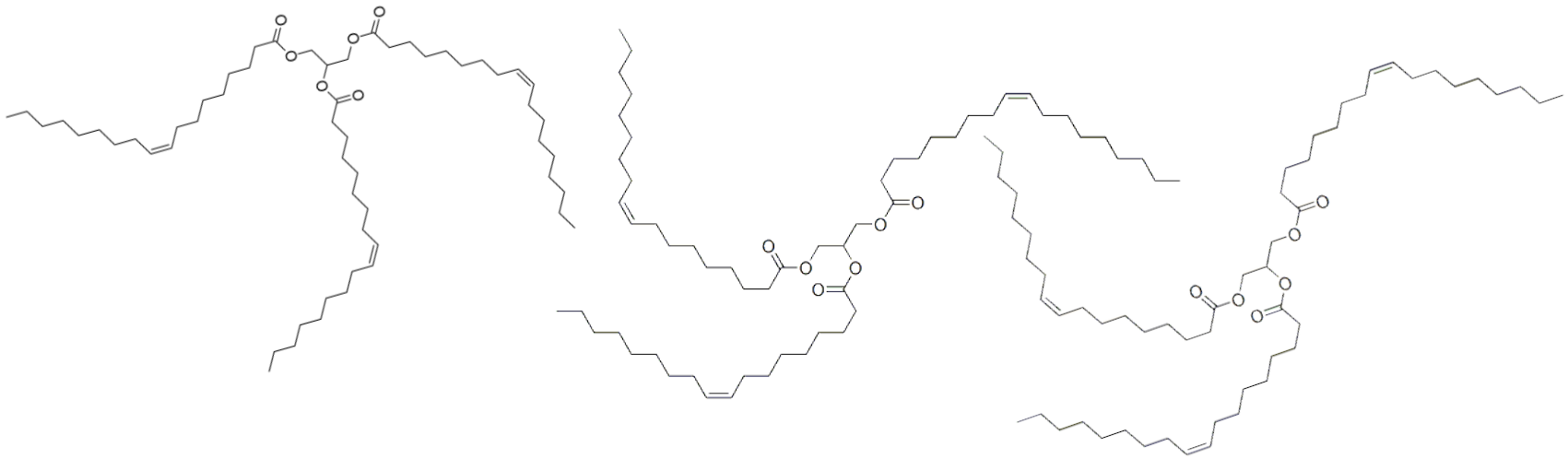
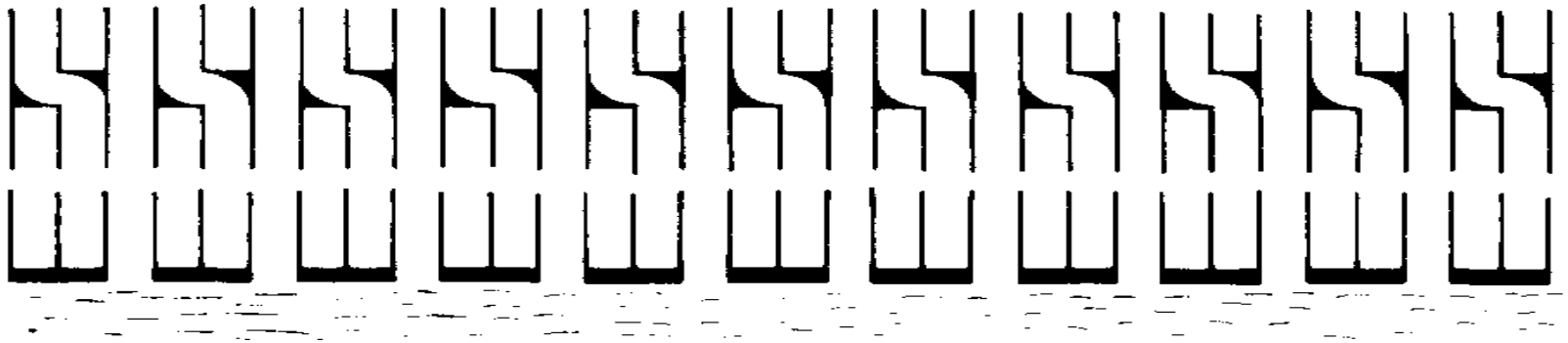
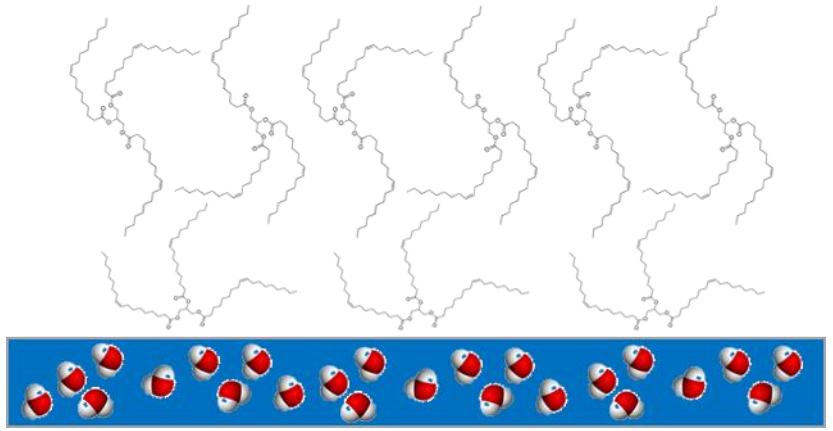
Introducing the substrate



Triolein: a symmetrical triglyceride derived from glycerol and three units of the unsaturated fatty acid, oleic acid. Triolein represents 4–30% of olive oil

