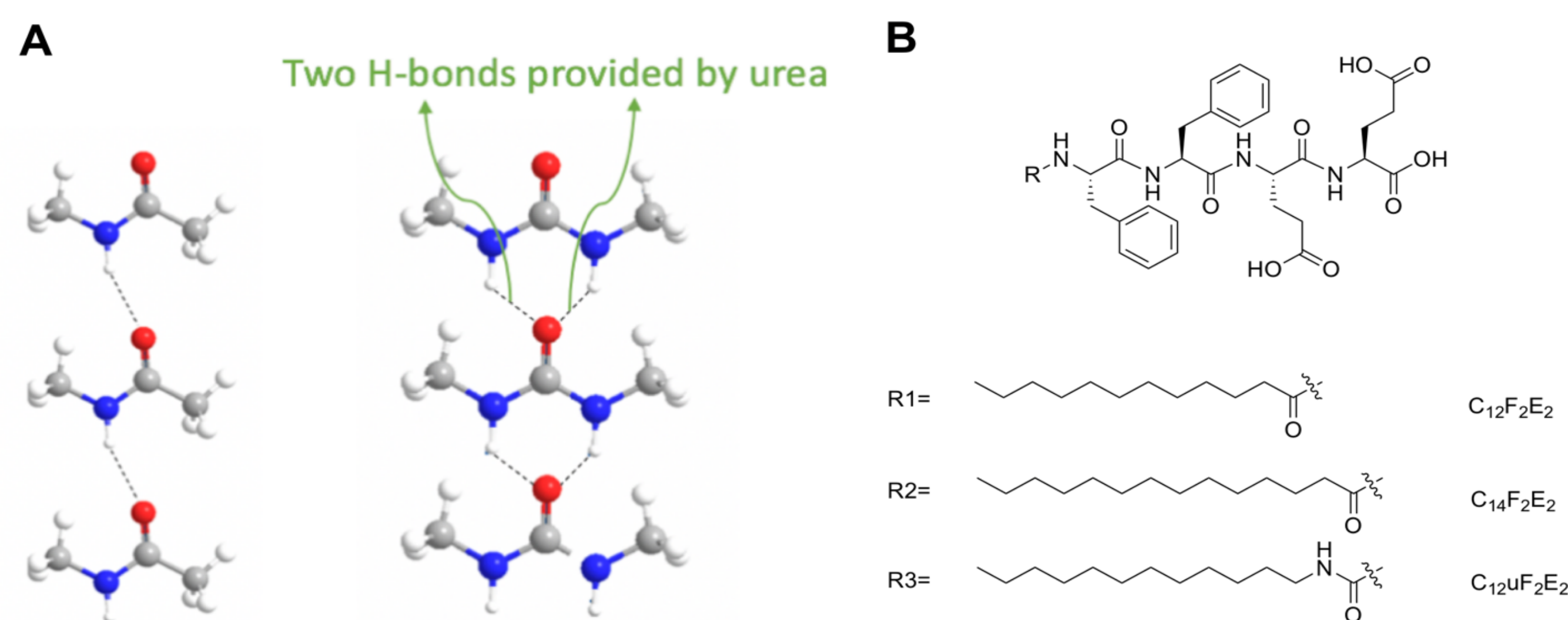


Exploring the Role of Urea Substitutions on Stabilizing Phenylalanine-based Peptide Amphiphiles Nanostructures

Huihua Xing,^[a] Agustín S. Picco,^[b] Cristián Huck-Iriart,^[c] Stacey Chin,^[d] Sieun Ruth Lee,^[d] Jeffrey Comer,^[e] Samuel I. Stupp,^[d,f] Martin Conda-Sheridan^{[a]*}

