دانشگاه علوم پزشکی و خدمات بهداشتی،درمانی کرمانشاه
Kermanshah University Of Medical Sciences

ULTRA-EFFECTIVE INHIBITION OF AMYLOID FIBRIL ASSEMBLY BY NANOBODY-GOLD NANOPARTICLE CONJUGATES

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Impact Factor 2019: 4.031 | Citations 2019: 15,877

Bioconjugate Chemistry

Communication

Ultra-Effective Inhibition of Amyloid Fibril Assembly by Nanobody-Gold Nanoparticle Conjugates

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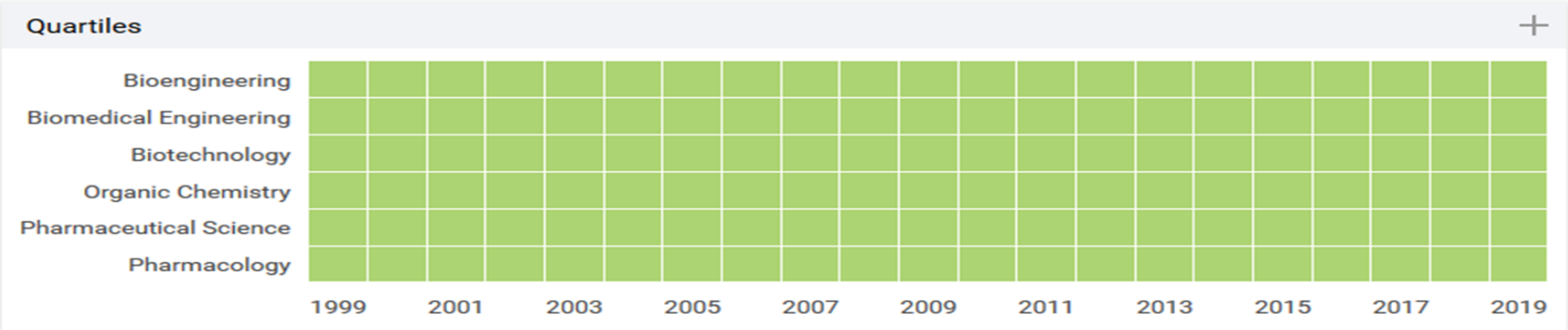
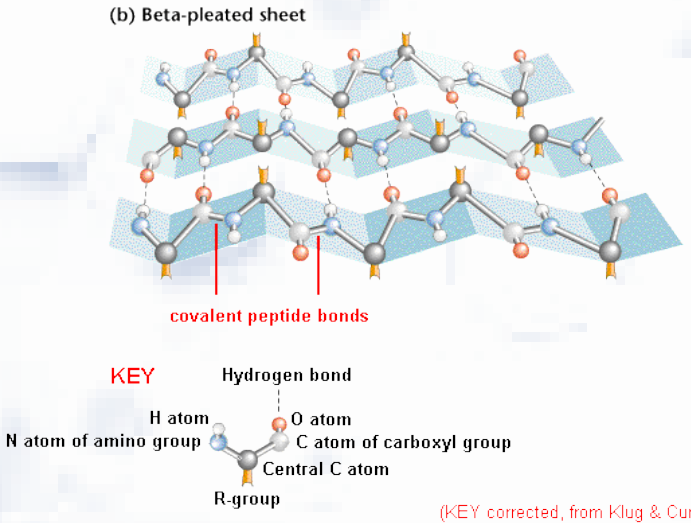
Bioconjugate Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.bioconjchem.8b00797 • Publication Date (Web): 18 Dec 2018



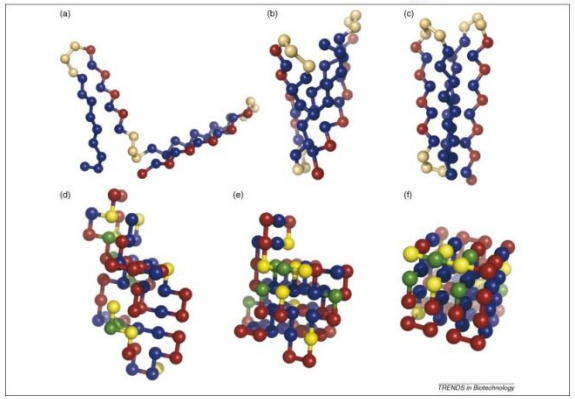
Introduction

The misfolding and aggregation of peptides and proteins into βsheet-enriched amyloid fibrils is linked to many human diseases, including Alzheimer's disease, Parkinson's disease, and Type 2 diabetes.

A number of strategies have been applied to the development of inhibitors for the amyloid fibril formation.

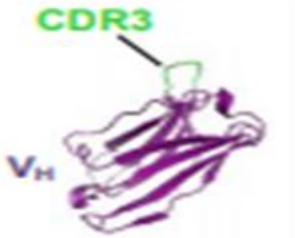


(KEY corrected, from Klug & Cummings 1997)



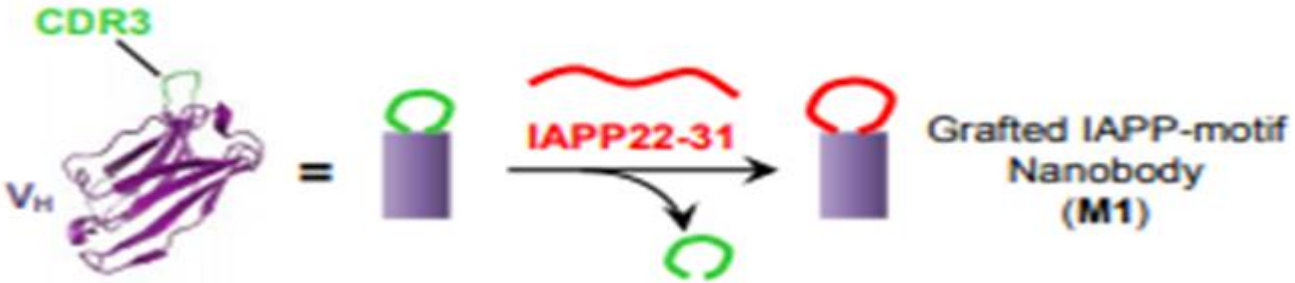
Introduction

- Many inhibitors, ranging from small molecules to peptides and proteins, are effective only at high or near stoichiometric concentrations.
- Improved inhibitors, as well as facile and generally applicable inhibitor development strategies are in great need.
- Nanobody, or single domain antibody, has recently emerged as a promising candidate for inhibitors of amyloid fibril formation.



