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ESC1:
Circular Dichroism:
best practice and data analysis

Lecture 5: The difficult measurement. What are the limits and how to push them?

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What is a difficult sample…

I found a spider in the table one midsummer morning…

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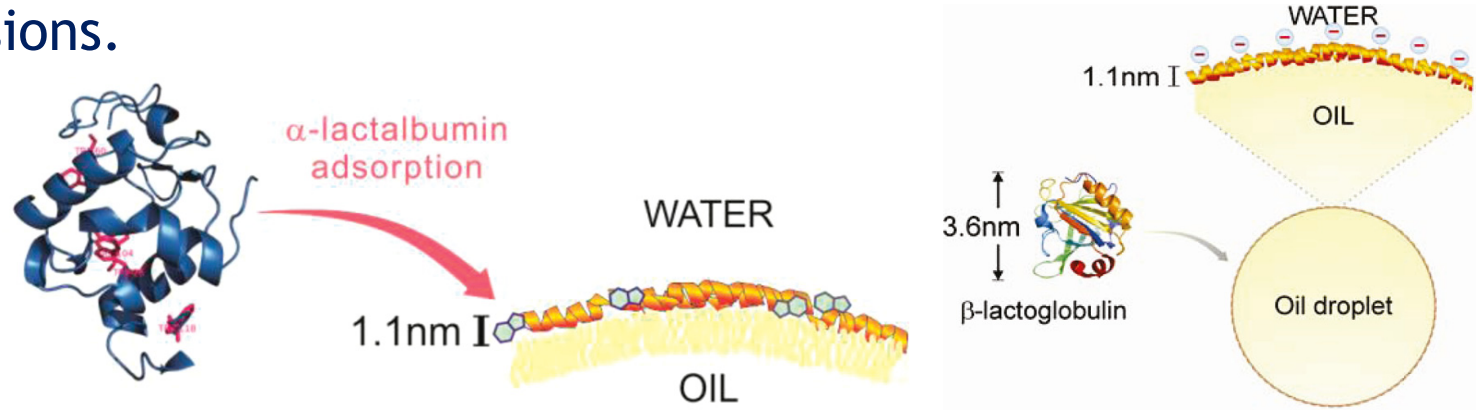
Oil-water interfaces

- The structure of proteins adsorbed at oil/water interfaces of emulsions.



- Major factor controlling the colloidal stability and quality of emulsion-based products

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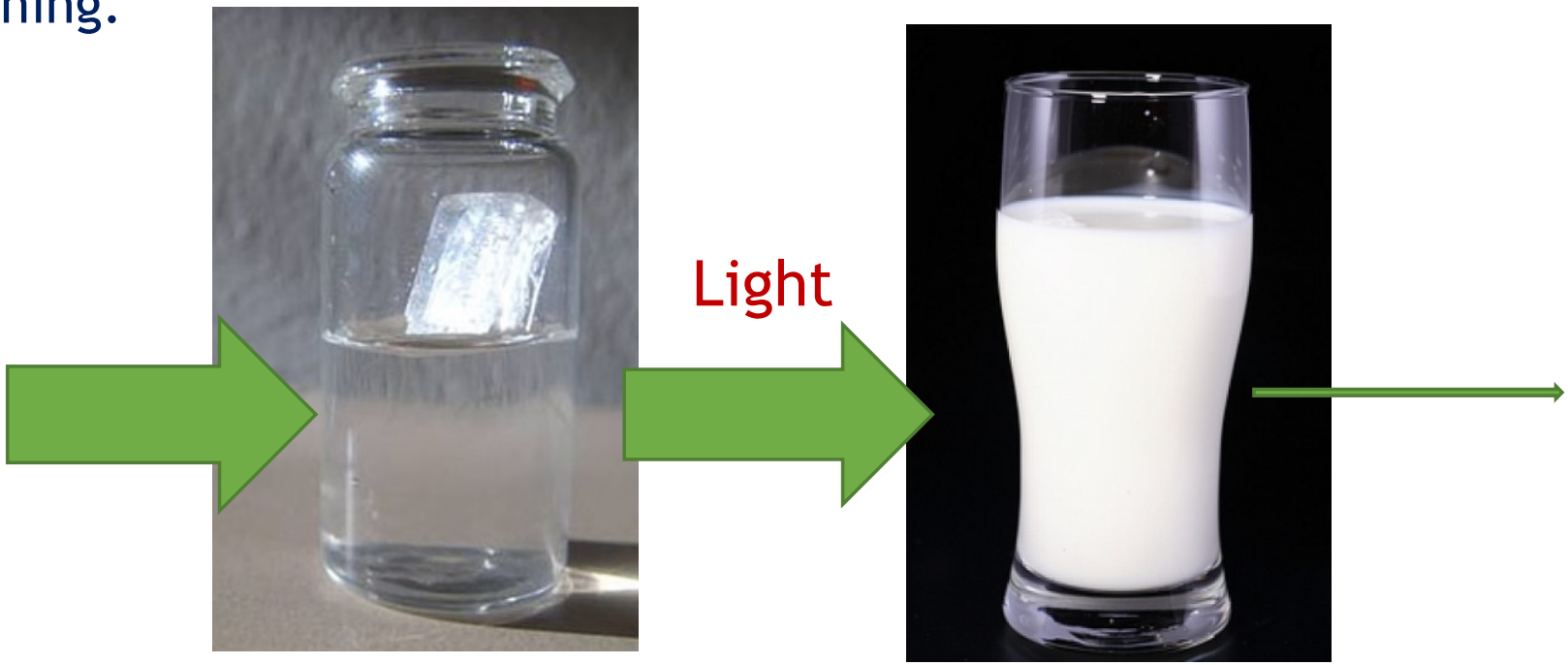
Oil-water interfaces

- Measurement directly on the emulsion without Refractive Index matching.



- Refractive index matching will transmit visible light, but absorbs a lot in the far UV

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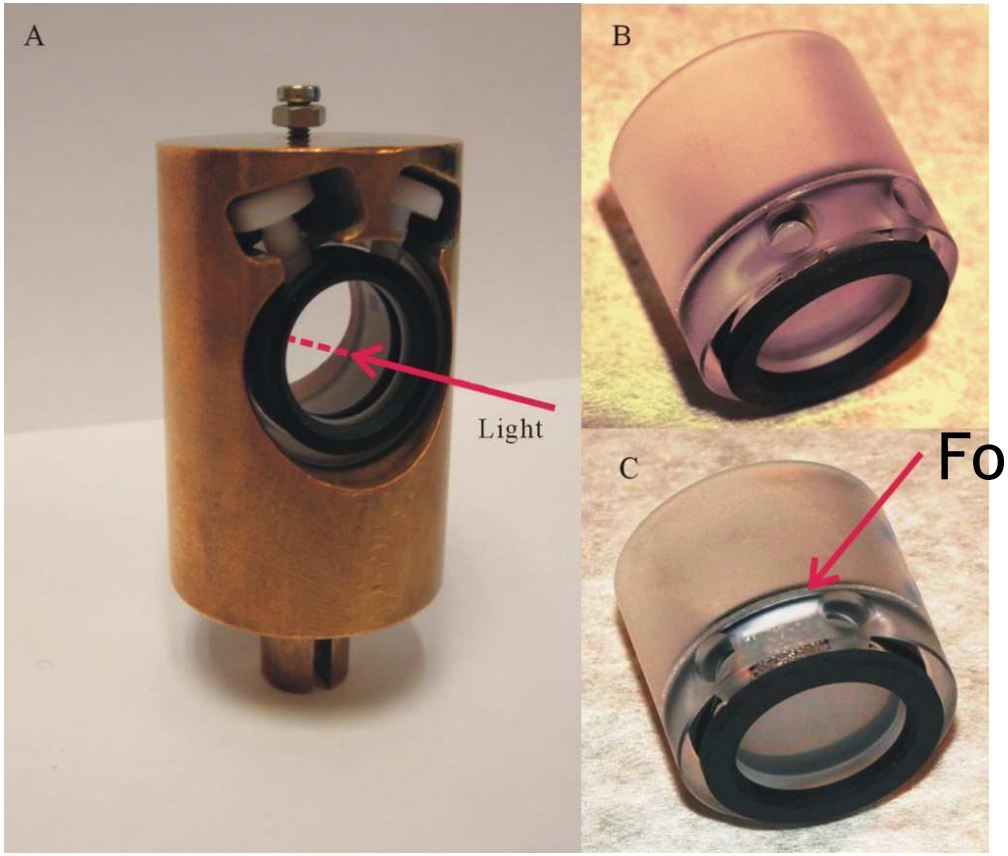

