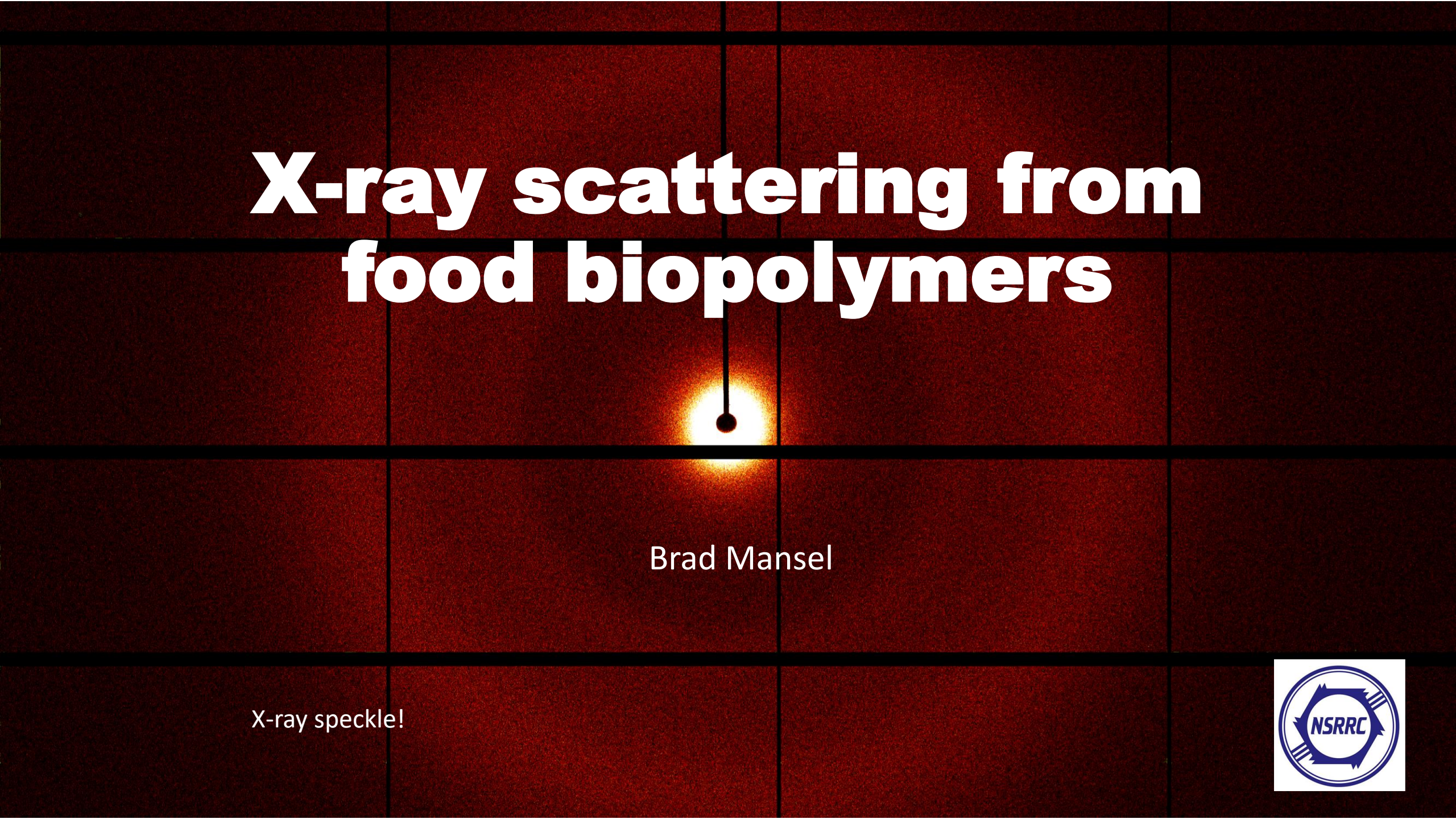
X-ray scattering from food biopolymers

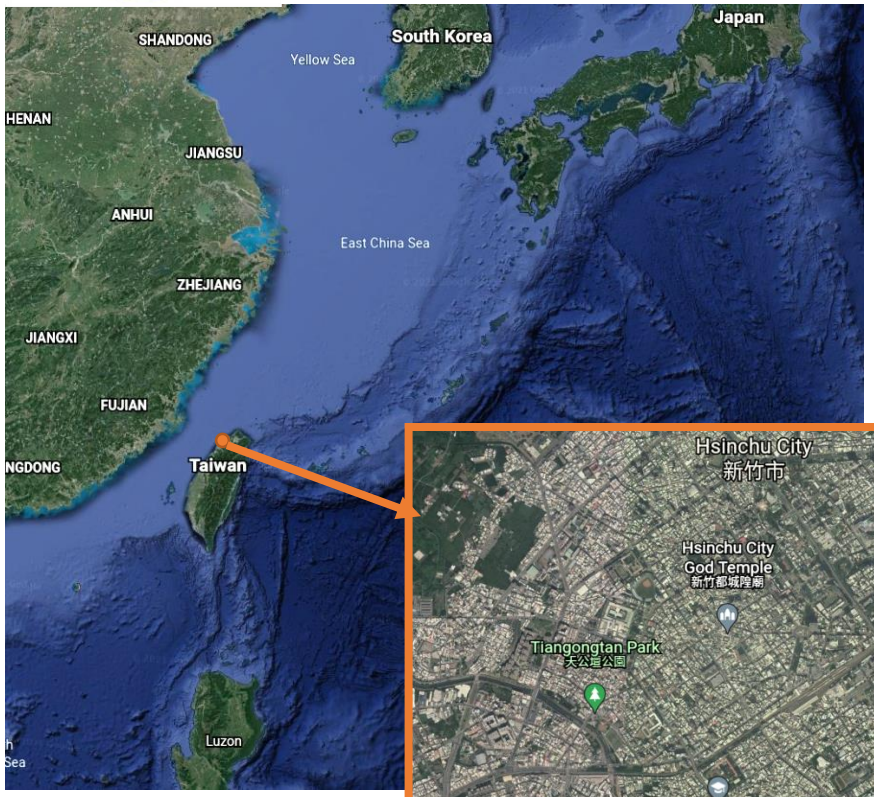


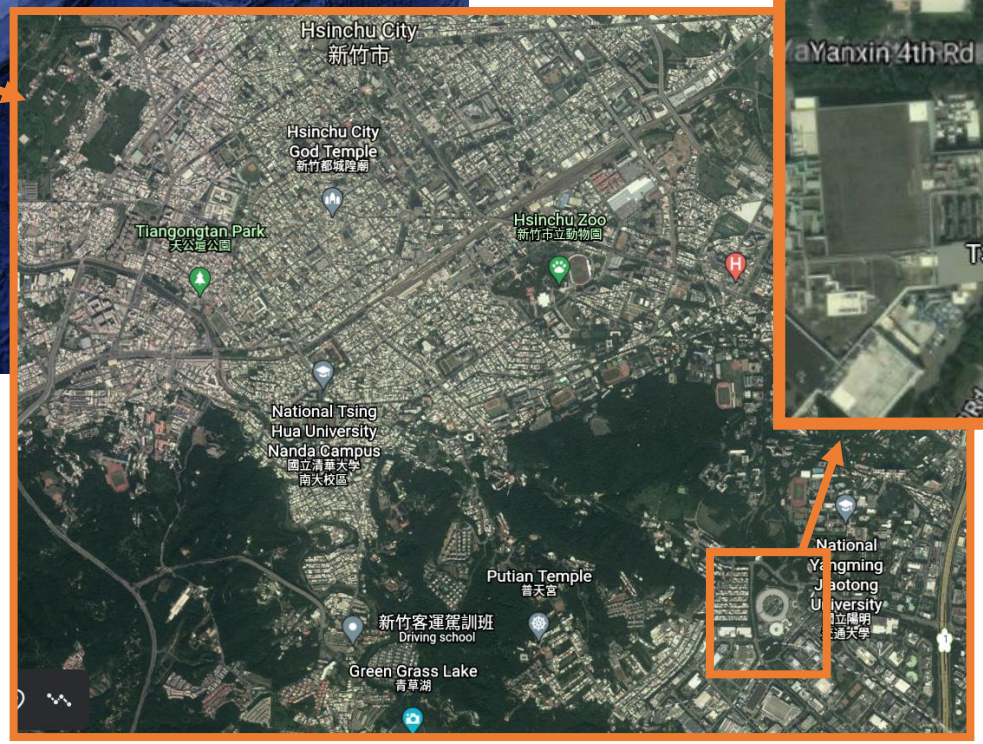
Brad Mansel

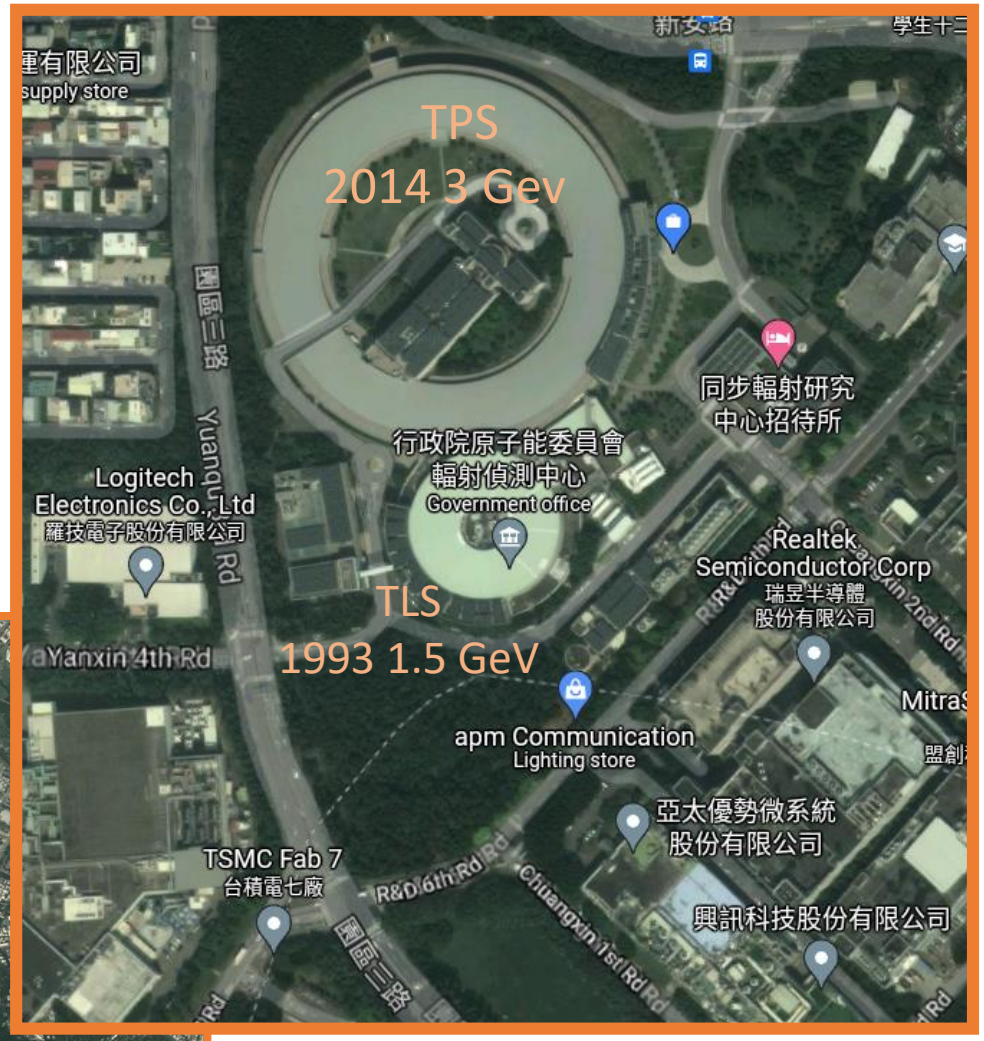
X-ray speckle!

