Neutrons for Biology and health: cyanobacterial circadian clockwork and other hot topics

Wojciech Zając

18 January 2023

Disclaimer

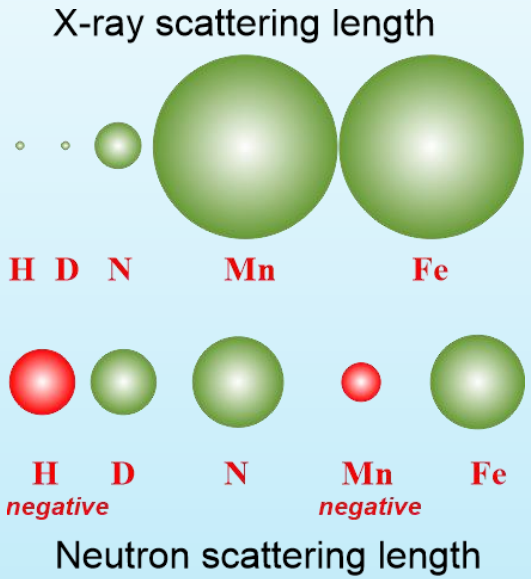
- I assume no responsibility or liability for any discomfort caused by my use of the term "complementarity" in anybody allergic to this.
- In fact,

  this talk will be about *complementarity*.

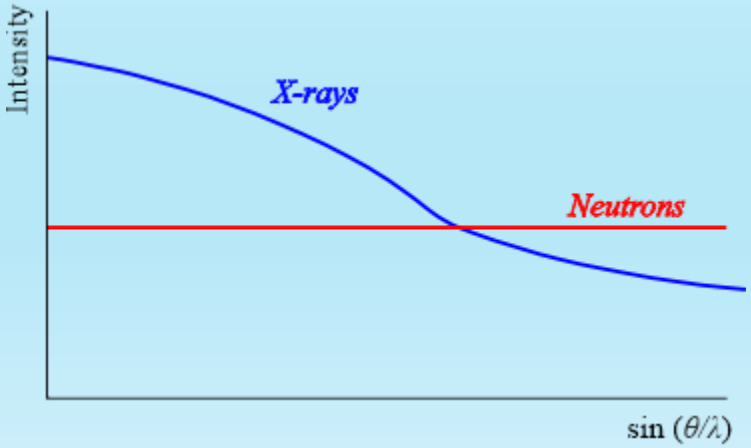
- Big tasks in complex systems' research require well-designed experimental approach by several methods.

Neutrons, or photons, or both?

Scattering lengths



Intensity (θ)



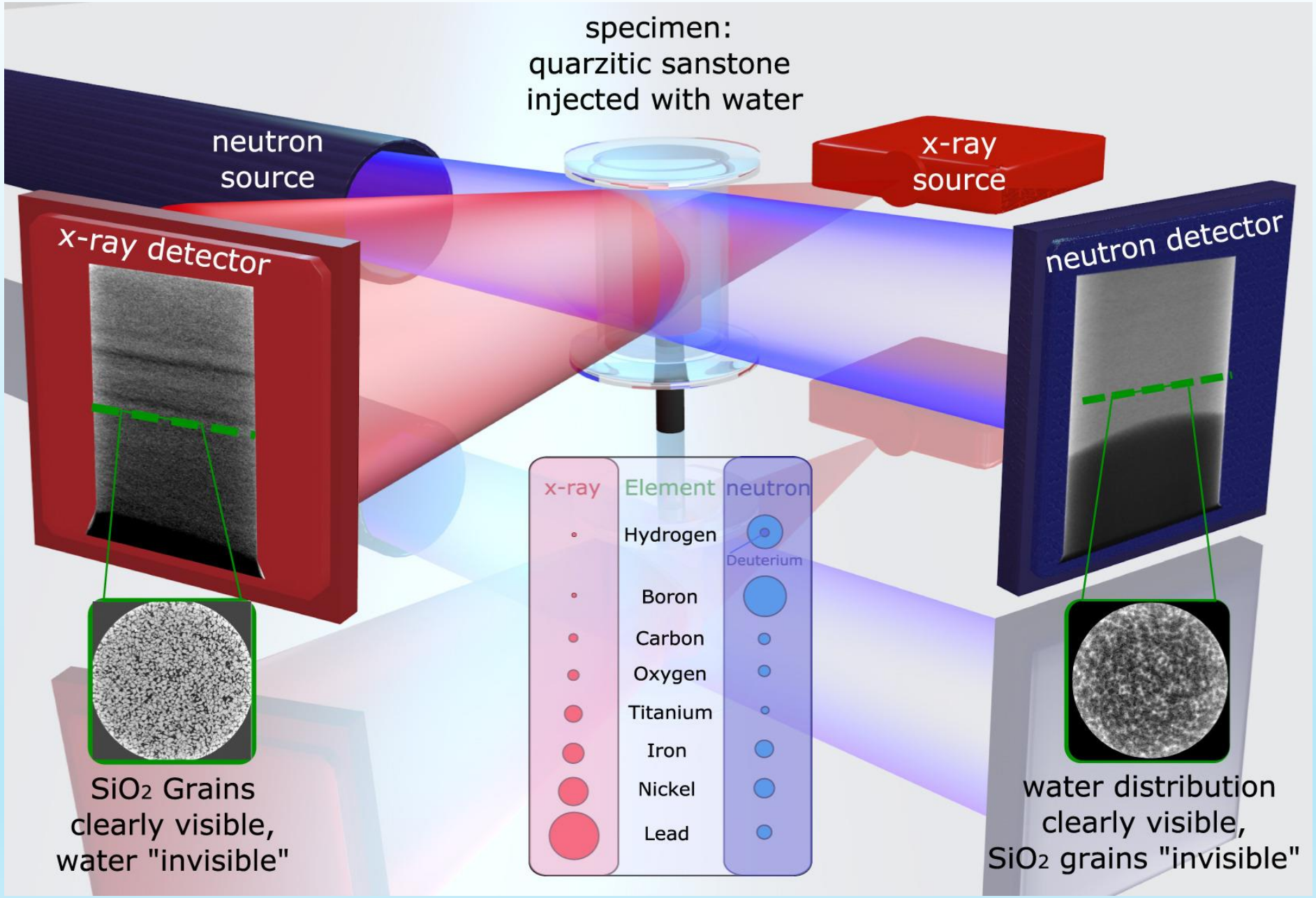
Photons

- high flux
- time resolution (ms)
- energy control (ASAXS)
- scanning micro- & nano-beams (diffraction, imaging)
- study of ultra-thin layers (GISAXS)

Neutrons

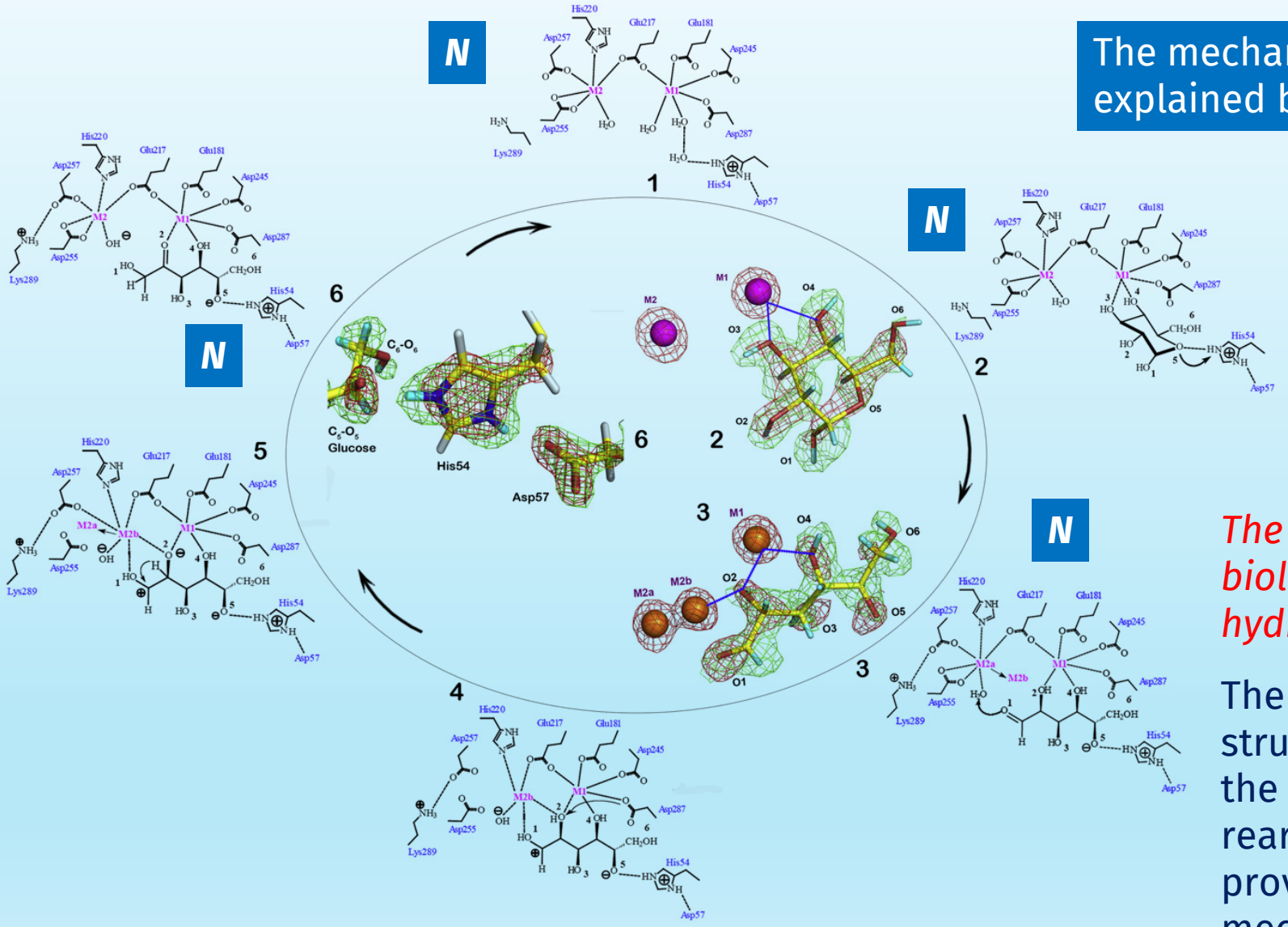
- sensitive to light atoms (polymers, biologic samples, soft matter, hydrogen in metals)
- differentiate between isotopes (multicomponent systems)
- no radiation damage
- deep penetration
  - very large samples (engineering)
  - difficult sample environments (p,T)
- can see magnetic contrast

Both!



NeXT-Grenoble, the Neutron and X-ray tomograph @ ILL

the lighter side of a sweet reaction

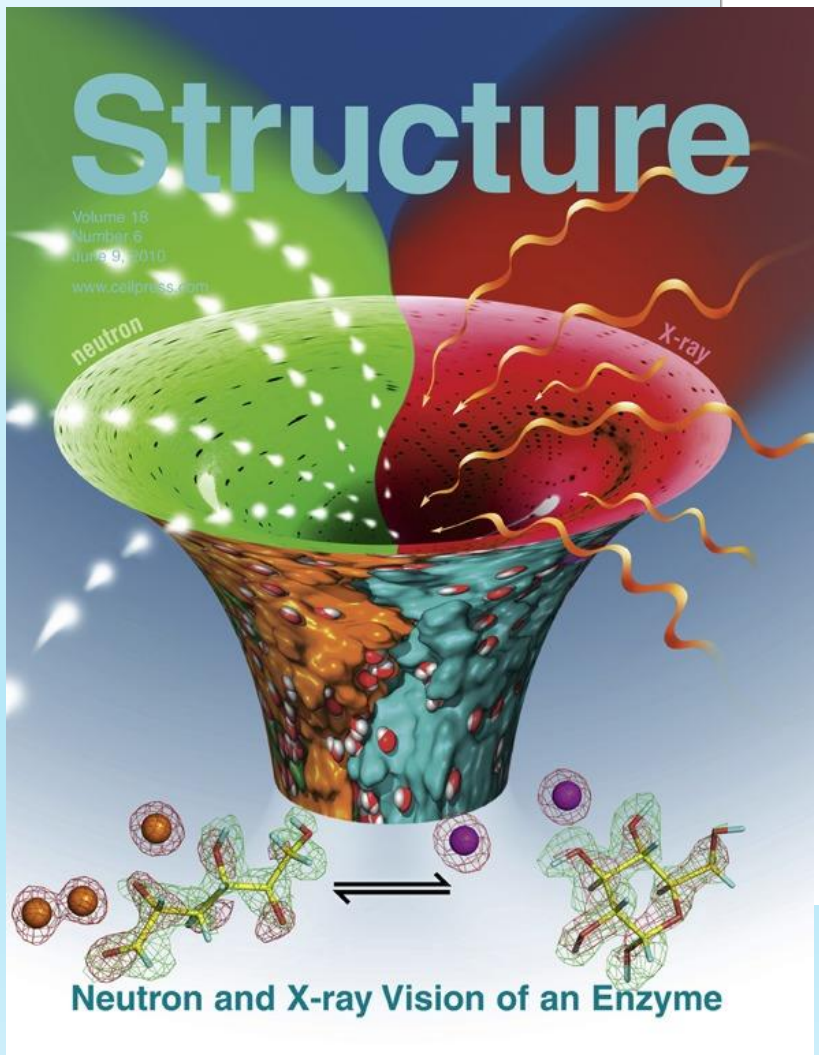


The mechanism of glucose to fructose reaction explained by neutron diffraction

The unique advantage of neutrons as biological probes is the ability to visualize hydrogen atoms in macromolecules.