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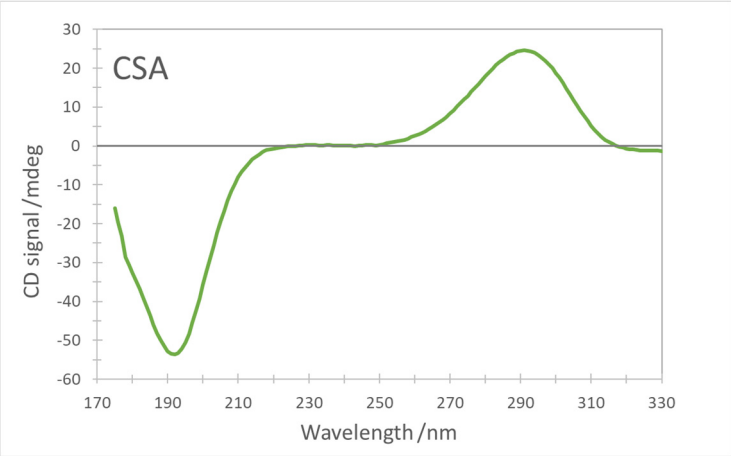
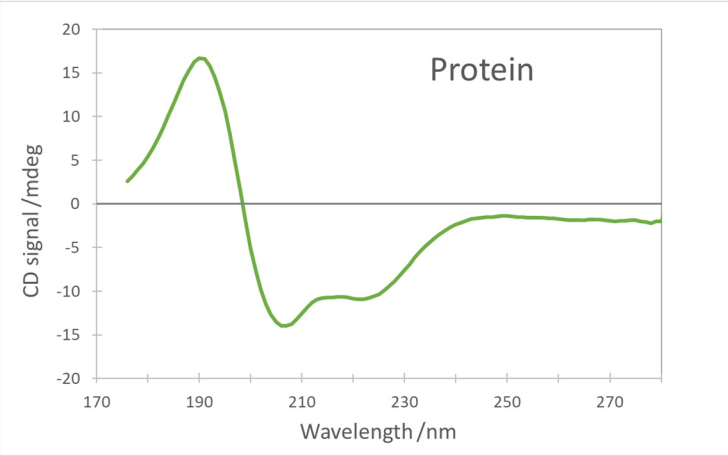
Oil-water interfaces – baseline match

Normal non-scattering samples. *What is a baseline?*





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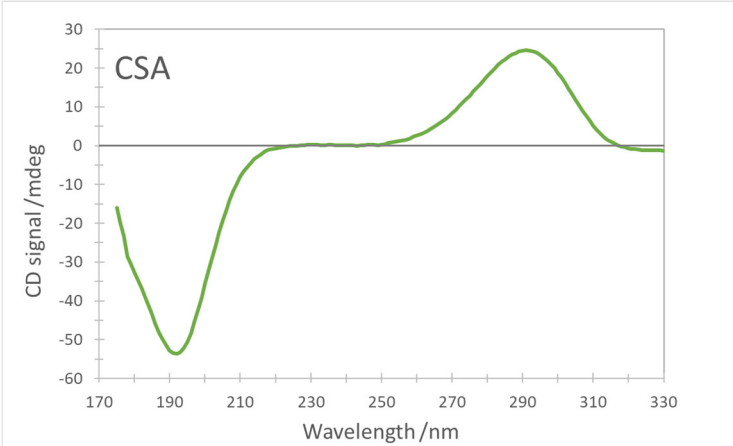

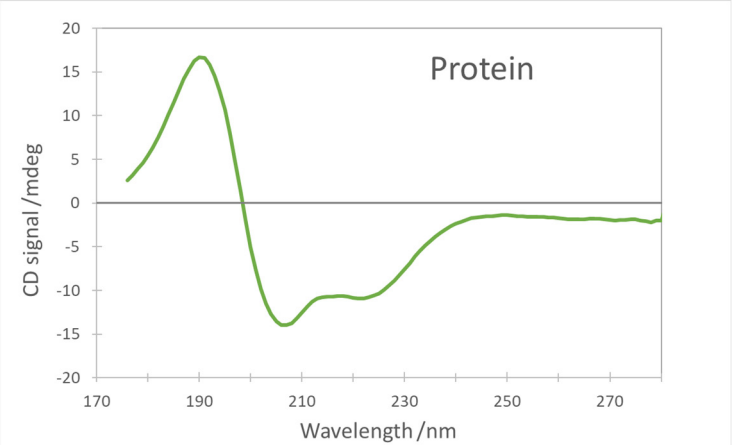

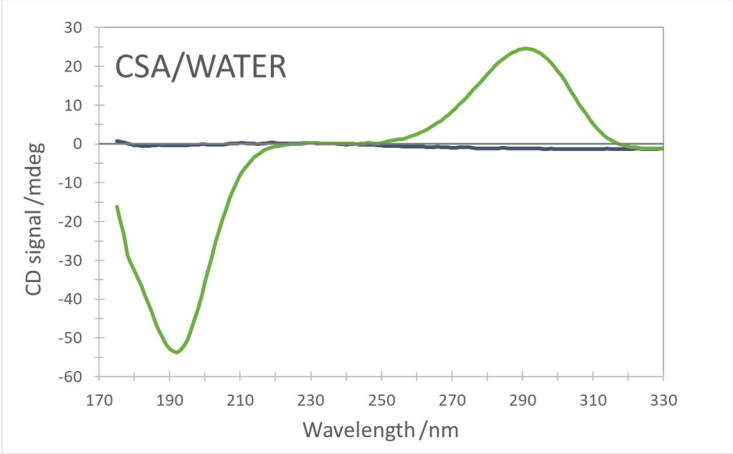

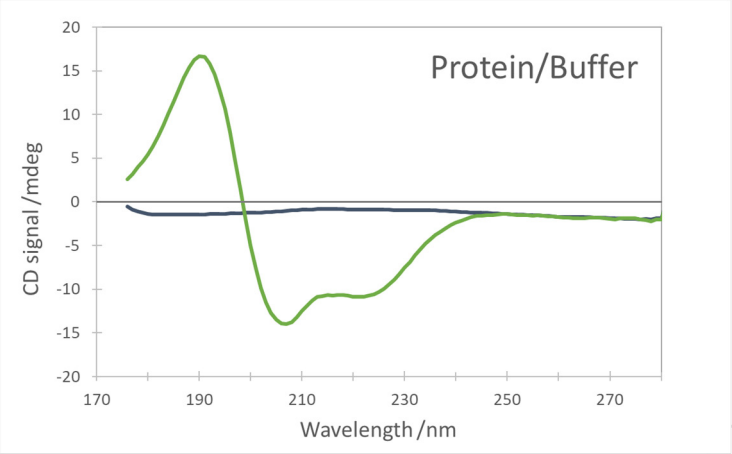

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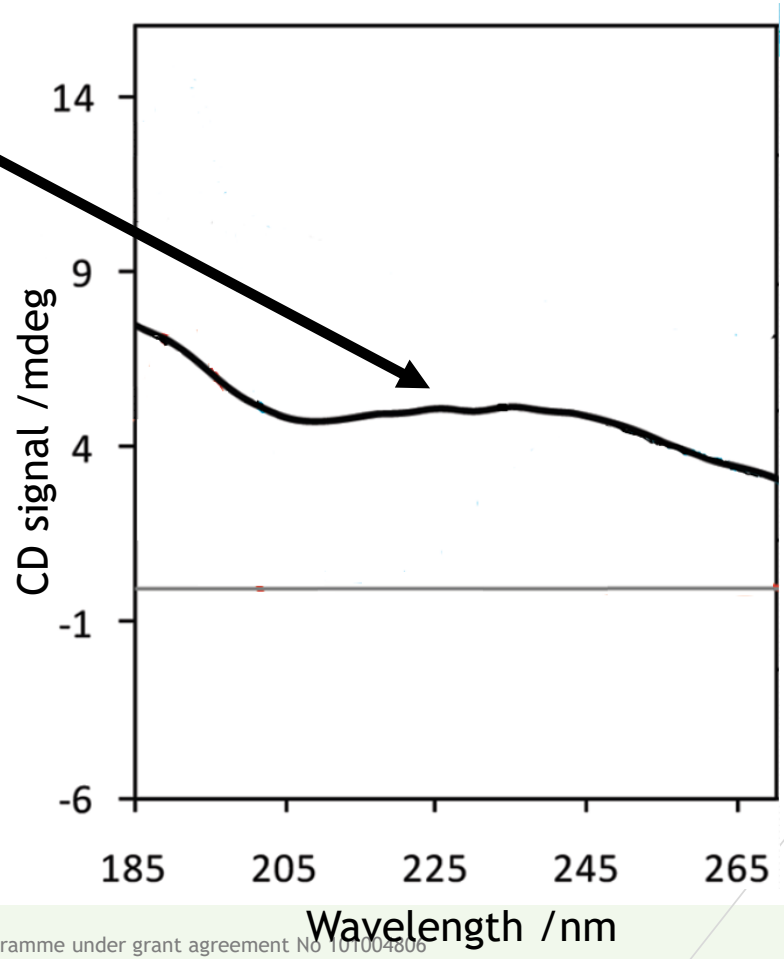
Oil-water interfaces – baseline match

Emulsion is stabilized by the protein:
What is the correct baseline?