NSRRC

Introduction





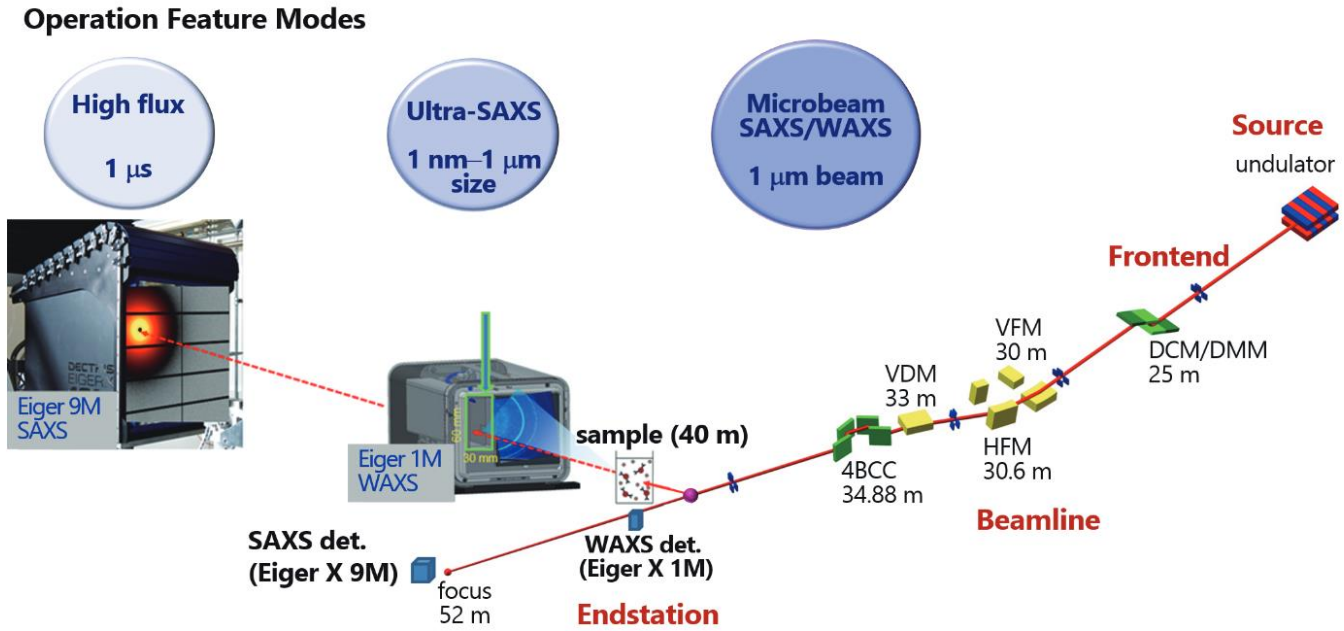


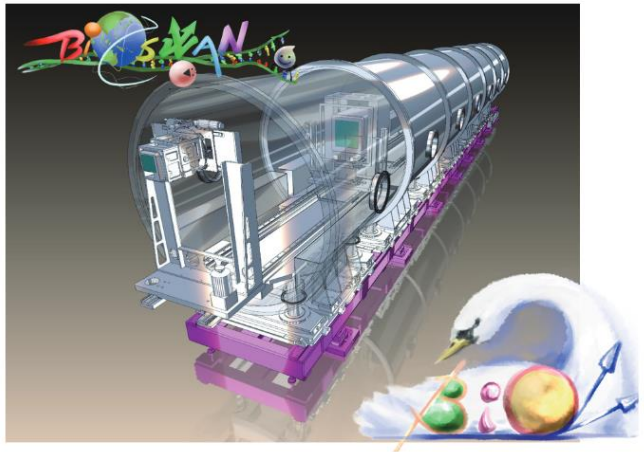
Beamlines at Spring-8 in Japan and Sika triple axis @ ANSTO

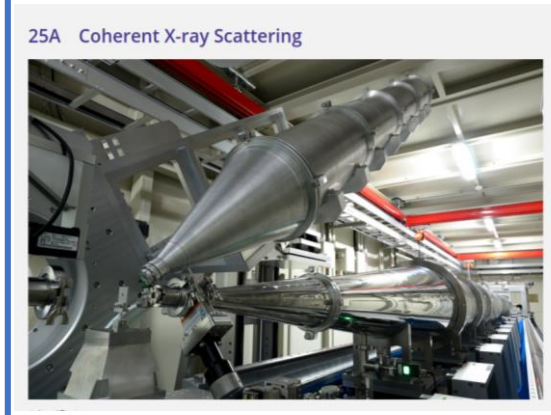
Taiwan accounts for >60 % global foundry revenue

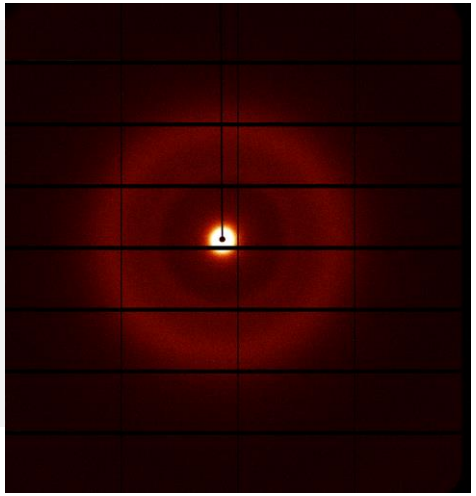
Many companies are in Hsinchu science park next to the NSRRC.

NSRRC – BIOSAXS and coherent X-ray scattering



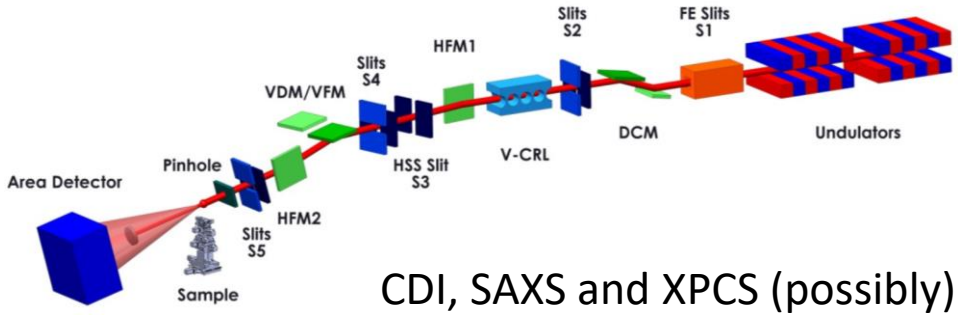






10^{10} ph/sec coherent flux
($COSAXS 10^{11}$ ph/sec)*

EIGER 16M



CDI, SAXS and XPCS (possibly)

Mansel, B. W., Chen, C. Y., Lin, J. M., Huang, Y. S., Lin, Y. C., & Chen, H. L. (2019). Hierarchical structure and dynamics of a polymer/nanoparticle hybrid displaying attractive polymer–particle interaction. *Macromolecules*, *52*(22), 8741-8750.

*Kahnt, M., Klementiev, K., Haghighat, V., Weninger, C., Plivelic, T. S., Terry, A. E., & Björling, A. (2021). Measurement of the coherent beam properties at the CoSAXS beamline. *Journal of Synchrotron Radiation*, *28*(6).

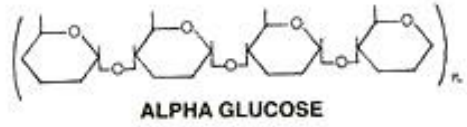
Overview

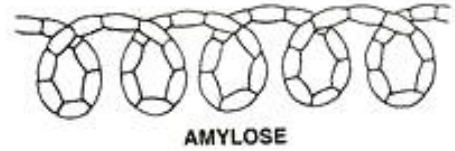
Cover 2 levels, some essential ideas with interesting examples which highlight polyelectrolyte effects, complexity and size ranges.

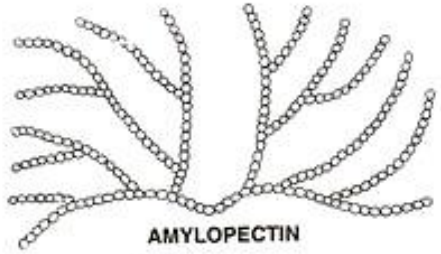
- Briefly introduce polymer scattering and modelling.
- Tips for preparing biopolymer samples and collecting data.
- Present solution examples from pectin, alginate and carrageenan.
- Example sol vs. gel.

Why are we interested?

- Used in thickening and stabilizing foods
  - Change mouth feel
  - Lower cost (a little biopolymer goes a long way)
  - Could have calorific or health benefits (slow digestion, satieting etc.)
- Understand the world around us.
  - Really amazing compact structures like starch and glycogen.
  - Interesting polymer physics / physical chemistry.







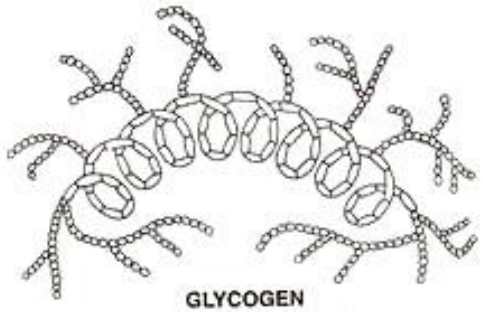
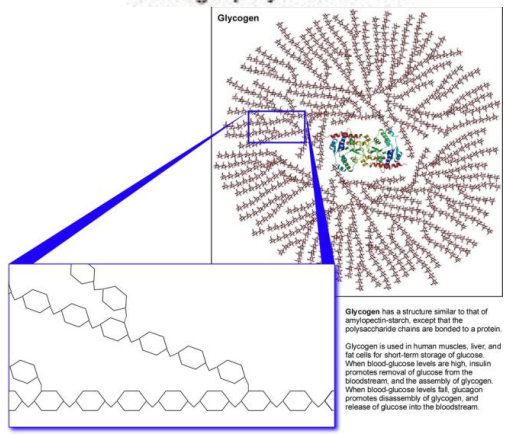
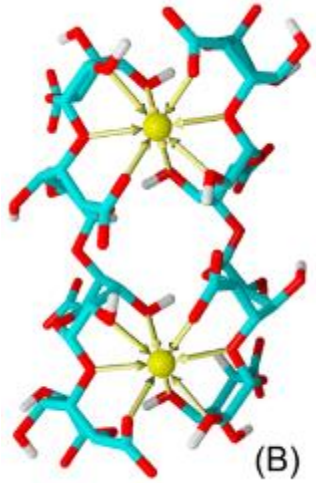


Fig. 9.7. Diagrammatic structure of storage polysaccharide.



