Unfolded and misfolded states of proteins and peptides explored by optical, vibrational and NMR spectroscopy"

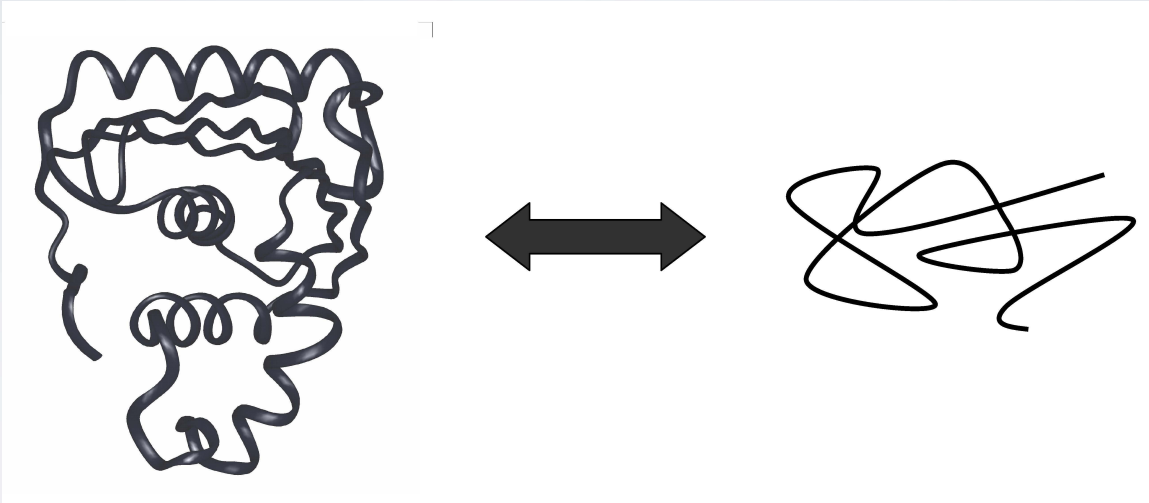
Maria Alessi, Omid Amidi, Laura Duitch, Stefanie Farrell, Emma Fradkin, Andrew M. Hagarman, Jodi Kraus, Dzmitry Malyshka, Thomas J. Measey, Leah A. Pandiscia, Reinhard Schweitzer-Stenner, Lee Serpas, Jonathan B. Soffer, E. Toal, Daniel J. Verbaro

Department of Chemistry
Drexel University
Philadelphia, PA 19104

FIRST SCIENTIFIC QUESTION: IS THE UNFOLDED STATE OF PEPTIDES AND PROTEINS REALLY RANDOM?

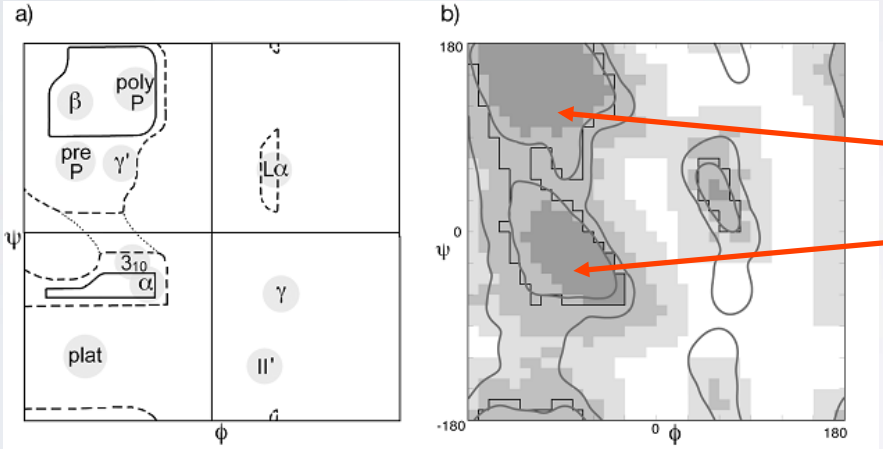
folded

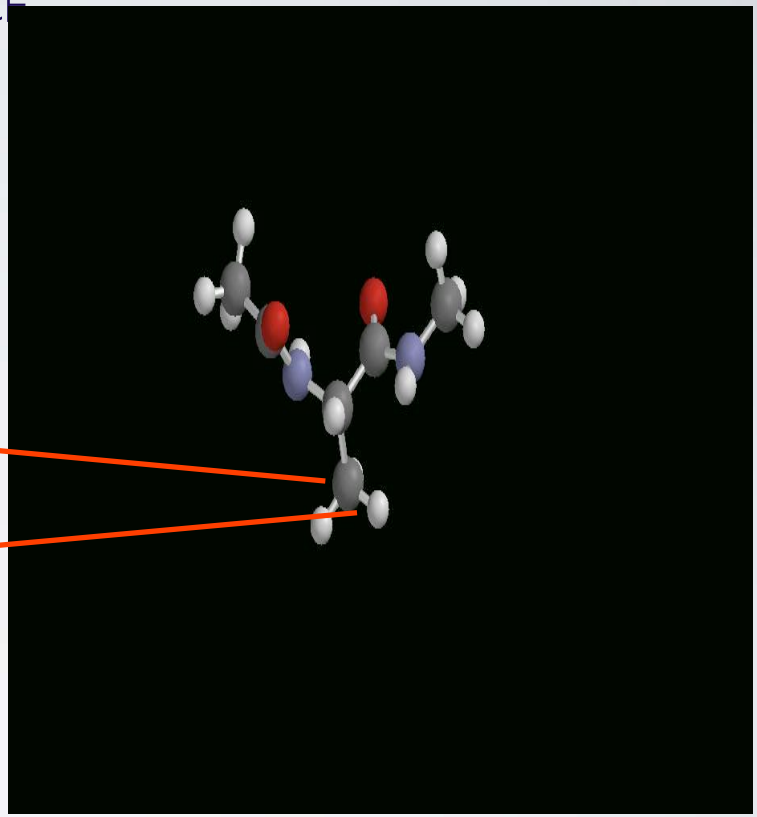
unfolded



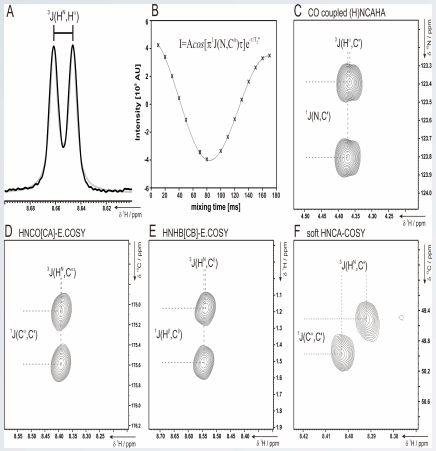
FLORY'S RANDOM COIL MODEL IS BASED ON THE ASSUMPTION THAT AMINO ACID RESIDUES SAMPLE THE ENTIRE ACCESSIBLE CONFORMATIONAL SPACE

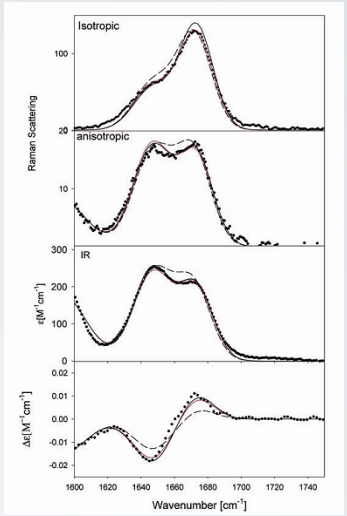
obtained for the alanine dipeptide →



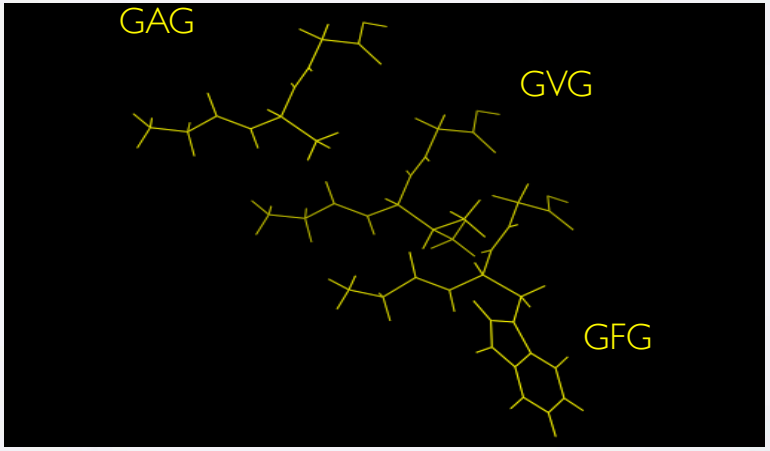


We combine IR, Raman vibrational and electronic circular dichroism and NMR to obtain for the first time experimentally determined conformational in the unfolded state.





Graf, Nguyen, Stock, Schwalbe, JACS, 129, 1179, 2007
Schweitzer-Stenner, JPC B, 113, 2992, 2009
Hagarman, Measey, Mathieu, Schwalbe JACS, 132, 540, 2010
Schweitzer-Stenner, **Hagarman, Toal,** Mathieu, Schwalbe, Proteins, 91, 955, 2013



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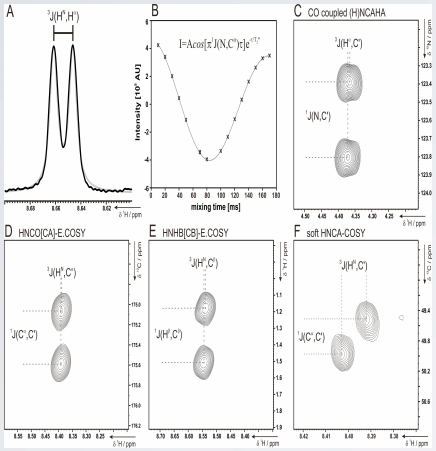