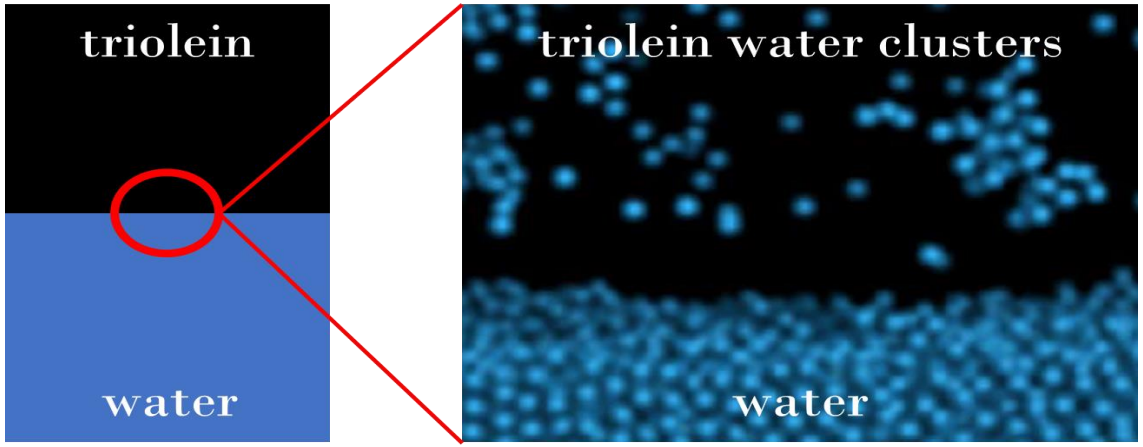




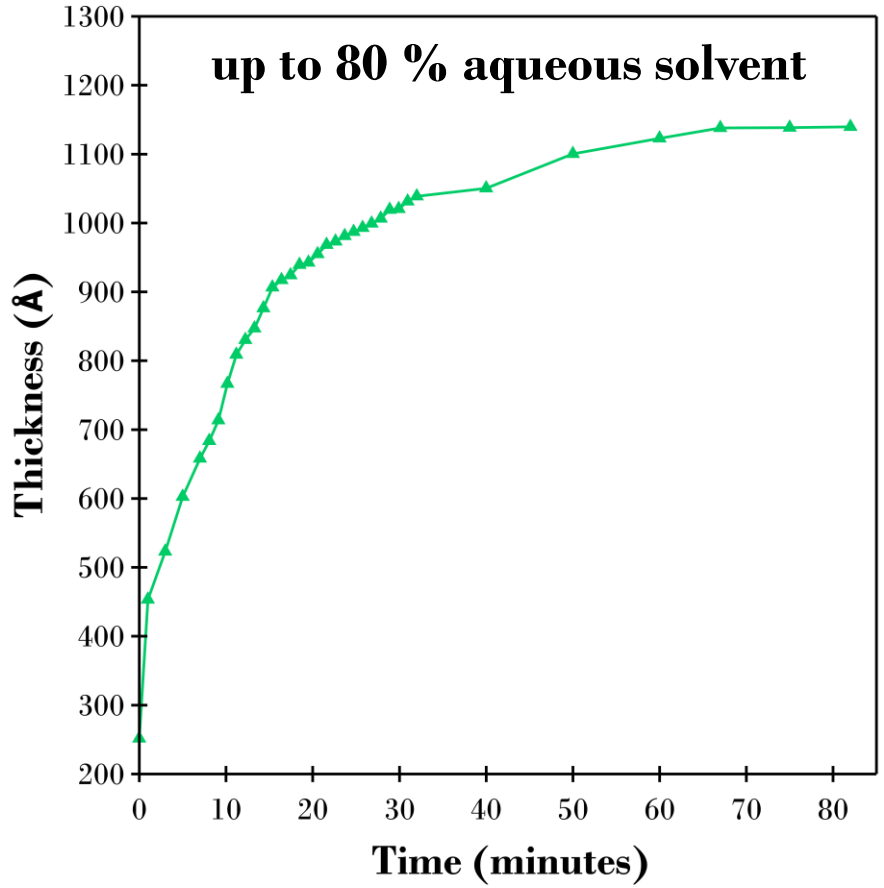
"It is reasonable to assume that the glyceride molecules in a fat-water interface are arranged according to the same principles as proposed for multilayers" [1]

(1) Brush *et al.*, *Chem Phys Lipids* **1968**, 2, 102

Uptake of water into the triolein phase



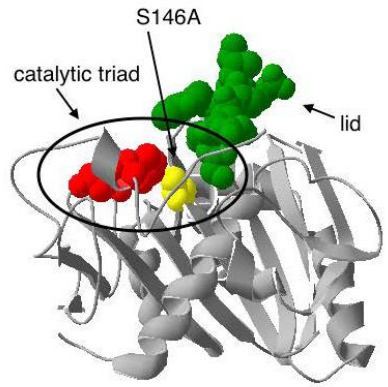
coarse grained simulations [1]



ellipsometry
(thin film ~250 Å dry) [2]

(1) Stamm *et al.*, *Chem. Phys. Lipids*, **2018**, *211*, 37. (2) Humphreys *et al.*, *Front. Soft. Matter* **2022** doi: 10.3389/frsfm.2022.929104

Lipolysis of triolein with thermomyces lanuginose

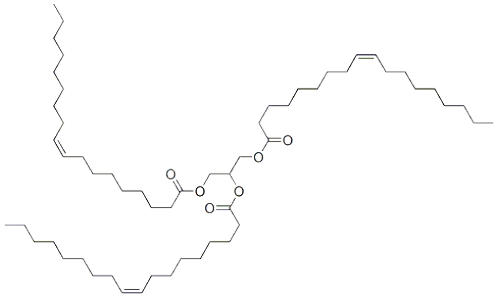


Thermomyces Lanuginose Lipase (TLL)

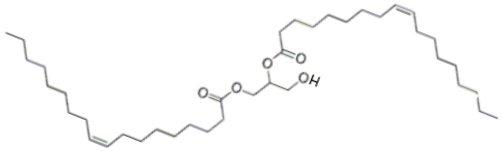
(supplied by Novozymes)

- Requires interfacial activation
- Selective lipase
  - Targets the Sn-1 and 3 positions of a glyceride [1]

triolein



H_2O → **TLL**

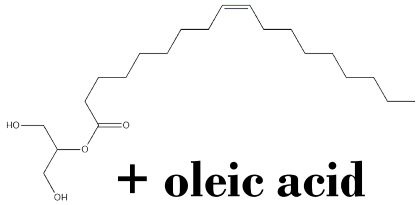


Sn-1/2 diolein

+ oleic acid

pK_a^{app}**~8** [2]

H_2O → **TLL**

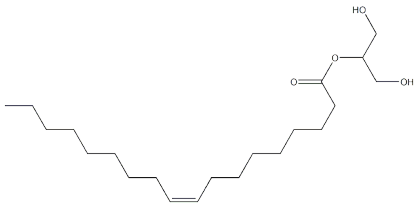


Sn-2 monoolein

+ oleic acid

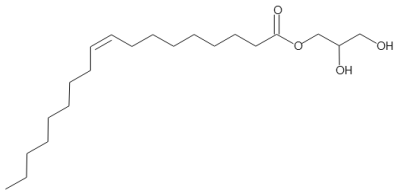
pK_a^{app}**~8**

Sn-2 monoolein



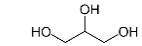
slow acyl migration [3] →

Sn-1 monoolein



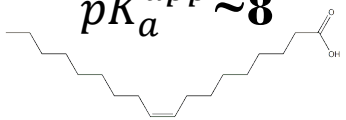
H_2O → **TLL**

glycerol



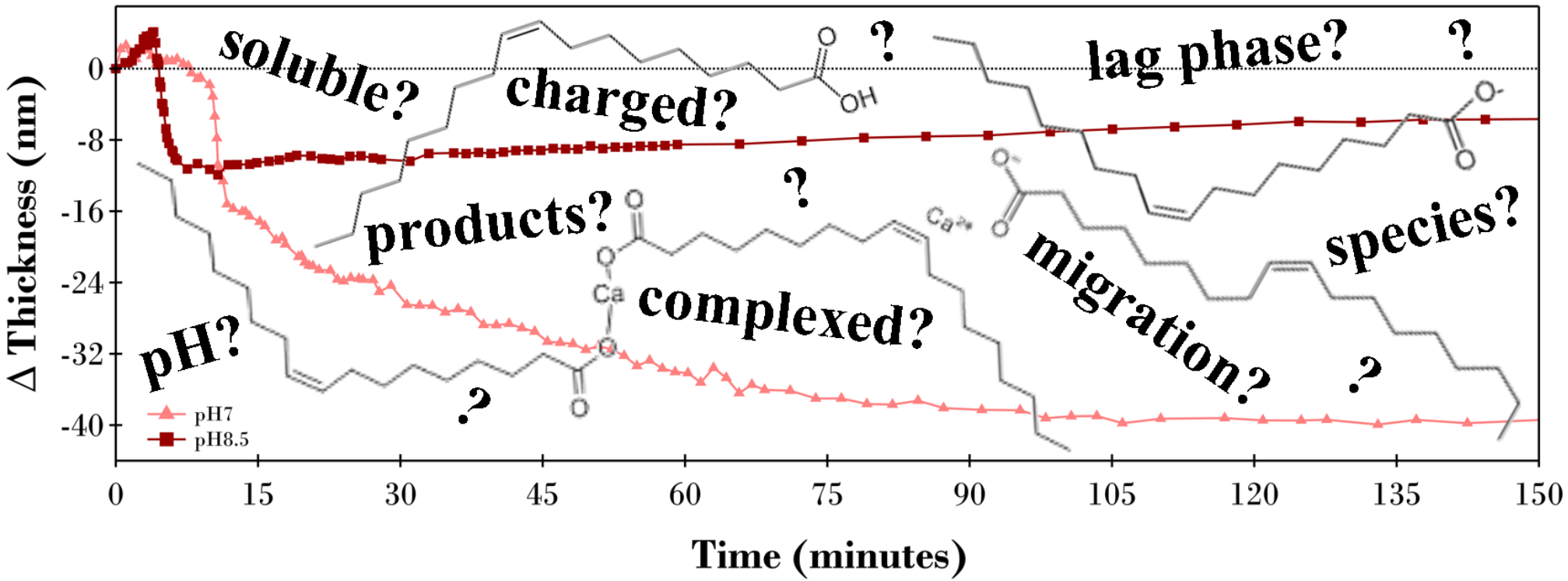
+ oleic acid

pK_a^{app}**~8**



(1) Fernandez-Lafuente, *J. Mol. Catal., B Enzym*, **2010**, 62, 197.(2) Cistola *et al.*, *Biochemistry*, **1988,** 27, 1881 (3) Li *et al.*, *Process Biochem*, **2010**, 45, 1888

Why investigate pH 7.0 and 8.5?