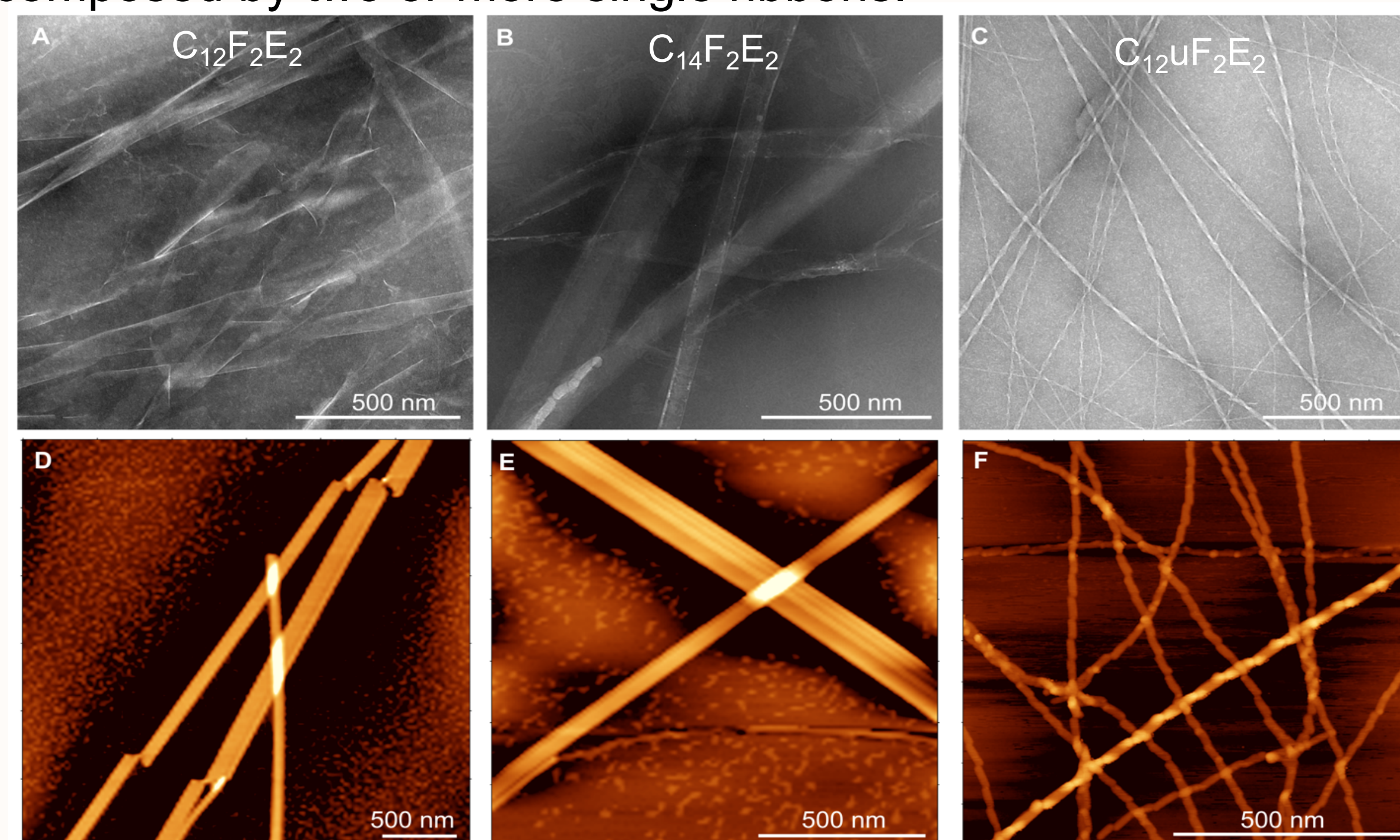
Introduction: Hydrogen bonding plays a critical role in the self-assembly of peptide amphiphiles (PAs).¹⁻³ Herein, we studied how to tune the macromolecular nanostructures properties by manipulating the PA's chemical structure with urea, a hydrogen bond donor. Herein, we designed and synthesized three PAs: urea modified PA, original PA and longer hydrophobic tail PA with peptide sequence FFEE. Our hypothesis is that the presence of the urea group, will enhance the stability of the formed nanostructures due to the additional hydrogen bond.