Stabilize the emulsion by SDS
(Scattering baseline)



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Oil-water interfaces – baseline match

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Stabilize the emulsion by SDS
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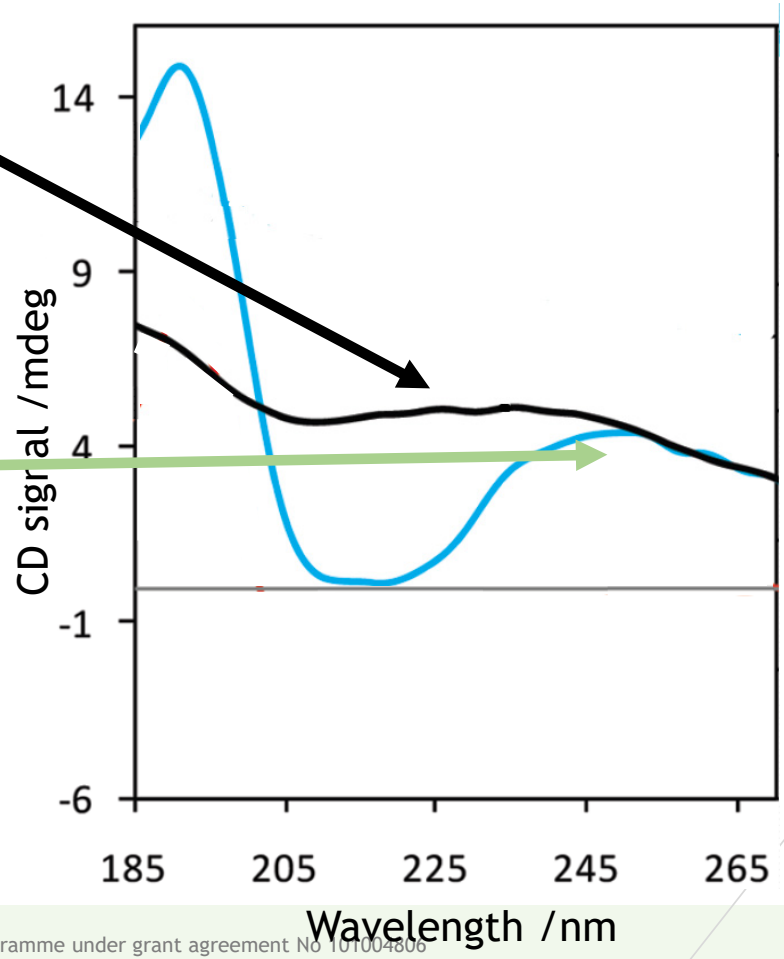
Nice baseline fit with
protein/emulsion spectrum



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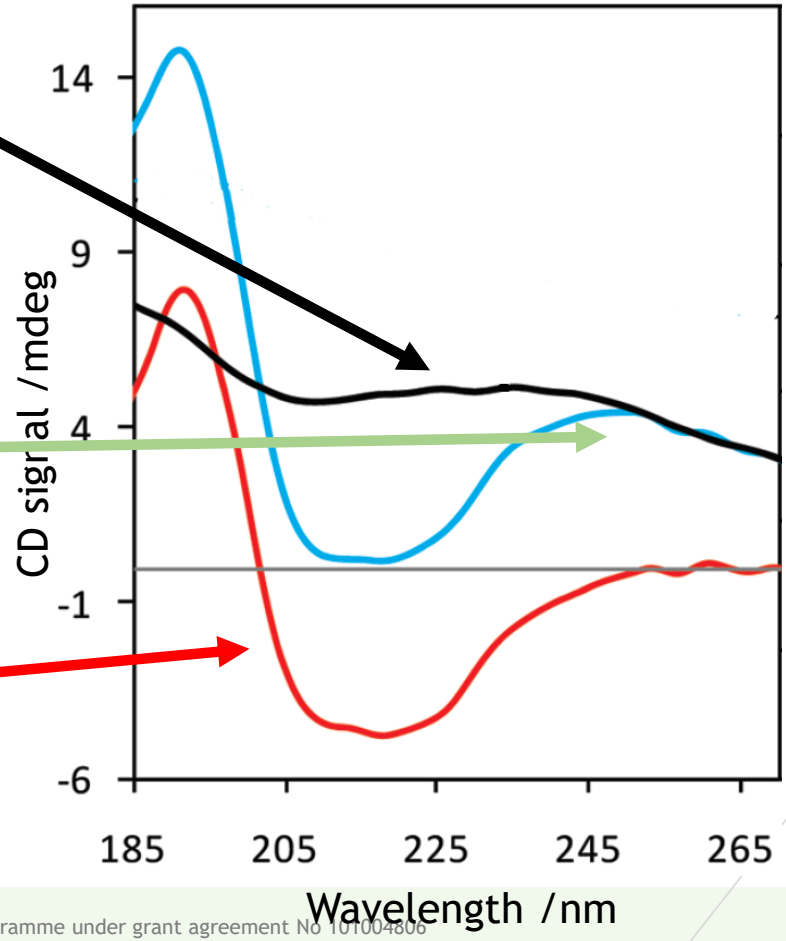
Oil-water interfaces – baseline match

Emulsion is stabilized by the protein:
What is the correct baseline?

Stabilize the emulsion by SDS
(Scattering baseline)

Nice baseline fit with
protein/emulsion spectrum

Final baseline subtracted
CD spectrum



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Oil-water interfaces

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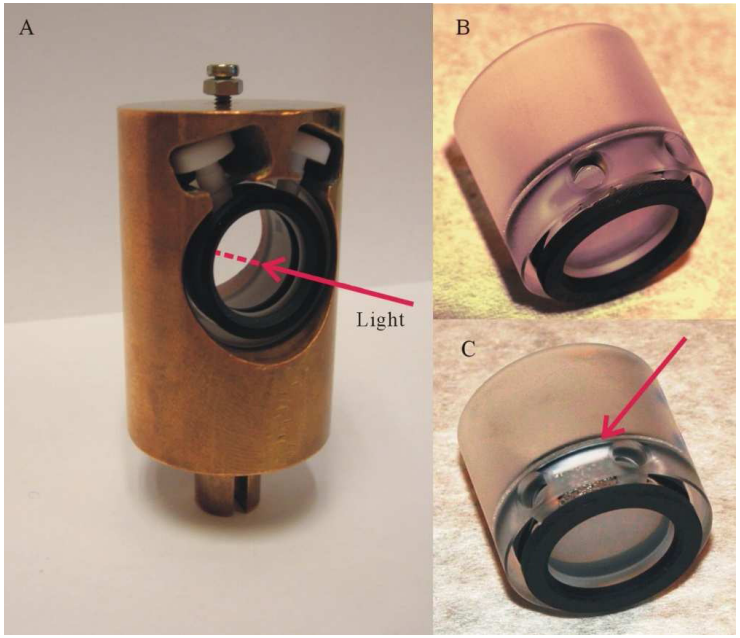

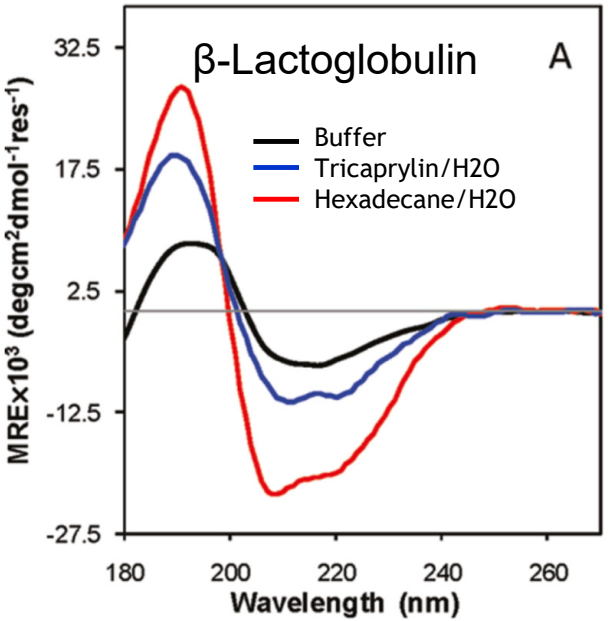

Zhai *et. al*. Langmuir 2011, 27, 9227–9236;
Day *et. al*. Food Hydrocolloids 34 (2014) 78e87;
Zhai *et. al*. Langmuir 2012, 28, 2357–2367;
Zhai *et. al*. *Biomacromolecules* 2010, *11*, 2136–2142

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Oil-water interfaces – absorption match