The ensemble of xylose isomerase structures by neutron crystallography, and the determination of hydrogen atom rearrangements during the catalytic cycle provides insight into the enzyme's mechanism.

the lighter side of a sweet reaction



Cell PRESS

Structure
Article

Metal Ion Roles and the Movement of Hydrogen during Reaction Catalyzed by D-Xylose Isomerase: A Joint X-Ray and Neutron Diffraction Study

Andrey Y. Kovalevsky,[1,*] Leif Hanson,[2] S. Zoe Fisher,[1] Marat Mustyakimov,[1] Sax A. Mason,[3] V. Trevor Forsyth,[3,4] Matthew P. Blakeley,[3] David. A. Keen,[5] Trixie Wagner,[1,7] H.L. Carrell,[6] Amy K. Katz,[6] Jenny P. Glusker,[6] and Paul Langan[1,2,*]

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DOI 10.1016/j.str.2010.03.011

SUMMARY

Conversion of aldo to keto sugars by the metalloenzyme D-xylose isomerase (XI) is a multistep reaction lent metal cations and catalyzes the interconversion of the aldo sugars D-xylose and D-glucose to the keto sugars D-xylulose and D-fructose, respectively. The reaction (Figure 1) is a multi-step process and is thought to involve hydrogen (H) transfer in

Structure over length scales from 0.1 to 100 nm

Soft condensed matter

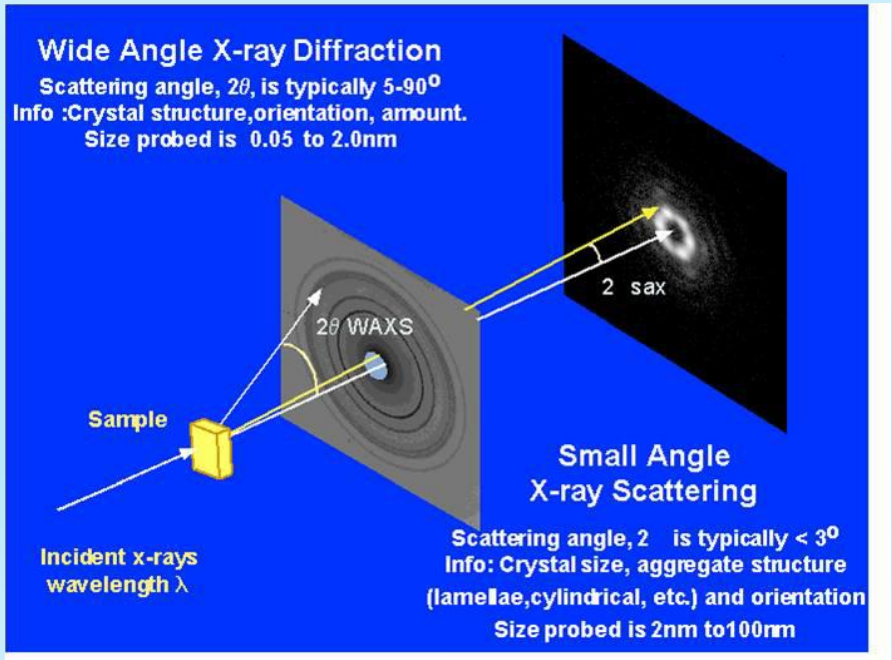
- emulsions
- micelles
- liquid crystalline structures
- organic nanoparticle (NP) dispersions
- + *many more*

Advanced functional materials

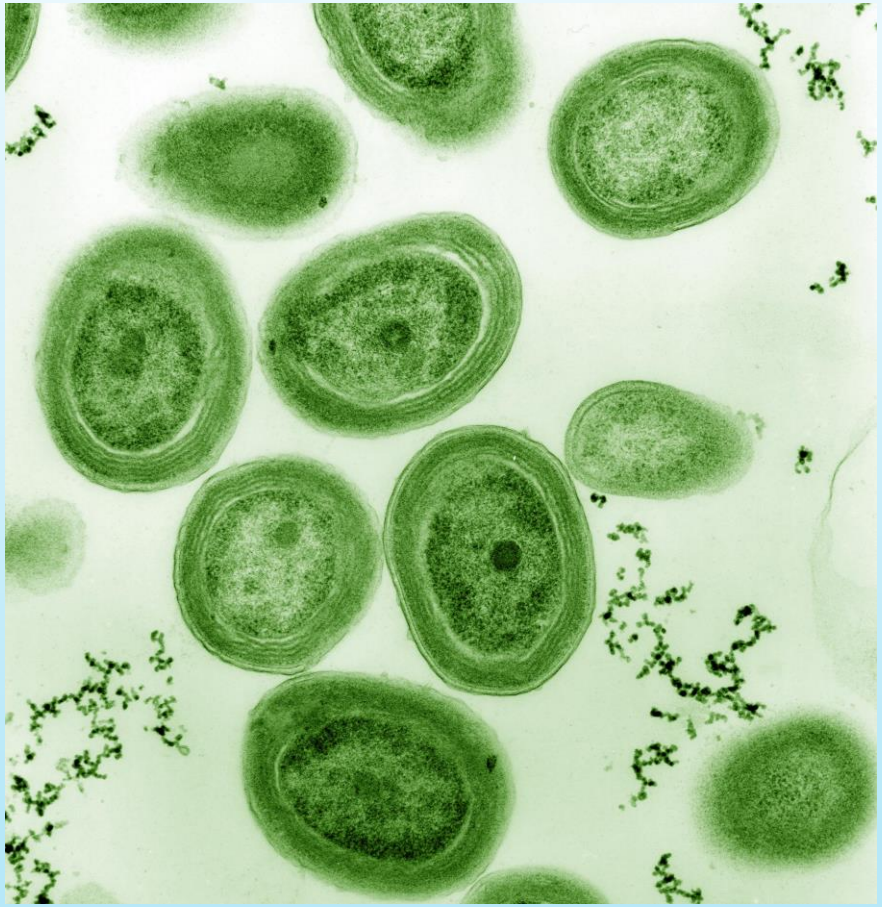
- sensors
- solar cells
- lithium-ion batteries
- formation, growth and stabilization of inorganic nanomaterials

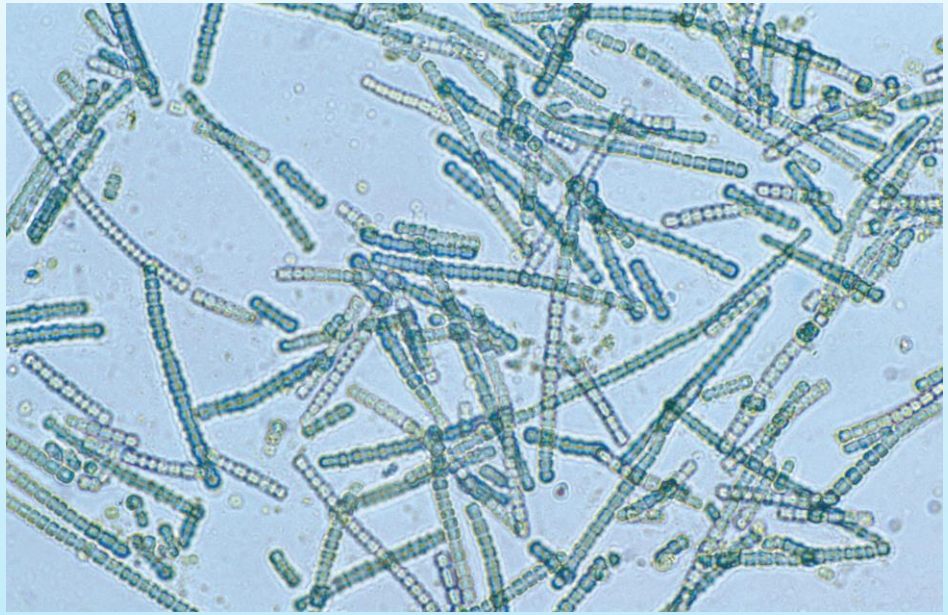
Modern biological applications

- structural characterization of
  - proteins
  - nucleic acids
  - lipids
- monitoring structural changes during:
  - protein folding
  - intrinsic disorder
  - conformational transitions
  - protein–protein assembly processes



Cyanobacteria *– a major model system for analyzing clock phenomena*

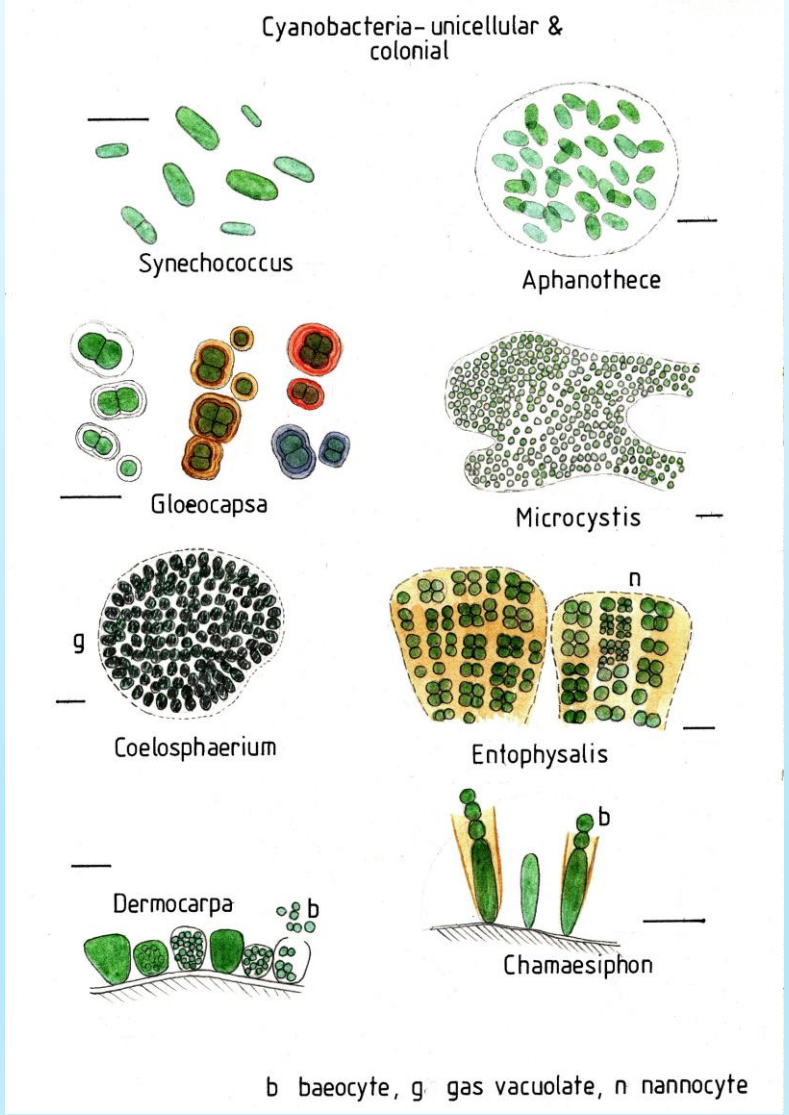




Cyanobacteria

- obtain energy via photosynthesis
- are the first organisms known to have produced oxygen
- produce (cyanotoxins) dangerous to humans and animals
- might help save the Planet
- owe their name to their colour

Cyanobacteria *–model system for analyzing clock phenomena*



Circadian rhythms a fascinating example of a biological timing mechanism

- provide an intracellular clock that estimates environmental time
- self-sustaining oscillators can maintain robust rhythms for years in the absence of any daily cue (such as light/dark or temperature cycles)

Cyanobacteria circadian rhythms

- truly global – essentially all genes are regulated by the circadian system
- the chromosome topology also oscillates and possibly regulates the rhythm of gene expression