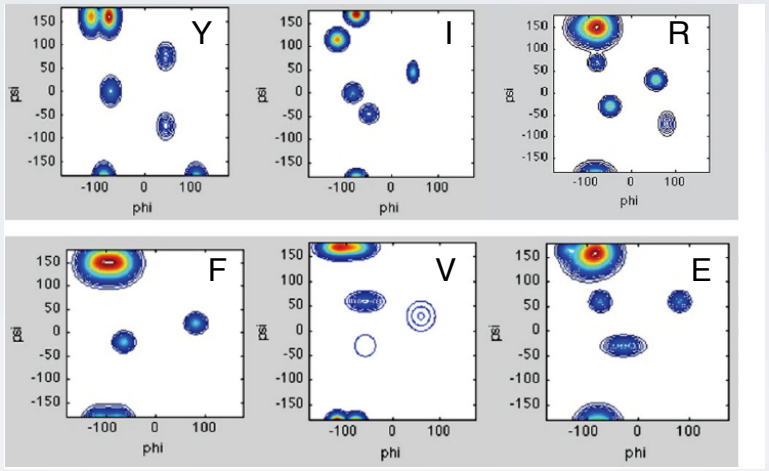

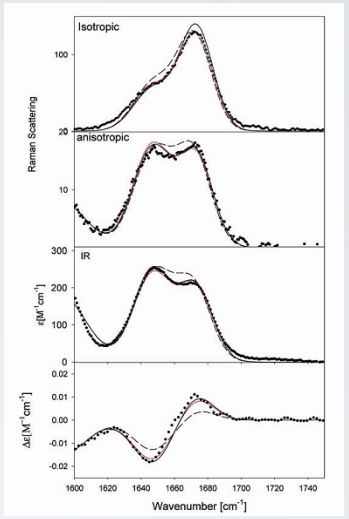

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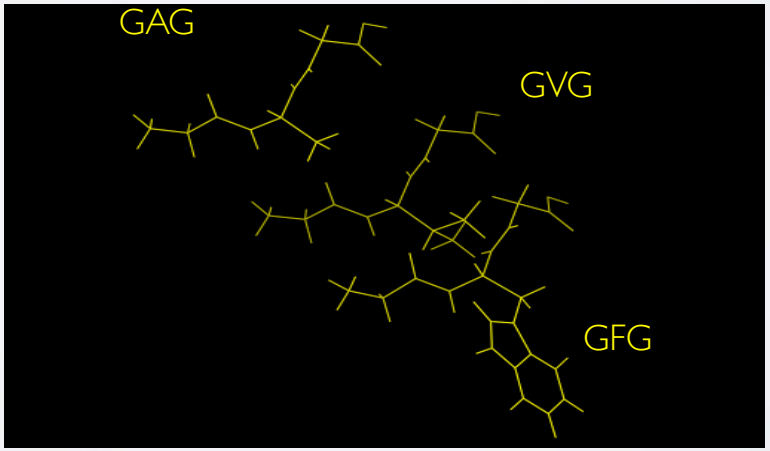
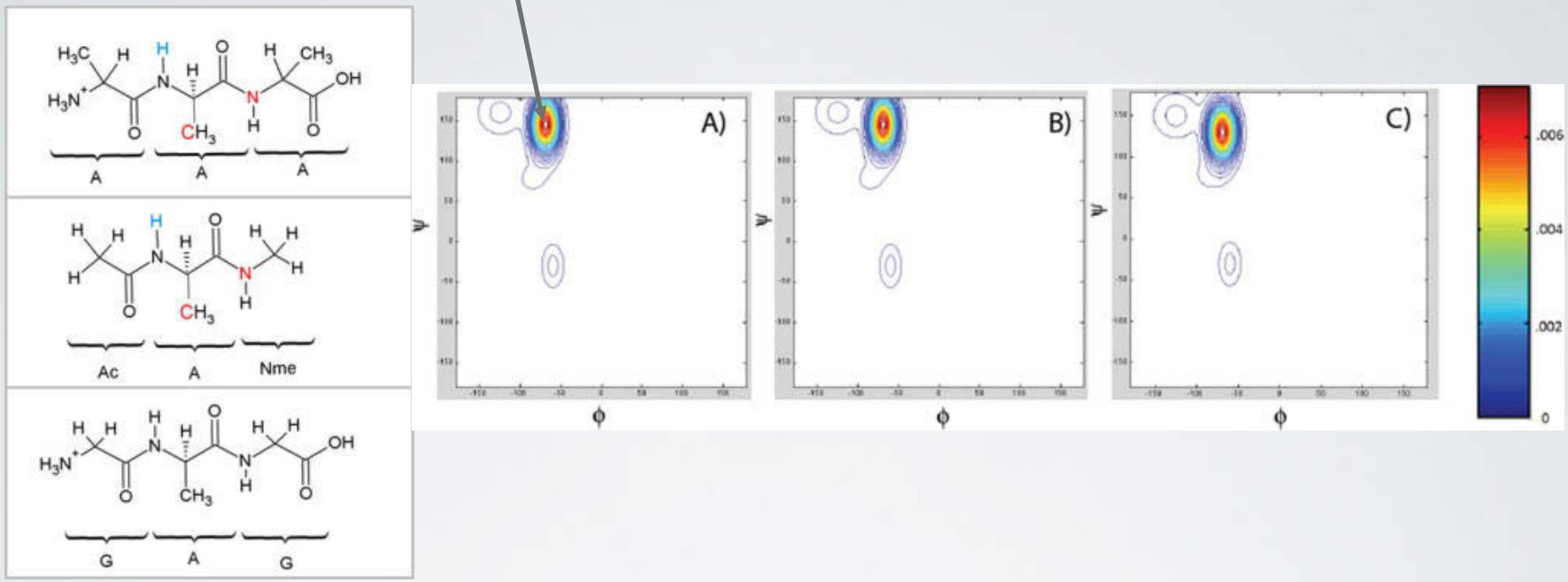
Schweitzer-Stenner, **Hagarman,**

Toal, Mathieu,

Schwalbe, Proteins, 91, 955, 2013



Alanine, a highly abundant amino acid in proteins, exhibits a totally unexpected preference for poly-proline II like conformations



Toal, Meral, Verbaro, Urbanc, Schwalbe, Schweitzer-Stenner, J. Phys. Chem. B. 117, 3689, 2013

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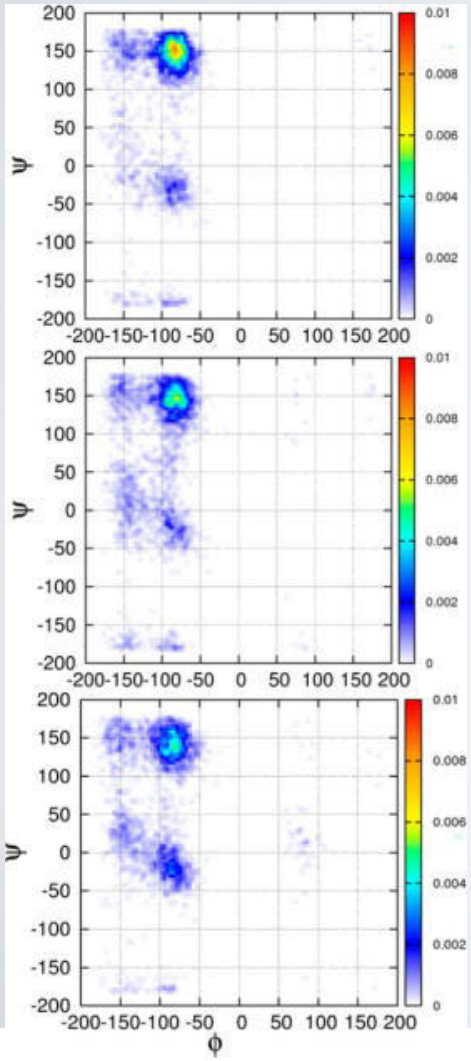
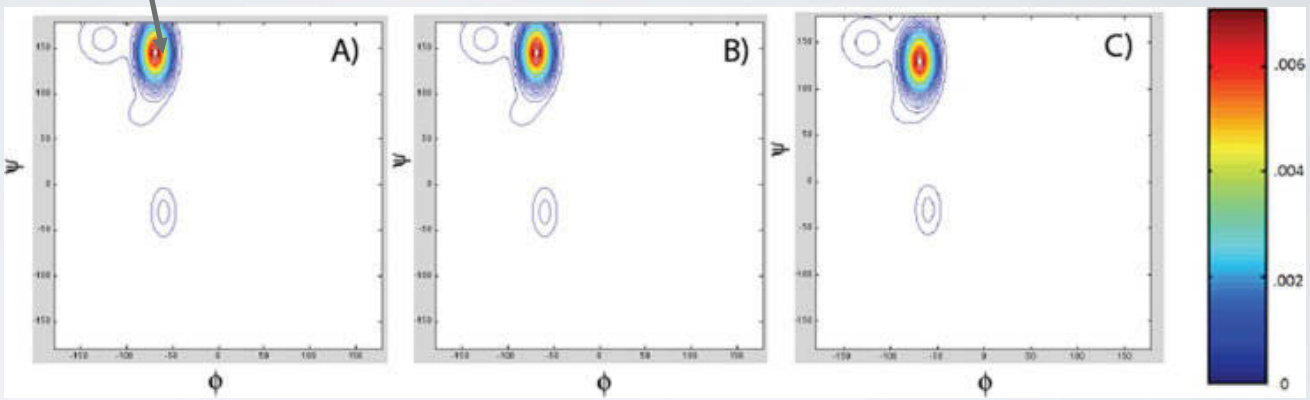
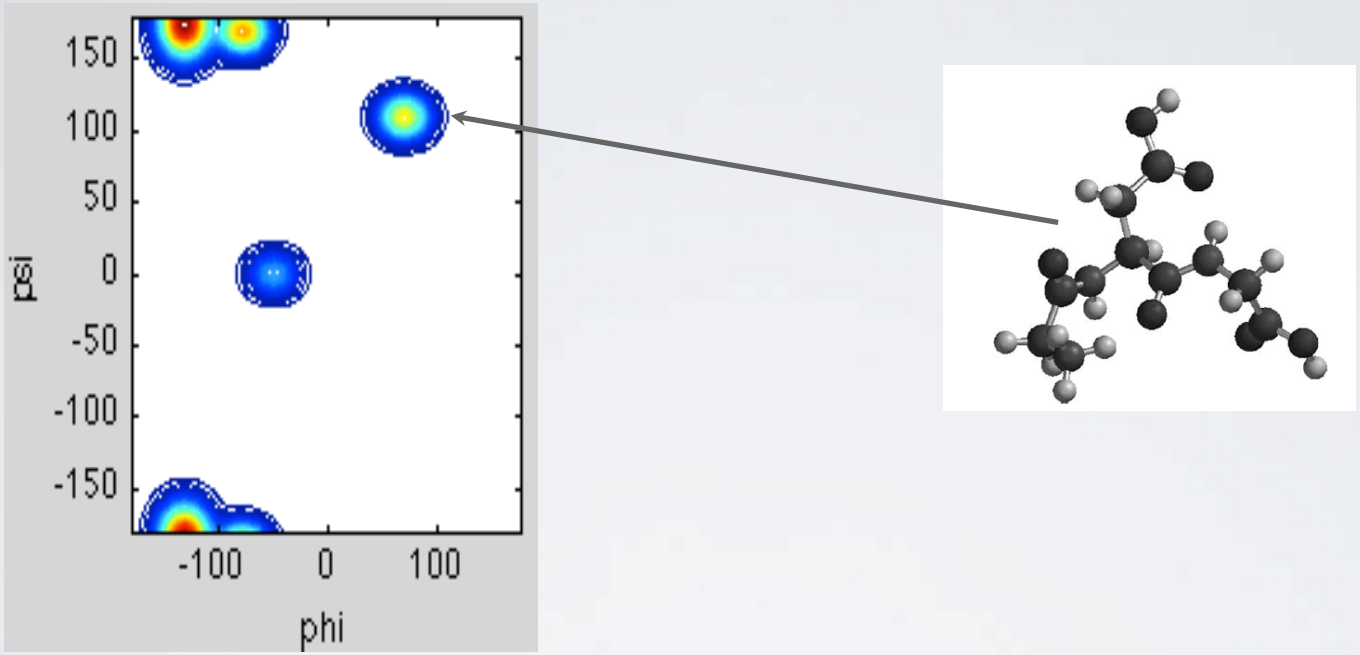




Reproduction of experimentally obtained distributions with MD simulations

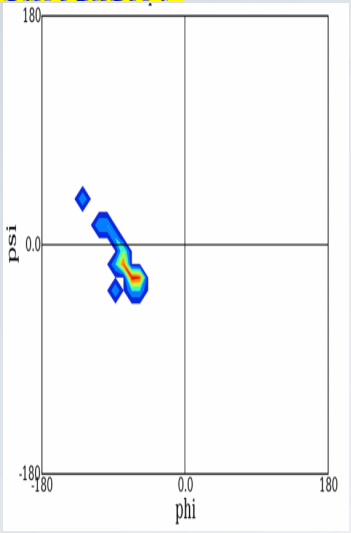
Toal, Meral, Verbaro, Urbanc, Schwalbe, Schweitzer-Stenner, J. Phys. Chem. B. 117, 3689, 2013

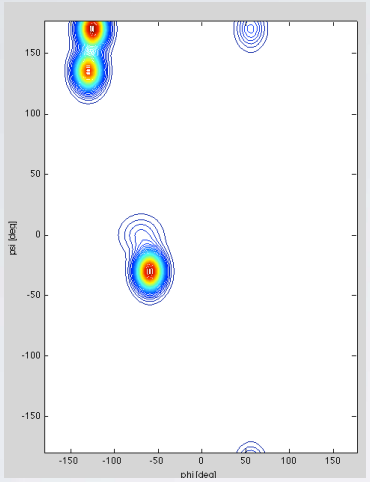
GxG with cysteine, aspartic acid and asparagine can form turn-like structures*