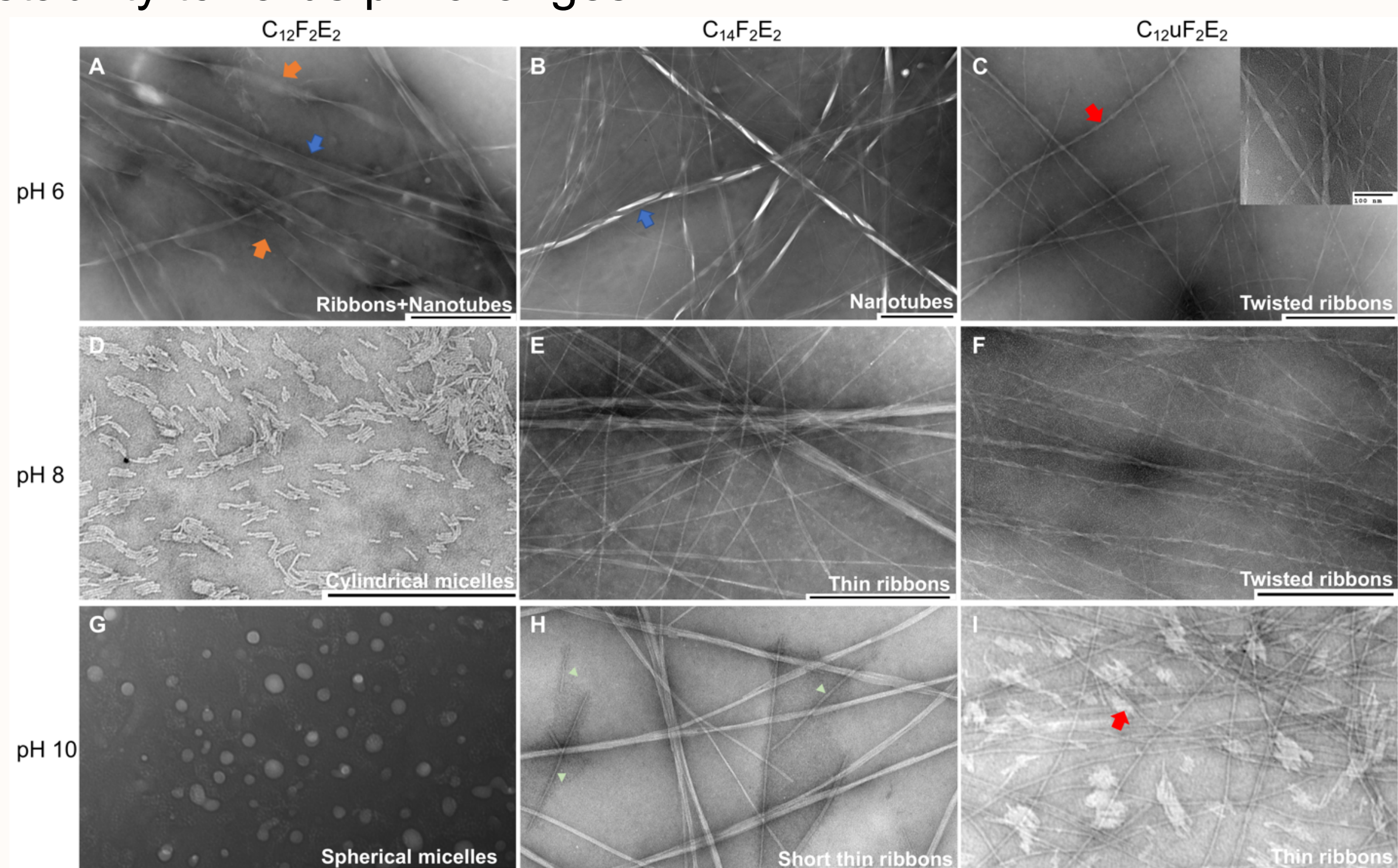
Methods The effect of pH and temperature on nanostructures were studied by Transition Electron Microscopy, Atomic Force Microscopy, Circular Dichroism and Small-Angle X-ray Scattering. Computational modeling revealed the packing and mechanism of PA-assembled nanostructures in the atomic level.