The underlying circadian mechanism

- consists a post-translational oscillator (PTO) and a transcriptional/translational feedback loop (TTFL)
- PTO can be reconstituted in vitro with three purified proteins (KaiA, KaiB, and KaiC) and ATP
- Kai… oscillator proteins were crystallized, their structure determined, *the only full-length circadian proteins to be so characterized*

Fully assembled cyanobacterial KaiABC circadian clock complex

KaiA, KaiB and KaiC periodically assemble into a large complex

- small-angle X-ray scattering (SAXS)
- size-exclusion chromatography
- inverse contrast matching small-angle neutron scattering (IC-SANS)
- extensive MD calculations

Starting point

- the KaiA-KaiB-KaiC is commonly referred to as **ABC complex**
- recently, a cryo-EM study revealed the structure of ABC complex (more precisely, $A_{12}B_6C_6$ complex)
- $_N$A terminal domains of KaiA were missing from the above suggesting they can dynamically fluctuate in $A_{12}B_6C_6$
- consequently: *the overall structure of $A_{12}B_6C_6$ complex still remained to be completely solved*

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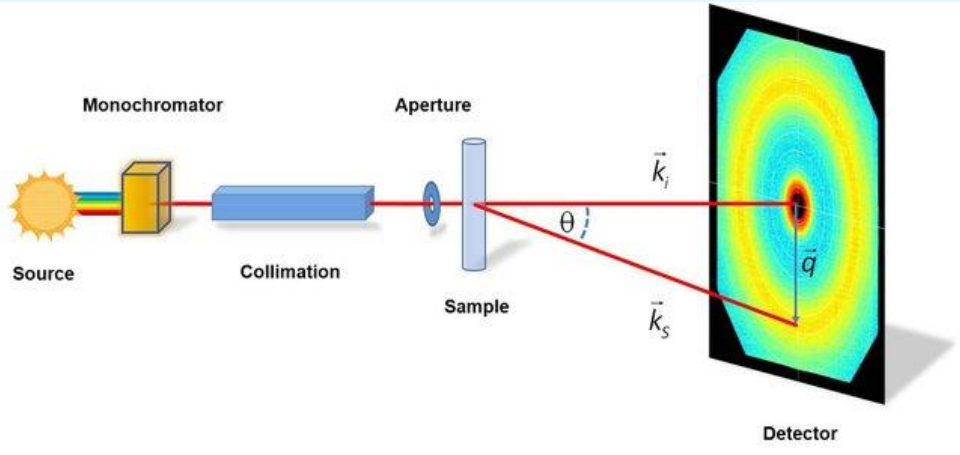
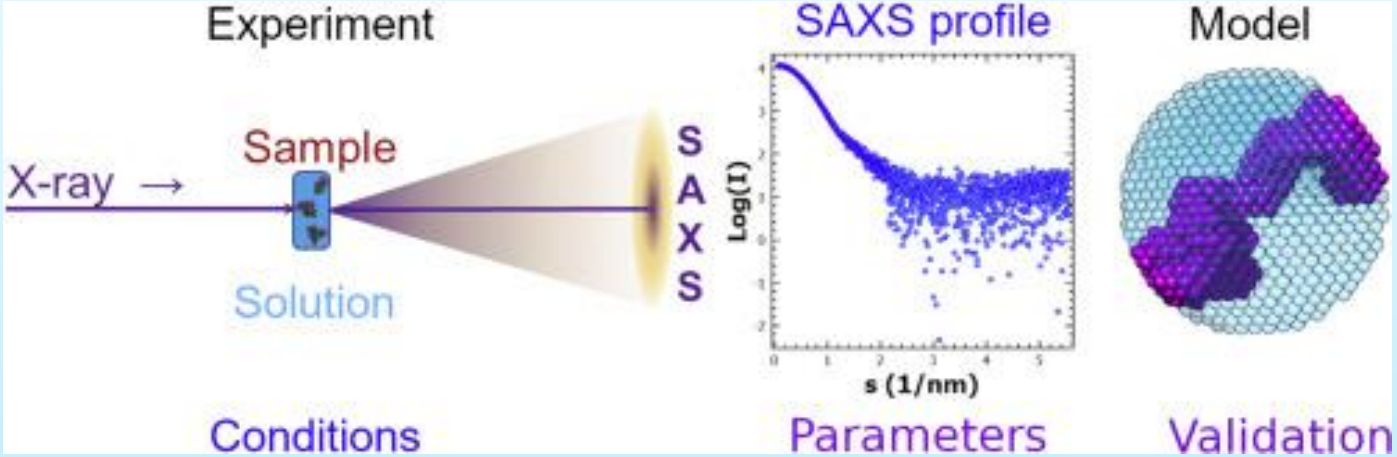
- $_N$A terminal domains of KaiA were missing from the above suggesting they can dynamically fluctuate in $A_{12}B_6C_6$
- previous mutational studies indicated that the $_N$A domain is essential for generation of circadian rhythm
- consequently: *the overall structure of $A_{12}B_6C_6$ complex still remained to be completely solved*

The KaiABC circadian clock complex – major research callenge

SAS for structure analysis



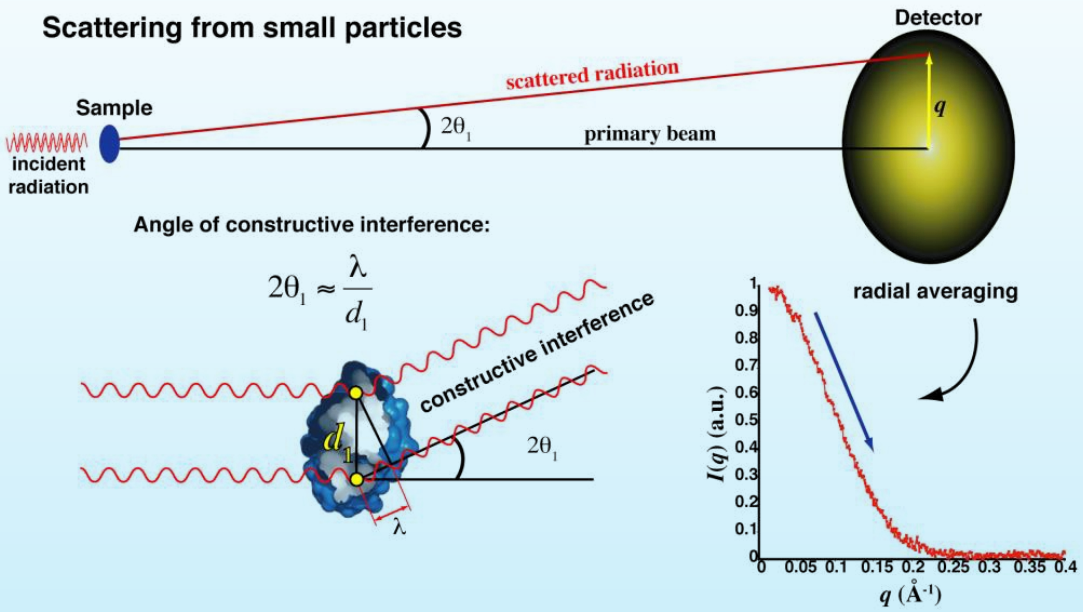


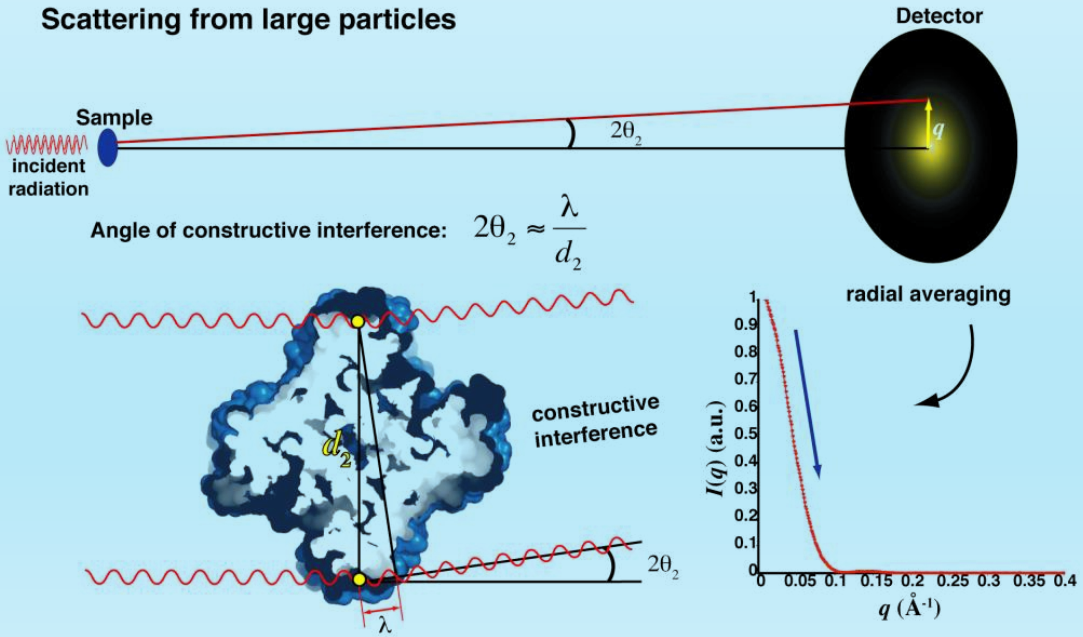
- small-angle scattering (SAXS and SANS) provide overall structural information of supramolecular complex in solution and can potentially be used for investigating a dynamic structure by combination with computational analysis,

three issues remain, however

1. how to selectively observe the scattering from the components of interest in a complex
2. how to eliminate contributions to scattering from undesirable components
3. how to build a three-dimensional structural model and characterize conformational dynamics of the large complex

SAS for structure analysis





Small-angle scattering occurs when a plane wave neutron passes through a particle and interacts with the nuclei in the particle giving rise to scattered wavelets of coherent, elastic scattering that can interfere.

The angle for constructive interference is inversely proportional to the size of the scattering object.

$$V^*(r) = \frac{2\pi\hbar^2}{m} b\delta(r)$$

Fermi pseudopotential

$$\rho(r) = \langle b_{coh} \rangle_V \approx \frac{2\pi}{10Q}$$

Scattering length density

$$\frac{\partial\sigma}{\partial\Omega}(Q) = N_p V_p^2 (\Delta\rho)^2 F(Q) S(Q) + B_{inc}$$

contrast factor — *form factor* — *sructure factor*

SAS for structure analysis

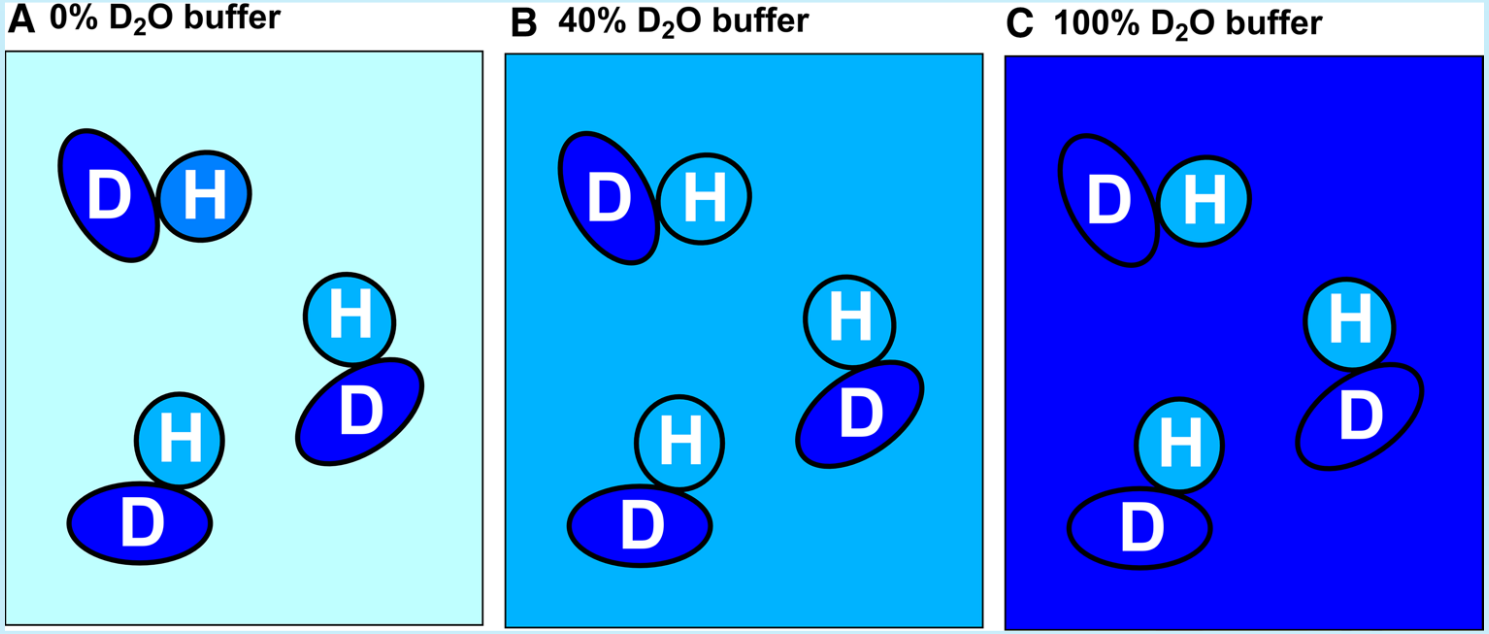
1. how to selectively observe the scattering originating from the components of interest in a complex

A single SAXS profile is not enough to analyze the structure of the large $A_{12}B_6C_6$ complex with the fluctuating $_NA$ domains and then it is required to edit scattering data.