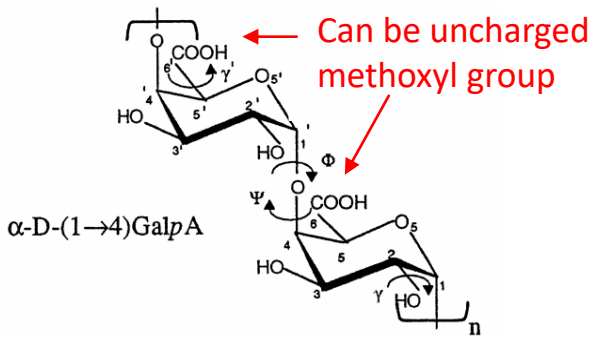
Introduction: pectin, alginate and carrageenan

- Polymers from land plants, brown algae and red seaweed, respectively
- Utilized extensively in food systems
- Pectin and alginate have similar gelation mechanisms involving the chelation of divalent ions, Ca^{2+} by carboxyl groups
- Carrageenan has a significantly different gelation mechanism which has been hotly debated. Can gel with different monovalent or divalent ions. Are sulphated.

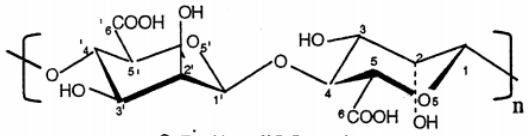


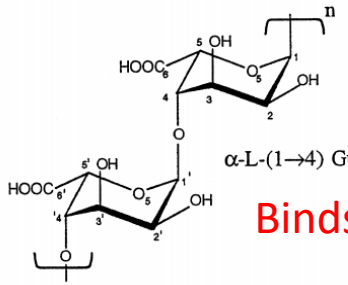
Plazinski, Wojciech, and Mateusz Drach. *The Journal of Physical Chemistry B* 117.40 (2013)

Pectin



Alginate

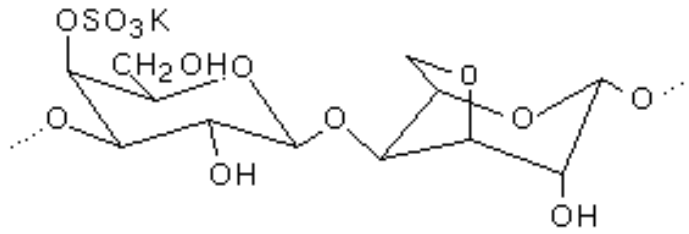




Binds Ca^{2+}

κ-carrageenan

(1-4)-linked β-D-galactopyranose-4-sulfate alternating
(1-,3)-linked 3,6-anhydro-α-D-galactopyranose



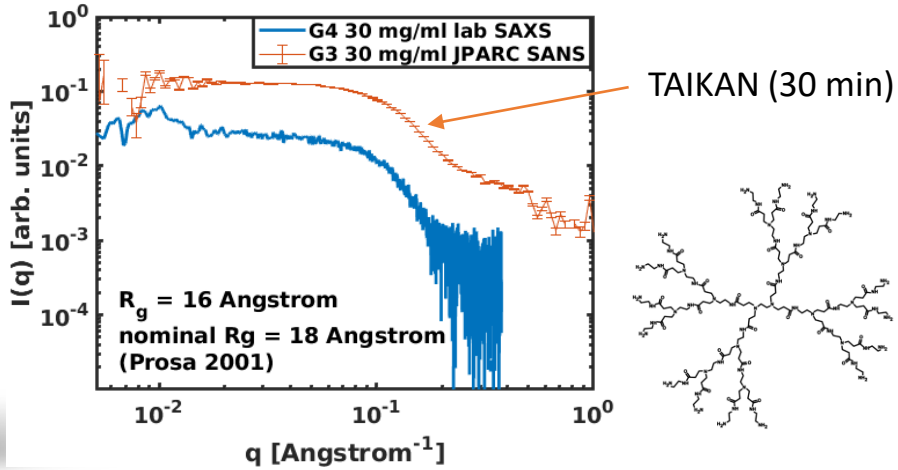
Specific challenges for x-ray scattering on biopolymers

- Very large range of length-scales of interest sub monomer to clusters of single chains (0.5 to +100's nm)
- Often have high viscosities (reduce mol. weight or concentration)
- Weakly scattering (not really an issue on modern bioSAXS beamlines)
- Gels are often of interest: cannot flow, beam damage, bubbles cannot escape, and accurately measuring background difficult
- Water soluble polymers are typically **highly charged** (carboxymethyl cellulose vs. cellulose)
- Purity, need biochemist pure not food grade pure, i.e. (BSA € € € vs commercial pectin €)
- Broad array of food biopolymers.
  - Can be broadly classified as polypeptides and polysaccharides.
  - Many have highly specific properties which must be known before the measurement
  - Solubility under different salts or temperatures.
  - Conditions can significantly change the local and global structures.

Choice of SAXS / WAXS instrument

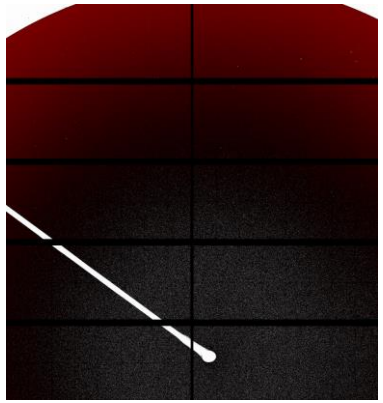
- Broad q-range and weak scattering makes most measurements unfeasible on a lab SAXS (for now).
- coSAXS 👍👍👍
- BioSAXS beamlines are the best choice.

30 mg/ml PAMAM dendrimer 4 hours



TAIKAN (30 min)

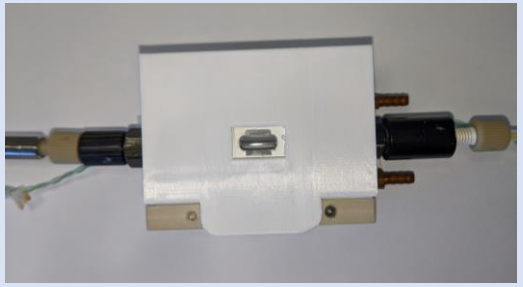
Synchrotron: 20 gels + 40 sols easy in 48 hours

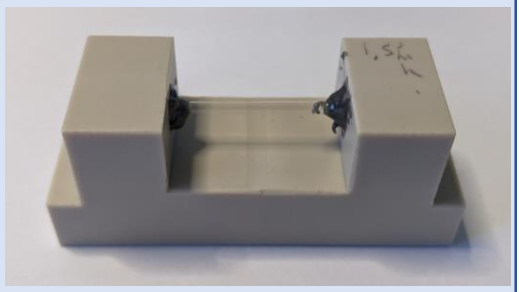


Sample cell

Solutions

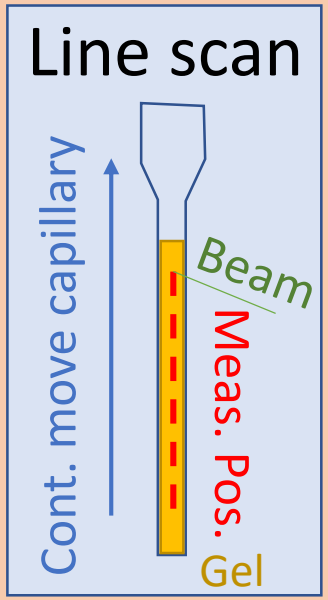
- Use flow cell (check viscosity).
- Flow reduces beamdamage.
- Accurate background subtraction.
- Auto loaders!! High throughput, reduction pipeline.
- Beamline scientist will help you optimize flux and flow rate.





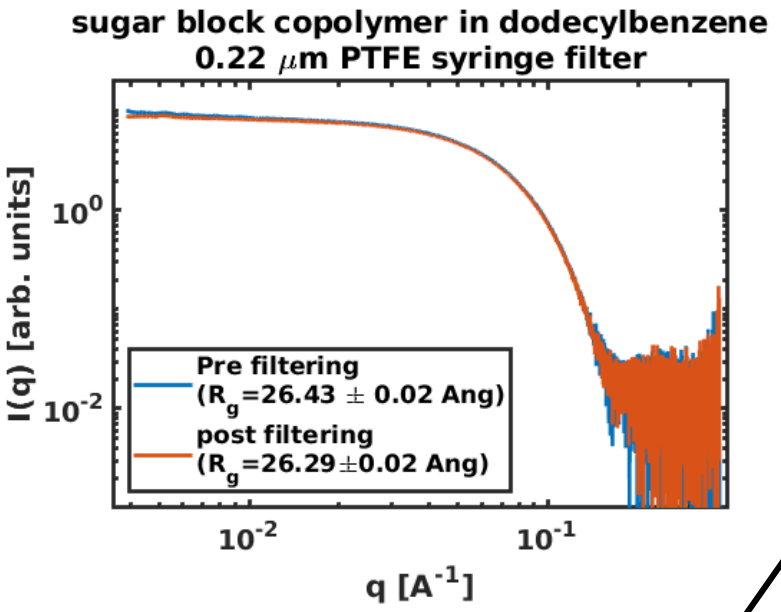
Gels

- Capillaries are more accurate than de mountable cells.
- De mountable cells have flexible windows, bad for background subtraction.
- Every capillary is unique. Measure the background at the **exact same position** on the **same capillary** which you measure the sample. (can be difficult)
- Use line scans down the capillary to minimize beamdamage.