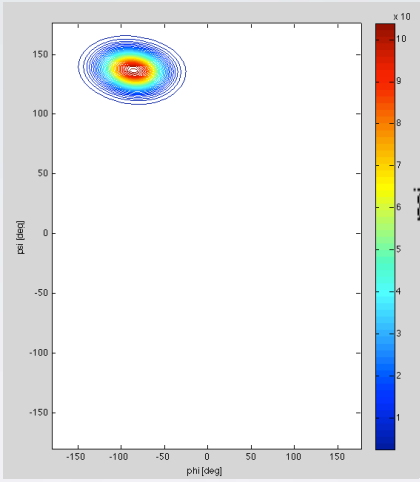


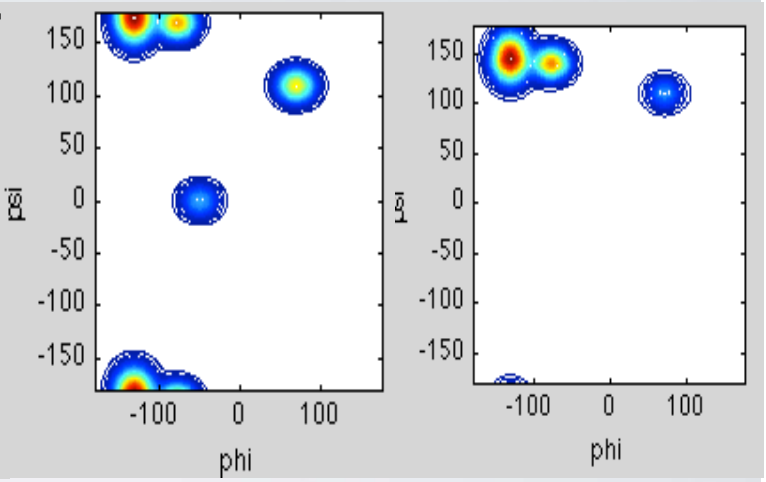
Hagarman, Mathieu, **Toal, Measey,** Schwalbe, Schweitzer-Stenner. Eur. J. Chemistry 17, 6789, 2011
Rybka, Mathieu, **Toal,** Schwalbe, Schweitzer-Stenner, Proteins. 81, 968, 2013

Comparing DDD distributions:



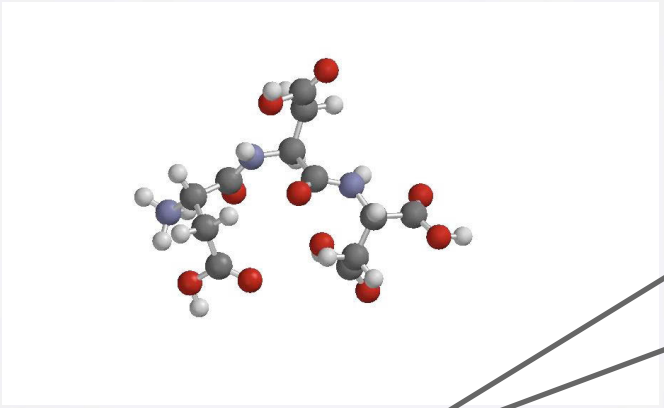


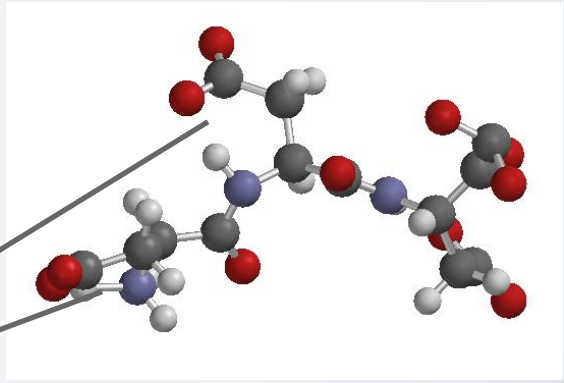




Sosnick's coil library

DDD^P DDD^i GDG^P GDG^i



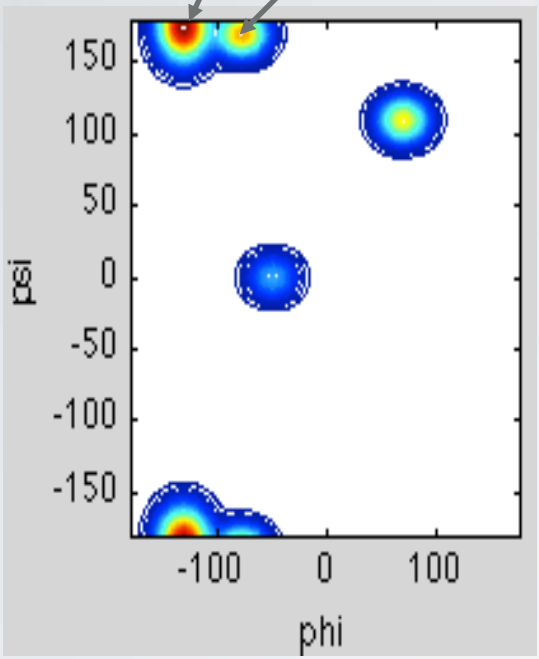


hydrogen bond

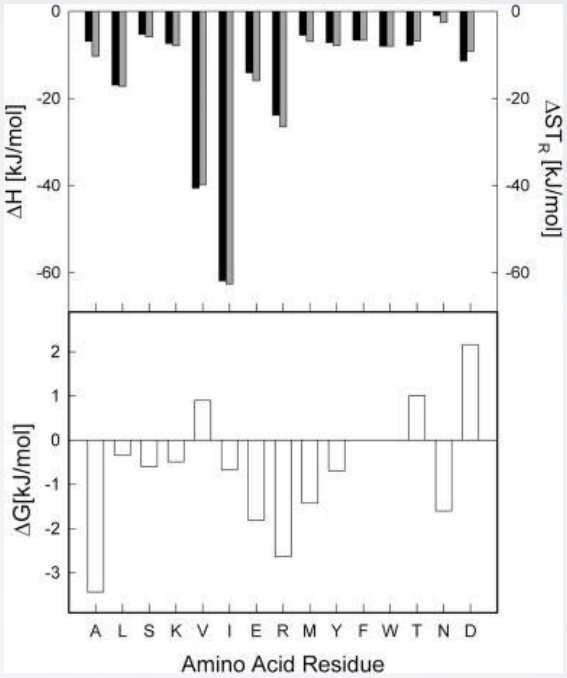
Duitch, Toal, Measey, Schweitzer-Stenner, JPC B 116, 5160-5171, 2012

Rybka, Mathieu, **Toal, Verbaro,** Schwalbe, Proteins 91, 968, 2013

We recently analyzed the thermodynamics of the β⇔pPII equilibrium for GxG peptides



We discovered a nearly perfect enthalpy-entropy compensation.



Toal, Verbaro, Schweitzer-Stenner, J. Phys. Chem B. 118, 1309, 2014

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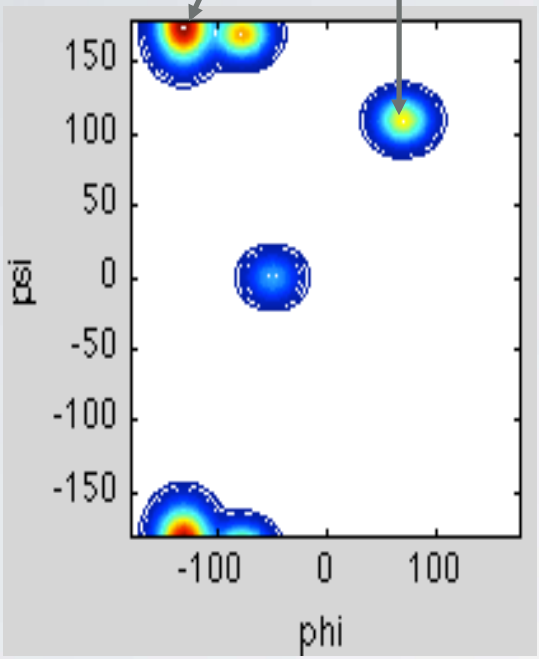

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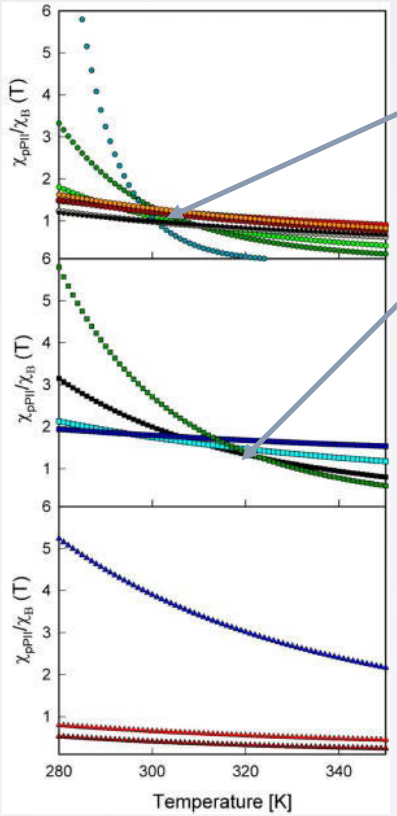
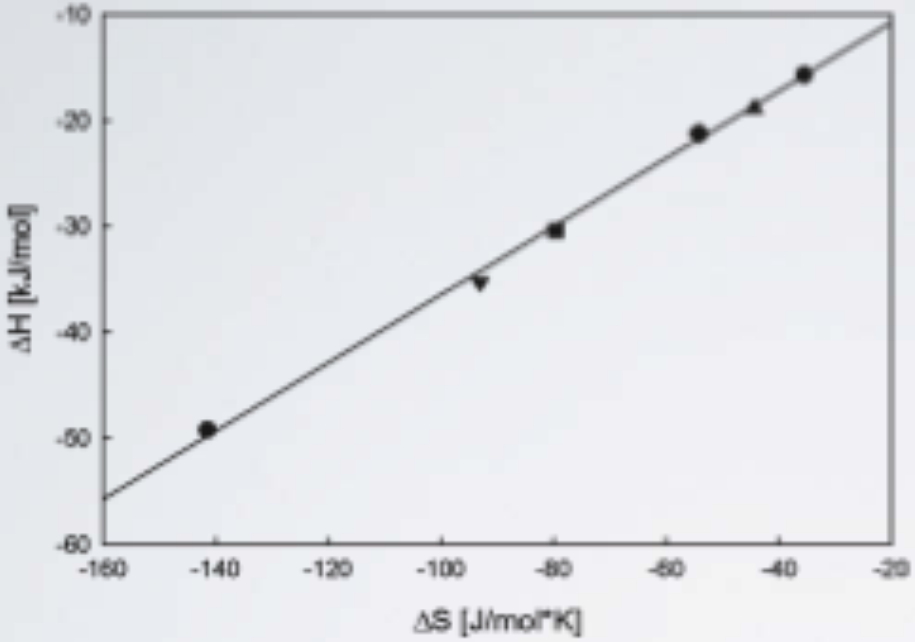


Two iso-equilibrium cluster

Points to peptide-water interactions as the key determinant of propensities

Toal, Verbaro, Schweitzer-Stenner, J. Phys. Chem B. 118, 1309, 2014

I. Thermodyanamic changes are governed by enthalpy entropy compensation

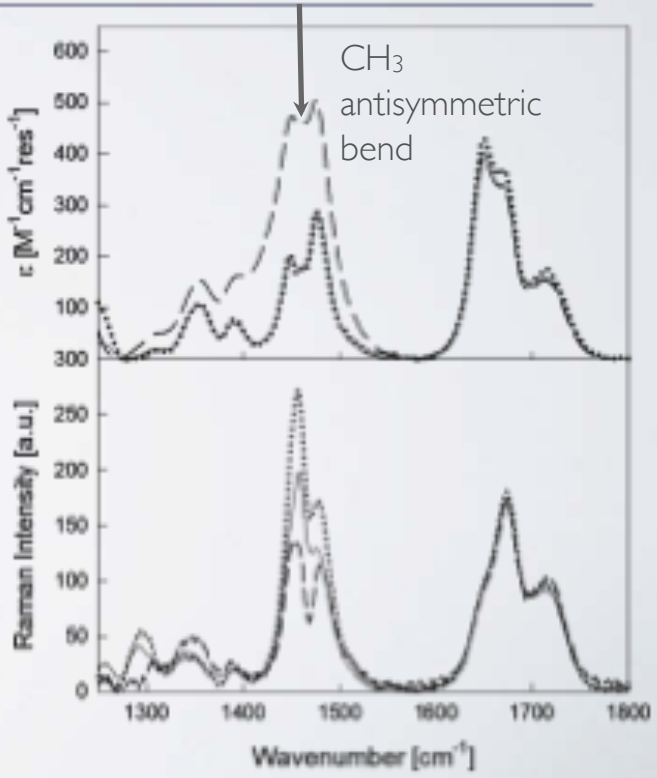


$$\Delta H = \Delta H_0 - T_R \Delta S$$

Table 3. Results Obtained from the Linear Fit of Enthalpy–Entropy Data[a]

residue	ΔH_0 [kJ/mol]	T_R [K]	R^2
N-terminal	−4.28	321.5	0.997
C-terminal	−1.96	309.2	0.989

[a] Transition temperature, zero-point enthalpy, and correlation coefficient for the N- and C-terminal.