Results From these studies, we found that the urea motif can change PA's morphology and secondary structure at neutral pH, while enhance the physical stability against pH and temperature changes. We clearly showing that there are greater numbers of hydrogen bonds for the PA with urea motif than for the others by a factor of 1.4. Furthermore, the urea-phenyl interaction makes the peptide portion more compact than it is in the absence of urea.

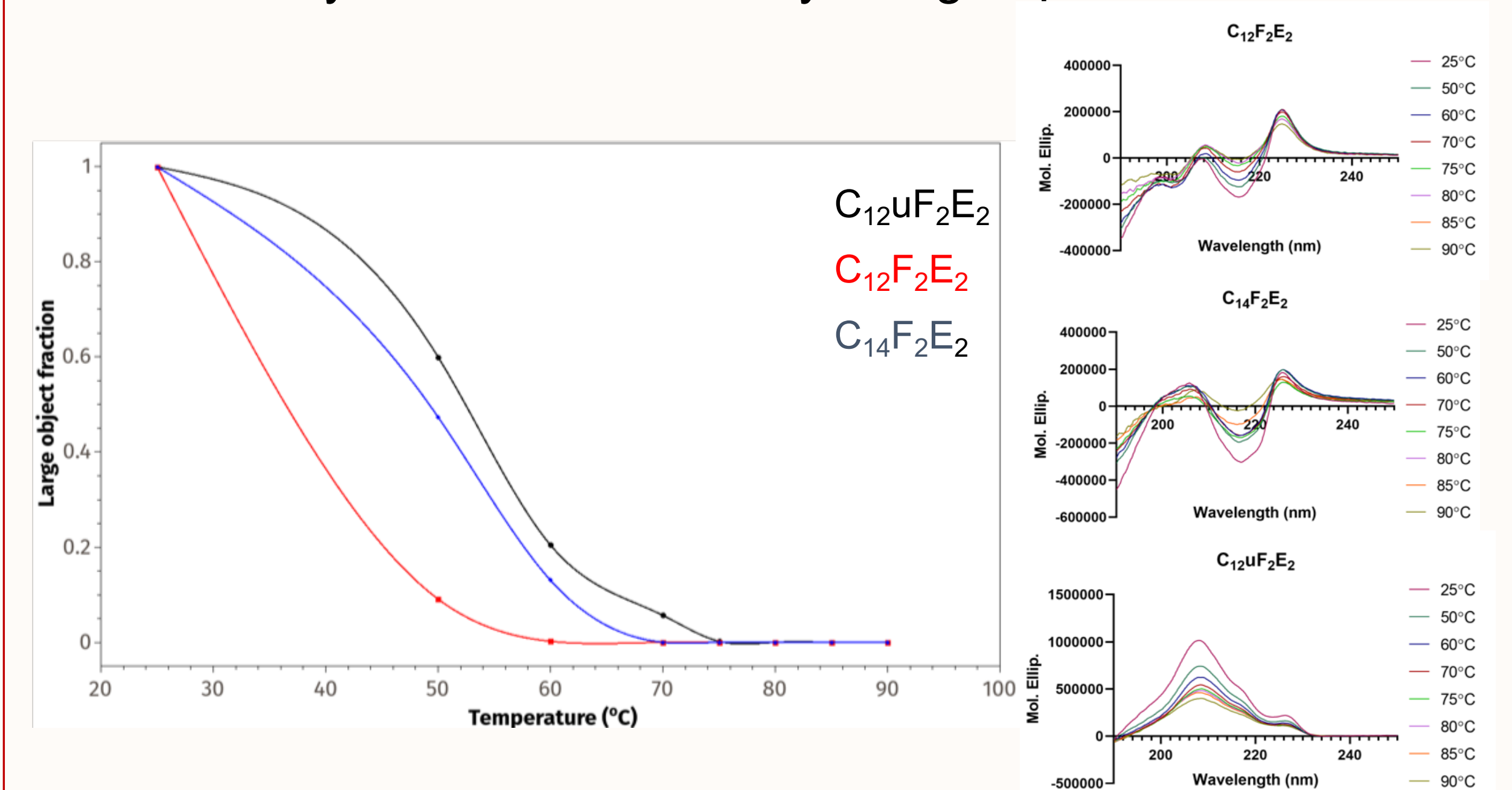
1. PA synthesis and nanostructure characterization C₁₂uF₂E₂ formed highly twisted ribbons (average width=19.76 ± 2.88 nm) indicating the effect of the urea motif in the self-assembled morphology, while the others formed nanobelts or nanotubes. composed by two or more single ribbons.



