Synchrotron for medical and public health research

Nano structural characterization

Nuntaporn Kamonsutthipaijit
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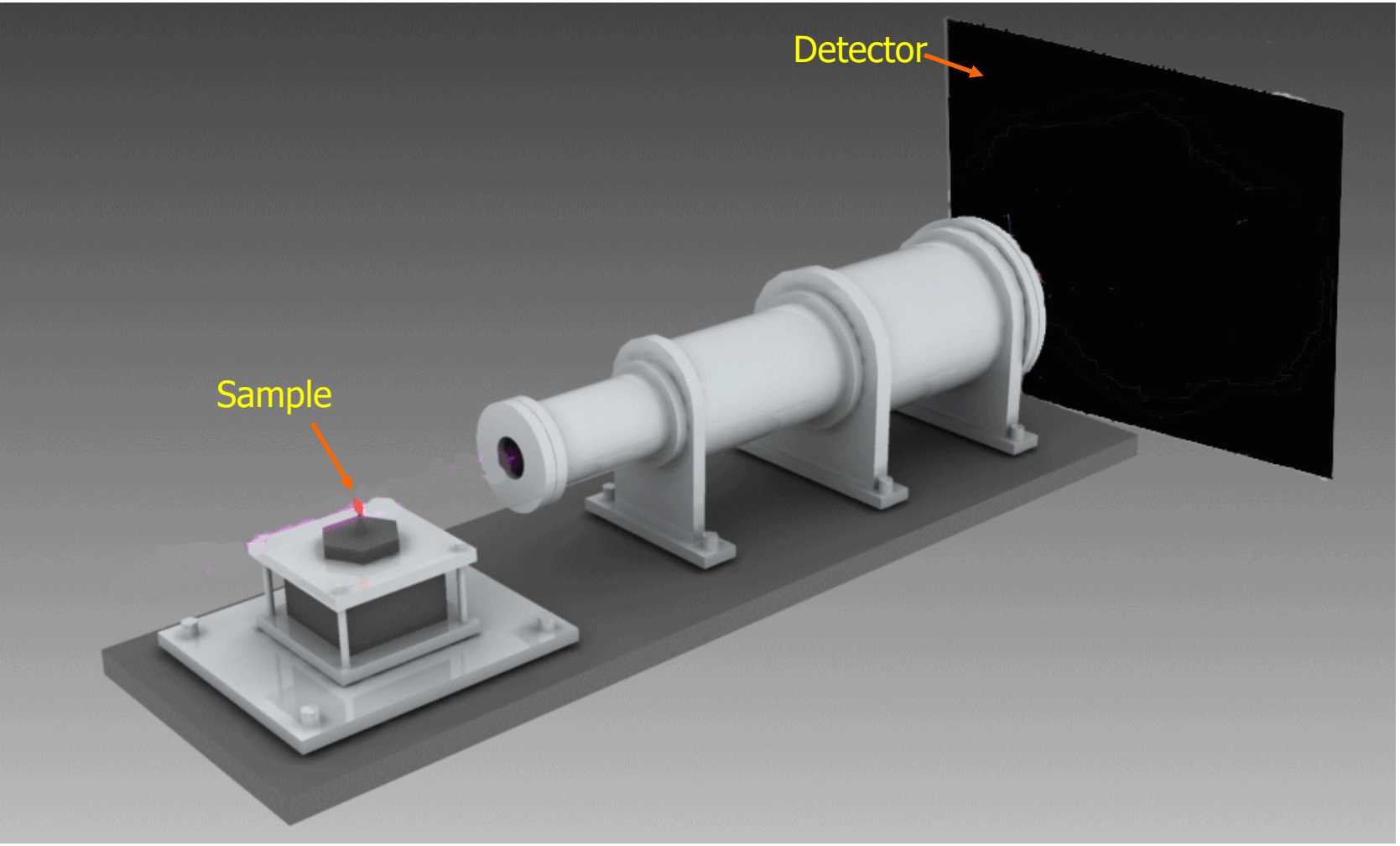
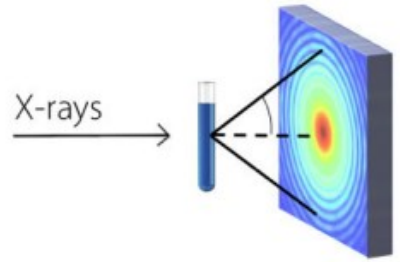
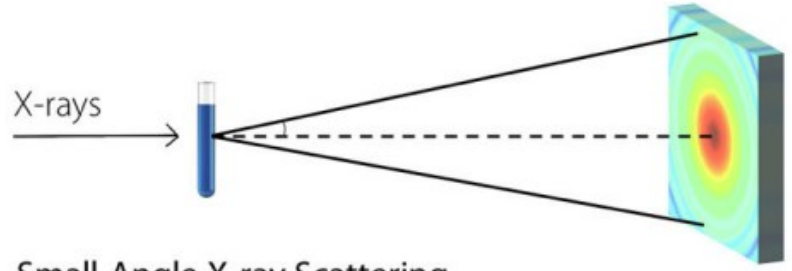
Small Angle X-ray Scattering (SAXS)





Wide-Angle X-ray Scattering



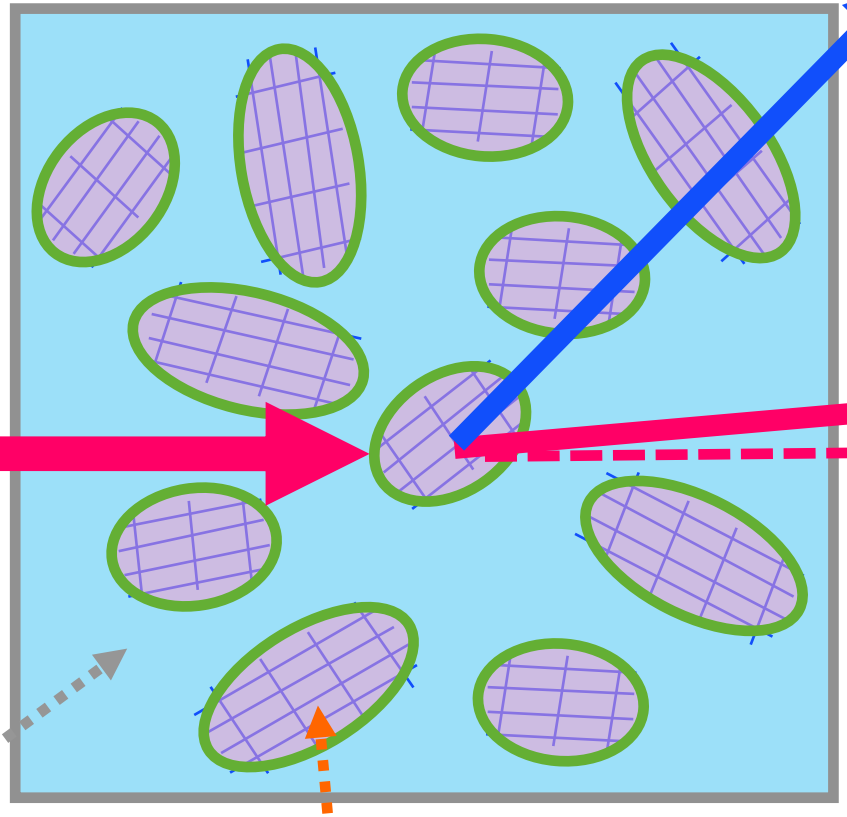
Small-Angle X-ray Scattering

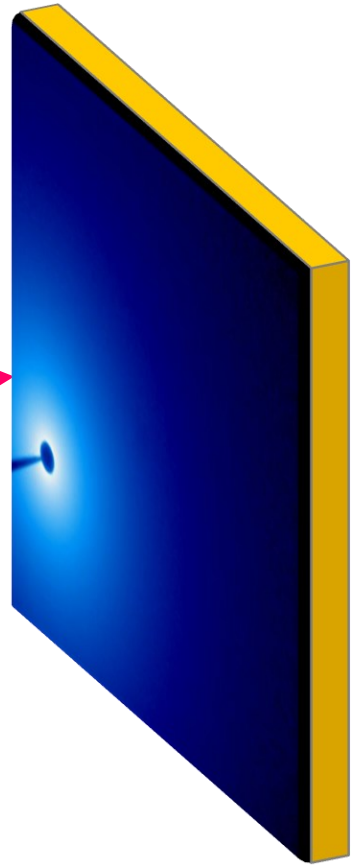
Small and Wide Angle

WAXS
Angles: 5-170°
atom-sized structures (crystal planes)

SAXS
Angle: 0-5°
scattering from whole particle (molecule)
Angle: 0-5°

INCIDENT X-RAY BEAM from Synchrotron

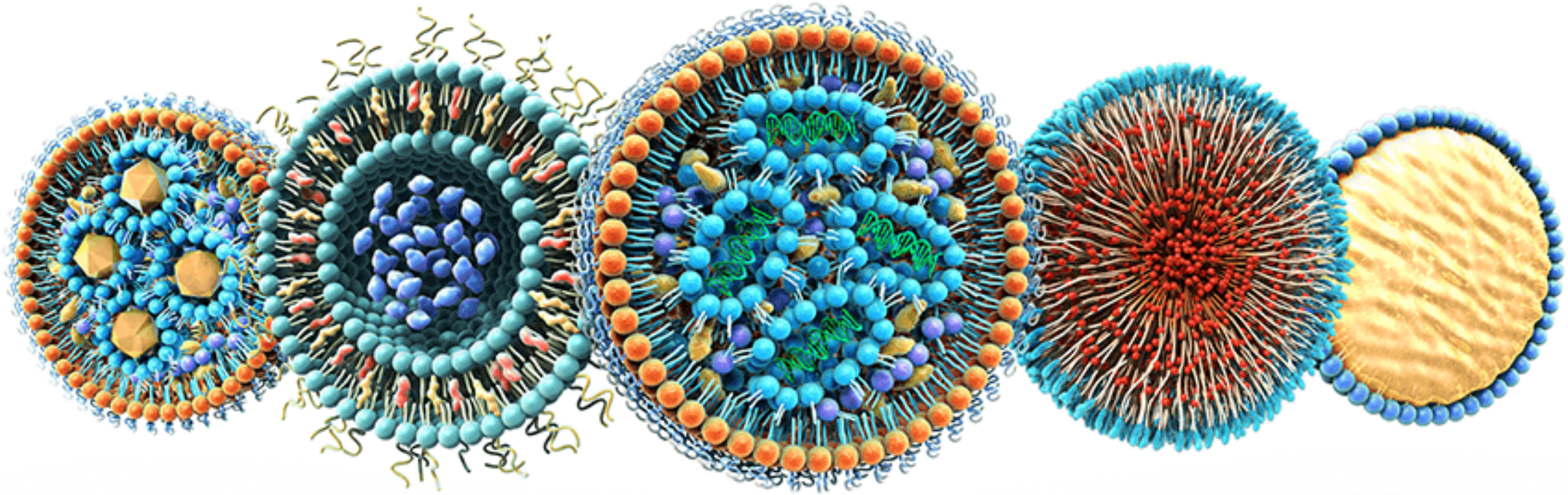




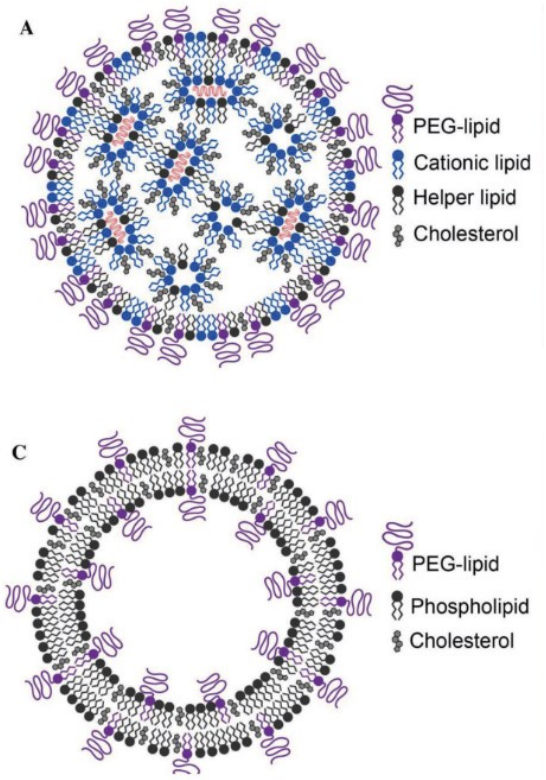
matrix (air, solid, fluid)

Particles, pores, molecules etc.

