Protein Characterizations by Chiral Vibrational Sum Frequency Generation Spectroscopy

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Yale University

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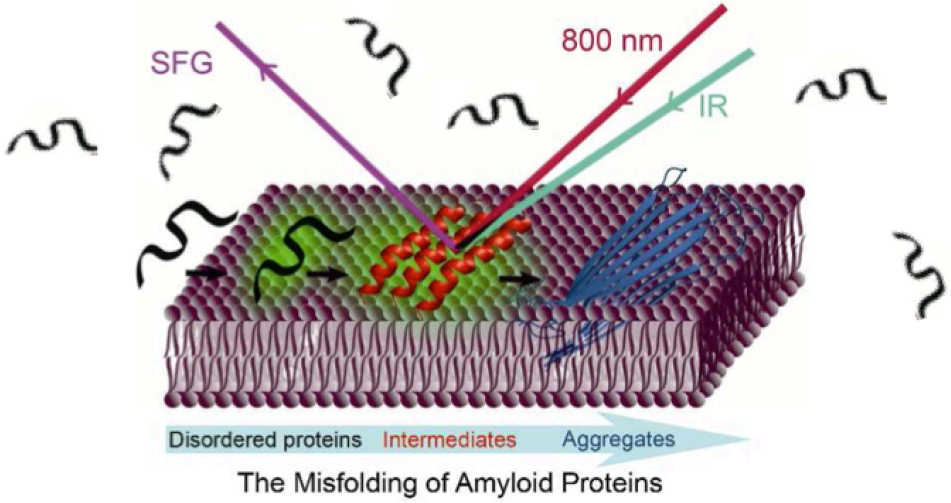
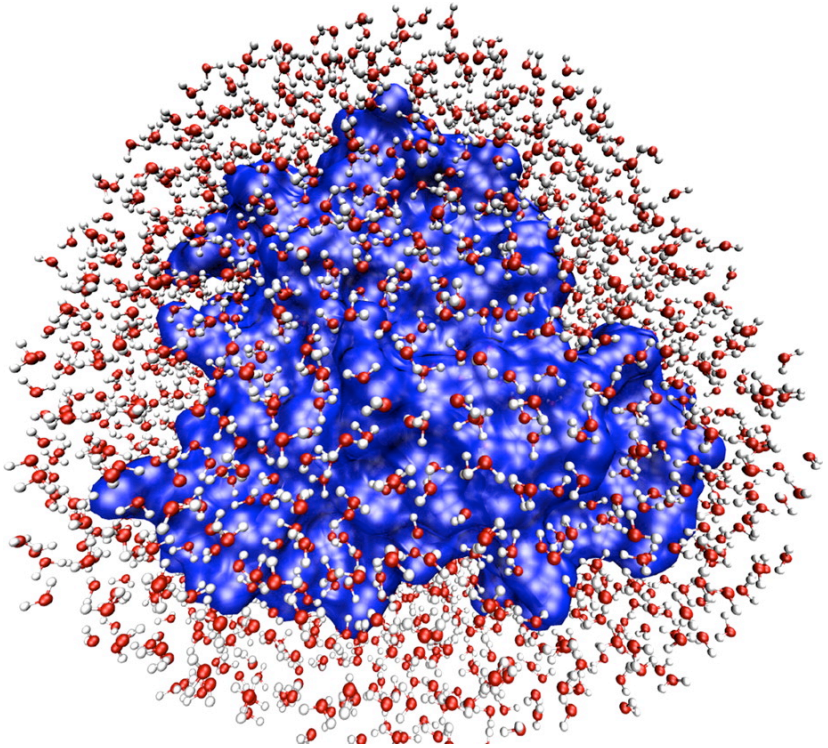
I. Charactering Protein Secondary Structures at Interfaces

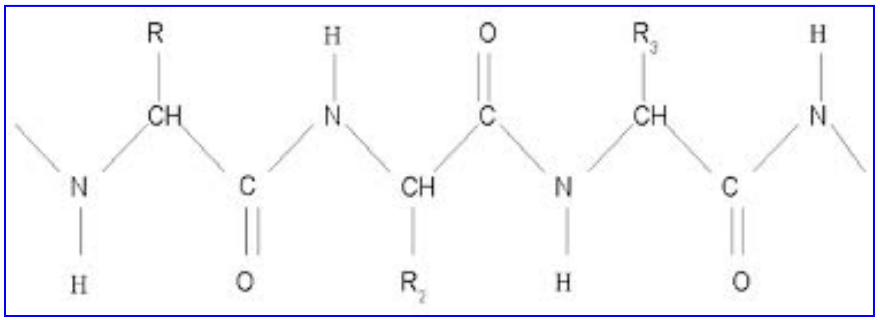


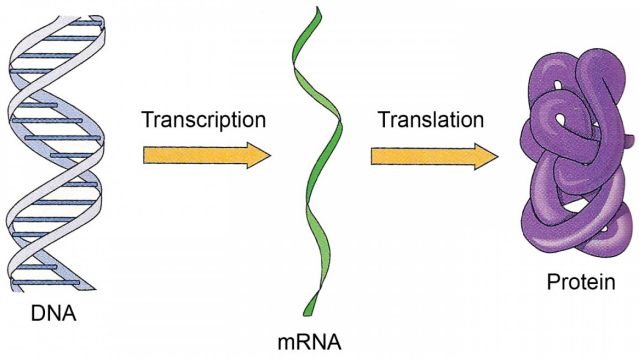
The Misfolding of Amyloid Proteins

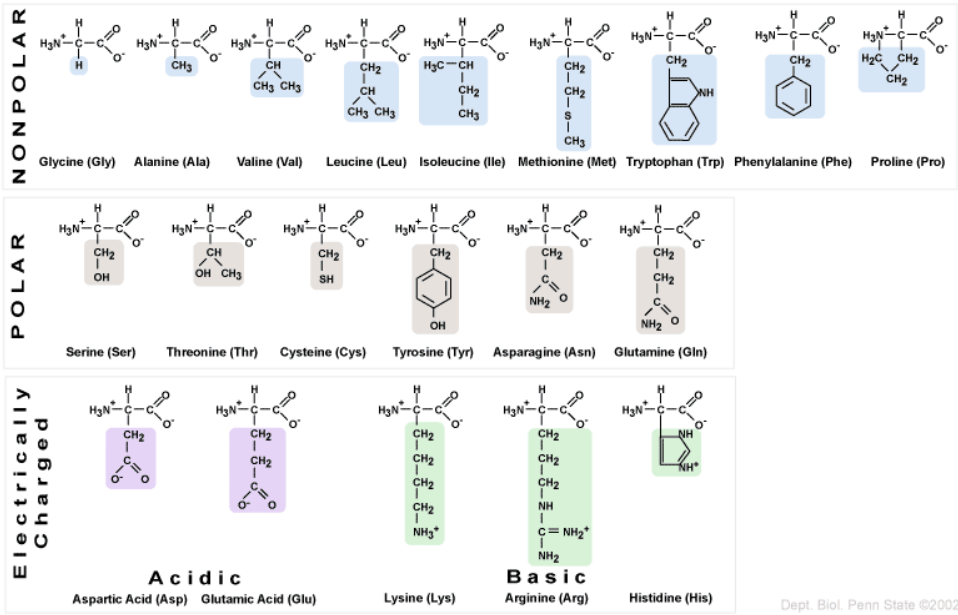
II. Probing Water Structures in Hydration Shell of Proteins

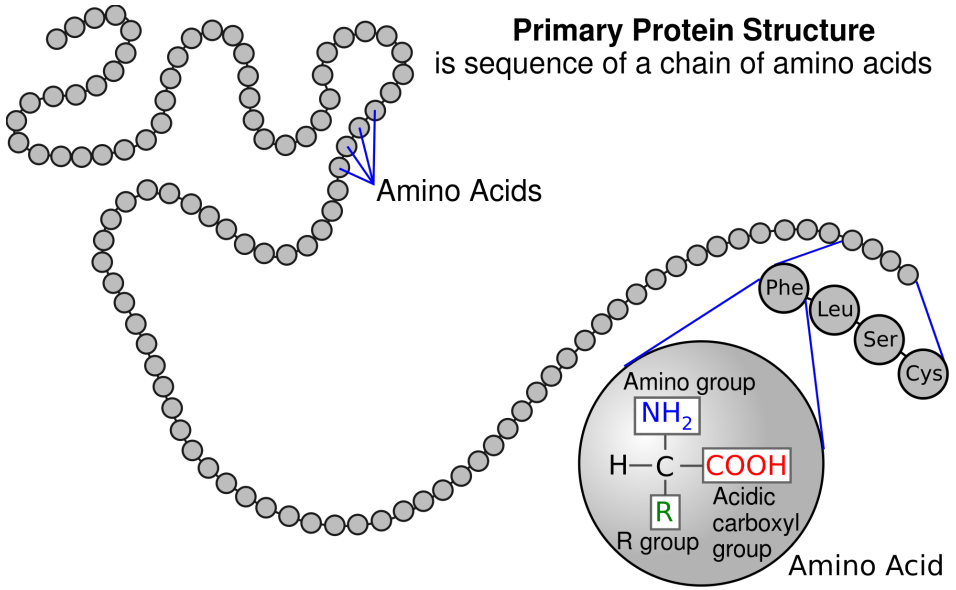


Motivation: Proteins

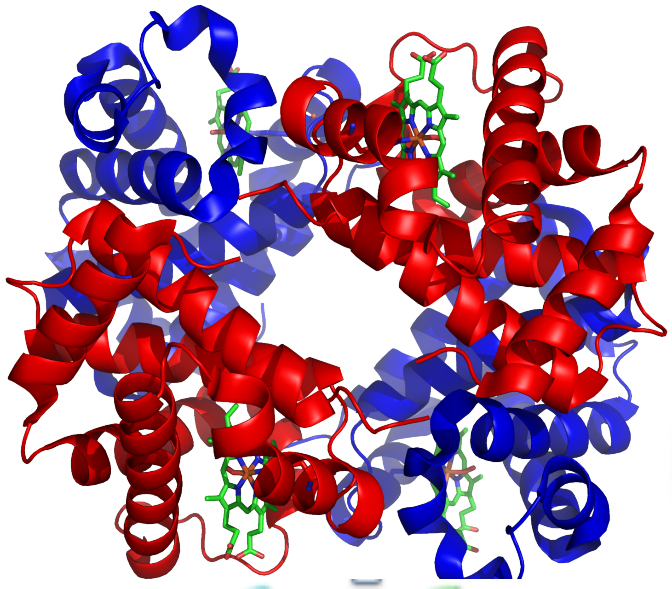


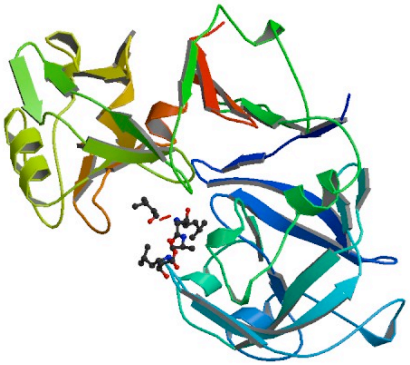


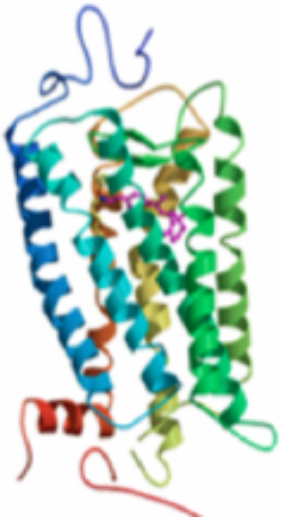


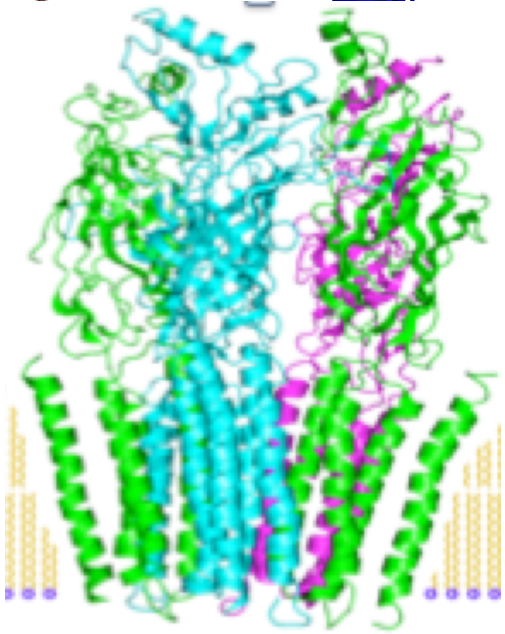


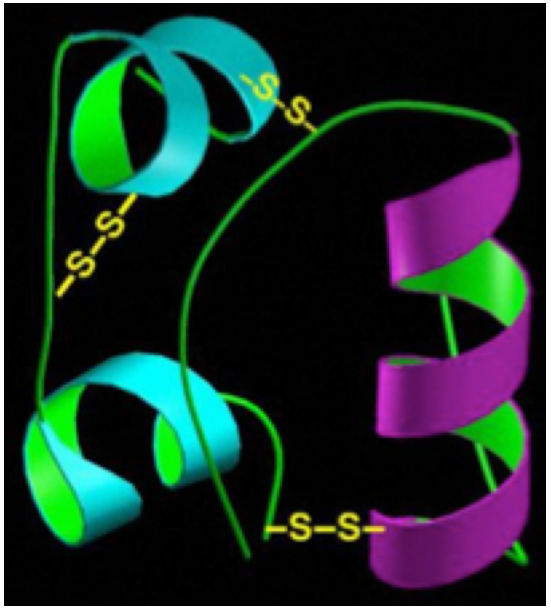
Motivation: Protein Structures and Functions

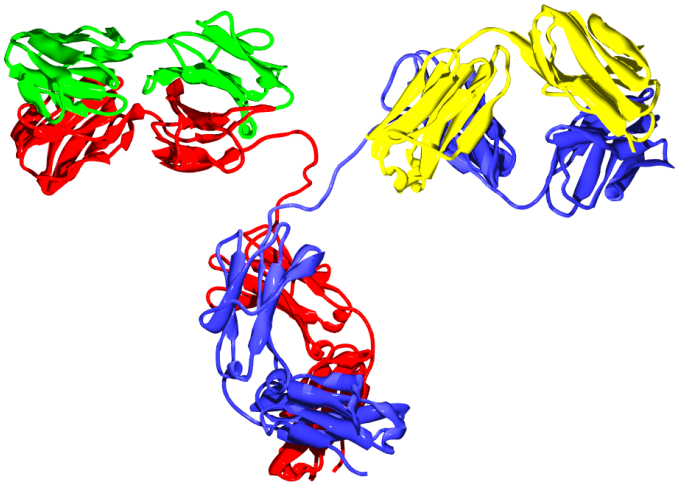












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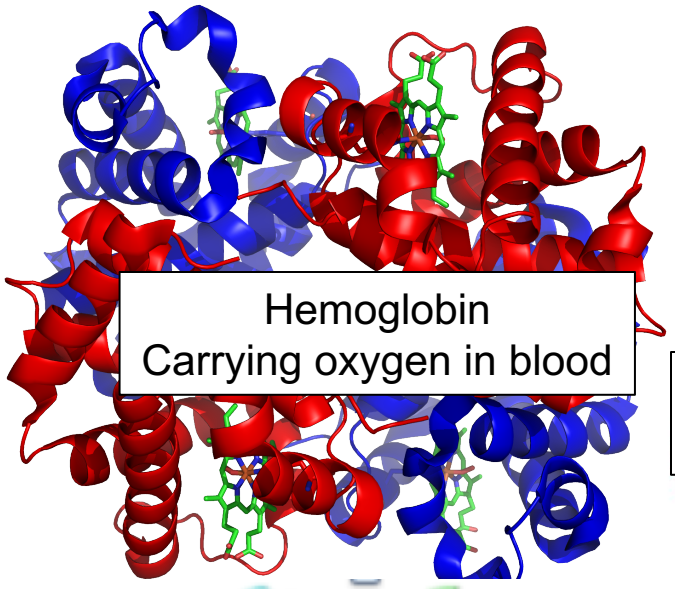

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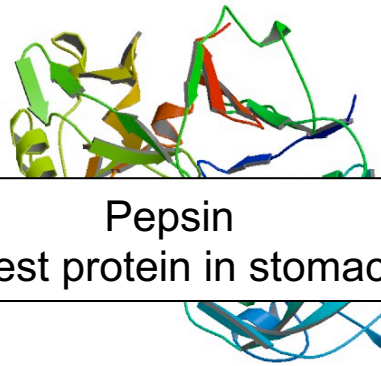
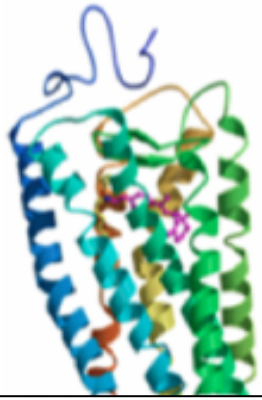
Carrying oxygen in blood

Filamin
Muscle contraction



Pepsin
Digest protein in stomach



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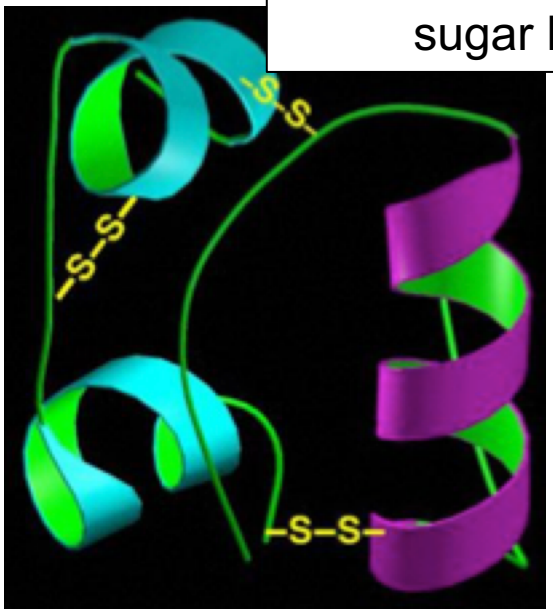
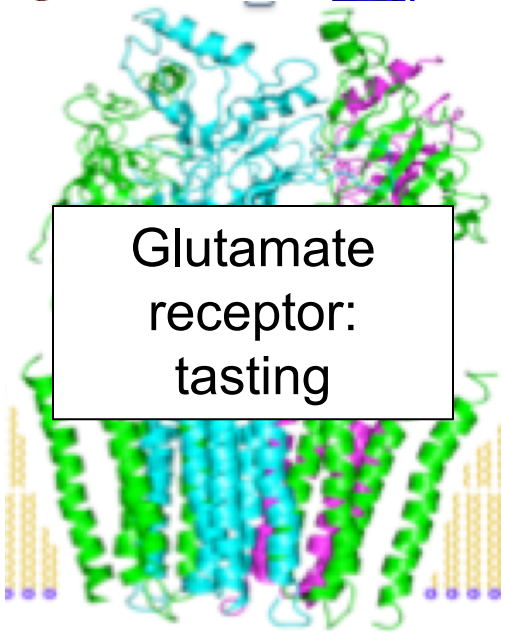
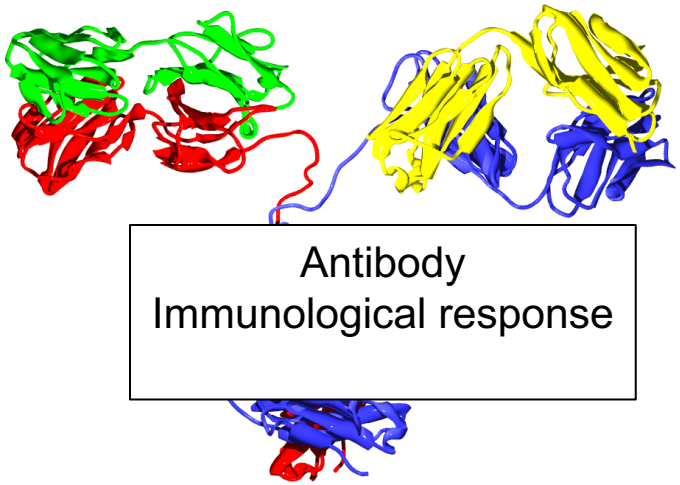
Light detection for vision

Insulin: hormone for controlling sugar level in blood





Glutamate receptor: tasting



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Immunological response

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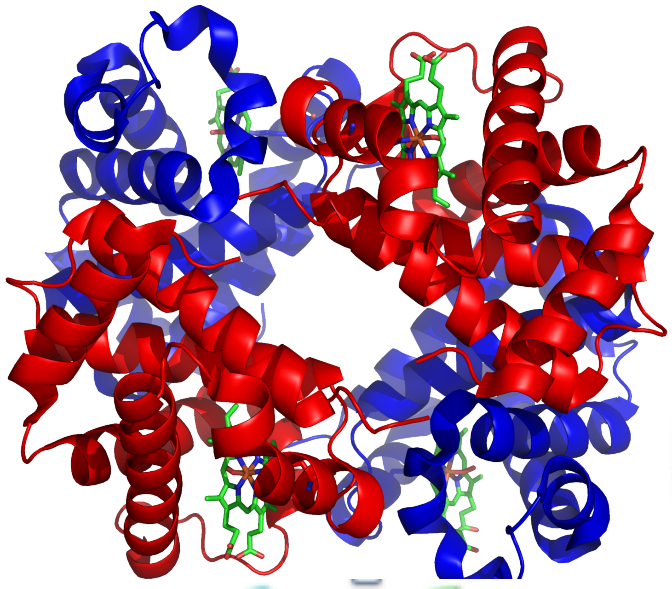

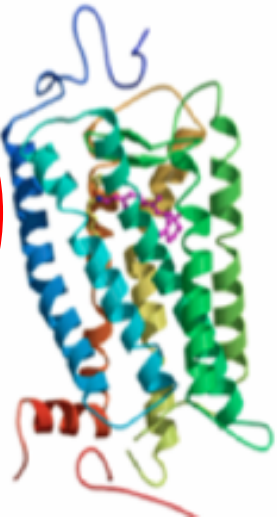

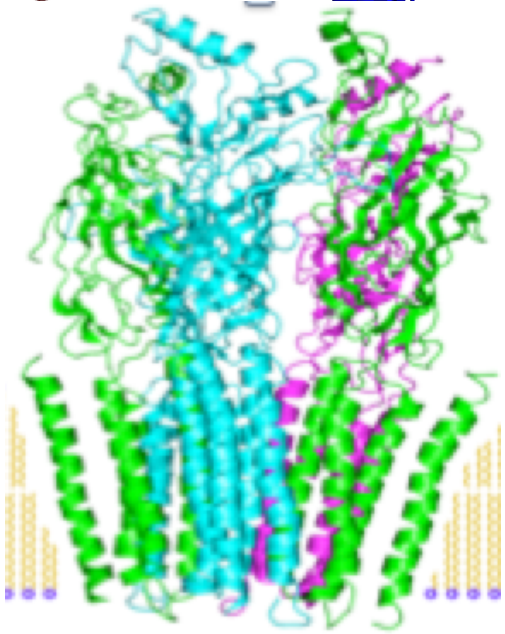

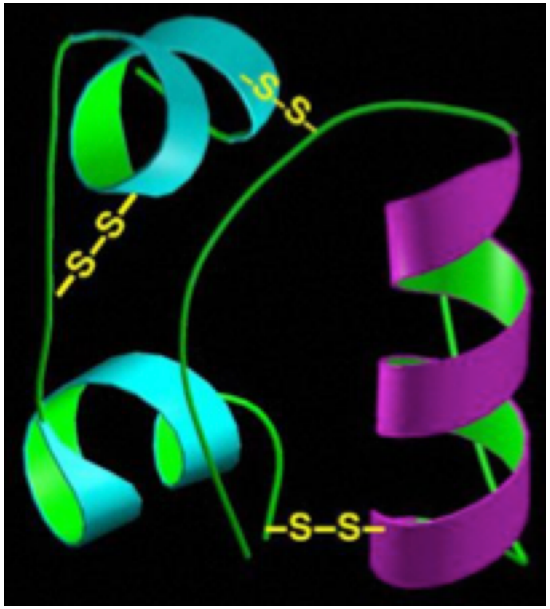

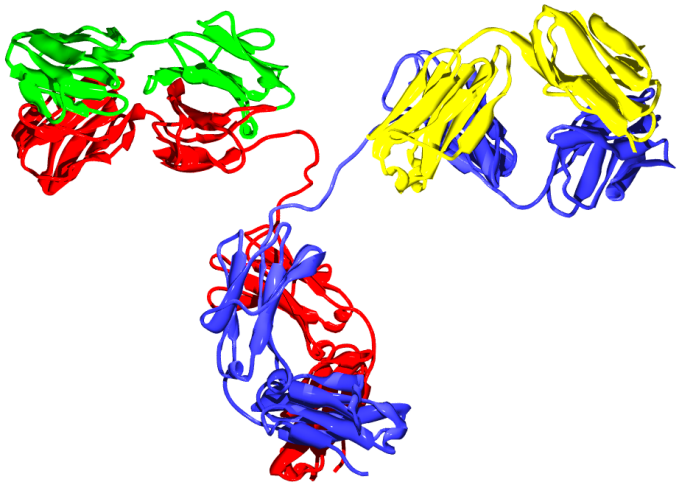

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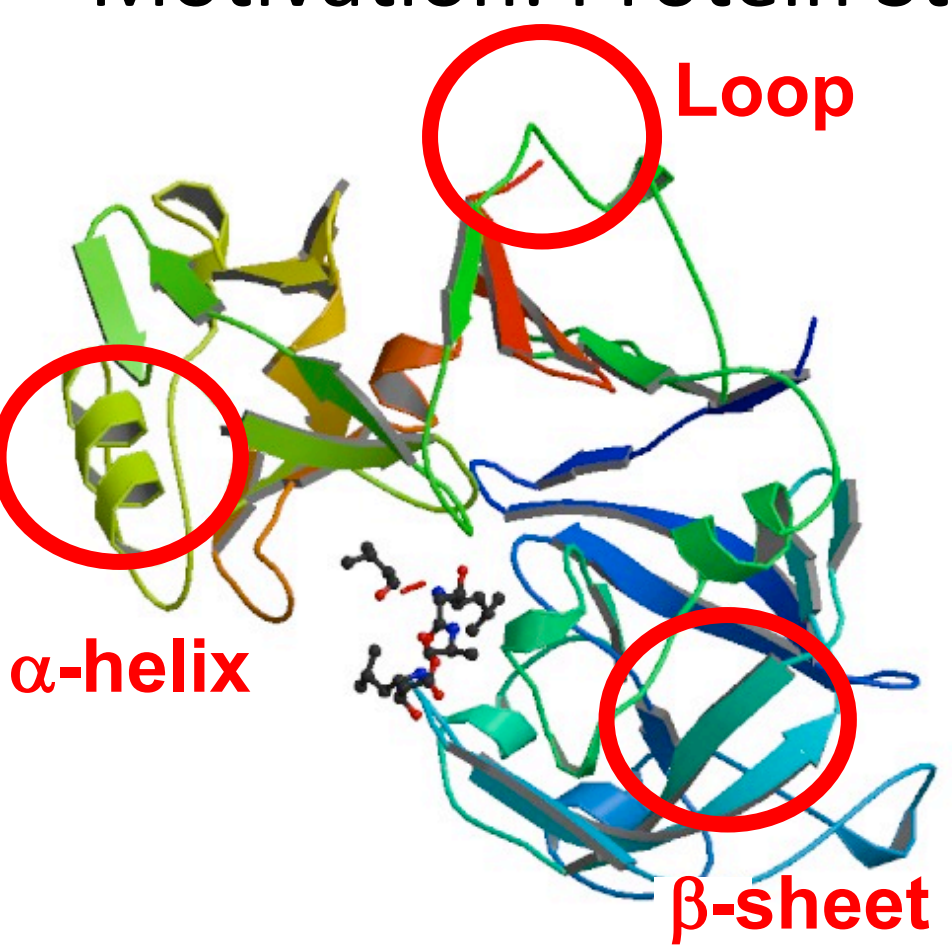


Protein Secondary structures:

1. Stability
2. Dynamics
3. Organization

⇒ Mechanism of protein functions

- To explore disease mechanism
- To develop therapeutics

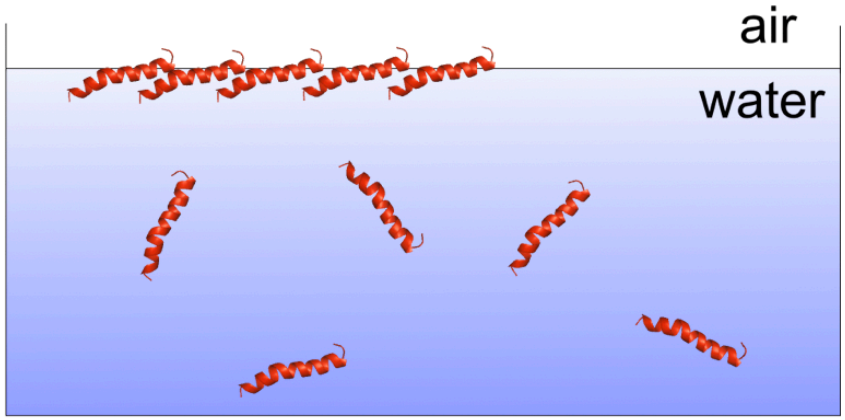
Conventional Methods:

1. Circular Dichroism Spectroscopy
2. NMR Spectroscopy
3. X-ray Crystallography

} => Not surface sensitive

Motivation: Protein Structures and Functions

Signals overwhelmed by
- Solvent
- proteins in the bulk



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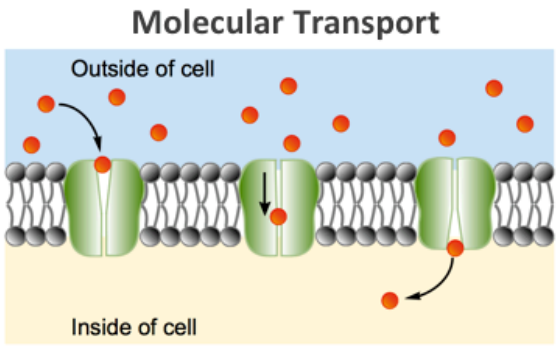
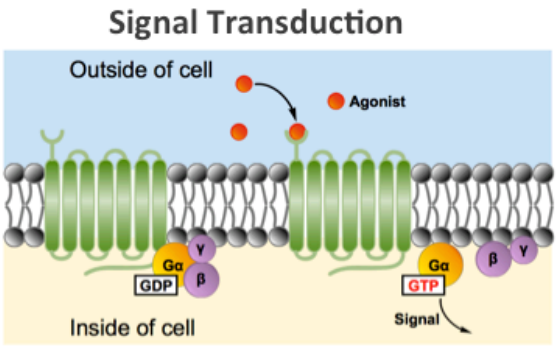
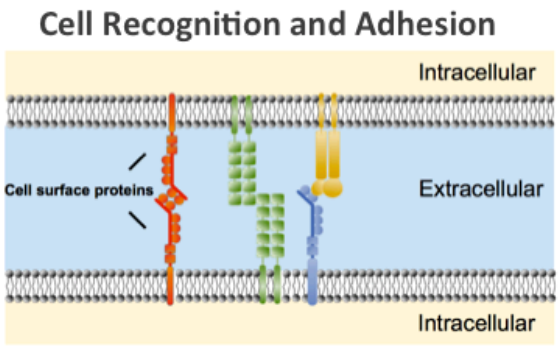
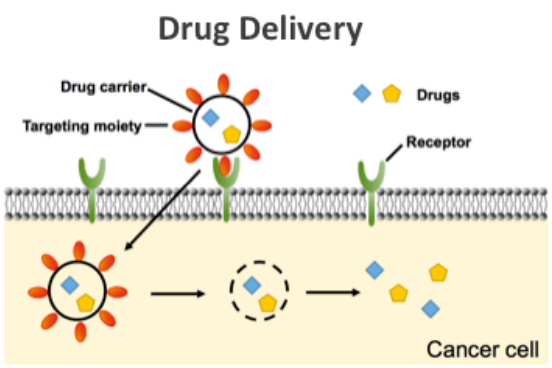
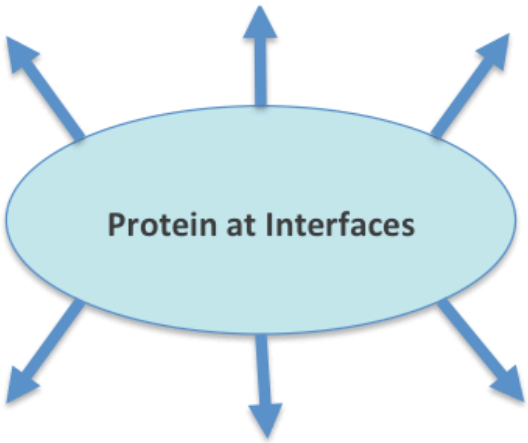
Motivation: Proteins at Interfaces

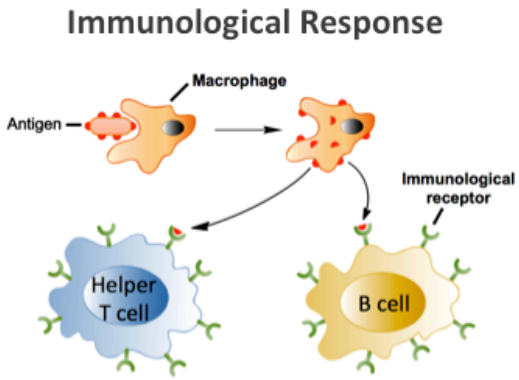


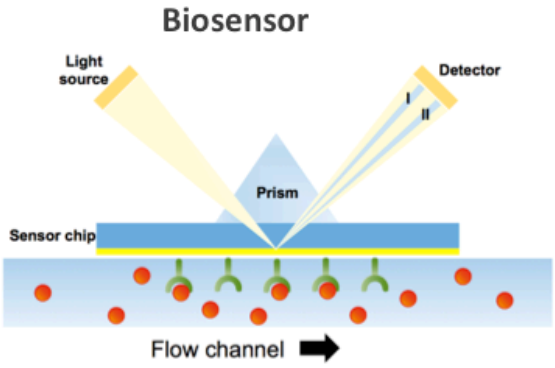


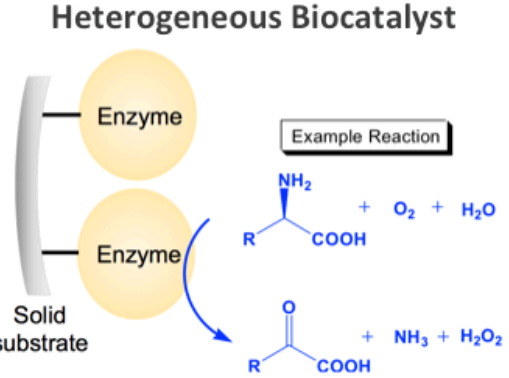


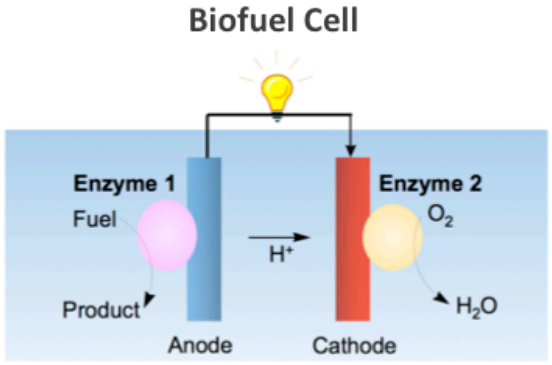












Motivation: Protein Structures at Interfaces

Interfaces and Bulk Media are **DIFFERENT!**

Air
Density 1.2 g/L
Refractive index: 1
Dielectric constant:1

? ? ?
? Interface ?
? ? ?