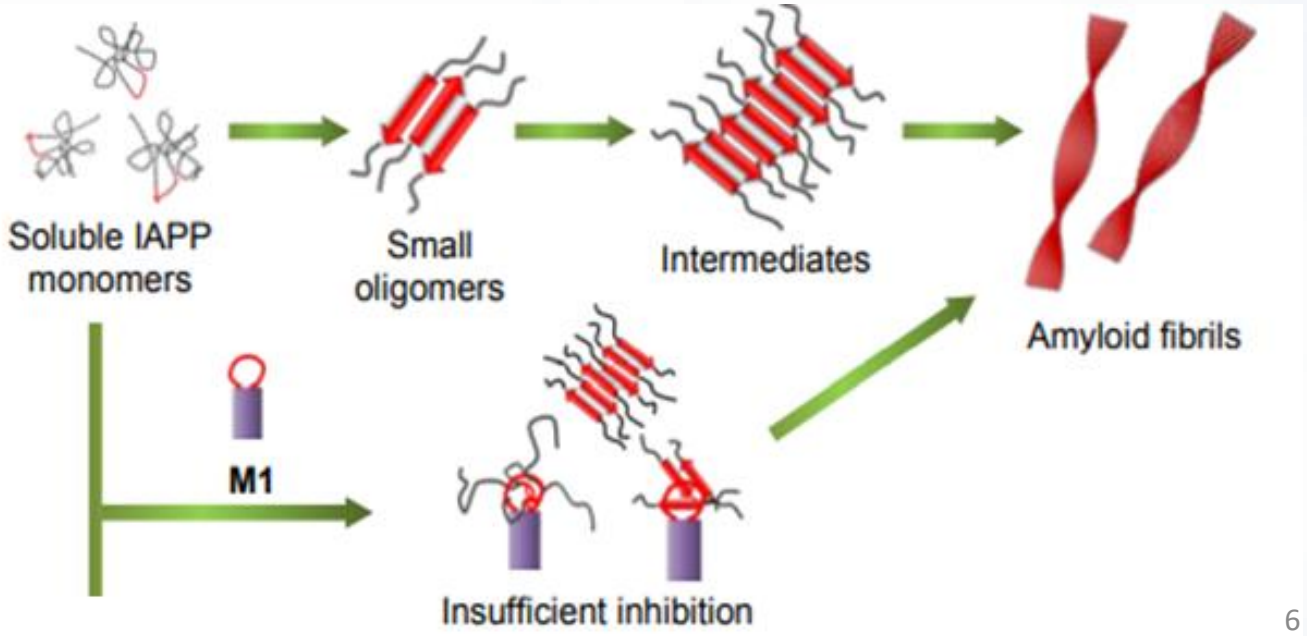
Introduction

- A nanobody inhibitor is generated by grafting a small segment of an amyloidogenic peptide into the third complementarity determining region (CDR3) of a parent single domain antibody.

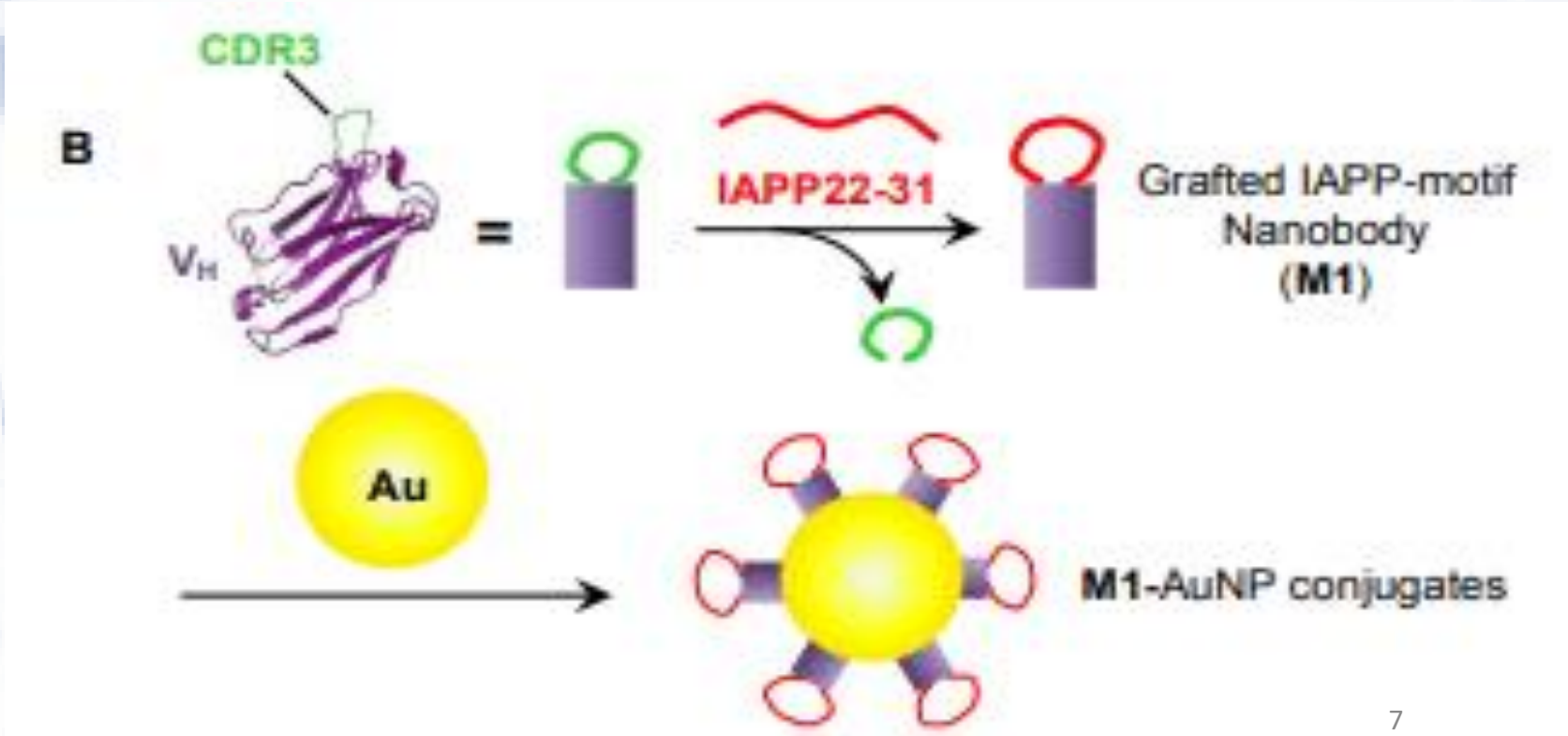


- It inhibits fibril assembly of the peptide through the homotypic interactions between the grafted motif on the nanobody and the same motif on the peptide.

- As a result, the nanobody can inhibit the aggregation of the peptide at low stoichiometric concentrations (1:10 nanobody inhibitor:peptide molar ratio).
- Unfortunately, further decreasing the stoichiometric concentrations of nanobody inhibitors eliminates their anti-aggregation capabilities.



➢Here, we demonstrated a convenient approach to boost the inhibition potency of nanobody inhibitors, by simply conjugating the inhibitors with gold nanoparticles (AuNPs).



Materials & methods

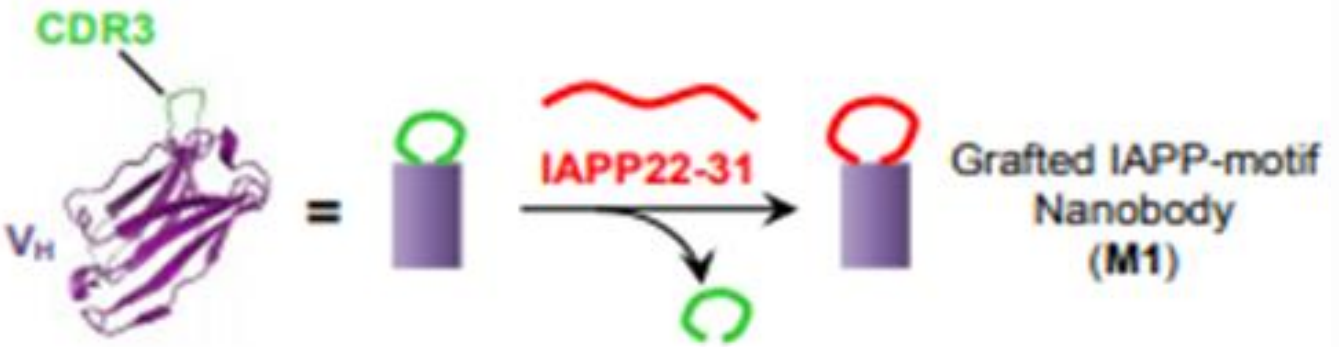
❑ M1-AuNP conjugates preparation

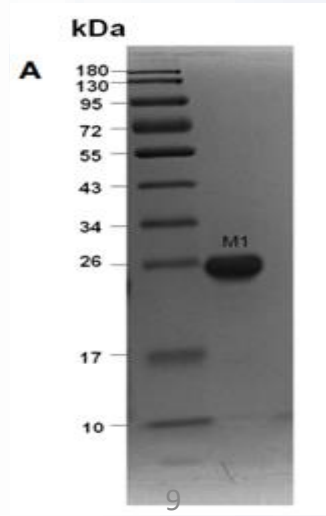
- We use human islet amyloid polypeptide (IAPP)
- IAPP is highly amyloidogenic and aggregates promptly in vitro, and the development of effective inhibitors of IAPP amyloid formation is particularly challenging.