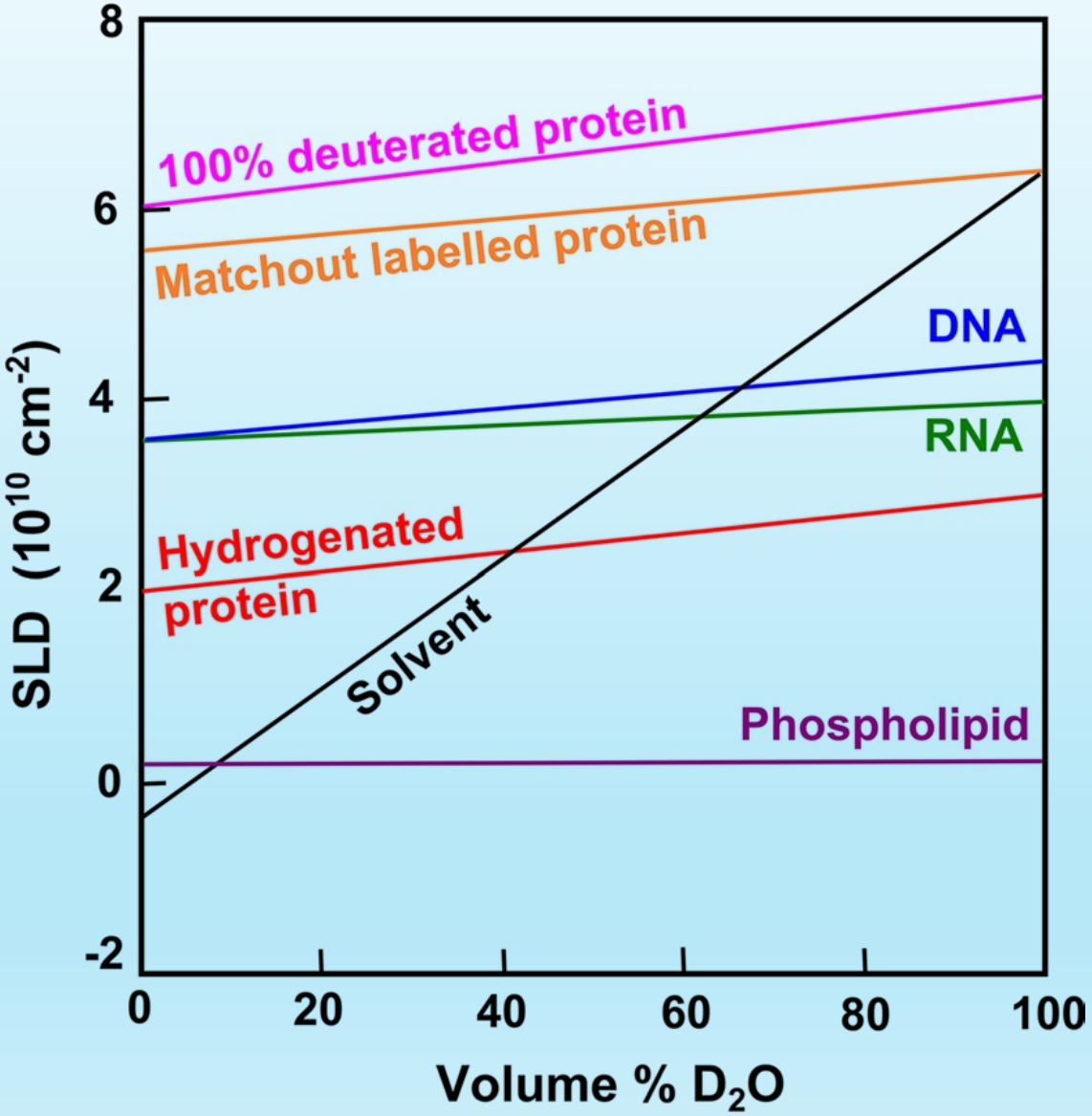
Remedy:

Inverse contrast-matching SANS (iCM-SANS):

100% D2O solvent together with
a well controlled fractional deuteration



digression: contrast matching for biophysical SANS expts.



Scattering length densities (SLDs) of the four major biomolecules as a function of the volume percentage of D_2O, assuming all labile hydrogen atoms are exchanged.

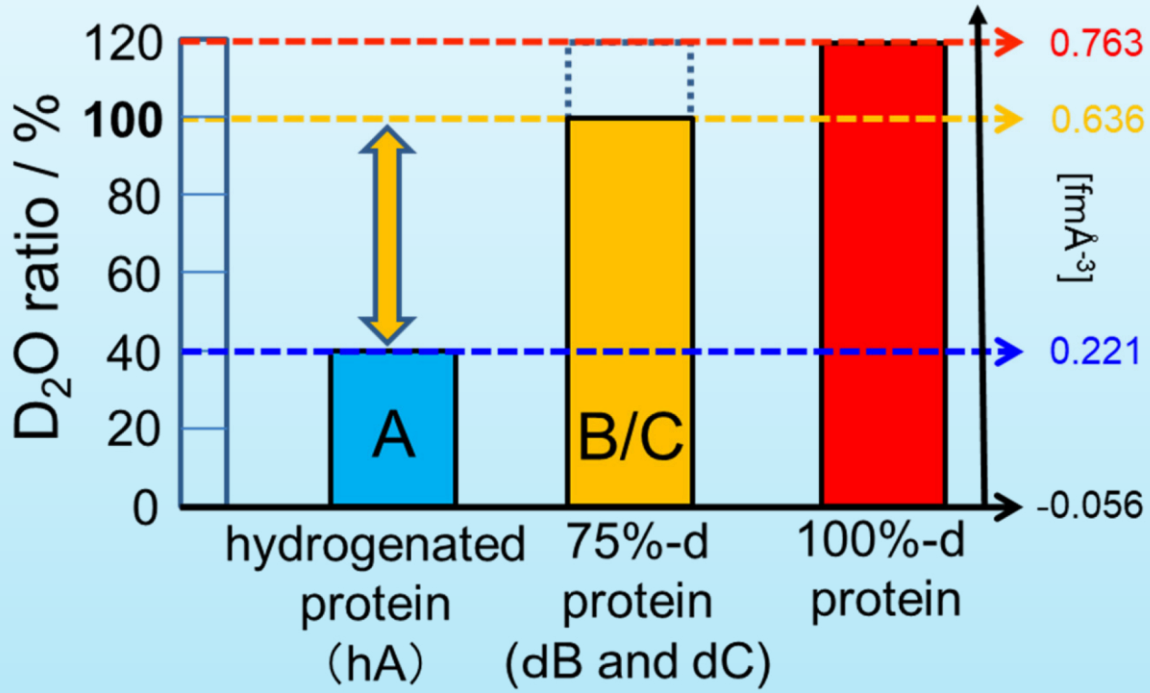
The black line represents the variation of the solvent SLD.

The match point of each biomolecule corresponds to the intersection of the solvent SLD with that for each biomolecule.

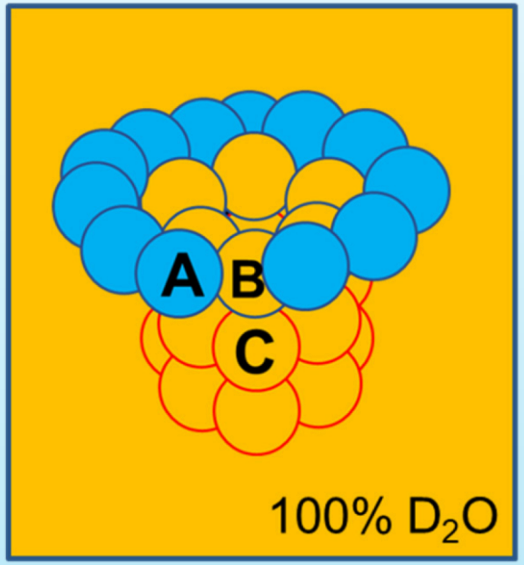
Perdeuterated protein, in which all the hydrogen atoms are replaced by deuterium, has an SLD that is higher than that of D_2O and cannot be solvent-matched.

Inverse Contrast Matching Small-Angle Neutron Scattering

(a) Scattering length density map



(b) Scattering visibility with iCM-SANS



Neutron SLD map.

There is no 120% D_2O solvent in reality, meaning that SANS cannot make 100%-deuterated protein invisible with any water solvent.

Selective observation of KaiA protomers in ABC complex. When KaiA, KaiB and KaiC are hydrogenated, 75%-deuterated and 75%-deuterated, respectively, only KaiA is visible in the ABC complex in 100% D_2O.

2. How to eliminate contributions to scattering from undesirable components

The $A_{12}B_6C_6$ complex stably exists only in a solution mixture with over-saturation of A_2, B_4, and B_6C_6 complexes.

The sample solution of $A_{12}B_6C_6$ complex inevitably includes non-integrated A_2, B_4, and/or B_6C_6 complexes and their aggregates as undesirable components, which interfere with SAS measurements.

Remedy:

To observe SAXS only from the $A_{12}B_6C_6$ complex in the multi-component solution, SAXS coupled with size-exclusion chromatograph (SEC-SAXS) was used.

besides,

SEC-SANS is also used.

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JB THE JOURNAL OF BIOCHEMISTRY

A feasibility study of inverse contrast-matching small-angle neutron scattering method combined with size exclusion chromatography using antibody interactions as model systems

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Nobuhiro Sato [1,†], Rina Yogo [2,3,4,†], Saeko Yanaka [2,3,4], Anne Martel [5], Lionel Porcar [5], Ken Morishima [1], Rintaro Inoue [1], Taiki Tominaga [6], Takao Arimori [7], Junichi Takagi [7], Masaaki Sugiyama [1,*] and Koichi Kato [2,3,4,‡]