2. pH effect on nanostructures C₁₂uF₂E₂ behaved differently from the other PAs, maintaining the twisted ribbons from pH 6 to pH 10. The data indicates the urea group provides enhanced stability towards pH changes.



3. Thermostability studies on PA nanostructures The most important observation is that C₁₂uF₂E₂ shows better stability against temperature changes than the added hydrophobic effect introduced by the additional methylene groups.

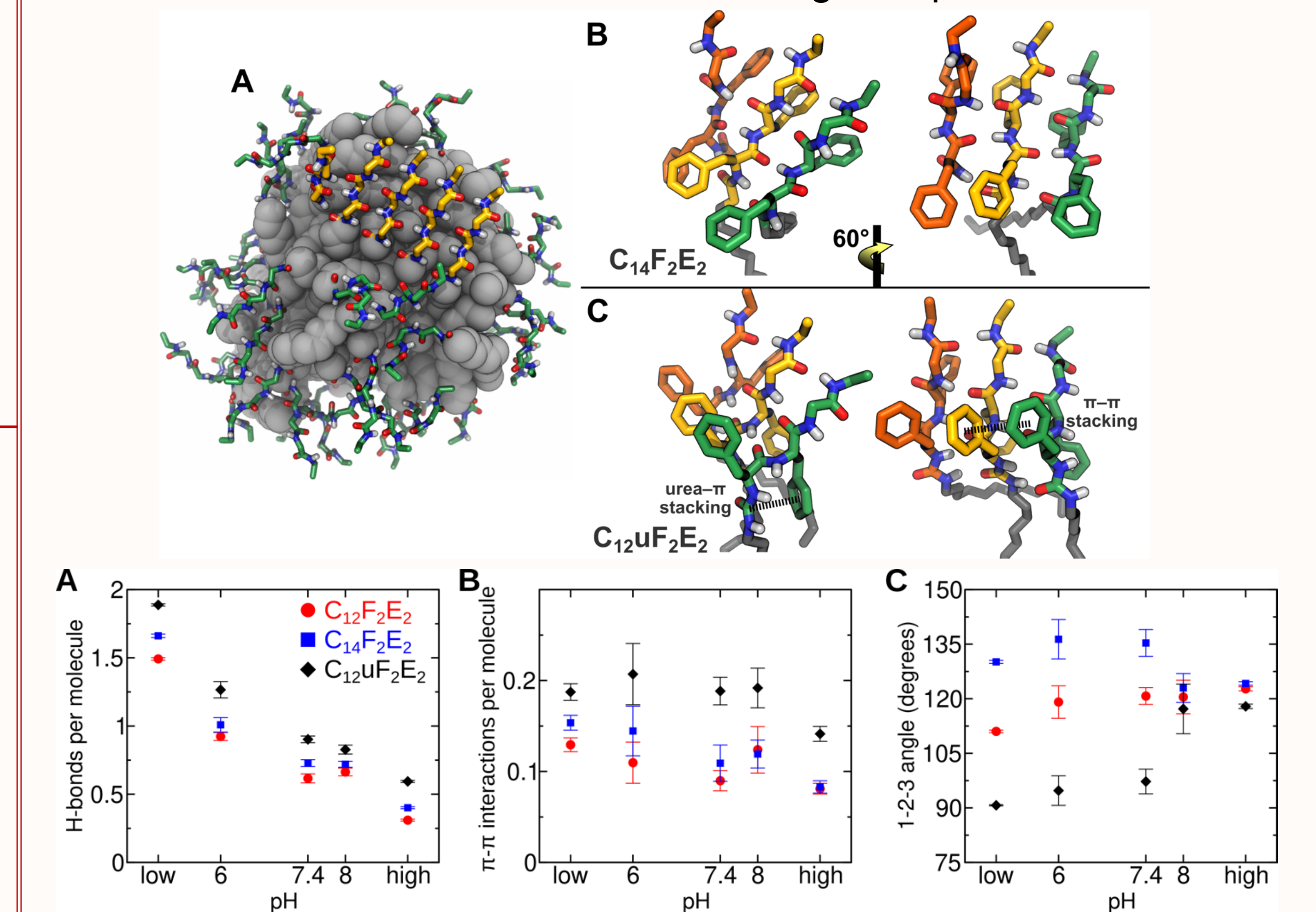


Conclusion We have demonstrated that adding a urea motif to get extra hydrogen bond is a viable option to increase the stability and alter the morphology of a supramolecular assembly. Studied showed the addition of the urea makes the nanossemblies stable to pH and temperature changes. Computer simulations indicates that the urea motif makes the nanostructure more compact and generate a urea- π interaction, which results in a different behavior on secondary structure and leads to a different morphology.

Reference

1. Paramonov, S. E.; Jun, H.-W.; Hartgerink, J. D., *Journal of the American Chemical Society* **2006**, *128* (22), 7291-7298.
2. Pashuck, E. T.; Cui, H.; Stupp, S. I., *Journal of the American Chemical Society* **2010**, *132* (17), 6041-6046.
3. Jiang, H.; Guler, M. O.; Stupp, S. I., *Soft Matter* **2007**, *3* (4), 454-462