Materials & methods

❑ M1-AuNP conjugates preparation

➢Grafting a 10-residue peptide segment of IAPP (22-NFGAILSSTN-31) into the CDR3 region of the heavy chain variable domain (VH) of a human antibody generates a grafted nanobody inhibitor of IAPP, or M1, that is highly pure, well expressed (>25 mg/L), and stable in its folded state

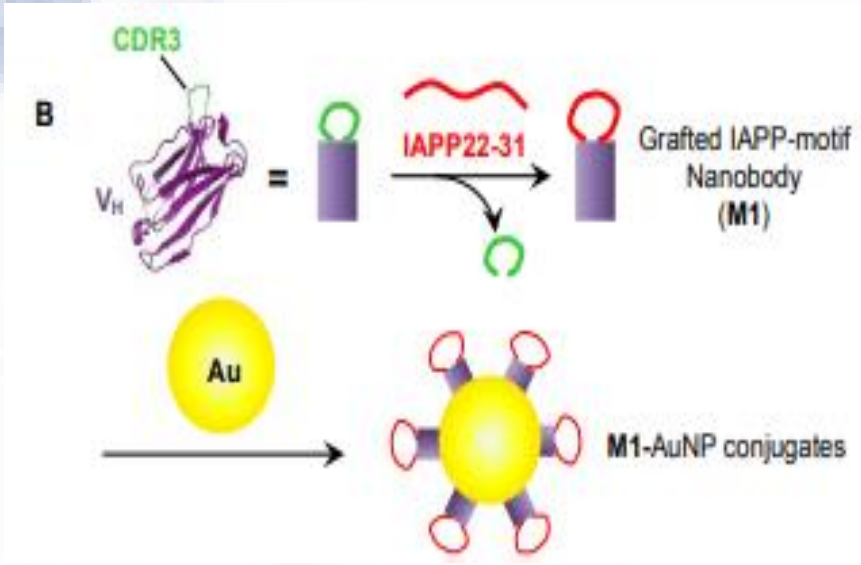


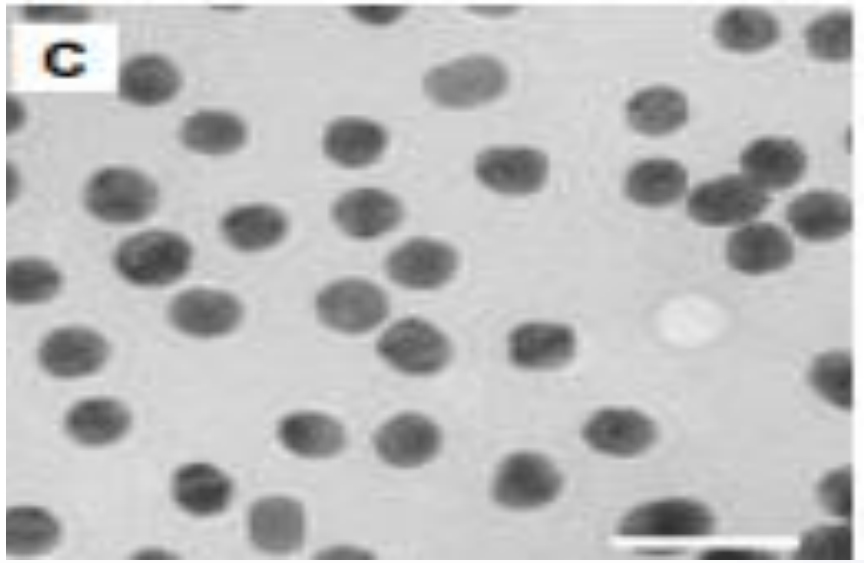


Materials & methods

❑ M1-AuNP conjugates preparation

➢Co-incubating M1 and citrate-covered AuNPs (15 nm in ter) yields M1-AuNP conjugates that are well dispersed in aqueous environments with uniform particle sizes.

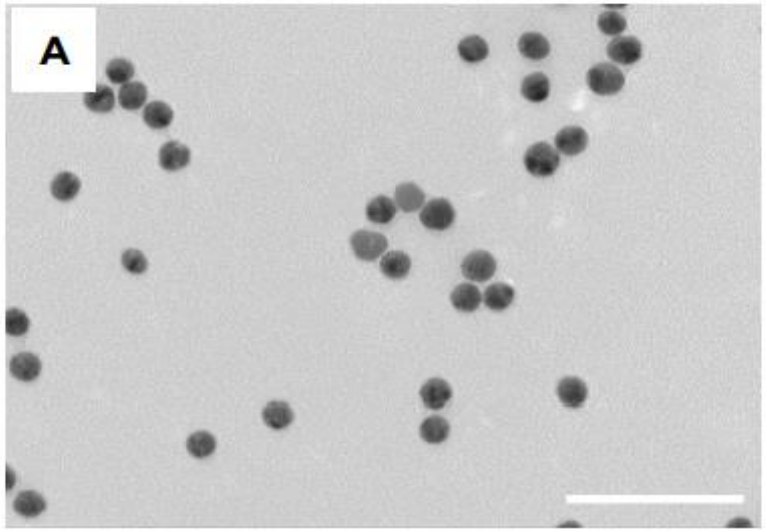


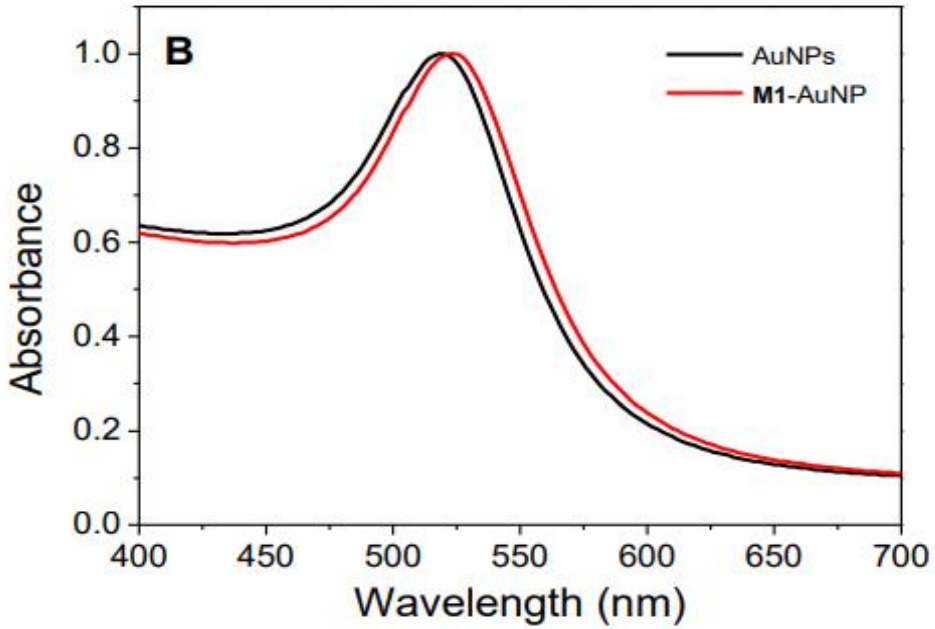


C) TEM image of the M1-AuNP conjugates. Scale bar: 50 nm.

