Accessible Solvent Effects on Spontaneous Diphenylalanine (FF/Phe-Phe) Nanostructure Formation for Undergraduates

Abstract

We report an adapted guide for the optimized selective synthesis of FF nanorods using a more accessible solvent, replacing HFP with DMSO.[1] This replacement of a significantly hazardous solvent with one far more suitable for undergraduate laboratory experimentation, allows undergraduate students an opportunity to experiment with the physical chemistry of organic nanostructures in a safer and cheaper lab environment.

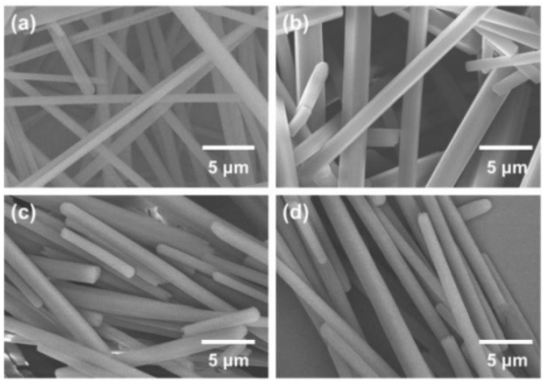

Fig 1). SEM images of 3 mg of FF assembled in different volume ratios of HFP/water solutions with ultrasonic treatment at room temperature for 20 min: (a) 10 μL/140 μL, (b) 30 μL/120 μL, (c) 70 μL/80 μL, and (d) 90 μL/60 μL. (Li. et. al.)[1]

In the field of drug design and development, the resilience and biocompatibility of FF nanostructures allow for their implementation as carrier vesicles or targeting scaffolds with controllable release through hydrogen bonding and π-stacking interactions.[1] The unique optical waveguide properties of FF nanorods can be leveraged thanks to their structural anisotropy for things like charge transport in light-harvesting systems.[1] Altogether, the versatility of the FF dimer underscores the value of research in bio-inspired systems as building blocks for future therapeutic drugs and optical devices.

Introduction

Brief History of Diphenylalanine

The FF dimer and it's relatively minimalistic β-amyloid conformation has emerged as a biocompatible tool for nanotechnology since its characterization in the 1990's as the mechanistic culprit behind Alzheimer's disease.[2] Since this pathological discovery, biochemists have launched a broader exploration into the unique physicochemical properties that cause such stable amyloid fibrils, progressing the disease.[1]

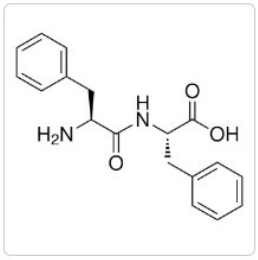

Fig 2). Single FF dimer.

The first big breakthrough came just a few years later in the early 2000s, when Gazit's lab demonstrated that FF peptides could self-assemble resilient, hexagonal nanotubes with remarkable thermal stability.[3] When studying these nanotubes, they observed not only intrinsic photoluminescence (thanks to excimers), but semiconductive behavior as well. As a waveguide, they're able absorb light in waves and propagate it for long distances without loss, where light is confined within the core and reflected at the core-cladding surface. In other words, these nanorods, dependent on selectable size and shape, can form standing wave modes to transfer light or charge, useful in biosensing, optical switches or energy harvesting. This came as a surprise, such amazing properties for such a simple and relatively

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[1], Fig 1). : Li, Q.; Jia, Y.; Dai, L.; Yang, Y.; Li, J. Controlled Rod Nanostructured Assembly of Diphenylalanine and Their Optical Waveguide Properties. ACS Nano 2015, 9 (3), 2689–2695. https://doi.org/10.1021/acsnano.5b00623.
[2]: The History of Alzheimer's Disease. https://atrinews.usc.edu/resources/history-of-alzheimers-disease (accessed 2025-05-14).
[3]: Mayans, E.; Casanovas, J.; Gil, A. M.; Jiménez, A. I.; Cativiela, C.; Puiggalí, J.; Alemán, C. Diversity and Hierarchy in Supramolecular Assemblies of Triphenylalanine: From Laminated Helical Ribbons to Toroids. Langmuir 2017, 33 (16), 4036–4048. https://doi.org/10.1021/acs.langmuir.7b00622.