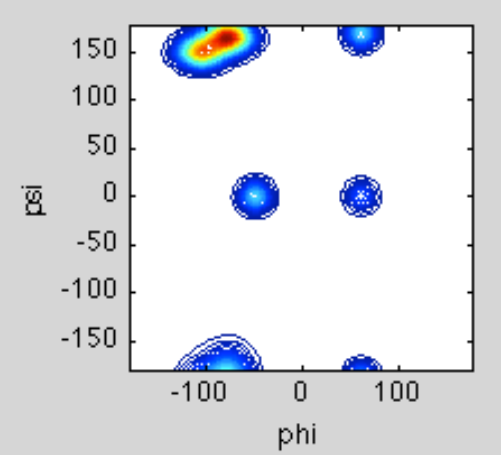


II. Spectroscopic data reveal glyerol-peptide interactions

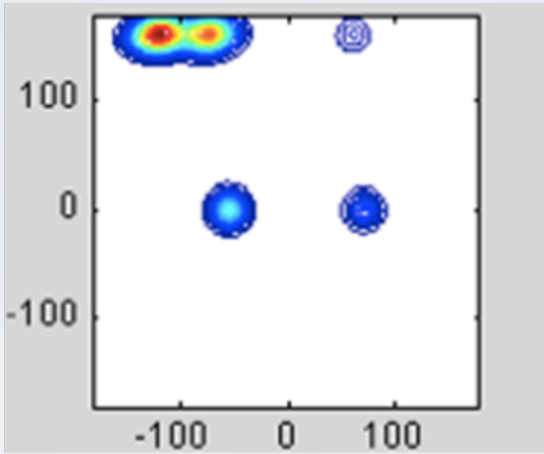
Toal, Amidi, Schweitzer-Stenner, JACS 133, 12728, 2011

Looking on GxyG peptides:
Comparing Ramachandran plots

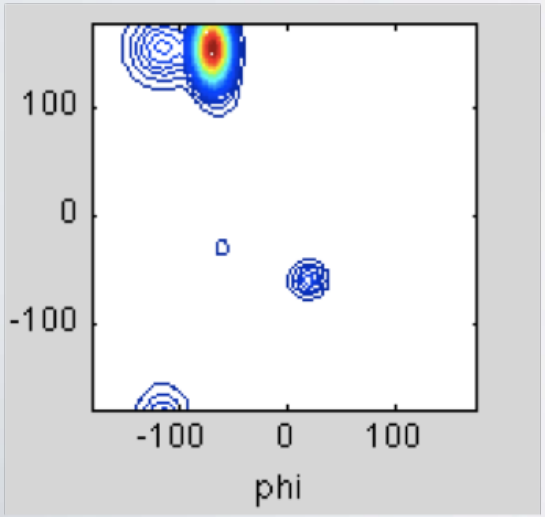
GSG



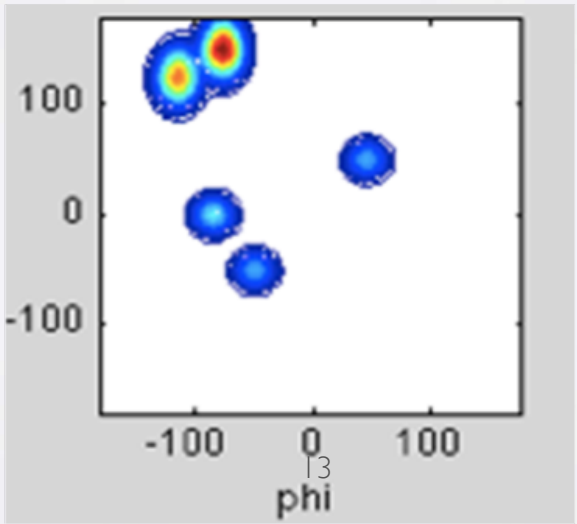
GSAG



GAG



GSAG



A direct comparison of the conformational distribution for S in GSAG and GSLG with S in GSG reveals that A and L cause the asx turn forming propensity of S (10%) to be mitigated to 2% in preference for more extended pPII and β structures which increase by 6%.

The populations of alanine conformations are only modestly changed (shift from pPII to β), but the position of pPII and particularly of β is shifted to lower ψ values

Toal, Rybka, Schwalbe, Schweitzer-stenner, unpublished

We are currently pursuing an investigation of a rather representative series of GxyzG peptides to study the influence of nearest neighbors.

We also study the influence of the solvent composition on the conformational manifold of short peptides.

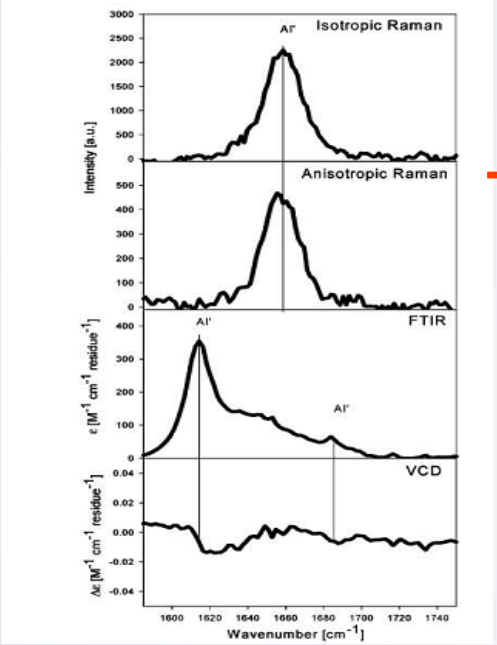
Investigating blocked peptides

Collaboration with Harald Schwalbes's group at the Johann Wolfgang Goethe University in Frankfurt (NMR) and Brigita Urbanc's group in Physics (MD simulations)

Siobhan Toal, Stefanie Farrel, Bridget Molorey

Second question: How can we probe self-aggregation and gel formation of peptides?

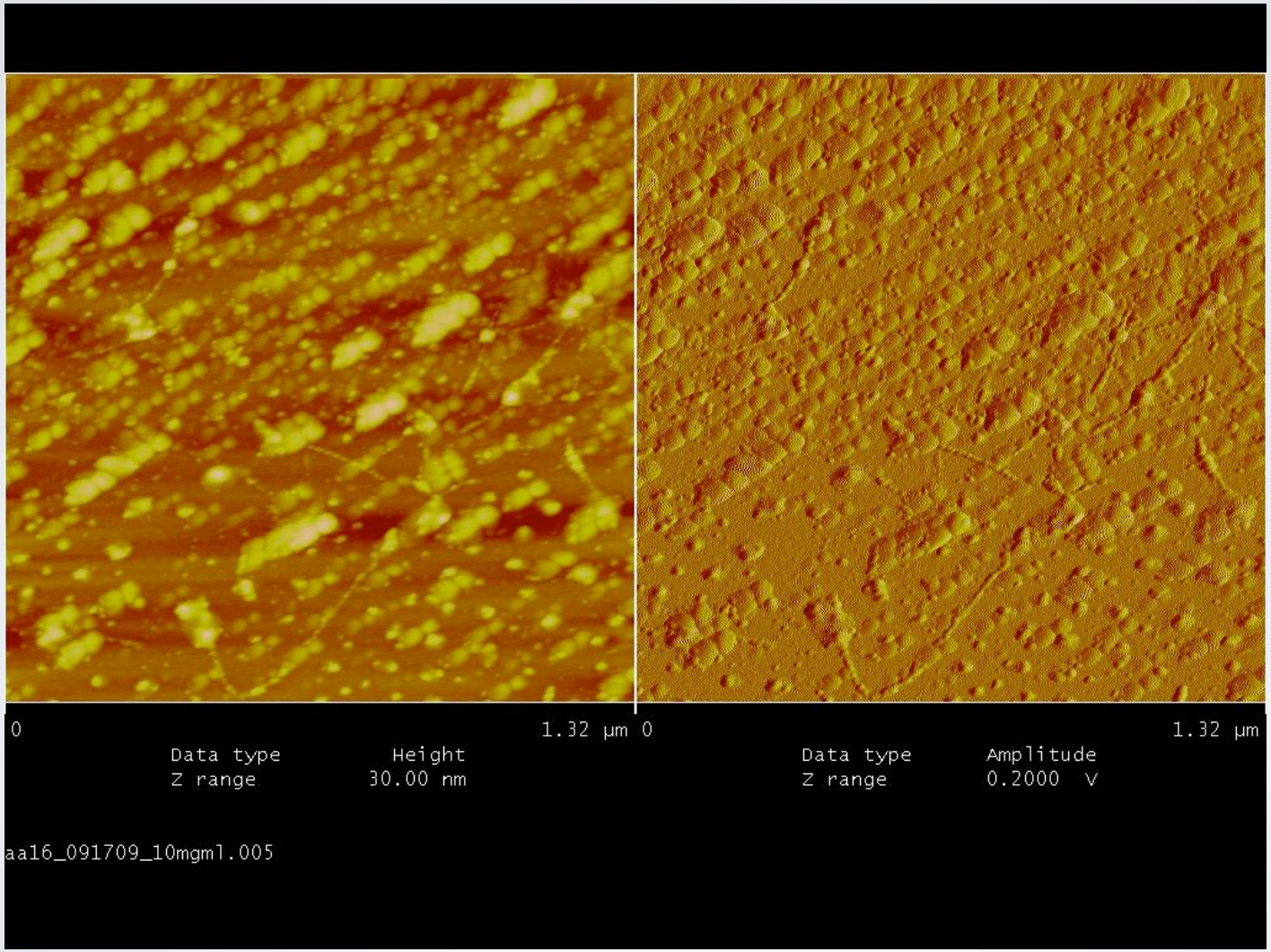
A recent discovery: the 16mer $(AAKA)_4$ forms a hydrogel at centimolar and acidic pH:



→ antiparallel β-sheet

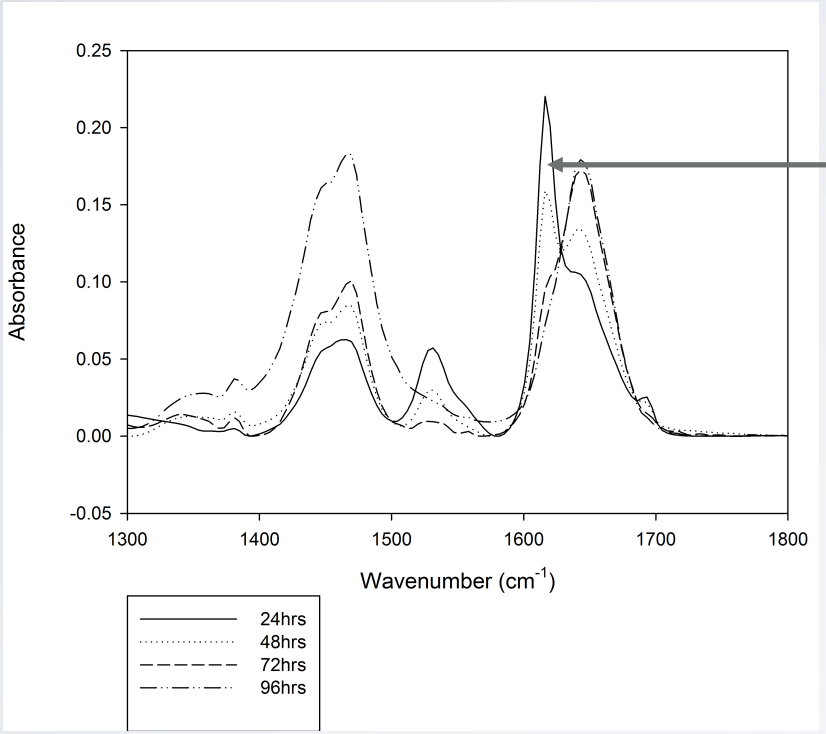
Measey, Schweitzer-Stenner, JACS 128, 13324, 2006

AFM suggests the formation of colloid like beads



Measey, Schweitzer-Stenner, Schweitzer-Stenner, Sa and Kornev. Macromolecules 43, 7800, 2010

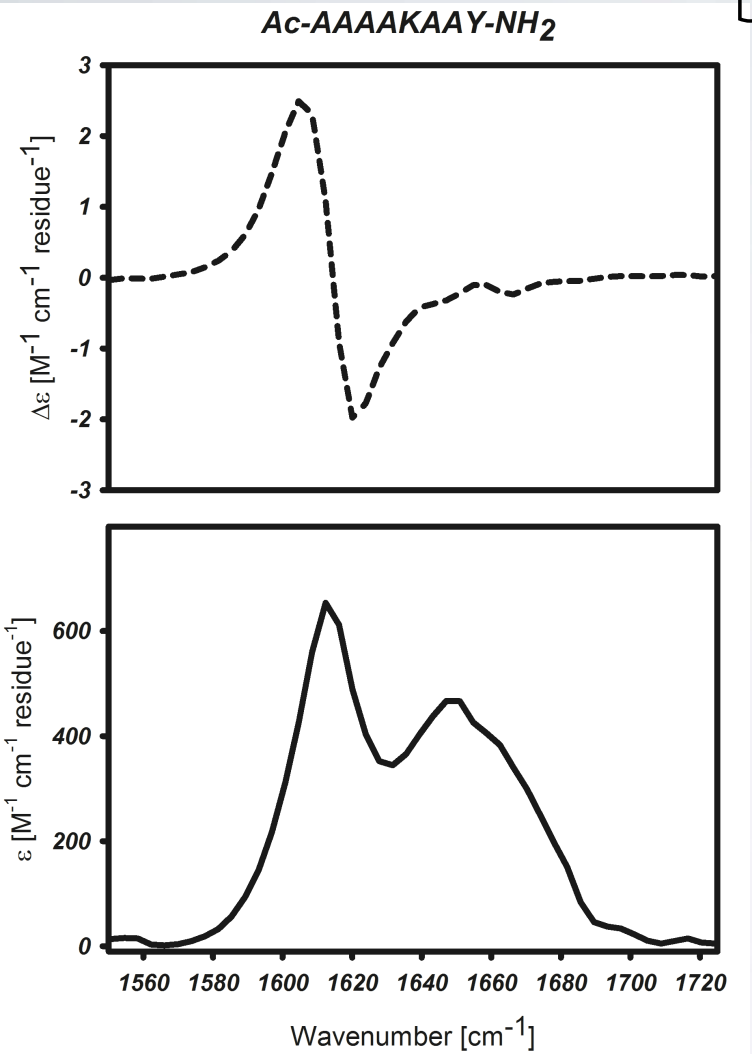
Contrary to many self-aggregating peptides, $(AAKA)_4$ decays over time



probed by FTIR spectroscopy

Kraus, Toal, Schweitzer-Stenner, unpublished

After incubating AAAAKAAY for obtained a rather rigid gel and the following amide I band profiles:



Measey, Smith, Decatur, Zhao, Yang, †,, Schweitzer-Stenner JACS 181, 18218, 2009

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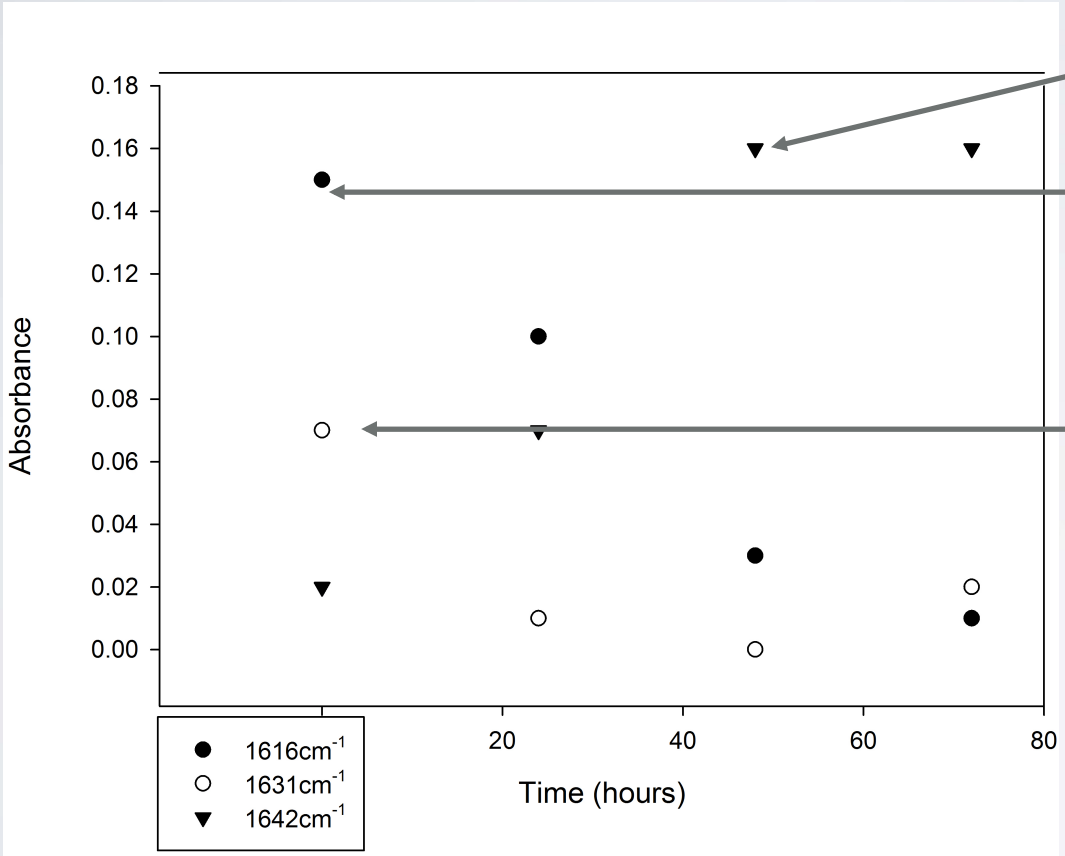


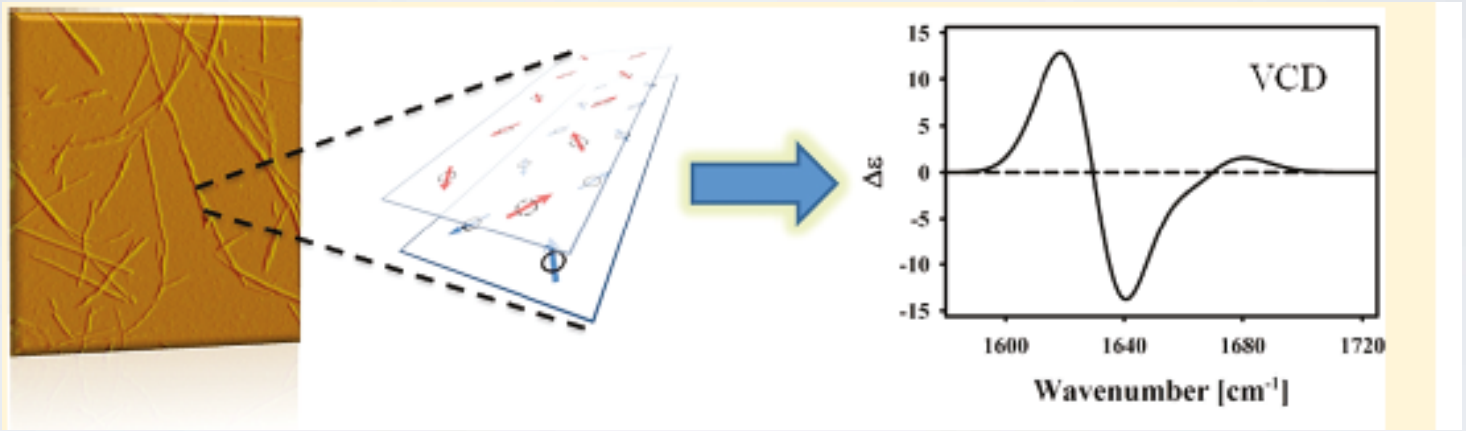
amorphous aggregates

long sheets, may be fibrils

oligomers

Kraus, Toal, Schweitzer-Stenner, unpublished

We calculated IR and VCD for a very simple 2-dim model of Frenkel excitons:



Measey and Schweitzer-Stenner, JACS 133, 1066, 2011

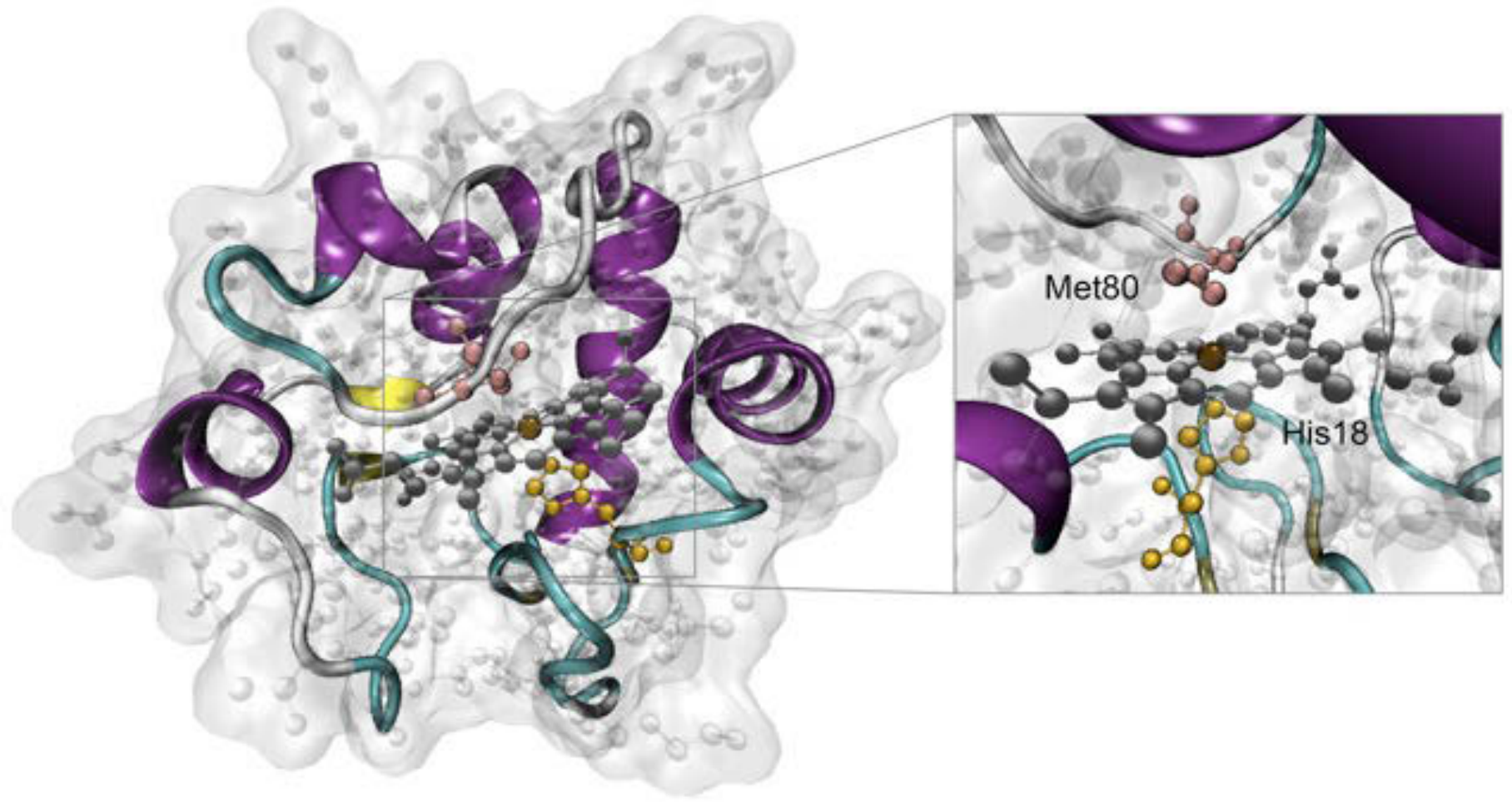
Future directions:

Use isotopic labeling and more accurate modeling to explore local structures of peptide fibrils.