Materials & methods

- M1-AuNP conjugates preparation & characterization





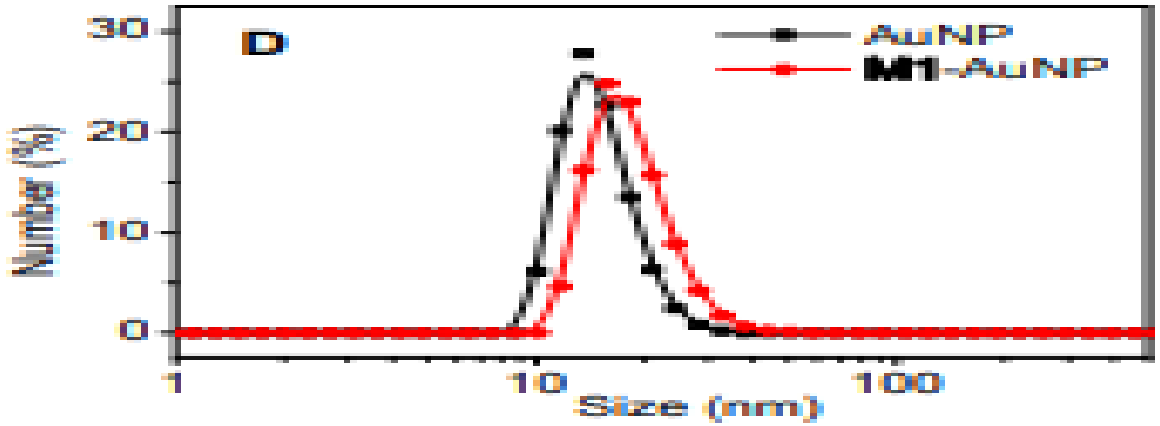
Characterization of AuNPs and M1-AuNP conjugates.

A) TEM image of AuNPs. Scale bar: 100 nm.

B) UV-Vis measurement of AuNPs and M1-AuNP conjugates.

Materials & methods

- M1-AuNP conjugates preparation & characterization
  - The dynamic light scattering (DLS) measurements show that the nanoparticle diameter increases from15 nm to 18 nm after conjugation.



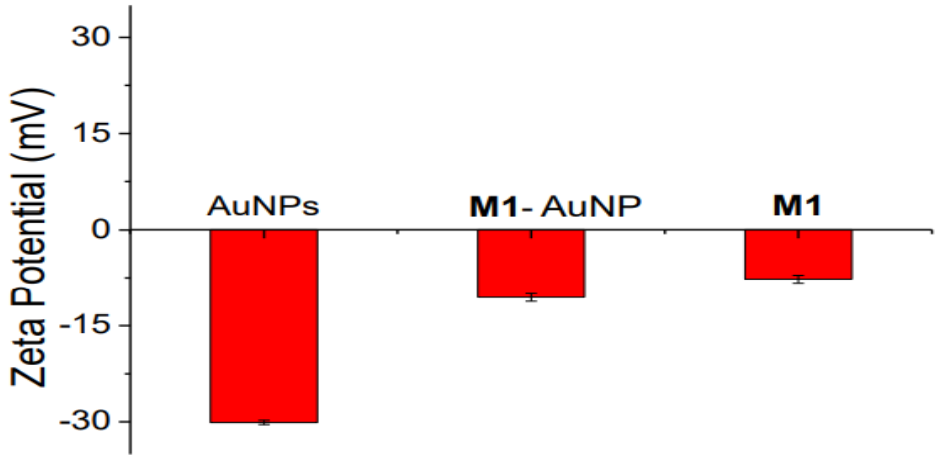
DLS evaluation of the size of AuNPs and M1-AuNP conjugates.

Dynamic light scattering (**DLS**) is a technique in physics that can be used to determine the size distribution profile of small particles in suspension or polymers in solution

Materials & methods

❑ M1-AuNP conjugates preparation & characterization

- ➢ The zeta potential of AuNPs changes from -30 mV to -10 mV , further indicating the formation of a protein layer on the Au surface.
- ➢ The number of M1 immobilized per AuNP is approximately 143 ± 12, determined by quantifying the free fluorescin-labe

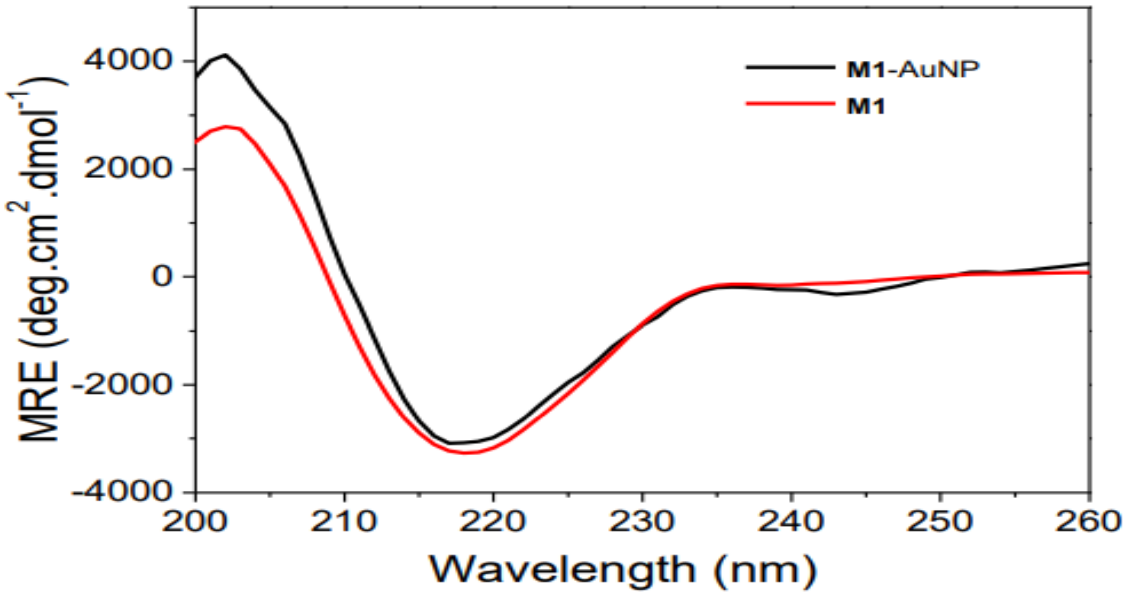


Zeta potential of AuNPs, M1-AuNP conjugates and free M1.

Materials & methods

❑ M1-AuNP conjugates preparation & characterization

➢The far-UV circular dichroism (CD) spectra between M1-AuNP conjugates and M1 are almost identical, suggesting that the conformation of M1 is persistent in the M1-AuNP conjugates.