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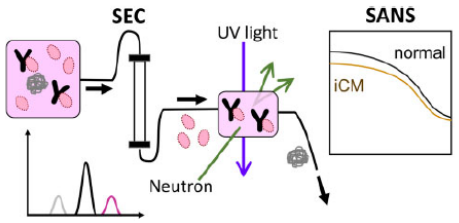
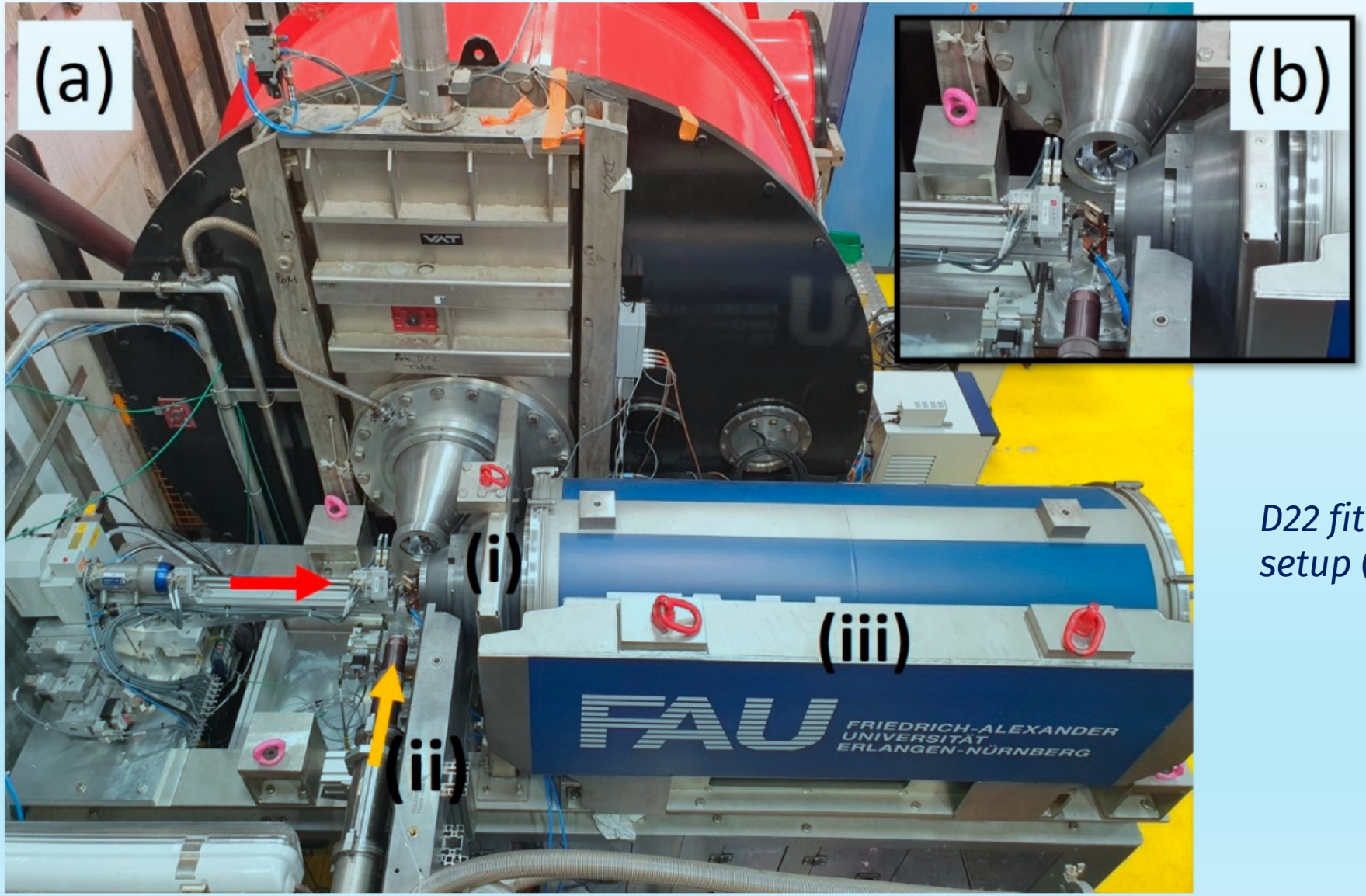
IgG-degrading enzyme of *Streptococcus pyogenes* and a soluble form of Fcγ receptor IIIb, and subjected to SEC–SANS as well as SEC–SAXS as reference. We successfully observe SANS from the non-deuterated IgG or Fc formed in complex with these binding partners, which were unobservable in terms of SANS in D_2O, hence demonstrating the potential utility of the SEC–iCM–SANS approach.

Graphical Abstract



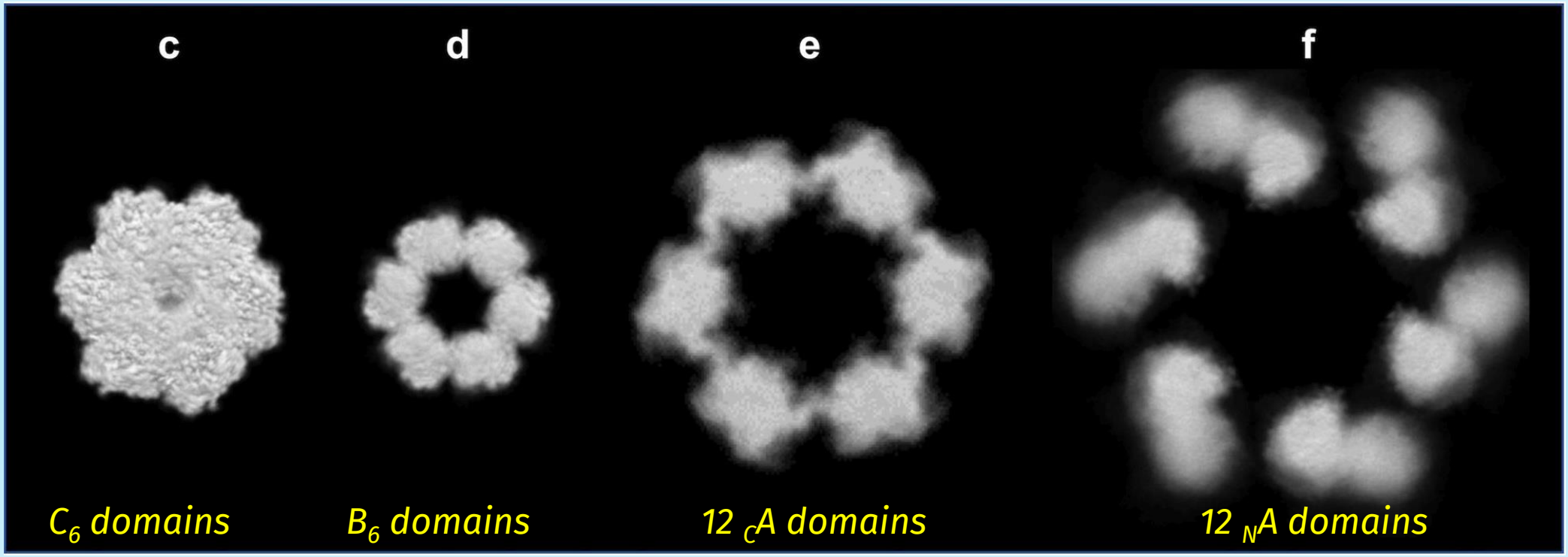
SAS / SANS cmplementarity on D22 @ ILL



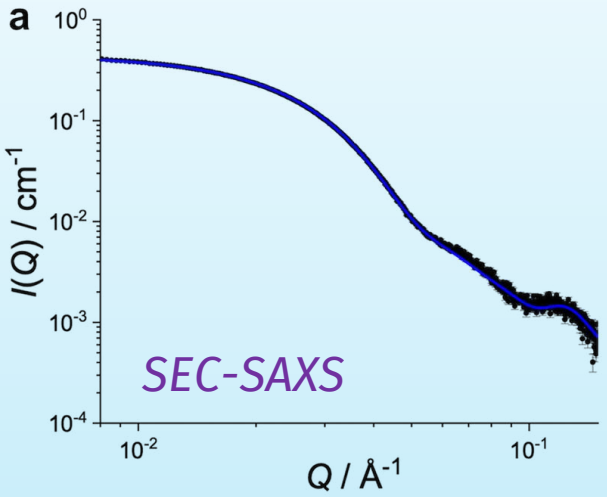
D22 fitted with removable SAS setup (X-ray optics from Rigaku)

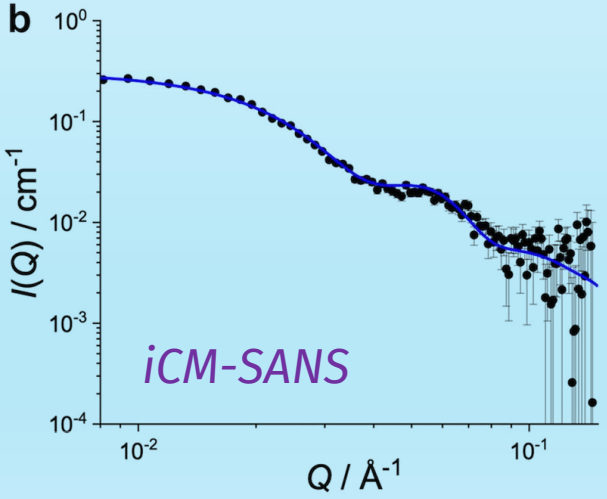
2. how to build a three-dimensional structural model and characterize conformational dynamics of the large complex

A vast array of computational models of the $A_{12}B_6C_6$ complex were generated based on the cryo-EM and X-ray crystallographic structures and subjected to screening based on the SEC-SAXS and SEC-iCM SANS data. Eventually, selected models were verified through molecular dynamics simulations.