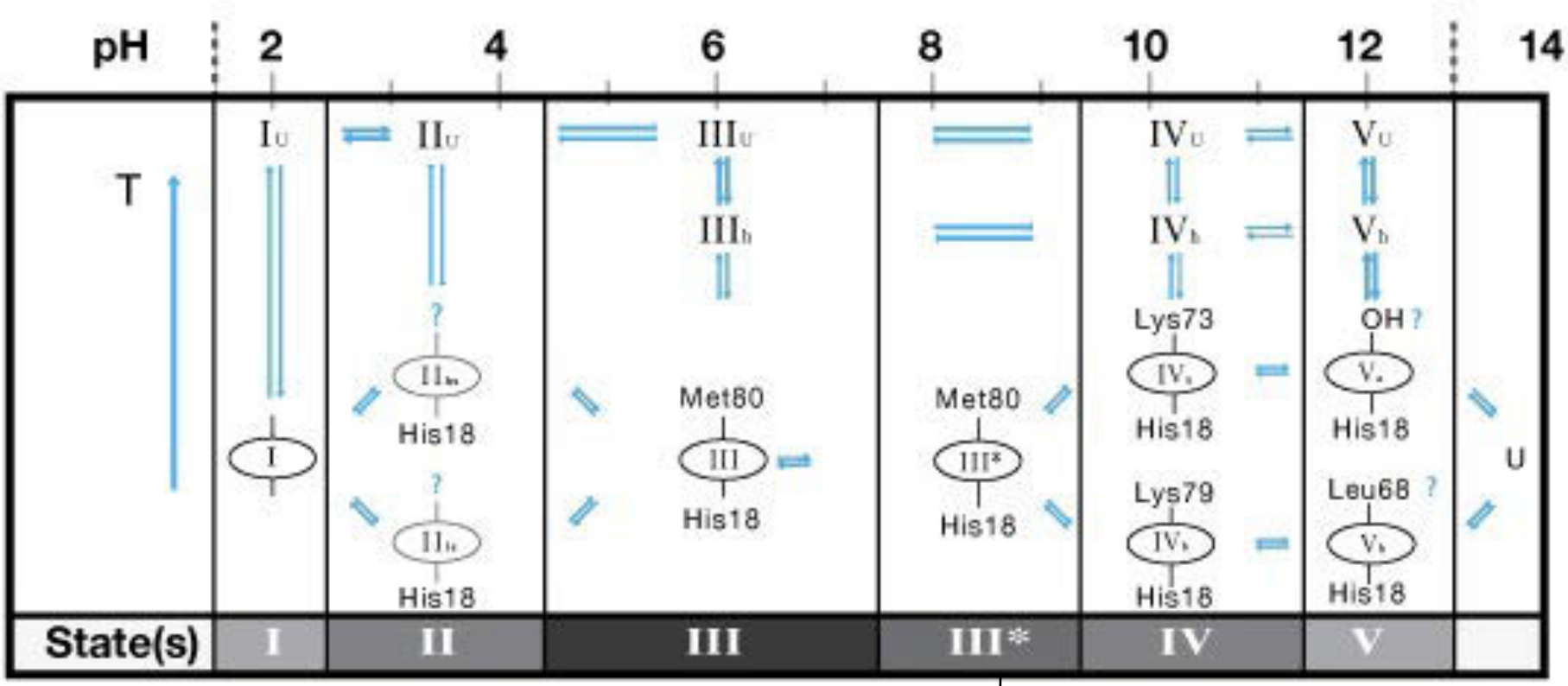
Siobhan E. Toal, Jodi Kraus

Collaboration with Sean Decatur, Obelin College

Third issue : Non-native states of cytochrome c: the 'linkage protein' for electron transfer in the respiratory chain



Changing the pH switches cytochrome into partially unfolded states:



discovered in our group*

Verbaro, Hagarman, Soffer, Schweitzer--Stenner, Biochemistry 48, 2990, 2009

II. Populating a non-native state under folding conditions

A new sample preparation protocol was utilized to avoid photo reduction upon samples exposure to the laser beam for our resonance Raman experiments:*

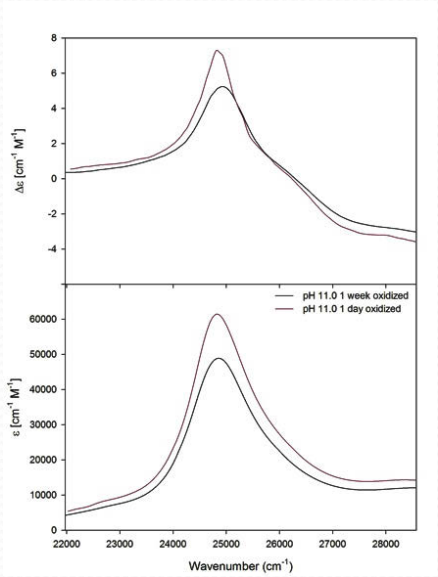
1. Adjust the pH of the cytochrome c sample to pH 11.5 (state V)

2. Oxidize the sample with potassium ferricyanide

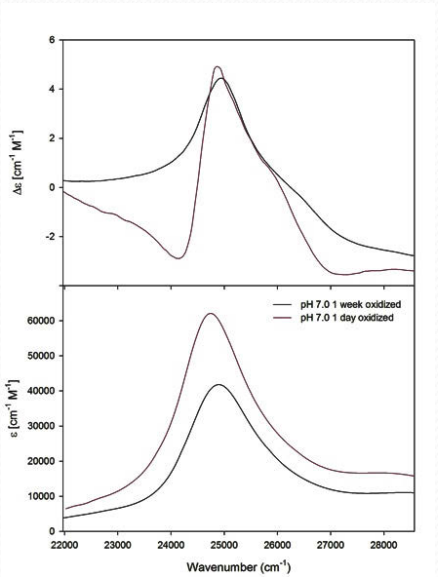
3. Running sample over a sephadex G-25 column to separate the protein from potassium ferricyanide

Alessi, Hagarman, **Soffer**, Schweitzer-Stenner; J. Raman Spectrosc. 42, 917, 2011

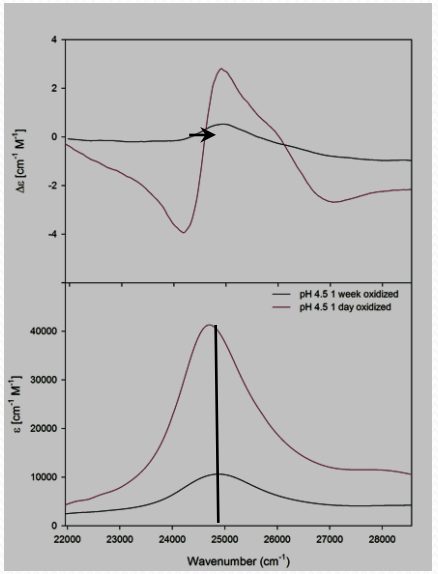
What happened with the heme environment? Comparison after 1 day and 1 week of oxidization



pH 11: same lost in oscillator strength



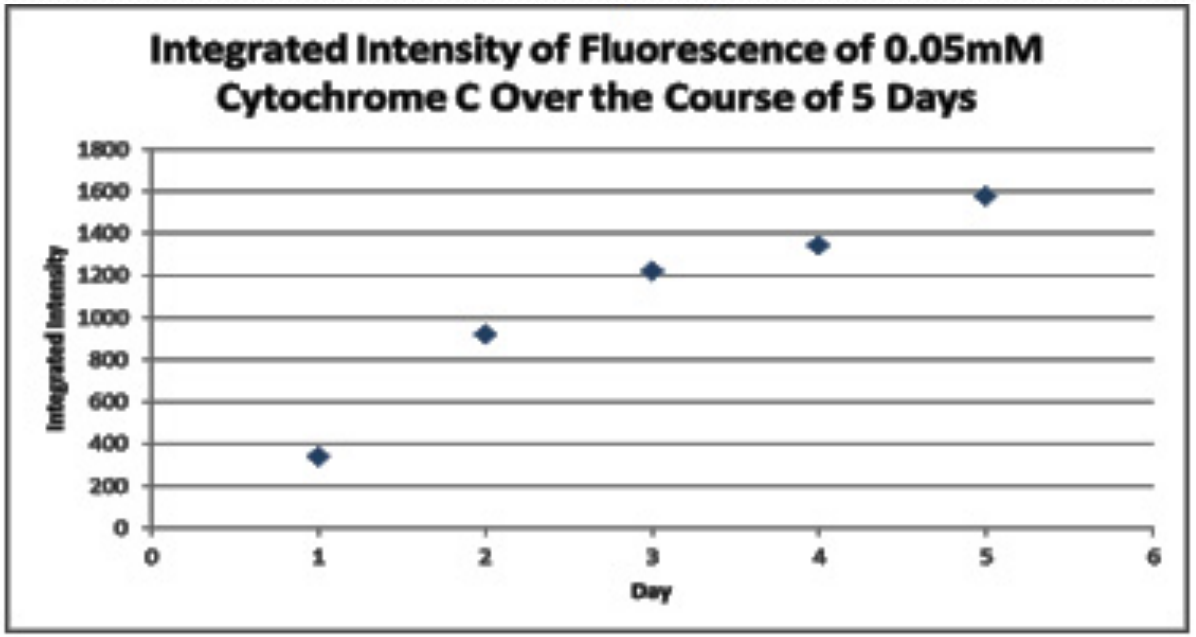
pH 7: After one day, it shows the couplet of native state III, after 7 days the positive Cotton band is diagnostic of a non-native low spin state



pH 4.5: conversion into a non-native state and aggregation after one week.