Water
Density 1000 g/L
Refractive index: 1.3
Dielectric constant: 80

Need:

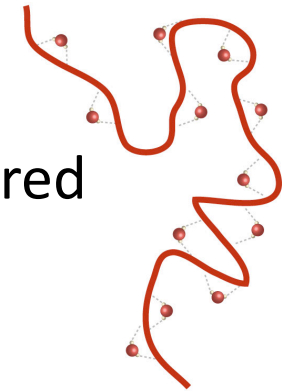
Characterization of Protein in situ at Interfaces

Motivation: Protein Secondary Structures—Basic Functional Units of Proteins

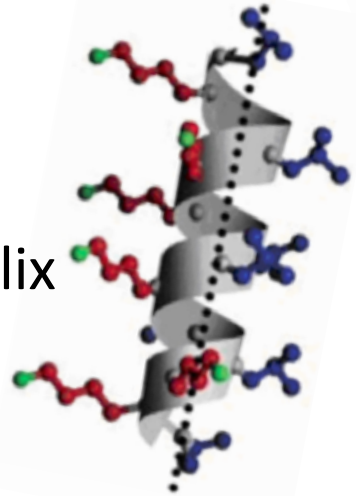
Parallel β-sheet

Antiarallel β-sheet

Disordered



α-helix



3_{10}-helix

In situ characterization of protein secondary structures at interfaces is important to fundamental biological sciences and engineering.

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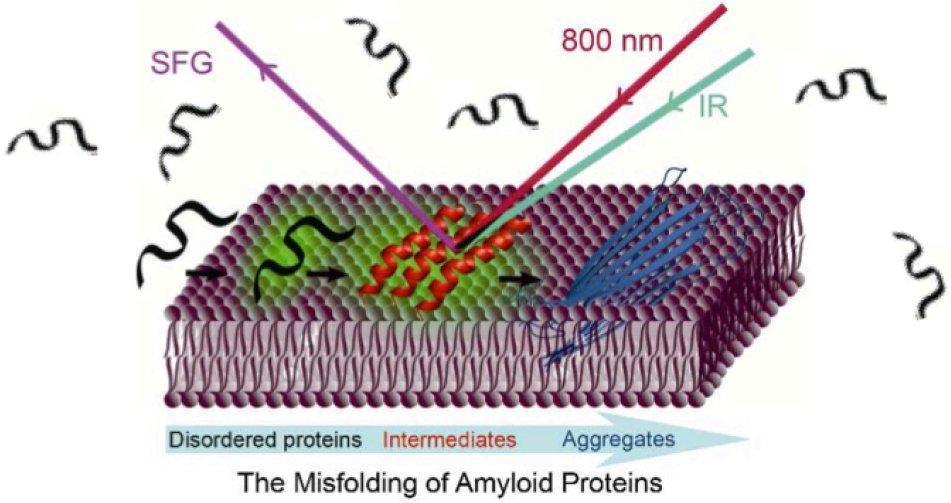

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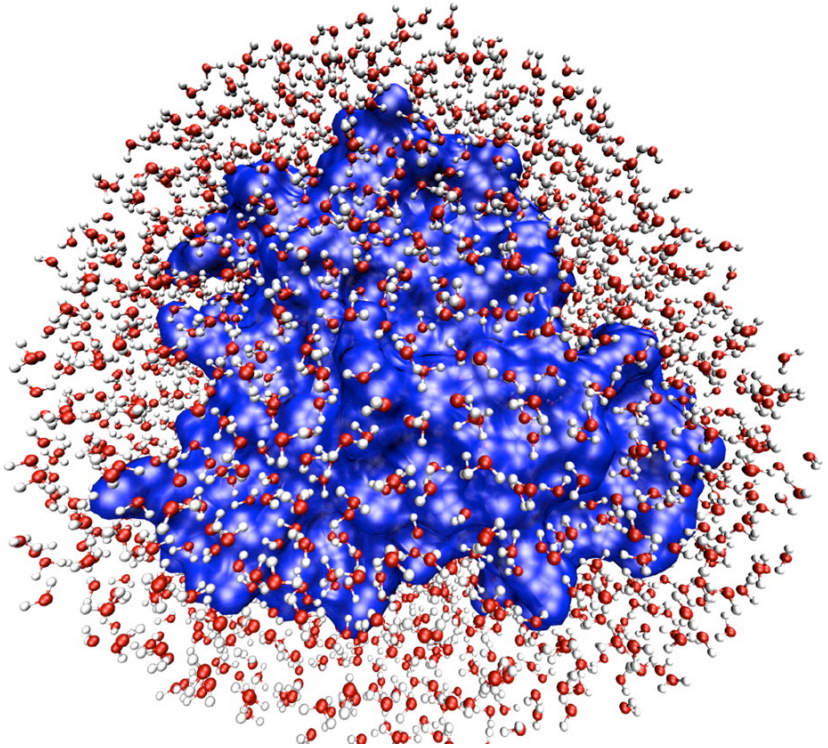

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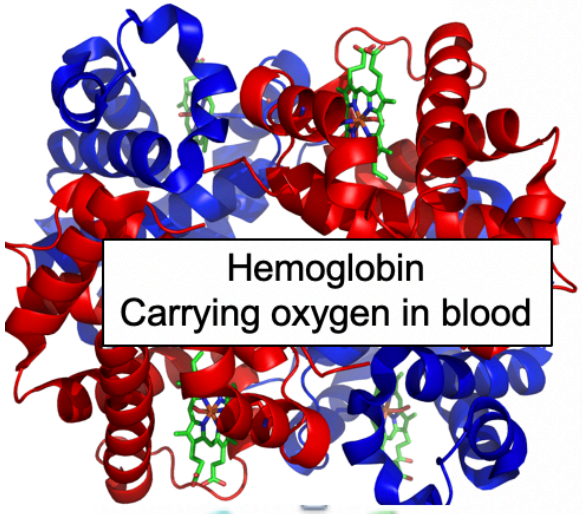

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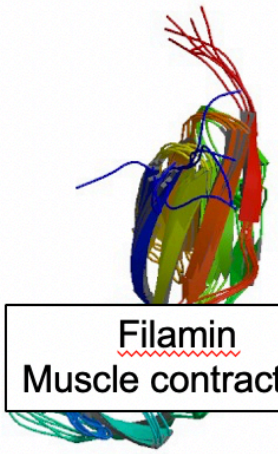

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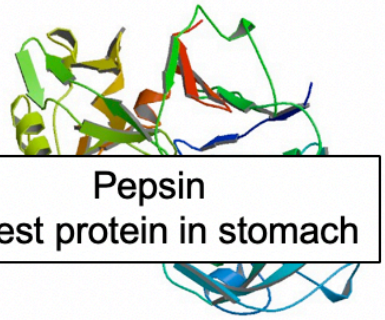
Muscle contraction



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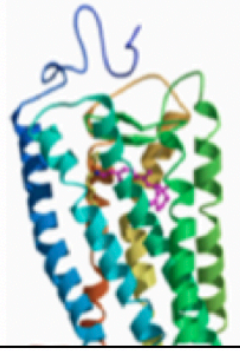

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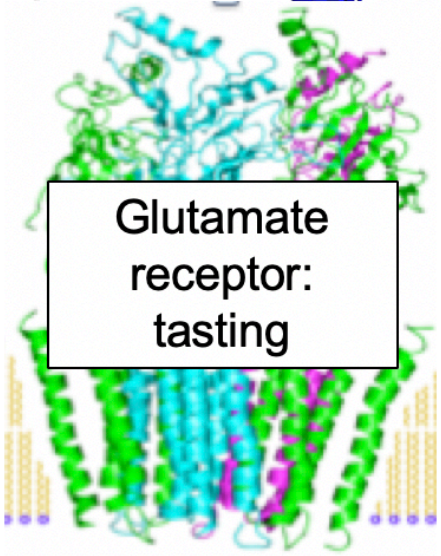

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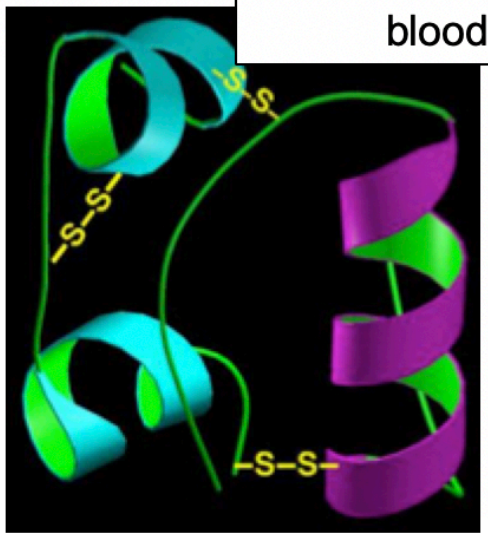
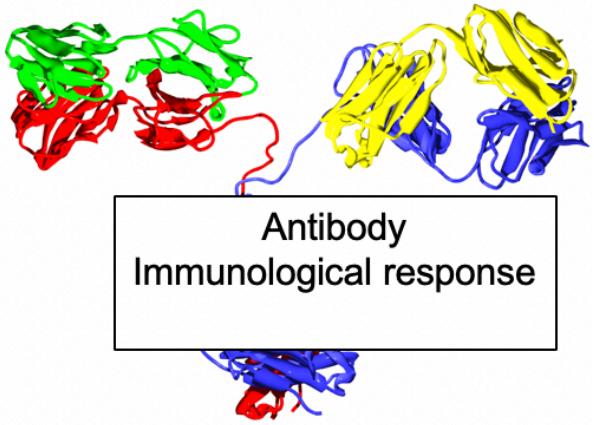
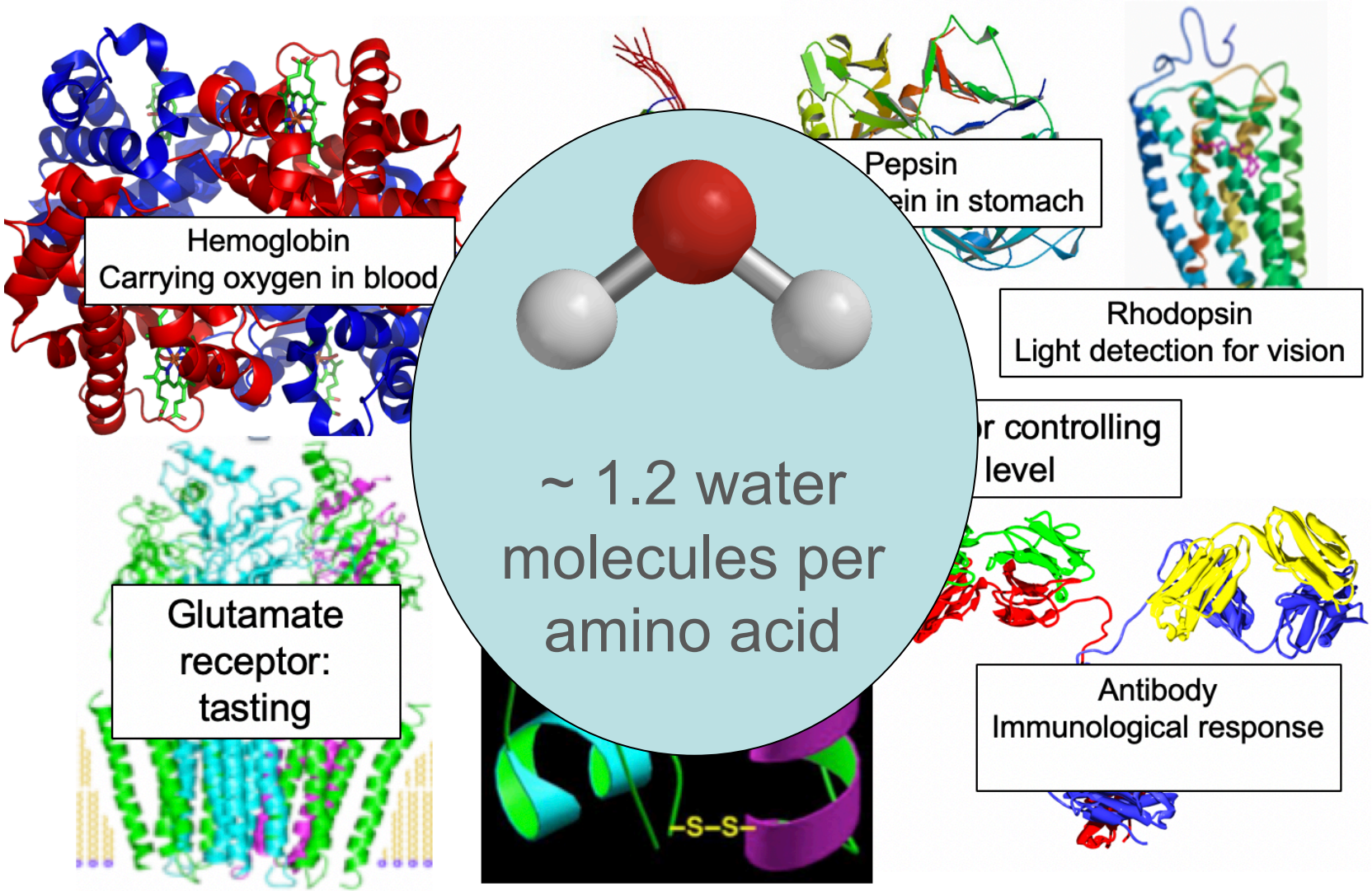




Antibody
Immunological response

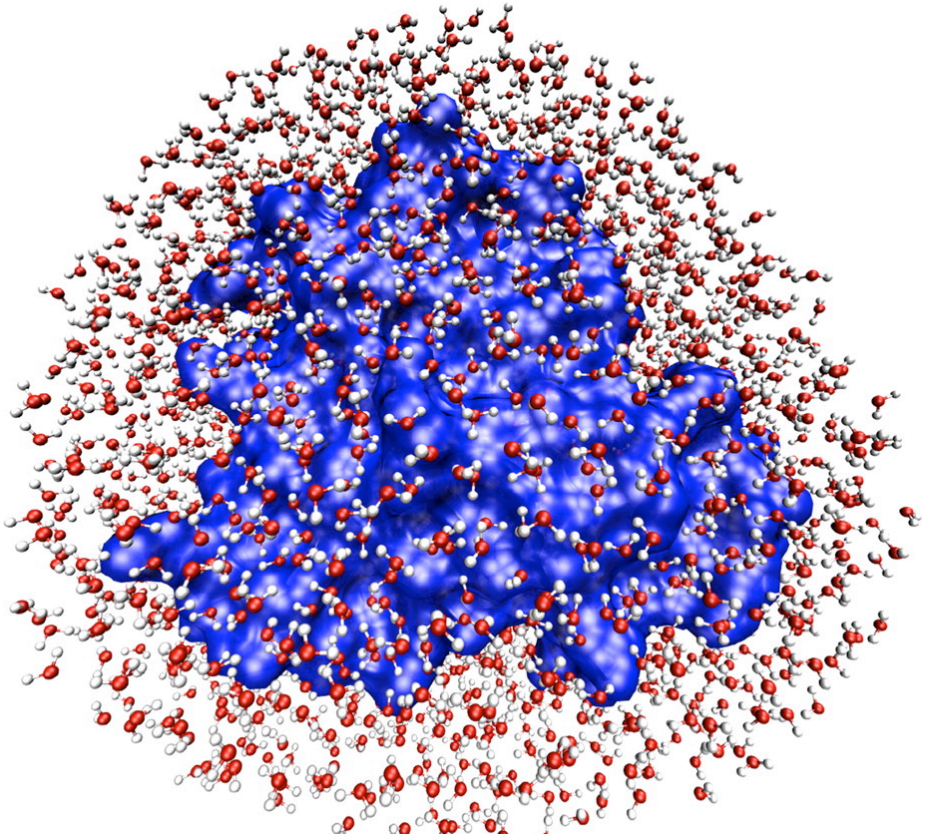
Motivation: Water Structures in Protein Hydration



Motivation: Water Structures in Protein Hydration

1. Protein dynamics
2. Protein folding/aggregation
3. Protein Interactions
4. Participation in biochemical reactions
5. Translocation ions across membrane

Water molecules are integral parts of proteins.



Motivation: Water Structures in Protein Hydration

1. Protein dynamics
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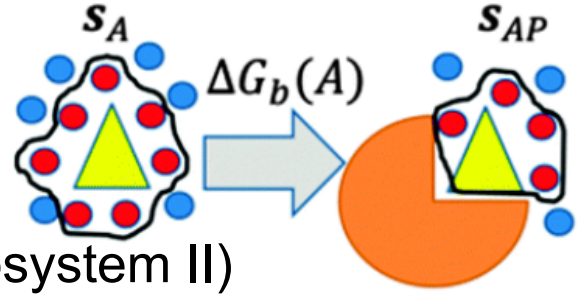
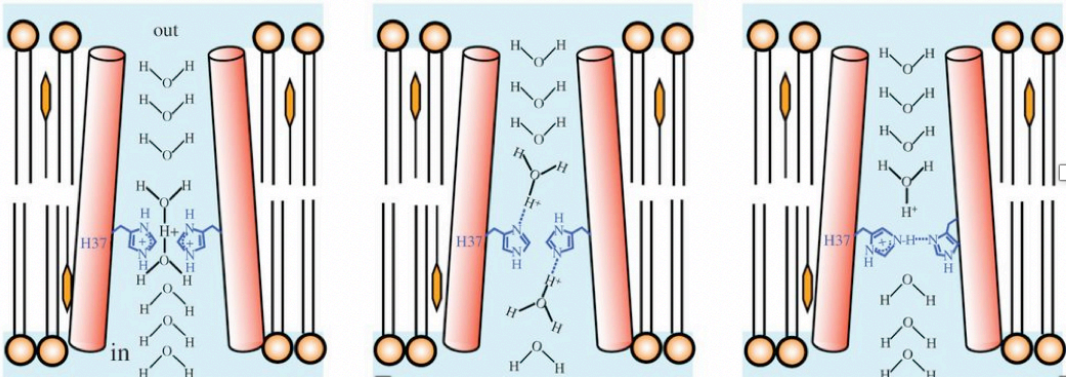
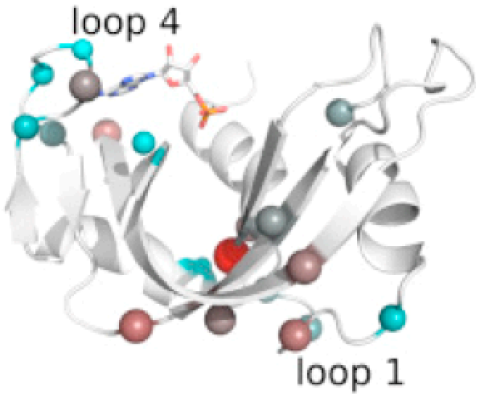
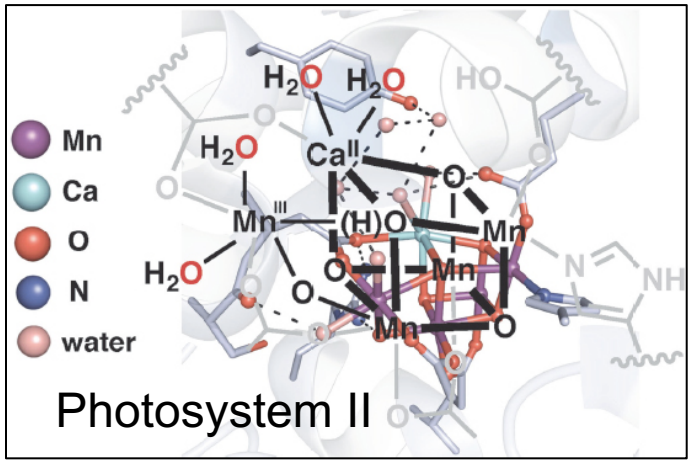
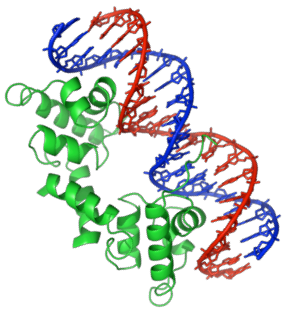
4. Participation in biochemical reactions (e.g., Photosystem II)
5. Involve in performing protein functions (e.g., ions transport)

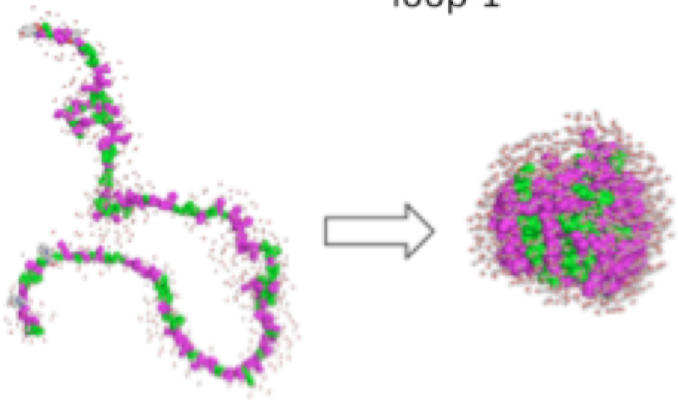












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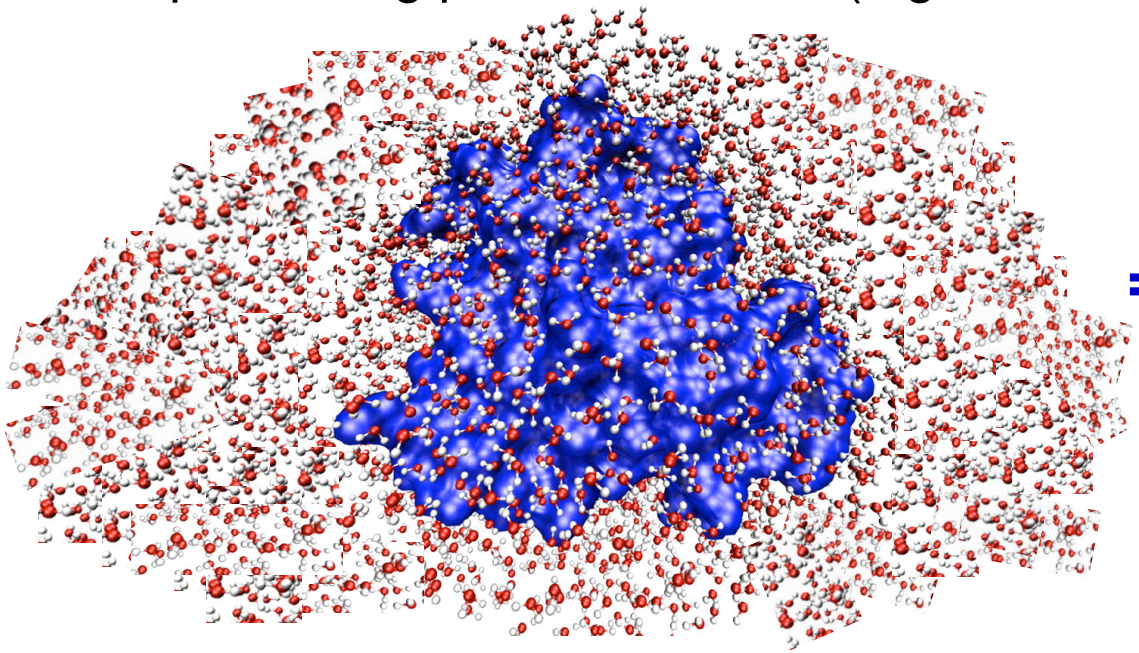

=> Structure-function correlation of proteins

Challenges:

Background from a large population of water molecules in bulk solution

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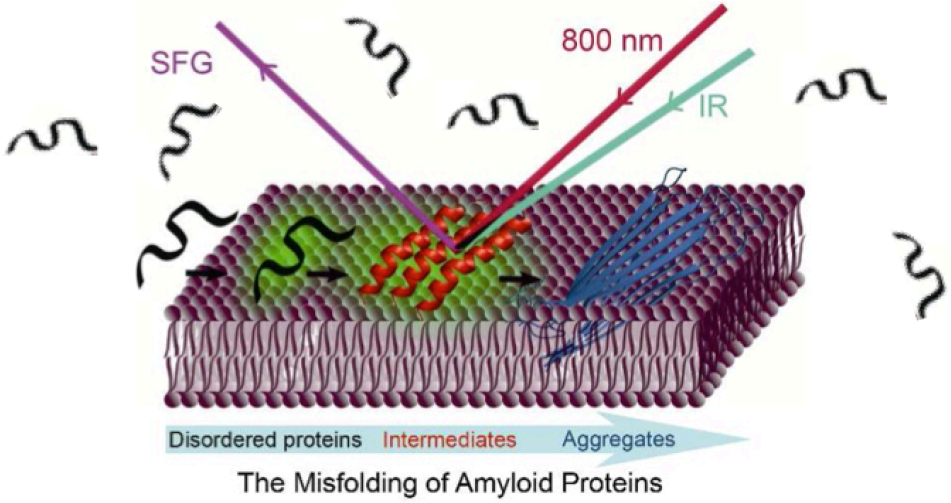
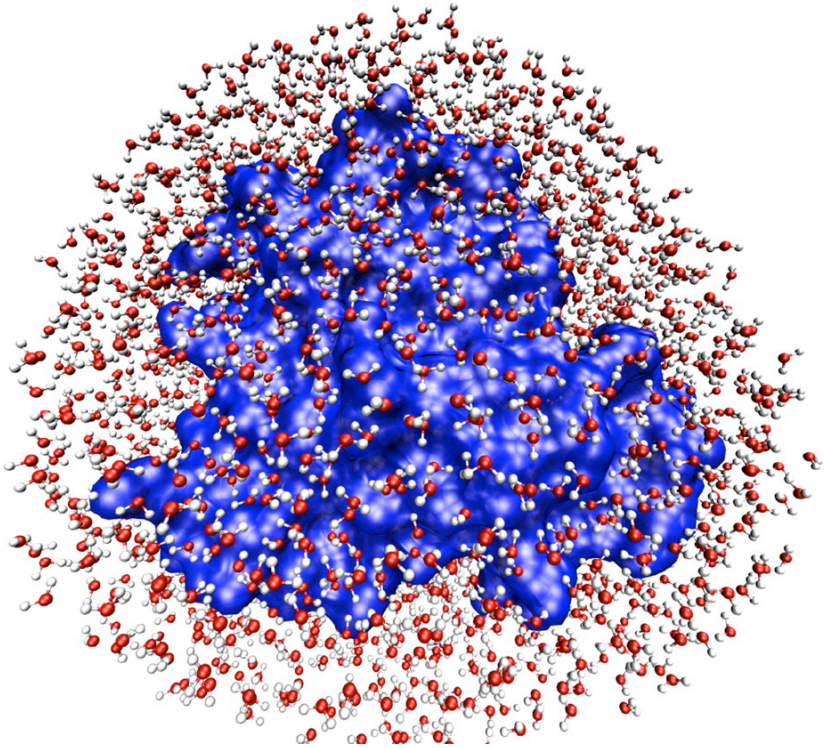
I. Charactering Protein Secondary Structures at Interfaces



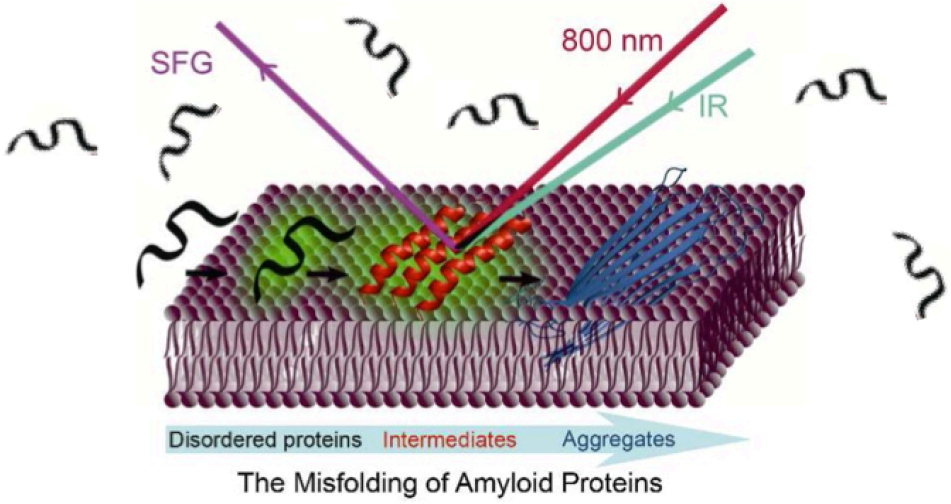
The Misfolding of Amyloid Proteins

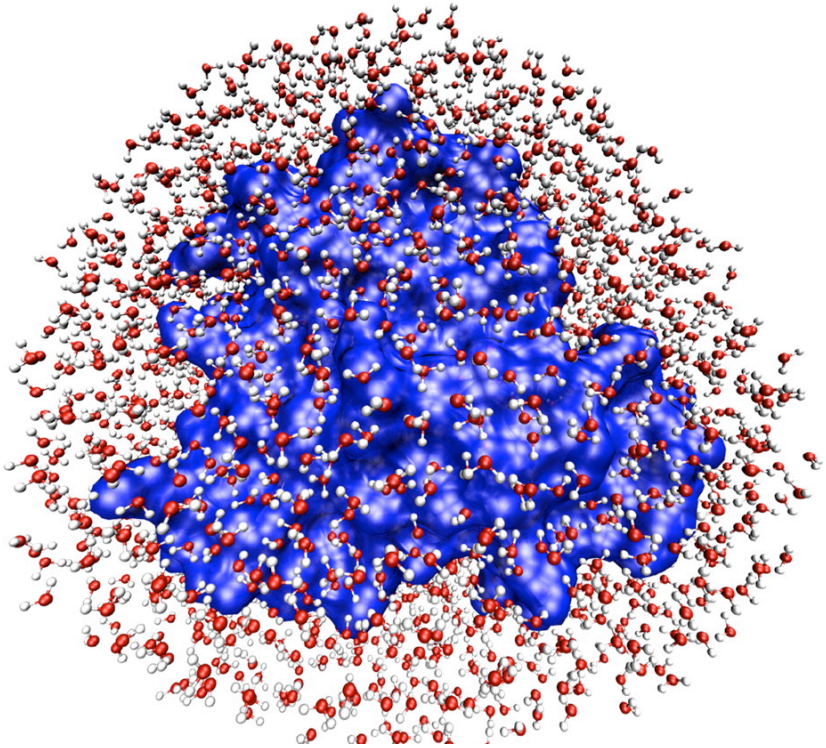
II. Probing Water Structures in Hydration Shell of Proteins



Outline

1. Introduction to Chiral SFG Spectroscopy
2. Characterizing Protein Secondary Structures at Interfaces
3. Probing Water Structures in Hydration Shell of Proteins