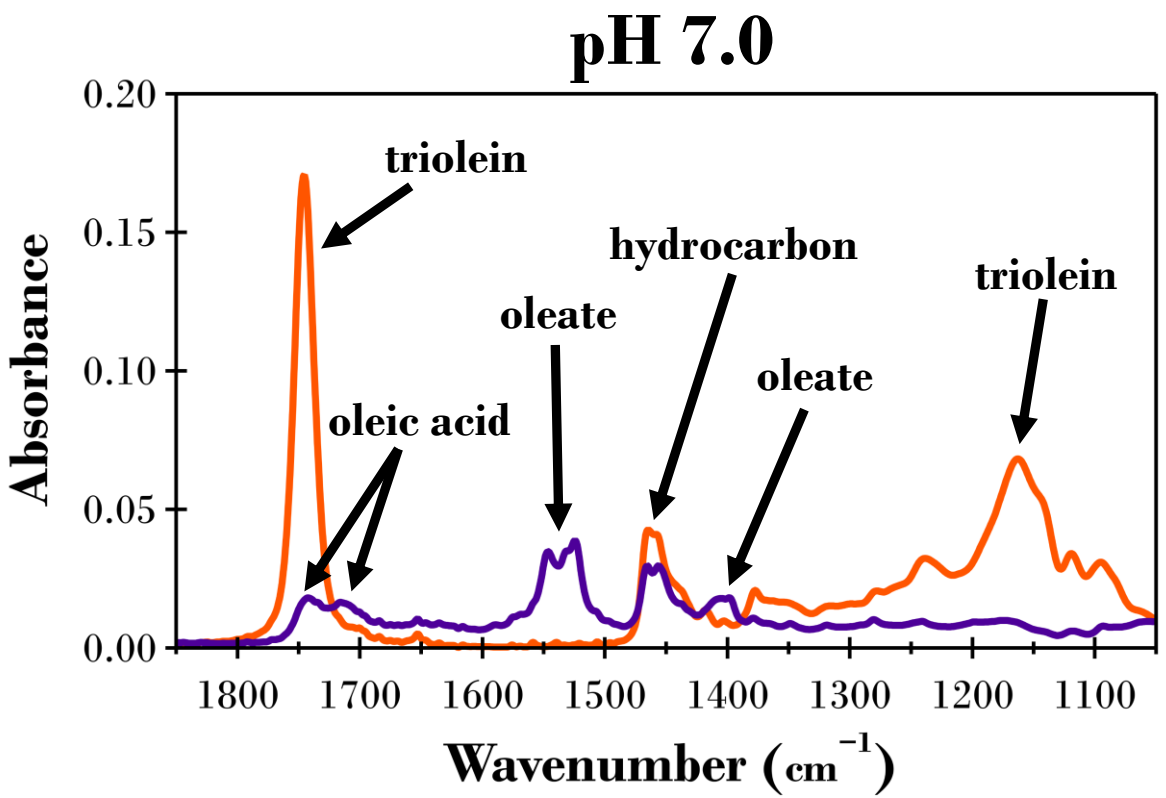


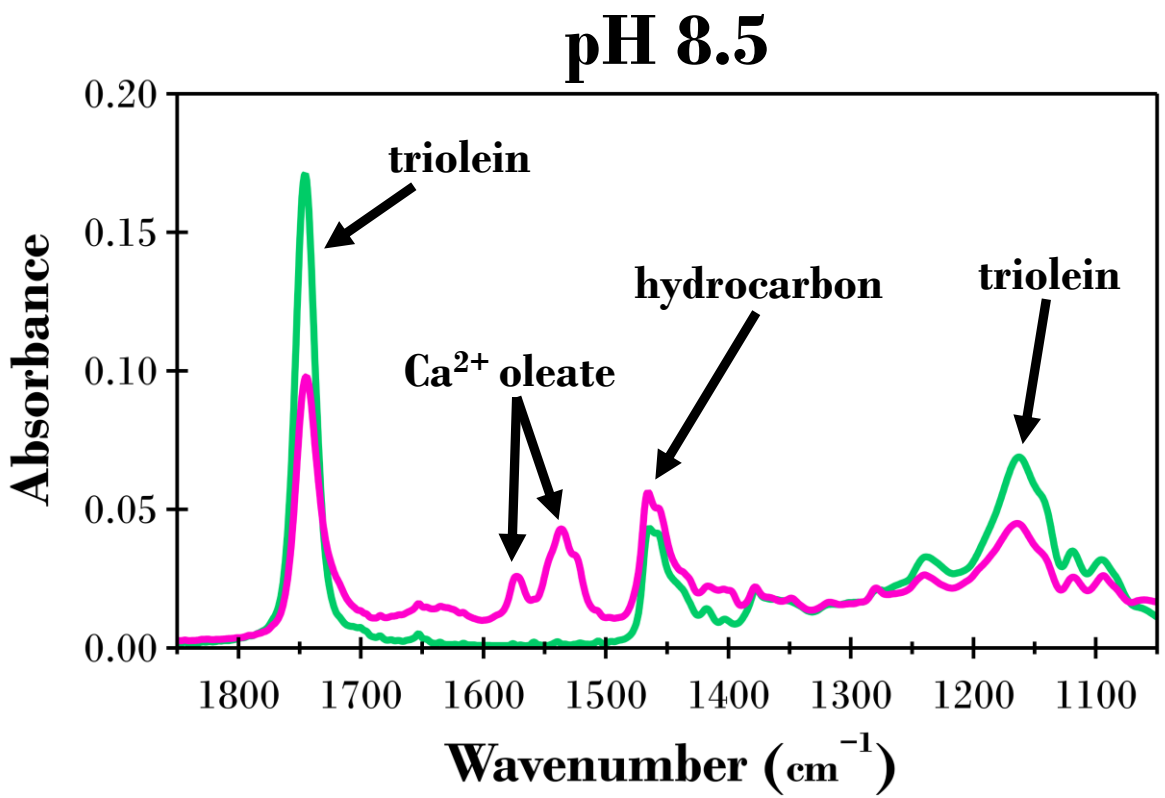
- Either side of the apparent pK_a of oleic acid produced in the reaction (pK_a^{app}~8) [1]
  - Influences the charged state of the product therefore;
    - Dictates the solubility of the products and how they interact with TLL [2,3]
    - Influences location of products (interface, aqueous phase or organic phase) [2]
    - If calcium complexes are formed [3]
  - pH can affect the kinetics of acyl migration [4]

(1) Cistola *et al.*, *Biochemistry*, **1988**, 27, 1881 (2) P. Reis *et al.*, *C. R. Chimie*, **2009**, 12, 163 (3) Li *et al.*, *Process Biochem*, **2010**, 45, 1888 (4) Snabe *et al.*, *Chem. Phys. Lipids*, **2003**, 125, 69

TLL digestion of a triolein film

ATR-FTIR





Summary of relevant infrared bands with approximate wavenumbers [2-6]

$_{(S)}$symmetric stretching mode; $_{(A)}$asymmetric stretching mode; $_{(B)}$bending mode $_{(m)}$monomeric oleic acid; $_{(d)}$dimeric oleic acid; $_{(u)}$unidentate complex; $_{(b)}$bidentate complex

infrared band	wavenumber (cm^{-1})
triolein ester	(C=O) $1745_{(S)}$ & (C–O–C) $1160_{(S)}$
oleic acid	(C=O) $1743_{(S,\,m)}$ & $1710_{(S,\,d)}$
Ca^{2+} oleate complex	(COO–Ca) $1575_{(A,\,u)}$ & $1540_{(A,\,b)}$
ionized oleic acid (oleate)	(COO^-) $1550_{(A)}$ & $1400_{(S)}$
hydrocarbon	(CH_2/CH_3) $1465_{(B)}$

(1) Humphreys *et al.*, *Front. Soft. Matter* **2022** doi: 10.3389/frsfm.2022.929104 (2) Lee *et al.*, J. Mater. Sci., **1999**, 34, 139 (3) Snabe *et al.*, Chem. Phys. Lipids, **2003**, 125, 69 (4) Sutton *et al.*, **2015**, Chem. Eur. J. 21, 6801
(5) Sutton *et al.*, **2015**, Acta Part A Mol. Biomol. Spectrosc. 134, 535 (6) Yang *et al.*, **1968**, J. Colloid Interface Sci. 113, 218