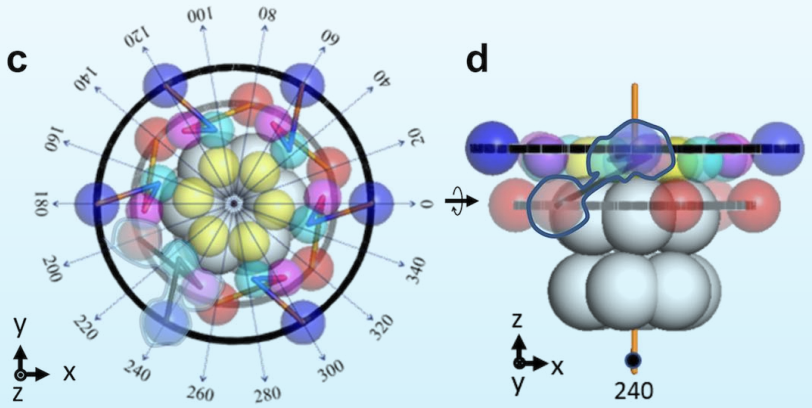


best-fit model

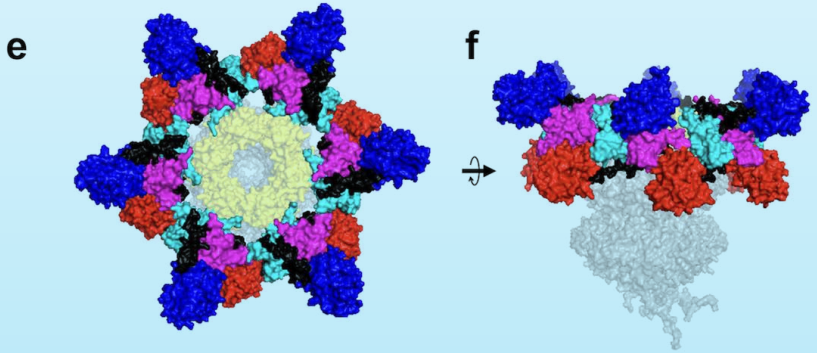




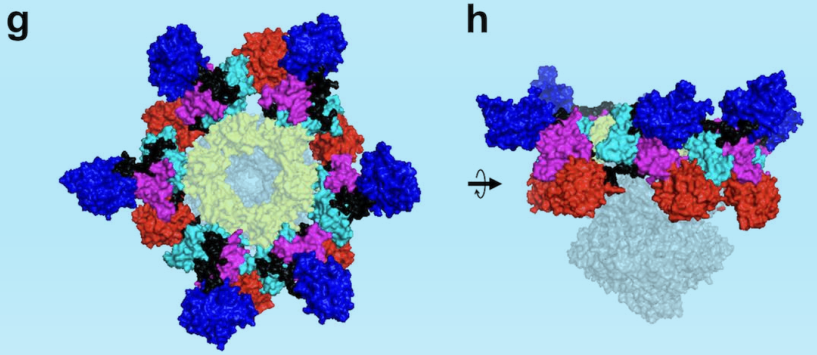
after 5000 MD-trajectories



schematic structure

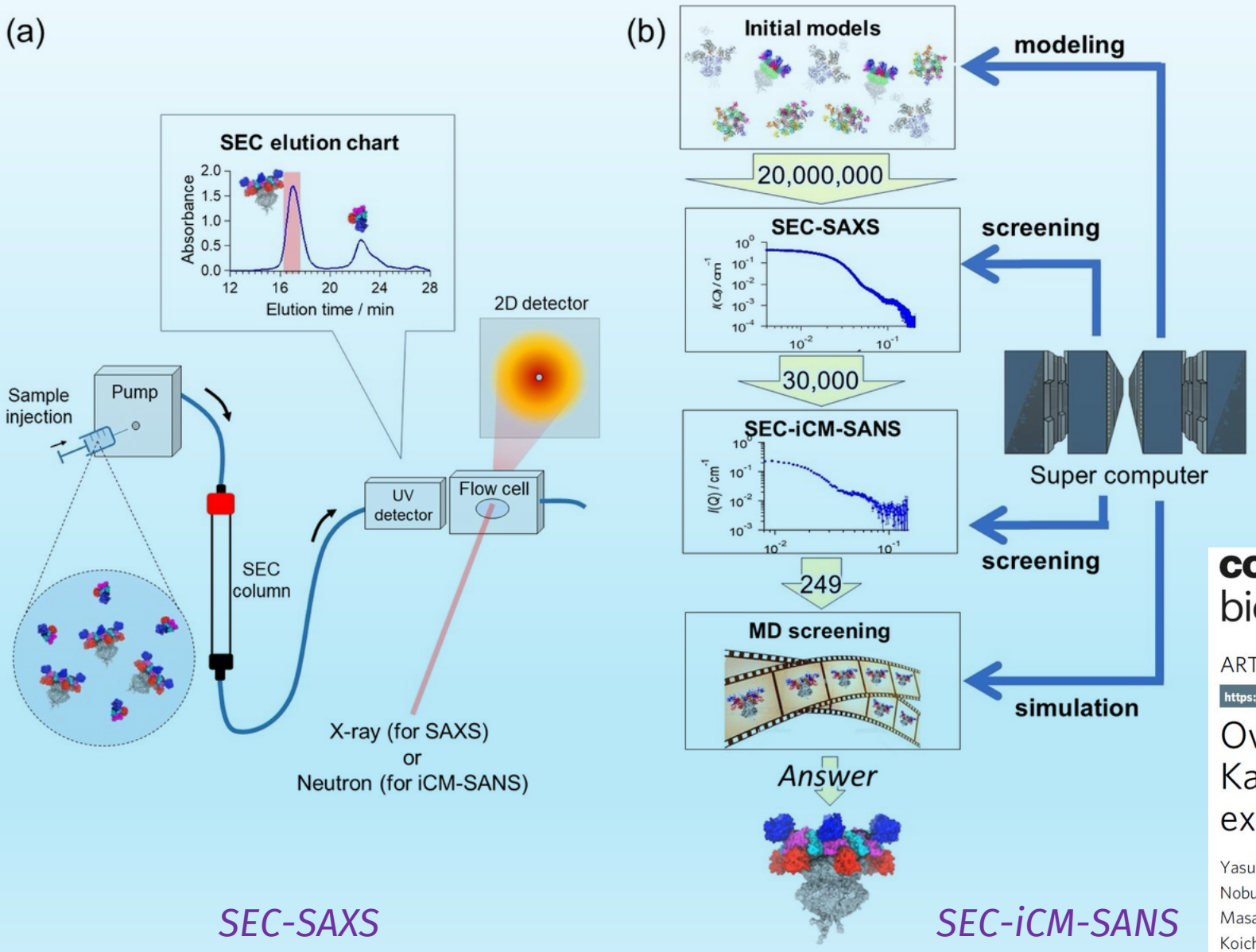


initial structure



Structure after 100 ns MD simulation

summary on cyanobacteria clockwork



SEC-SAXS

SEC-iCM-SANS

communications biology

ARTICLE

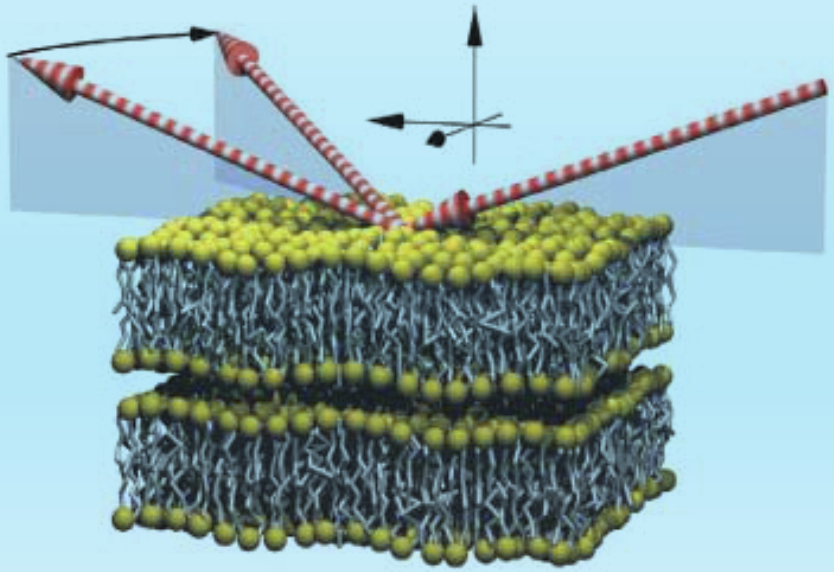
https://doi.org/10.1038/s42003-022-03143-z OPEN

Overall structure of fully assembled cyanobacterial KaiABC circadian clock complex by an integrated experimental-computational approach

Yasuhiro Yunoki[1,2,8,10], Atsushi Matsumoto[3,10], Ken Morishima[4], Anne Martel[5], Lionel Porcar[5], Nobuhiro Sato[4], Rina Yogo[1,2,9], Taiki Tominaga[6], Rintaro Inoue[4], Maho Yagi-Utsumi[1,2], Aya Okuda[4], Masahiro Shimizu[4], Reiko Urade[4], Kazuki Terauchi[7], Hidetoshi Kono[3✉], Hirokazu Yagi[2✉], Koichi Kato[1,2✉] & Masaaki Sugiyama[4✉]

thin films, multilayers, interfaces… ***neutron reflection***

- Below an angle of incidence called the **critical angle**, neutrons are perfectly reflected from a smooth surface
- Specular reflection is used:
  - In neutron guides
  - In multilayer monochromators and polarizers
  - To probe surface and interface structure in layered systems
- Above the critical angle reflectivity is determined by the variation of scattering length density perpendicular to the surface, i.e. we can determine the “average” density profile normal to the surface of a film on the surface
- Diffuse scattering gives information on surface & interface roughness



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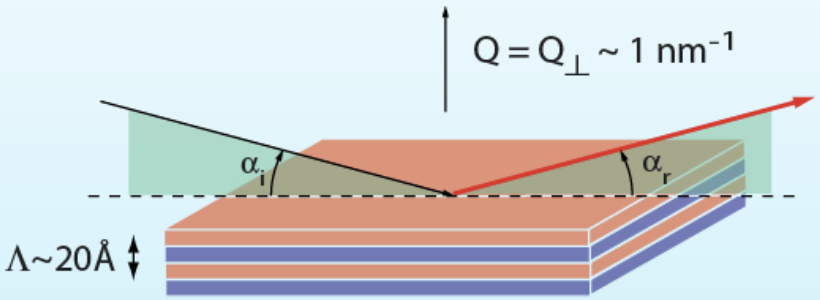
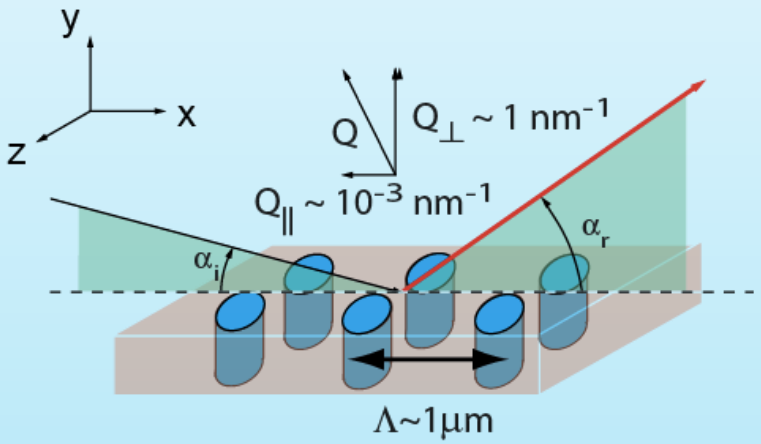


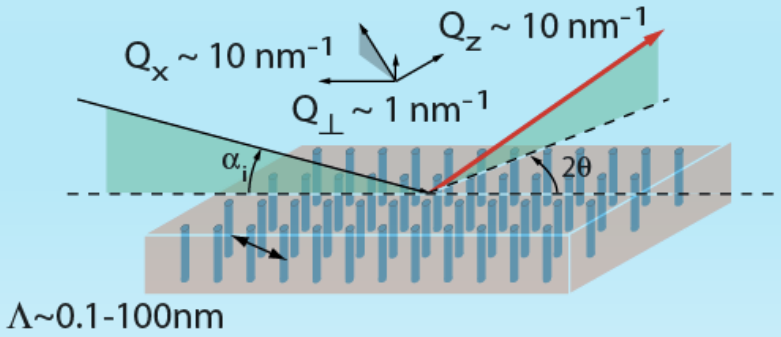
Specular refletometry

- depth profiles, nuclear or magnetic



Off-specular (diffuse) scattering

- in-plane correlated roughness
- magnetic strips
- phase separation (polymers)



Glancing incidence diffraction

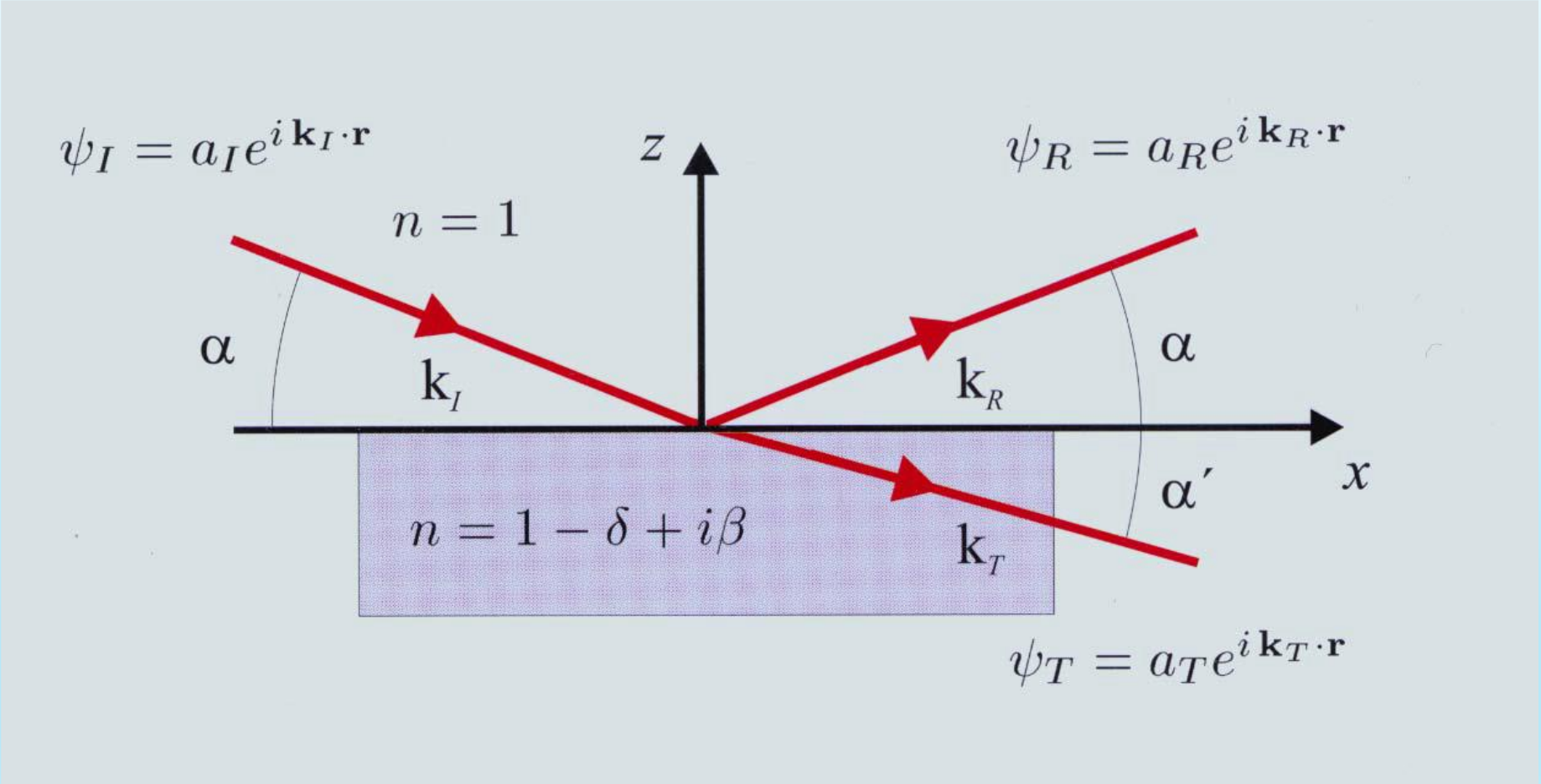
- ordering in liquid crystals
- atomic structures near surfaces
- nanodots interactions