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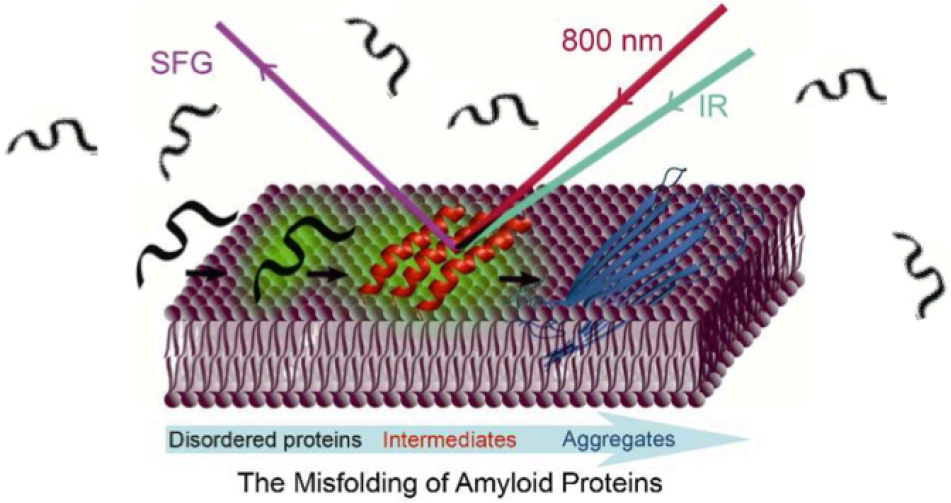

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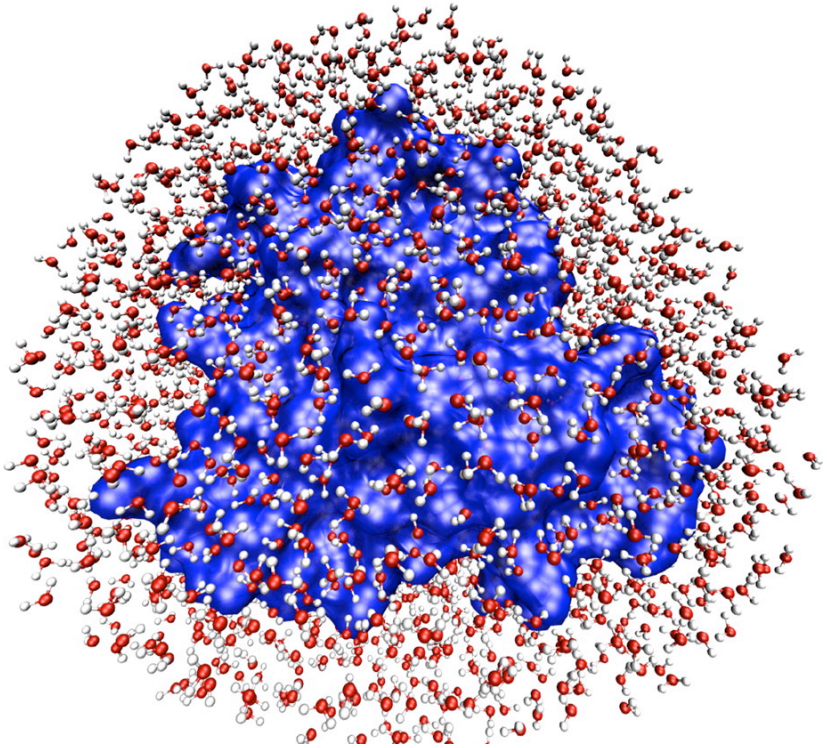

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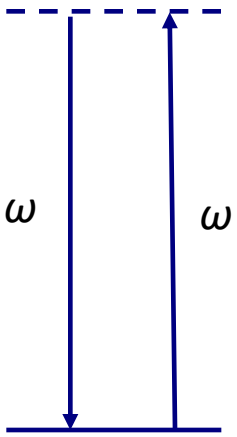
3. Probing Water Structures in Hydration Shell of Proteins

Development of Chiral SFG

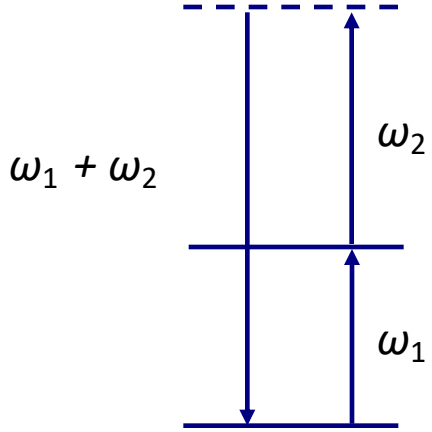
1. Shen (Berkeley): Observation of chiral SFG from pure chiral bulk liquid (Belkin et al, *Phys. Rev. Lett.*, 2000)
2. Simpson (Purdue): Theory of surface-sensitive chiral SFG for probing macroscopic chiral structures (Simpson, *ChemPhysChem*, 2004)
3. Chen (Michigan): First application to study amide I of a β-sheet on polymer surface (Wang *et al.*, *PNAS*, 2005)
4. Geiger (Northwestern): First application to study double helix DNA on glass surface (Stoke *et al.*, *JACS*, 2007,)

Introduction to Chiral SFG Spectroscopy

SFG Spectroscopy



Linear optical process

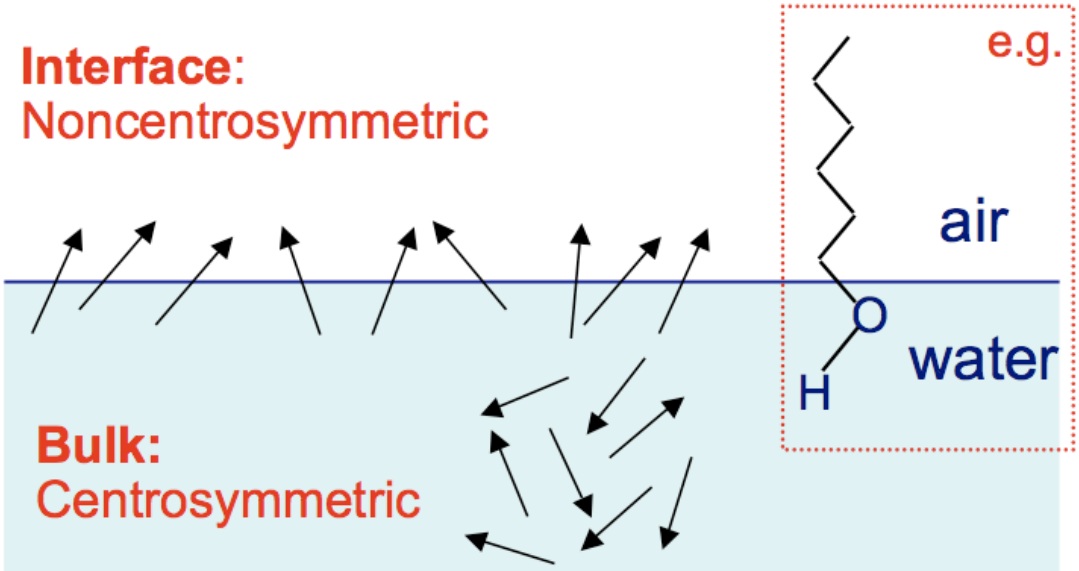


Second-order optical process

A second-order optical technique

Introduction to Chiral SFG Spectroscopy

SFG Spectroscopy



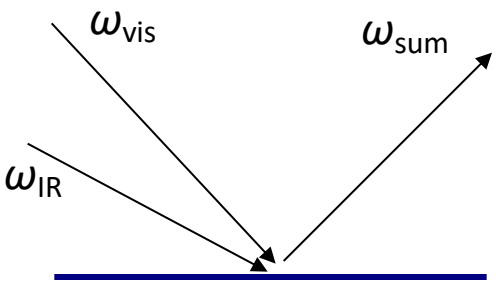
Noncentrosymmetric Interface => Surface-selectivity

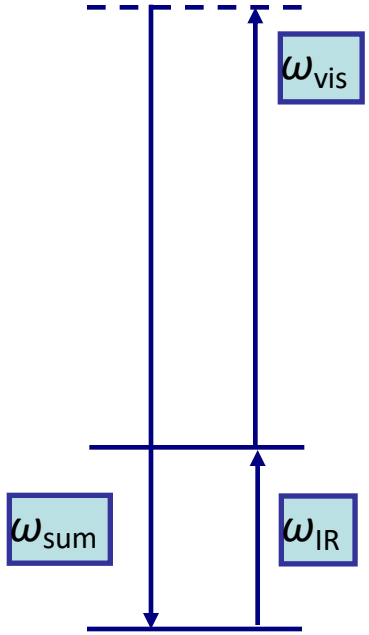
Introduction to Chiral SFG Spectroscopy

SFG Spectroscopy

SFG

$\omega_{vis} + \omega_{IR} = \omega_{sum}$





IR in resonance with surface vibrational structures

=> SFG enhancement

SFG: a surface-specific vibrational spectroscopy

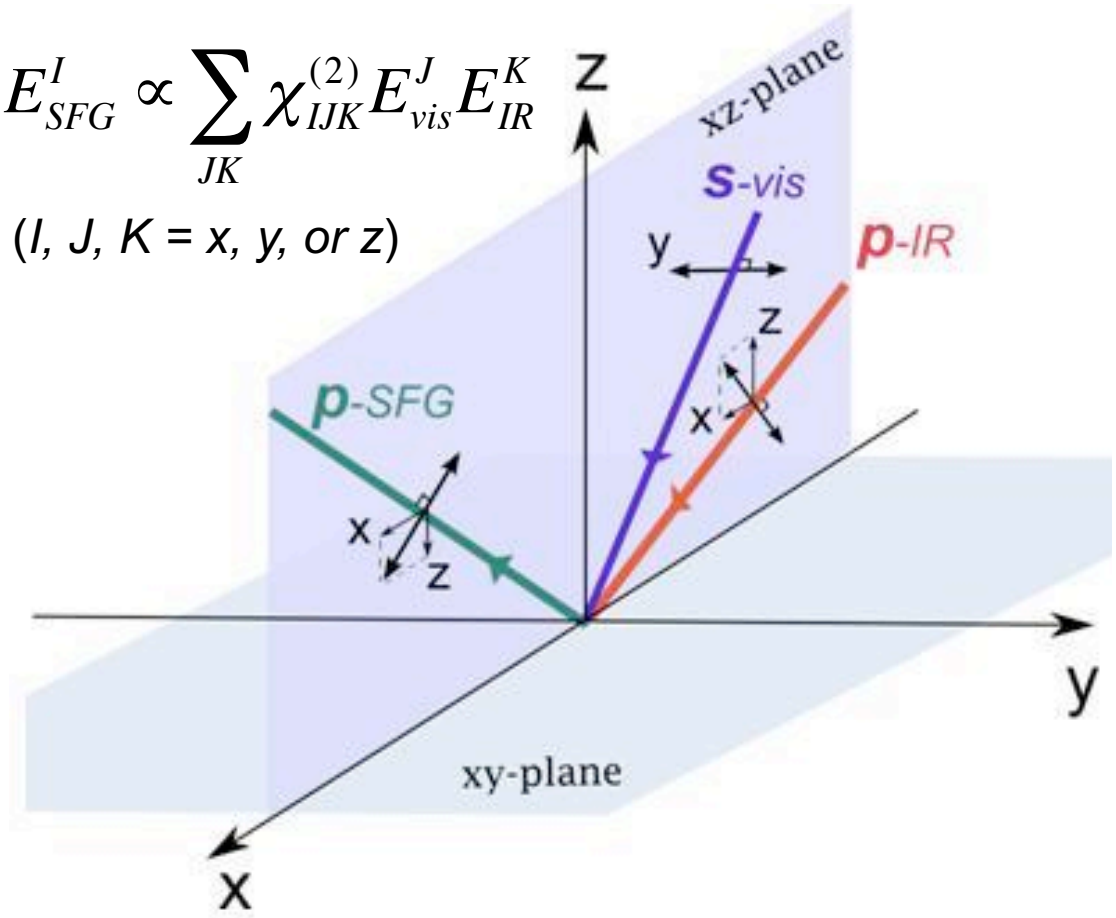
Introduction to Chiral SFG Spectroscopy

$$I_{SFG} = \left|\vec{E}_{SFG}\right|^2$$

$$\vec{E}_{SFG} = E^x_{SFG}\hat{x} + E^y_{SFG}\hat{y} + E^z_{SFG}\hat{z}$$

$$E^I_{SFG} \propto \sum_{JK} \chi^{(2)}_{IJK} E^J_{vis} E^K_{IR}$$

(*I*, *J*, *K* = *x*, *y*, or *z*)



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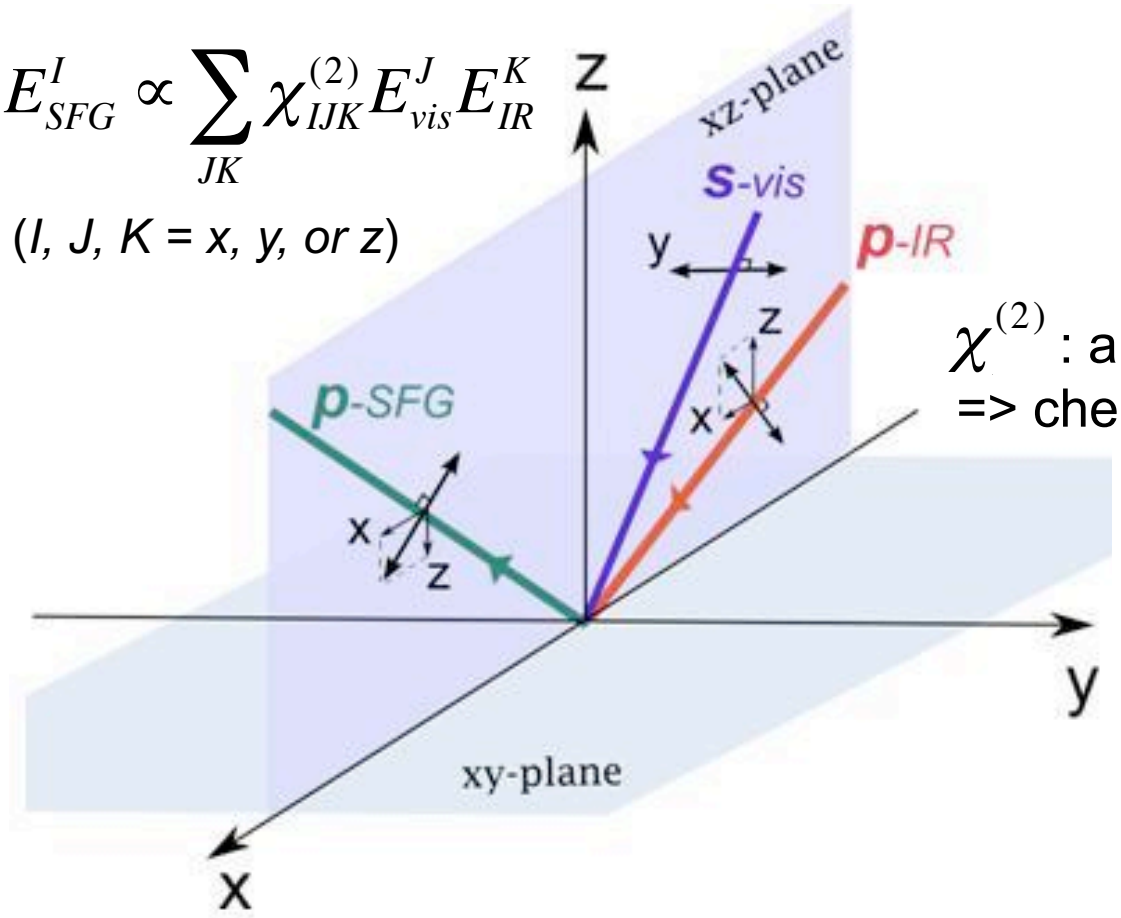

$\chi^{(2)}$: a tensor with 27 elements
=> chemical & structural information

Individual tensor elements can be measured by different combinations of polarization settings.

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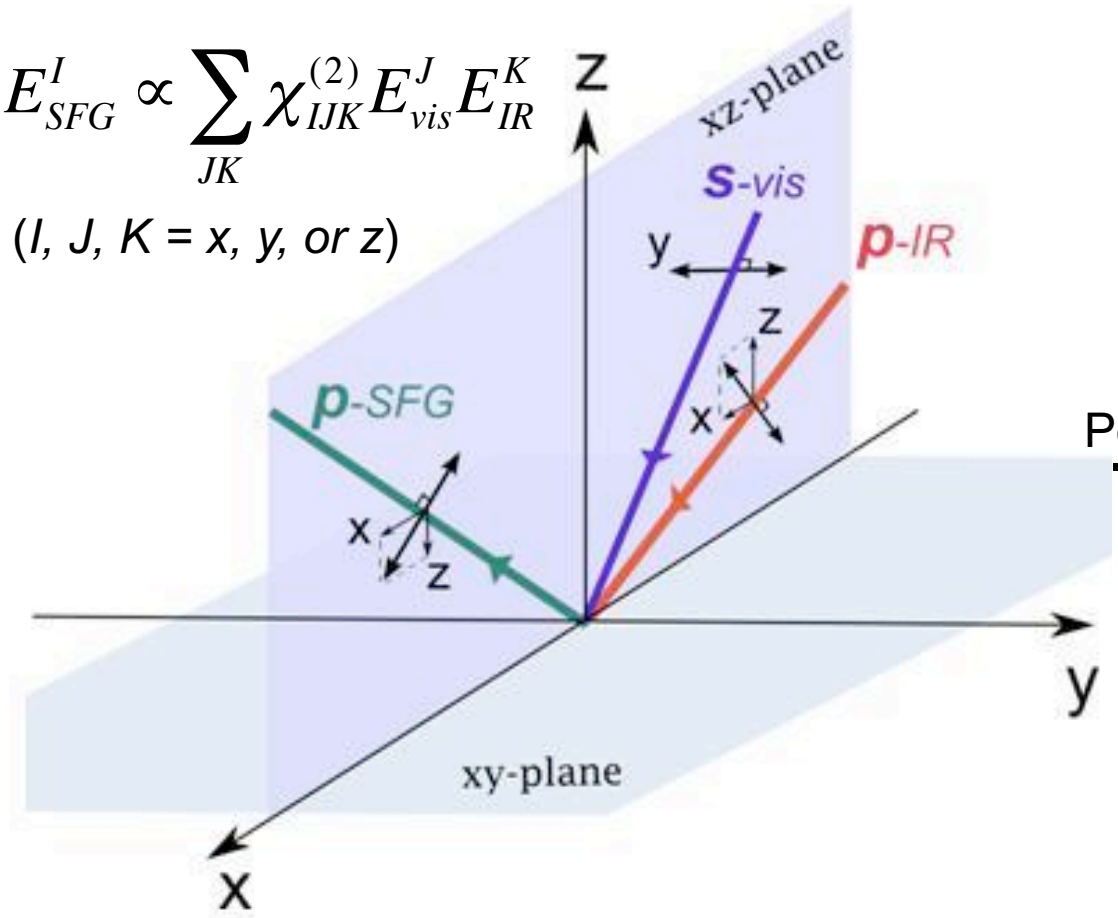
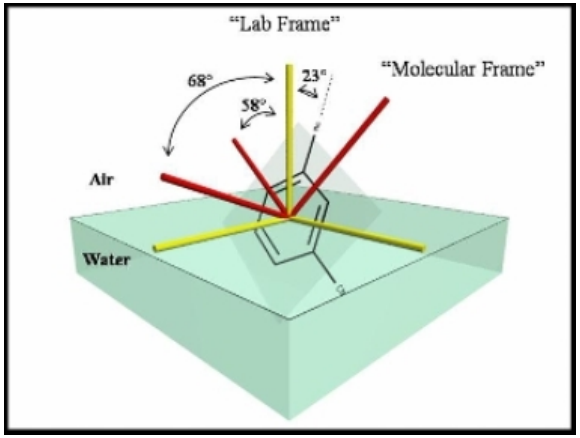
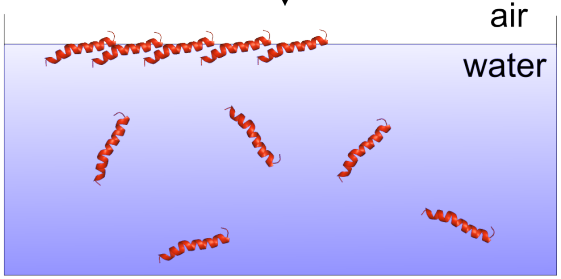


Molecular Orientation



Polarization of beams ↕ $\chi^{(2)}_{IJK}$



Surface chirality

Introduction to Chiral SFG Spectroscopy

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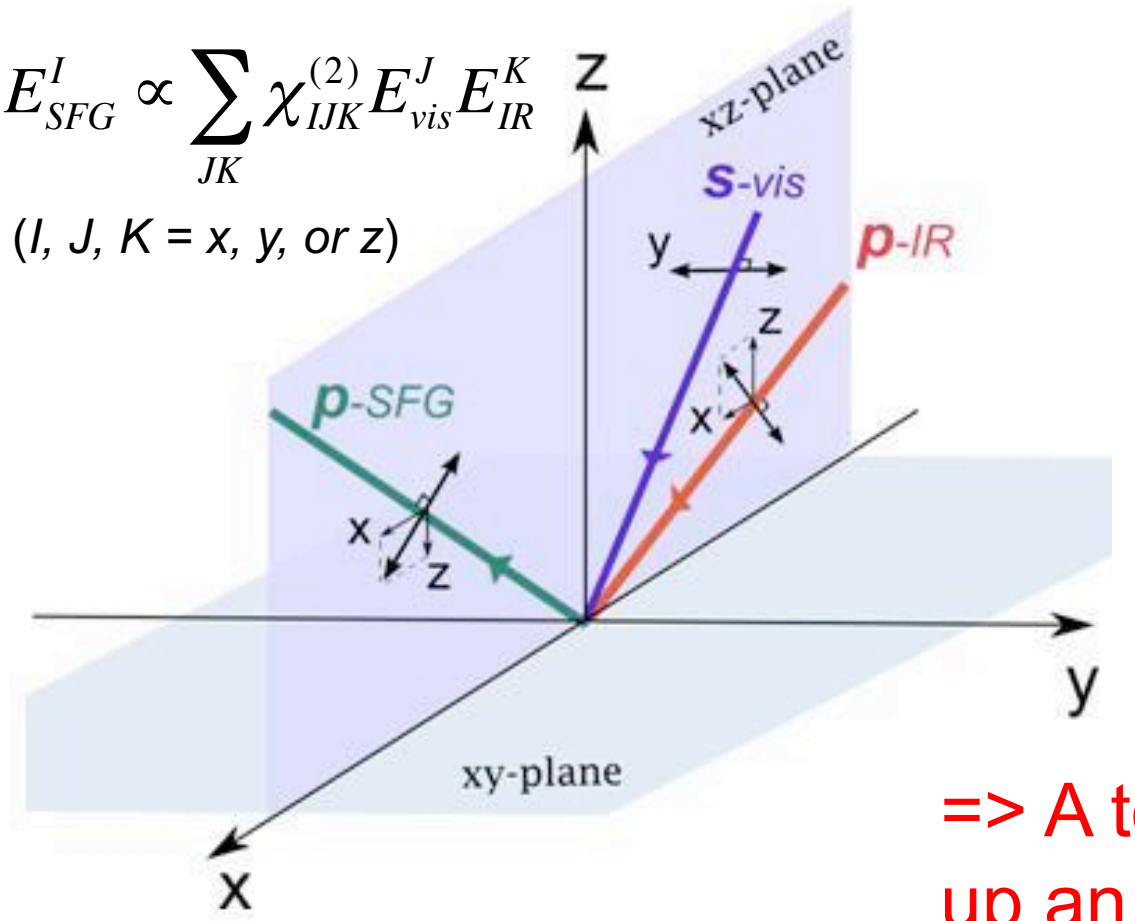

SFG Vis IR

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psp
ppp
pss
sps
ssp
sss

=> A total of 8 ways to set up an SFG experiment

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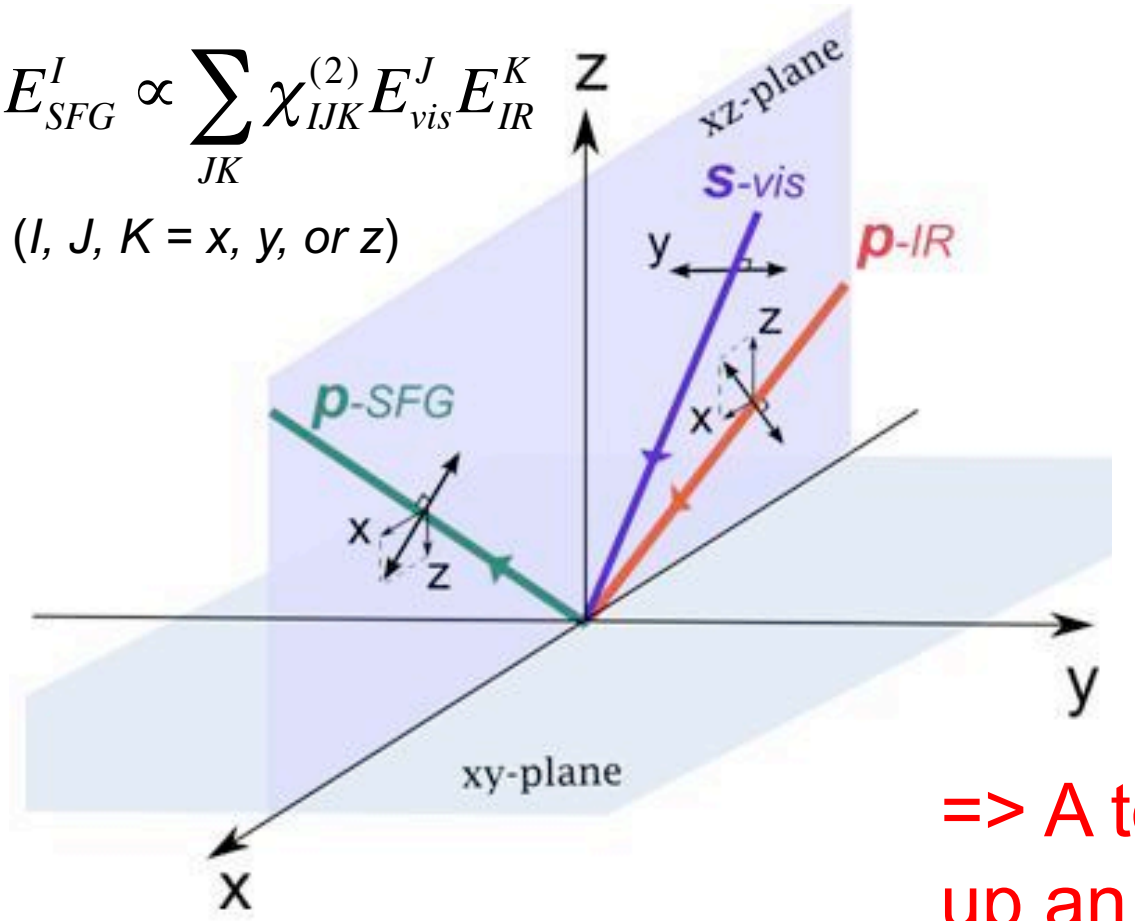

SFG Vis IR

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Chiral SFG

=> A total of 8 ways to set up an SFG experiment

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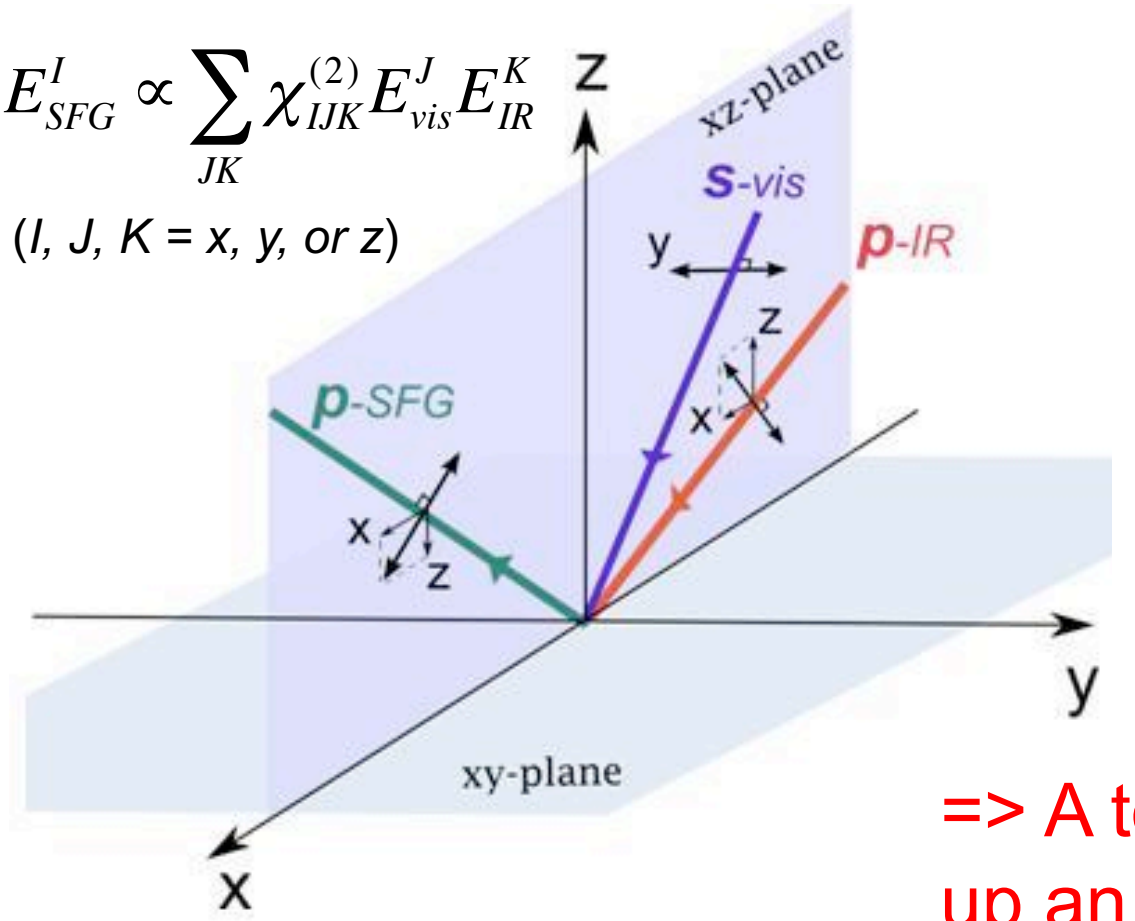

SFG Vis IR

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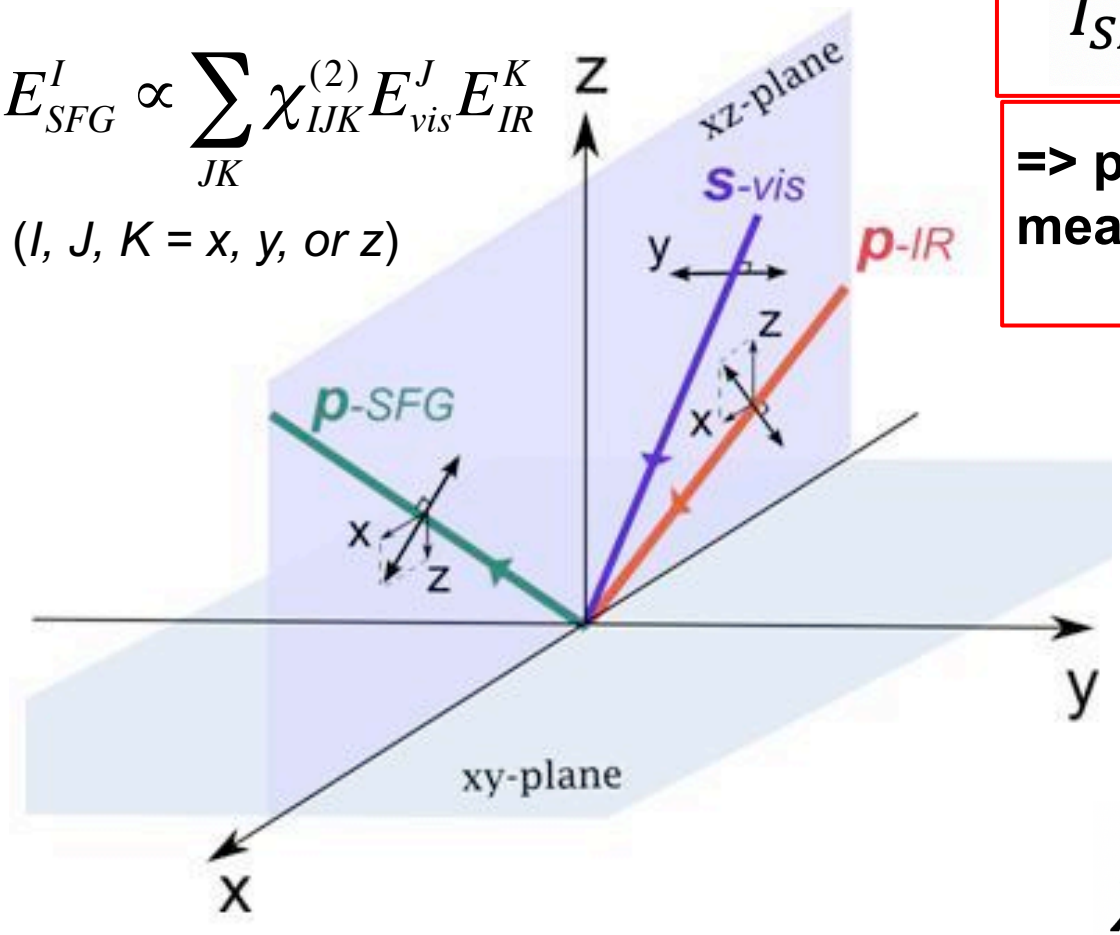
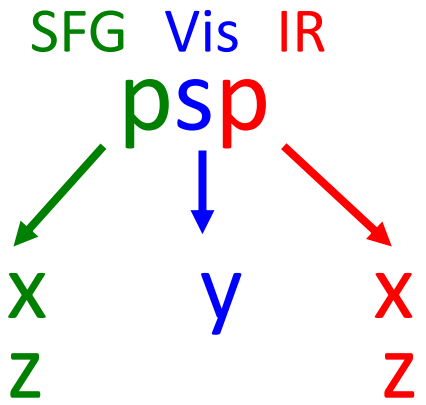