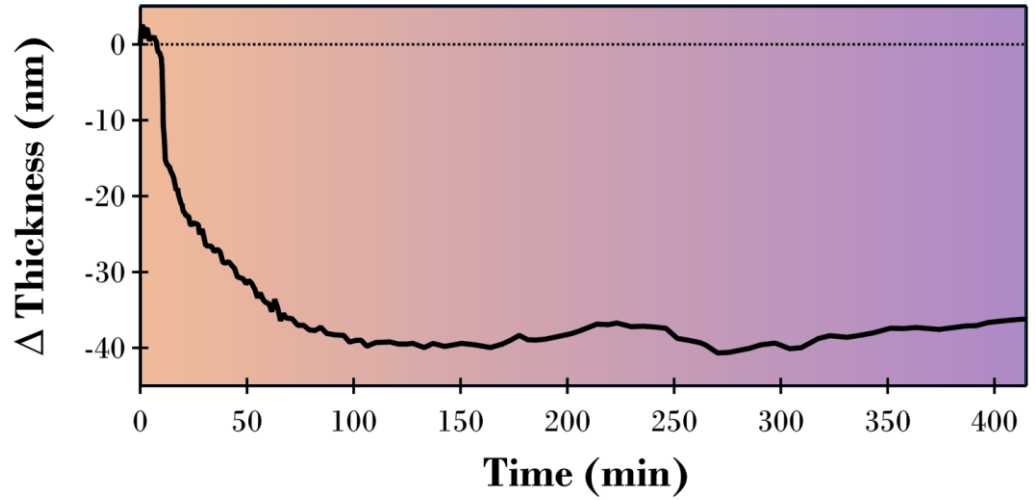
TLL digestion of a triolein film

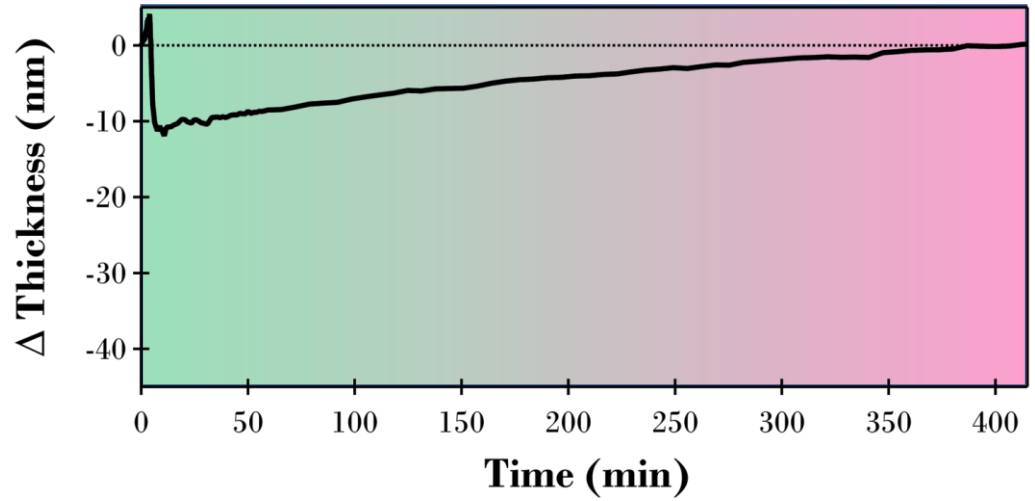
Ellipsometry & QCM-D

pH 7.0

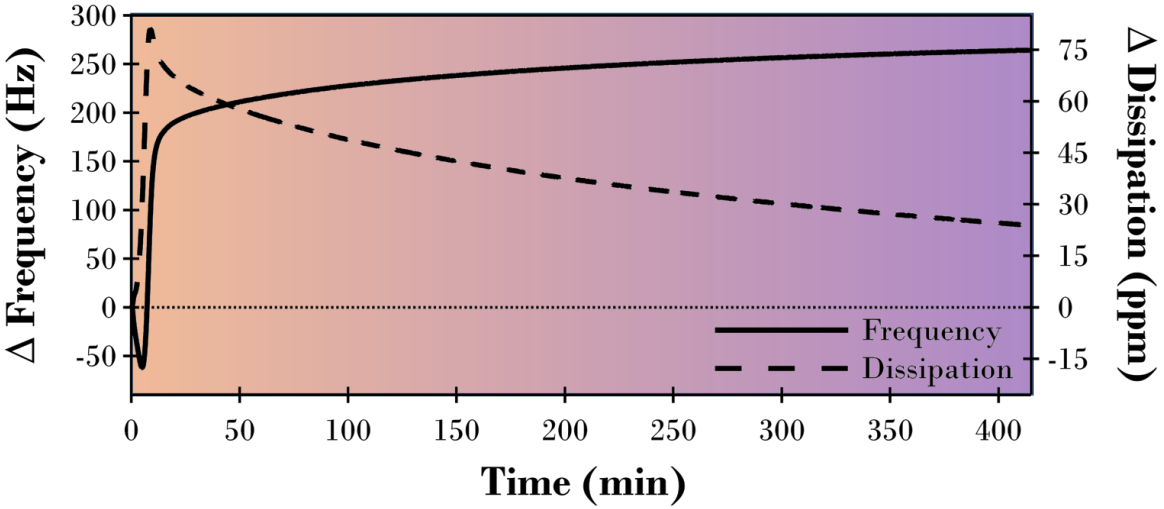
pH 8.5

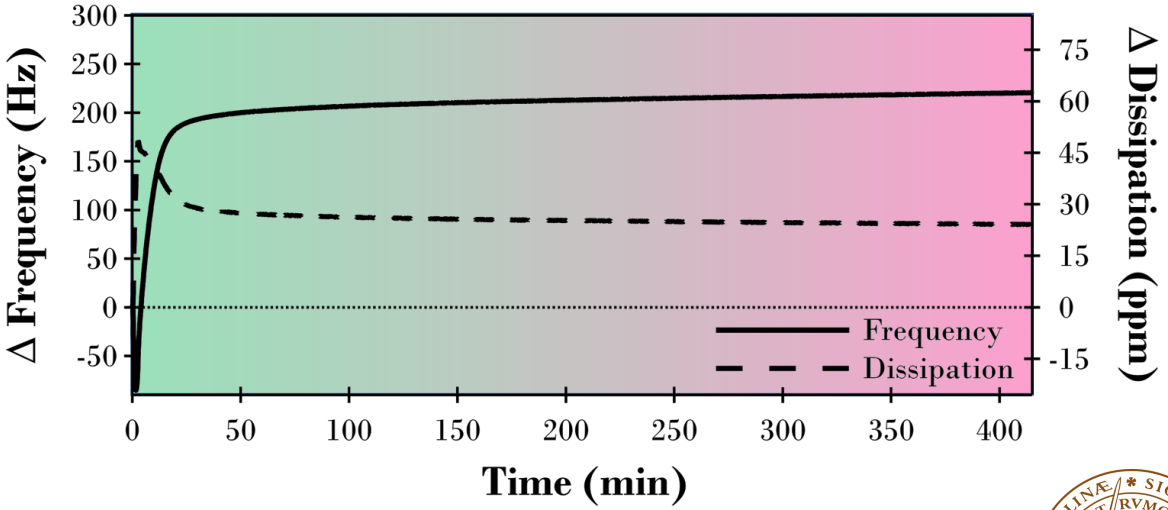