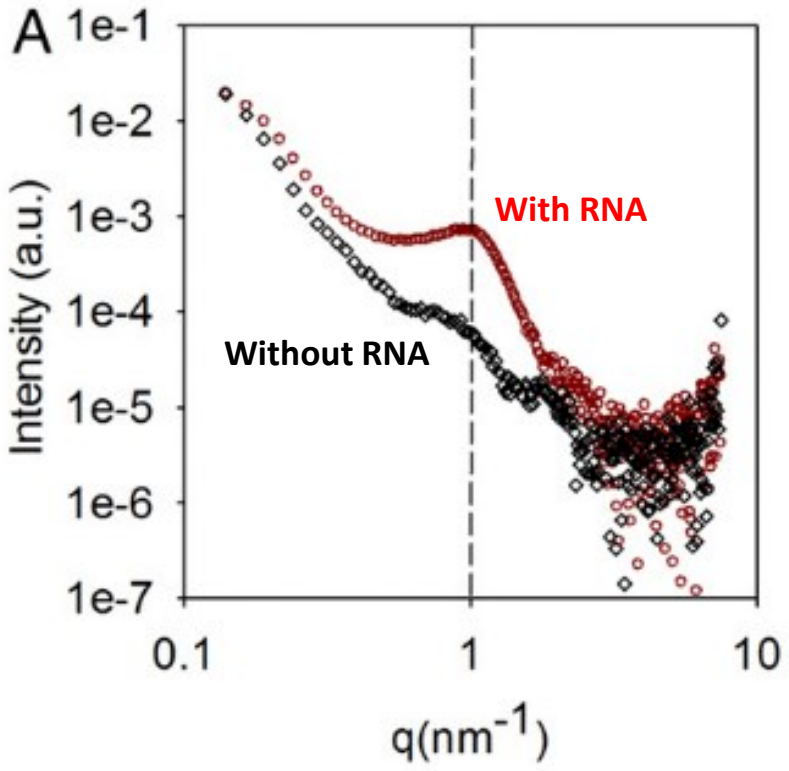
SAXS for Vaccines

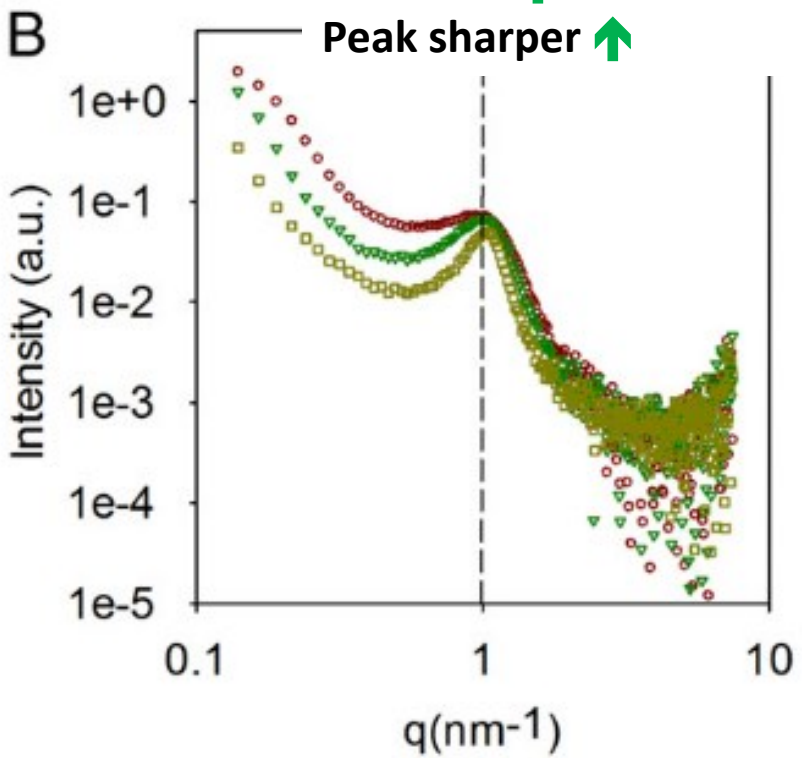


SAXS characterization of LNPs



%PEG ↓ (red-yellow)
Size LNPs ↑
Peak sharper ↑

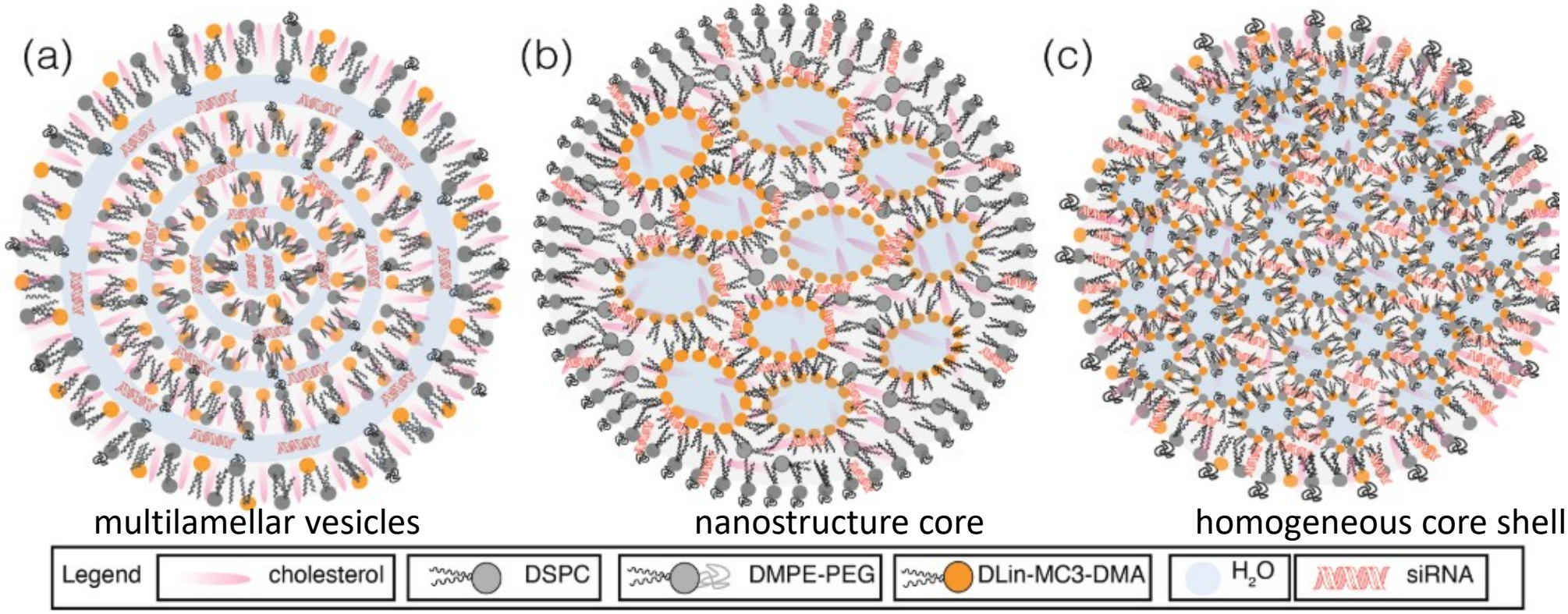




Arteta MY, Kjellman T, Bartesaghi S, Wallin S, Wu X, Kvist AJ, Dabkowska A, Székely N, et al (2018) Successful reprogramming of cellular protein production through mRNA delivered by functionalized lipid nanoparticles. *Proc Natl Acad Sci U S A* **115**, E3351–60.

Proposed models for siRNA-LNP and mRNA-LNP structure

SAXS measurements reveal structural differences between lipid nanoparticles with and without RNA and the evolution of RNA filled LNPs as a function of temperature and pH



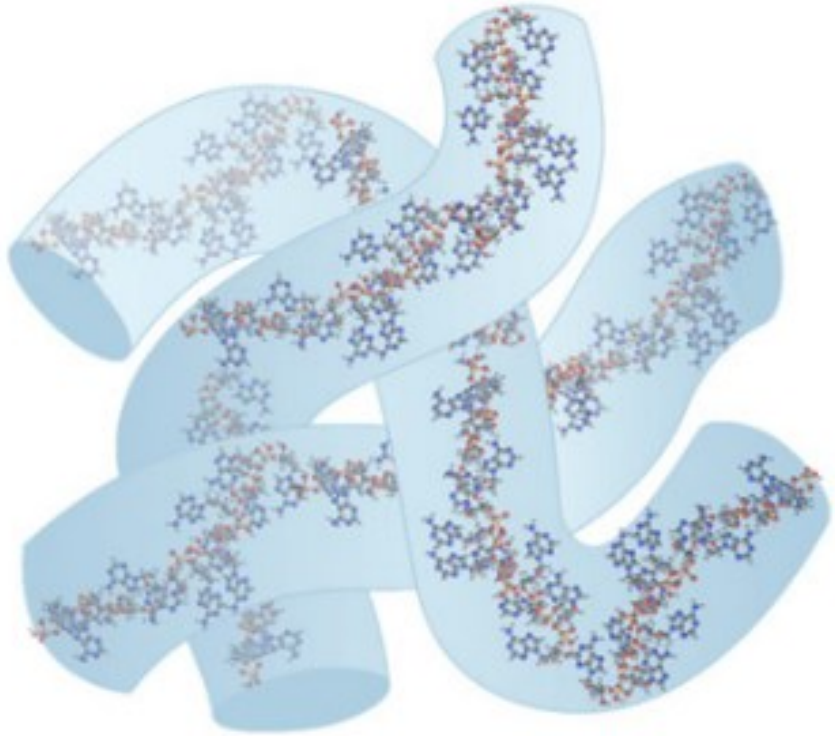
Schoenmaker L, Witzigmann D, Kulkarni JA, Verbeke R, Kersten G, Jiskoot W, Crommelin DJA (2021) mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability. *Int J Pharm* **601**, 120586.