hydrophobic dipeptide, encouraging greater research into investigations of their role in nanoelectronics.[1]

This interest was further sparked by biomedical research into their drug encapsulation potential, providing specific delivery thanks to their responsiveness to pH and temperature.[1] Intrigue into their vesicle and nanotube structures quickly followed, alongside research into surface modification, allowing them to target specific cellular environments. Smart, self-regulating delivery by pharmacokinetics that auto-assemble, where structural variance is just a matter of solvents and sonication time, what more could a lazy chemist want?[1]

Mechanism(s) of Action

Hydrophobic Interactions

While the FF dimer does exhibit remarkable solubility in water thanks to its amide group and carboxylic acid group's ability to hydrogen bond, remains steadily hydrophobic. In this case, its lack of solubility accelerates aggregation, pushing the dimers together, where intermolecular forces can take over and drive selective aggregation.

Hydrogen Bonding

Again, these amide groups steadily bring the dimers together, reinforcing linear stacking and further enabling the formations of tertiary structures in aqueous solutions. We can then analyze the solution's optical properties to determine which structures the dimers form by looking for the specific response signal of certain tertiary structures, such as the β-amyloid.

Π-Stacking Interactions

Thanks to the FF dimer's twin phenyl groups, the dimers in solution begin to rapidly aggregate together, forming a soupy yet solid hydrophobic pill of aggregate, almost like centrifugation of biological samples. The phenyl groups begin to create a conjugation web between molecules, known as π-stacking thanks to the rings of π electrons above and below the benzene rings being displaced up and down the stack.

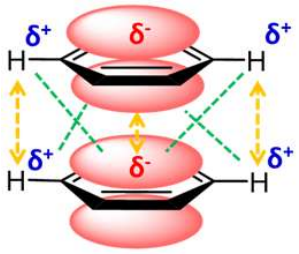

Fig 3). Visual representation of pi-stacking interactions.

Pivot to DMSO

Our experimentation centers around the replacement of the hazardous hexafluoropropylene (HFP) with a more tame and widely accessible solvent, DMSO, and thus were left with two key questions. First, we tested whether DMSO resulted in similar nanorod growth when compared to HFP, before investigating what concentration range resulted in the most aggregation.

Methods

Synthesis of FF Nanorods

Diphenylalanine nanorods were prepared by a modified literature method.[1] Briefly, 5 mg of dipeptide were combined with a variable volume of DMSO and shaken till suspended in seven individually sealed 25 mL test tubes. Variable volumes for the solutions were determined by

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Fig 3). 6.5.1: Host-Guest Chemistry and π-π Stacking Interactions. Chemistry LibreTexts. https://chem.libretexts.org/Bookshelves/Inorganic_Chemistry/Inorganic_Chemistry_(LibreTexts)/06%3A_Acid-Base_and_Donor-Acceptor_Chemistry/6.05%3A_Intermolecular_Forces/6.5.01%3A_Host-Guest_Chemistry_and_-_stacking_interactions (accessed 2025-05-15).

observed relative dissolution time and are listed in Figure 4. After suspension was reached, 2.4 mL of MilliQ water was added to each tube before being sonicated for 16 minutes to select for nanorods.[1]

Improvements to Data Collection

Our methods of weighing the peptide were heavily flawed, only late into the experiment did we finally figure out a proper procedure to rule out human error and ensure that our 5 mg was completely accurate, dismissing instrumental error margins. Utilization of a fume or vacuum hood during peptide measurements also would've helped reduce the instrumental error, as the peptide could possibly have absorbed water vapor from the surrounding atmosphere. In addition, for our initial tests with UV-Vis we failed to properly take the light blank of the changing DMSO additions, likely leading to inflated absorption numbers in the lower concentration trials (200-600 μL), causing us to exclude them from this report, potentially critical data.

Physical Characterization

Room temperature UV-Vis and Fluorescence absorption spectra were taken for all aqueous suspensions of nanorods in 1.0 cm path length, crystalline cuvettes using a Black Comet UV-Vis (Stellar Net) spectrometer between 184-850 nm wavelengths and a Beckman-Coulter PA800 with laser-induced fluorescence detectors from 300-800 nm respectively. Blanks were taken dark and with DMSO and MilliQ water and between collections all cuvettes were rinsed thoroughly with analyte prior to analysis. Full spectrum room temperature FTIR spectra was then run on the same aqueous suspensions.

