SAND2014-18722D

Implementation of FTIR spectroscopy to characterize self-assembly in unique peptide sequences

Alina M. Martinez, Brad H. Jones, Jill S. Wheeler, Erik D. Spoerke

Electronic, Optical, and Nano Materials Department, Sandia National Laboratories, Albuquerque, NM

Sandia National Laboratories

Abstract

Background

- Select peptides can undergo spontaneous self-assembly in solution.
- This occurs due to hydrophobic and aromatic interactions, hydrogen bonding and electrostatics.
- These interactions lead to characteristic secondary structures – sheets and helices.
- Secondary structures can then define nanostructured morphologies such as ribbons, tubes, rods, sheets, or spheres.

Practical Approach

- Improving our understanding of these self-assembly processes has the potential for broad impact on fields ranging from neurodegenerative diseases to templated materials synthesis. This poster describes our use of FTIR spectroscopy to characterize secondary structures of peptides, highlighted by examples from a model amphiphilic peptide synthesized in our lab.

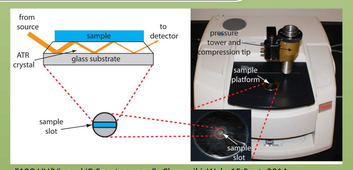
How?

- Through the use of FTIR spectroscopy enabled by an attenuated total reflectance (ATR) cell, we are able to study self-assembly upon drying peptide solutions, yielding powerful insight into the dynamics of these processes. These results are complemented by scanning electron microscopy (SEM).

FTIR and Peptide Self-Assembly

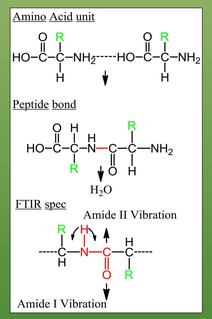
Fourier transform infrared spectroscopy detects unique bonds in molecules to determine behavior and identity. Coupled with an ATR crystal, the infrared signal can be detected.

- Infrared radiation is emitted and passes through the sample.
- The amount of light absorbed or transmitted in wavenumbers by the sample will be unique to each molecule.
- Absorption is proportional to the concentration of absorbing species.

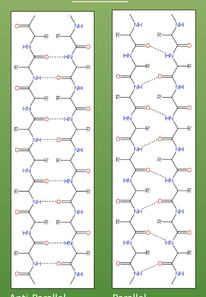


"10C UV/Vis and IR Spectroscopy"- *Chemwiki*. Web. 15 Sept. 2014. http://chemwiki.ucdavis.edu/Analytical_Chemistry/Analytical_Chemistry_2.0/10_Spectroscopic_Methods/10C_UV%2F%2FVis_and_IR_Spectroscopy

Primary Structure



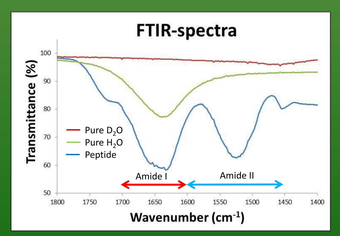
Secondary Structure



"Beta Sheet." *Wikipedia*. Wikimedia Foundation, 24 Aug. 2014. Web. 15 Sept. 2014. http://en.wikipedia.org/wiki/Beta_sheet

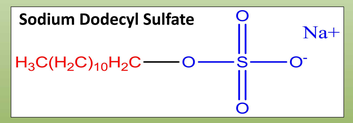
"Alpha Helix." *Wikipedia*. Wikimedia Foundation, 14 Sept. 2014. Web. 15 Sept. 2014. http://en.wikipedia.org/wiki/Alpha_helix

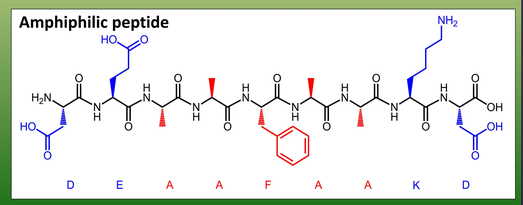
FTIR interpretation: Peptide Behavior	
1600-1700 cm^{-1} Amide I Region (stretching vibrations of C=O bonds)	
1450-1600 cm^{-1} Amide II Region (bending vibrations of N-H bonds)	
Wavenumber (cm^{-1})	**Secondary Structure**
1610-1630	β-sheet
1640	Disorder
1650-1660	α-Helix
1670-1700	Turns