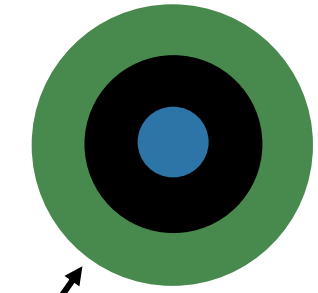


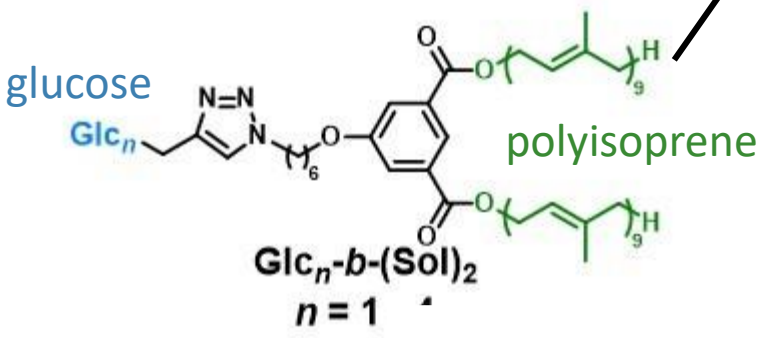
Sample preparation: dust and aggregation

Filtration

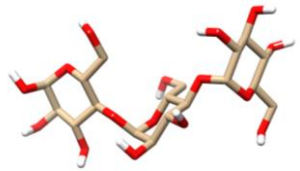


Glucose in center of micelle

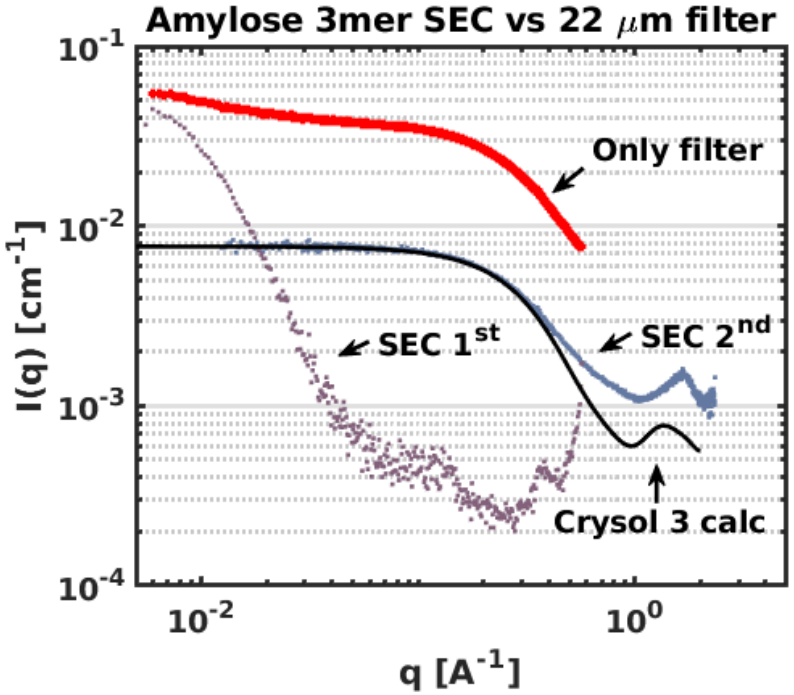




SEC



Filter:
R_g = 5.33 ± 0.02 Å
SEC:
R_g = 4.74 ± 0.01 Å



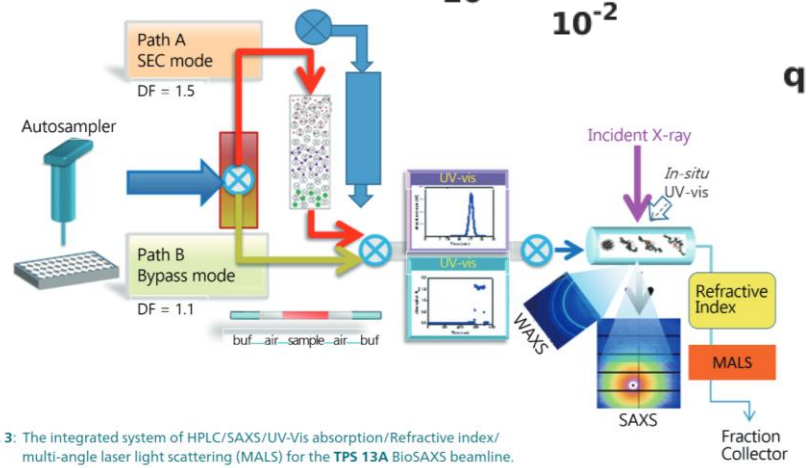
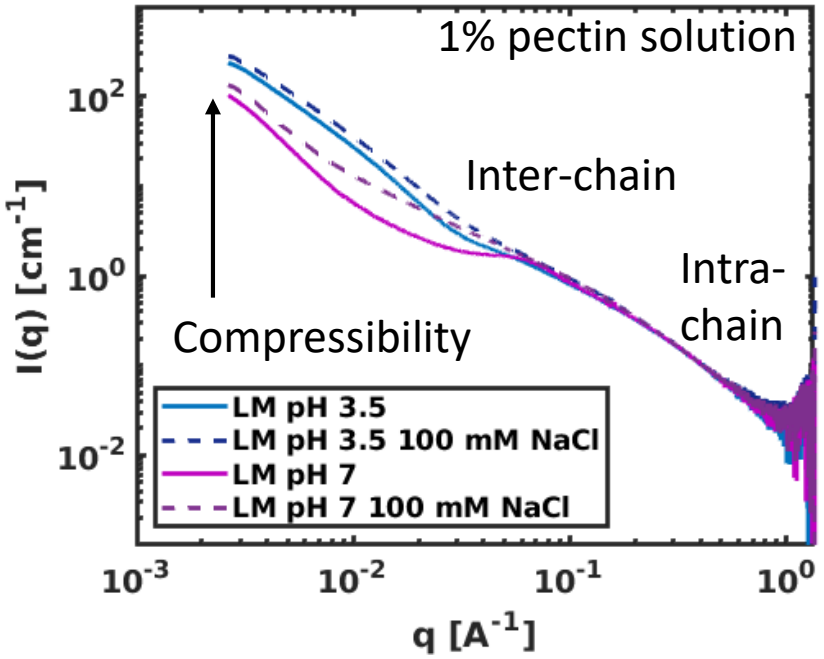


Fig. 3: The integrated system of HPLC/SAXS/UV-Vis absorption/Refractive index/ multi-angle laser light scattering (MALS) for the **TPS 13A** BioSAXS beamline.

Sample preparation: controlling salt and charge (polyelectrolyte effect)

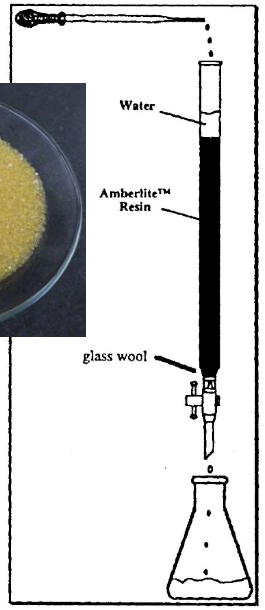


- pH 3.5, close to the pKa of carboxyl groups, structure factor is present.
- Moderate amounts of salt also significantly change the mid to low-q scattering
- Negligible changes above q=0.1 Å^{-1}
- Ion exchange column or dialysis can greatly improve data quality.

More info:
M. Muthukumar, A Perspective on Polyelectrolyte Solutions, amcromolecules 2017

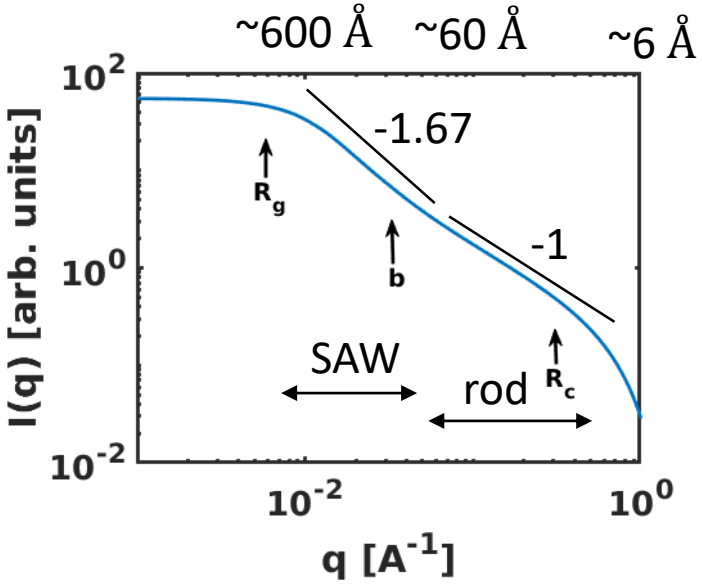
Ion exchange chromatography

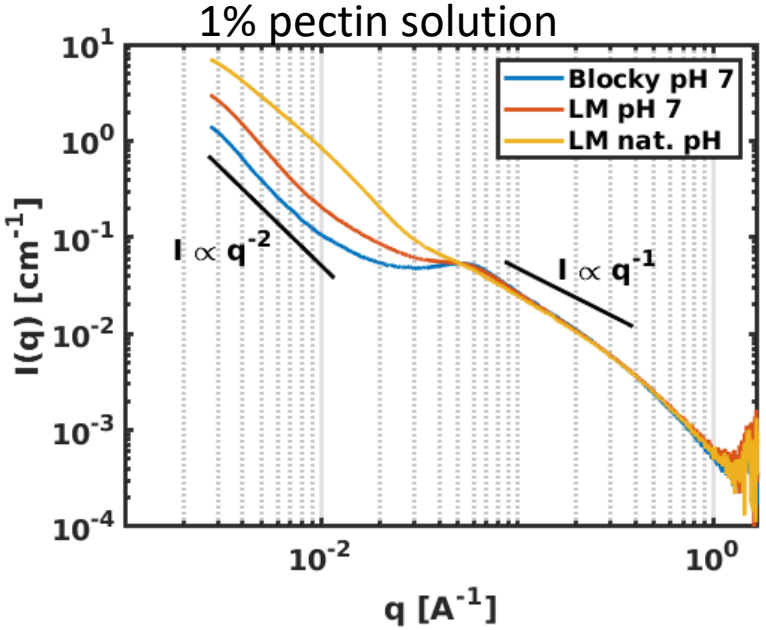
1. charge up column (HCL)
2. run biopolymer through
3. Wash out polymer with DI water
4. Titrate back to require pH (record amount of ions)
5. Freeze dry
6. Dissolve at correct concentration



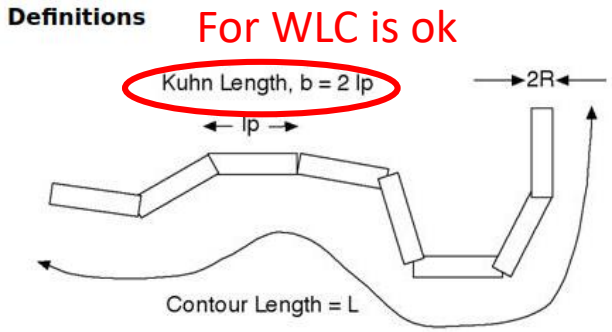
Wikipedia and www.vanderbilt.edu/AnS/Chemistry/courses/chem104/experiment4/ionexchange.htm

Real data vs phenomenological models





Author's work, unpublished



SASView manual

$$q = \frac{4\pi}{\lambda}\sin\left(\frac{\theta}{2}\right) = 2\pi s,\ d = \frac{2\pi}{q} = \frac{1}{s}$$

$$1\ \text{Å} = 0.1\ \text{nm}$$

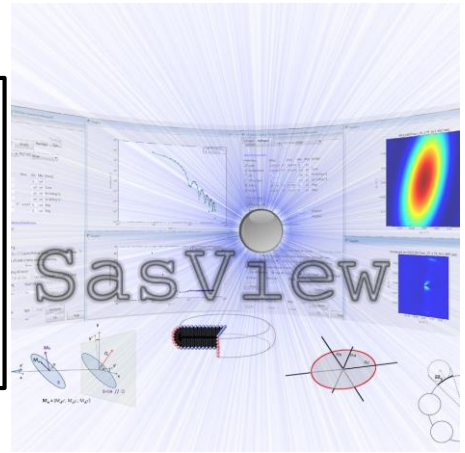
Fractals are useful!
Phenomenological models are helpful but typically cannot describe our materials.