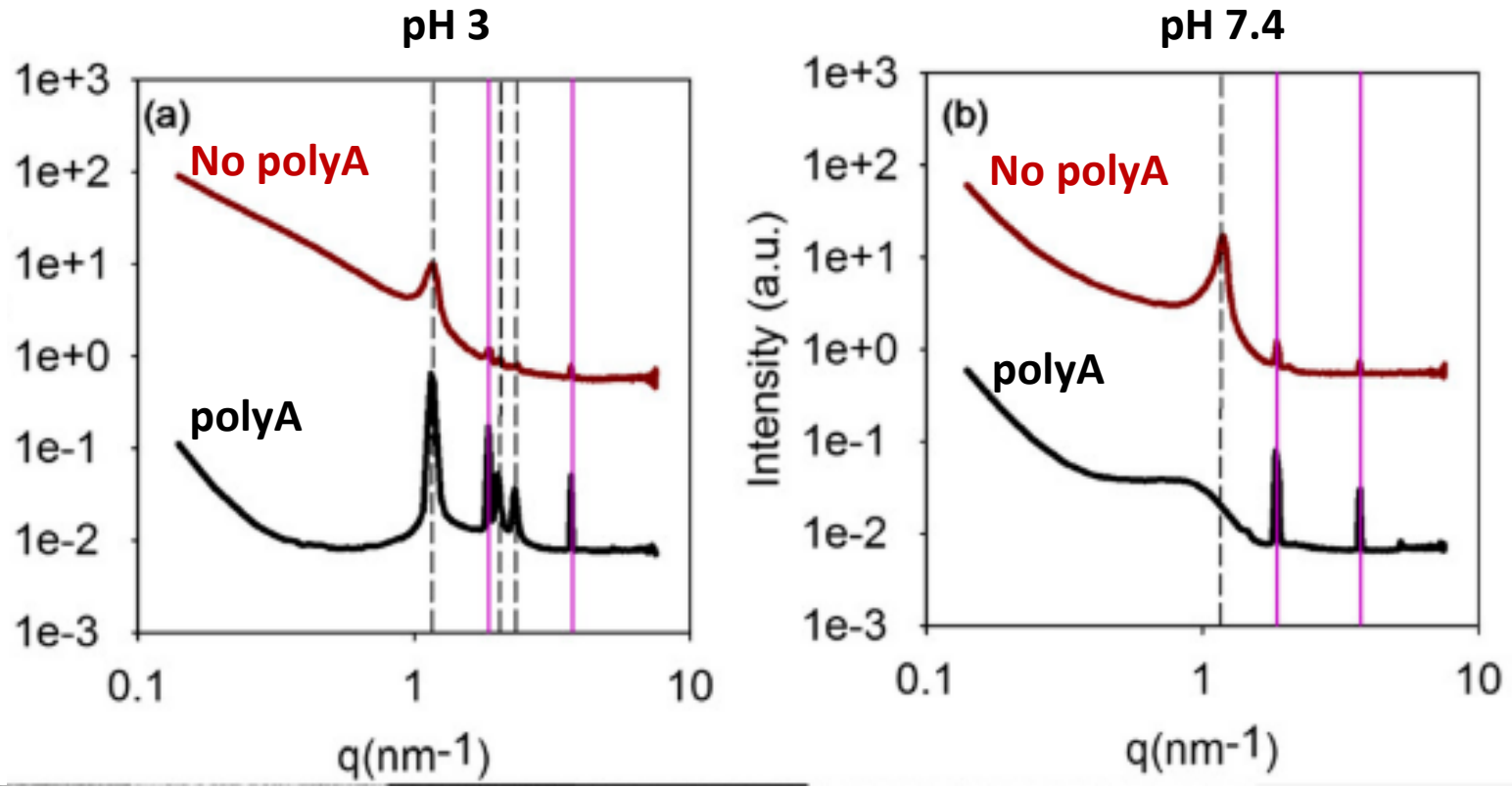
Core structure of the LNPs and cholesterol solubility by SAXS

---- Hexagonal peak

—— Cholesterol content

Inverse hexagonal



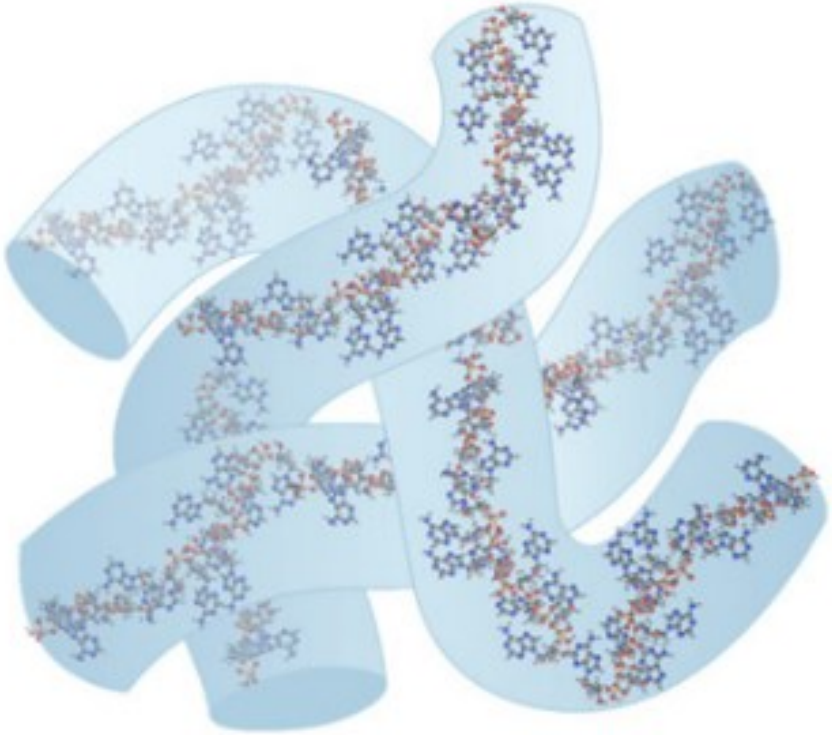


mRNA is located inside water cylinders, which are surrounded by cationic lipids

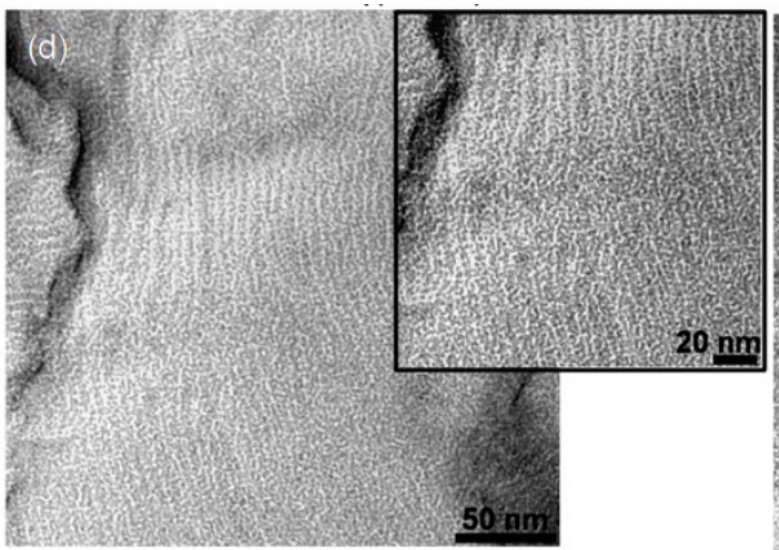
Arteta, M. Y. et al. Successful reprogramming of cellular protein production through mRNA delivered by functionalized lipid nanoparticles. Proc. Natl. Acad. Sci. U. S. A. 115, E3351–E3360 (2018).

Core structure of the LNPs and cholesterol solubility by TEM

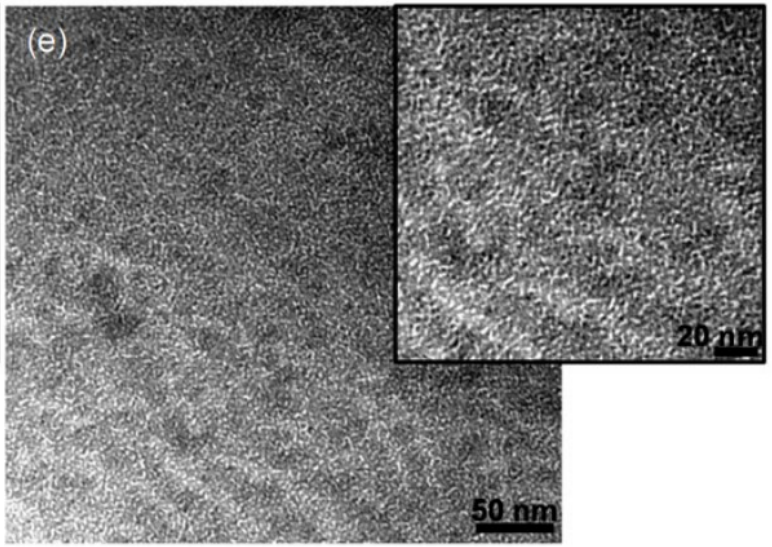
Inverse hexagonal



pH 3



pH 7.4



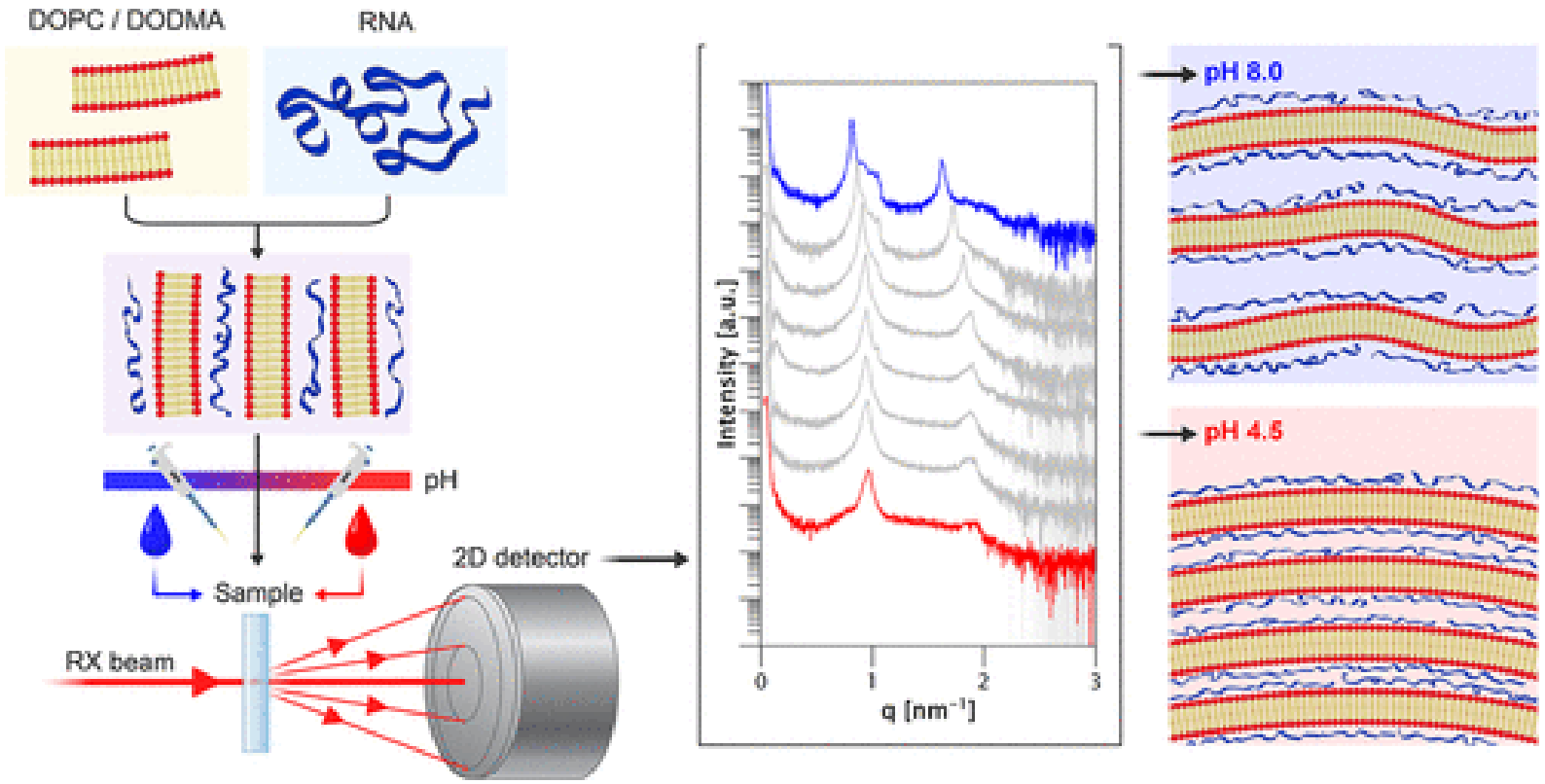
Arteta, M. Y. et al. Successful reprogramming of cellular protein production through mRNA delivered by functionalized lipid nanoparticles. Proc. Natl. Acad. Sci. U. S. A. 115, E3351–E3360 (2018).