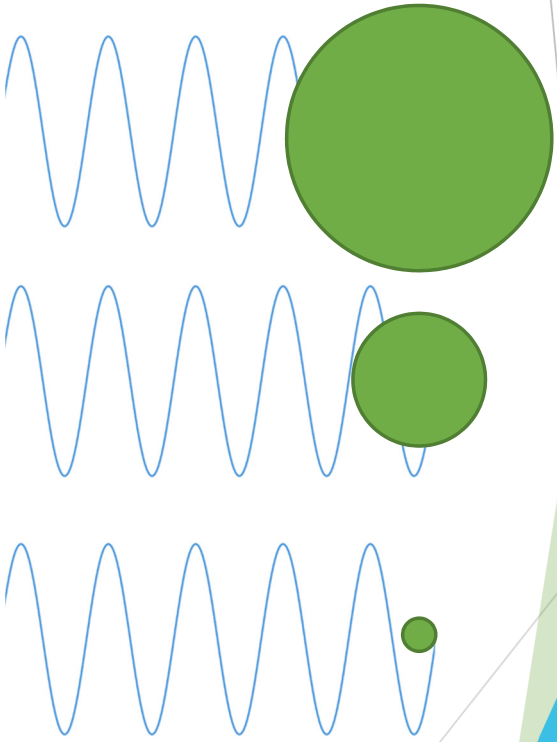
The scattering of light in absorption measurements can be modelled

Scattering depends strongly on the particle size compared to the wavelength

A large particle blocks the light

Size comparable to wavelength gives rise to *Mie scattering*

Size smaller than the wavelength gives rise to *Rayleigh scattering*



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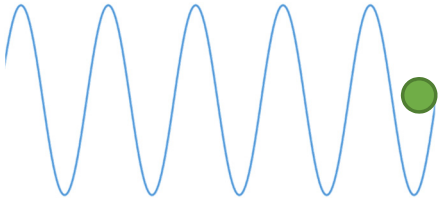

$$I_s = I_0 \times c \times \lambda^{-4}$$

Fun fact: the strong wavelength dependence on the scattering give rise to the blue sky

$$A_{\text{scattering}} = \log_{10}\left(\frac{I_0}{I_0 - I_s}\right) = \log_{10}\left(\frac{1}{1 - c\lambda^{-4}}\right)$$

To model the scattering of your sample it is important to ***measure out to long wavelengths***

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Oil-water interfaces – absorption match

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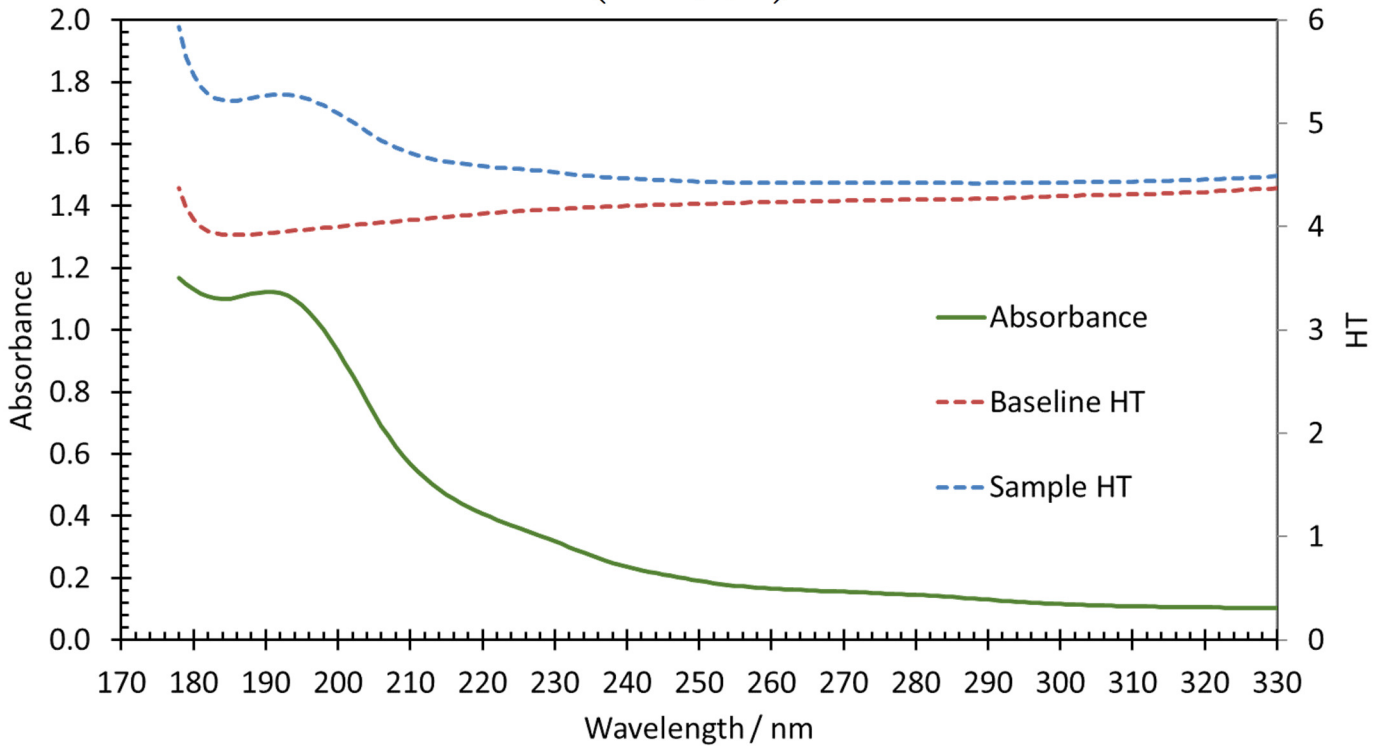

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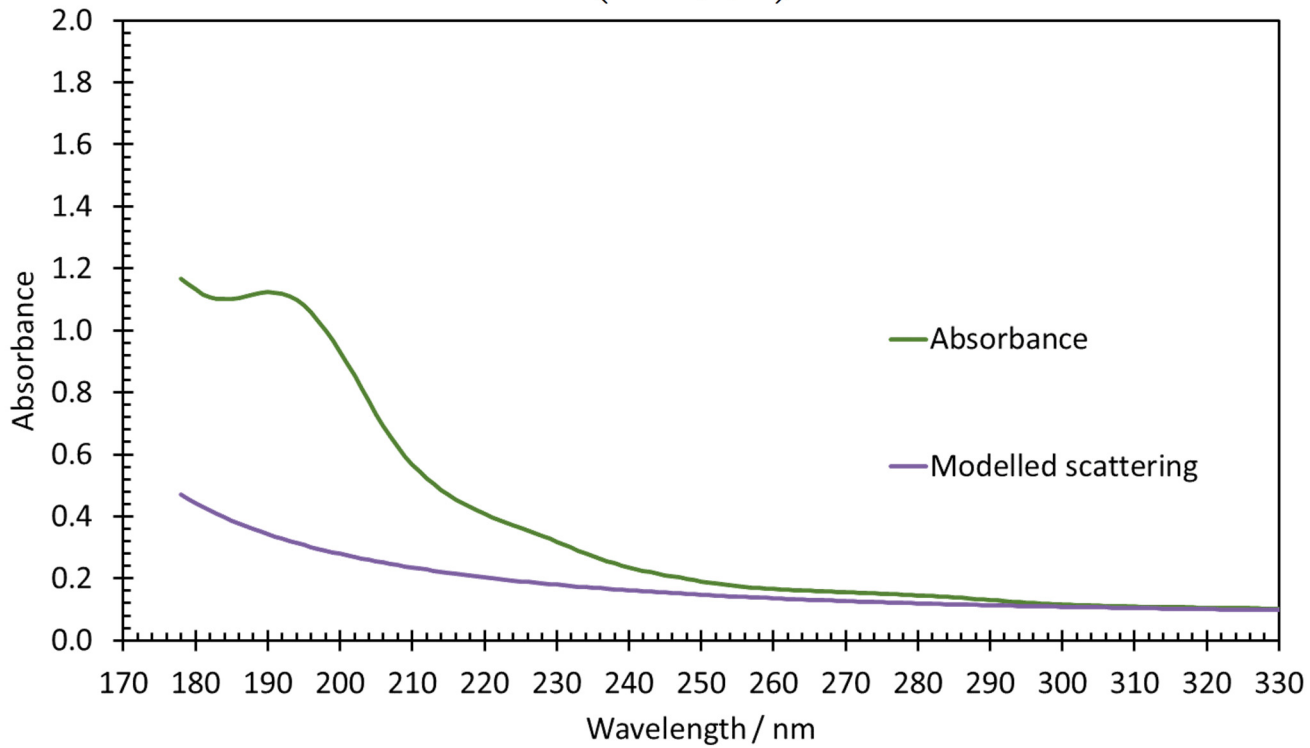