Experimental observable:

$$I_{SFG} \propto |E_{SFG}|^2 \propto \left|\chi^{(2)}_{zyx}\right|^2$$

=> psp polarization selectively measuring $\chi^{(2)}_{zyx}$



$$\chi^{(2)}_{ijk}: \overset{0}{\chi^{(2)}_{xyx}}, \overset{0}{\chi^{(2)}_{xyz}}, \chi^{(2)}_{zyx}, \overset{0}{\chi^{(2)}_{zyz}}$$

Introduction to Chiral SFG Spectroscopy

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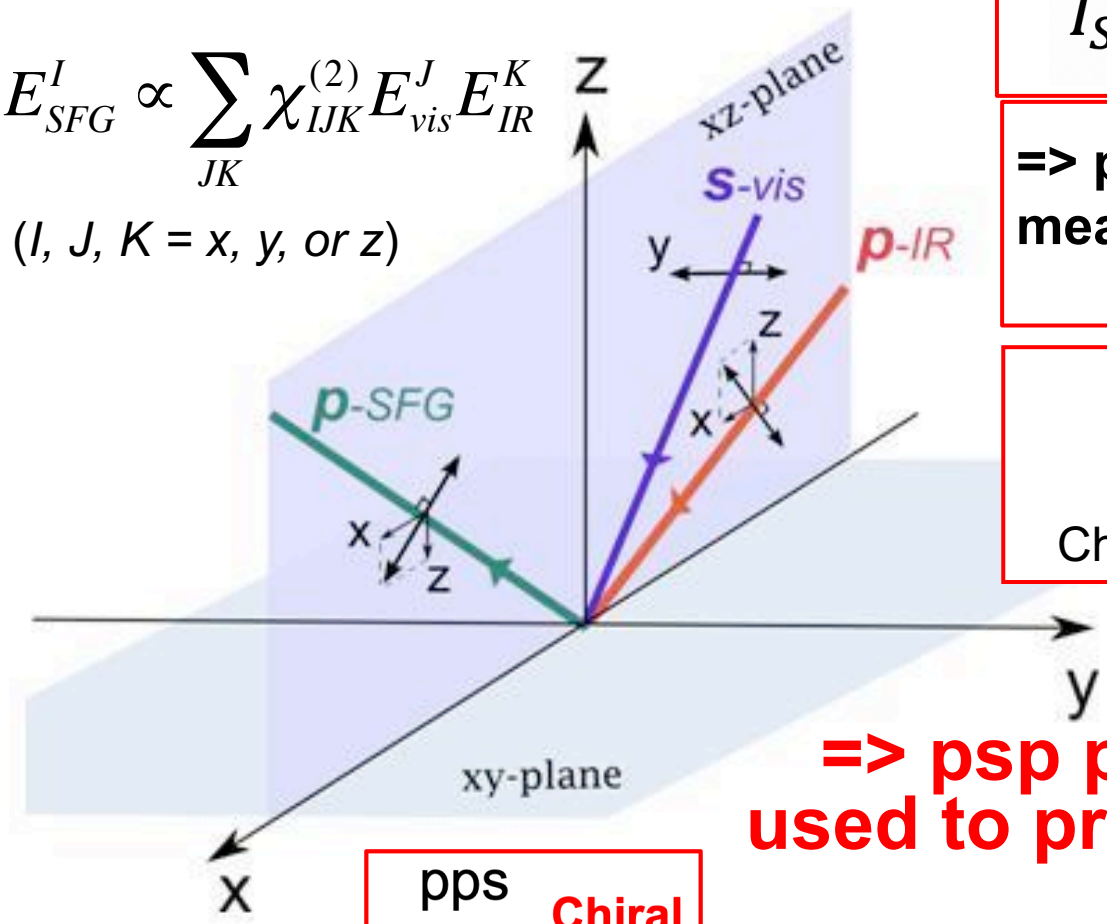


Experimental observable:

$$I_{SFG} \propto |E_{SFG}|^2 \propto \left|\chi^{(2)}_{zyx}\right|^2$$

=> psp polarization selectively measuring $\chi^{(2)}_{zyx}$

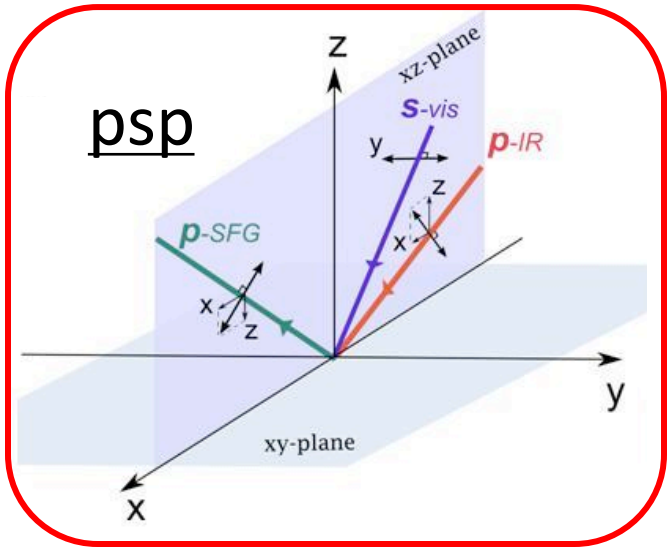
$\chi^{(2)}_{IJK}$ ($I \neq J \neq K$):
Orthogonal $\chi^{(2)}$ Element
Characteristics of Chiral Surfaces

=> psp polarization can be used to probe surface chirality

pps
spp
psp

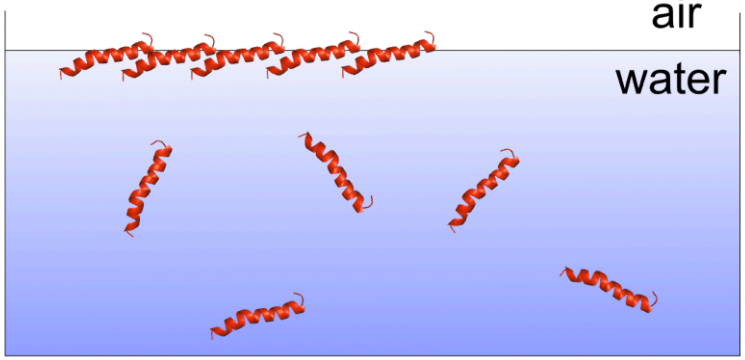
Chiral SFG

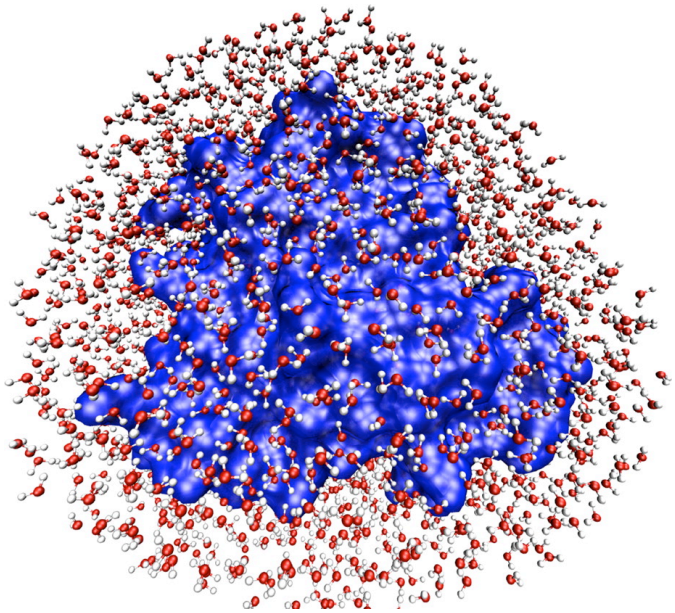
Introduction to Chiral SFG Spectroscopy



I. Protein Secondary Structures at Interfaces

II. Water Structures in Protein Hydration





Protein Characterizations by Chiral Vibrational Sum Frequency Generation Spectroscopy

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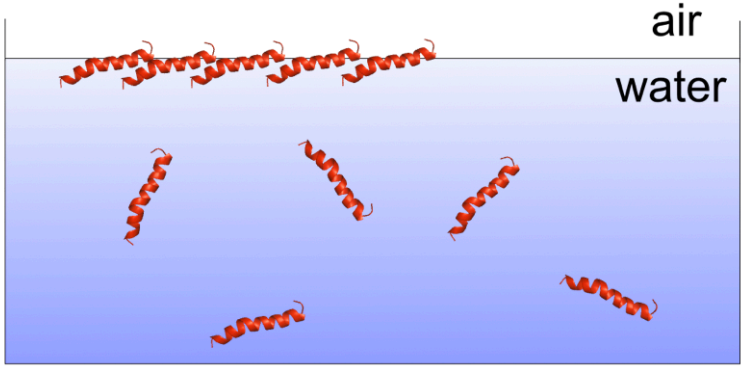

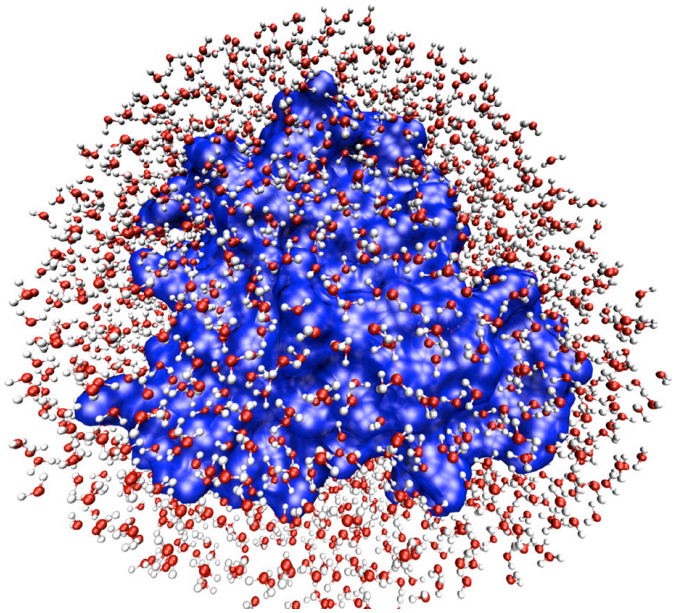

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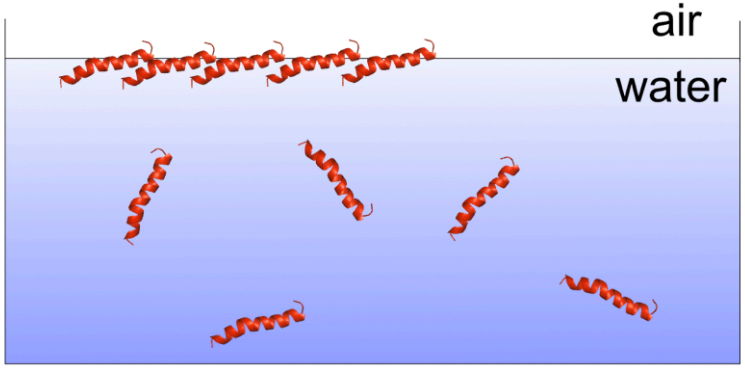
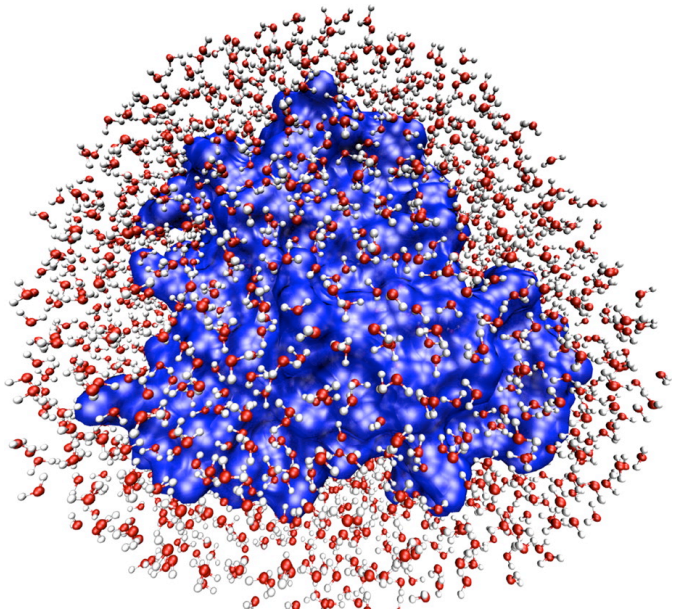
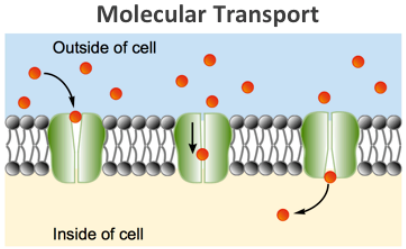
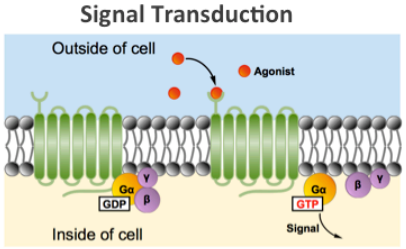
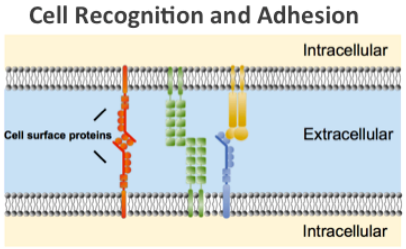


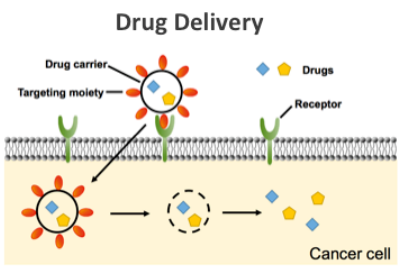


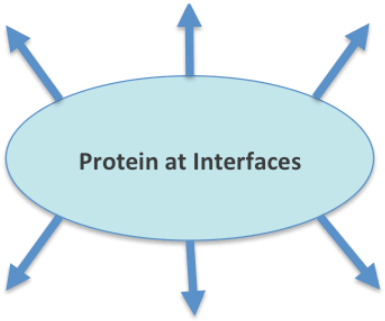
Protein Secondary Structures at Interfaces

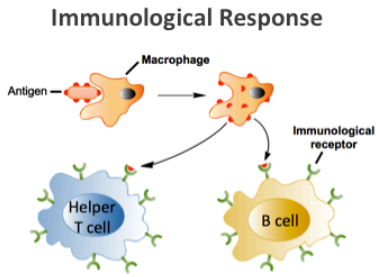


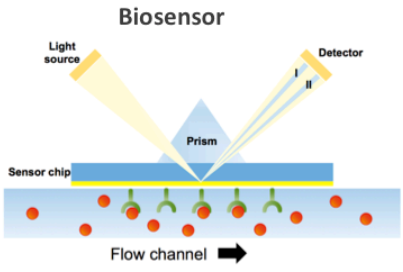


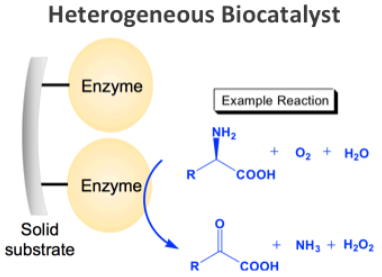


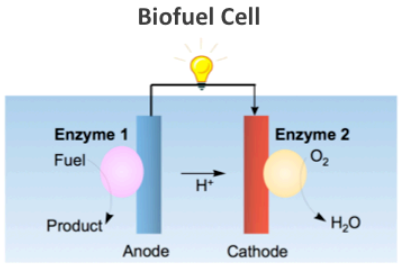








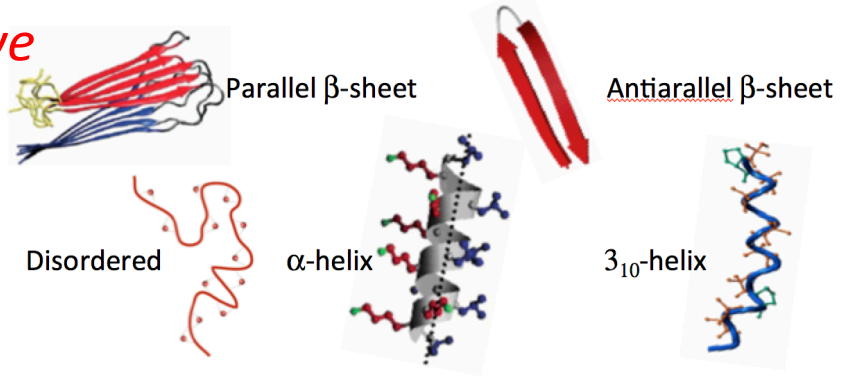




Challenge: Selectivity to
(1) Interfaces
(2) Secondary Structures

Conventional Methods—*Not surface selective*

1. Circular Dichroism Spectroscopy
2. NMR Spectroscopy
3. X-ray crystallography



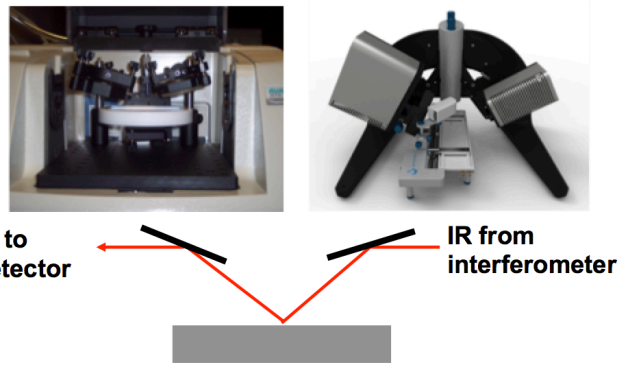
Protein Secondary Structures at Interfaces

Challenge: Selectivity to

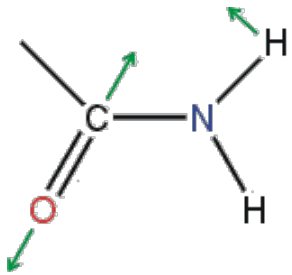
(1) Interfaces

(2) Secondary Structures

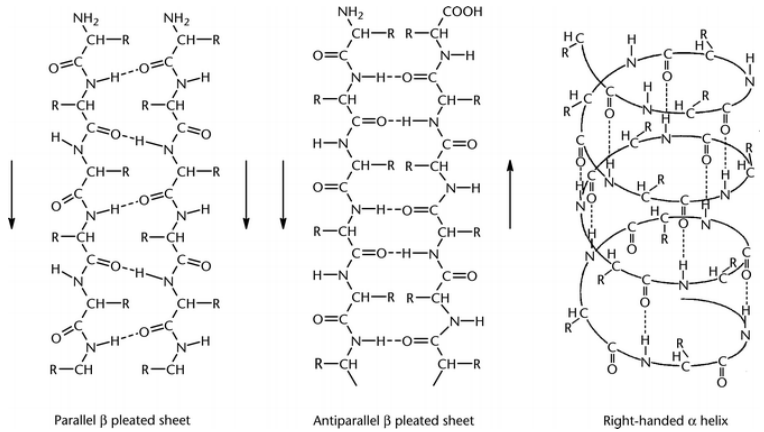
Infrared Reflection Absorption Spectroscopy (IRRAS)



Amide I



Secondary structure	Band position in H_2O/cm^{-1}	
	Average	Extremes
α-helix	**1654**	1648-1657
β-sheet	1633	1623-1641
β-sheet	1684	1674-1695
Turns	1672	1662-1686
Disordered	**1654**	1642-1657



Spectral overlapping

- **Deconvolution**
- **Overlap with water bending**
- **D_2O as solvent**

Protein Secondary Structures at Interfaces

Challenge: Selectivity to

(1) Interfaces

(2) Secondary Structures

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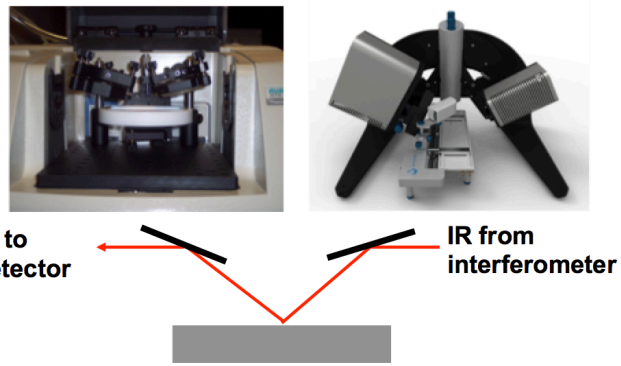
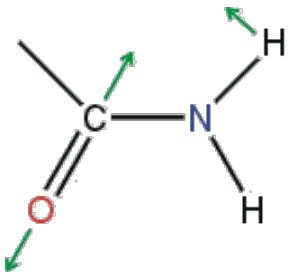
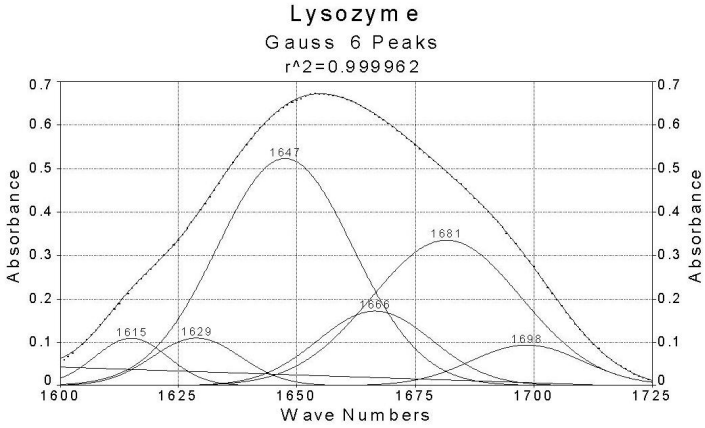


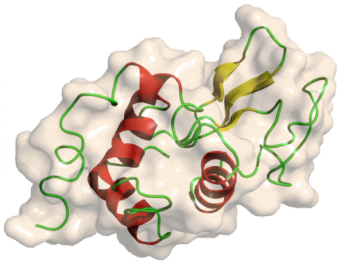
metal surface; air-water interface; etc

Amide I



Secondary structure	Band position in H_2O/cm^{-1}	
	Average	Extremes
α-helix	**1654**	1648-1657
β-sheet	1633	1623-1641
β-sheet	1684	1674-1695
Turns	1672	1662-1686
Disordered	**1654**	1642-1657





Spectral overlapping

- **Deconvolution**
- **Overlap with water bending**
- **D_2O as solvent**

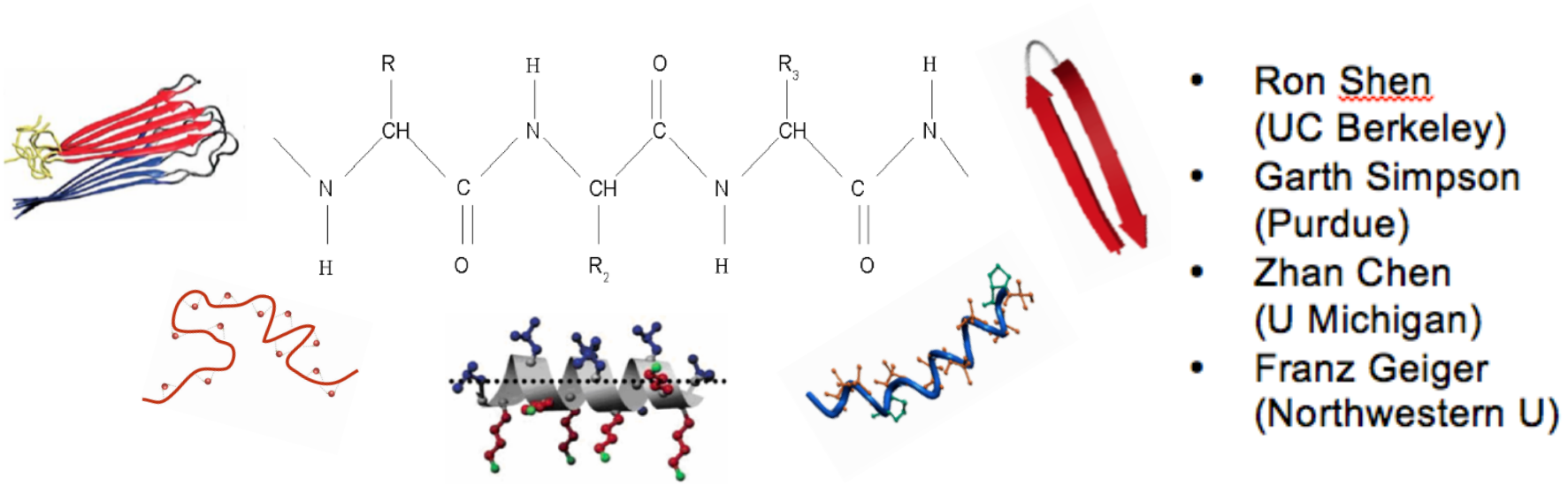
Protein Secondary Structures at Interfaces

Challenge: Selectivity to

(1) Interfaces

(2) Secondary Structures

Chiral Sum Frequency Generation Spectroscopy



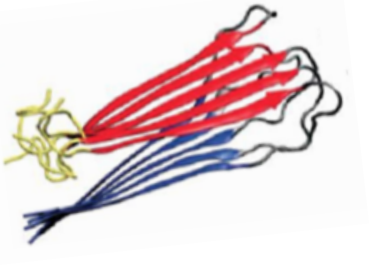
- Ron Shen (UC Berkeley)
- Garth Simpson (Purdue)
- Zhan Chen (U Michigan)
- Franz Geiger (Northwestern U)

Protein Backbone (Amide I and N-H Stretch) Chiral Vibrational Structures

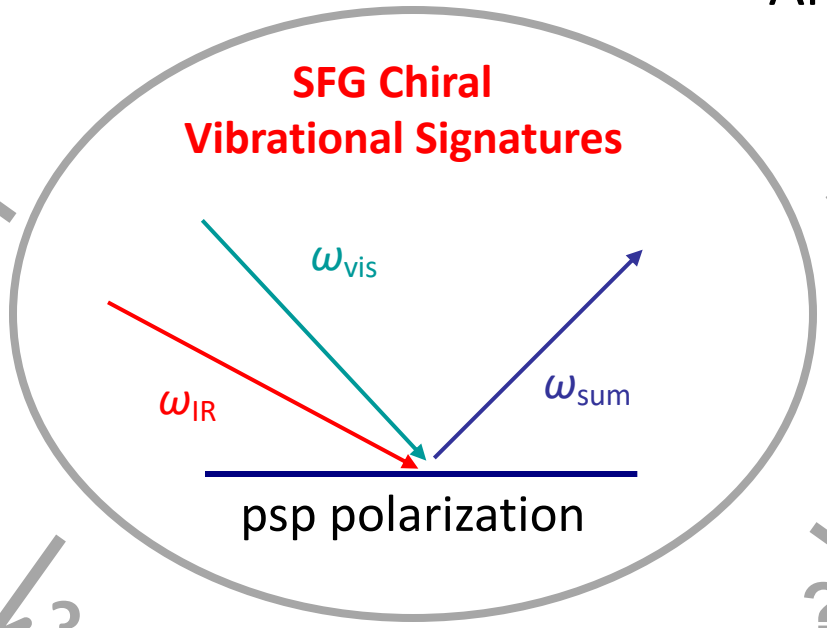
Characterization of Protein Secondary Structures at Interfaces