For aqueous peptide solutions, the use of D_2O is necessary to avoid solvent interference with the characteristic amide I absorptions

Model Peptide System





Experimental

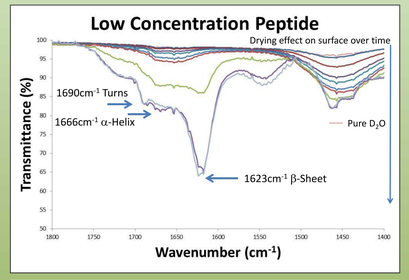
Three regimes of peptide self-assembly behavior discovered:

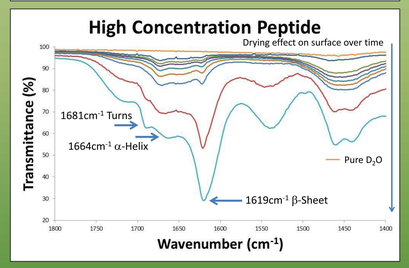
- Low concentration of peptide in D_2O
- High concentration of peptide in D_2O
- Low concentration of peptide , 15mM SDS in D_2O

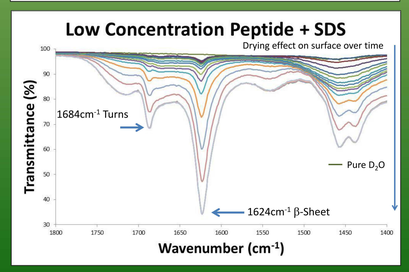
Results

FTIR Solution Spectra

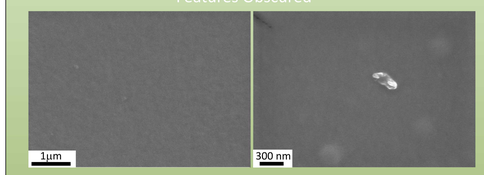
SEM images of peptide in solution ambiently dried onto surface of silicon substrate





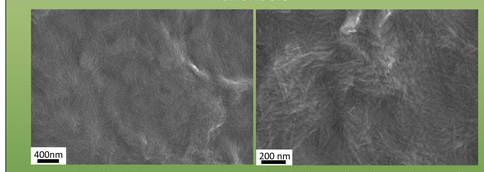


Features Obscured



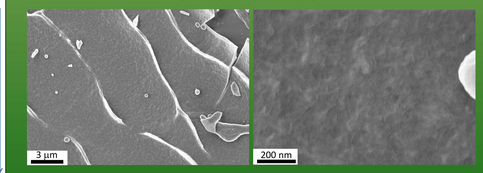
- Based on FTIR spectra, a secondary structure has been achieved.
- SEM images suggest there is unobservable secondary structure interactions due to low concentration of peptide in solution. Aggregations in this solution are weak and therefore, may not be large enough to see.

Nanofibers



- Based on FTIR spectra, a secondary structure has been achieved immediately on the first scan.
- SEM images shows evidence of a tertiary structure in the form of nanofibers. Possibly due to higher concentration, there is a visible amount of peptide interaction.

Unknown structure



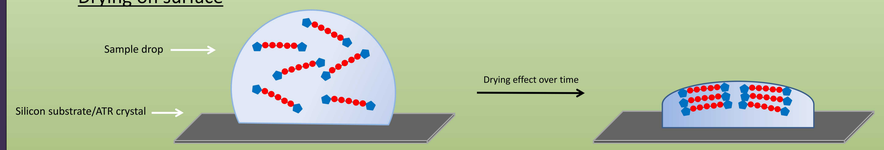
- Based on FTIR spectra, a secondary structure has been achieved immediately on the first scan.
- SEM shows evidence of a tertiary structure in an unidentified form differing from the "High Concentration" experiment. This could be due to the missing peak in the 1650-1660cm^{-1} range that yields α-Helices.