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[1]: 1200 μL vial was sonicated for 20 minutes.

Materials

Dimers of phenylalanine (98%, s) and dilute DMSO were purchased from Sigma Aldrich. MilliQ water was prepared in-lab with a MilliQ benchtop lab purifier from Sigma Aldrich.

Results and Analysis.

FTIR

We first tested whether DMSO would work as a competent substitute for HFP in nanostructure synthesis. According to our adapted literature, if tertiary structures had formed, we expected to see a peak in the range of ~1630-1610, characteristic of a β sheet structure.[1,4] While we could not confirm whether this structure was indeed our desired nanorods or not, we found a peak quite close to the published range, possibly confirming that DMSO was a viable replacement as a dissolution agent for our desired synthesis reaction.

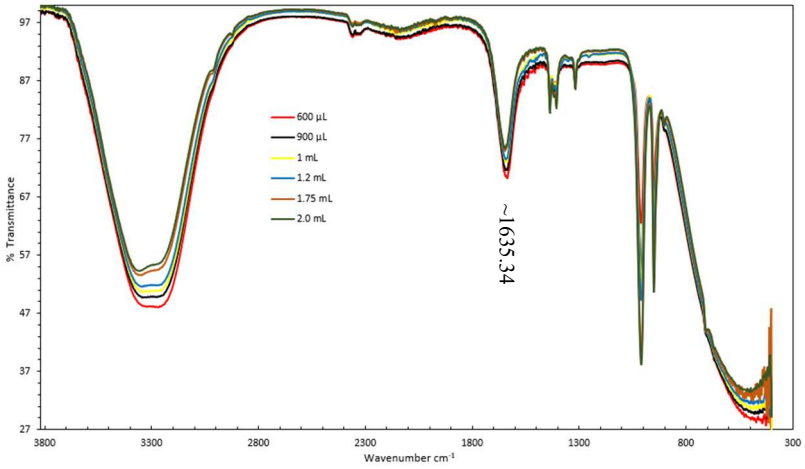

Fig 4). Observed FTIR Spectra of each combinataion of 5 mg peptide, 2.4 mL MilliQ water and the variable addition of DMSO.

[4]: Ultraviolet and visible spectroscopy. Chemistry LibreTexts. https://chem.libretexts.org/Courses/Oregon_Institute_of_Technology/OIT%3A_CHE_333_-_Organic_Chemistry_III_(Lund)/New_Page/4%3A_Structure_Determination_I-_UV-Vis_and_Infrared_Spectroscopy_Mass_Spectrometry/4.4%3A_Ultraviolet_and_visible_spectroscopy (accessed 2025-05-14).

UV-Vis

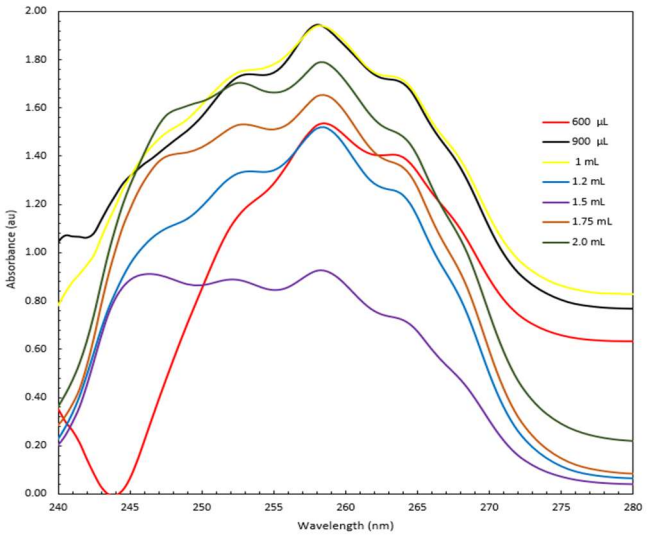

Fig 4). Observed UV-Vis spectra of 5 mg peptide and 2.4 mL MilliQ water with the variable addition of DMSO in solution.

