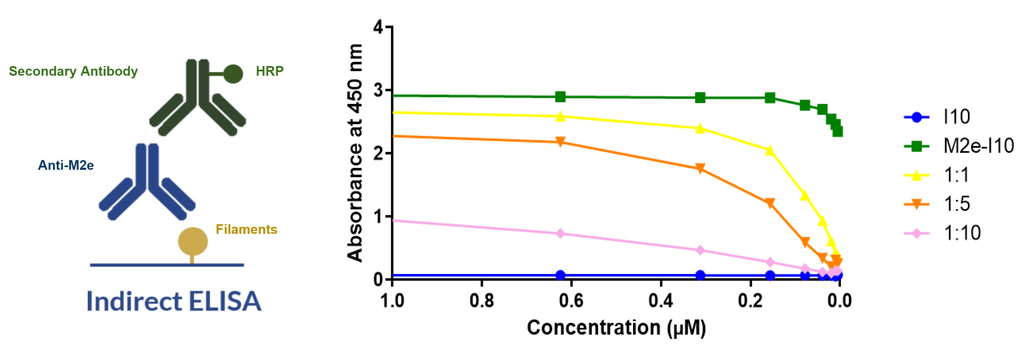


Figure 2 : Co-assembled nanofilaments show different antigens densities on their surface. Nanofilaments were absorbed at different concentrations to the bottom of a 96-well plate, then the density of the antigen exposed on the surface was monitored by ELISA, using an anti-M2e antibody (14C2) diluted to 1/500 as a primary antibody, and a anti-IgG coupled to HRP diluted to 1/10 000.

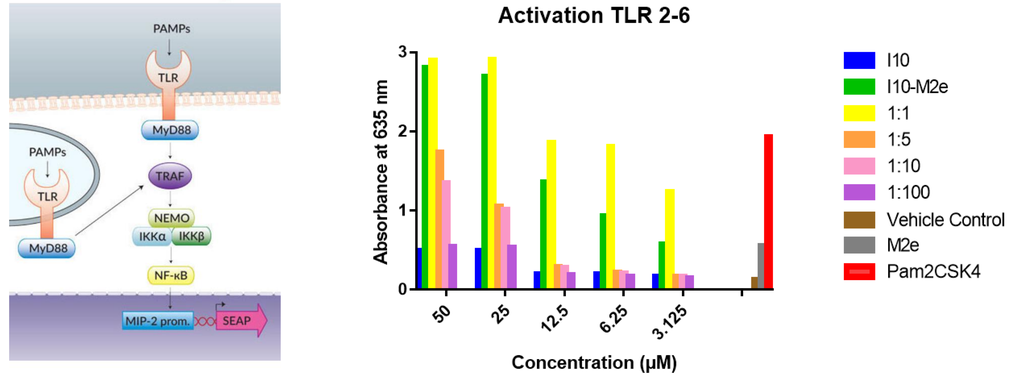


Figure 3 : Nanofilaments activate the innate immune receptor TLR2/6. HEK-Blue hTLR2-TLR6 cells were stimulated for 16h with increasing concentrations of nanofilaments (ranging from 3.125 to 50 µM), 100 µg/ml M2e peptide, 100 ng/ml Pam2CSK4 or with the vehicle control (50 mM Tris buffer pH 7.5). NF-kB-induced SEAP activity was quantified using HEK-Blue detection medium and spectroscopy at 635 nm.

Conclusion

This study indicates that the density of a given epitope and other bioactive molecules on the nanofibril surface can be precisely controlled through molecular co-assembly, ultimately fine regulating the amplitude of the immune responses.

References

[1] Zottig, X., et al., (2020). Protein Supramolecular Structures: From Self-Assembly to Nanovaccine Design. Nanomaterials (Basel).10(5).

[2] Zottig, X., et al., (2019). Guiding the Morphology of Amyloid Assemblies by Electrostatic Capping: from Polymorphic Twisted Fibrils to Uniform Nanorods. Small, 1901806. doi:10.1002/smll.201901806

Acknowledgments