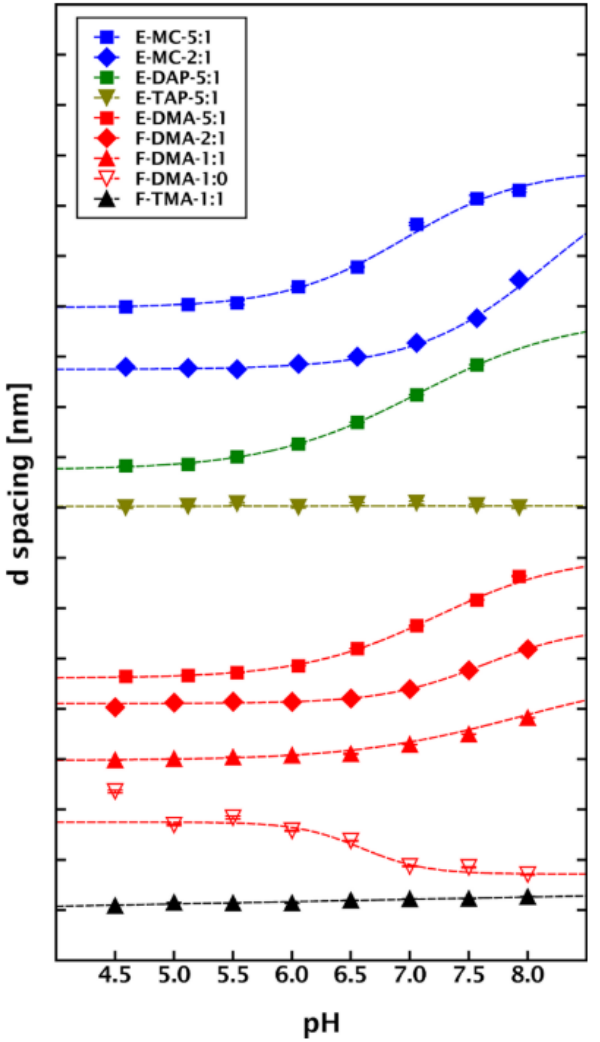
Synchrotron (EMBL facilities) support development of RNA vaccines

Biotechnology company BioNTech and Johannes Gutenberg University Mainz conduct collaborative research with EMBL scientists at the beamline P12 in Hamburg

The beamline P12 at EMBL Hamburg allows studying the structure of molecules in solution using small-angle X-ray scattering (SAXS) technique. Svergun group/EMBL

Investigation of pH-Responsiveness inside Lipid Nanoparticles for Parenteral mRNA Application Using Small-Angle X-ray Scattering





Uebbing, L. et al. Investigation of pH-Responsiveness inside Lipid Nanoparticles for Parenteral mRNA Application Using Small-Angle X-ray Scattering. Langmuir 36, 13331–13341 (2020).

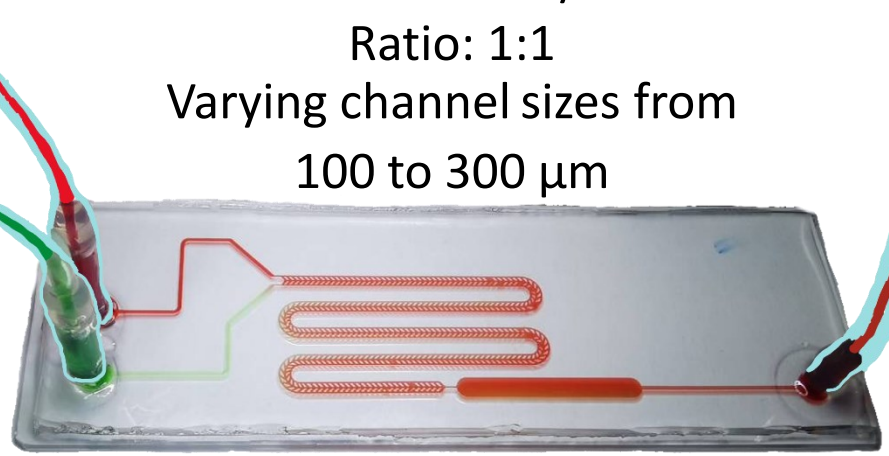
Lipid Nanoparticles for RNA Vaccine

DI water

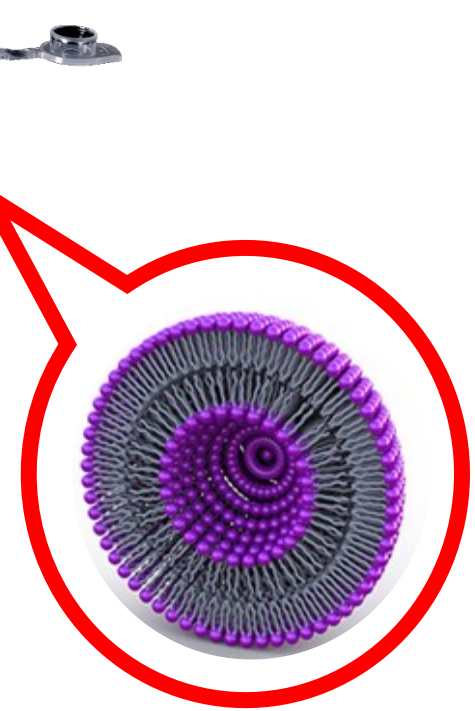
Lipids in EtOH

syringe pump

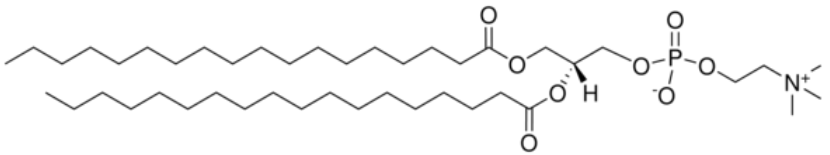
Flowrate: 1 mL/min
Ratio: 1:1
Varying channel sizes from 100 to 300 µm