Notice the importance of measuring out to long wavelengths

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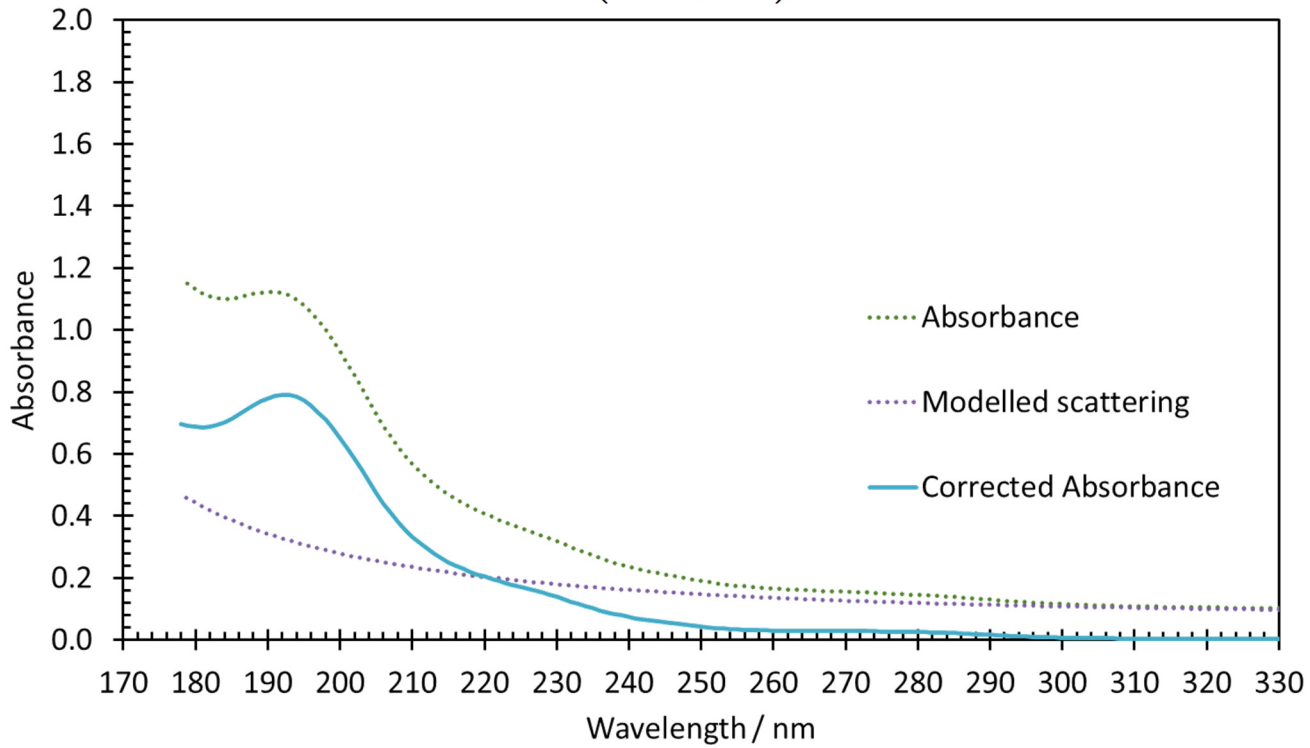

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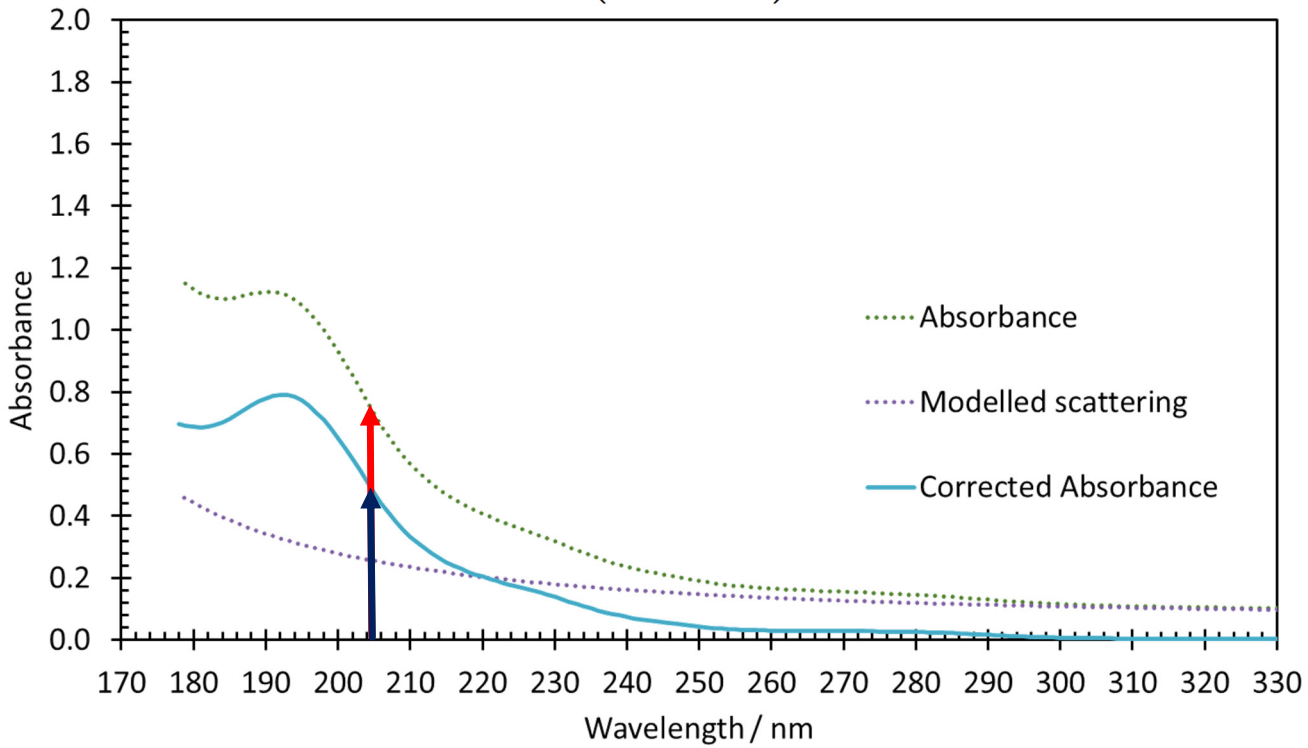

A significant effect on the 205 nm absorption: protein concentration

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Oil-water interfaces – absorption match

The scattering of light in absorption measurements can be modelled

$$A_{\text{scattering}} = \log_{10}\left(\frac{1}{1 - c\lambda^{-4}}\right)$$

Other model in the literature

$$A_{\text{scattering}} = a\lambda^{-k}$$

B. Nordén, A. Rodger and T. Dafforn. Linear Dichroism and Circular Dichroism. A Textbook on Polarized-Light Spectroscopy, 2010, Royal Society of Chemistry, Cambridge

A.J. Miles and B.A. Wallace. Chem. Soc. Rev., 45, 2016, 4859-4872

- The parameter *k* may be varied to get a better fit (Nordén k=3.5)
- Be very careful using this model as it changes the correction at low WL a lot
- In reality this model is the same as Rayleigh for $c\lambda^{-4}$ << 1, NOT true for low WL

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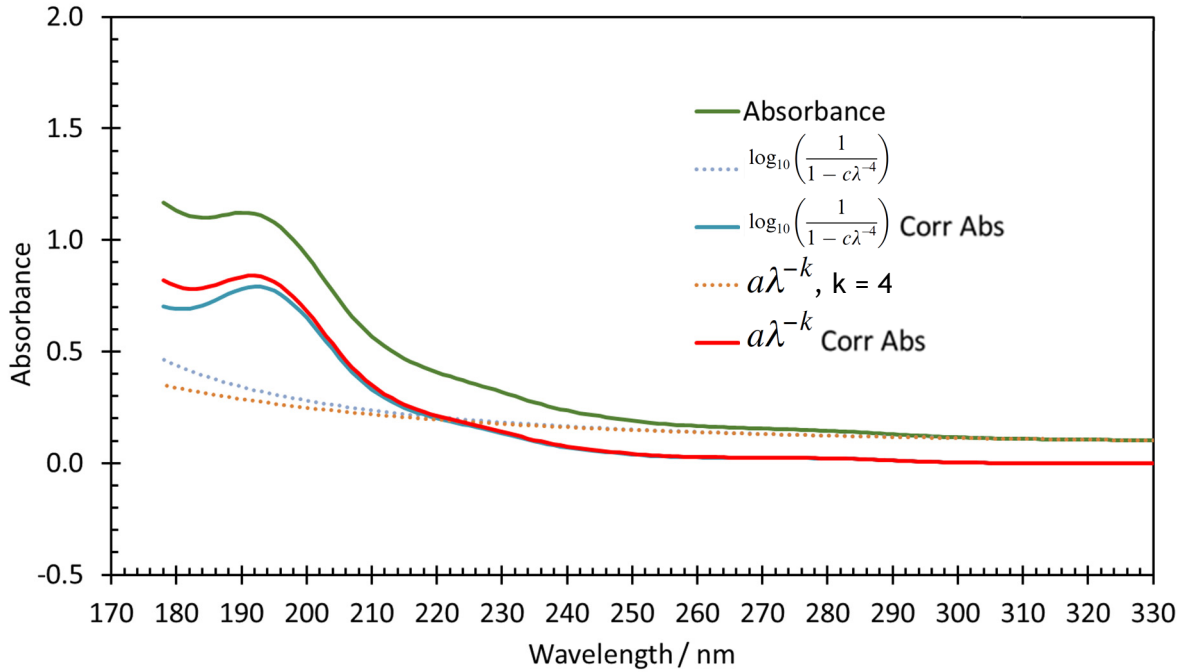
Oil-water interfaces – absorption match