WARNING!!

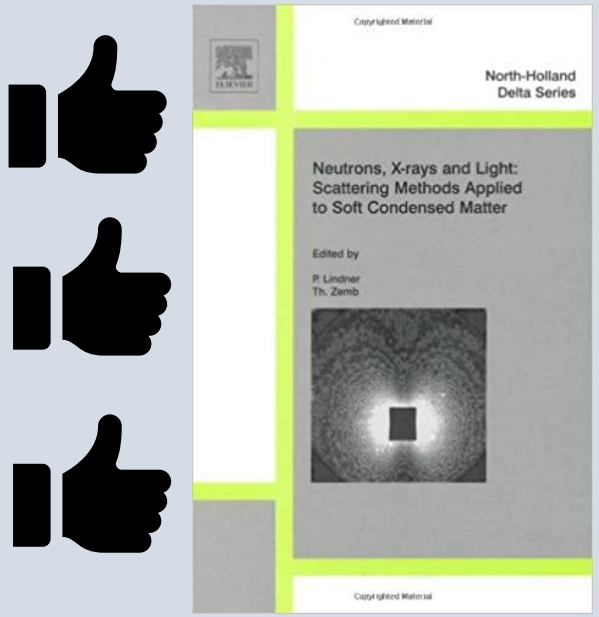
$I(\mathrm{q}) \propto P(\mathrm{q})S(\mathrm{q})$

Only for centrosymmetric objects.

Not for polymers!



Neutrons, X-rays and Light: Scattering Methods Applied to Soft Condensed Matter

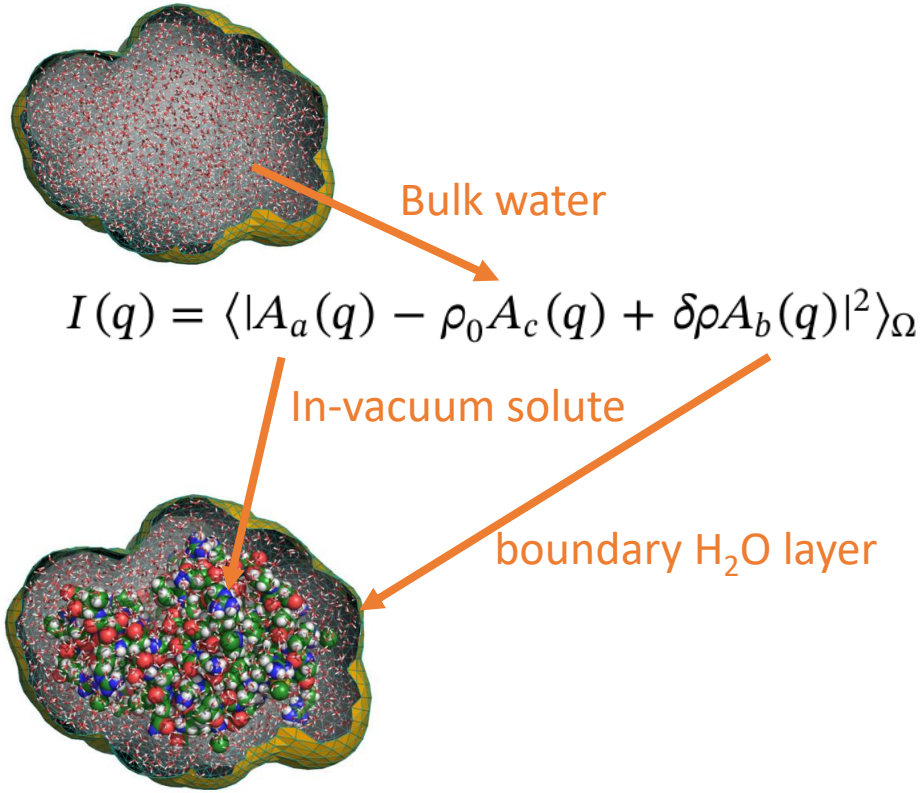


- **Pedersen, Jan Skov, and Peter Schurtenberger. "Scattering functions of semiflexible polymers with and without excluded volume effects." *Macromolecules* 29.23 (1996): 7602-7612.**
- **Chen, Wei-Ren, Paul D. Butler, and Linda J. Magid. "Incorporating intermicellar interactions in the fitting of SANS data from cationic wormlike micelles." *Langmuir* 22.15 (2006): 6539-6548.**

Atomistic models

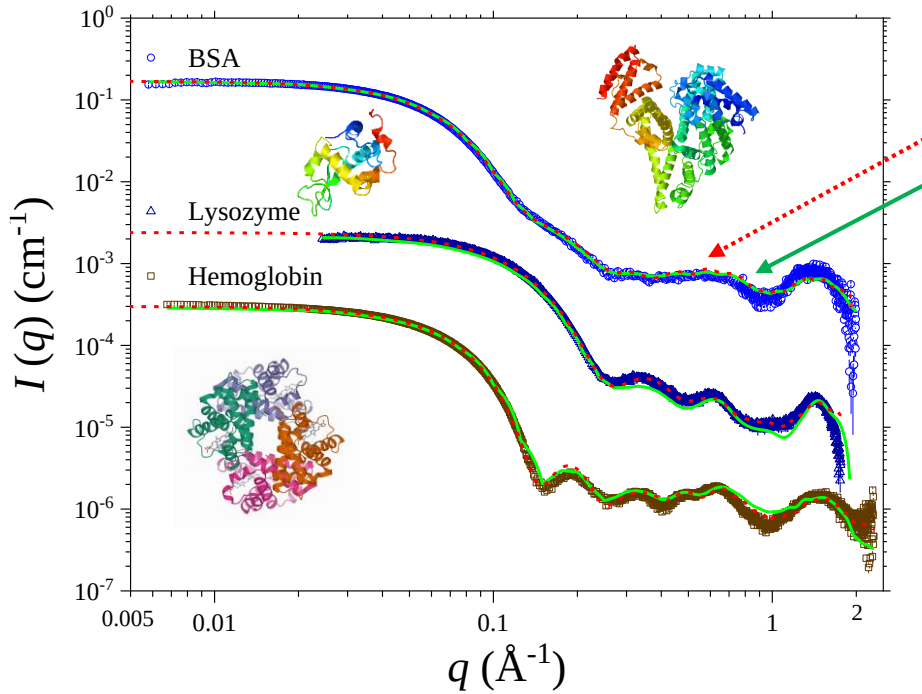
$$\langle |A_a(q)|^2 \rangle_\Omega \equiv I_{eu}(q) = Nf^2 + f^2 \sum_i^N \sum_{i \neq j}^N \frac{\sin(qr_{ij})}{qr_{ij}}$$

P Debye, *Ann. Physik* 1915



Knight and Hub, *Nucleic Acids Research* 2015

- Atomistic models can be the most accurate way to model SAXS / WAXS data
- For biopolymers typically dynamics must be taken into account.
- For biopolymers can model the local structure.
- Combining with molecular dynamics can help with modelling or reveal shortcomings of the force field.



Software:

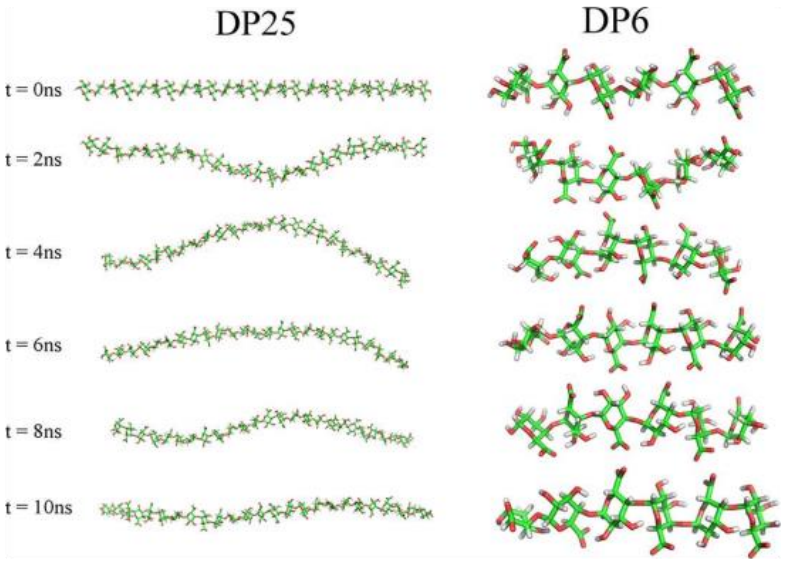
- Crysol
- WAXSIS
- FOXS
- SASSIE
- AXES

etc

Shih et al submitted *to J. Appl. Cry.* 2021
Latest 13A data

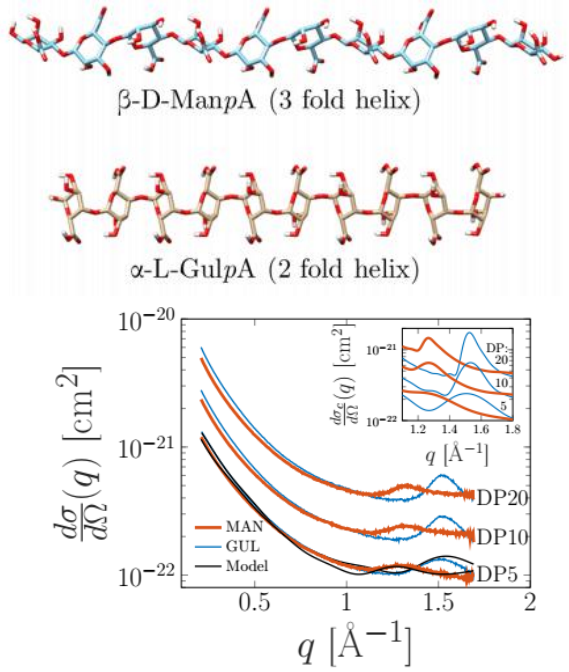
High-q (>0.1 Å) local chain information and monomer spacing