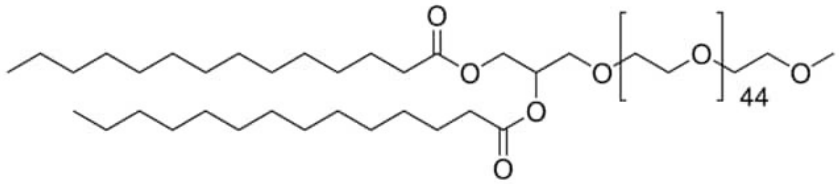
Chip

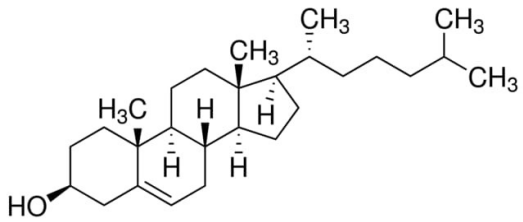


DPSC



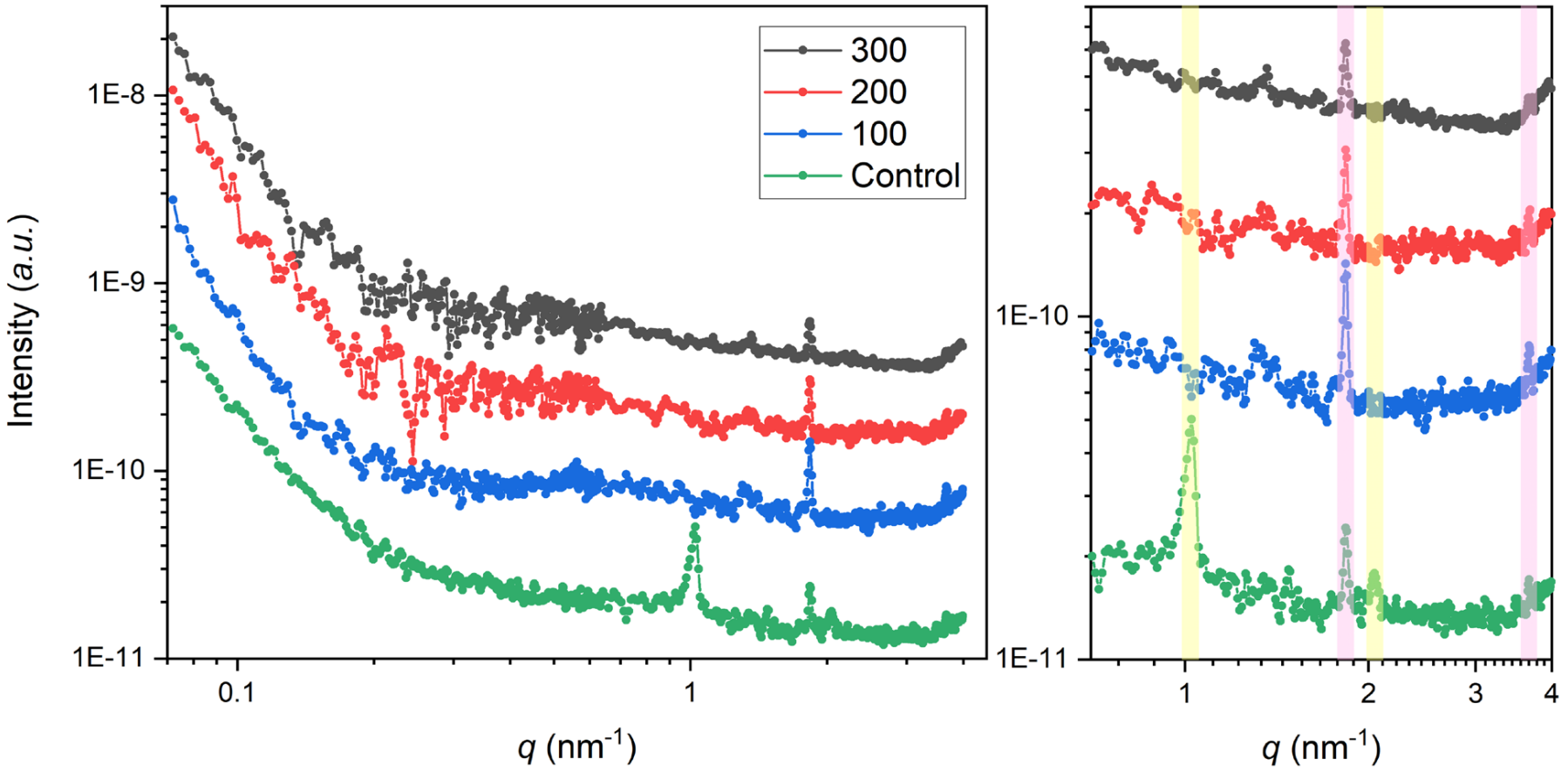
DMG-PEG2000

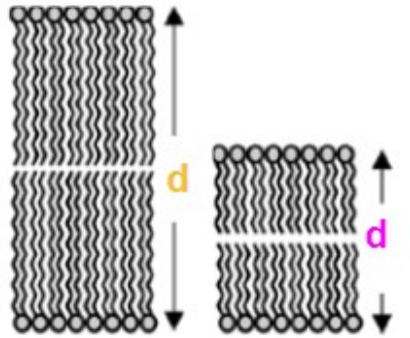




Cholesterol

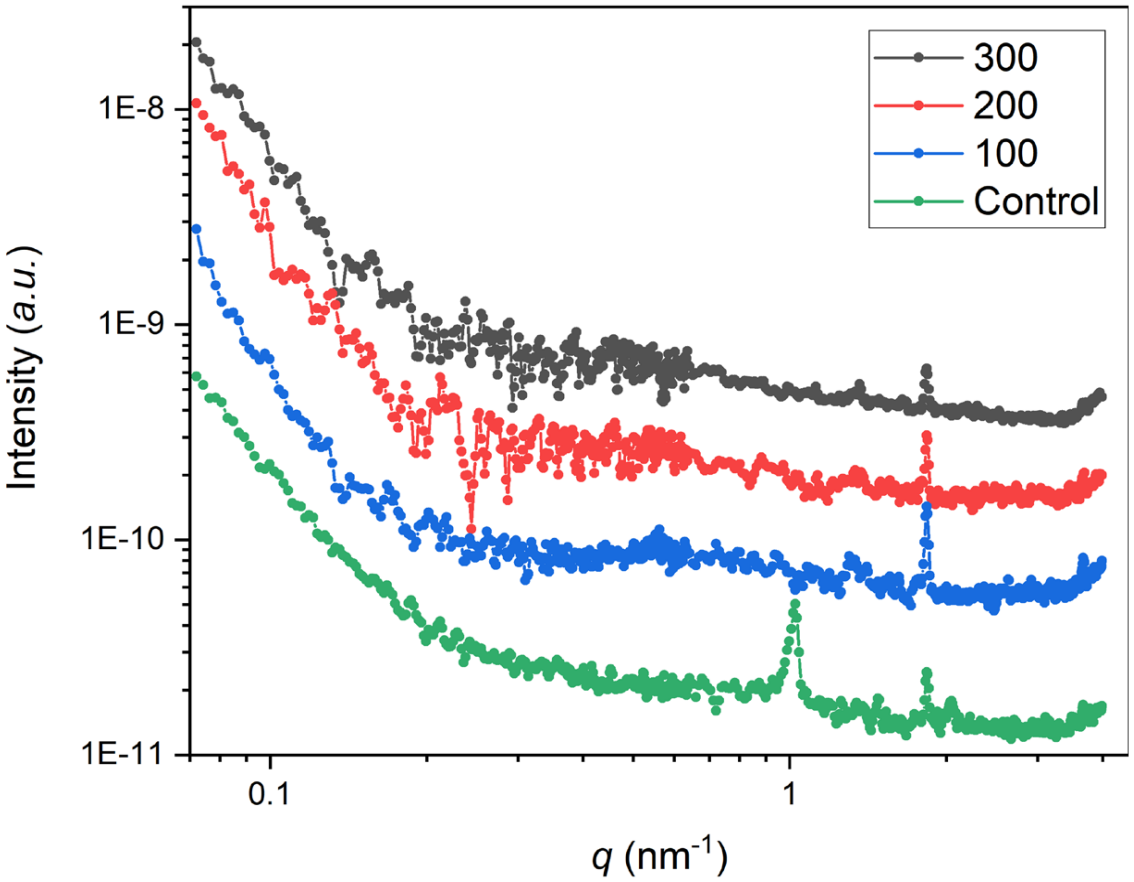
Lipid Nanoparticles for RNA Vaccine



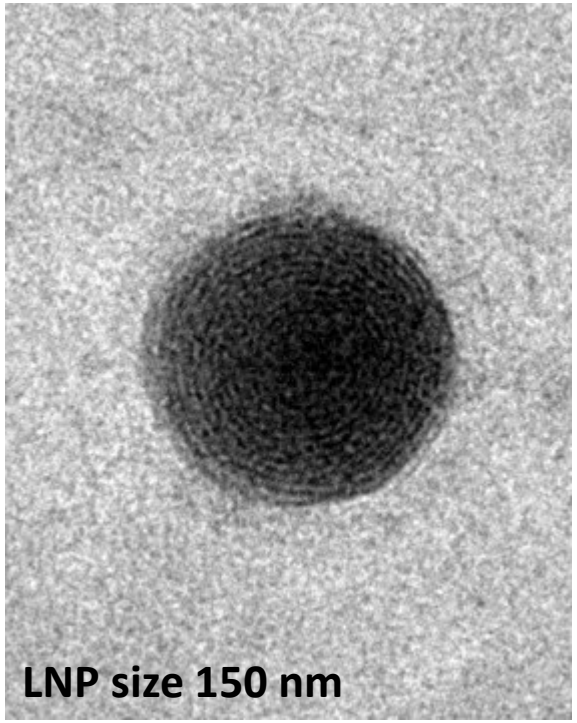


SAXS

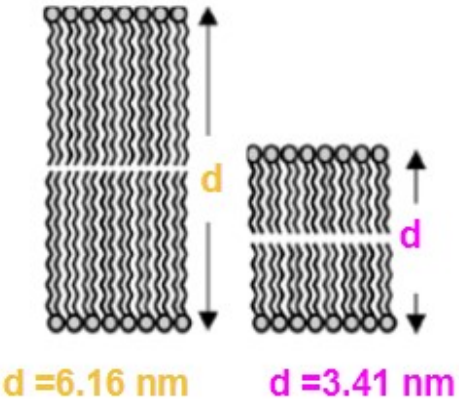
Lipid Nanoparticles for RNA Vaccine



SAXS



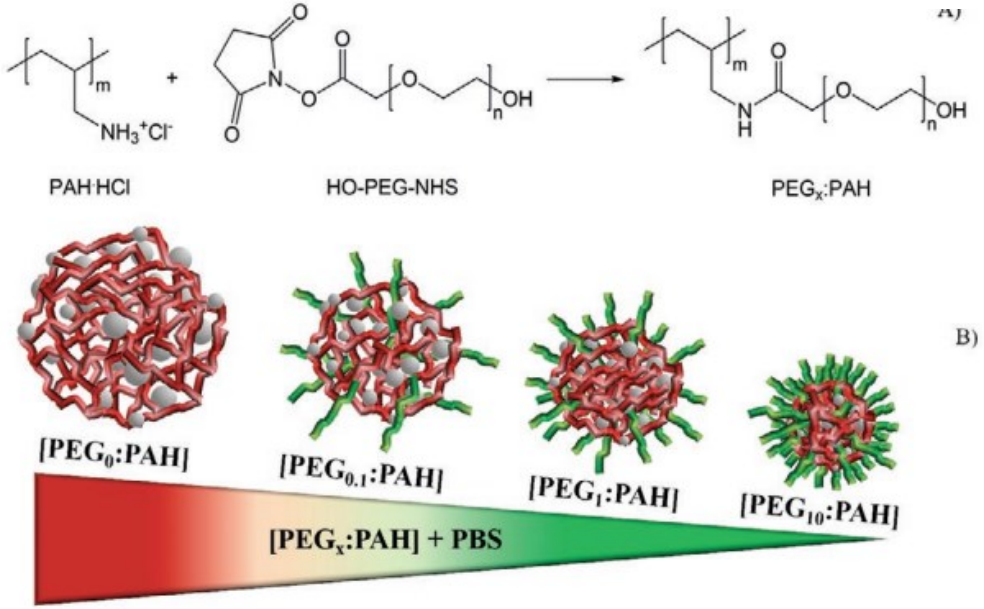
TEM



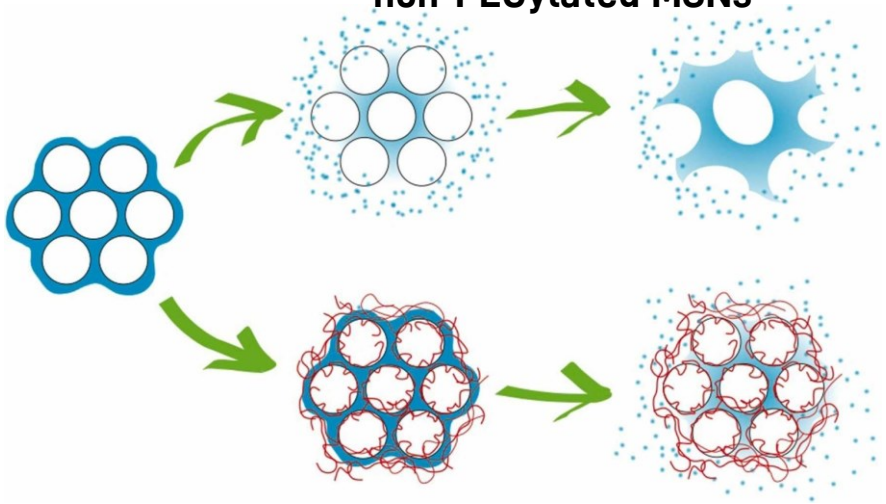
SAXS for Supramolecular Polyamine, Drug delivery

CIC biomaGUNE
MEMBER OF BASQUE RESEARCH & TECHNOLOGY ALLIANCE

Soft Matter Nanotechnology Lab



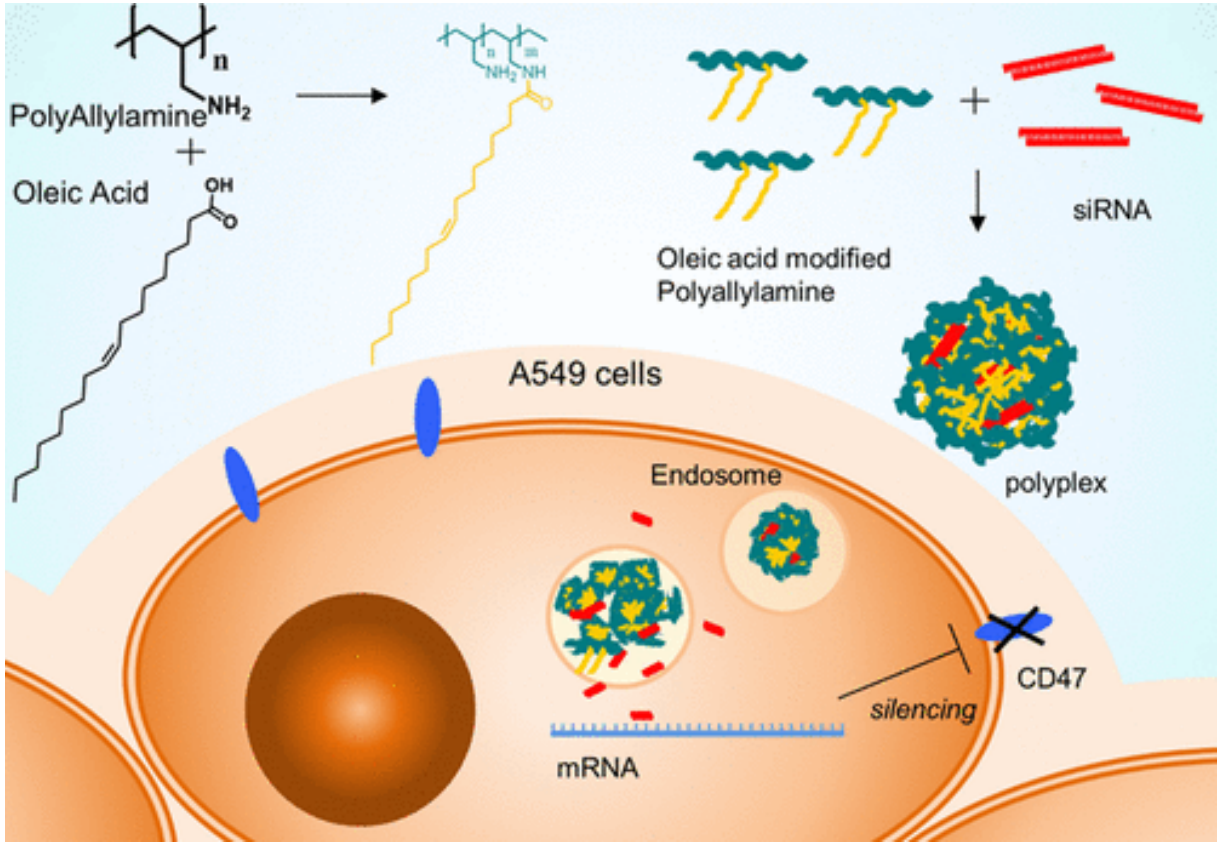
Scheme 1. Synthetic procedure followed for the preparation of the PEGylated PAH NPs. On top PEGylation of PAH. On the bottom, scheme of the PANs obtained for the different PEG_x:PAH polymers in presence of PBS.