Characterization of Secondary Structures at Interfaces

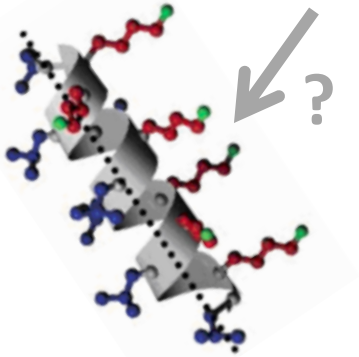
Parallel β-sheet

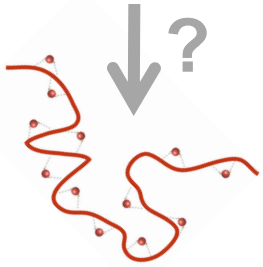


Antiparallel β-sheet

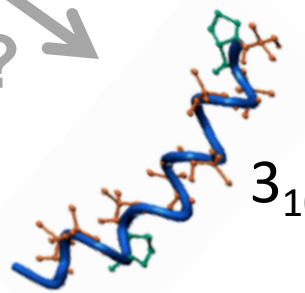


α-helix





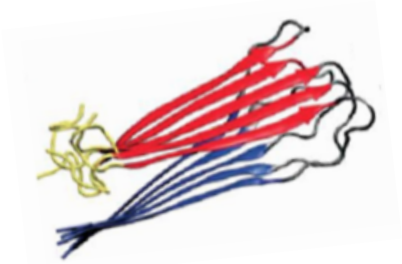
Unstructured



3_{10}-helix

Characterization of Secondary Structures at Interfaces

Parallel β-sheet

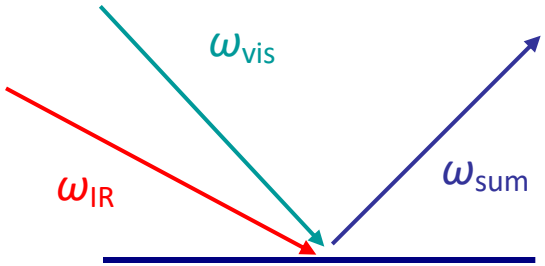


Model:
The hIAPP aggregate

Antiparallel β-sheet

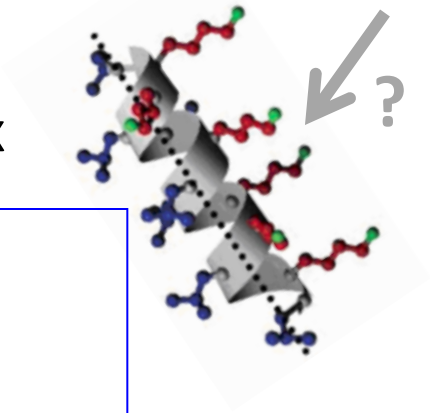
Model:
Tachyplasin I
$LK_7\beta$

SFG Chiral Vibrational Signatures



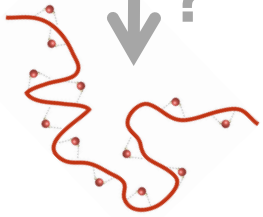
psp polarization

α-helix



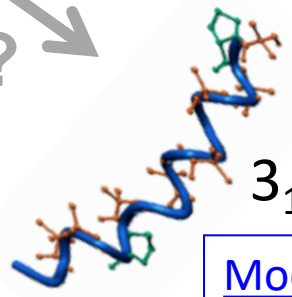
Model:
Rhodopsin
pHLIP
$LK\alpha_{14}$

Unstructured



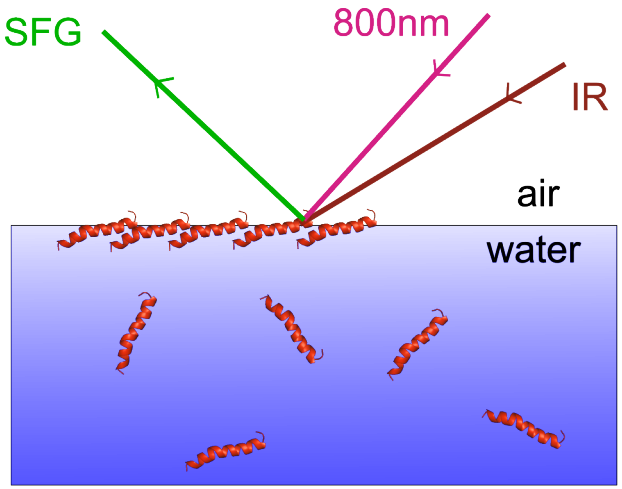
Model:
disordered rIAPP

3_{10}-helix

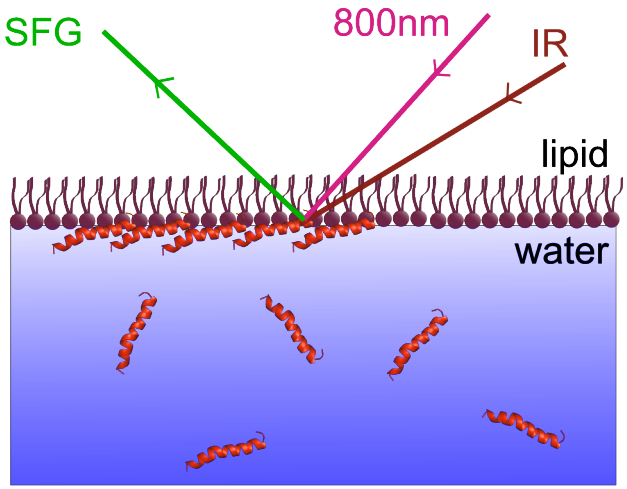


Model:
Alamethicin

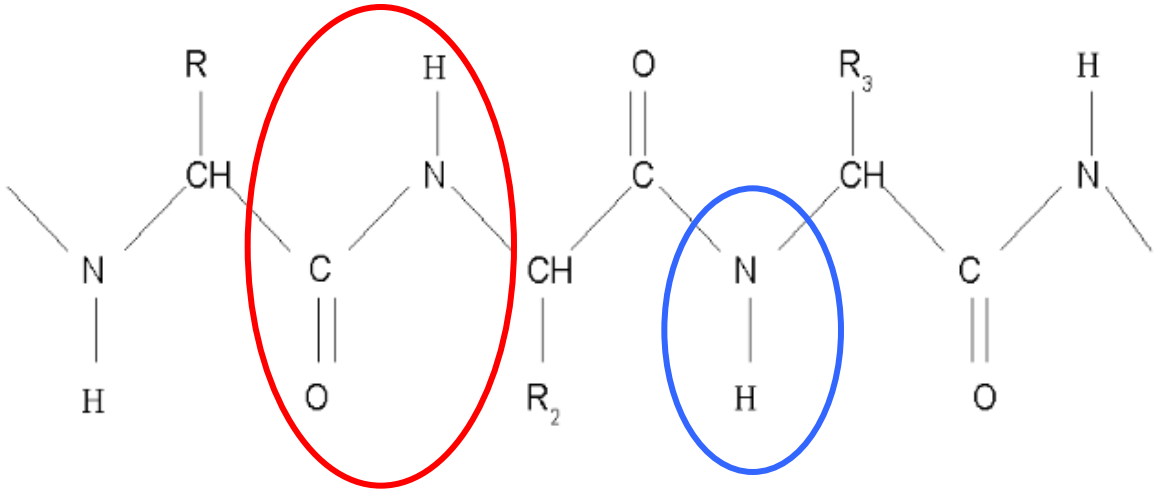
Chiral SFG Experiments—psp Polarization



or



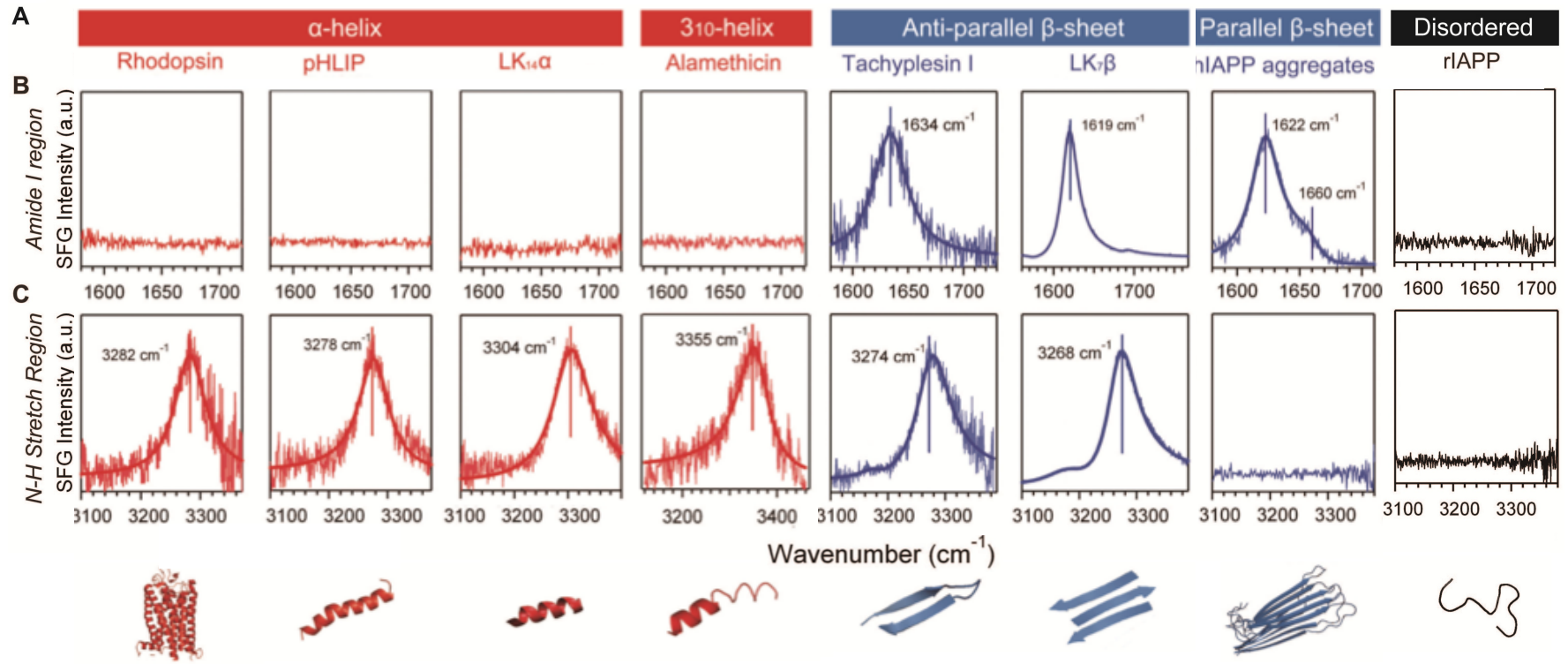
Peptide Backbone



The Amide I Band (1600-1700 cm^{-1})

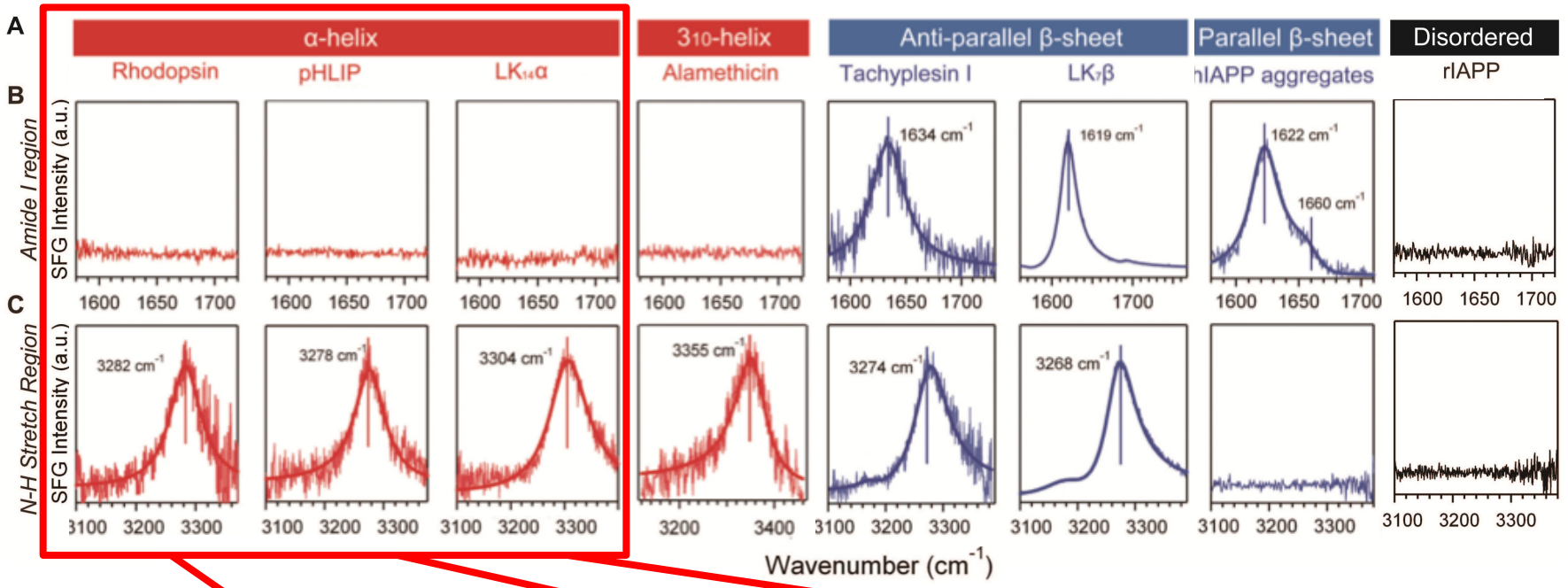
The N-H Stretch (3000-3400 cm^{-1})

cSFG Vibrational Signatures for Secondary Structures

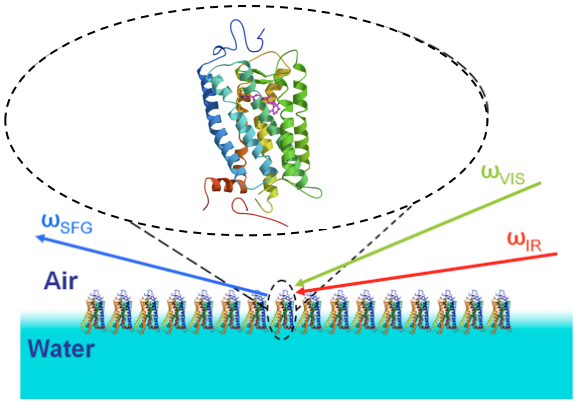


- Yan *et al Chem. Rev.* 2014
- Fu *et al Int. J. Mol. Sci.* 2011
- Yan *et al. J. Phys. Chem. B* 2015
- Wang *et al. Langmuir*, 2015

cSFG Vibrational Signatures for Secondary Structures

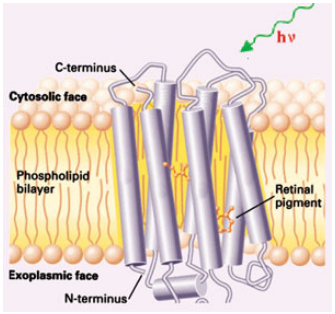


Rhodopsin



(Lavoie *et al. Biochemistry*, 2002)

pHLIP

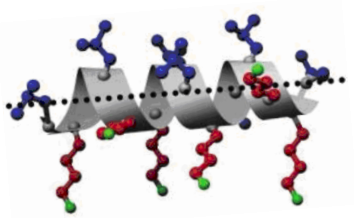


Helix 3 of Bacteriorhodopsin

(Englman and coworkers, PNAS, **2010**)

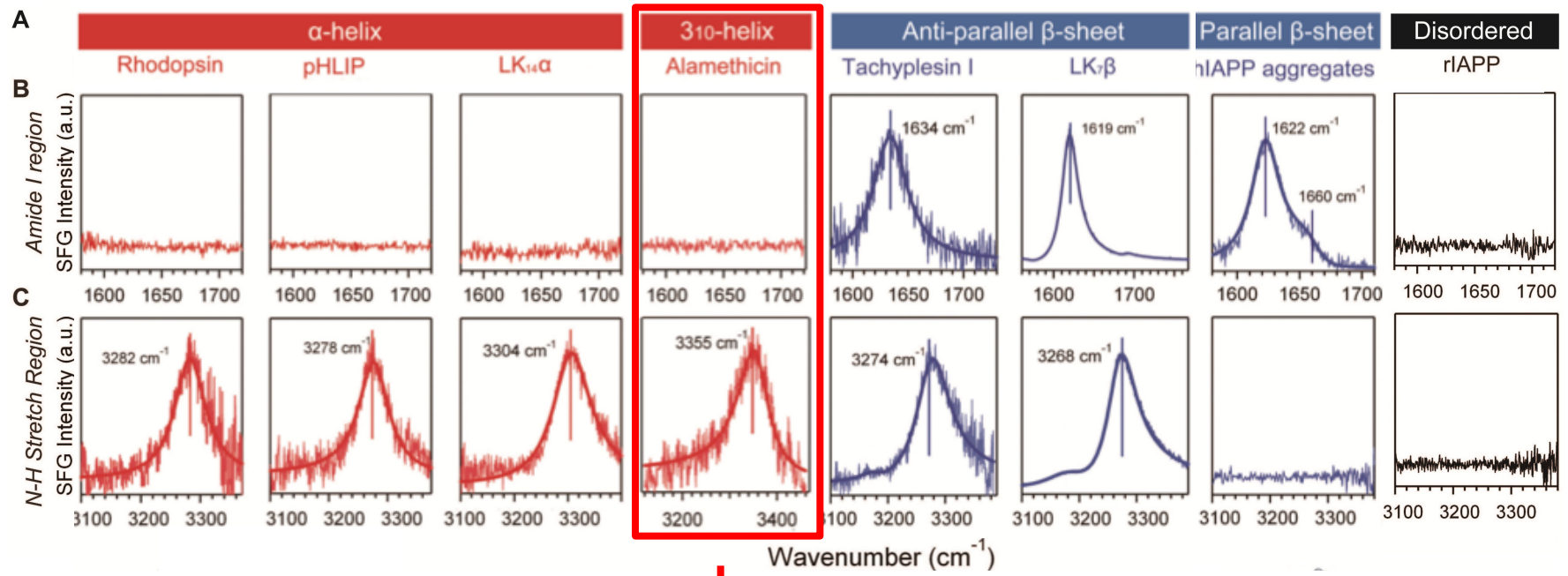
$LK_{14}\alpha$

LKKLLKLLKKLLKL

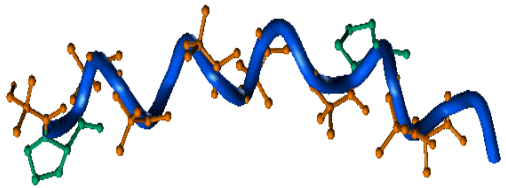


(DeGrado and Coworkers *JACS*, **1985**)

cSFG Vibrational Signatures for Secondary Structures



A mixture of α-helix & 3_{10}helix



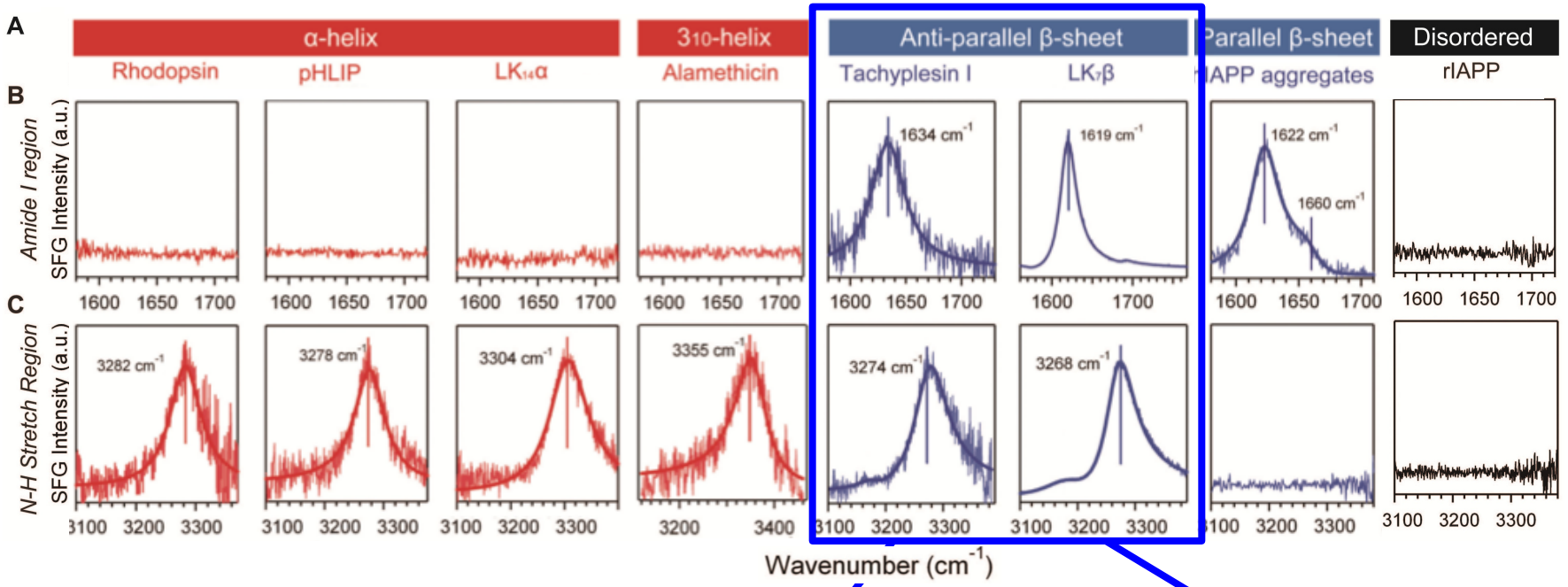
(Ionov et al. *J. Phys. Chem.* 2004)

Alamethicin

Ac-Aib-L-Pro-Aib-L-Ala-Aib-L-Ala-L-Gln-Aib-L-Val-Aib-Gly-L-Leu-Aib-L-Pro-L-Val-Aib-Aib-L-Glu-L-Gln-L-Phol

- Yan *et al Chem. Rev.* 2014
- Fu *et al Int. J. Mol. Sci.* 2011
- Yan *et al. J. Phys. Chem. B* 2015
- Wang *et al. Langmuir*, 2015

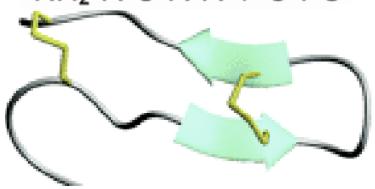
cSFG Vibrational Signatures for Secondary Structures



Tachyplesin I

H-K-W-C-F-R-V-C-Y-R

NH_2-R-C-R-R-Y-C-I-G



(Nakamura *et al. J. Biol. Chem.* **1988**)

(Chen and coworker, *PNAS*, **2005**)

$LK_7\beta$

LKLKLKL

(DeGrado and coworker, *JACS* **1985**)

- Yan *et al Chem. Rev.* 2014
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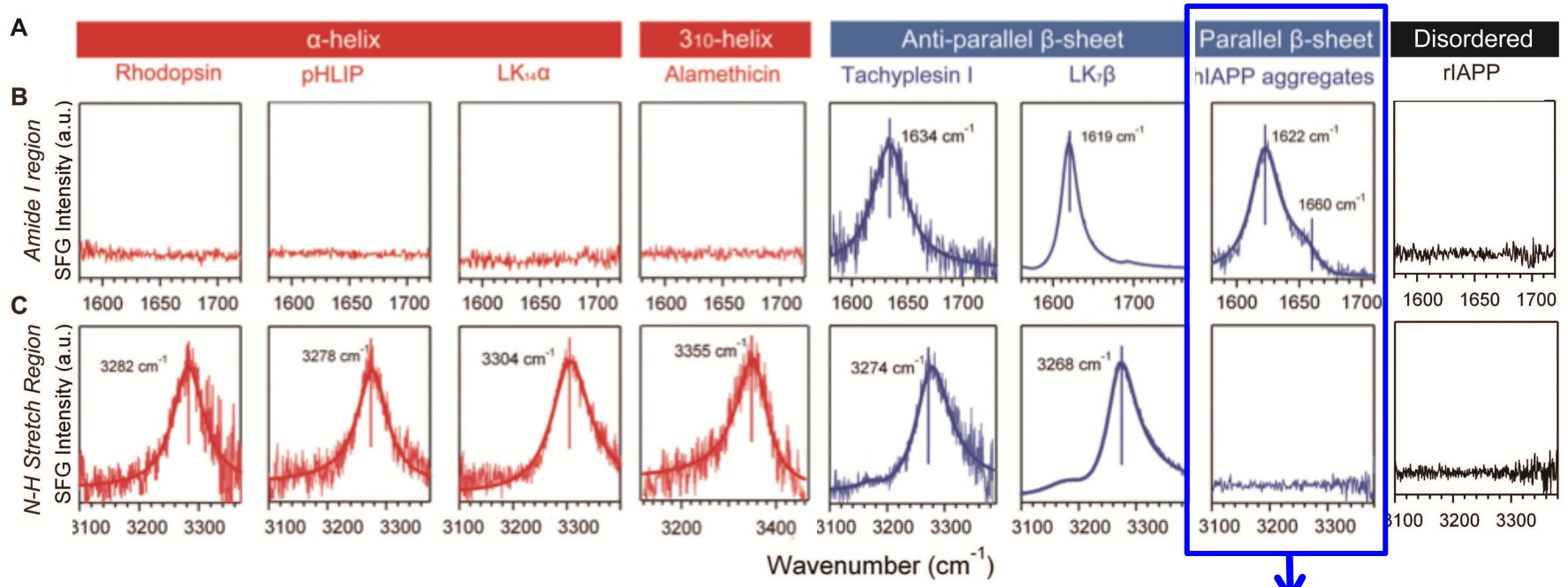
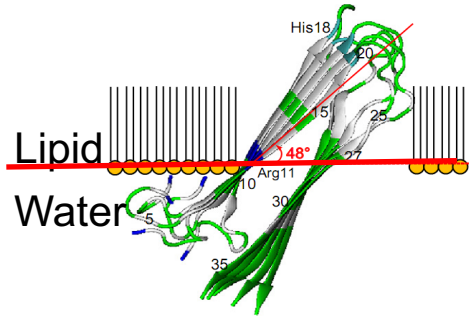
- Wang *et al. Langmuir*, 2015

cSFG Vibrational Signatures for Secondary Structures

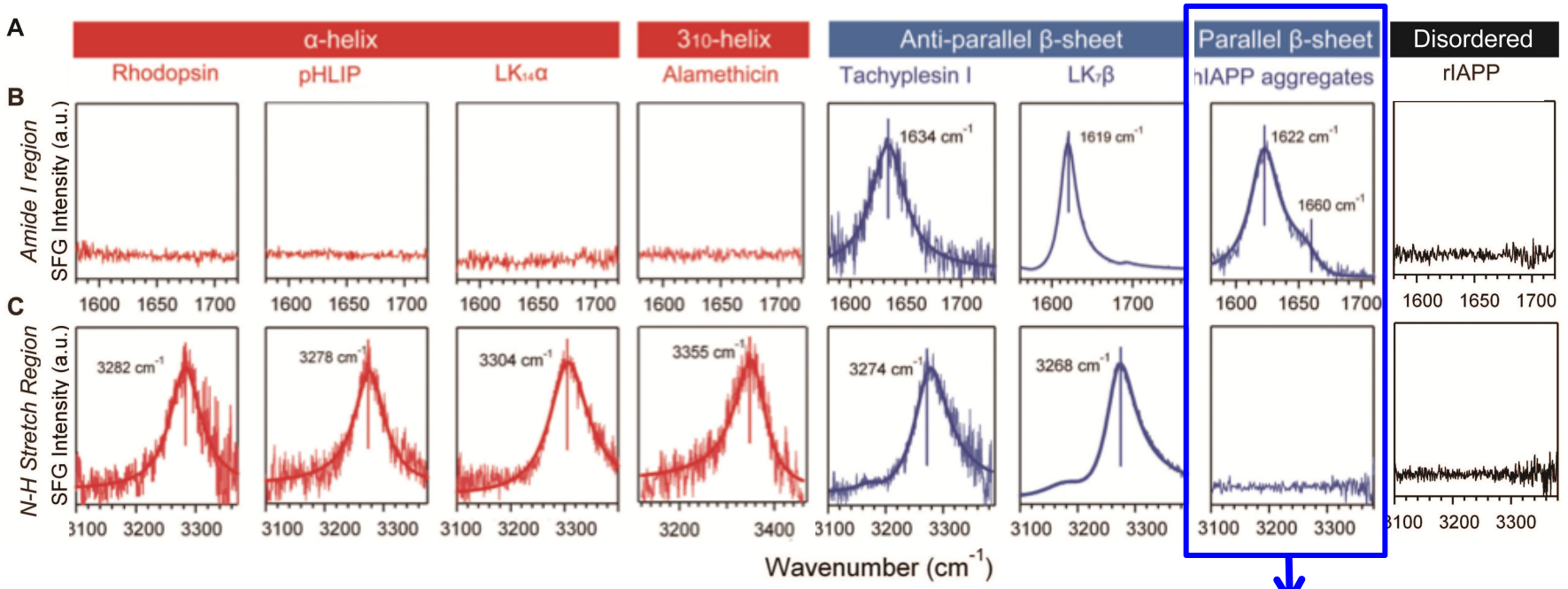


hIAPP aggregate

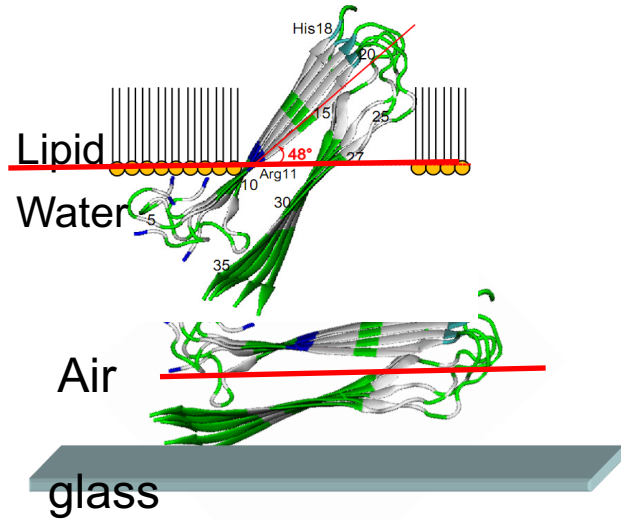


- Yan *et al Chem. Rev.* 2014
- Fu *et al Int. J. Mol. Sci.* 2011
- Yan *et al. J. Phys. Chem. B* 2015
- Wang *et al. Langmuir*, 2015

cSFG Vibrational Signatures for Secondary Structures

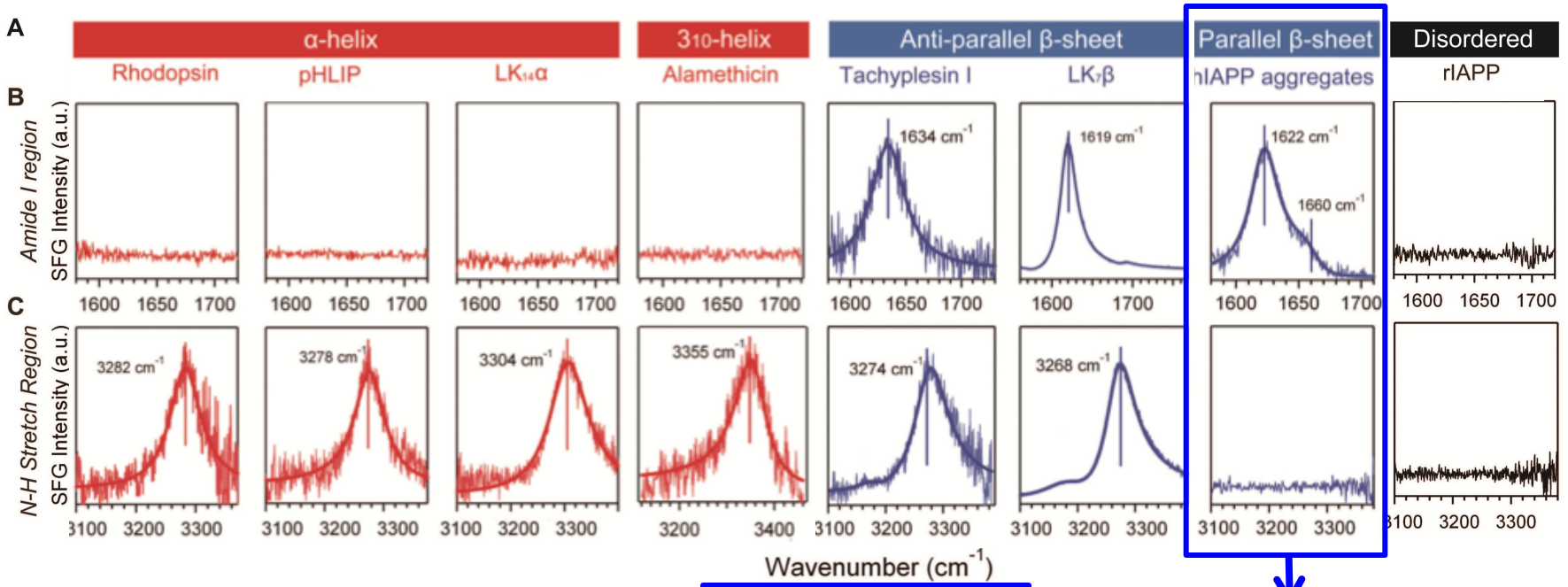


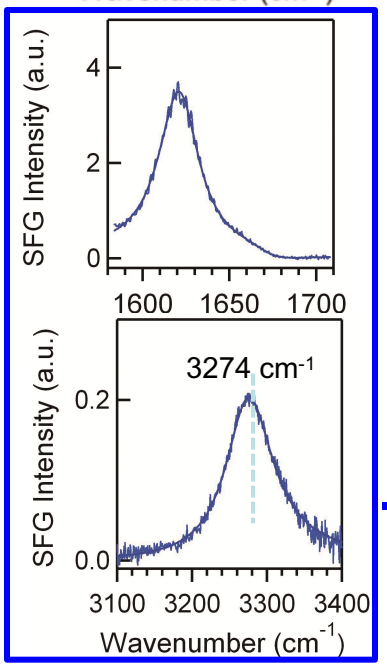
hIAPP aggregate



- Yan *et al Chem. Rev.* 2014
- Fu *et al Int. J. Mol. Sci.* 2011
- Yan *et al. J. Phys. Chem. B* 2015
- Wang *et al. Langmuir*, 2015

cSFG Vibrational Signatures for Secondary Structures





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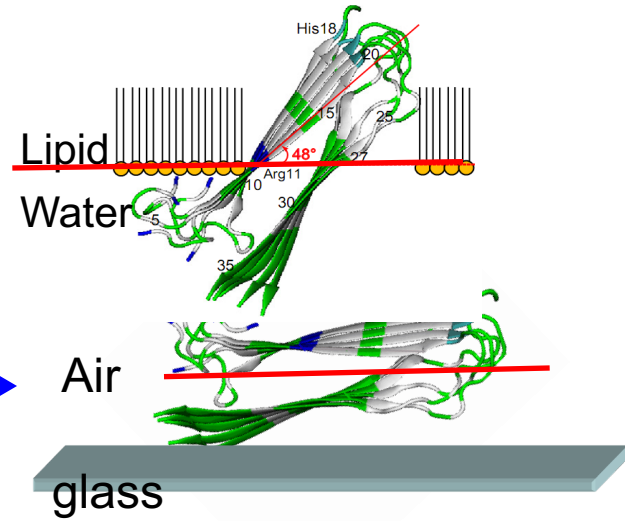