We initially aimed to identify changes in absorbance at around ~258-265 nm wavelengths, where π-stacking absorption can be typically observed. Our original hypothesis was that the higher the concentration of DMSO in solution, the greater the stimulus to aggregation due to the higher rate of dissolution. Evidently, we underestimated the impact of hydrophobic interactions on the dimers, where the higher the DMSO concentration past 1000 µL, the weaker the absorption. This disaggregation stimulus by excessive dissolution was reinforced by the changing spectral shape, possibly indicating structural changes in solution. In more dilute trials, shoulders and splits become more visible, possibly indicating reduced or confused aggregation, as more aggregated structures tend to have overlapped, merging electronic transitions due to coupling interactions and non-homogeneous environments. We believe that the reason the 600 µL solution is so low is due to the peptide's continued struggle to dissolve, as we still observed considerable suspension quite late into the sonication process.

Fluorescence Spectroscopy.

Continuing with the disapproval of our high DMSO, high aggregation hypothesis, we conducted FL spectroscopy on the same combinations of peptide, MilliQ water and DMSO. Here, we found a massive emission peak ~500 nm with a smaller shoulder ~575 nm. The massive spike we determined to be a product of RAMAN or Raleigh scattering in the spectrometer due to it's immediate fall-off beyond 1000 µL. The shoulder, however exhibited massive red-shift from the typical peptide peaks in the 300-400 nm range. The diminishing shoulder could represent a diminishing red-shift as we further dilute with DMSO past 1000 µL, consistent with disaggregation. Aggregated systems typically exhibit broader peaks as opposed to sharp, monomeric points and as opposed to excimers, which although possible in such a heavily π-stacked system, usually exhibit broad plateaus in the spectra. In other words, we predict that the higher the concentration of DMSO beyond ~1 mL, the weaker the hydrophobic interactions driving the peptide together, and the weaker the aggregation stimulus.

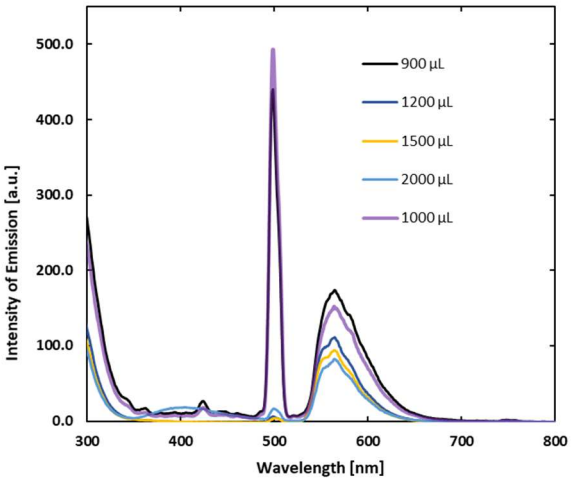

Fig 4). FL spectra of the same combinations of peptide, MilliQ water and DMSO.

Conclusion

We highlight the potential for selective peptide nanocrystal formation for drug design and photonic applications in superconductivity, providing a safer and more accessible method for working with dipeptide nanocrystals. Through our FTIR spec. investigations, we managed to

prove our initial hypothesis that DMSO, a more accessible and less hazardous solvent, could be used as a replacement for HFP in nanocrystal formation thanks to it's ability to solubilize the FF dimer without excessive hydrophilic interference (Fig 4). The range we found to maximize the aggregation was approximately 900-1000 μL of DMSO, past which we observed a diminishing red-shift, consistent with disaggregation. This was fairly consistent with our UV-Vis data, where the spectra became increasingly vague, consistent with a change in structure in the solution. Concentrations of DMSO below 900 μL restrict the solubility of the dimer, where concentrations above 1000 μL restrict the hydrophobic interactions that seem to drive aggregation and crystal formation. These results are promising, allowing for future undergraduate level physiochemical experimentation with our now stockpile of the FF dimer with an optimized synthesis regimen for continued research. Future investigations could dive into chemical entrapment in vesicles, their unique optical waveguide properties and further optimizations for their synthesis or selection.

Bibliography

Scientific References

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Figure References

1. Li, Q.; Jia, Y.; Dai, L.; Yang, Y.; Li, J. Controlled Rod Nanostructured Assembly of Diphenylalanine and Their Optical Waveguide Properties. *ACS Nano* 2015, *9* (3), 2689–2695. https://doi.org/10.1021/acsnano.5b00623.

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