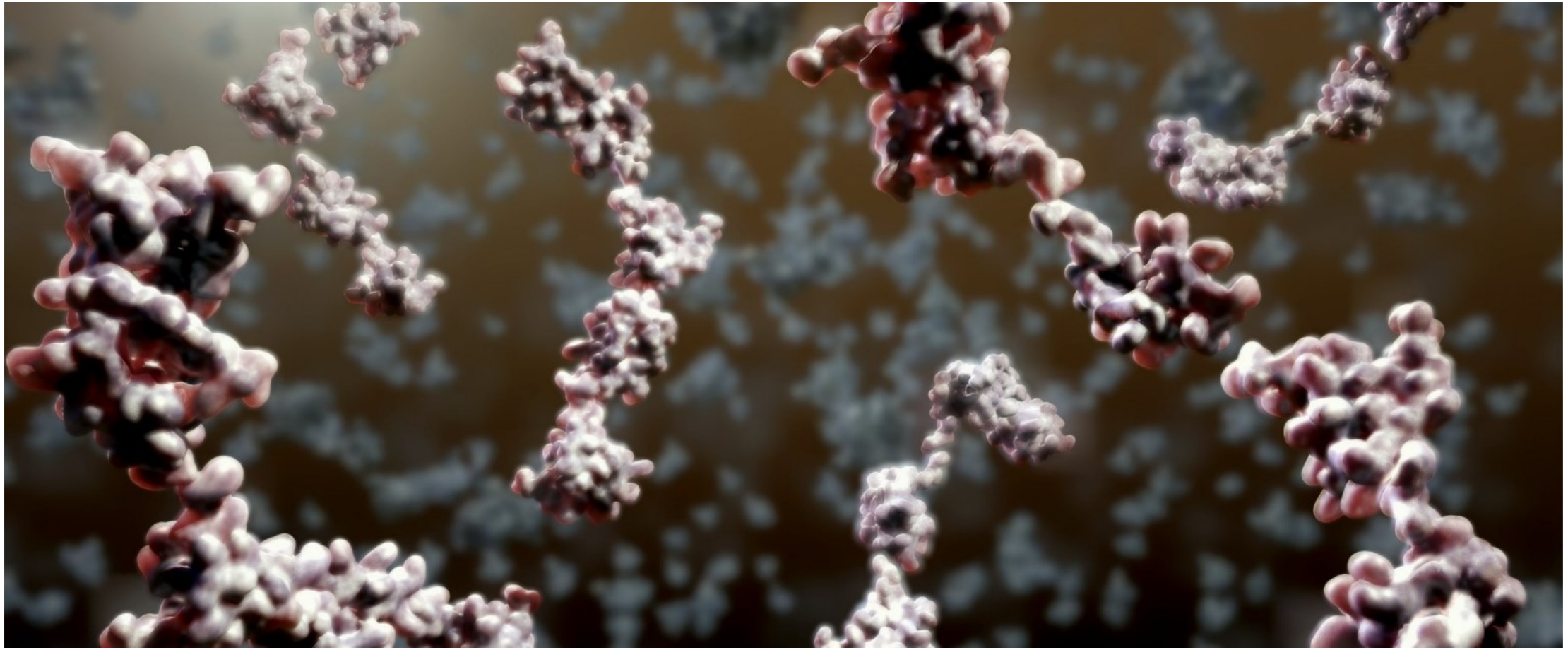
Sergio Moya

SAXS for Supramolecular Polyamine, Drug delivery

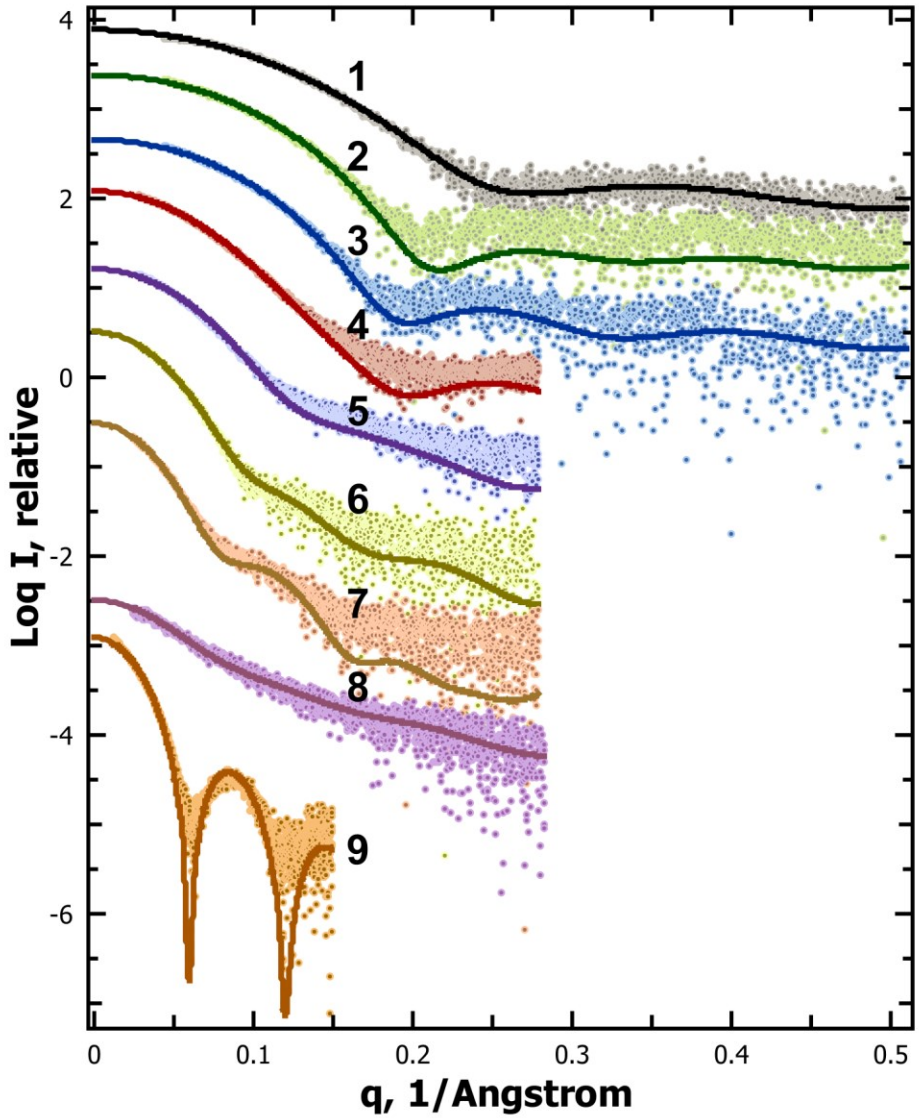
@suprogenproject researchers Paolo and Bruno from @CICbiomaGUNE analyze their polymer carriers at the SAXS beamline at the @elettrasincro in Trieste.

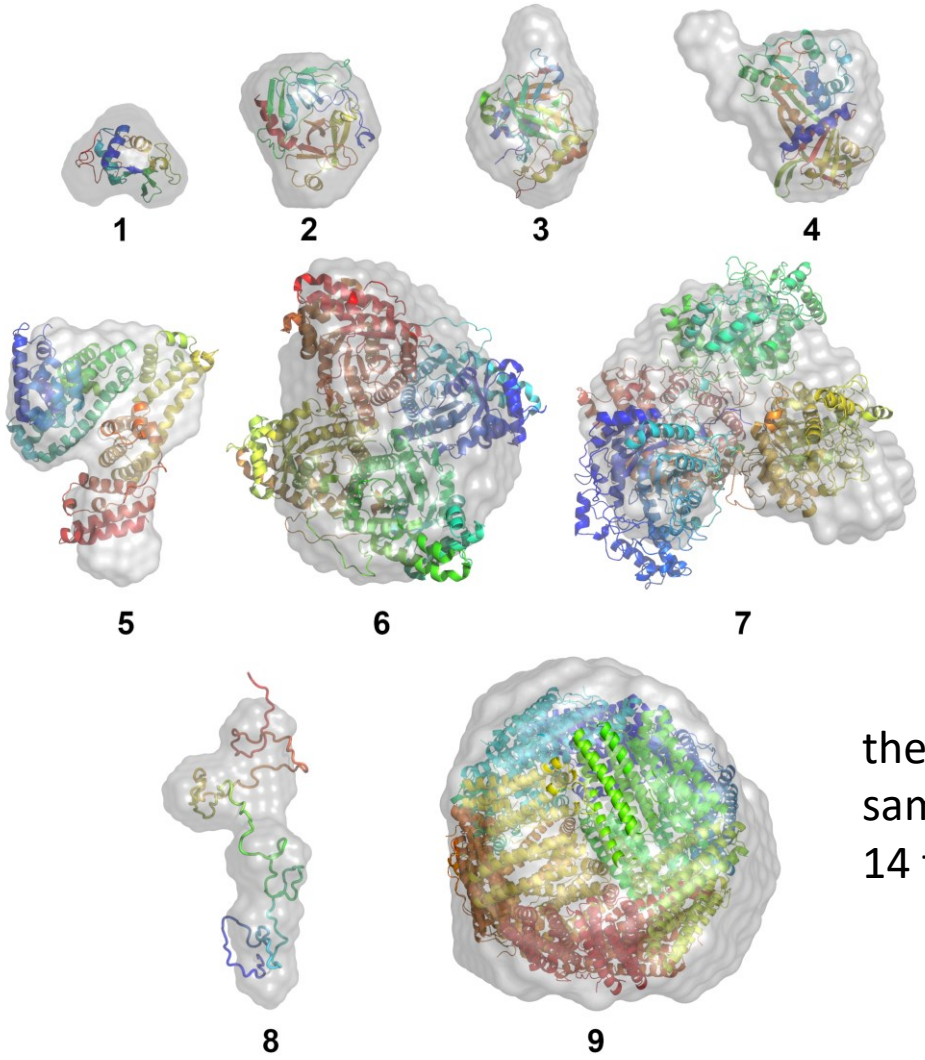


SAXS for Structural Protein in Solution



SAXS for Structural Protein in Solution





the MWs of protein samples range from 14 to 400 kDa

Kamonsutthipaijit, N. *et al. ScienceAsia* **2022**, *48* (4), 399–405.