The scattering of light in absorption measurements can be modelled

$A_{\text{scattering}} = a\lambda^{-k}$ vs. $A_{\text{scattering}} = \log_{10}\left(\frac{1}{1 - c\lambda^{-4}}\right)$



Fit to 330-310 data: same curve above 230 nm, but not below

Absorbance difference at 205 nm is about 5%

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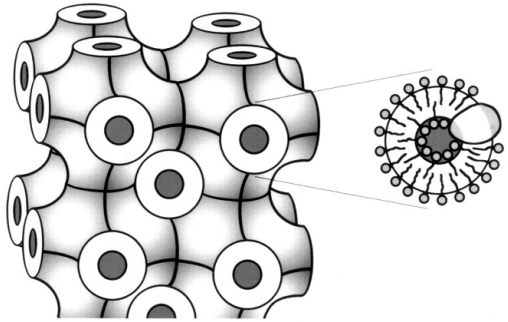
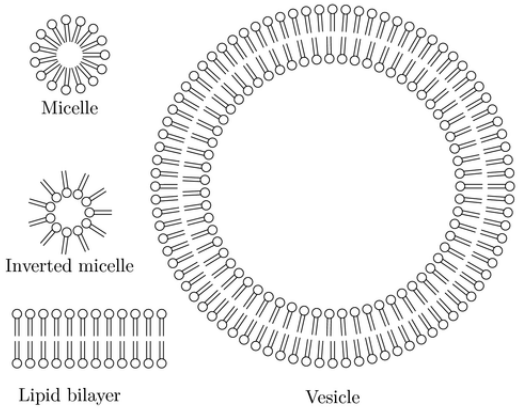
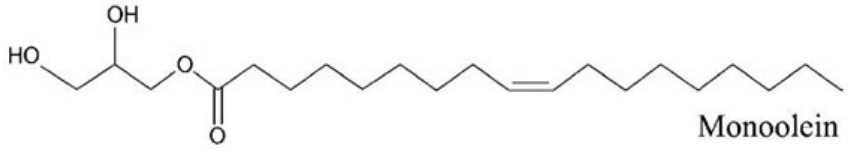
CD spectra of challenging samples

Cubic phase lipids with peptides

Particle size
comparable to
UV wavelength







Monoolein

Looks
like
milk

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CD spectra of challenging samples

Cubic phase lipids with peptides

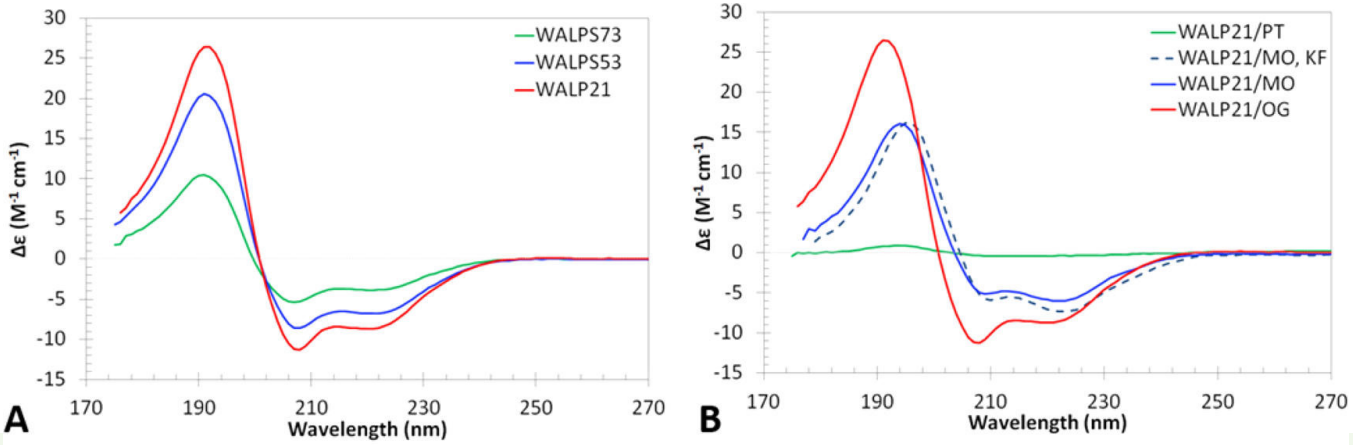
LANGMUIR Article
pubs.acs.org/Langmuir

How Peptide Molecular Structure and Charge Influence the Nanostructure of Lipid Bicontinuous Cubic Mesophases: Model Synthetic WALP Peptides Provide Insights

Leonie van 't Hag,[†,‡,⊥] Xu Li,[†,‡] Thomas G. Meikle,[‡,⊥,§] Søren V. Hoffmann,[#] Nykola C. Jones,[#] Jan Skov Pedersen,[▽] Adrian M. Hawley,[○] Sally L. Gras,[†,‡,∥] Charlotte E. Conn,[*,^] and Calum J. Drummond[*,⊥,^]

DOI: 10.1021/acs.langmuir.6b01058
Langmuir 2016, 32, 6882−6894

These samples can only be measured on our SRCD facility.



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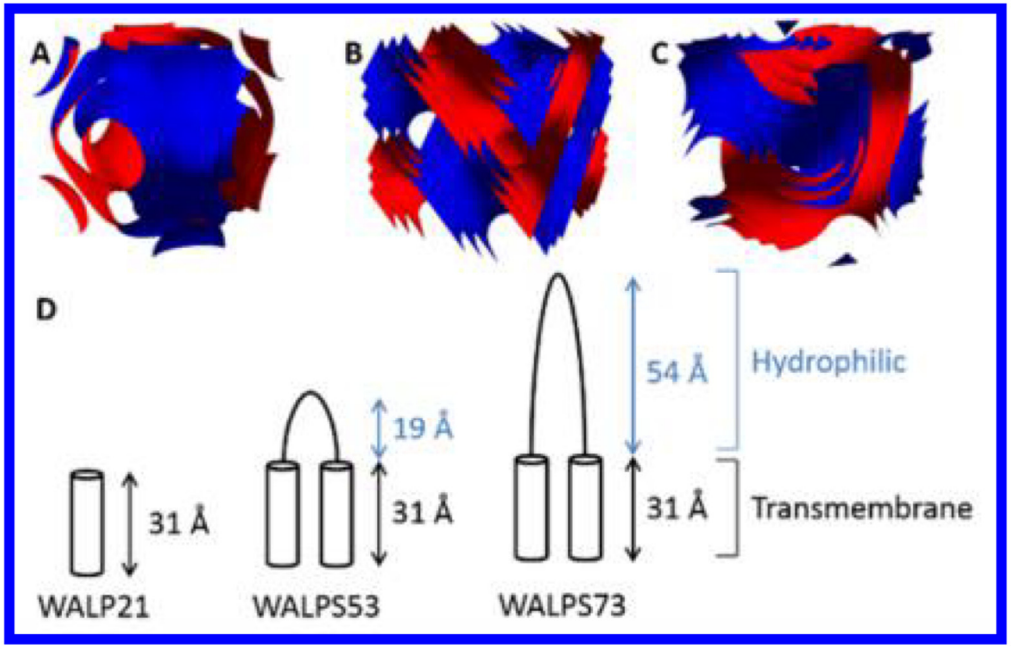

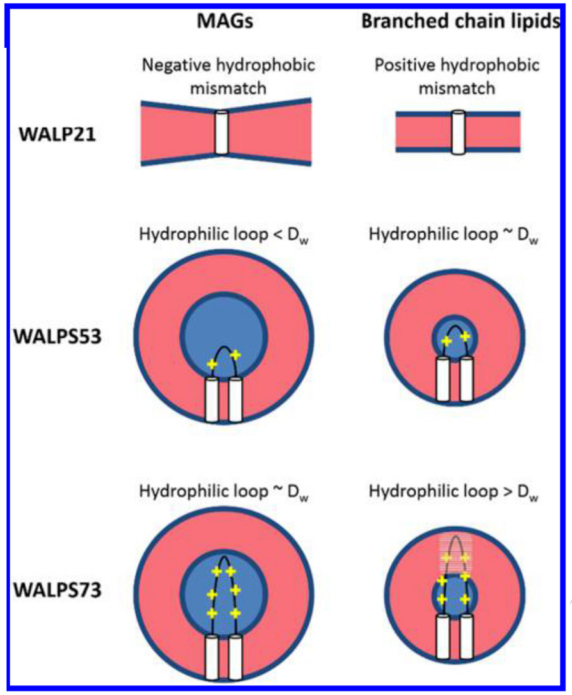

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CD spectra of challenging samples