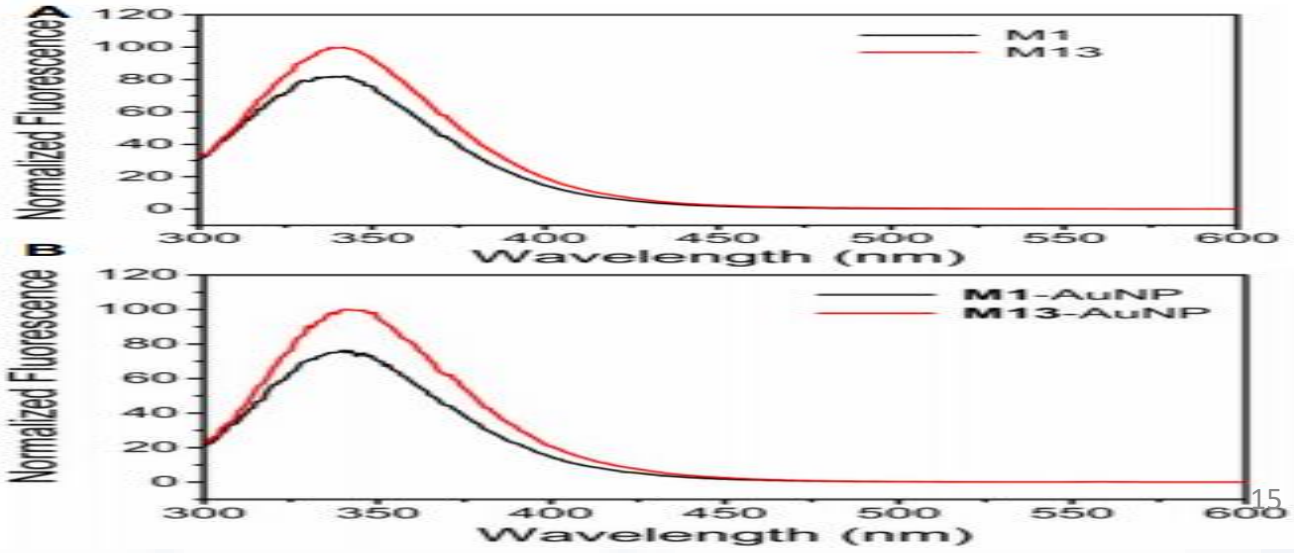


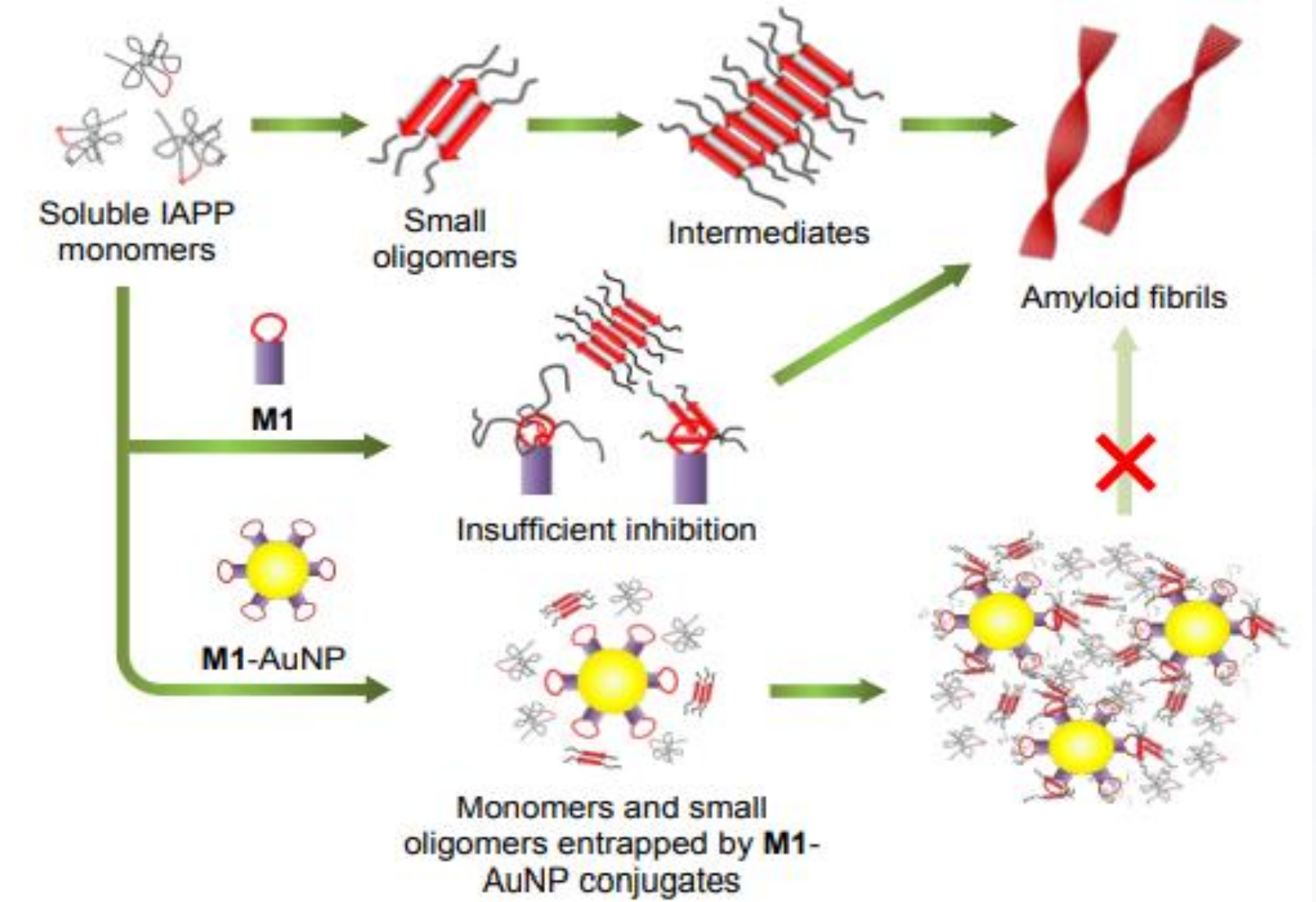
CD spectra of M1 and M1-AuNP conjugates.

Materials & methods

❑ M1-AuNP conjugates preparation & characterization

- ➢to confirm that the grafted region of M1 is solvent-exposed in the conjugates, we have constructed another nanobody M13, the CDR3 of which is grafted by a fluorescent peptide segment.
- ➢The relative fluorescence intensity of M13 to M1 almost keeps unchanged before and after conjugation , **indicating that the CDR3 region of the nanobody is accessible to the target epitopes after conjugation.**