thin films, multilayers, interfaces… ***neutron reflection***

reflectance & transmittance

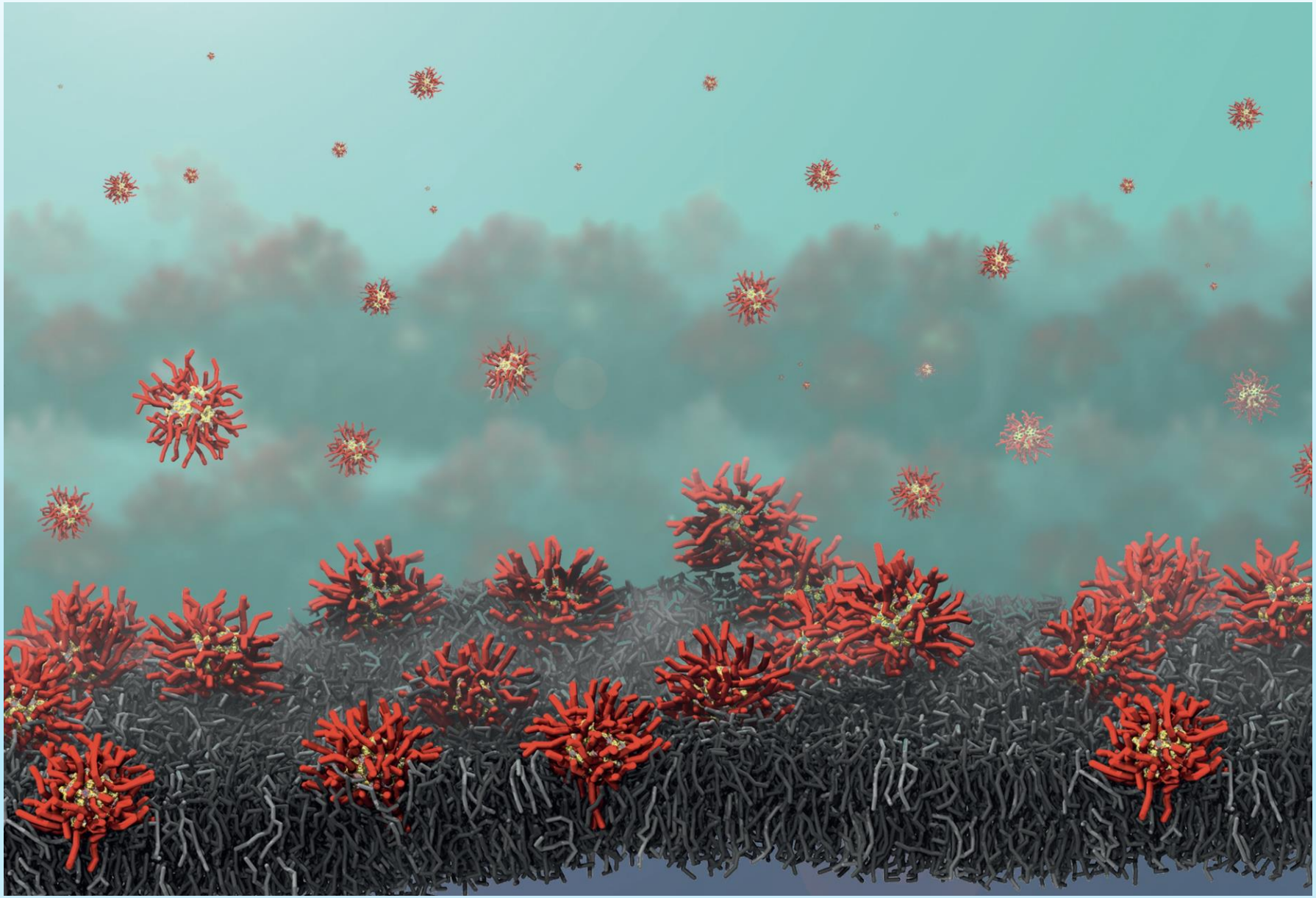
$$r = \frac{a_R}{a_I} = \frac{(k_{Iz} - k_{Tz})}{(k_{Iz} + k_{Tz})}$$

$$t = \frac{a_T}{a_I} = \frac{2k_{Iz}}{(k_{Iz} + k_{Tz})}$$



More on this in an excellent promer by Roger Pynn (available on the internet)

gold nanoparticles and cell membranes



AuNPs

- have a range of biomedical applications and are an important tool for drug delivery
- temperature and membrane charge are revealed to play a key role

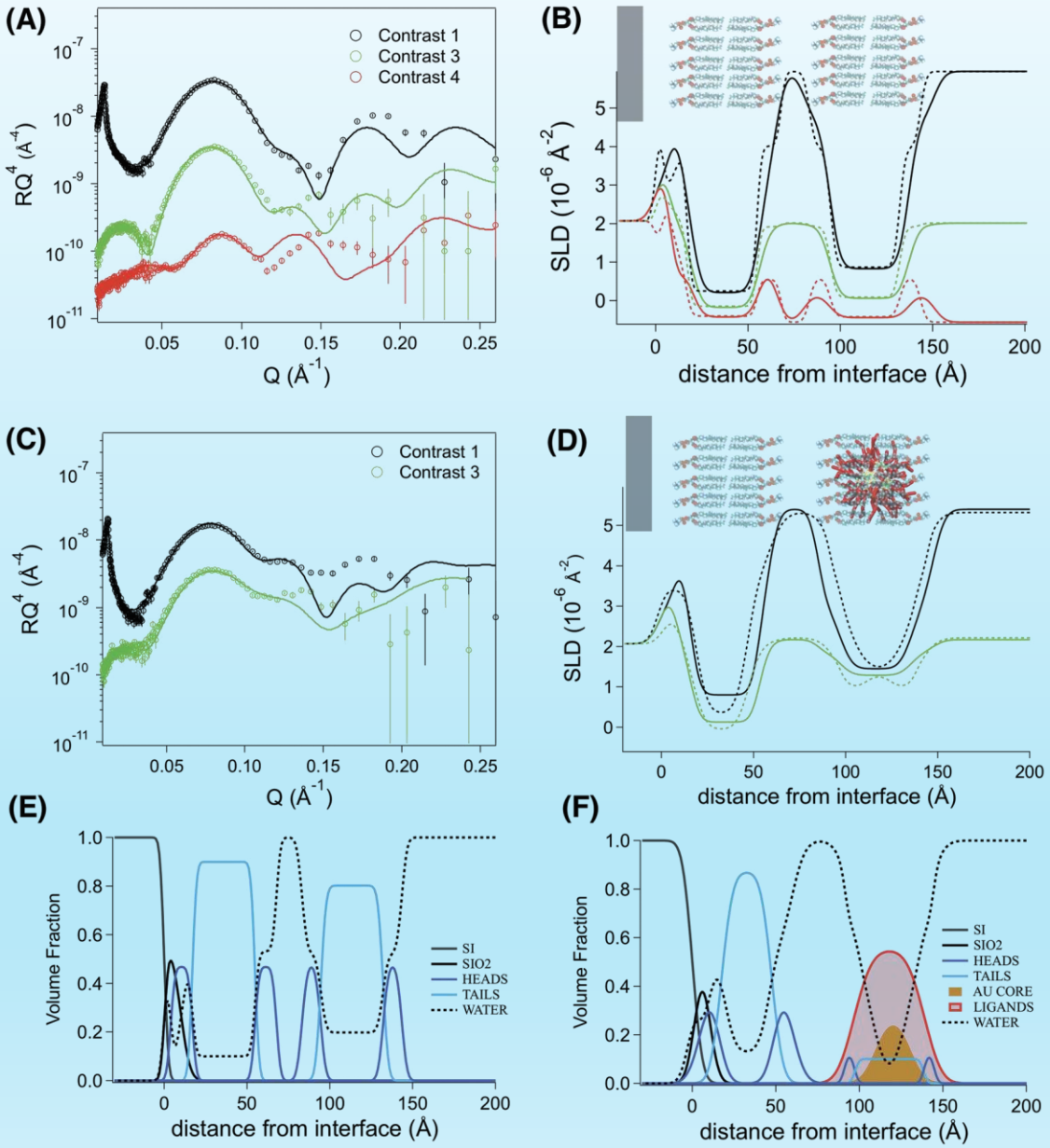
case study: intake/uptake of cationic AuNPs into lipid bylayers

- three lipid bilayers studied:
  - DSPC[(1)]
  - dDSPC/DSPG[(2)] (3:1)
  - DSPC/DSPG (9:1)
- floating bilayers prepared to avoid interactions with substrate
- cationic Me_3N^+AuNPs used
- measurements were made at different solvent deuteration grades (contrast variation)
- (instrument: D17 @ ILL)

[(1)]*DSPC: 1,2-distearoyl-sn-glycero-3-phosphocholine*

[(2)]*DSPG: 1,2-distearoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (sodium salt)*

neutron reflectometry results – DSPC bilayer



DSPC bilayer (refl. profile + SLD profile)

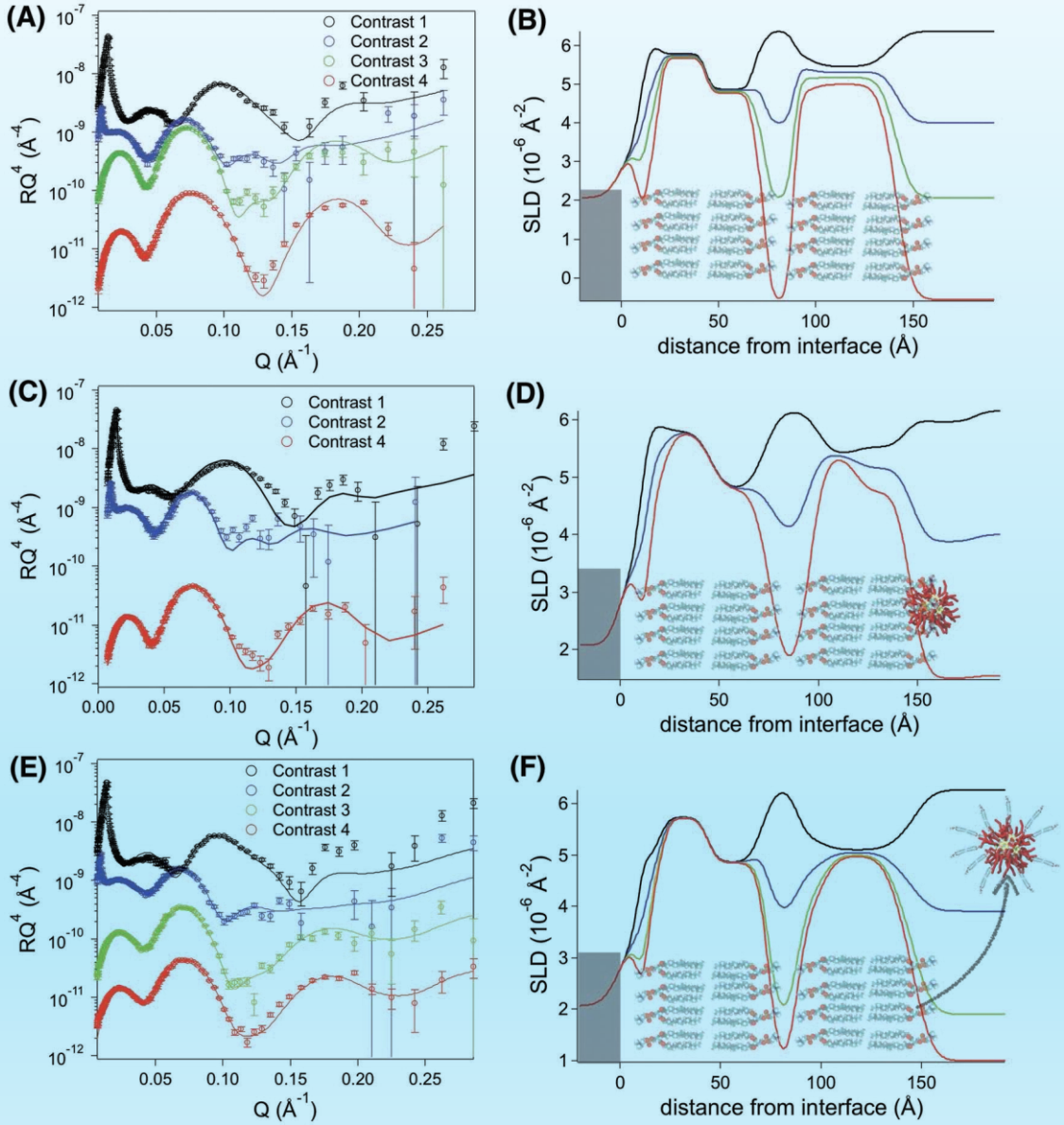
DSPC bilayer after the exposure to cationic Me_3N^+AuNPs (refl. profile + SLD profile)

volume occupancy profile of the molecular components present in the pristine membrane system:

pure

after interaction with Me_3N^+AuNPs

neutron reflectometry results – dDSPC/DSPG (3:1)