Ellipsometry





QCM-D

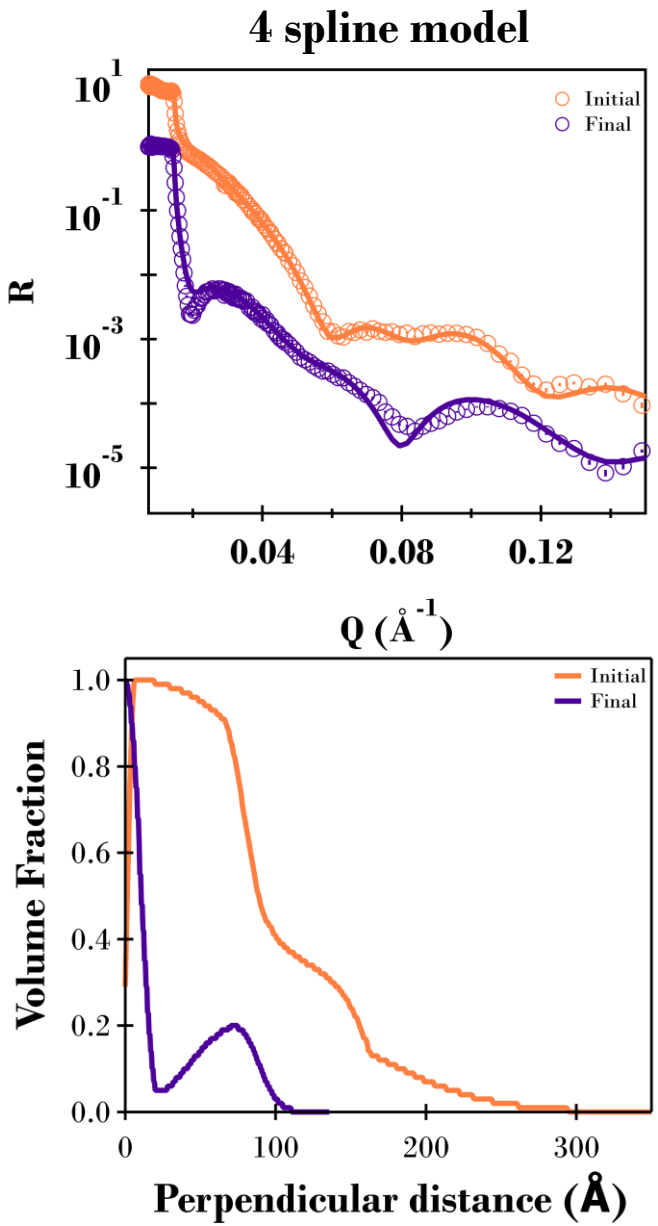


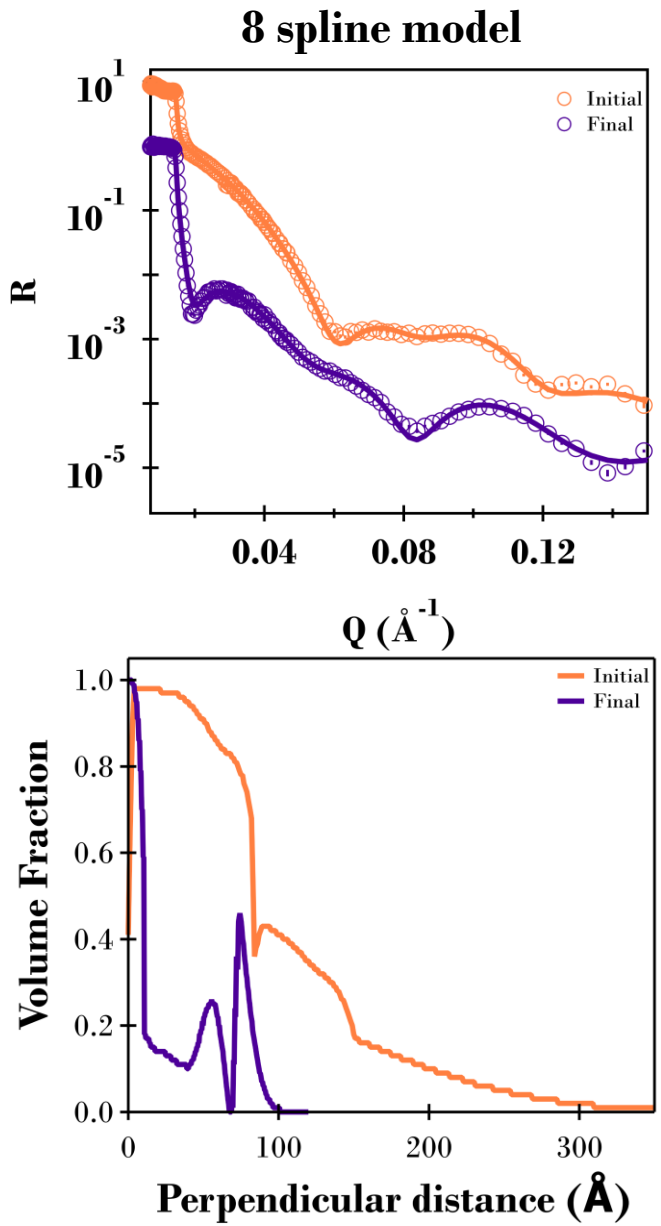


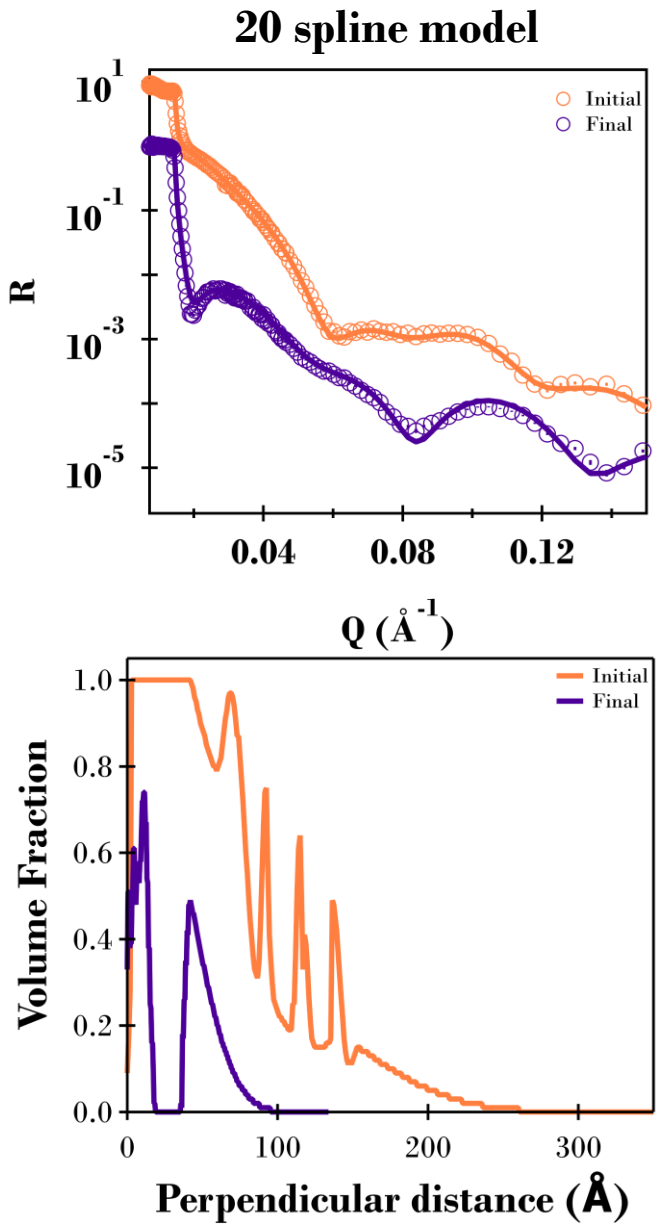
Humphreys *et al.*, *Front. Soft. Matter* **2022** doi: 10.3389/frsfm.2022.929104