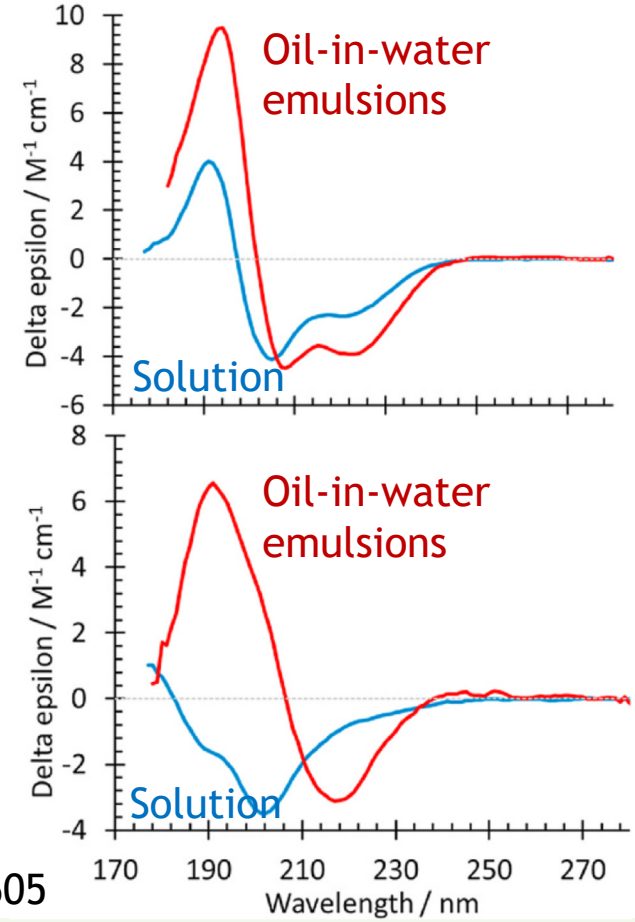
Potato peptides stability of fish oil-in-water emulsions

Some potato peptides adopt predominantly **α-helical conformation**

- leading to **poor** inter-peptides interactions as well as weak and stretchable interfaces

Other potato peptides adopt a highly **ß-strand structure**

- significantly **higher degree** of interfacial inter-peptide interaction, resulting in stiff and solid-like interfaces



P.J. García-Moreno *et al.* Food Hydrocolloids 115 (2021) 106605

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CD spectra of challenging samples

- In the *hands-on* today, you will try to measure a highly scattering sample – milk!
- You will see the effect of scattering and how to identify it

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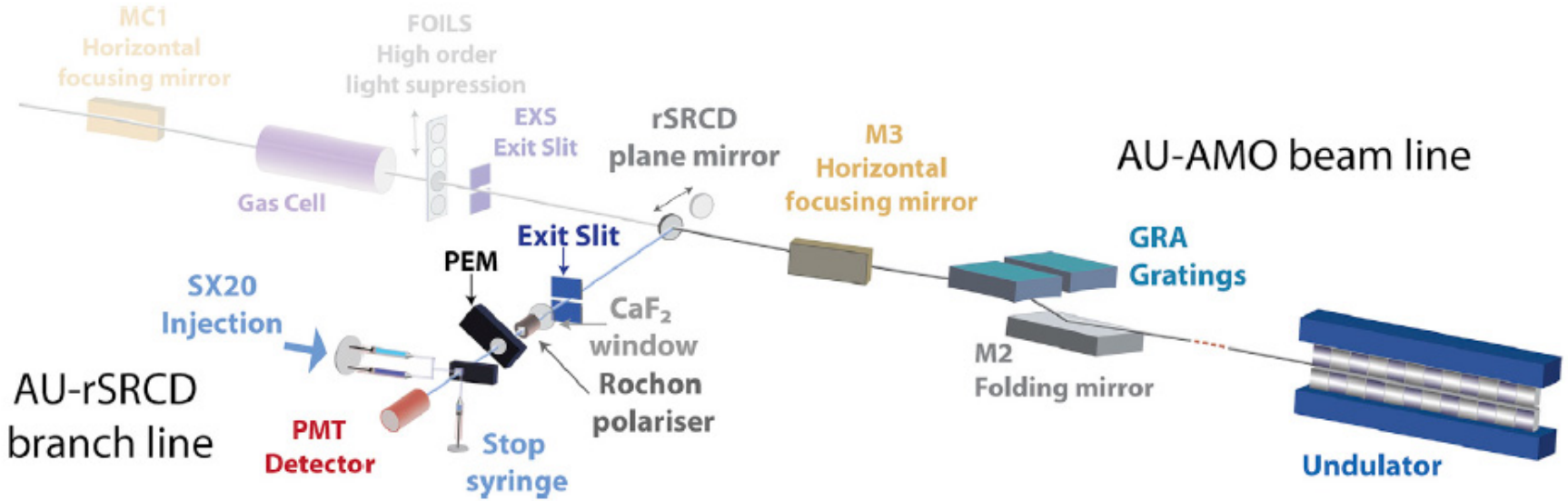
rSRCD/AMO beam line on ASTRID2

Stopped flow using the intense AMOLine



Two liquid samples are mixed, and the reaction is followed on *ms* scale

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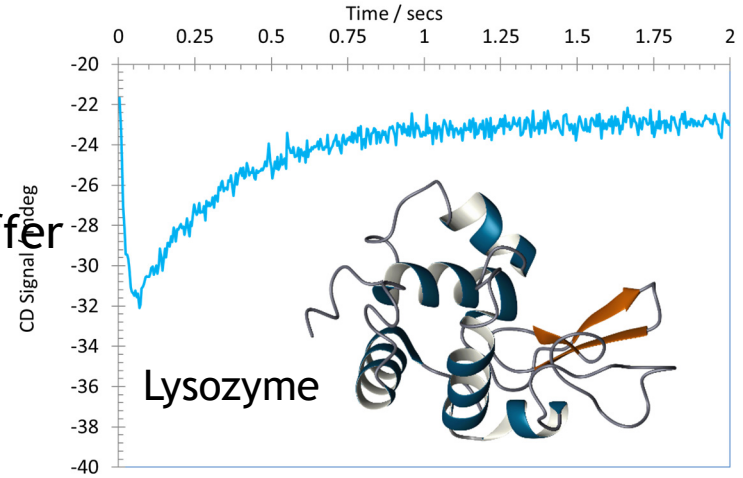
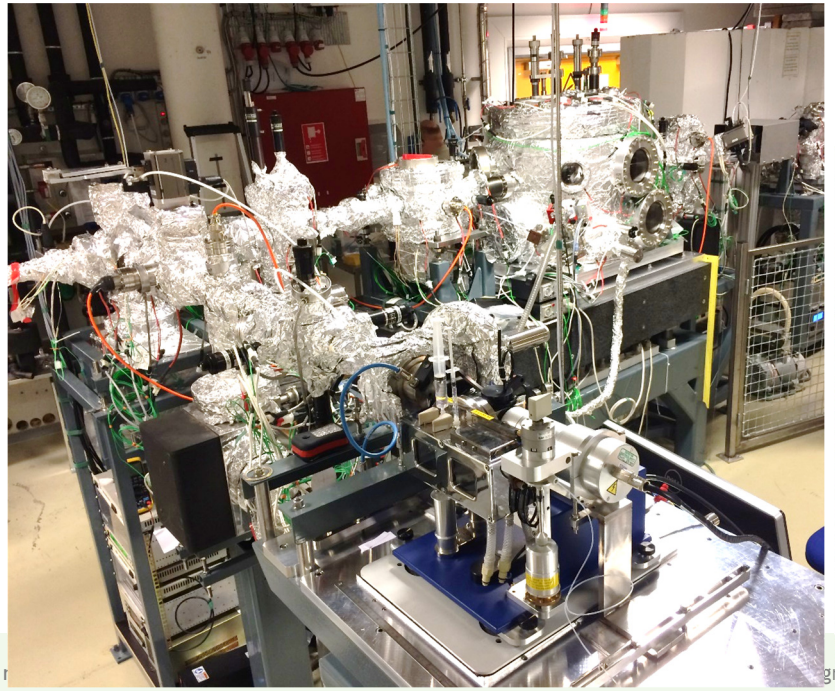
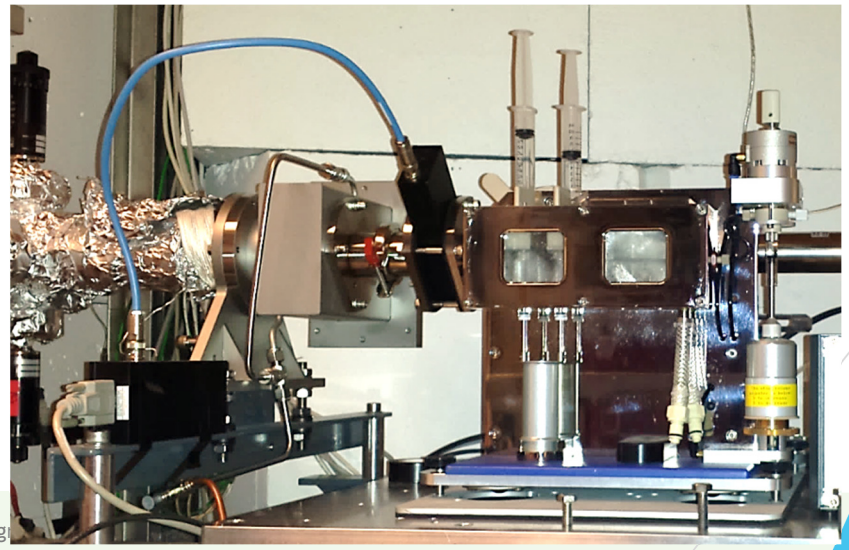
Rapid SRCD (rSRCD)/Stopped flow