- High-q $0.1 < q < 1$ Å shows local chain structure and can be fitted to atomistic models.
- $1 < q < 2$ Å for certain biopolymers can reveal local ordered structure and information about flexibility.
- Highly purified oligomers @ 10-30 mg/ml

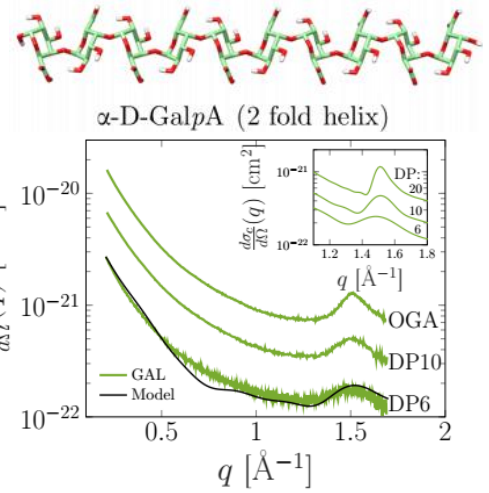


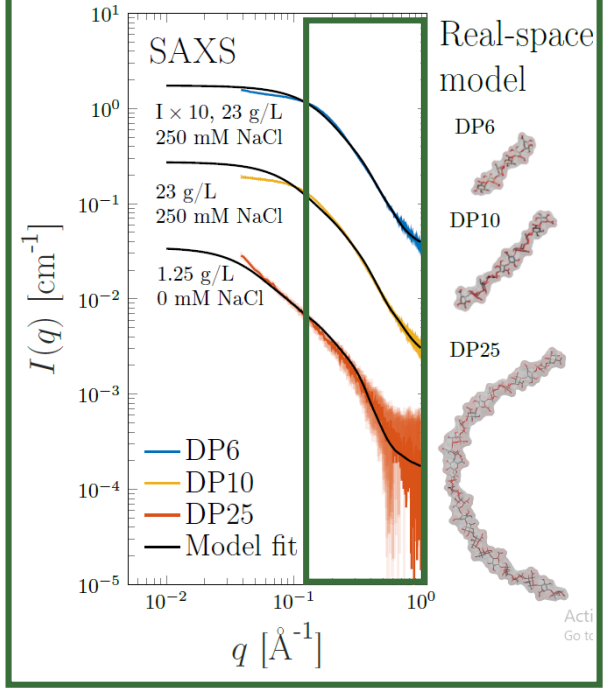
Sugar rings act as regularly spaced high electron density regions, broad diffraction peak.

Alginate



Pectin



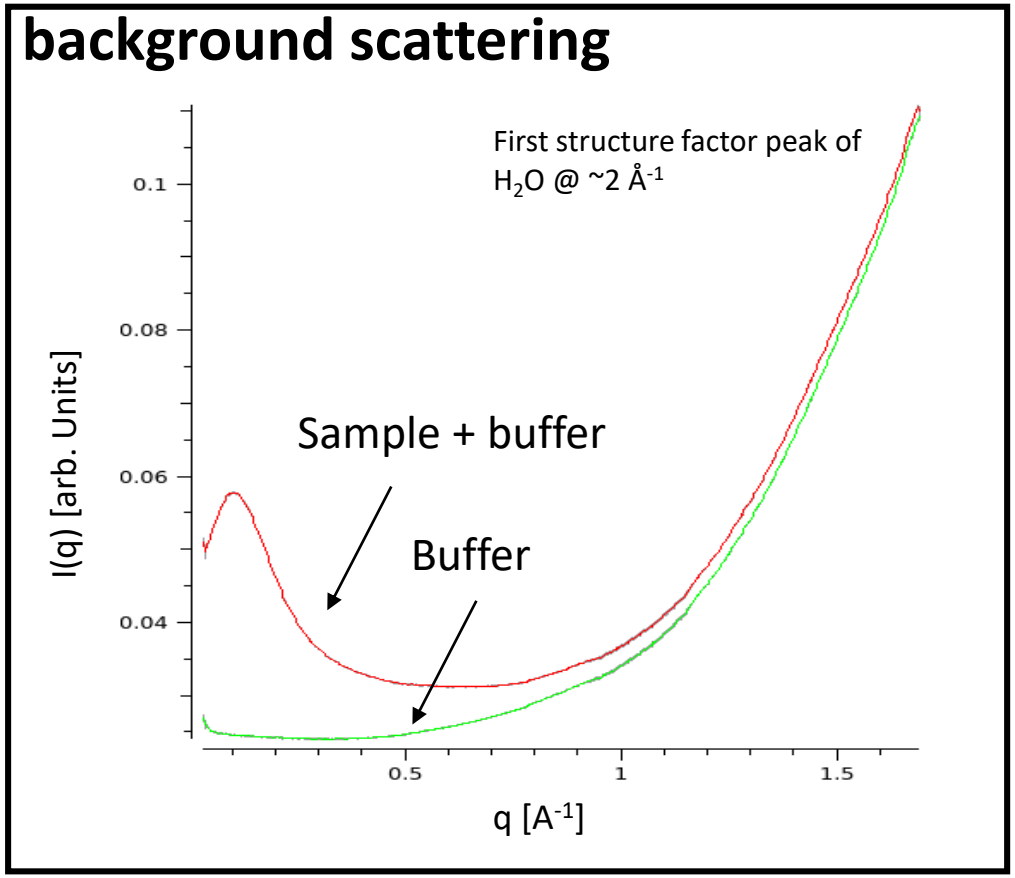


Mansel, B. W., Ryan, T. M., Chen, H. L., Lundin, L., & Williams, M. A. (2020). *Chemical Physics Letters*, *739*, 136951.

Mansel, B. W., Irani, A. H., Ryan, T. M., McGillivray, D. J., Chen, H. L., & Williams, M. A. (2019). *The European Physical Journal E*, *42*(2), 1-10.

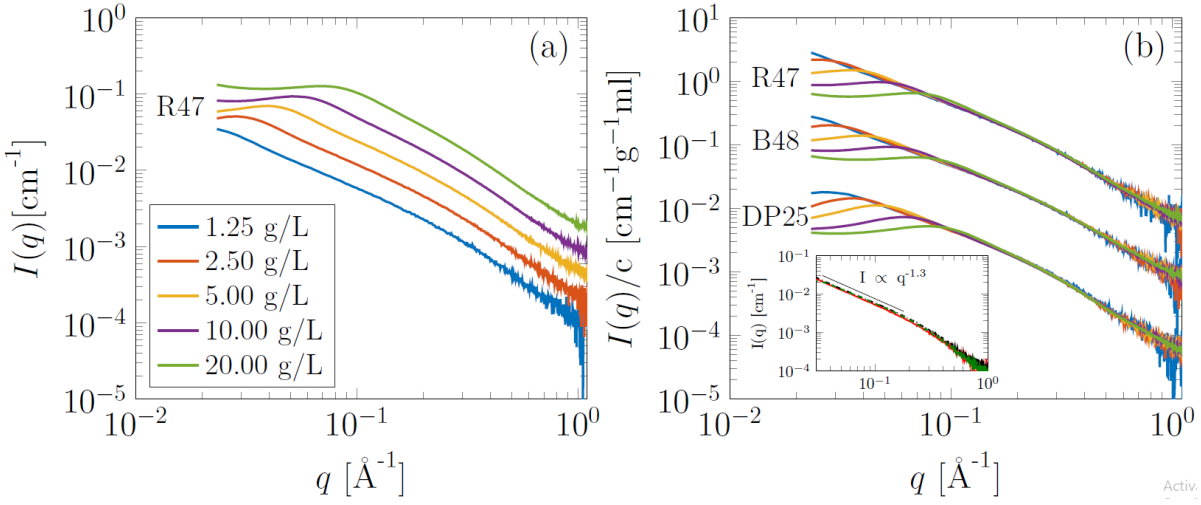
High-q: must take care!

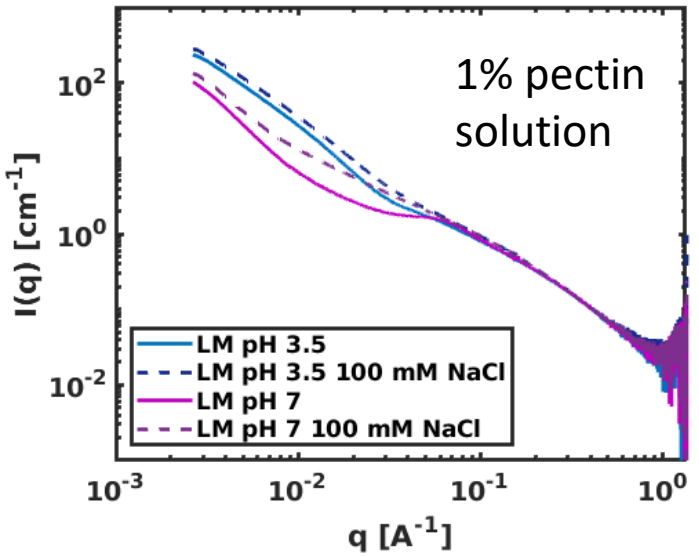
- Must have exact buffer for subtraction.
- Beamline transmission detector must be very accurate
- Sample concentration must be fairly high.
- In vacuum everything is best!



Intermediate-q (0.01 > q > 0.1 Å^{-1}) inter-chain correlation and / or flexibility

- Under low salt conditions significant structure factor effects makes obtaining useful information difficult or impossible
- If no structure factor the transition from rod-like to SAW or RW fractals can show either distance between chains or l_p at high molecular weights.
- Great care must be taken to extract l_p accurately.