Proposed Mechanisms

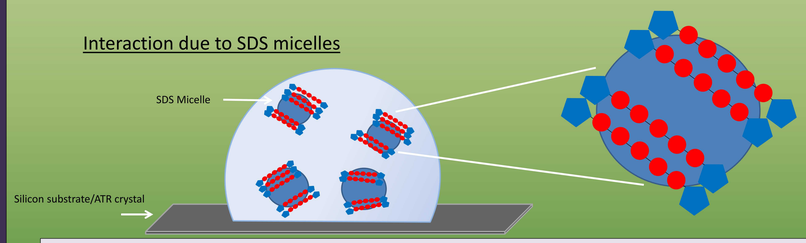
Self-assembly may occur due to two possible mechanisms:

1.) Concentration Effect: This phenomenon displays that as there is less of the water environment to interact with, the peptide will be able to interact with itself. Surface area to peptide ratio increased.

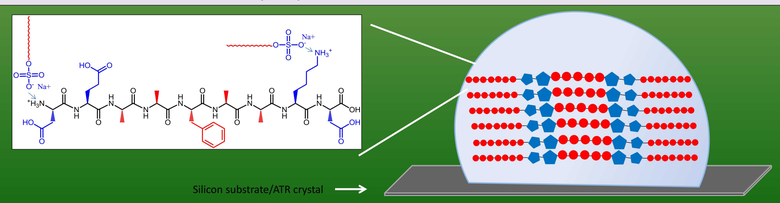
Drying on surface



Interaction due to SDS micelles



2.) Neutralization of charges: Addition of SDS neutralizes charges on the peptide, which in turn increases amphiphilicity. The peptide is more likely to self assemble with less hydrophilic interactions and an increase of hydrophobic interactions.



Conclusion and Path Forward

- **The observation of secondary structure by FTIR does not guarantee the observation of a discernible tertiary assembly**
  - Our weakly-aggregating, model peptide forms ordered secondary structure only when extremely concentrated
  - Ordering occurs near the limit of solubility, *i.e.*, the transition from a liquid drop to a solid film, which is ultimately the effect of drying.
  - Tertiary formation is dependent on initial conditions of peptide in solution.
- **The ionic surfactant SDS can be used to induce secondary structure in a weakly-aggregating peptide**
  - Micellization of SDS provides extensive polar-nonpolar interface, which may aid secondary structure formation
  - Neutralization of excess peptide charge by surfactant head group may increase effective amphiphilicity of peptide, further aiding secondary structure formation
- **FTIR and SEM show secondary and tertiary structure from SDS-induced peptide assembly is distinct from neat, aqueous systems**
- **Additional work is necessary to determine the exact nature of SDS-induced peptide assembly**
  - Compare secondary structure above and below critical micelle concentration
  - Study secondary structure in presence of nonionic surfactants
  - Investigate tertiary structure by alternative characterization techniques, *e.g.*, x-ray scattering

Additional Reading

- Byler, D. Michael, and Heino Susi. "Examination of the Secondary Structure of Proteins by Deconvolved FTIR Spectra." *Biopolymers* 25.3 (1986): 469-87.
- Gallagher, Warren. "FTIR Analysis of Protein Structure." 2-8.
- "Introduction to Fourier Transform Infrared Spectrometry." *Thermo Nicolet* (2001): 1-7.
- Rangachari, Vijayaraghavan. "Secondary Structure and Interfacial Aggregation of Amyloid-β(1-40) on Sodium Dodecyl Sulfate Micelles." *Biochemistry* 45.28 (2006): 8639-648.

This research was supported by the U.S. Department of Energy, Office of Basic Energy Sciences, Division of Materials Sciences and Engineering, Project KC0203010.
Sandia is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the United States Department of Energy's National Nuclear Security Administration under Contract DE-AC04-94AL85000

U.S. DEPARTMENT OF ENERGY

National Nuclear Security Administration