Lysozyme test:

Re-folding from 6M Guanidine-HCl
after *10-fold* dilution into phosphate buffer







This project has r

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Stopped flow

Short Aß fragment (Alzheimer's) peptide folding:

- Fast self-assembling molecule
- Reaching macroscopic (i.e., mm) size in ***seconds***

ACS NANO

Cite This: *ACS Nano* 2018, 12, 5408−5416

www.acsnano.org

ARTICLE

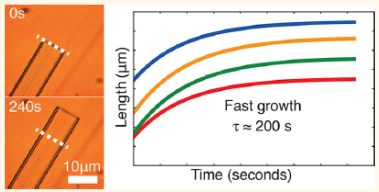
Rapid Growth of Acetylated Aβ(16−20) into Macroscopic Crystals

Christian Bortolini,[†,‡] Lasse Hyldgaard Klausen,[†,§] Søren Vrønning Hoffmann,[∥] Nykola C. Jones,[∥] Daniela Saadeh,[⊥] Zegao Wang,[†] Tuomas P. J. Knowles,[‡] and Mingdong Dong*[,†,§]

[†]Interdisciplinary Nanoscience Center, Aarhus University, Aarhus 8000, Denmark
[‡]Department of Chemistry, University of Cambridge, Cambridge CB2 1TN, U.K.
[§]Department of Chemistry, Stanford University, Stanford, California 94305, United States
[∥]ISA, Department of Physics and Astronomy, Aarhus University, Aarhus 8000, Denmark
[⊥]Centre for Astronomy & Particle Theory, University of Nottingham, Nottingham NG7 2RD, U.K.

Supporting Information

ABSTRACT: Aberrant assembly of the amyloid-β (Aβ) is responsible for the development of Alzheimer's disease, but can also be exploited to obtain highly functional biomaterials. The short Aβ fragment, KLVFF ($Aβ_{16-20}$), is crucial for Aβ assembly and considered to be an Aβ aggregation inhibitor. Here, we show that acetylation of KLVFF turns it into an extremely fast self-assembling molecule, reaching macroscopic (*i.e.*, mm) size in seconds. We show that KLVFF is metastable and that the self-assembly can be directed toward a crystalline or fibrillar phase simply through chemical modification, *via* acetylation or amidation of the peptide. Amidated KLVFF can form amyloid fibrils; we observed folding events of such fibrils occurring in as little as 60 ms. The ability of single KLVFF



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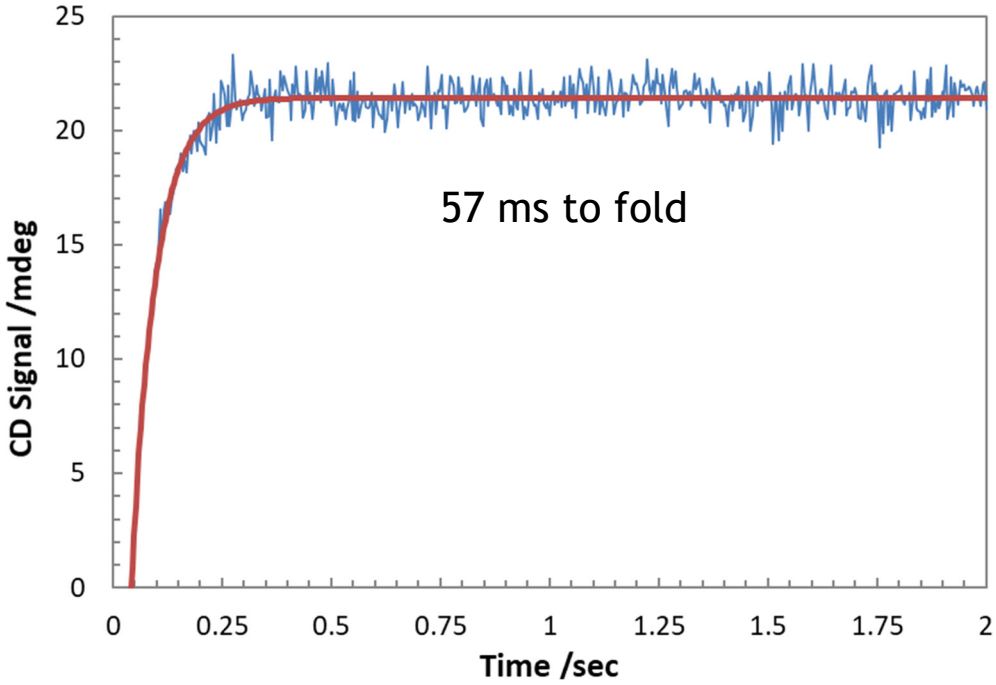
Stopped flow

Short Aß fragment (Alzheimer's) peptide folding:

- Fast self-assembling molecule
- Reaching macroscopic (i.e., mm) size in ***seconds***



Initial folding is extremely fast.

Measured on peptide in highly absorbing buffer at ***190 nm***

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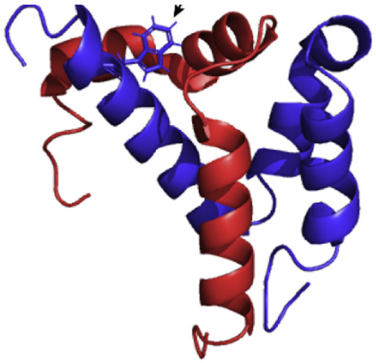
Stopped flow

IDP gain structure upon binding to their target proteins

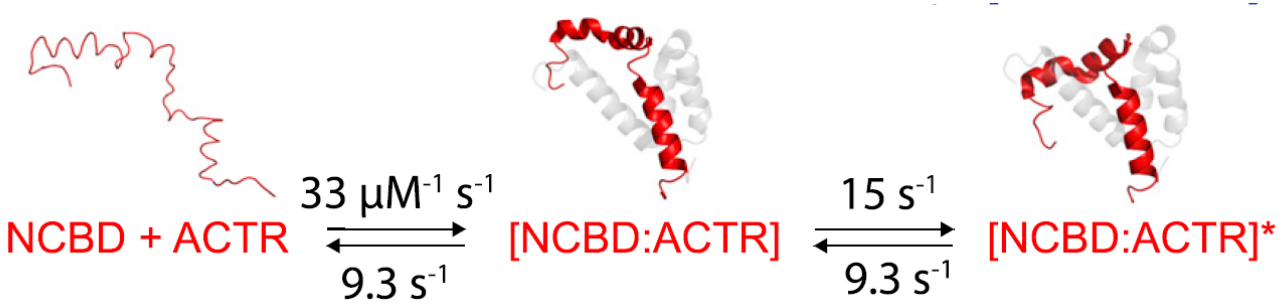
SRCD stopped flow shows that *almost all helicity is formed upon initial association of the proteins*

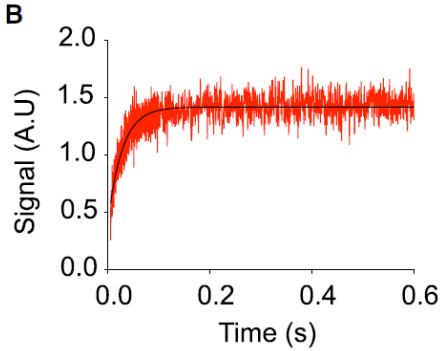


Final product of NCBD (blue) in complex with ACTR(red)

How is it formed?

Follow the fast folding of a protein to a structure which allows binding and model it.





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