TLL digestion of a triolein film at pH 7.0

Neutron Reflectometry

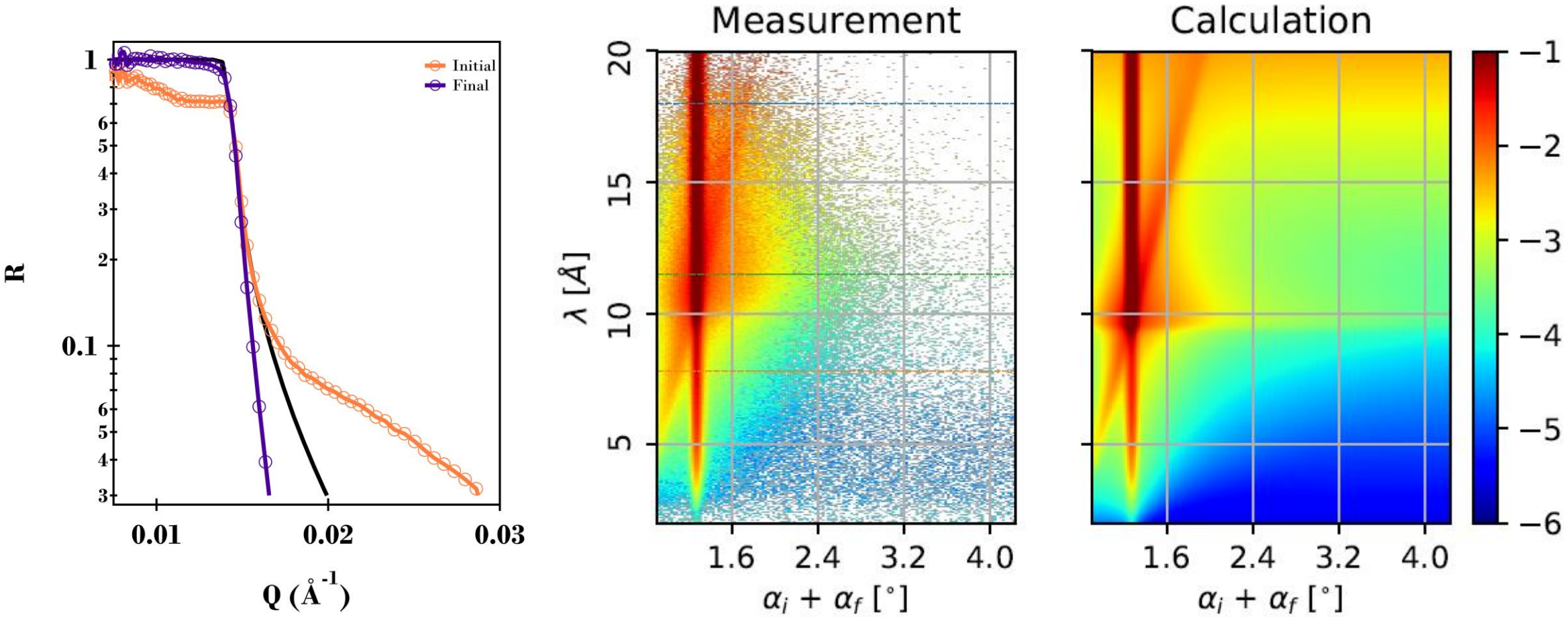






Off specular scattering observed

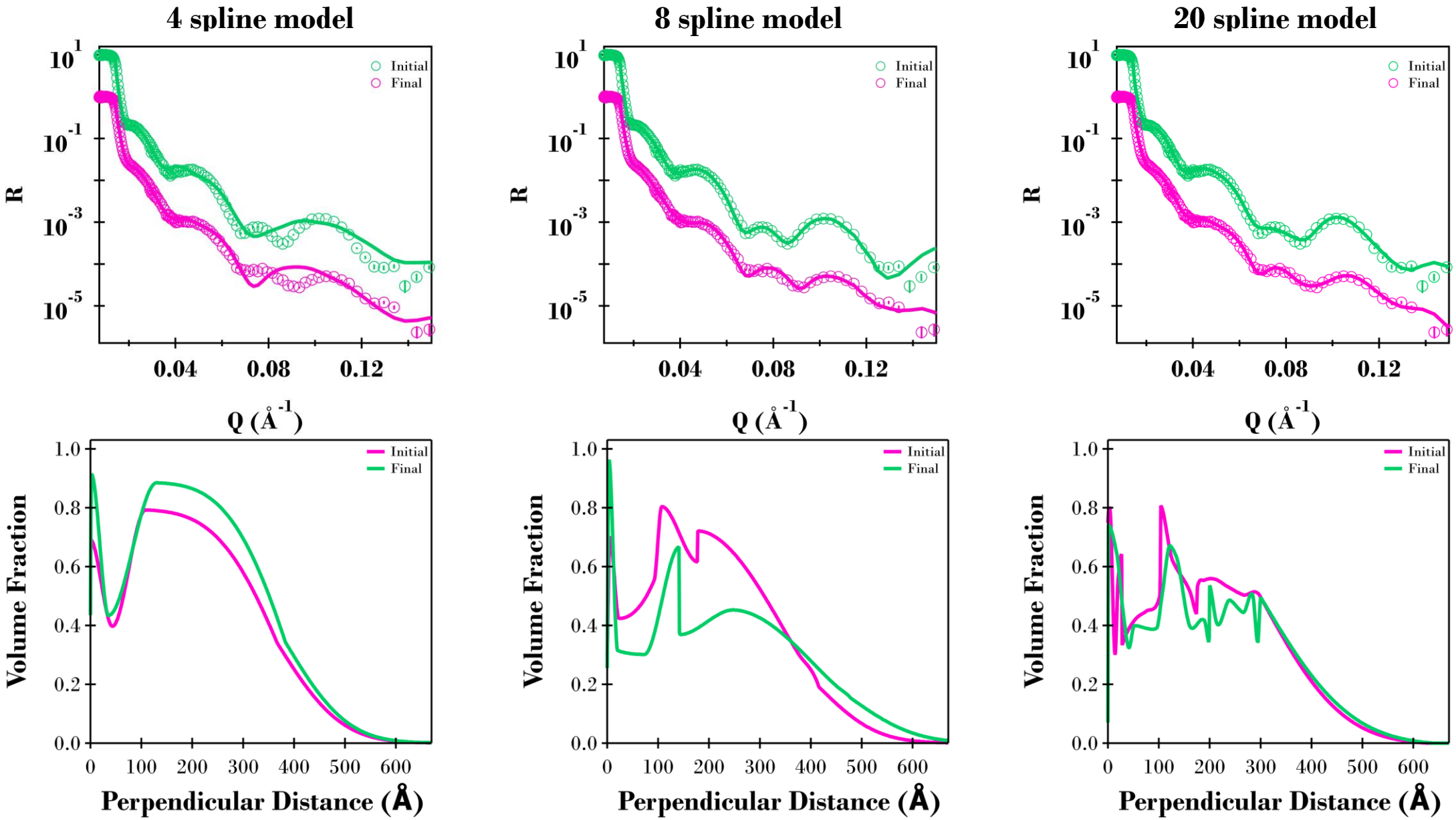
Neutron Reflectometry



Small clusters of dry triolein (sub-micron sized in-plane) separated by large areas of D_2O (in the tens of microns range) within the triolein layer(s)

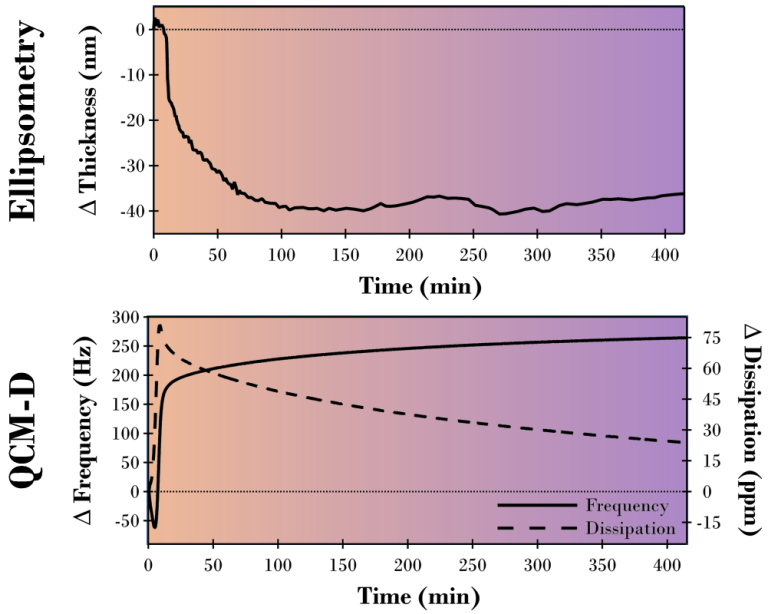
TLL digestion of a triolein film at pH 8.5