Archives of Biochemistry and Biophysics 653 (2018) 24–38

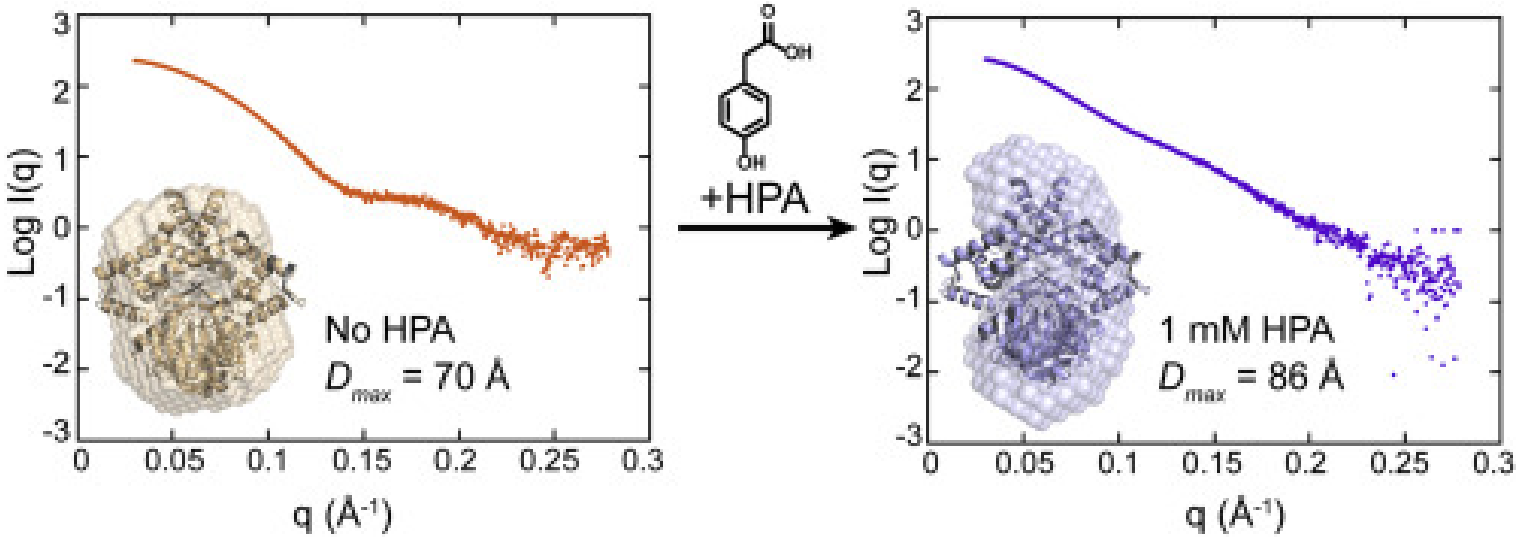
Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Crystal structure of the flavin reductase of *Acinetobacter baumannii* p-hydroxyphenylacetate 3-hydroxylase (HPAH) and identification of amino acid residues underlying its regulation by aromatic ligands

Anan Yuenyao[a], Nopphon Petchyam[a,1], Nuntaporn Kamonsutthipaijit[b], Pimchai Chaiyen[a,c], Danaya Pakotiprapha[a,*]



Hightlights:

- **C1 is a flavin reductase that is regulated by p-hydroxyphenylacetate (HPA).**
- **C1 contains an N-terminal flavin reductase domain and a C-terminal MarR domain.**
- **Binding of HPA to MarR domain causes structural change that relieves autoinhibition.**
- **In the context of C1, MarR domain is repurposed for control of enzyme activity.**

SAXS was used to probe the BSA dimerization in the solution

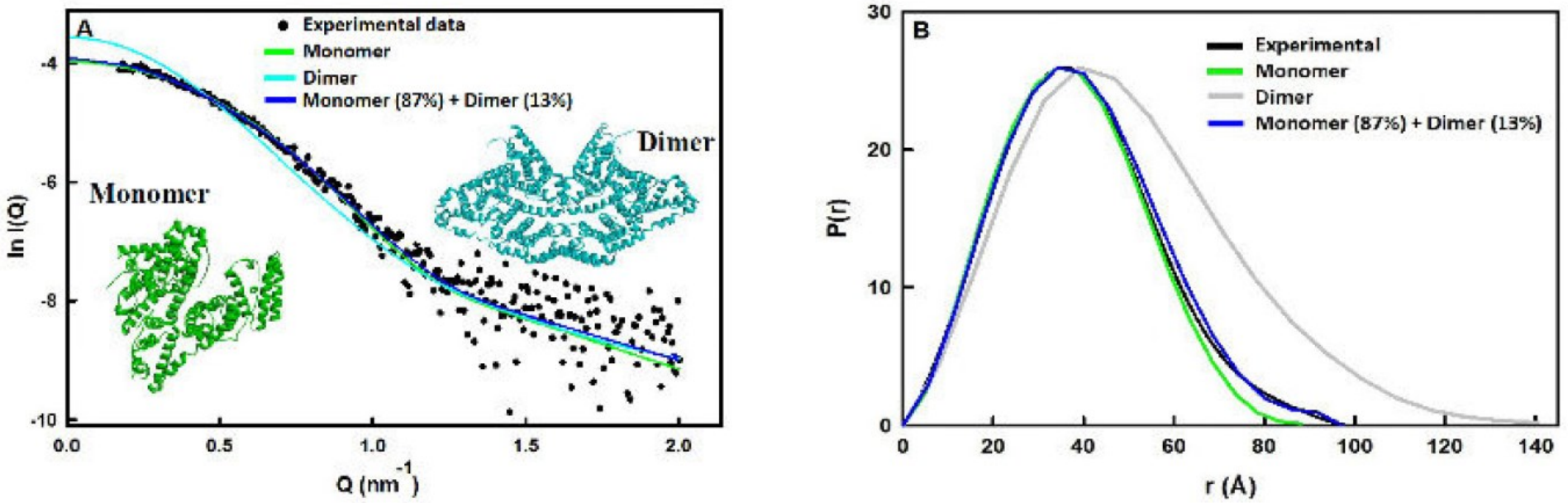
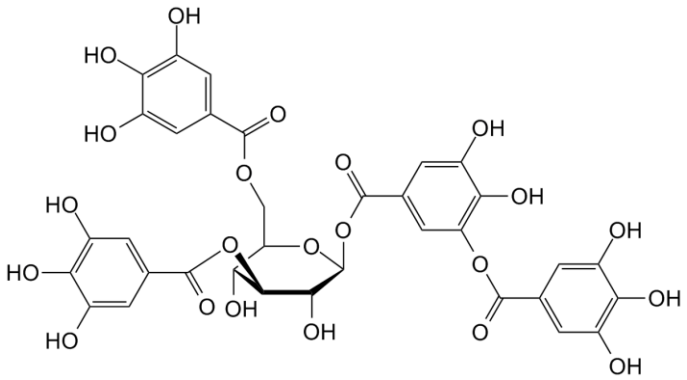


Figure 3. SAXS modeling curve fits and the *P*(*r*) distance distribution function. (A) Depicts the *I*(*Q*) curve fits for BSA monomer and dimer crystal structures (4f5s) and the monomer/dimer merged curve. (B) Shows *P*(*r*) distance distribution fits. The blue lines correspond to the merged curves of monomer and dimer using scattering weights of 87/13%.

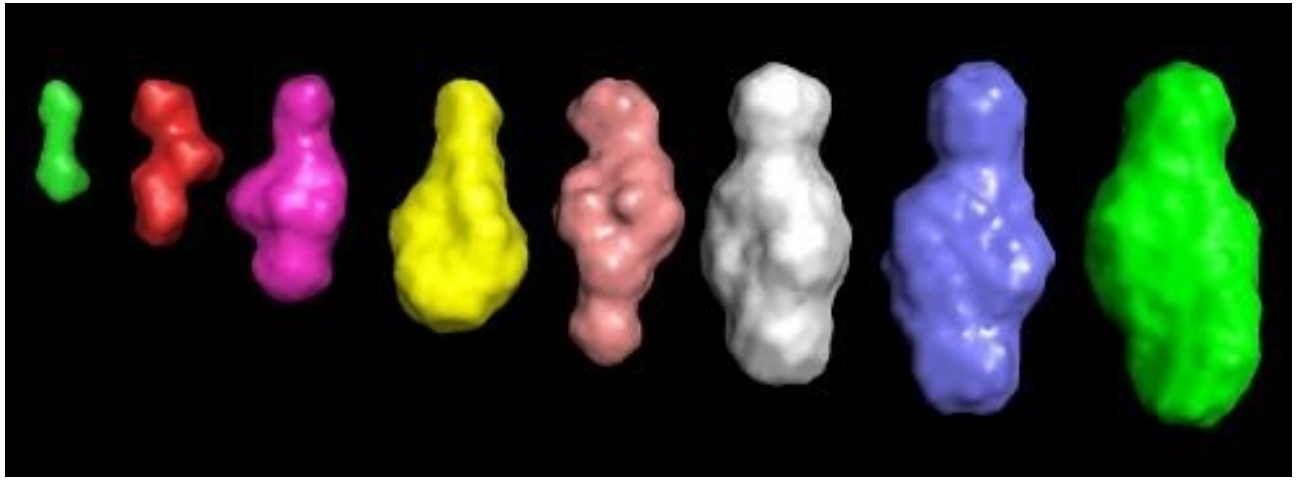
Wine and SAXS

We don't know tannins – or polyphenols – influence wine flavour, ageing, colour and colour stability.



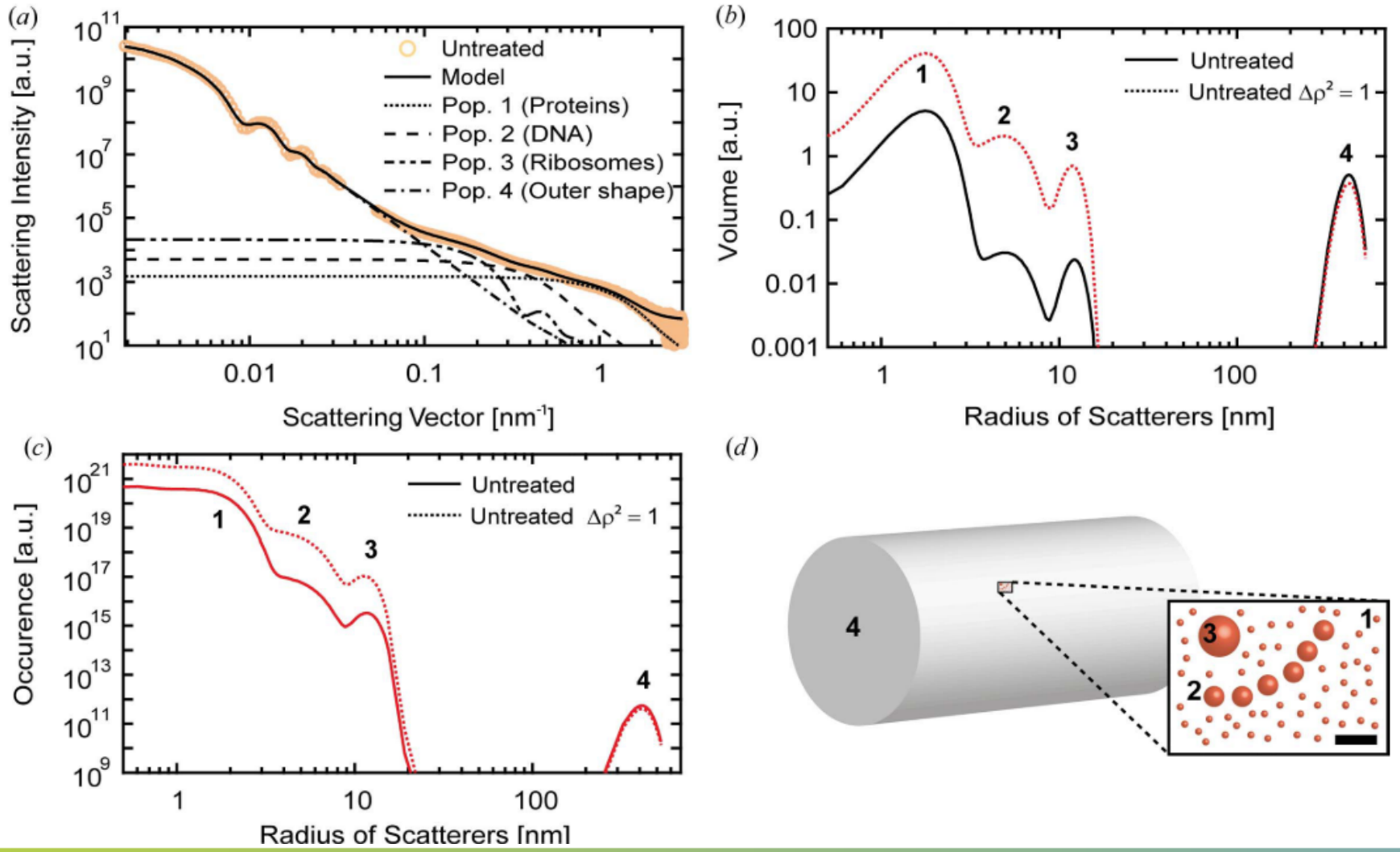
Tannin

What tannin molecules actually look like when dissolved in water. The size and shape of these tiny molecules play a part in how a wine tastes.