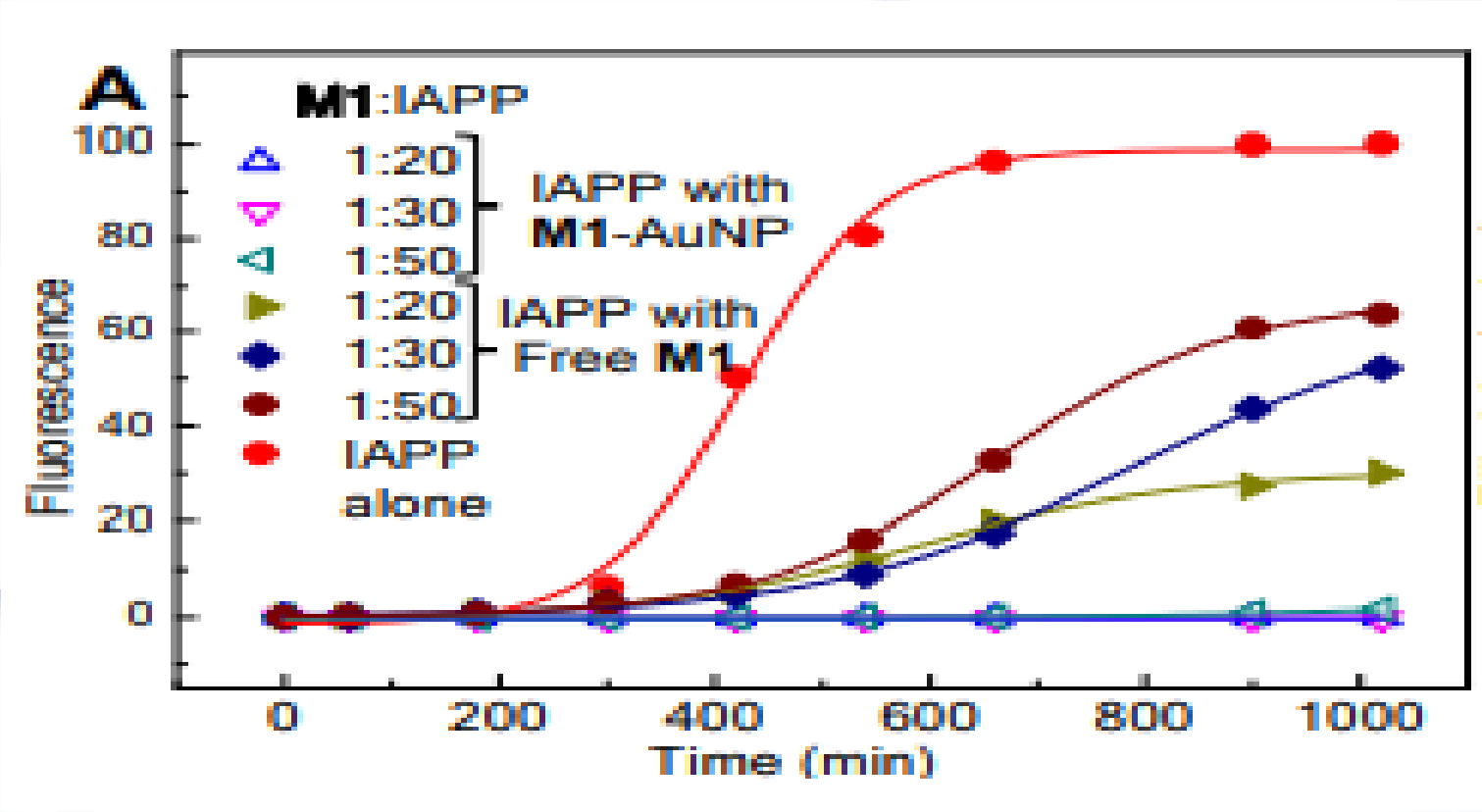


Representative scheme of hypothetic inhibition mechanism of ultra-low stoichiometric concentration of free M1 and M1-AuNP conjugates on IAPP fibrillation process.

Result and Discussion

- M1-AuNP conjugates evaluation

  - Thioflavin-T (ThT) fluorescence assays monitored the kinetics of amyloid formation

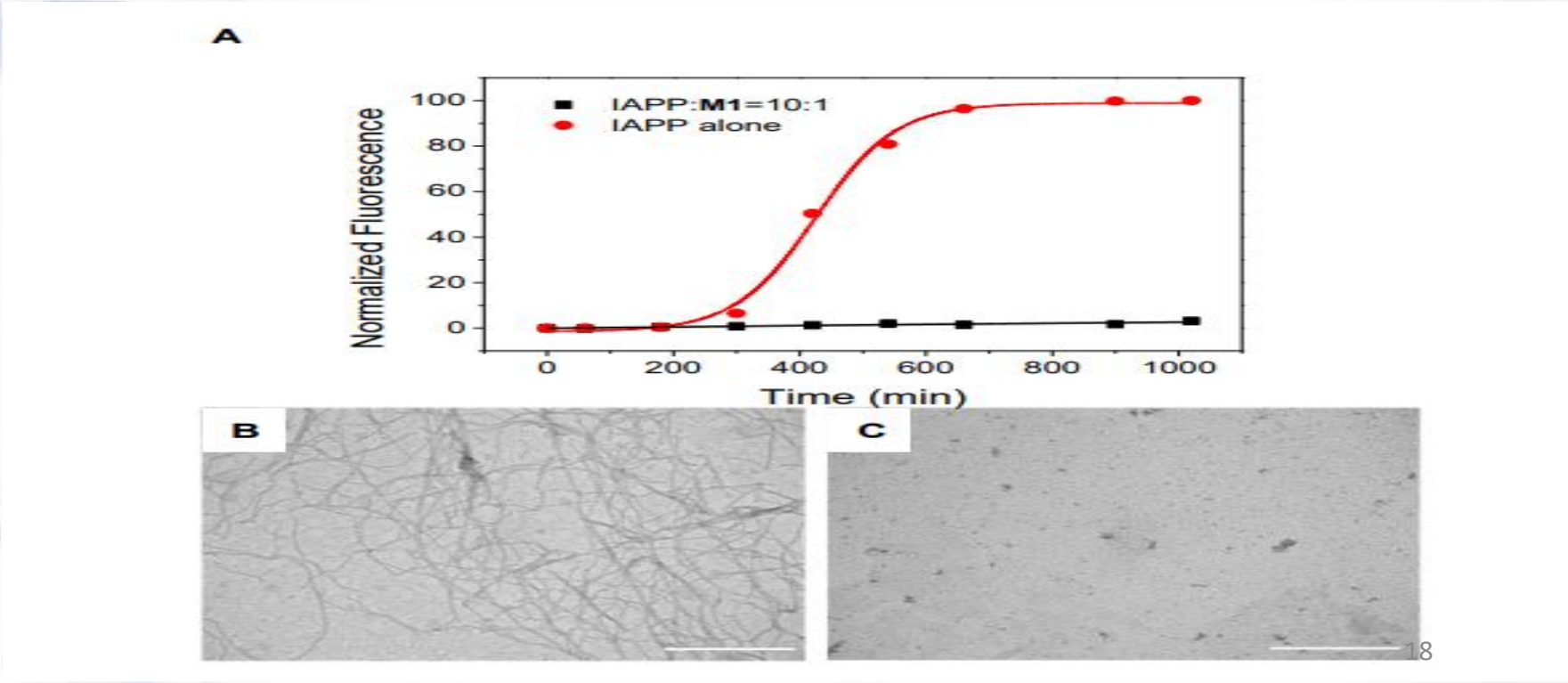


Kinetics of IAPP amyloid fibrillar assembly monitored by ThT assays. IAPP concentration in all experiments: 16 µM.

Materials & methods

❑ M1-AuNP conjugates preparation & characterization

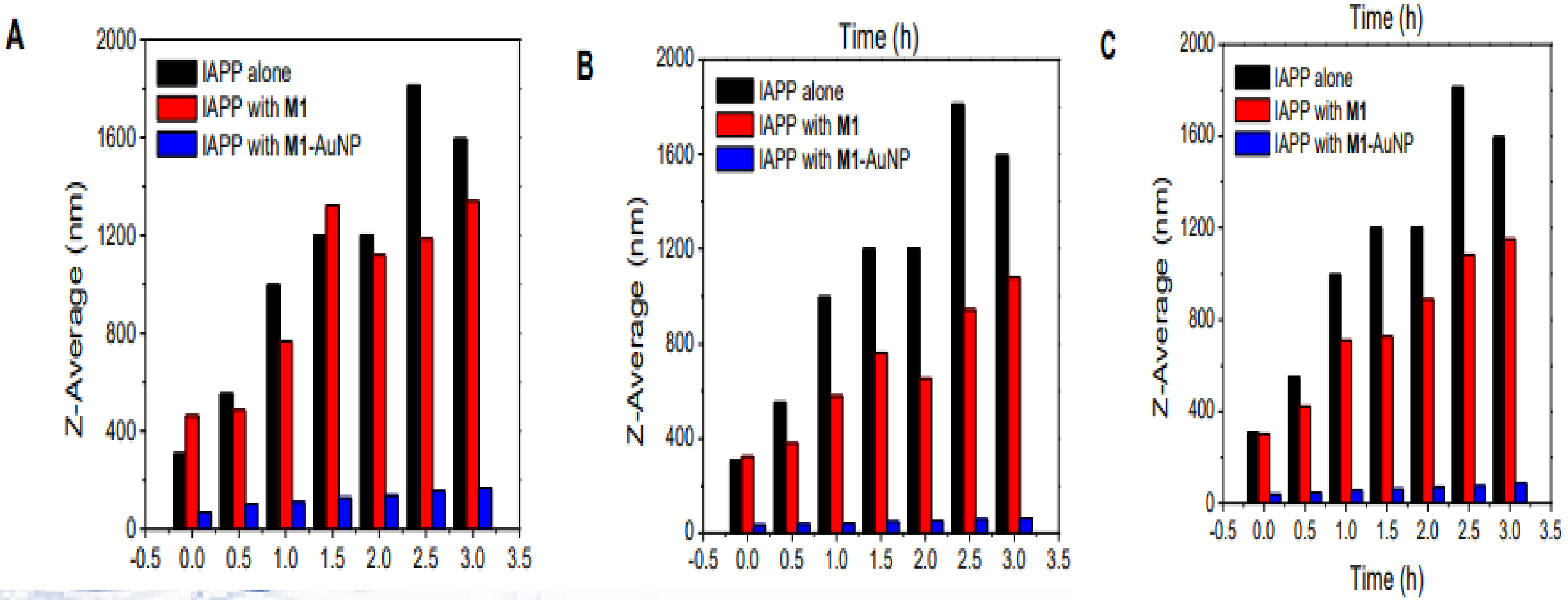
➢When the M1:IAPP molar ratio reaches 1:10, the amyloid fibril formation can be completed inhibited during the experiment time, consistent with the literature result.