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- Yan *et al. J. Phys. Chem. B* 2015
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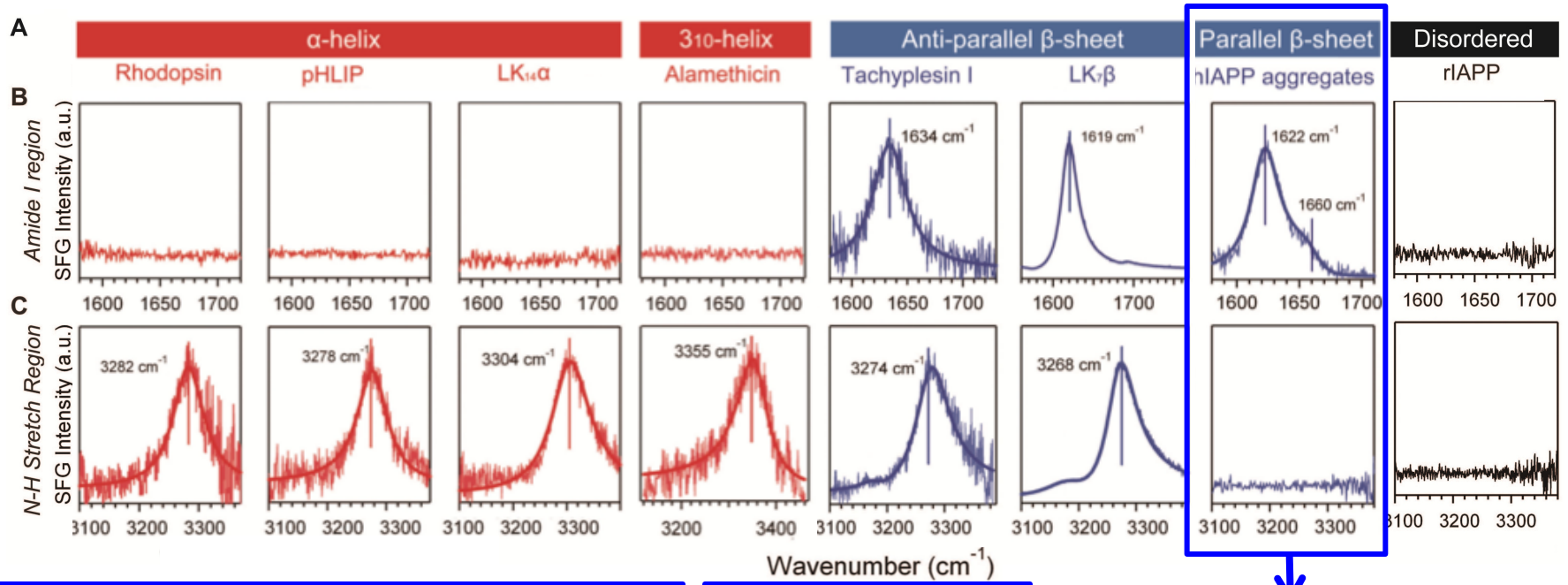
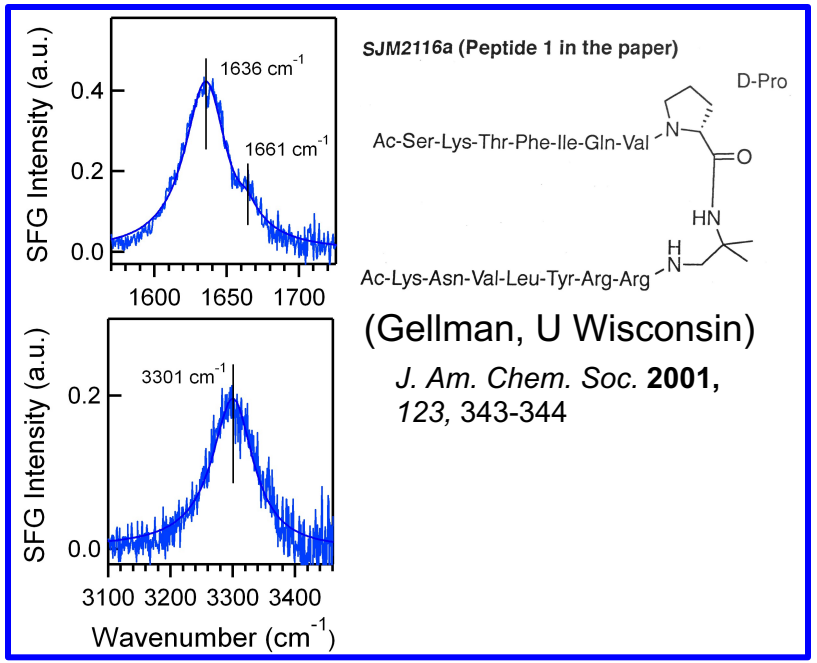
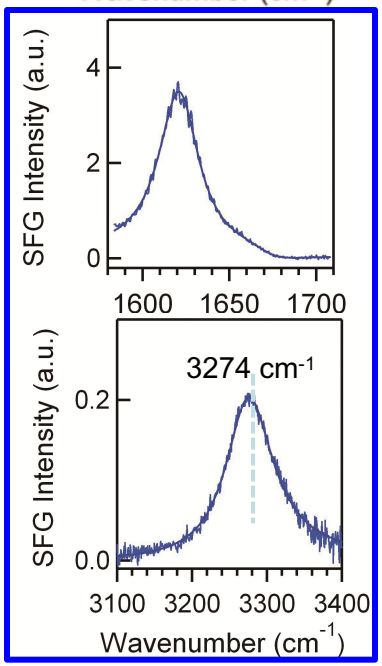
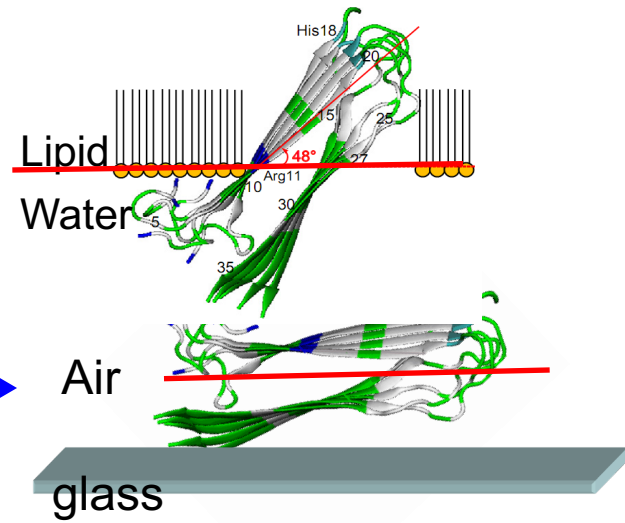
cSFG Vibrational Signatures for Secondary Structures



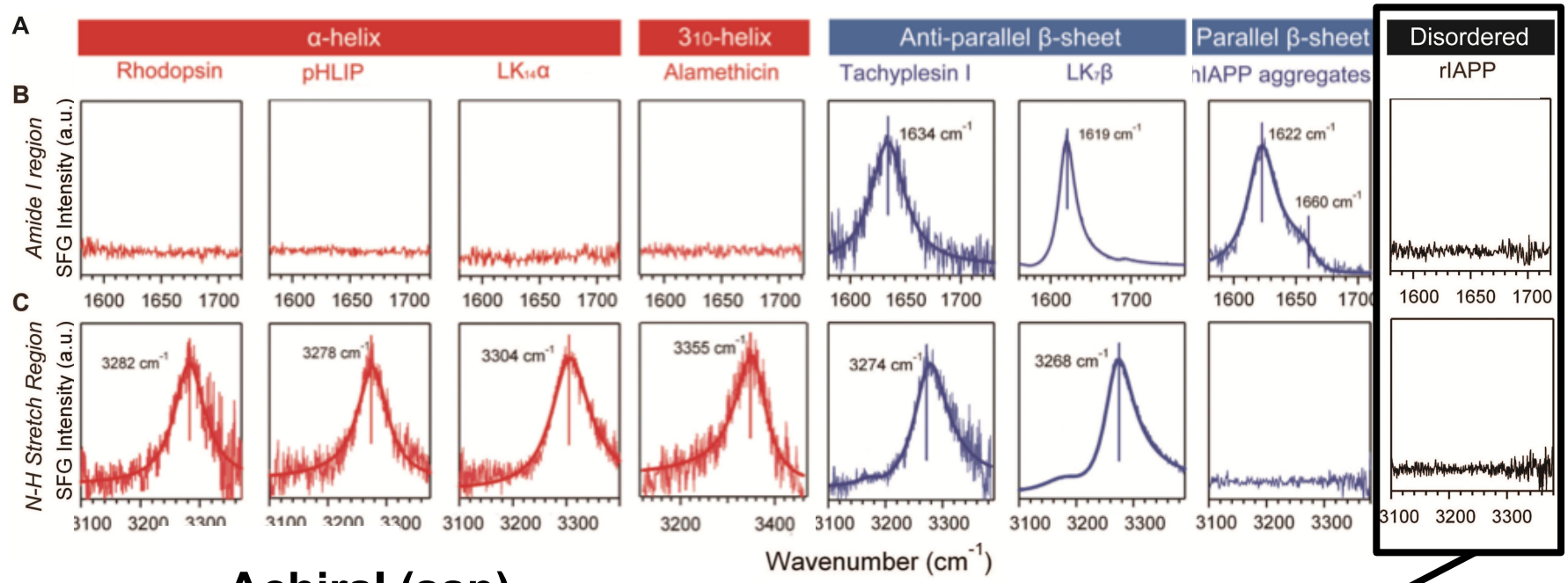




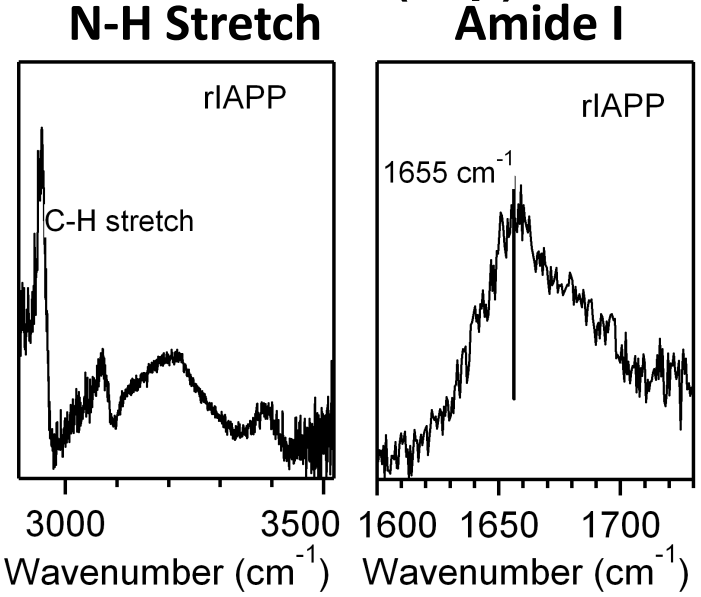
hIAPP aggregate



cSFG Vibrational Signatures for Secondary Structures



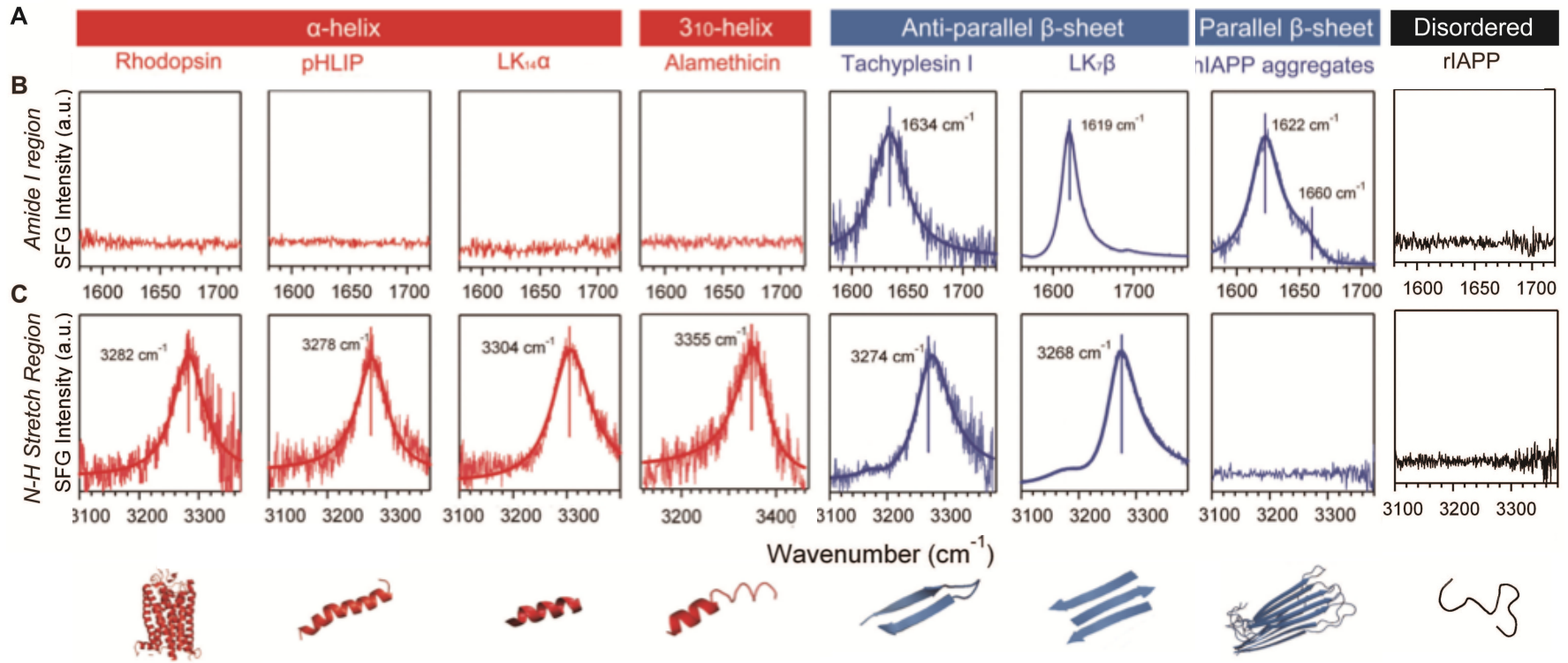
Achiral (ssp)



Rat Islet Amyloid Polypeptide

Disordered

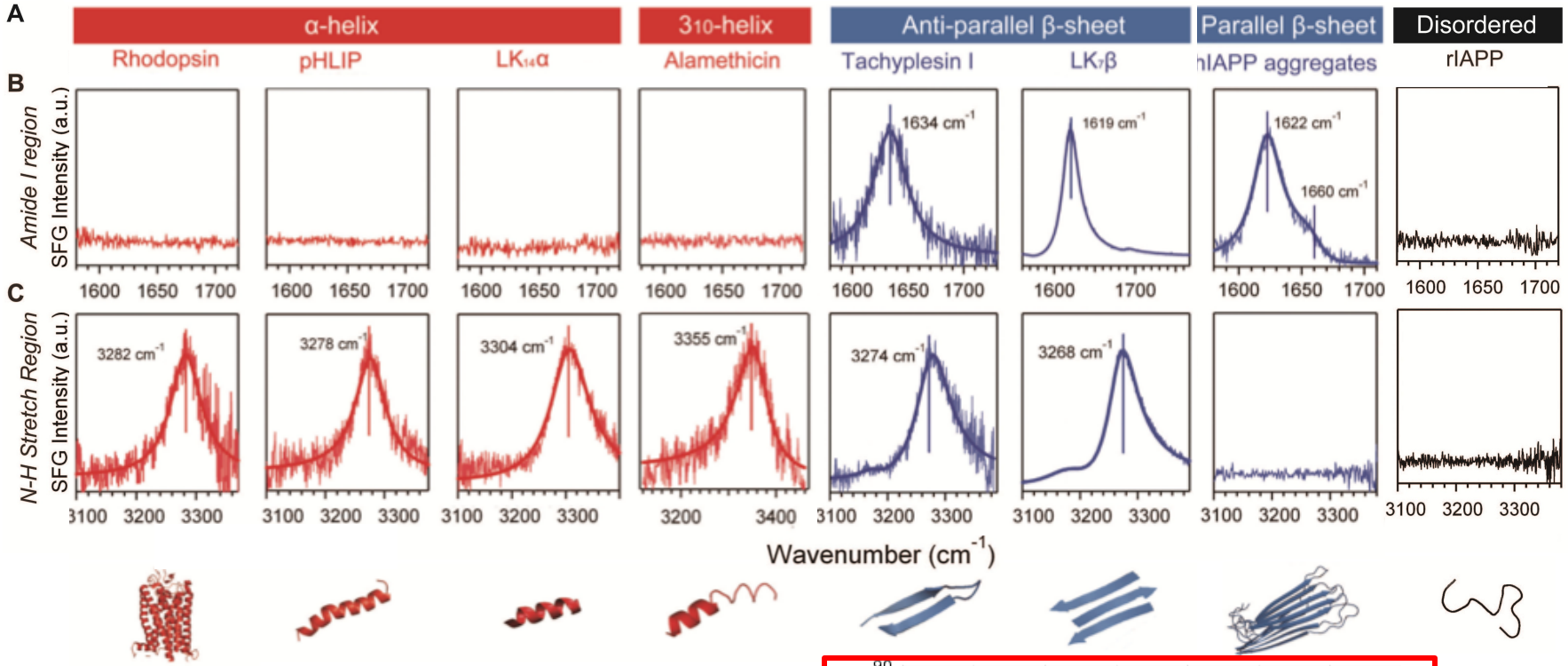
cSFG Vibrational Signatures for Secondary Structures



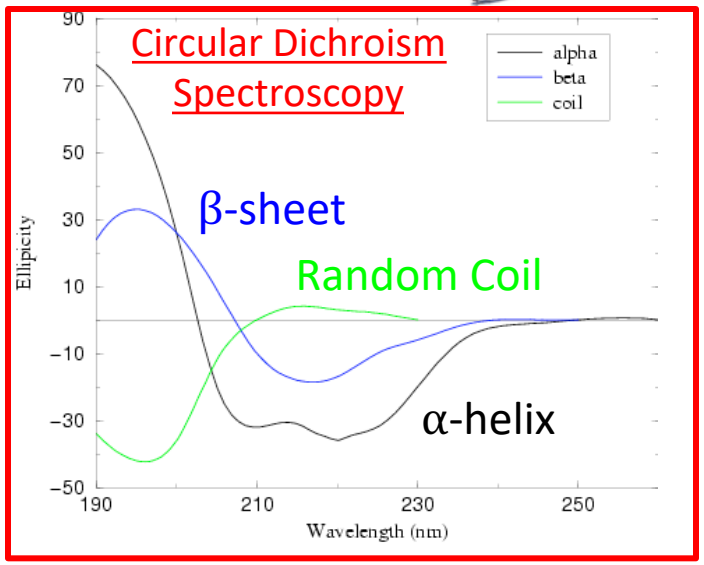
Chiral SFG: characterize protein secondary structures at interfaces

- Yan *et al Chem. Rev.* 2014
- Fu *et al Int. J. Mol. Sci.* 2011
- Yan *et al. J. Phys. Chem. B* 2015
- Wang *et al. Langmuir*, 2015

cSFG Vibrational Signatures for Secondary Structures



Chiral SFG: characterize protein secondary structures at interfaces



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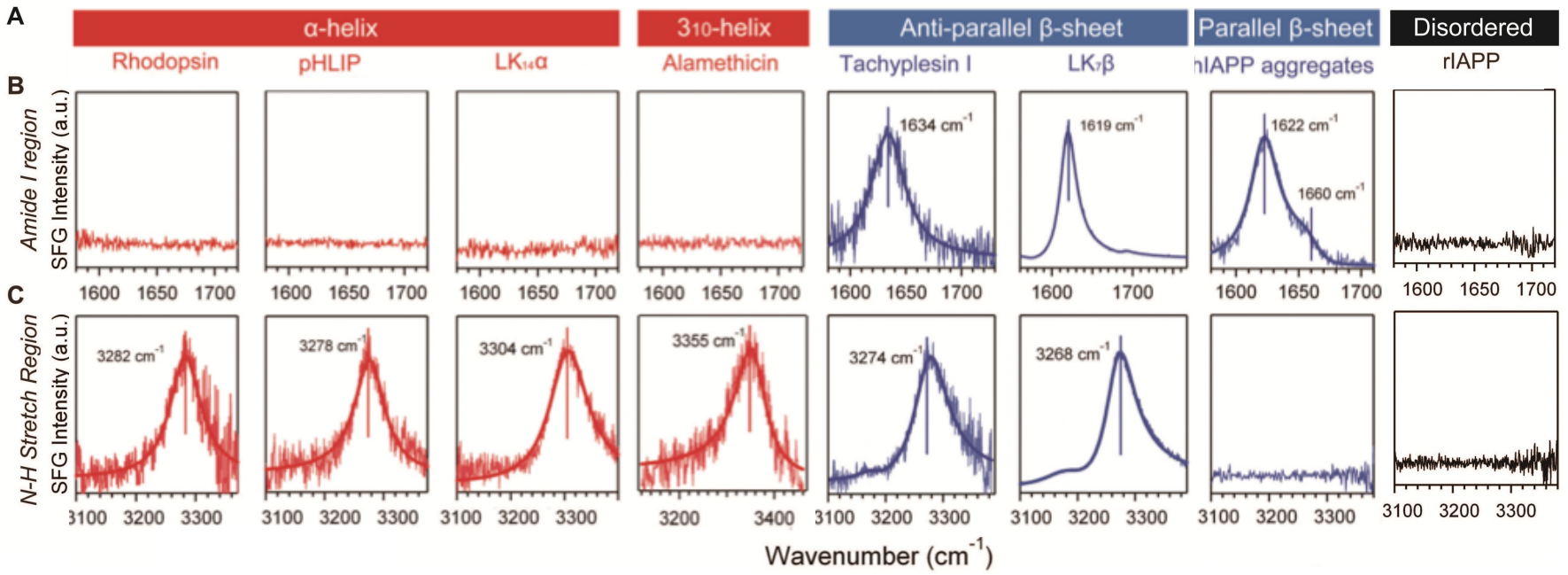
cSFG Vibrational Signatures for Secondary Structures



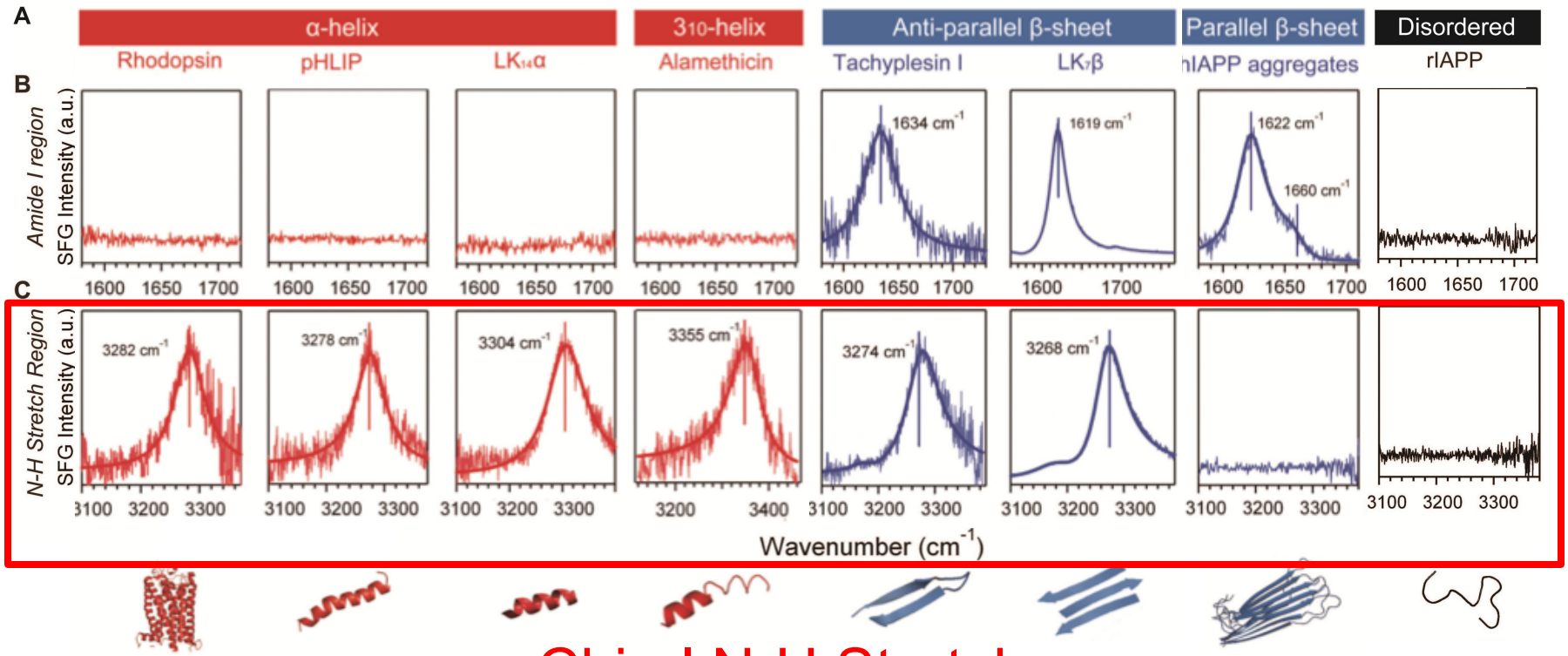
Compared to IR Spectroscopy

Secondary structure	Band position in H_2O/cm^{-1}	
	Average	Extremes
α-helix	1654	1648-1657
β-sheet	1633	1623-1641
β-sheet	1684	1674-1695
Turns	1672	1662-1686
Disordered	1654	1642-1657

Chiral SFG

1. Spectrally well separated
2. Background Free from water
3. Optically clean
4. H_2O as solvent

cSFG Vibrational Signatures for Secondary Structures

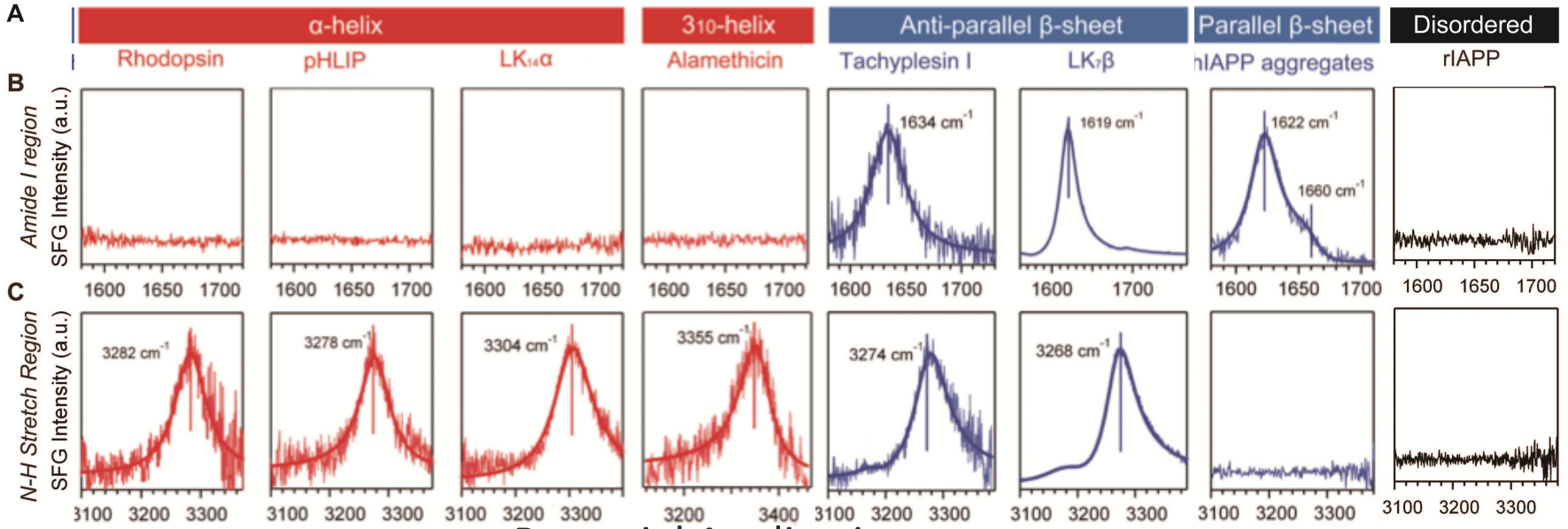


Chiral N-H Stretch

- Chiral SFG—Background-free from water O-H stretch
- Chiral SFG—Background-free from proteins in disordered structures
- N-H frequency—Secondary structures (Stabilization by H-bonds at N-H) => Characteristic of secondary structures

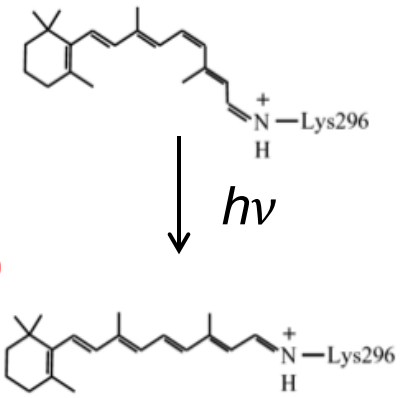
- Yan *et al Chem. Rev.* 2014
- Fu *et al Int. J. Mol. Sci.* 2011
- Yan *et al. J. Phys. Chem. B* 2015
- Wang *et al. Langmuir*, 2015

cSFG Vibrational Signatures for Secondary Structures

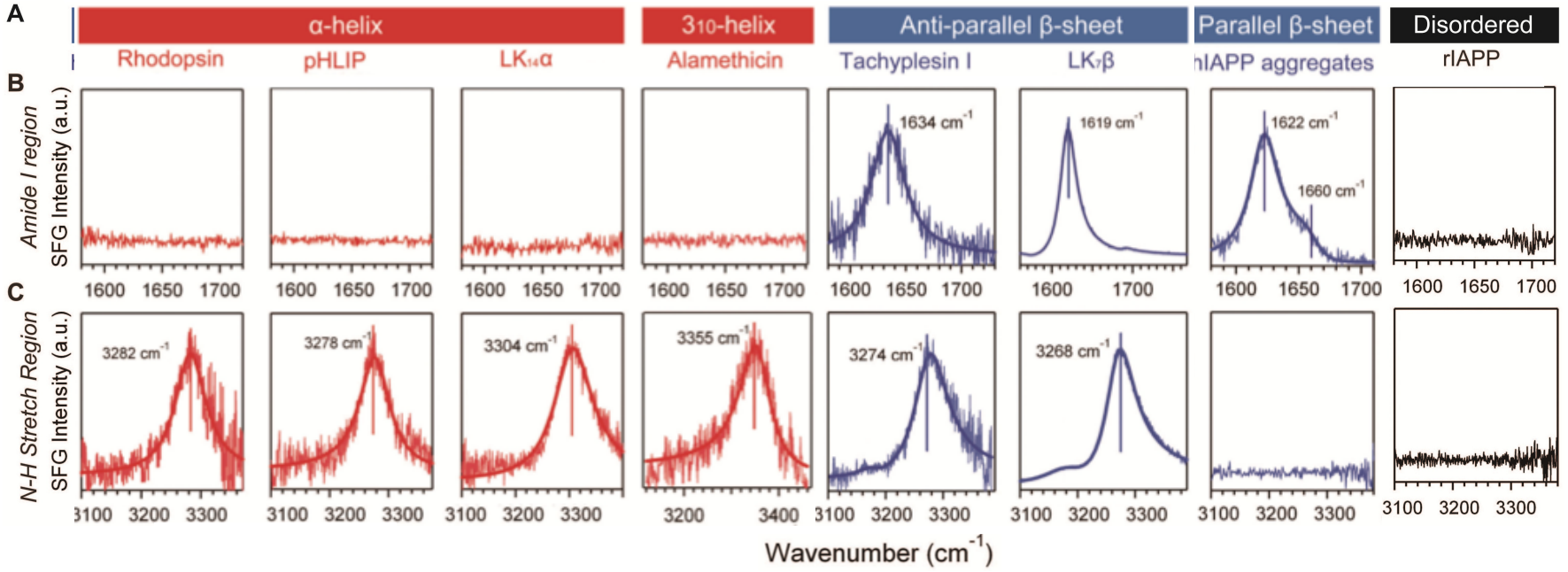


Potential Applications

- Changes in Secondary Structures
  - => Protein Folding at Interfaces
- Changes in Conformation and Orientation
  - => Protein Functions at Interfaces
- Surface Sensitive—Monolayer of Proteins
  - => Membrane Proteins--Small Sample Size (~μg)
- Background Free
  - => Imaging of Biological Samples
- Ultrafast laser & Vibrational Optical Signal in Visible Region
  - => Ultrafast Dynamics—IVR, Photobiological Systems



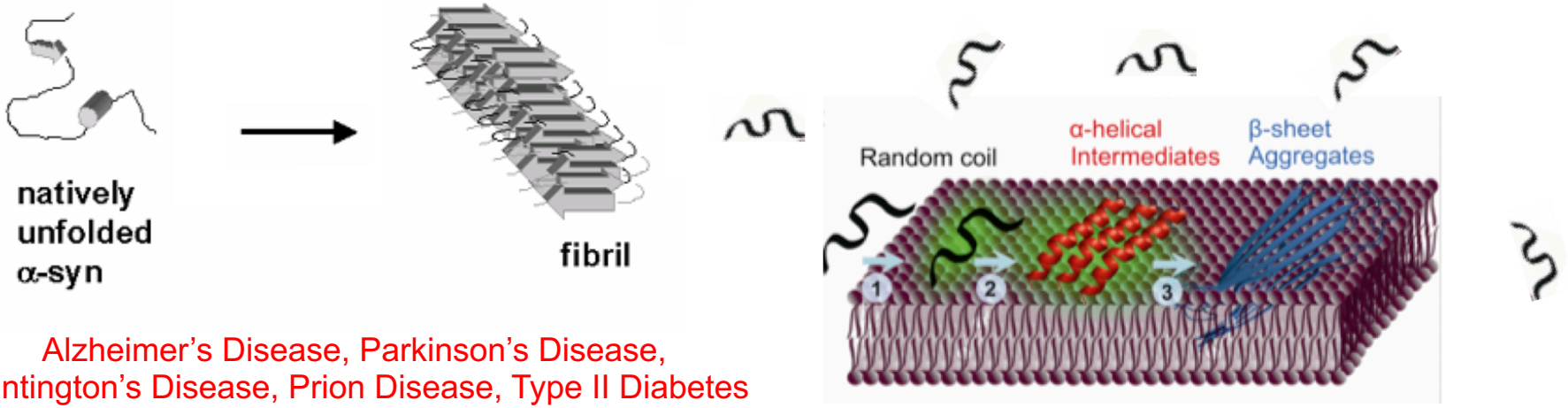
cSFG Vibrational Signatures for Secondary Structures



Chiral SFG Studies of Proteins at Interfaces

1. Amyloid Aggregation at Lipid/Water Interface (Li et al, *JACS*, 2010, 2011)
2. Proton Exchange of Proteins at Interfaces (Li et al, *JACS*, 2013)
3. Orientation of Proteins at Interfaces (Li et al, *JPCL*, 2015; Xiao et al *JMB*, 2012)
4. Functions of Biofilms Proteins (Wang et al. *Chem Comm*, 2016; Liu et al, *Langmuir* 2017)
5. Crowding Effect on Protein Folding at Interfaces (Liu et al. *PCCP*, 2018)

Aggregation of Amyloid Proteins



Alzheimer's Disease, Parkinson's Disease,
Huntington's Disease, Prion Disease, Type II Diabetes