The bigger the tannin, the greater its astringency

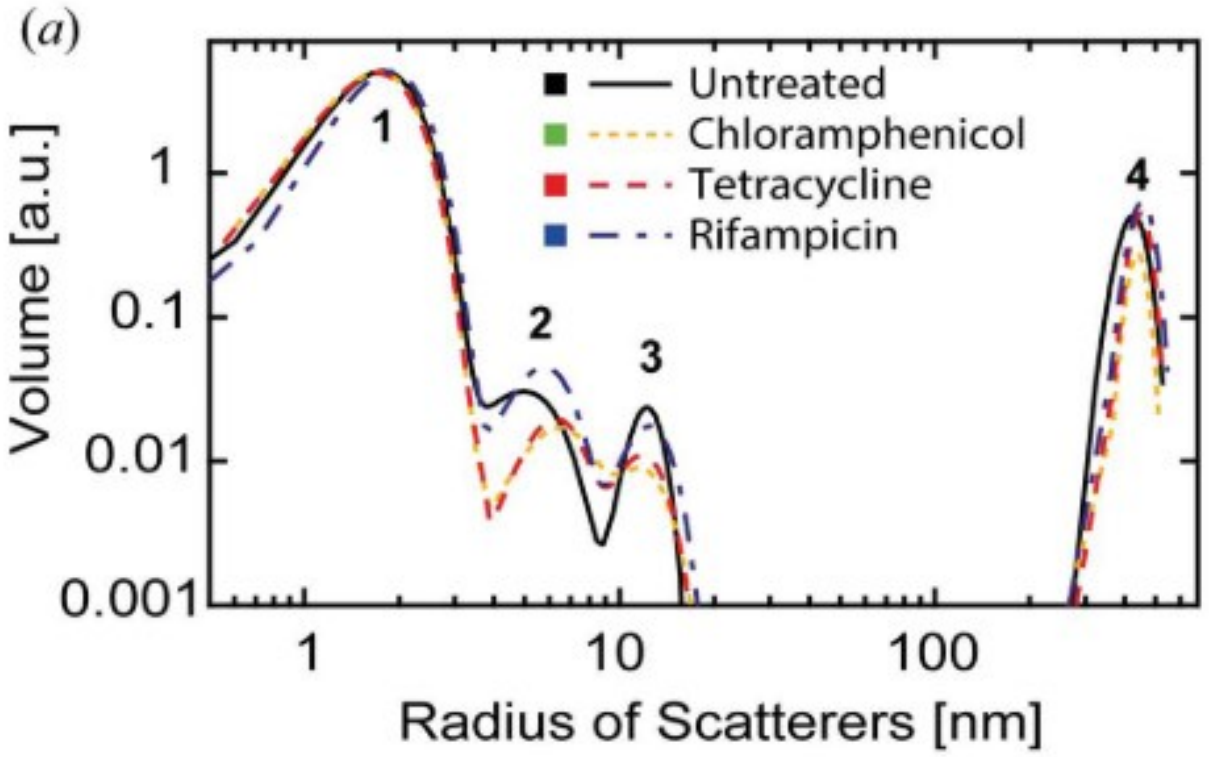
SAXS to resolve intracellular structure changes of E. coli cells induced by antibiotic

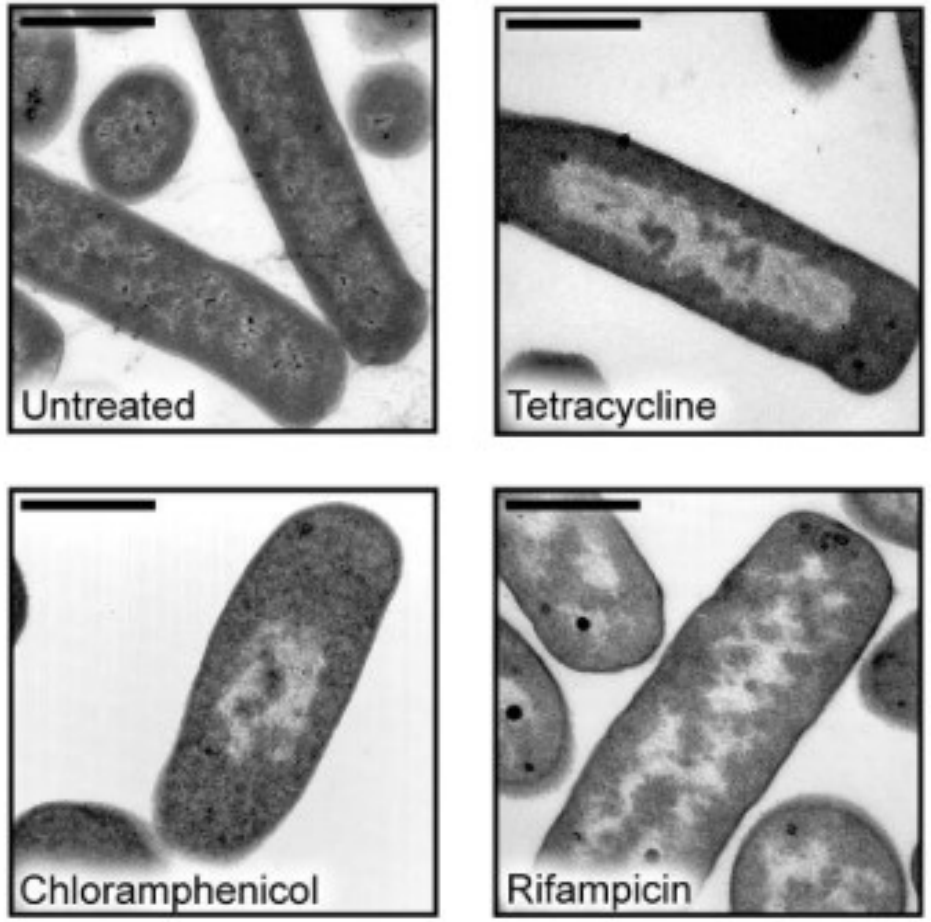


For covering the outer size of *E. coli* one needs to cover size ranges up to 5 µm, which is only possible if scattering at small angles is recorded at **ultra-small-angle** scattering instruments.

Von Gundlach, A. R.; Garamus, V. M.; Willey, T. M.; Ilavsky, J.; Hilpert, K.; Rosenhahn, A. *J. Appl. Crystallogr.* **2016**, *49* (6), 2210–2216.

SAXS to resolve intracellular structure changes of E. coli cells induced by antibiotic

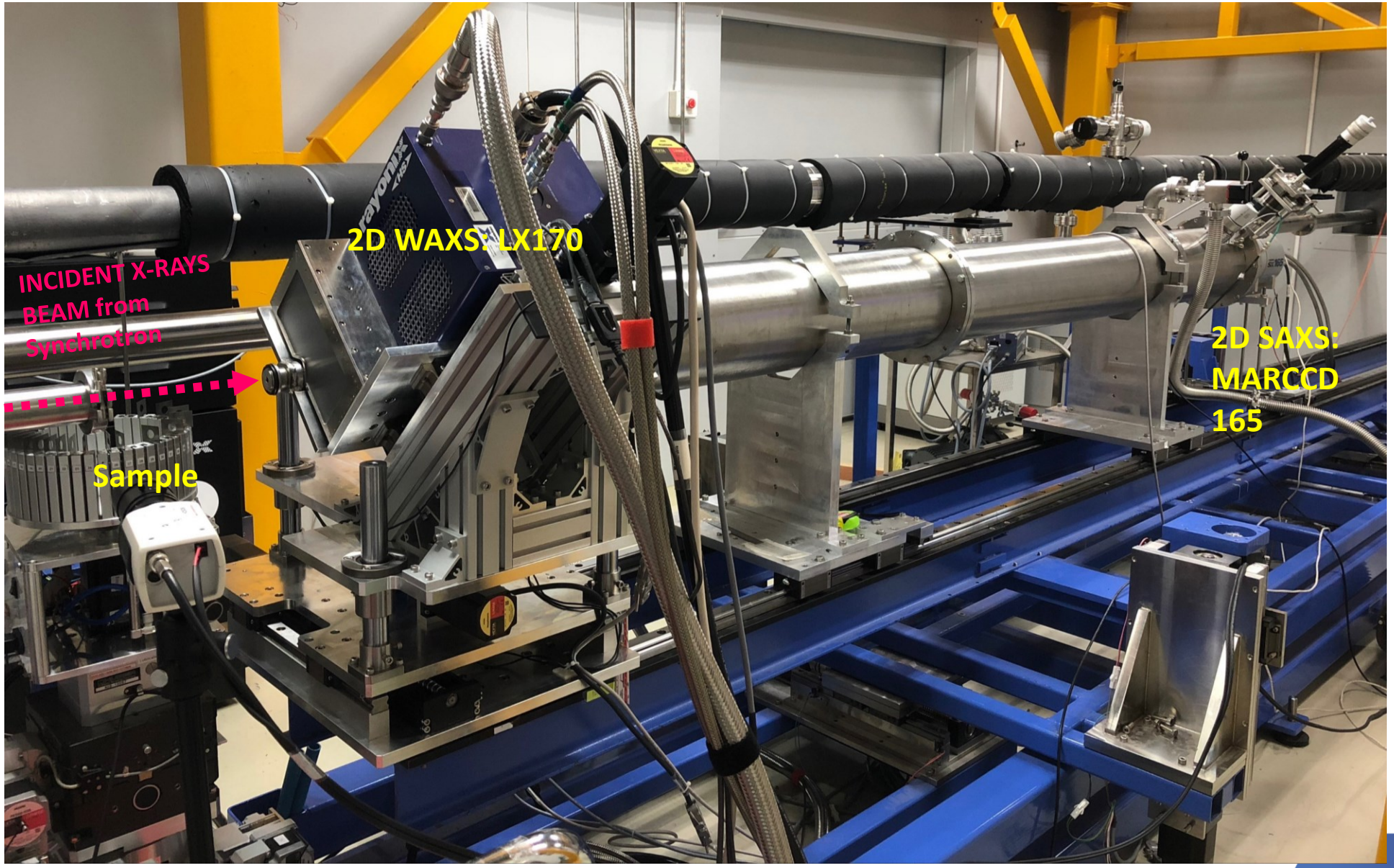




The key benefits of SAXS/WAXS are:

- Suitable for all types of samples (i.e. liquid, solid, gel, fiber…)
- No tedious sample preparation required
- Near native state measurements
- Can be done in a wide concentration range
- Static and dynamic sample manipulation possible (i.e. temperature, shear, humidity…)
- Low sample volumes of down to 60 µl
- Measurements can be automated with a sample changer
- Supplements of high-resolution structural determination tools (e.g. MX, NMR, Cryo-EM, etc)

BL1.3W: SAXS/WAXS at SLRI



THANK YOU

nuntaporn@slri.or.th

THAI
SYNCHROTRON
NATIONAL LAB