Materials & methods

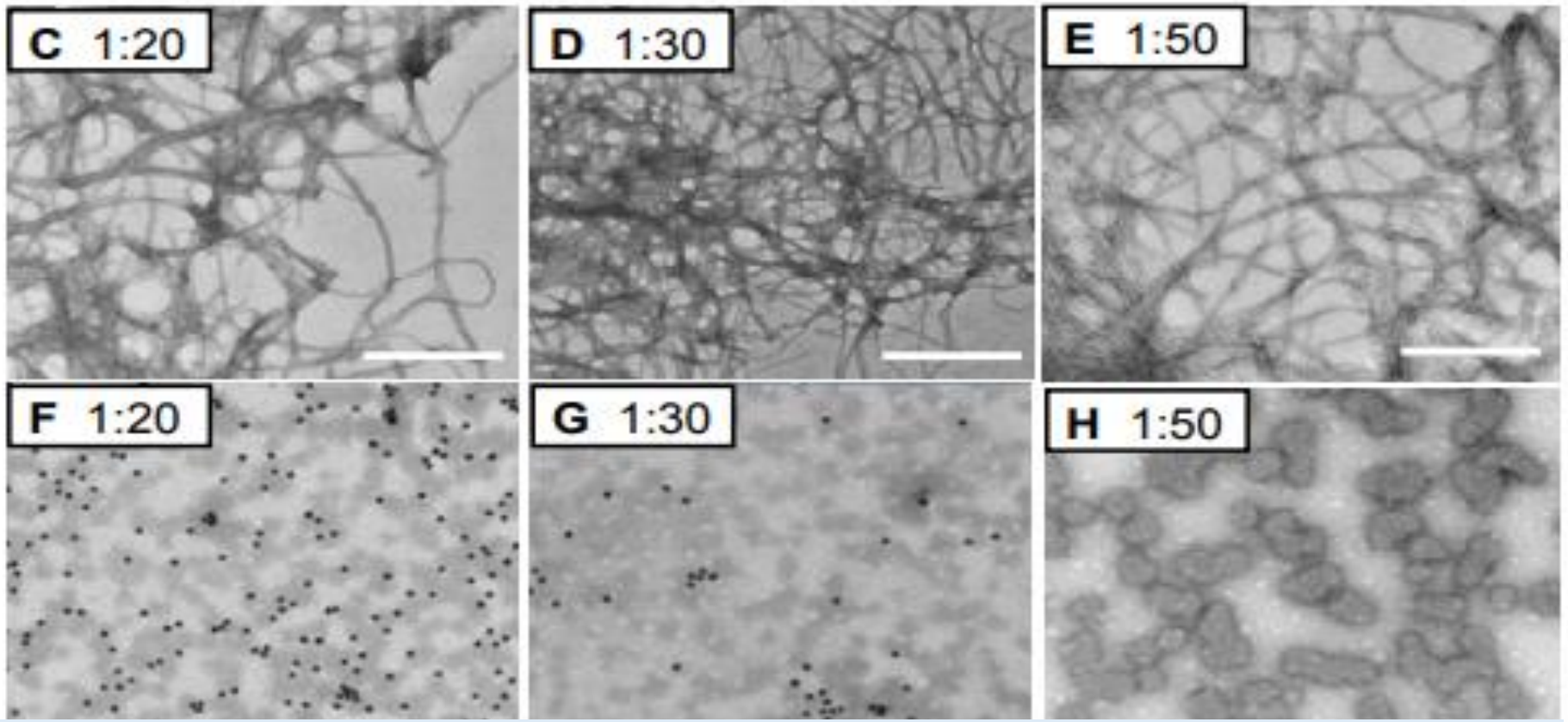
❑ M1-AuNP conjugates preparation & characterization

- we have employed DLS to monitor IAPP aggregation kinetics , the results of which are consistent with those of the ThT assays. IAPP alone or in the presence of free M1 aggregate very fast, whose hydrodynamic size increases to micron level within hours. However, adding M1-AuNP conjugates keeps the particle sizes under 200 nm during the experiment time.



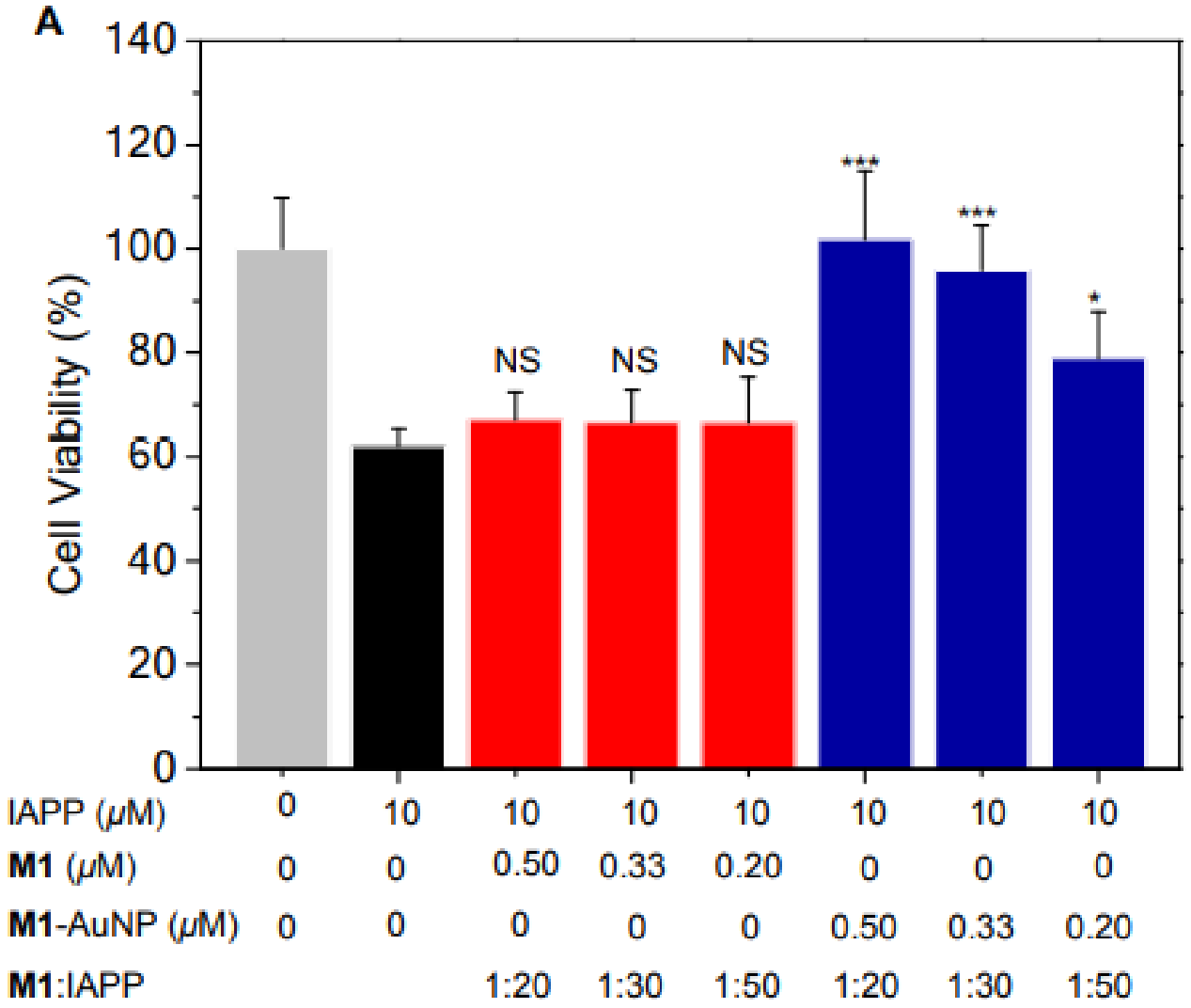
IAPP aggregation kinetics monitored by DLS with or without M1/ M1-AuNP at different time points. M1:IAPP molar ratios: (A) 1:20; (B) 1:30; (C) 1:50. The concentration of IAPP in all samples is 16 µM.