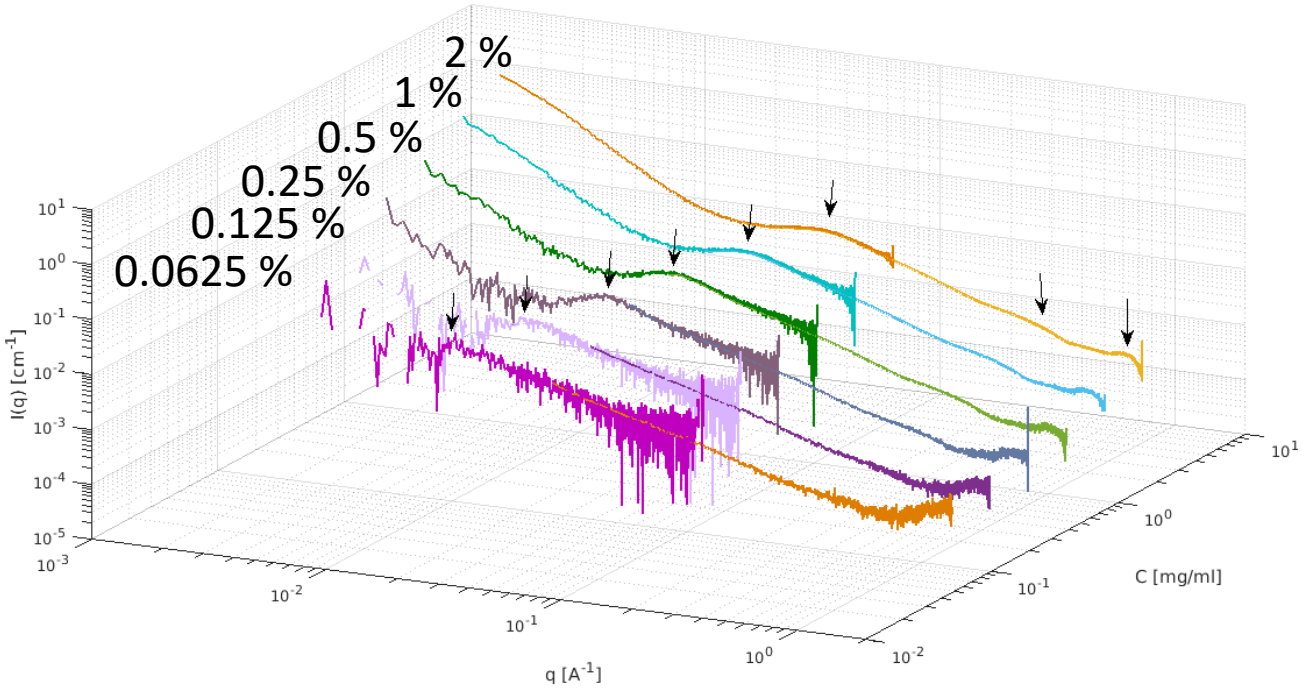


Mansel, B. W., Irani, A. H., Ryan, T. M., McGillivray, D. J., Chen, H. L., & Williams, M. A. (2019). *The European Physical Journal E*, *42*(2), 1-10.

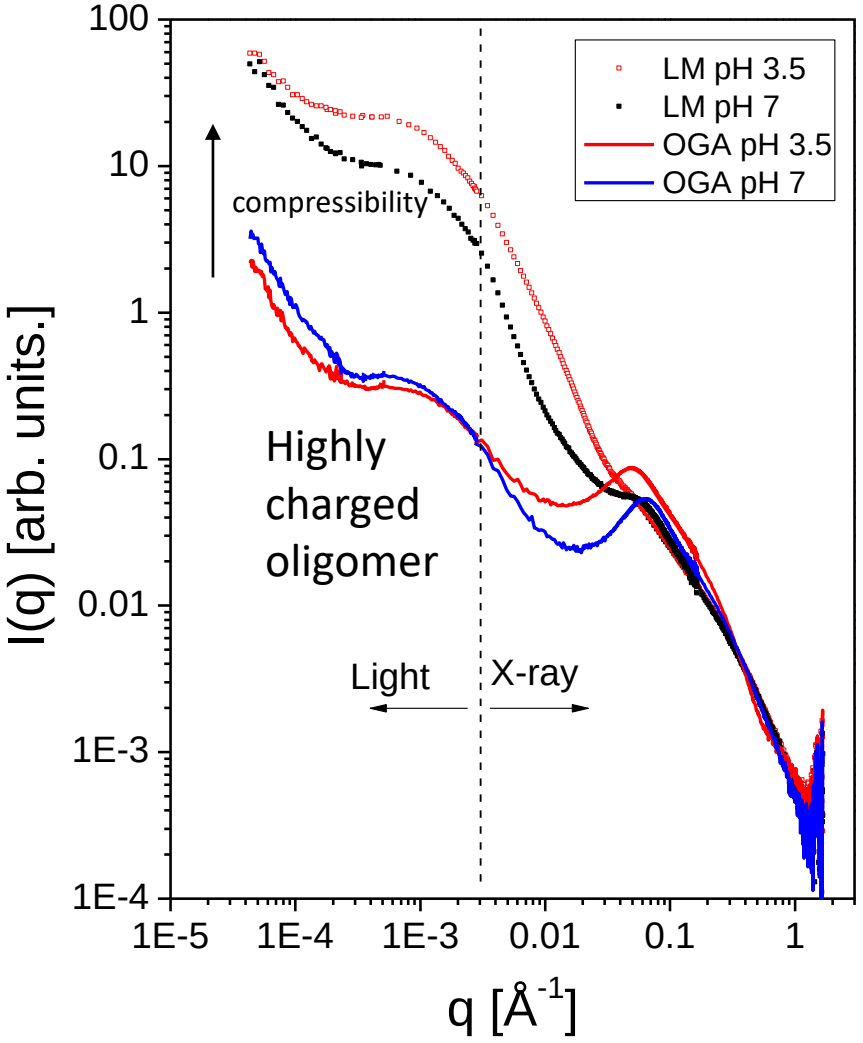
Low-q (q < 0.01 Å^{-1}) space filling properties or inter-chain effects

- Low-q provides insights into large length-scale polymer conformation, compressibility and inter chain effects.
- Clustering can be seen as a slope steeper than -2.
- Can be more difficult to measure due to beamline optics reducing S/N.

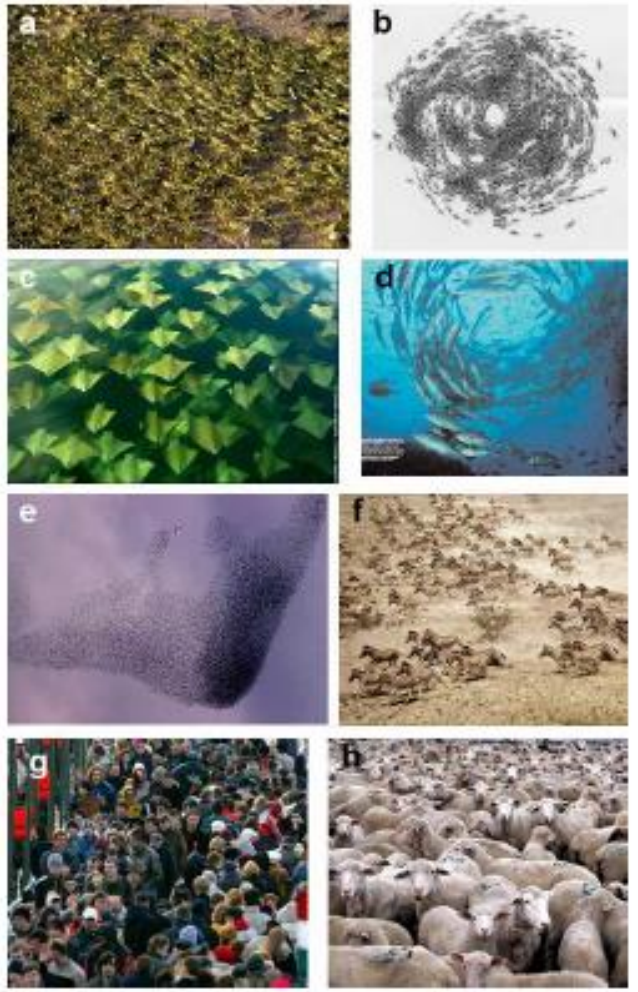
Australian synchrotron 7 & 0.7m S-D



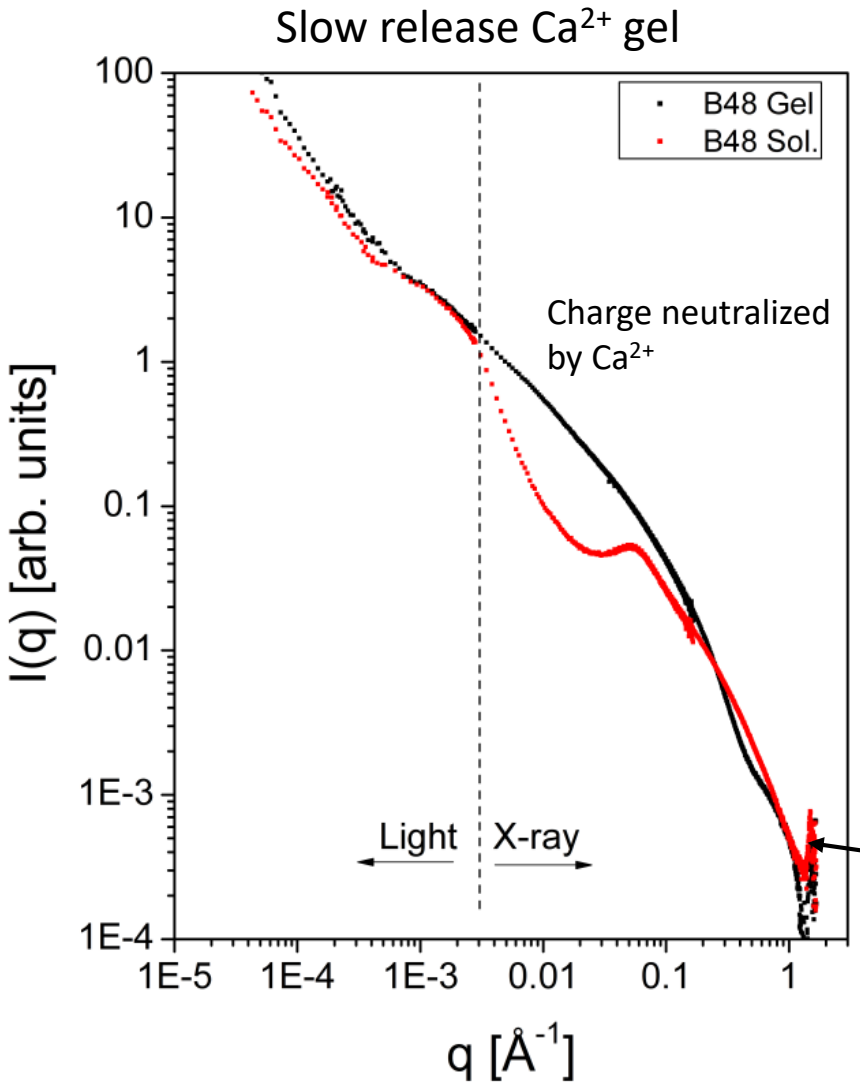
Putting it together: last solution(s)



- Low-q very complex!
- Compressibility plays a large role.
- Interpretation not easy.
- Can we use the low-q spatial inhomogeneity to make gels with different properties?

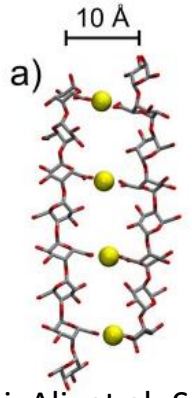


Sol to gel: What are the structural changes?