4. Molecular simulations of PA assemblies While β -sheet structures appeared for all three PA types, the urea-linked PA (C₁₂uF₂E₂) exhibited very different peptide conformations than the amide-linked analogues (C₁₂F₂E₂ and C₁₄F₂E₂). The trend for H-bonding (C₁₂uF₂E₂ > C₁₄F₂E₂ > C₁₂F₂E₂) was maintained over all pH values from the fully protonated to fully deprotonated states, despite the overall number of H-bonds diminishing with pH.



Conflicts of Interest The authors declare no conflicts of interest.

Acknowledgements The authors acknowledge a CONICET-NIH Level 1 Bilateral Cooperation Grant. M.C.-S. acknowledges support from the National Science Foundation (CAREER Award # 1941731).

Contact information email address: huihua.xing@unmc.edu

- ^[a] College of Pharmacy, University of Nebraska Medical Center, Omaha, NE 68105, USA
^[b] Instituto de Investigaciones Físicoquímicas Teóricas y Aplicadas, INIFTA-CONICET-UNLP, La Plata, Argentina
^[c] Laboratorio de Cristalografía Aplicada, Escuela de Ciencia y Tecnología, Universidad Nacional de General San Martín, Buenos Aires, Argentina
^[d] Department of Chemistry, Northwestern University, Evanston, IL 60208, USA
^[e] Institute of Computational Comparative Medicine, Kansas State University, Manhattan, KS 66506, USA
^[f] Department of Materials Science & Engineering, Chemistry, Biomedical Engineering, Medicine, and Simpson Querrey Institute, Northwestern University, Evanston, IL 60208, USA