➢We further confirmed the inhibitory effect of the conjugates by recording TEM images of the aliquots at the end of the ThT kinetics experiments



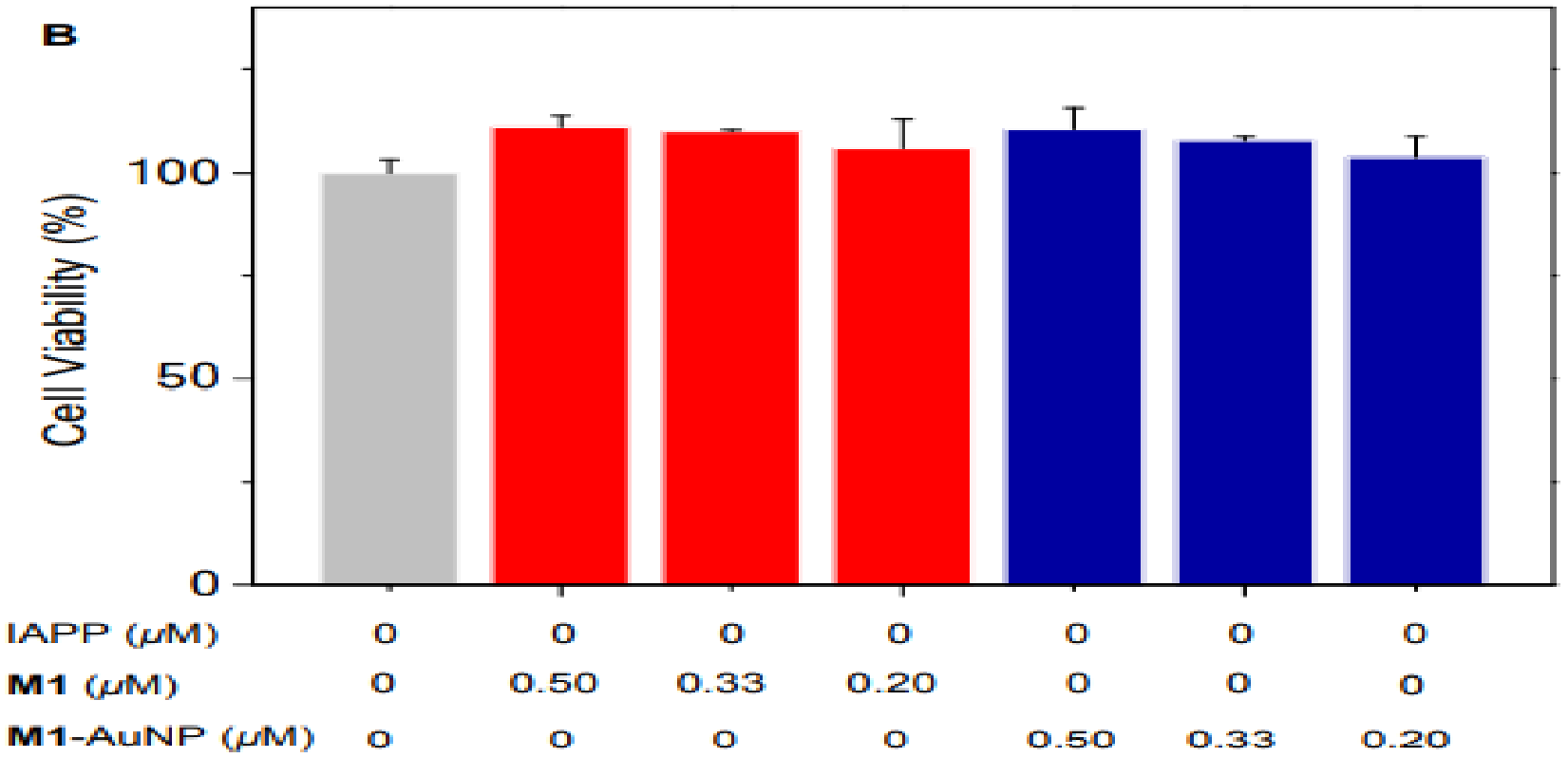
C-H) TEM images of IAPP samples at the end of the kinetics experiments. C-E: IAPP with free M1; F-H: IAPP with M1-AuNP conjugates. Scale bars of all TEM images: 500 nm.

The biocompatibility of M1-AuNP conjugates



A) Reduction of IAPP cytotoxicity by free M1 and by M1-AuNP conjugates

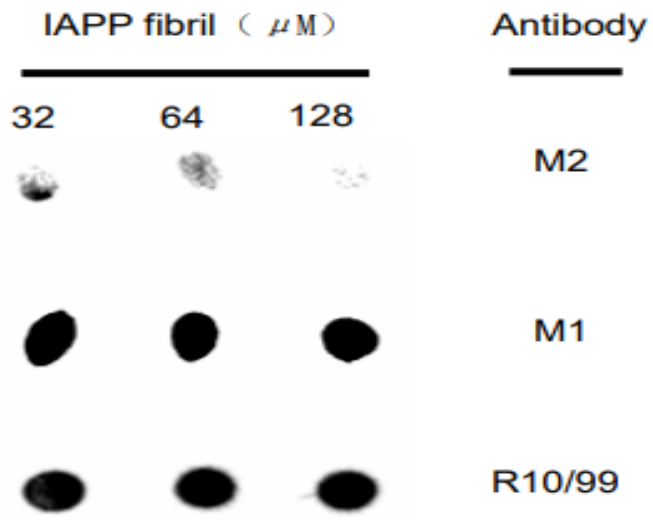
Biocompatibility evaluation of M1 and M1-AuNP conjugates



- For M1-AuNP, concentrations are based on M1 in M1-AuNP. Cell viability was measured by MTT assays
- **We conclude that M1-AuNP conjugates not only are nontoxic themselves, but also can substantially reduce IAPP-induced cytotoxicity by generating benign discrete nanostructures.**

Validation of the sequence-specific homotypic interaction

- To validate the sequence-specific homotypic interaction between IAPP and M1-AuNP conjugates, we have also prepared the conjugates between AuNP and a nanobody grafted with a non-IAPP targeting peptide sequence of α-synuclein 69-78 (M2-AuNP). Immunoblot results demonstrate that IAPP binds specifically to M1 but not to M2.



Immunoblotting analysis of the binding activity between IAPP and antibodies.

Conclusion

- The composition of the discrete nanostructures and the detailed inhibition mechanism of the M1-AuNP conjugates have not been clearly determined yet.
- Our design concept is expected to be generally applicable for developing a wide variety of highly potent and selective amyloid fibrillation inhibitors. Ongoing experiments include expanding this strategy to other disease-related amyloid inhibitor development, as well as optimizing the inhibitor efficacy by this strategy

- Thanks For Your Attention