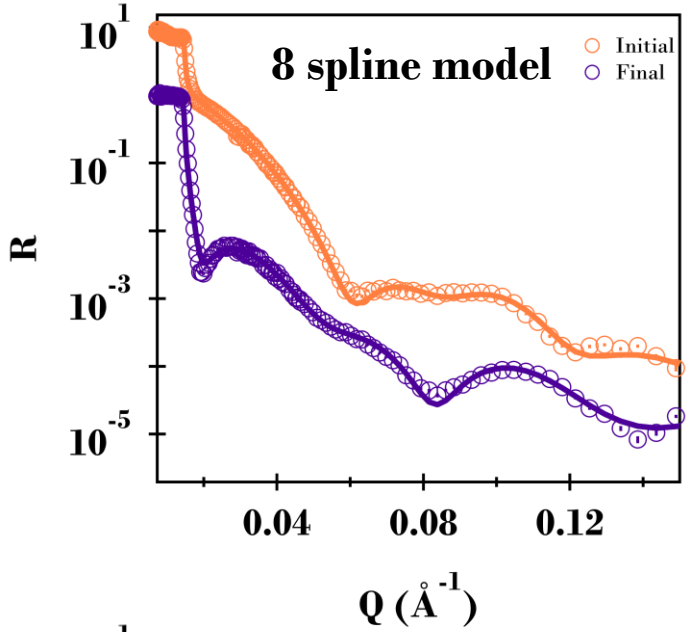
Neutron Reflectometry

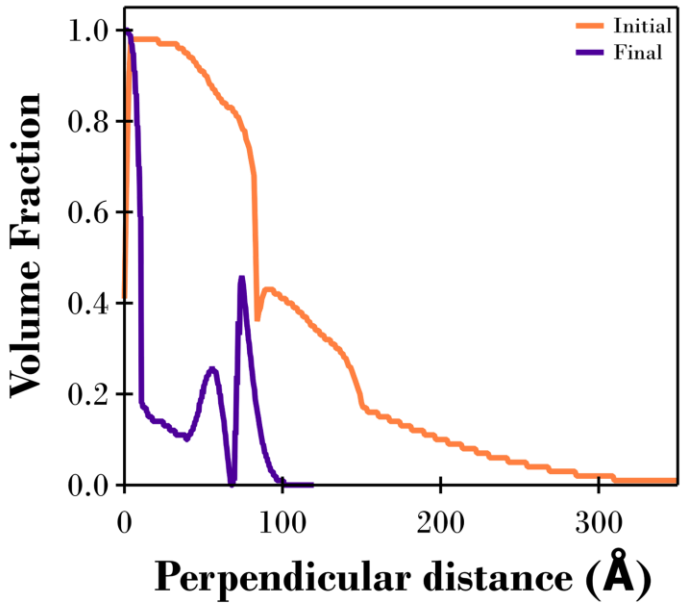


Summary of Results

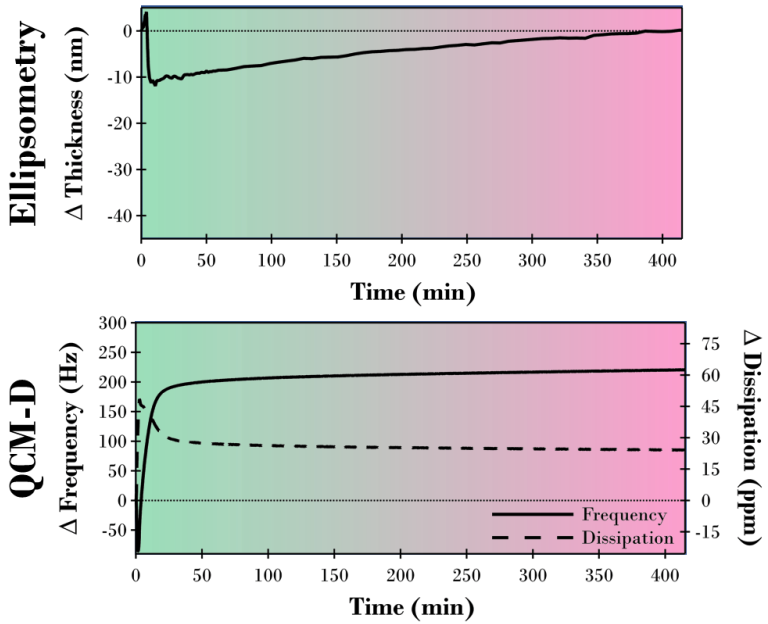
pH 7.0

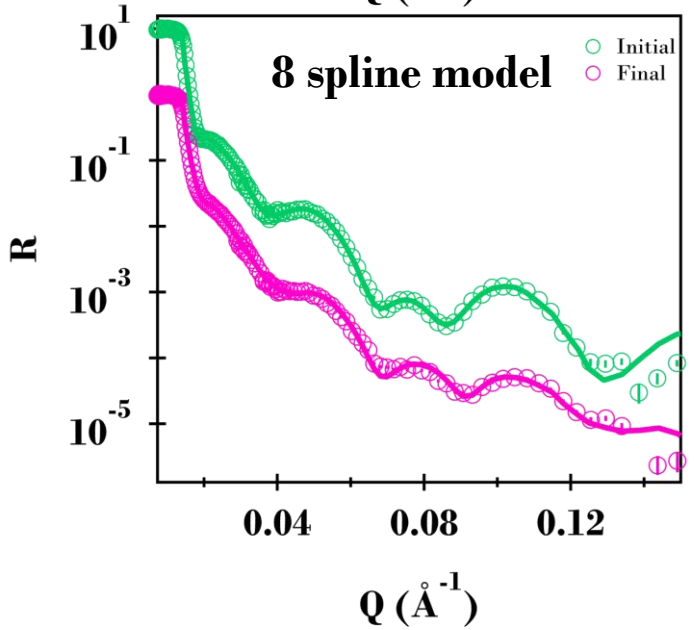


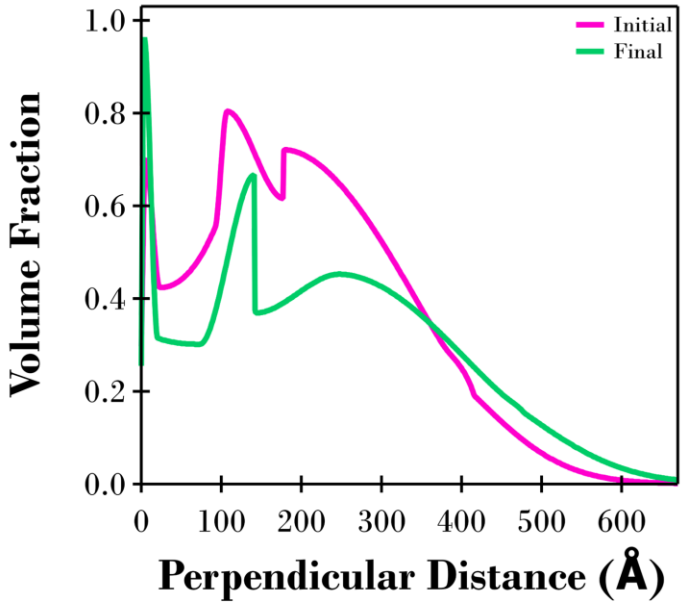




pH 8.5



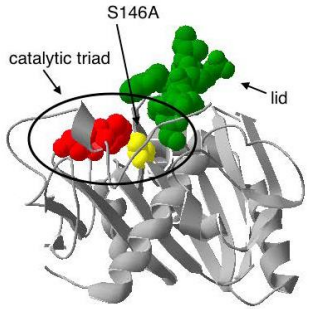


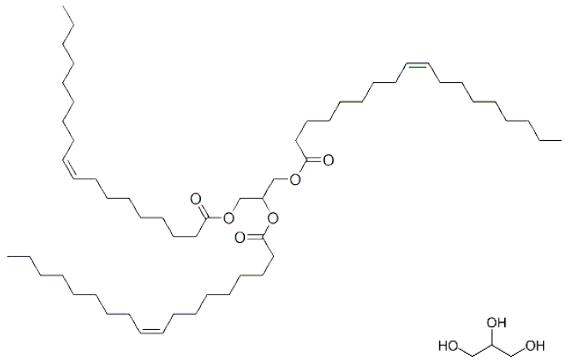


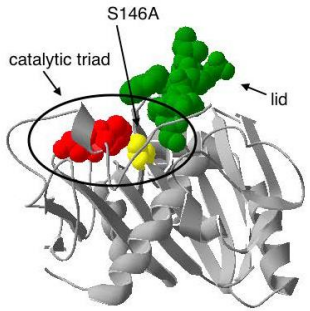
Key findings

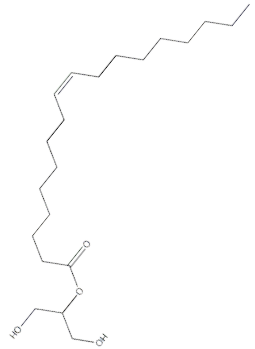
- pH influenced the lipolysis of triolein
  - Lag phase was increased at pH 7.0
  - TLL activity impeded at pH 8.5
    - Calcium oleate complexes play a significant role