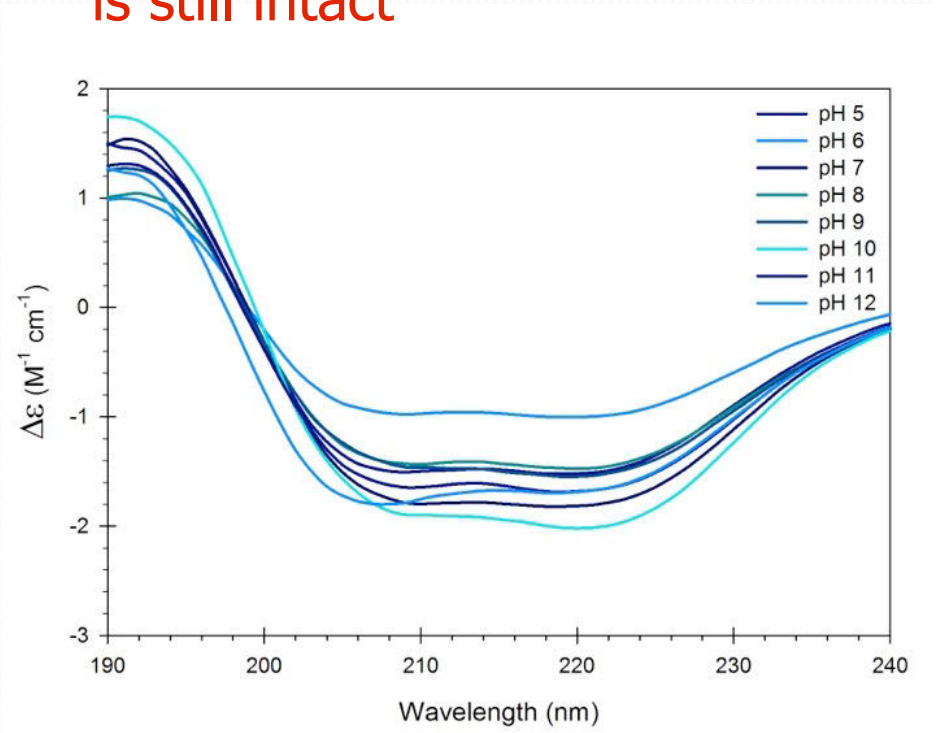
Soffer,Fradkin, Pandiscia, Schweitzer-Stenner, Biochemistry 92, 1397, 2013

Fluoresence monitor the slow structural change occurring at pH 11.5



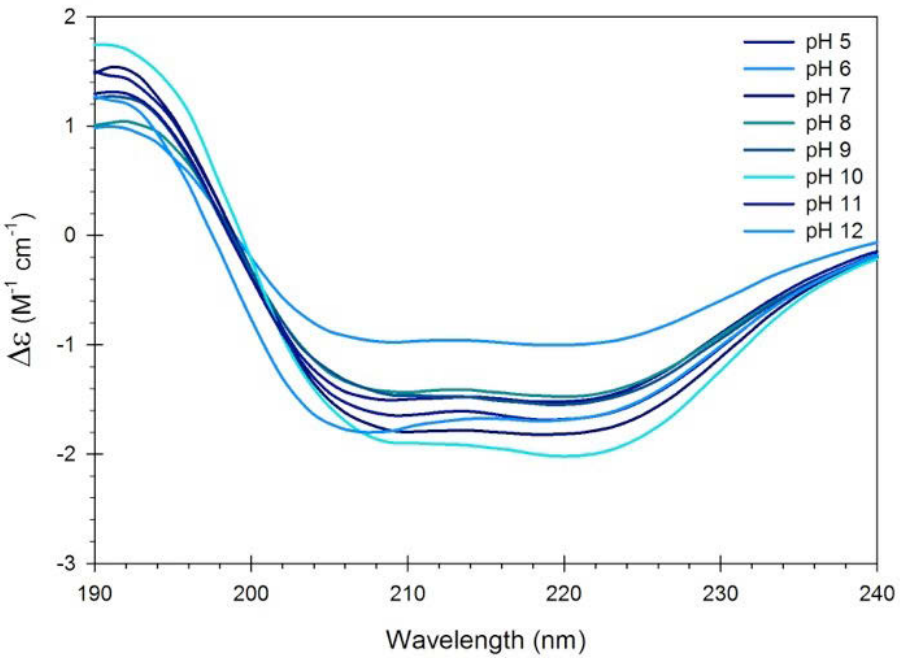
Serpas, Pandiscia, Schweitzer-Stenner, unpublished

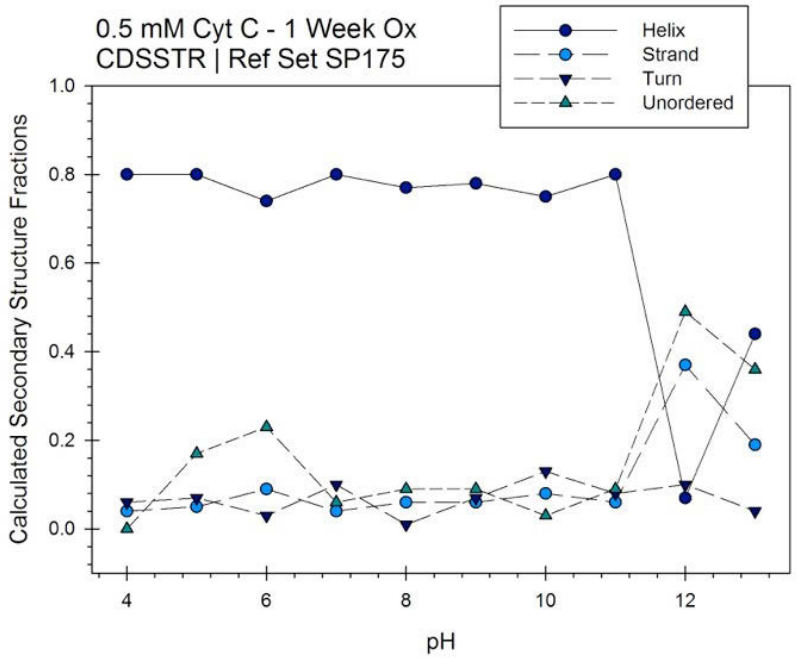
The UV CD spectra indicate that the secondary structure is still intact



Soffer,Fradkin, Pandiscia, Schweitzer-Stenner, Biochemistry 92, 1397, 2013

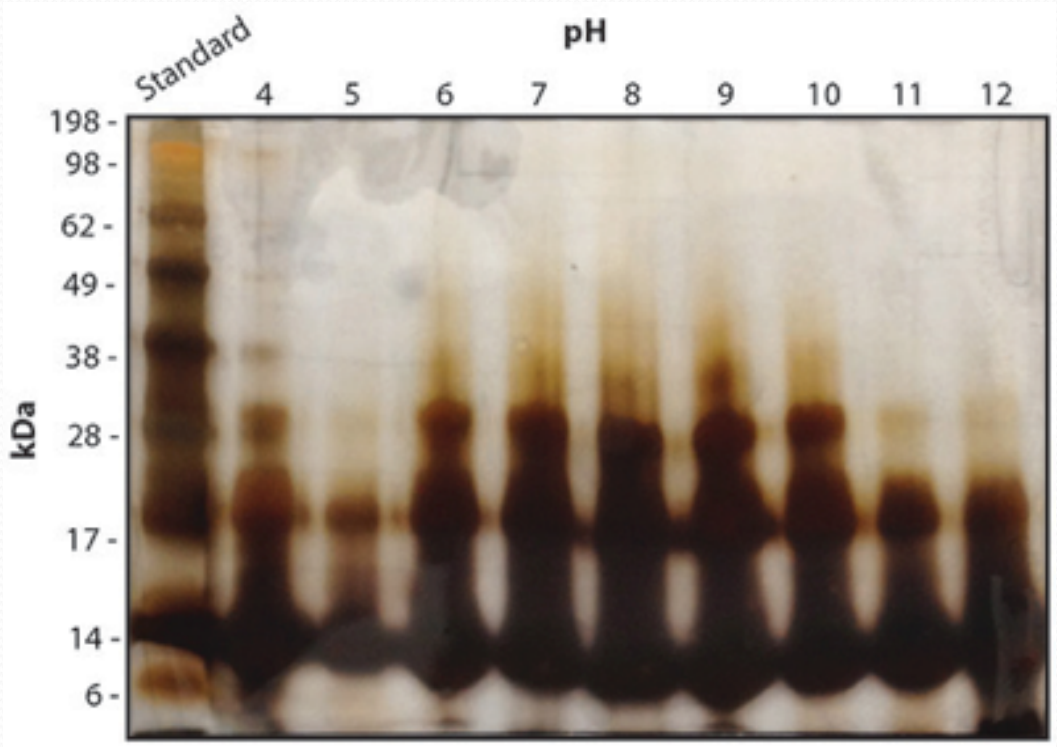
The UV CD spectra indicate that the secondary structure is still intact





Soffer,Fradkin, Pandiscia, Schweitzer-Stenner, Biochemistry 92, 1397, 2013

Gel electrophoresis suggests a monomer-dimer-trimer equilibrium at 0.5 M and pH values between 6 and 10:



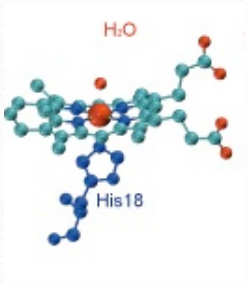
dimer

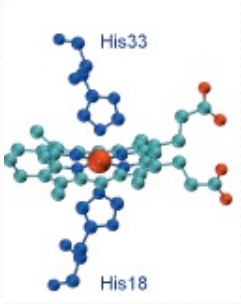
monomer

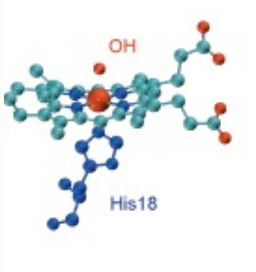
Soffer,Fradkin, Pandiscia, Schweitzer-Stenner, Biochemistry 92, 1397, 2013

Hypothetical model:

$$\begin{bmatrix} III_n & \Leftrightarrow & IV_n & \Leftrightarrow & V_n \\ & & & & \downarrow \\ PcQM & \Leftrightarrow & B_2(HH) & \Leftrightarrow & V_n^* \Leftrightarrow \end{bmatrix}$$



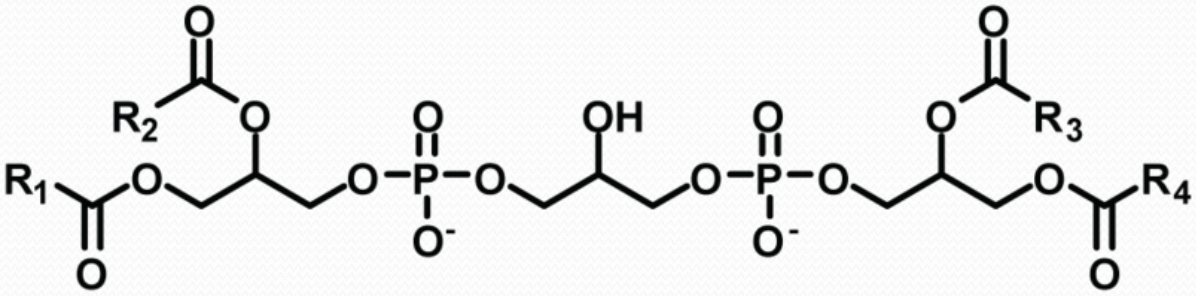


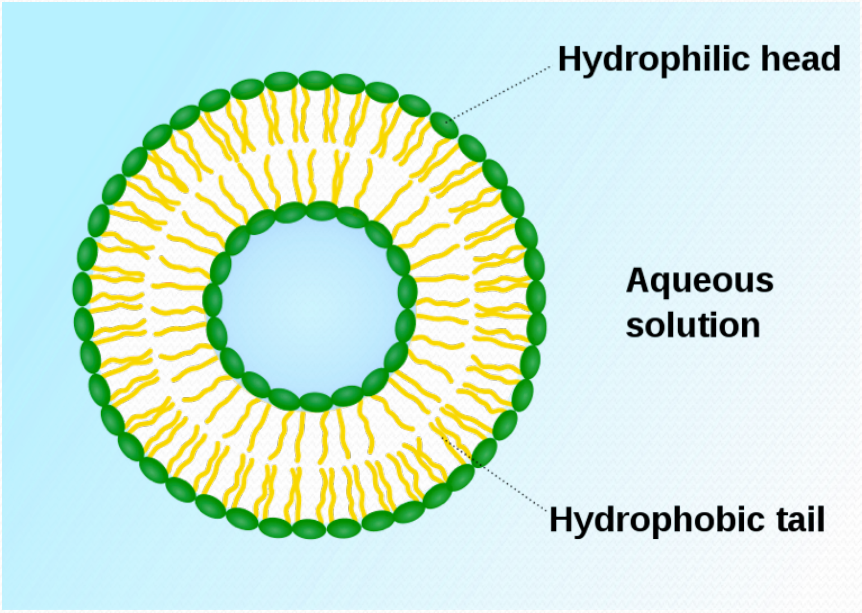


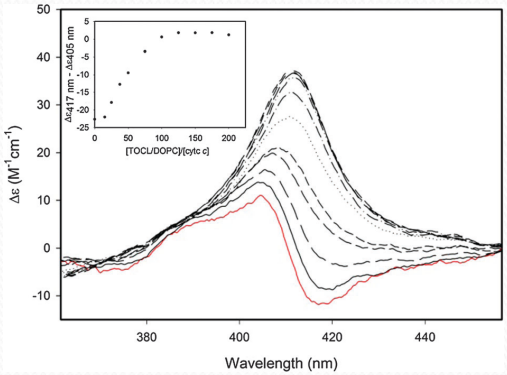
A physiological pH the misligated state B_2(HH) becomes populated, which in solution only appears as kinetic intermediate. At acidic pH, His 33 dissociate and a peroxidase like pentacoordinated state with a quantum mixed spin state is produced.