1. Small oligomer interrupt cell membrane and cause cytotoxicity:
2. Aggregation catalyzed by interactions with lipid membrane

Challenge: Selectivity to
(1) Interfaces
(2) Secondary Structures

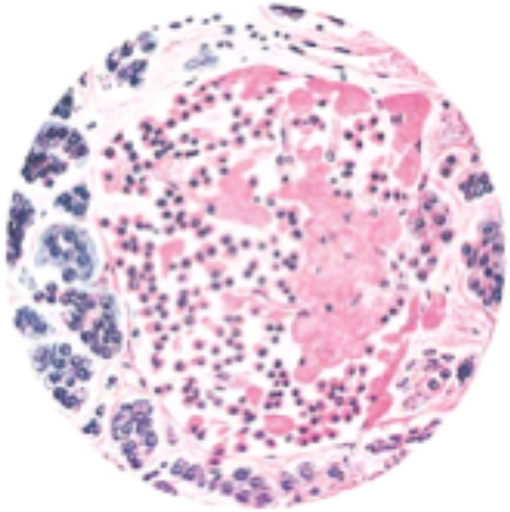
- NMR, EPR, CD—not surface-selective
- Reflective IR—spectral overlapping

Aggregation of Amyloid Proteins

Human Islet Amyloid Polypeptide (hIAPP)

```
          ┌S–S┐
hIAPP  KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY
```

Pathology of type II diabetes



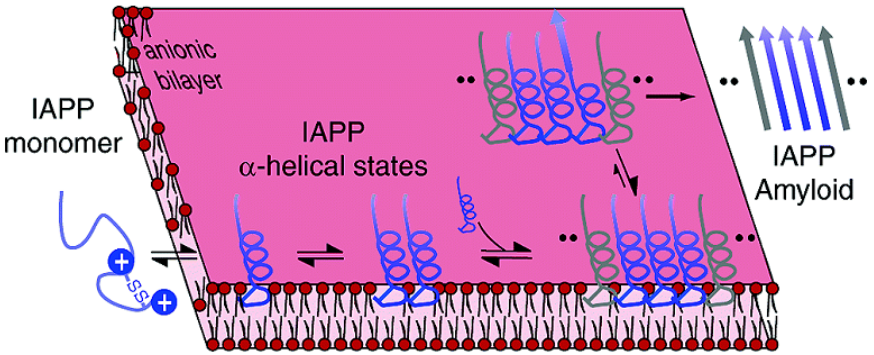
(Jo et al. *The New England Journal of Medicine)*

- IAPP forms fibrils in pancreas
- IAPP aggregation

  => Death of β cells

  => Production of Insulin

Model of IAPP aggregation

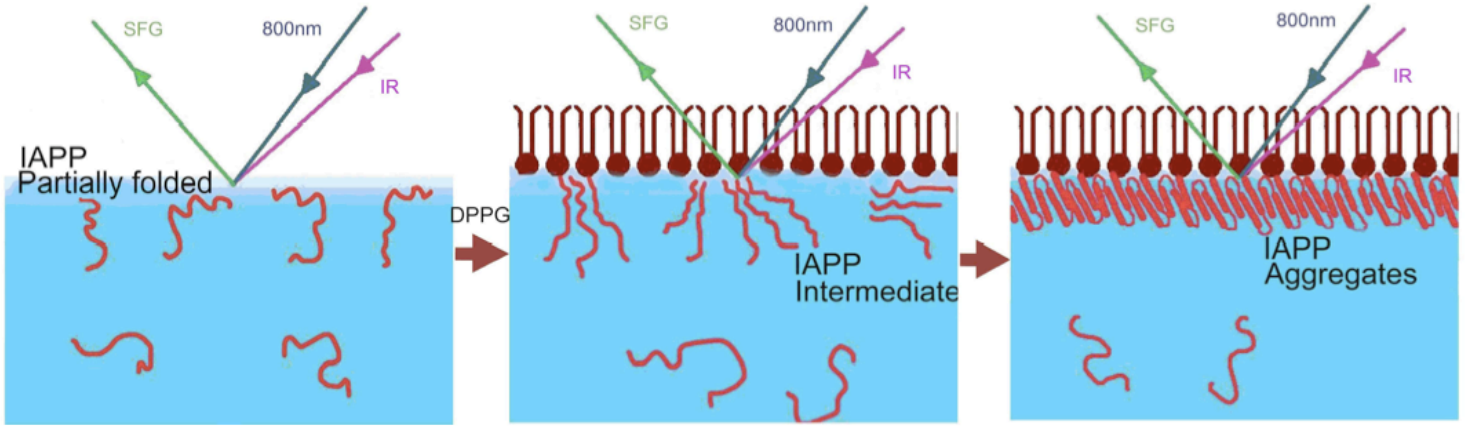


(Knight *et al Biochemistry* **2006**)

- Adsorption as α-helices
- Alignment of α-helices
- Conversion to β-sheets

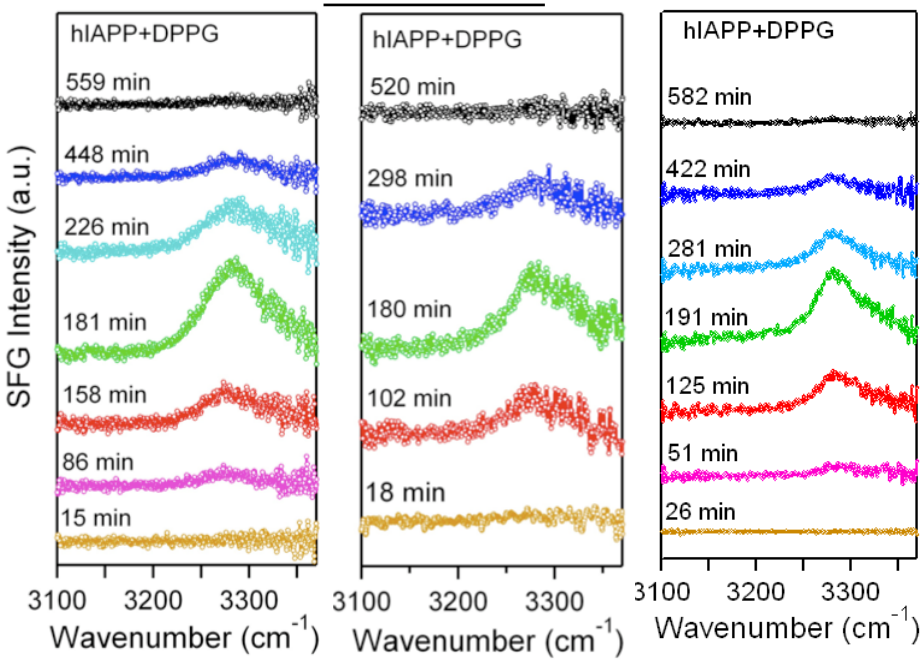
Aggregation of Amyloid Proteins

Kinetics: Chiral N-H vs. Chiral Amide I

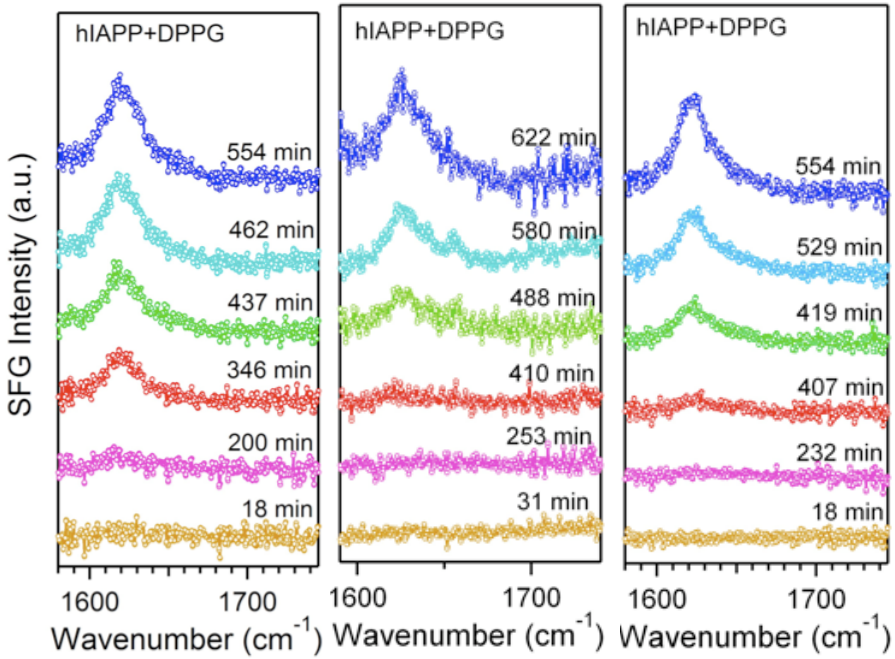


(Fu, L; Liu, J; Yan, ECY *JACS.*, 2011)

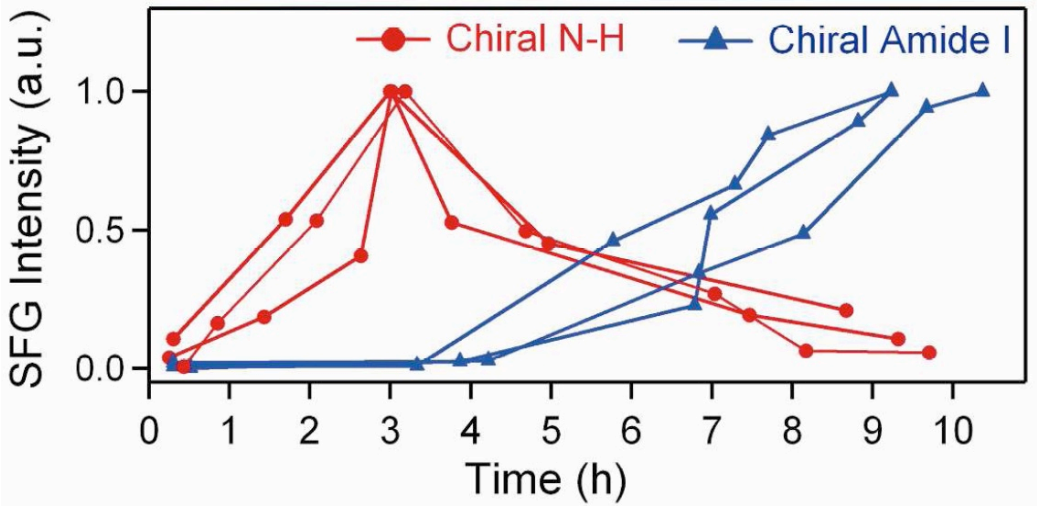
Chiral N-H



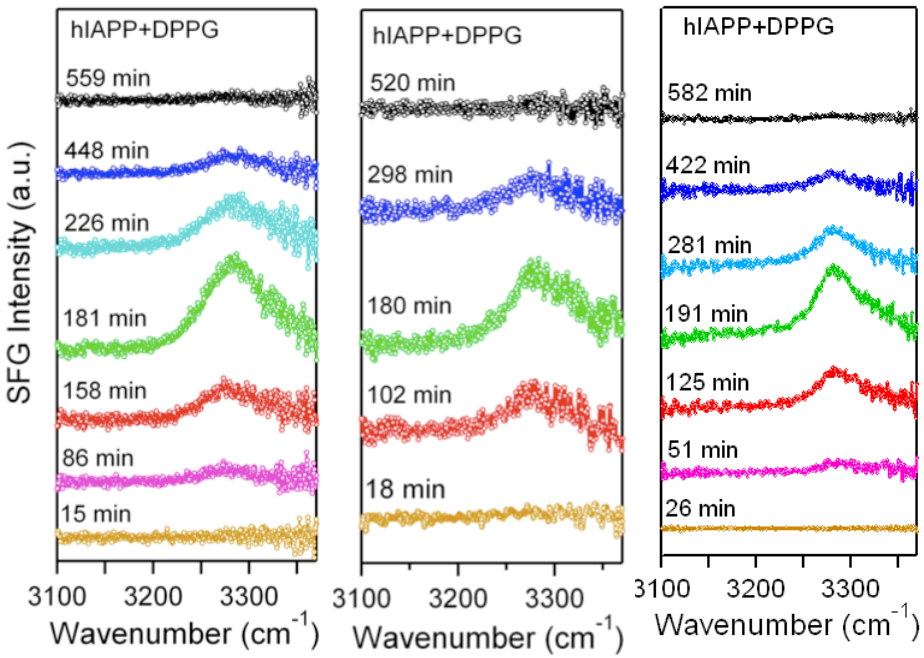
Chiral Amide I

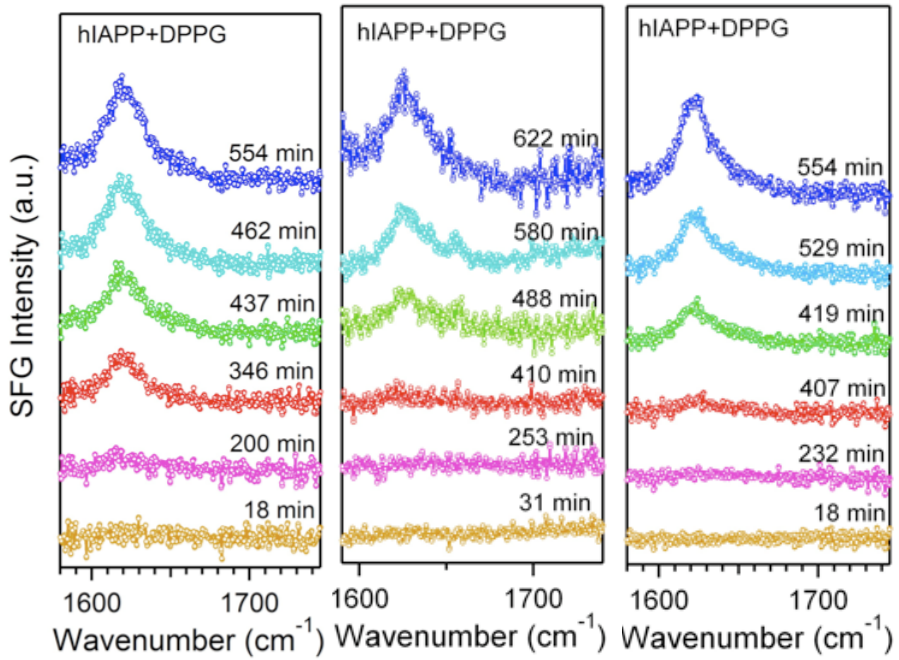


Aggregation of Amyloid Proteins

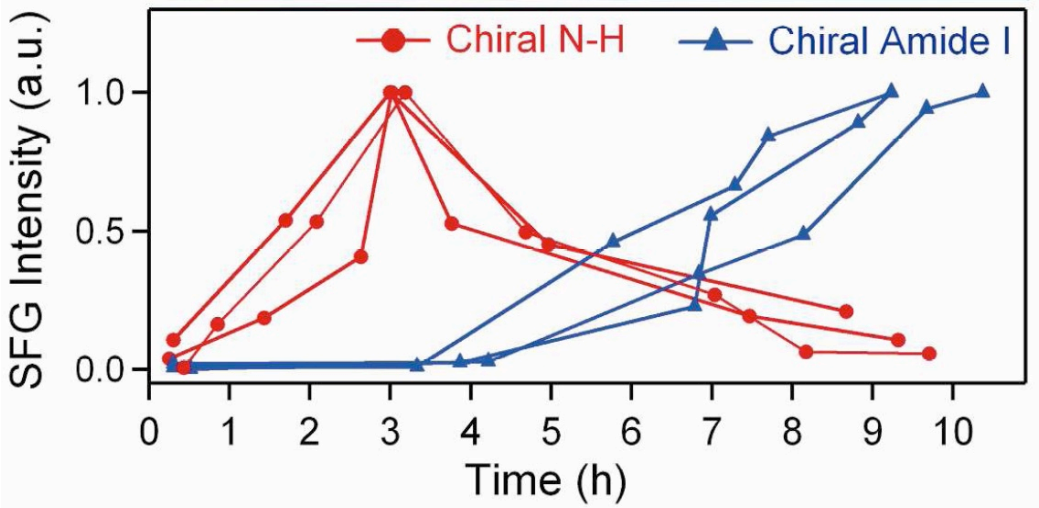


(Fu, L; Liu, J; Yan, ECY *JACS.*, 2011)



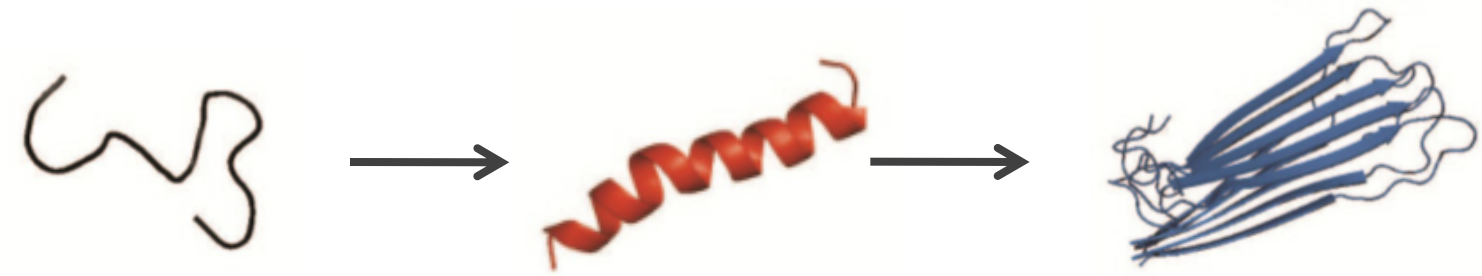


Aggregation of Amyloid Proteins



Seeding/Propagation | Aggregation

α-helix **β-sheet**

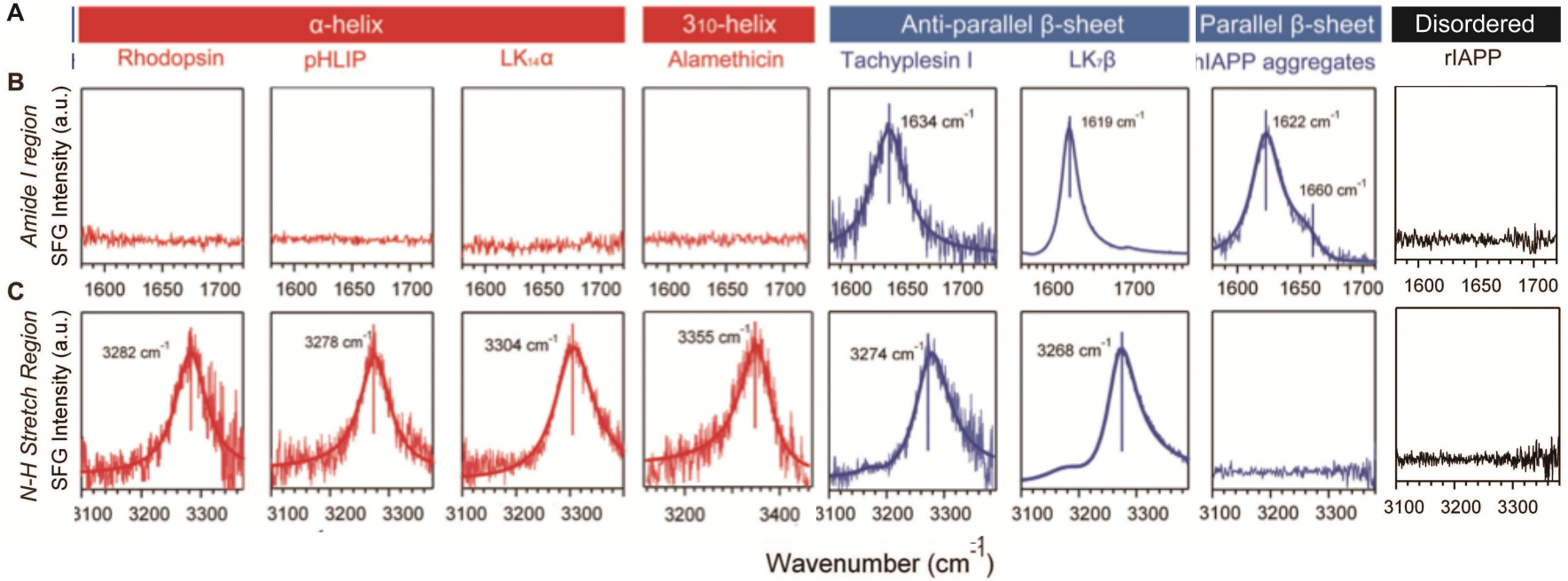


Disordered α-helix parallel β-sheet

(Fu, L; Ma, G; Yan, ECY *J. Am. Chem. Soc.*, 132, 5405, 2010)

(Fu, L; Liu, J; Yan, ECY *J. Am. Chem. Soc.*, 133, 8094, 2011)

Summary: Characterization of Protein Secondary Structures at Interfaces

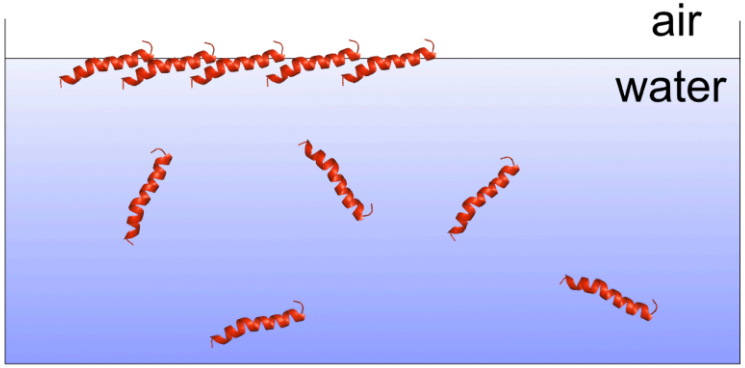


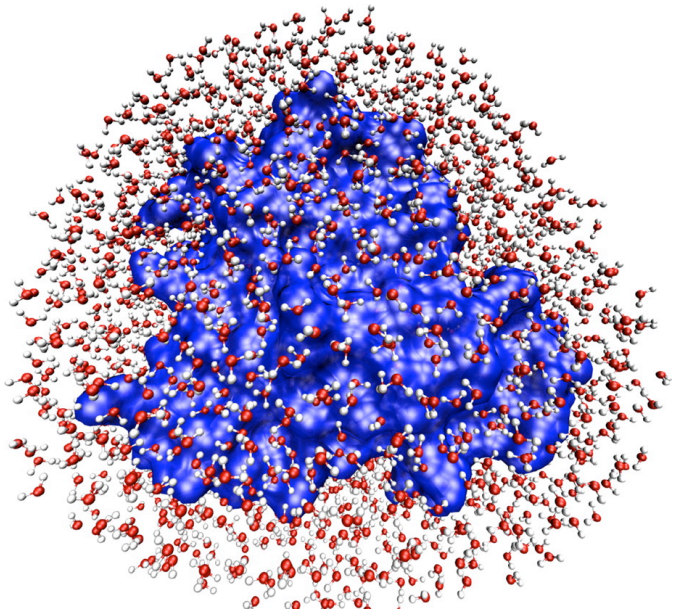
1. Amyloid Aggregation at Lipid/Water Interface (Li et al, *JACS*, 2010, *JACS* 2011)
2. Orientation of Proteins at Interfaces (Li et al, *JPCL*, 2015; Xiao et al *JMB*, 2012)
3. Proton Exchange of Proteins at Interfaces (Li et al, *JACS*, 2013)
4. Functions of Biofilms Proteins (Wang et al. *Chem Comm*, 2016; Liu et al, *Langmuir* 2017)
5. Crowding Effect on Protein Folding at Interfaces (Liu et al. *PCCP*, 2018)

Protein Characterizations by Chiral Vibrational Sum Frequency Generation Spectroscopy

Outline

1. Introduction to Chiral SFG Spectroscopy
2. Charactering Protein Secondary Structures at Interfaces
3. Probing Water Structures in Hydration Shell of Proteins





Protein Characterizations by Chiral Vibrational Sum Frequency Generation Spectroscopy

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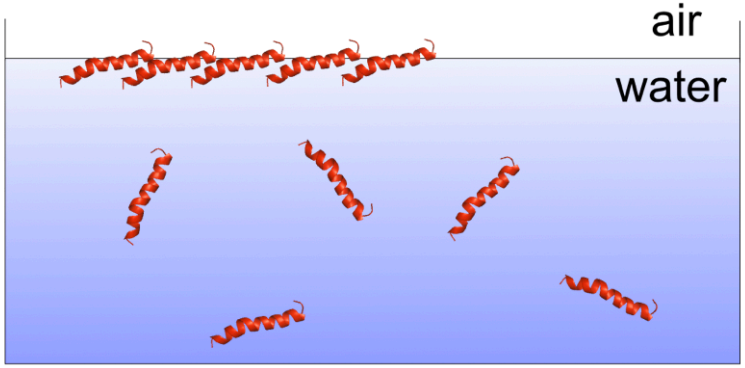
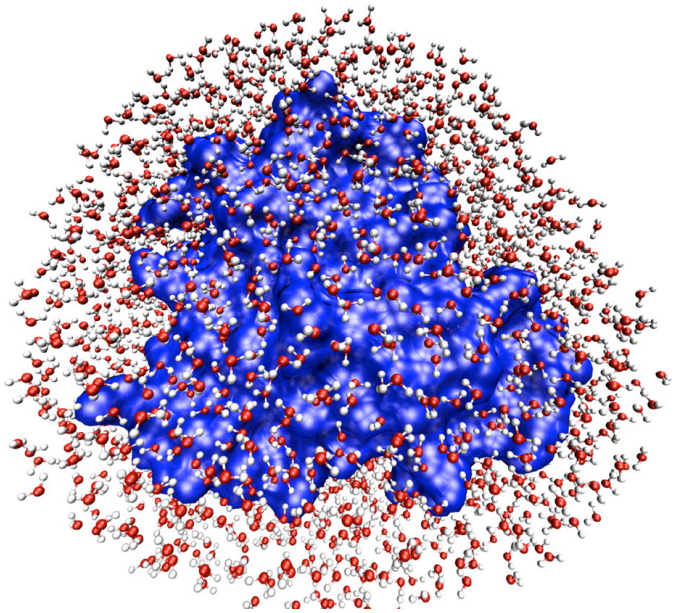




Chiral Water Superstructures Imprinted by DNA Detected by Chiral SFG

ACS central science

Research Article

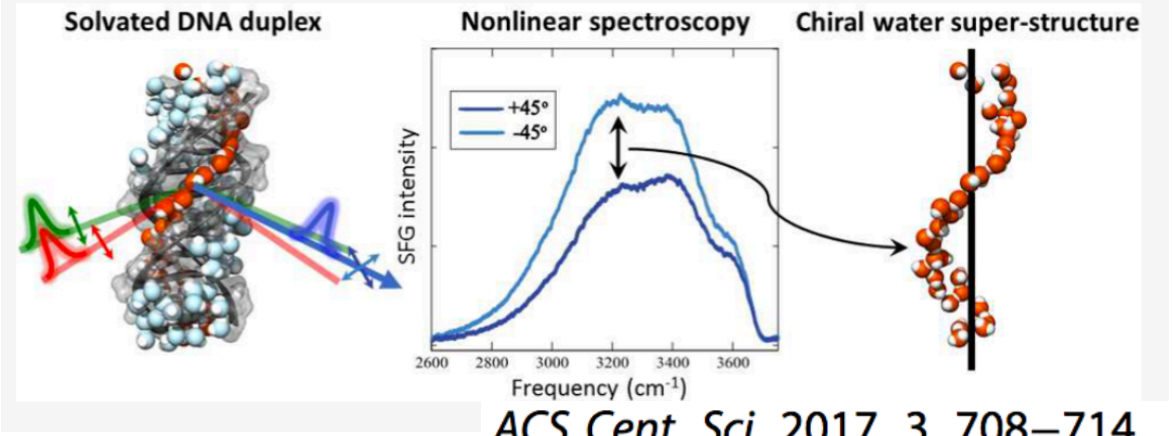
http://pubs.acs.org/journal/acscii

DNA's Chiral Spine of Hydration

M. Luke McDermott,[†] Heather Vanselous,[†] Steven A. Corcelli,[‡] and Poul B. Petersen*,[†]

[†]Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York, United States

[‡]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana, United States



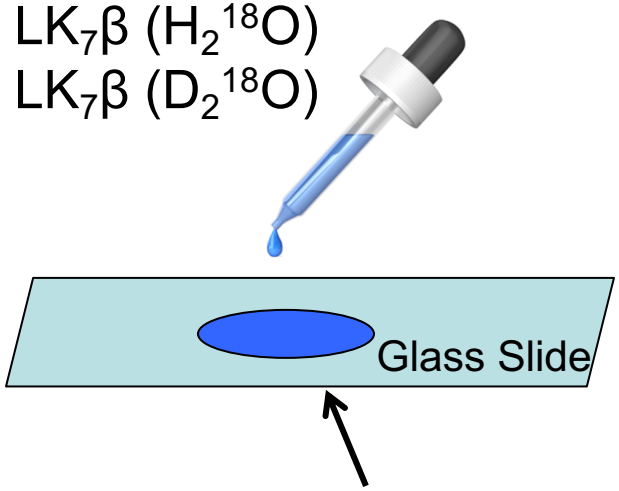
ACS Cent. Sci. 2017, 3, 708−714

Can proteins orient waters into chiral superstructures?
Can these structures be observed by chiral SFG?