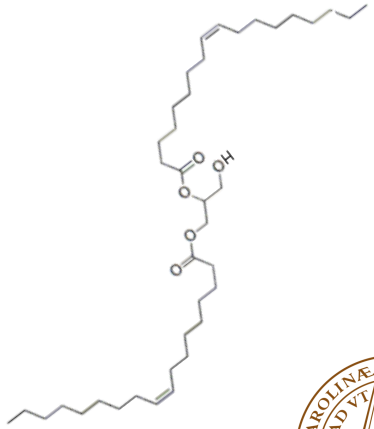
- Neutron reflectivity
  - Internal structure is very complex
  - Model suggests highly disorganised, stratified structure
  - Off specular scattering present at pH7.0











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ILL France
- Dr Philipp Gutfreund

ESS Sweden
- Dr Thomas Arnold

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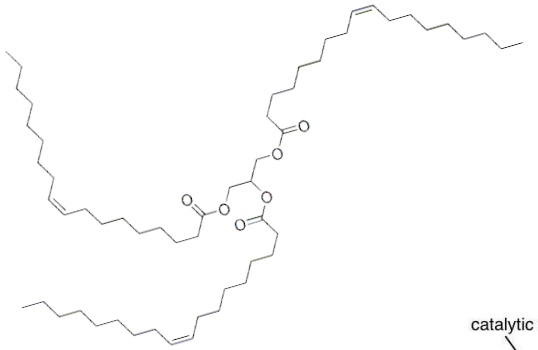
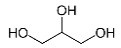
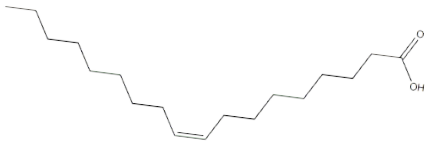
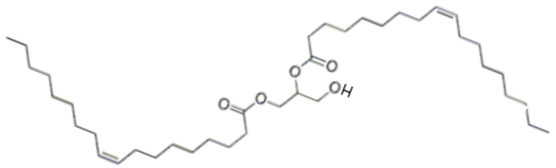
Thank you for your attention

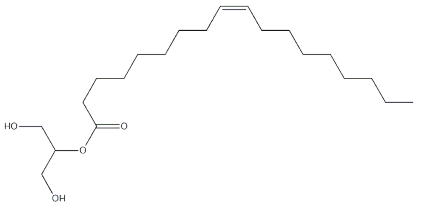
Questions???

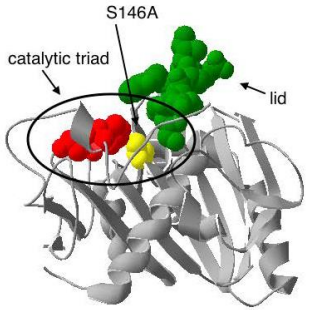


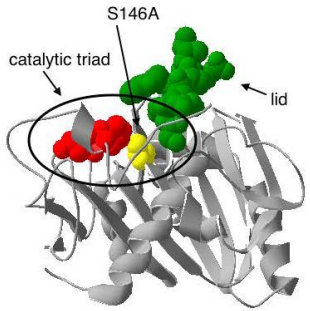






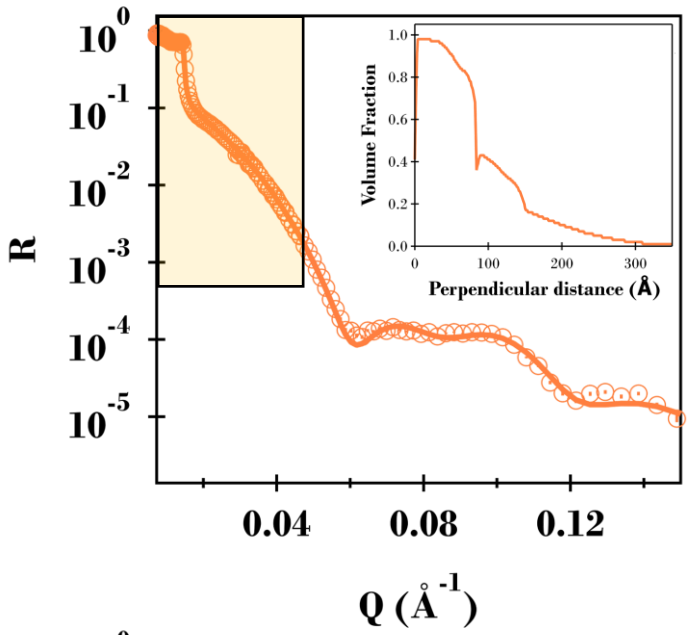


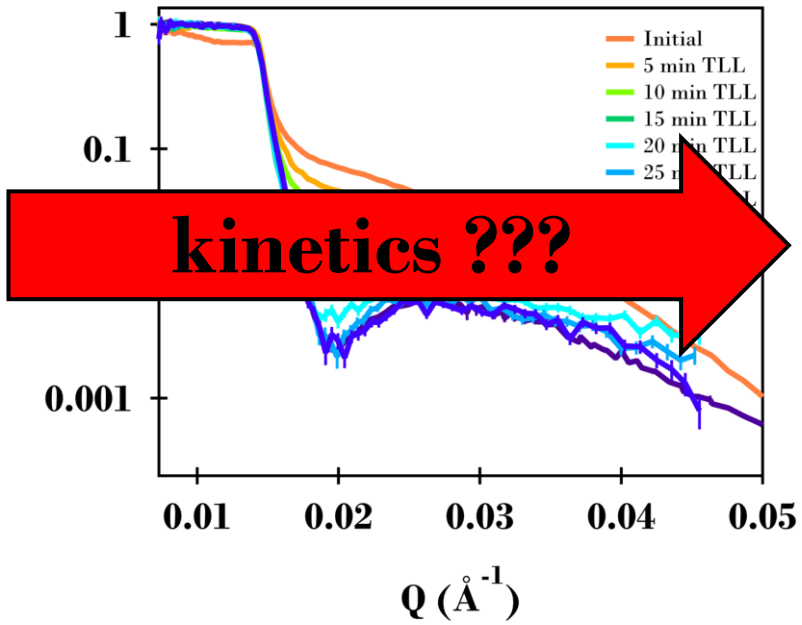


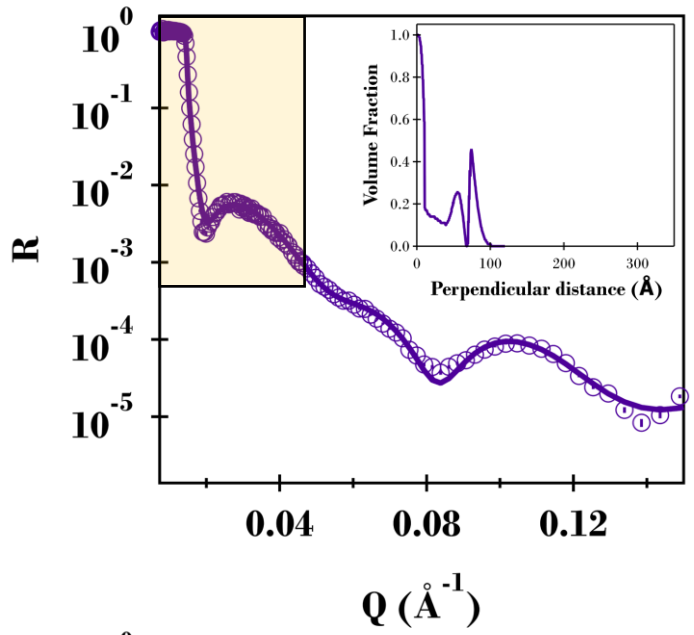


Kinetics of triolein film lipolysis with neutrons

pH 7







pH 8.5