This state is also populated on liposomes if the protein density is high

Cytochrome c binding to cardiolipin containing liposomes

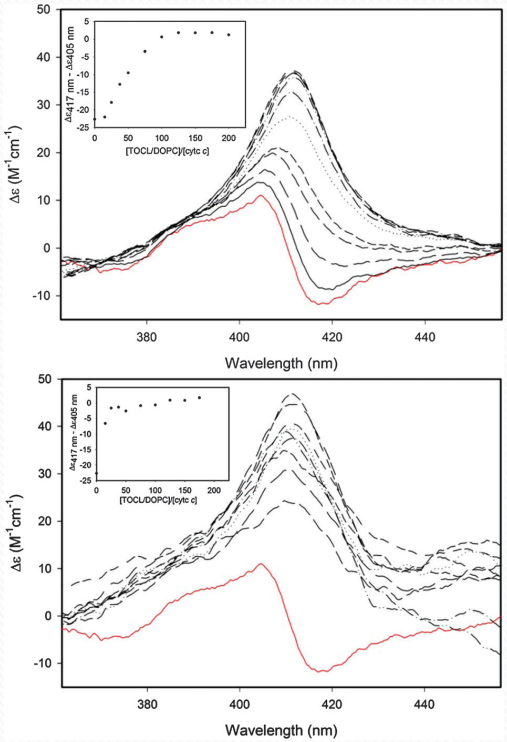






Binding of cytochrome to CL/DOPC liposomes change the tertiary structure in the heme pocket

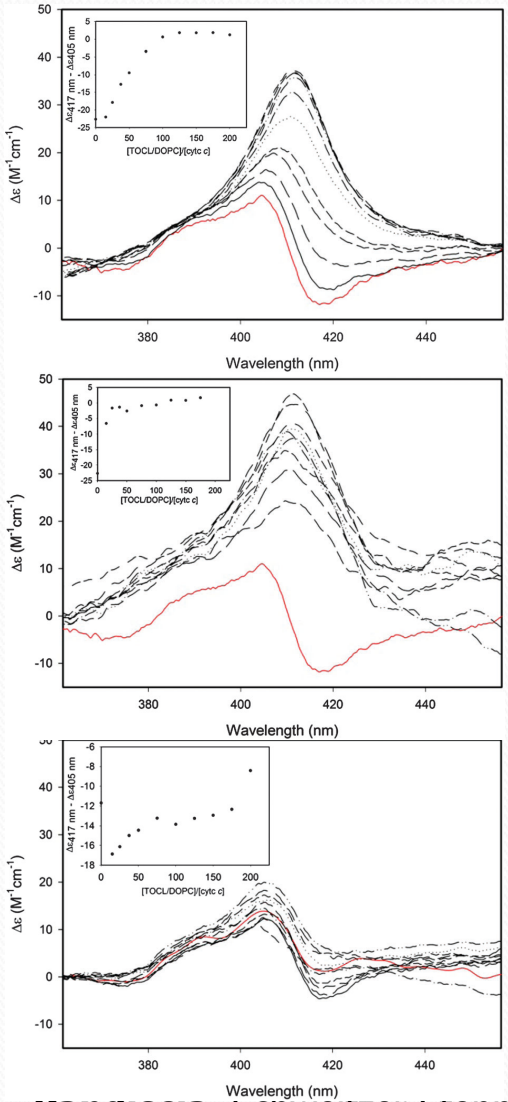
Pandiscia, Schweitzer-Stenner, Chem. Comm, in press, 2014



Binding of cytochrome to CL/DOPC liposomes change the tertiary structure in the heme pocket

Cytochrome c in the supernatant: structural change not reversible

Pandiscia, Schweitzer-Stenner, Chem. Comm, in press, 2014



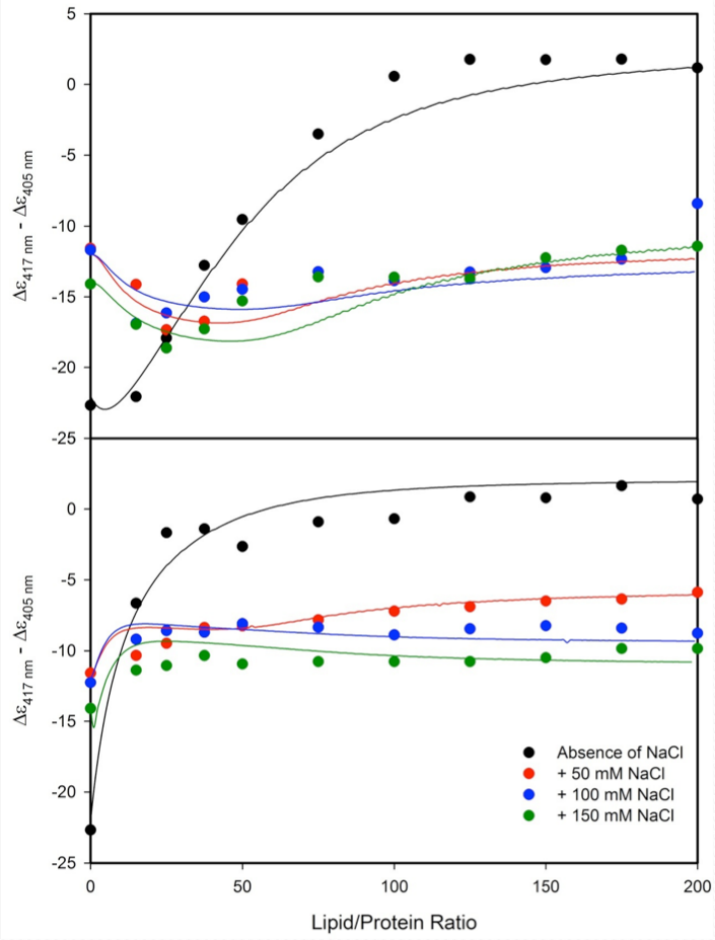
Binding of cytochrome to CL/DOPC liposomes change the tertiary structure in the heme pocket

Cytochrome c in the supernatant: structural change not reversible

In the presence of 100 mM NaCl, the structure becomes native again.

Pandiscia, Schweitzer-Stenner, Chem. Comm, in press, 2014

Currently, we are analyzing the different isotherms for cytochrome binding to CL/ DOPC liposomes

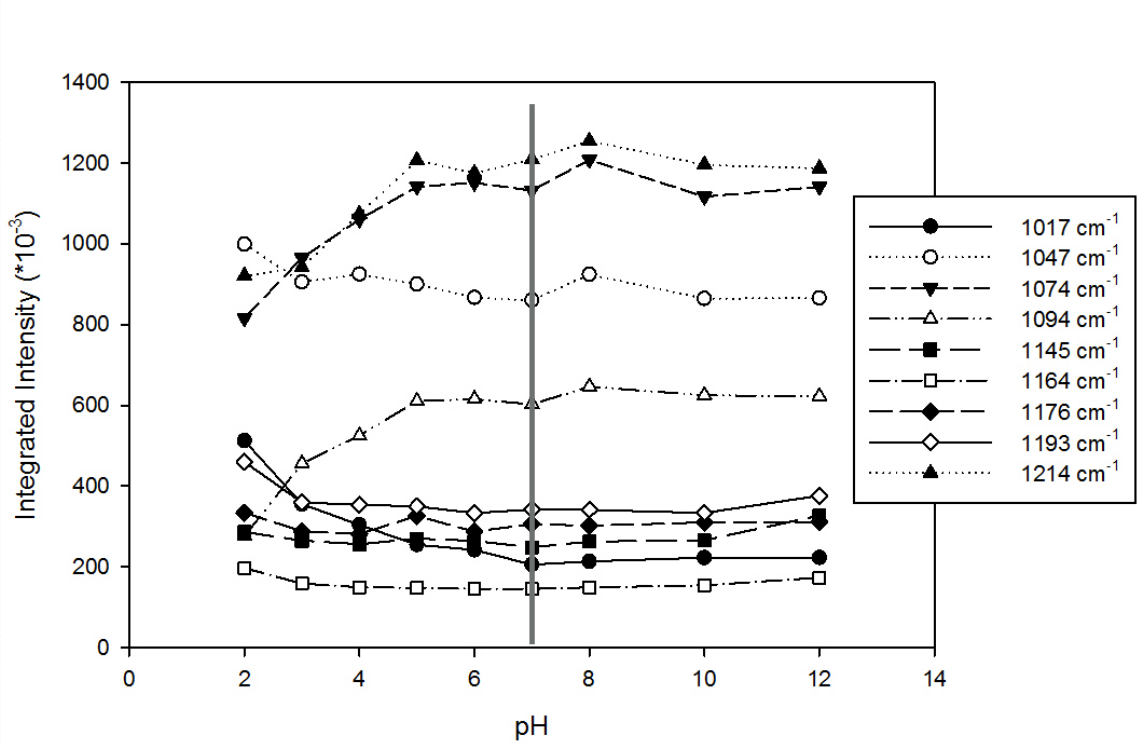


Cytochrome c + liposomes at different salt concentrations probed by visible CD

Cytochrome c in the supernatant at different salt concentrations probed by visible CD

Pandiscia, Schweitzer-Stenner, in preparation.

Are there two protonation states of two phosphate groups coexisting?



An IR study of CL liposomes suggests that this is not the case.

Malyshka, Pandiscia, Schweitzer-Stenner, manuscript in prepapration

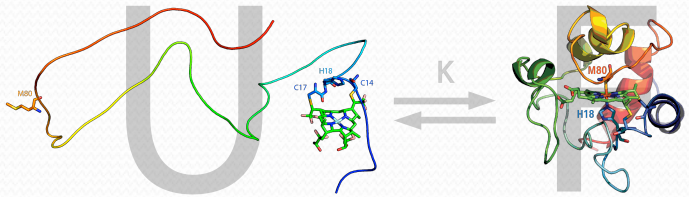
We identified three different modes of cytochrome c binding to CL-containing liposomes. Two of them lead to major structural change

Currently, we are analyzing the different isotherms for cytochrome binding to CL/ DOPC liposomes

Malyshka, Pandiscia, Schweitzer-Stenner, in preparation

differ in the degree of unfolding

not due to coexisting protonation state of the two phosphate groups



Future directions:

Characterizing the 'new' misfolded state in more detail. Does it have peroxidase activity?

Identifying and analyzing different cytochrome c conformations on liposomes, testing their function

Leah Pandiscia, Dzmitry Malyshka, Lee Serpas (returns for the 2014 fall term)

www.schweitzer-stenner.com