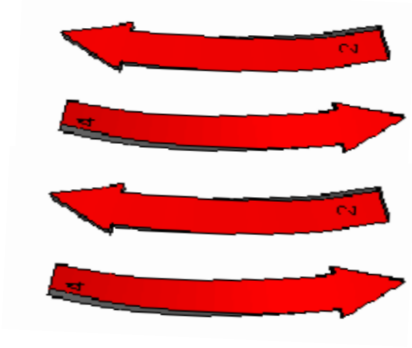
Chiral SFG Experiments: Chiral Water Superstructures Around Proteins

Model peptide, $LK_7\beta$ (LKLKLKL): antiparallel β-sheet

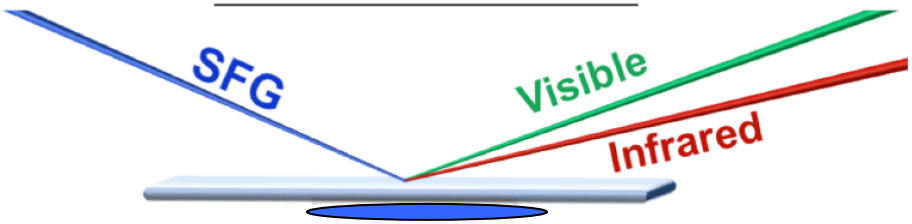
$LK_7\beta$ (H_2O)
$LK_7\beta$ (D_2O)
$LK_7\beta$ ($H_2{}^{18}O$)
$LK_7\beta$ ($D_2{}^{18}O$)



Dried overnight to obtain a thin film of $LK_7\beta$ adsorbed on a glass slide



PSP Polarization

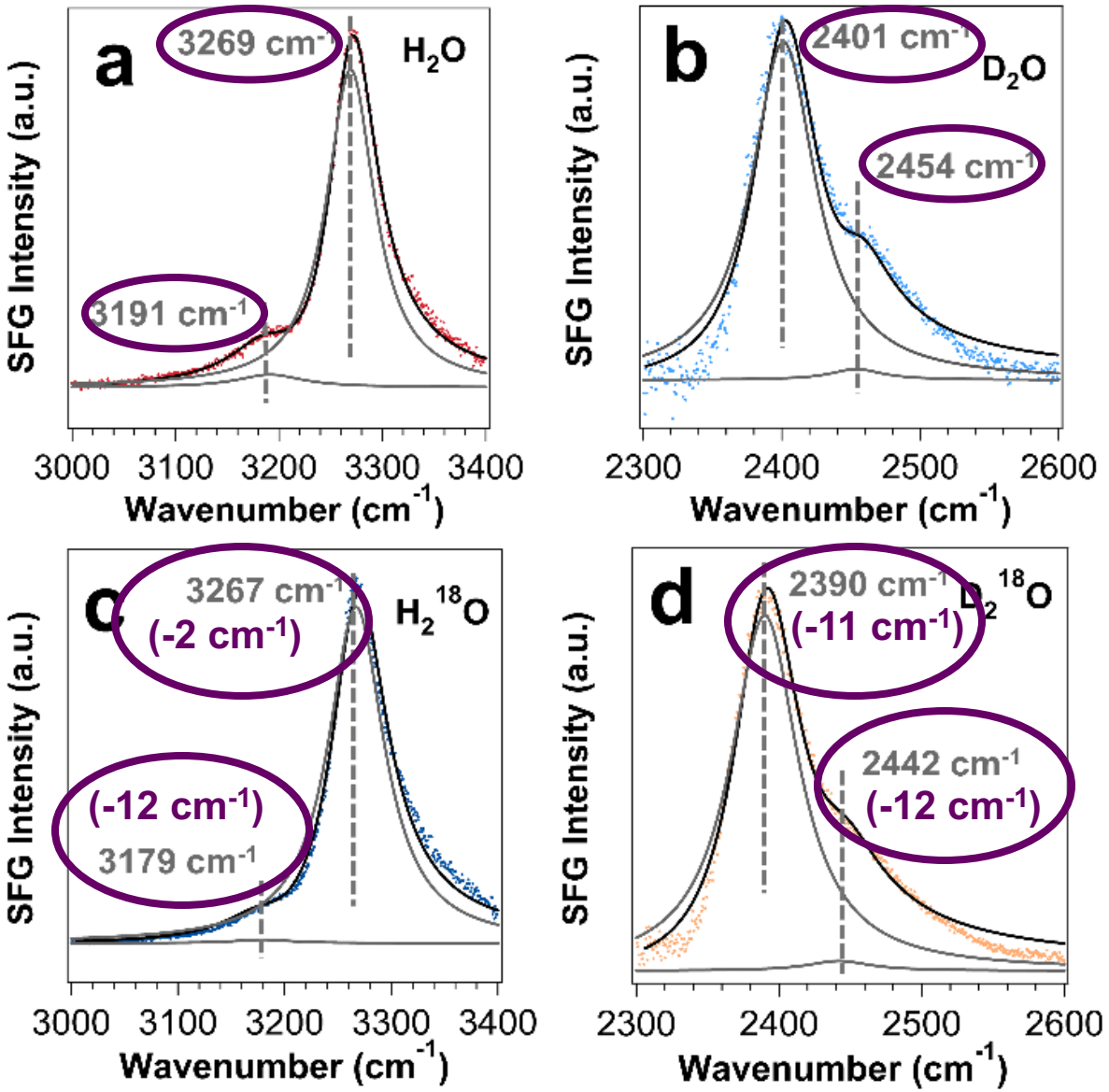


Chiral SFG spectra
1. **~3300 cm^{-1} (O-H Stretch)**
2. **~ 2400 cm^{-1} (O-D stretch)**

(Perets et al.
J. Phys. Letts, 2019)

Isotopic Shifts of $LK_7\beta$ Thin Film Spectra

One peak shifted by $H_2{}^{18}O$ **Both peaks shifted by $D_2{}^{18}O$**



(Perets et al.
J. Phys. Letts, 2019)

Isotopic Shifts of $LK_7\beta$ Thin Film Spectra

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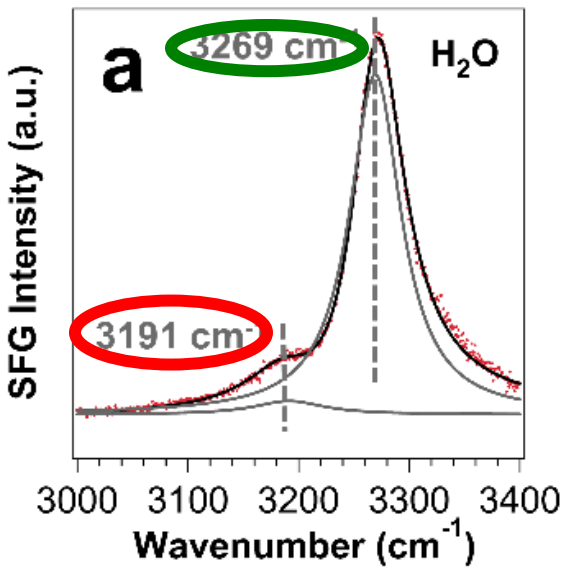
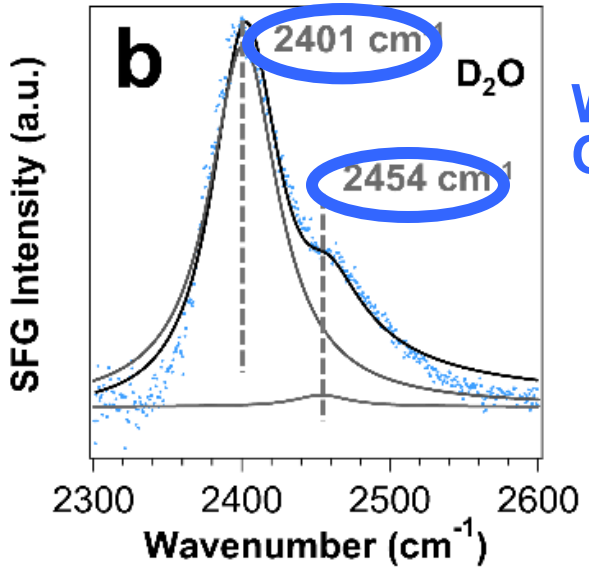
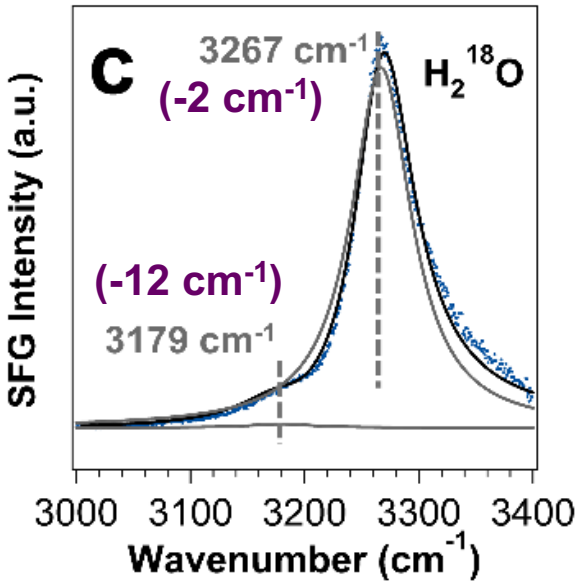
Peptide N-H Stretch

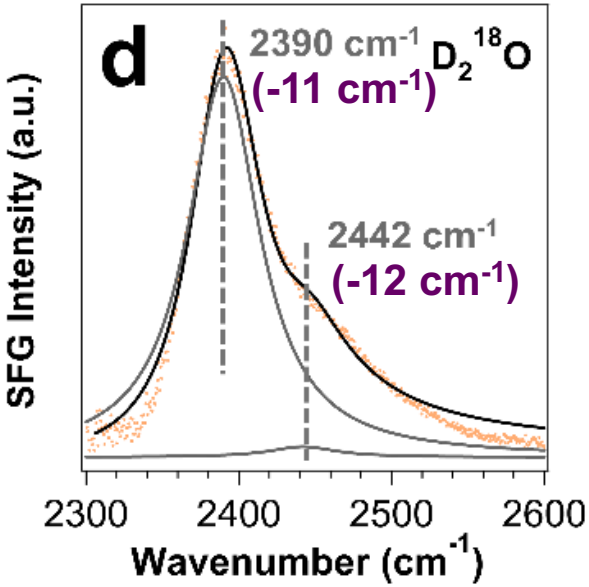
Water O-H Stretch





Water O-D Stretch

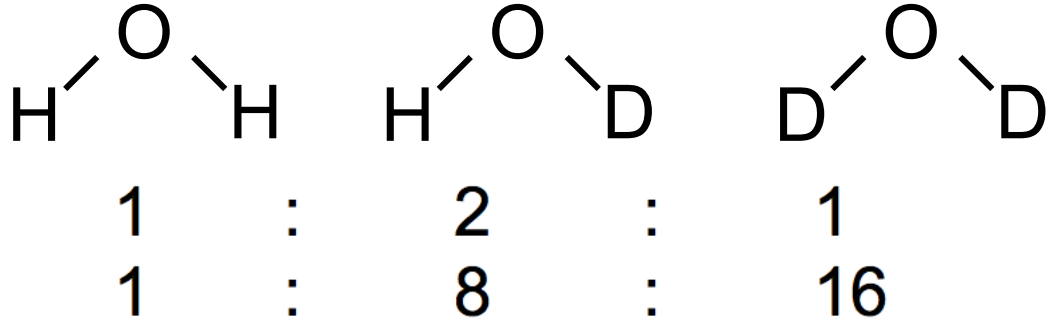


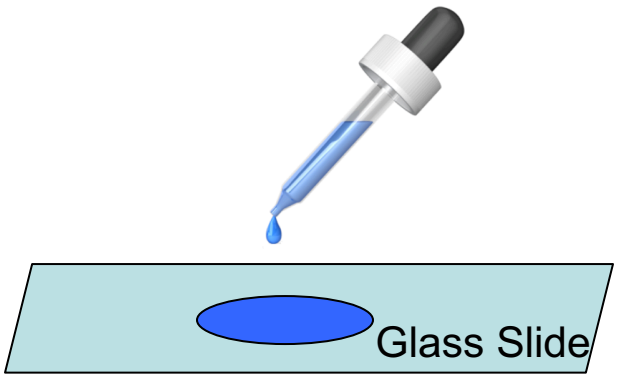


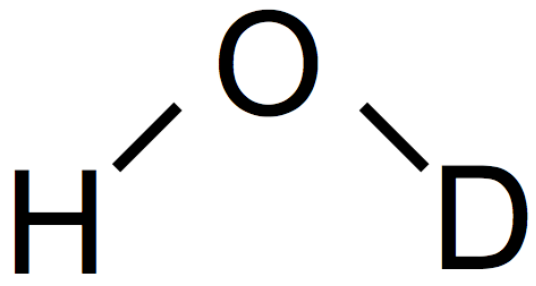
(Perets et al. *J. Phys. Letts*, 2019)

Isotopic Dilution

$LK_7\beta$ (H_2O)
$LK_7\beta$ (D_2O)
$LK_7\beta$ (H_2O:D_2O 1:1)
$LK_7\beta$ (H_2O:D_2O 1:4)



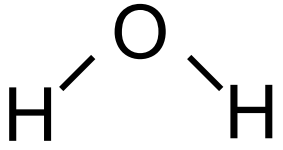
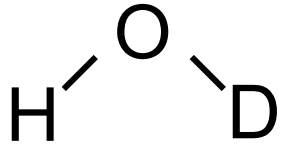
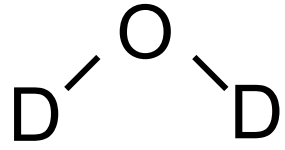


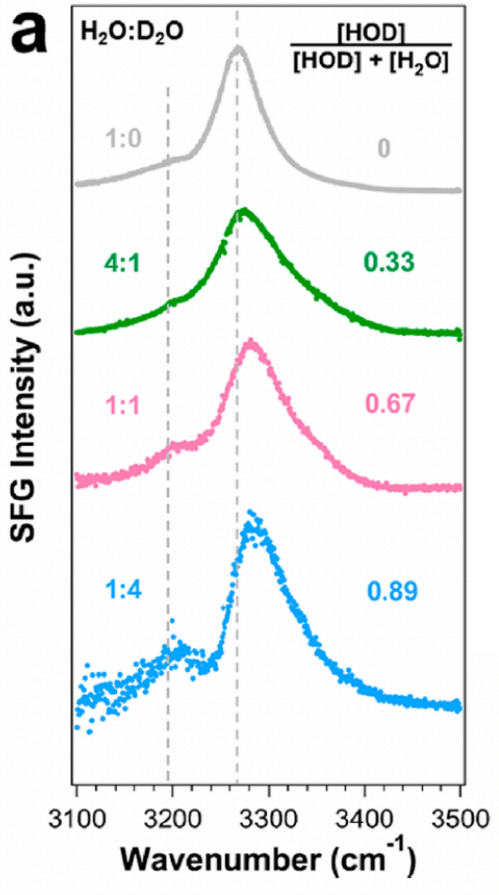


- Removing intramolecular coupling of two O-H stretches
- Changing the O-H stretch vibrational spectrum

(Perets et al.
J. Phys. Letts, 2019)

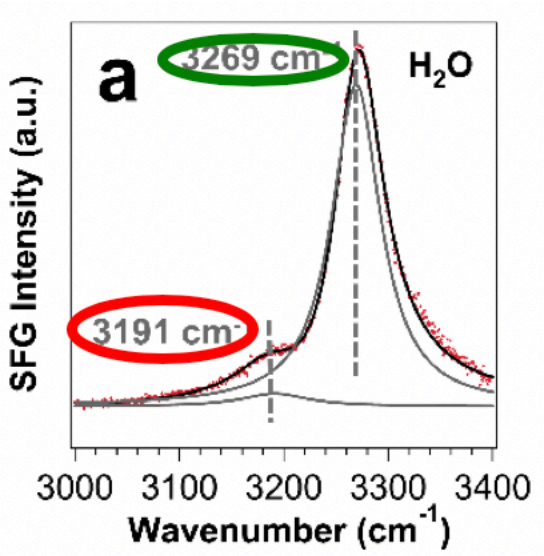
Isotopic Dilution

$LK_7\beta$ (H_2O)						
$LK_7\beta$ (D_2O)						
$LK_7\beta$ (H_2O:D_2O 1:1)	1	:	2		:	1
$LK_7\beta$ (H_2O:D_2O 1:4)	1	:	8		:	16

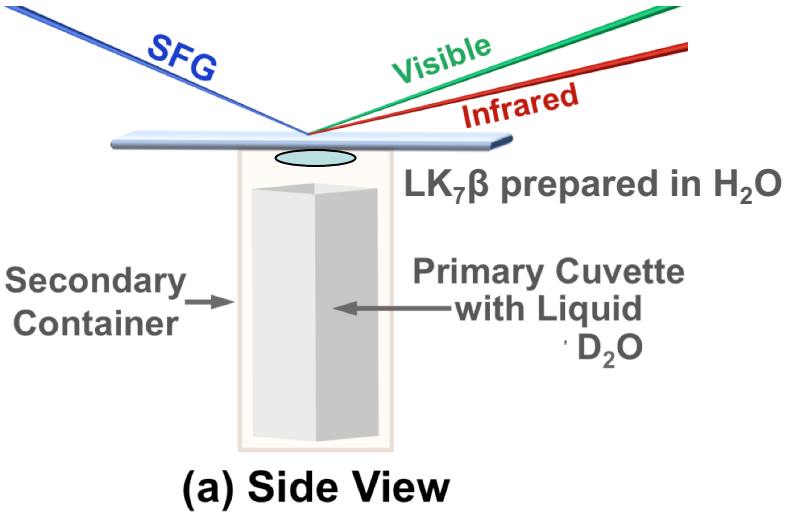


Peptide N-H Stretch

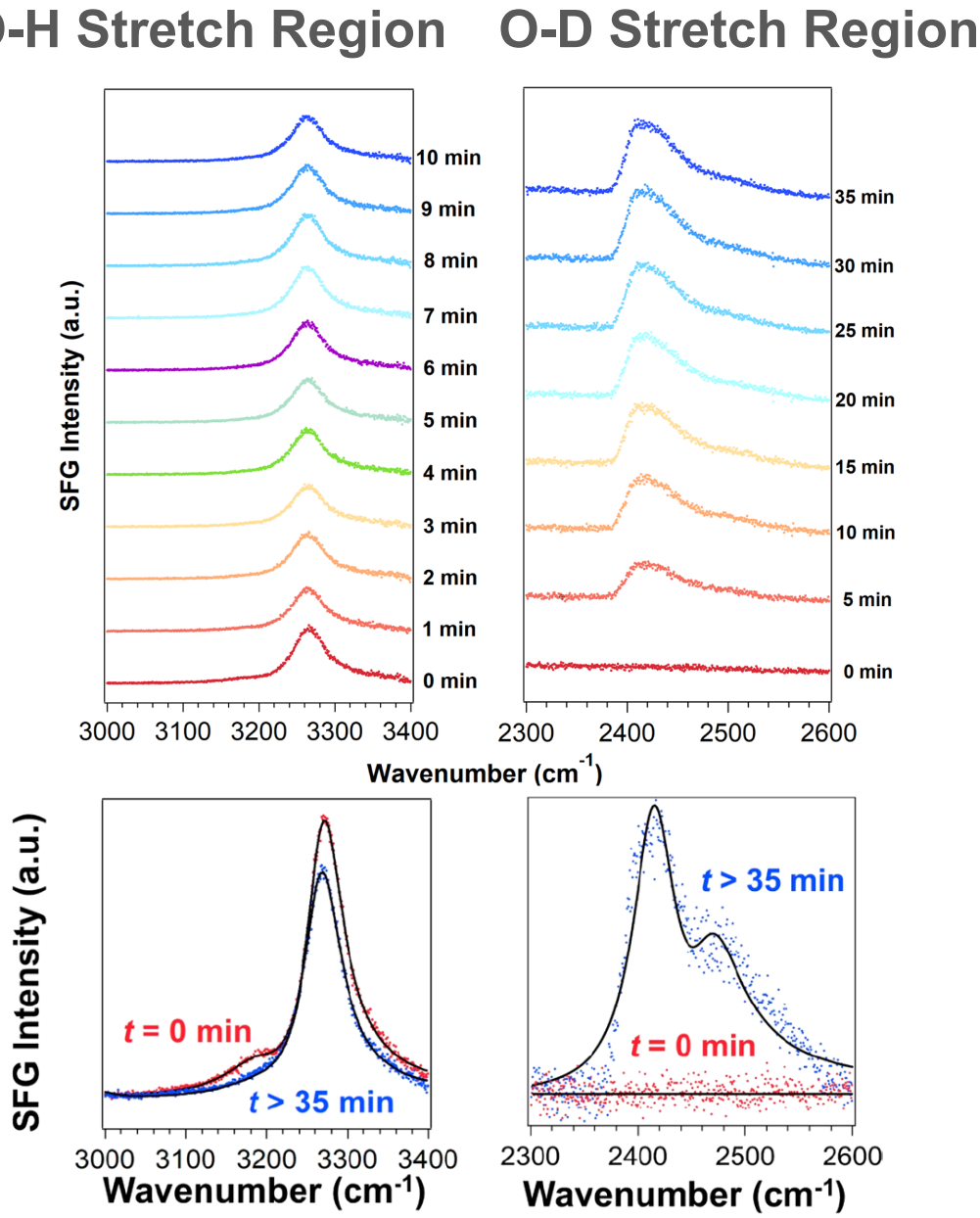
Water O-H Stretch



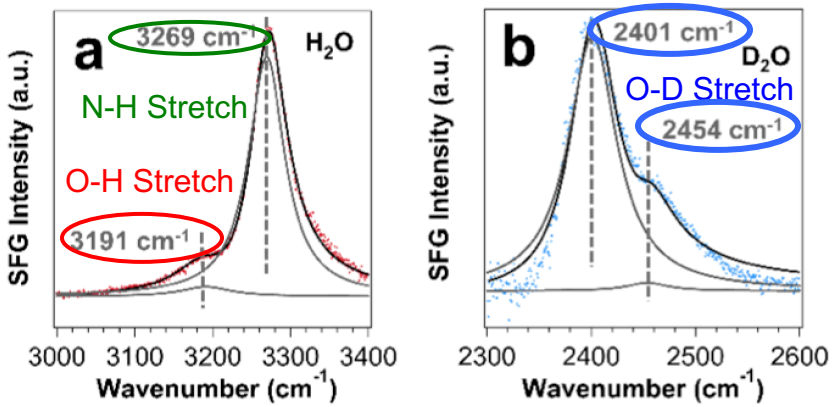
Kinetics of H_2O-D_2O Exchange

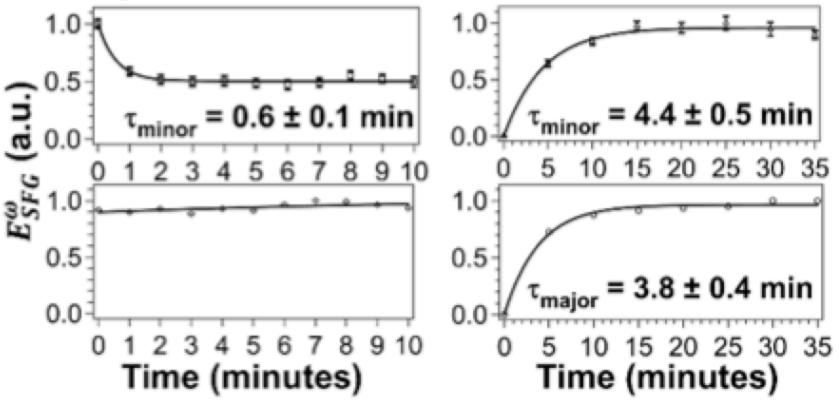


(a) Side View



Kinetics of H_2O-D_2O Exchange



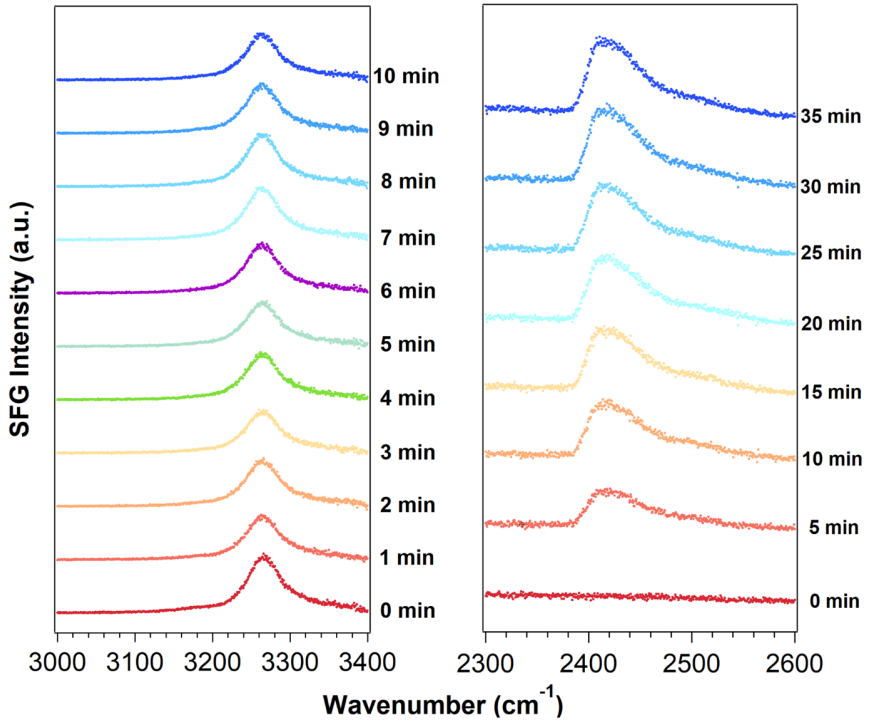


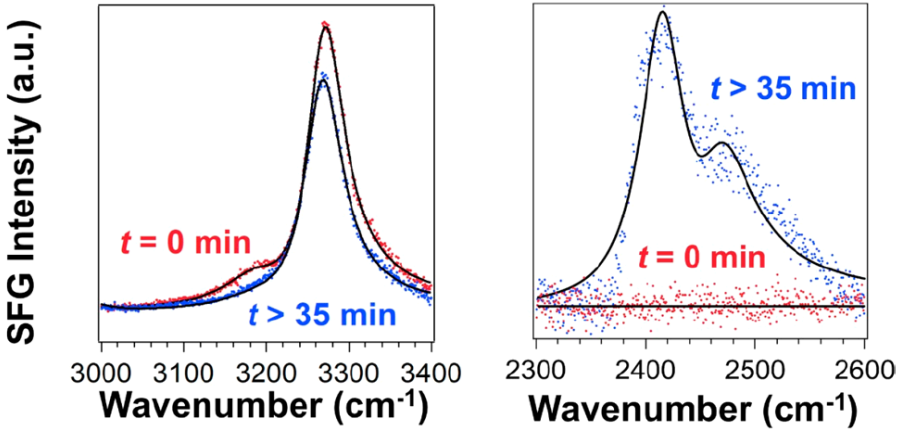
Two peaks from two different species

Possible that both peaks from a single species

O-H Stretch Region

O-D Stretch Region

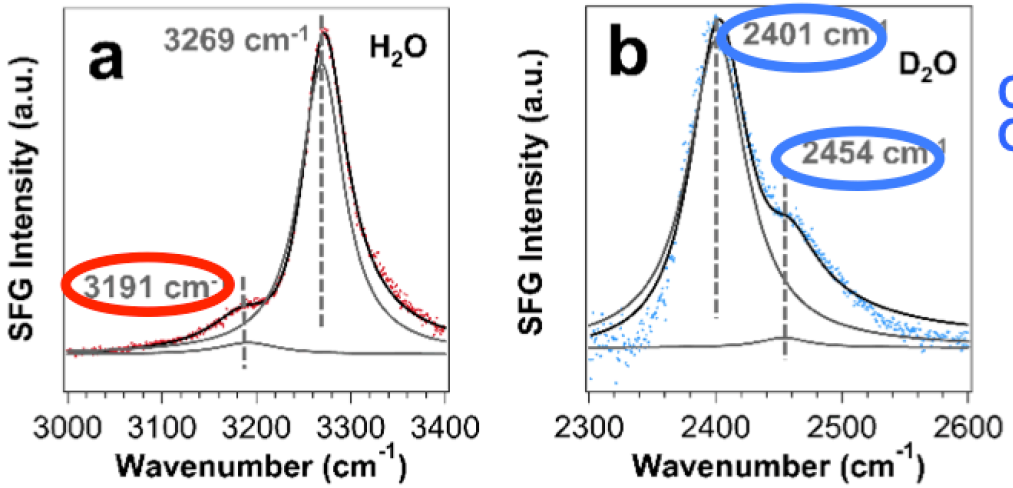




Chiral Water Superstructures observed by Chiral SFG

(1) Isotopic Shift, (2) Isotopic Dilution, (3) H_2O-D_2O Exchange

Chiral Water O-H Stretch



Chiral Water O-D Stretch

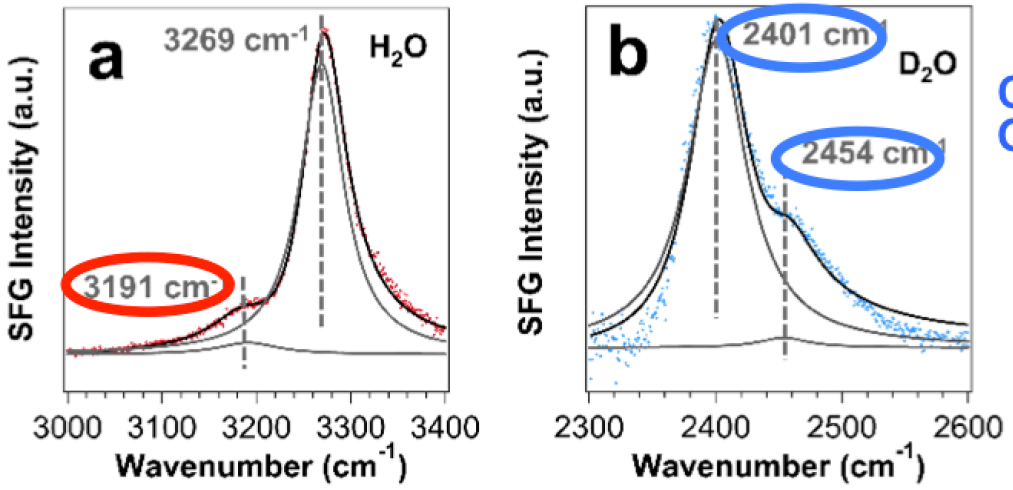
Implication:

1. Individual water molecules are achiral.
2. A protein secondary structure can imprint its chirality onto surrounding water
3. Water molecules form chiral supermolecular structures.

Chiral Water Superstructures observed by Chiral SFG

(1) Isotopic Shift, (2) Isotopic Dilution, (3) H_2O-D_2O Exchange

Chiral Water O-H Stretch



Chiral Water O-D Stretch

In progress:

1. Phase measurements using peptides made with L- and D-formed amino acids.
2. Protein denaturation mechanism.
3. MD simulations and computational studies:
   - to simulate the chiral SFG spectra
   - to explore the structures of water surrounding the peptide

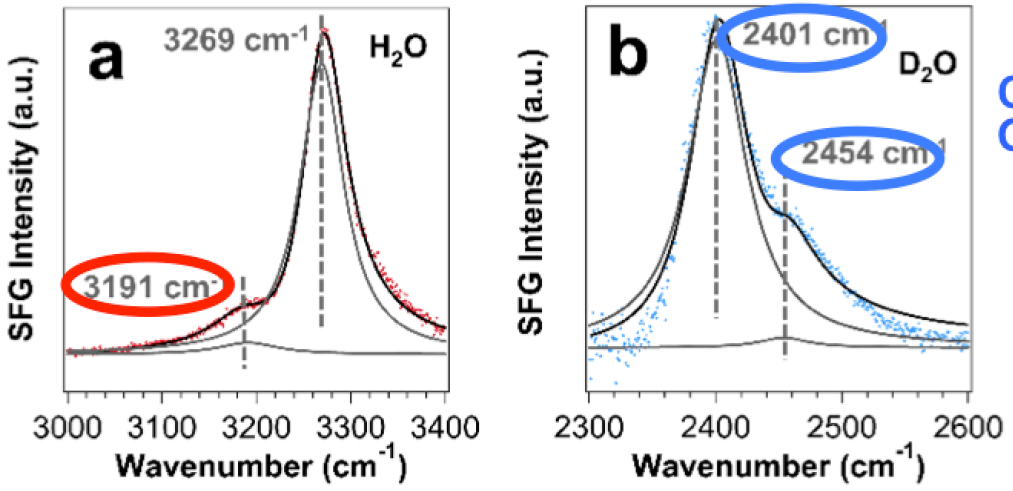
Prof. Sharon Hammes-Schiffer

Dan Konstantinovsky

Chiral Water Superstructures observed by Chiral SFG

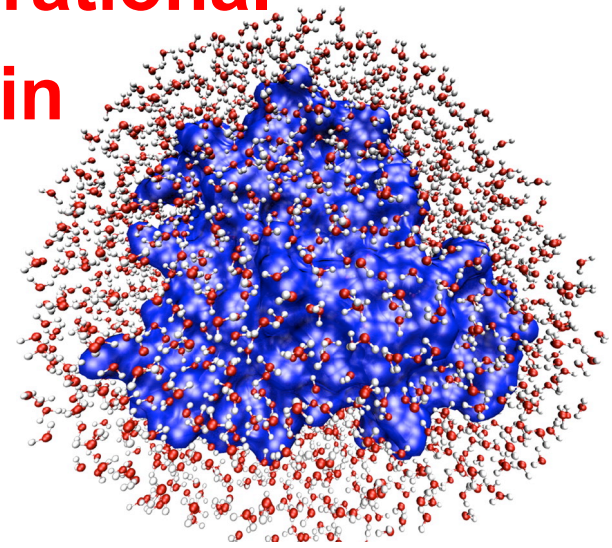
(1) Isotopic Shift, (2) Isotopic Dilution, (3) H_2O-D_2O Exchange

Chiral Water O-H Stretch



Chiral Water O-D Stretch

Chiral SFG for studying water vibrational structures and dynamics in protein hydration



Summary

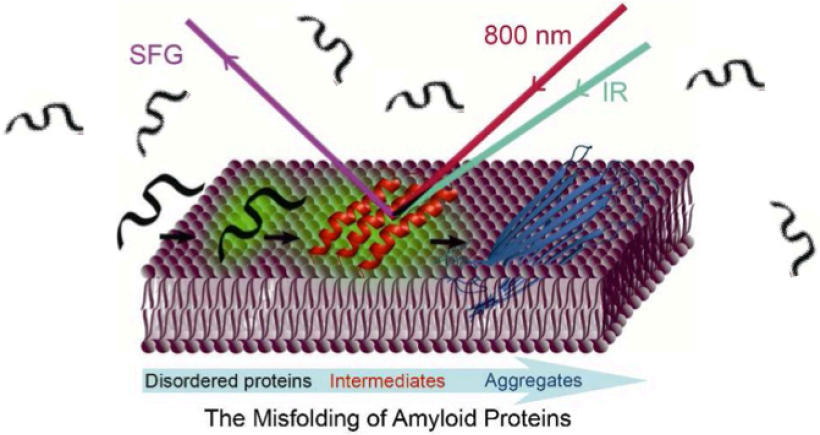
I. Introduction to Chiral SFG Spectroscopy

II. Protein Secondary Structures at Interfaces

III. Water Structures in Protein Hydration

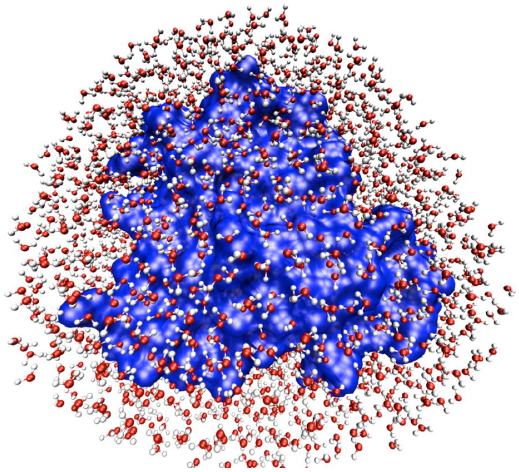
Conclusion

Protein Secondary Structures at Interfaces



The Misfolding of Amyloid Proteins

Water Structures in Protein Hydration



Chiral SFG can introduce some new opportunities to probe protein structures and dynamics

Acknowledgements

Yan Lab Member

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- **Ethan Perets**
- **Jaintao Chen**
- **Zachary Taitz**
- **Dan Konstantinovsky**

Yan Lab Alumni

- **Zhuguang Wang**
- **Li Fu**
- Ying Guo
- **Jian Liu**
- **Gang Ma**
- Victoria Mooney
- Yuting Liu
- **Wei Liu**
- Yingying Cai
- **Dr. Zahra Sophrabpour**
- Kelly Culhane

Collaborators

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- Prof. John Tully
- Prof. Sharon Hammes-Schiffer
- Prof. Roger Leblanc (U. Miami)

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