Author's work, unpublished

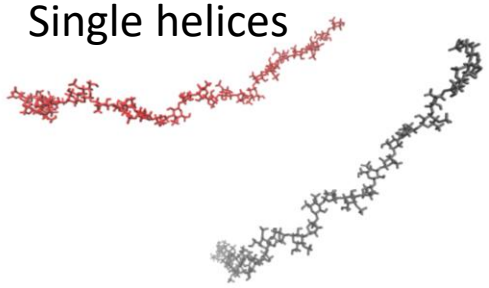
- Significant changes to the mechanical properties are not always related to significant changes in the scattered intensity.
- Repulsion to attractive interactions
- Pectin Ca^{2+} gels can be fine stranded.
- Essentially an arrested liquid.
- High-q shows some features of junction zones, although need to perform MD modelling to accurately characterize
- Phenomenological models not much use here



Assifaoui, Ali, et al. *Soft Matter* (2015)

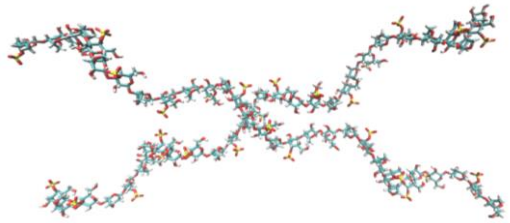
Carrageenan: loose single to double helix transition scattering vs MD

Single helices



No salt

100 ns

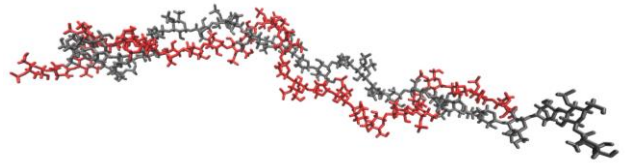


Starting structure

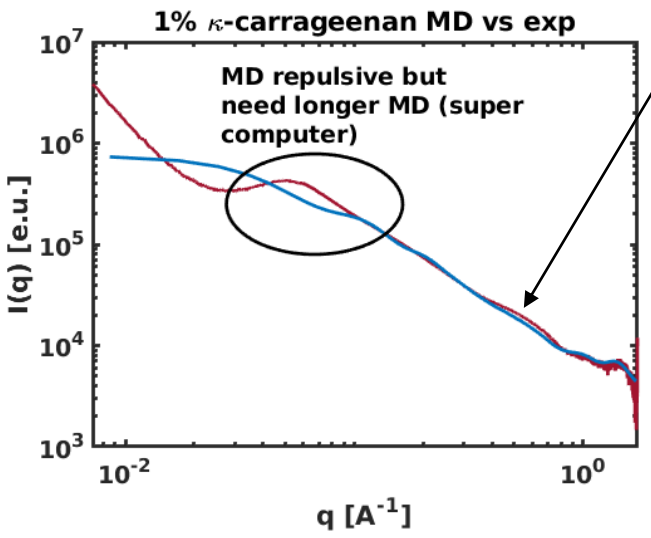
NaI

100 ns

Double helix

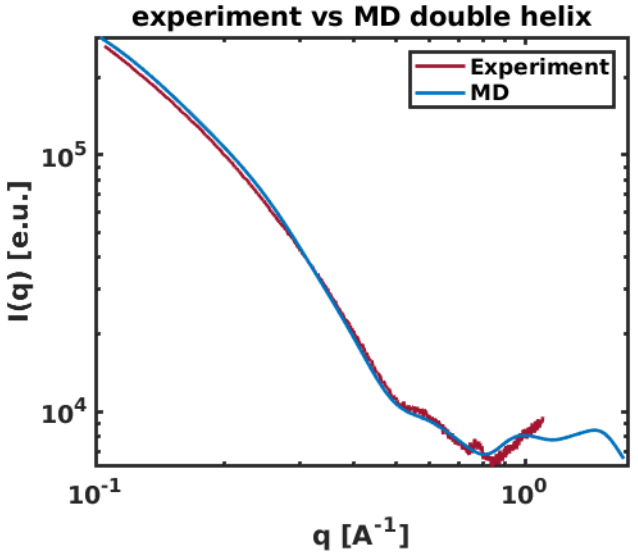


CRYSOL & <>



- In solution carrageen exists as well defined but loose single super helix (lots of dodgy literature on this!!!)
- Adding the correct ions makes assembly into double helix and subsequent gelation
- Ben has even better agreement between MD and scattering coming in the literature soon!!

CRYSOL & <>

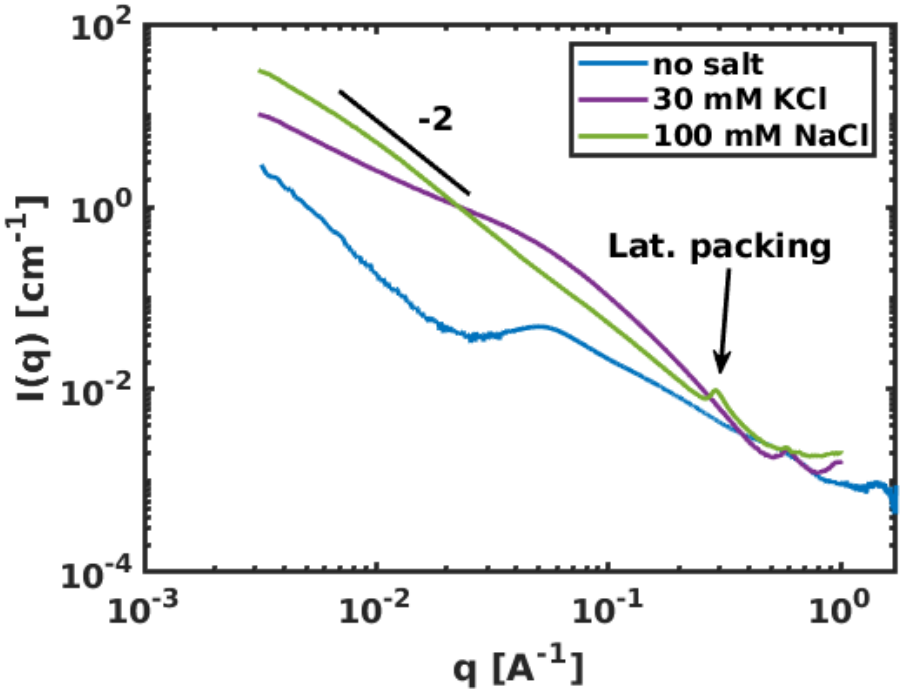


Ben Westberry's PhD work

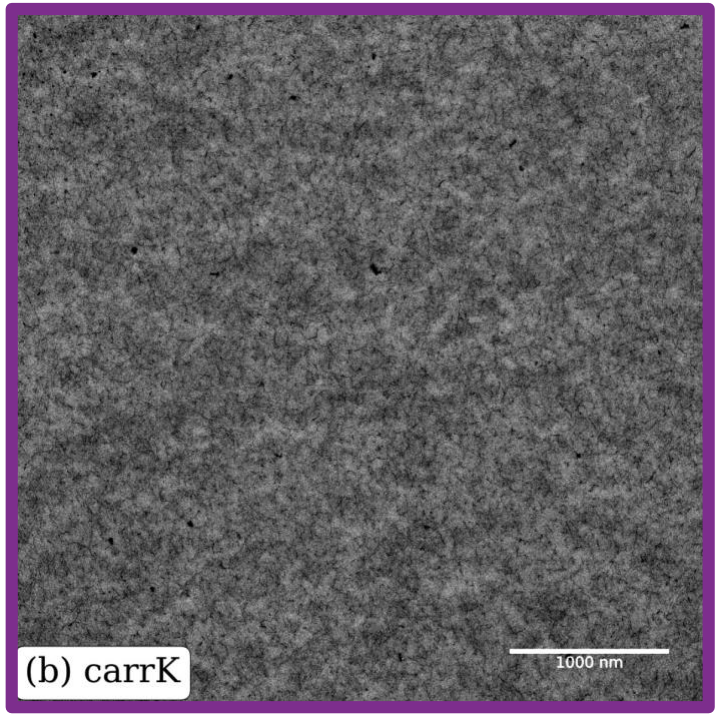
MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

The MacDiarmid Institute
for Advanced Materials and Nanotechnology

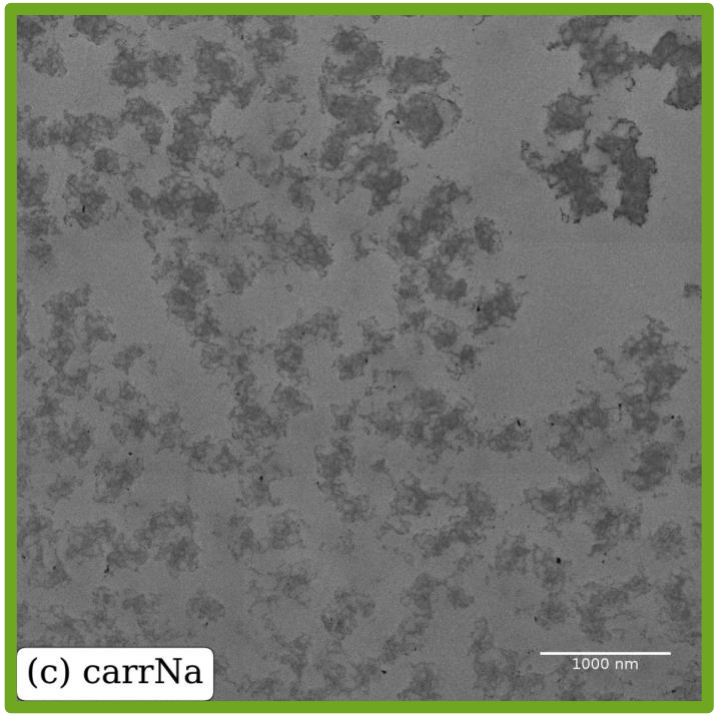
Carrageenan gel scattering ion effect



Ben's future MD shows this



Fine strand mostly double helix gel



Fractal aggregates, lateral packing in SAXS

Hernandez-Cerdan, **Leis, A**. et. al. (2018). Structural analysis of polysaccharide networks by transmission electron microscopy: comparison with small-angle X-ray scattering. *Biomacromolecules*, *19*(3), 989-995.

Conclusions

- Highly pure samples are key to success. Measurements while varying conditions can help tease out complex scattering.
- X-ray scattering from biopolymers is complex, inter- and intra- chain length scales overlap.
- Polyelectrolyte effects really dominate at low-q.
- Gelation can involve significant structural rearrangement from solution state or little, it depends on polymer and gelation method.
- Carrageenan exists as a loose helix in solution which transitions to double helix with the addition of ions.

Thanks for listening! Questions?

Further questions etc. mansel.bradley@nsrrc.org.tw