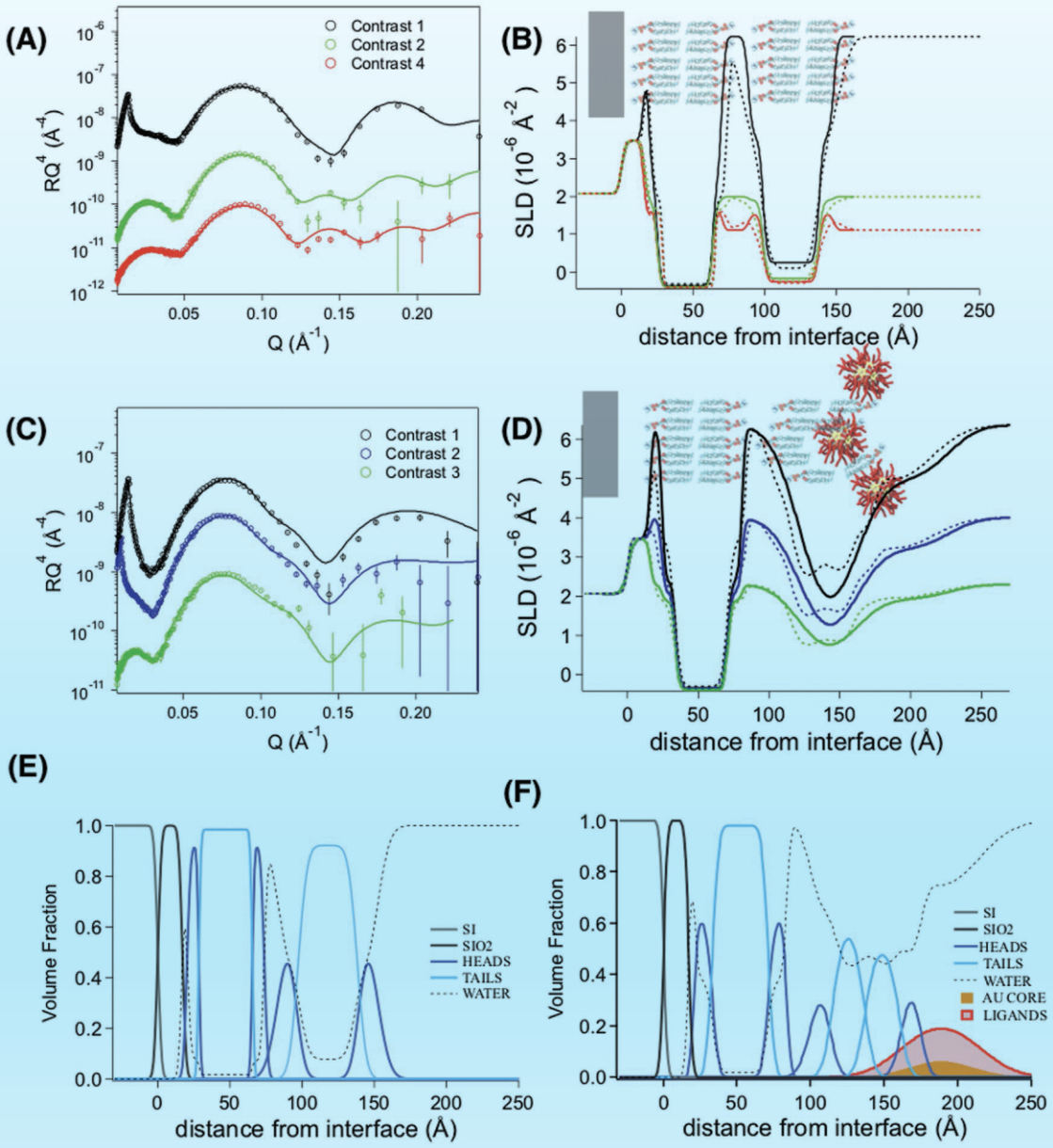


reflectivity and SLD profiles @ 25°C

reflectivity and SLD profiles after the exposure to cationic Me_3N^+AuNPs @ 25°C

reflectivity and SLD profiles after the exposure to cationic Me_3N^+AuNPs and annealing @ 65°C

neutron reflectometry results –DSPC/DSPG (9:1)



reflectivity and SLD profiles @ 25°C

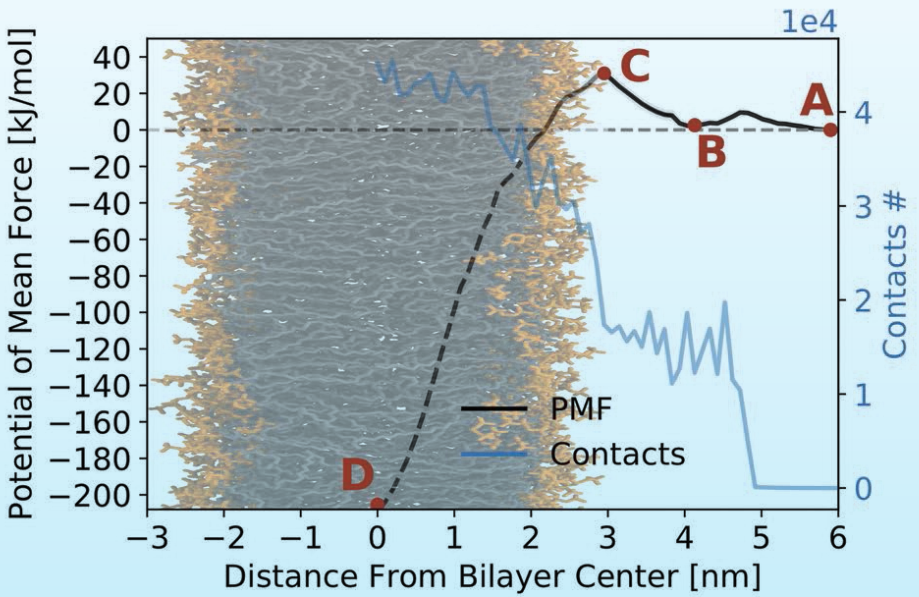
reflectivity and SLD profiles after the exposure to cationic Me_3N^+AuNPs. measurement @ 25°C after annealing @ 65°C

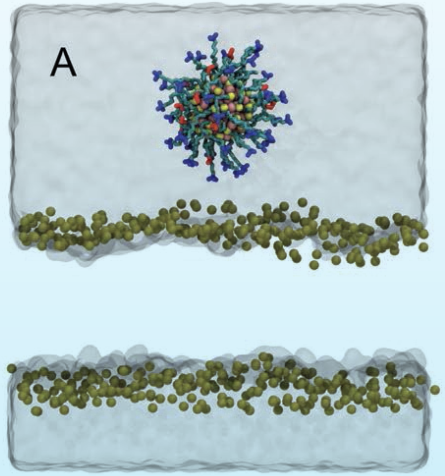
volume occupancy profile of the molecular components present in the pristine membrane system:

pure

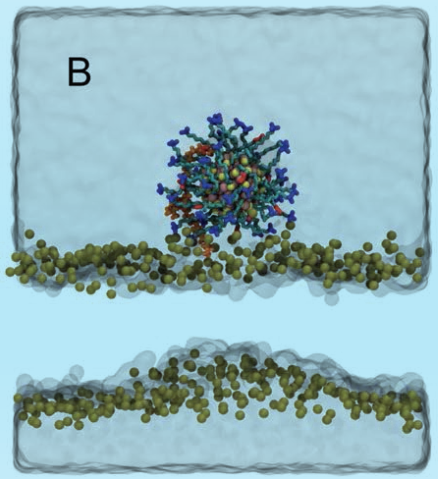
after interaction with Me_3N^+AuNPs

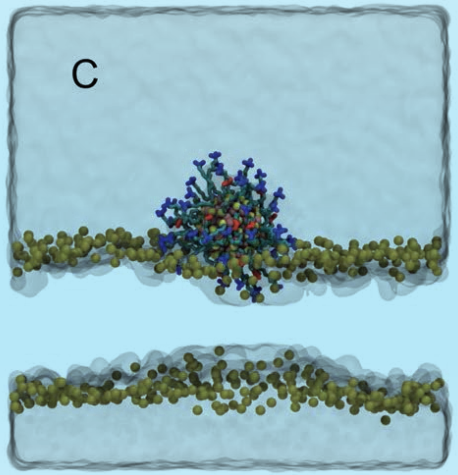
four key steps of the insertion pathway

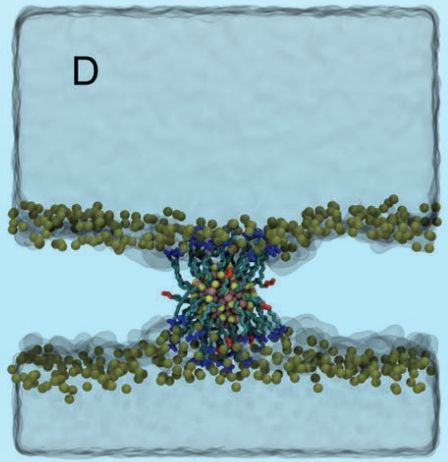




equilibrium position in the water phase







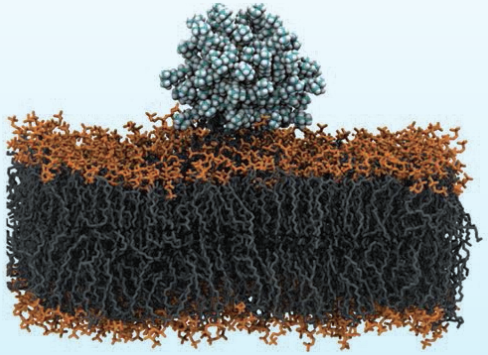
circular patch formation at the membrane surface

partial insertion

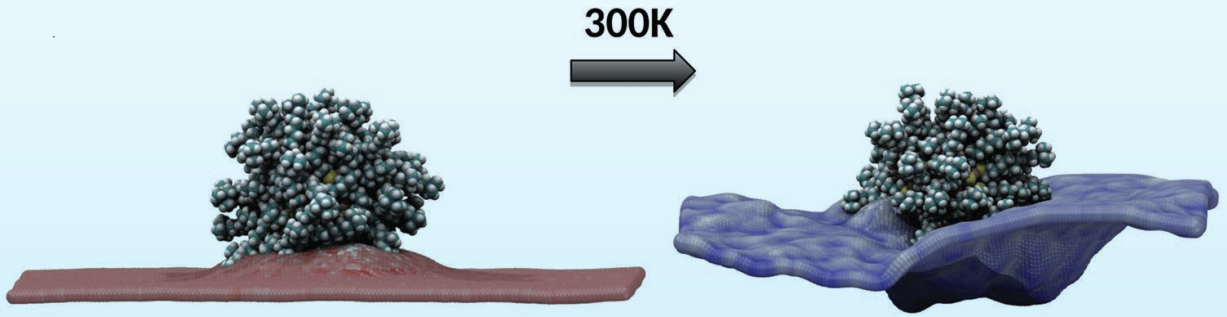
position inside a membrane with AuNP chains rearranged to expose the polar head groups to water on both sides of the bilayer

AuNP intake described by the free energy profile

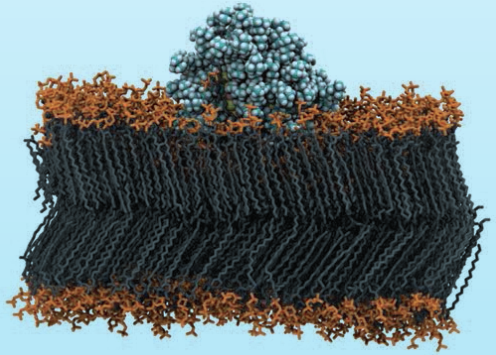
temperature effect (DSPC membrane)



AuNP interacting with the DSPC membrane surface at 343 K after 200 ns (liquid phase)

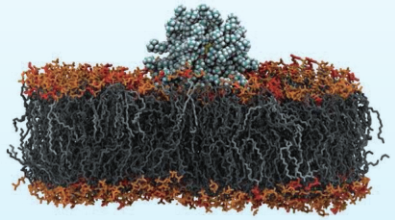


visualization of the local membrane curvature around AuNP averaged over the last 50 ns at 343 K (left) and 300 K (right)

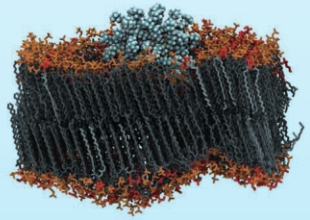


AuNP interacting with the DSPC membrane surface taken after 200 ns of cooling down the simulation to 300 K (gel phase)

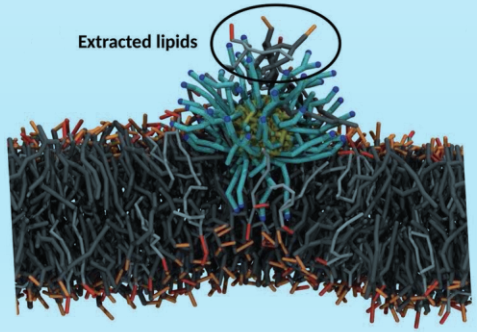
temperature effect (DSPC/DSPG (3:1) membrane)



AuNP interacting with the DSPC/DSPG (3:1) membrane surface at 343 K after 200 ns (liquid phase)

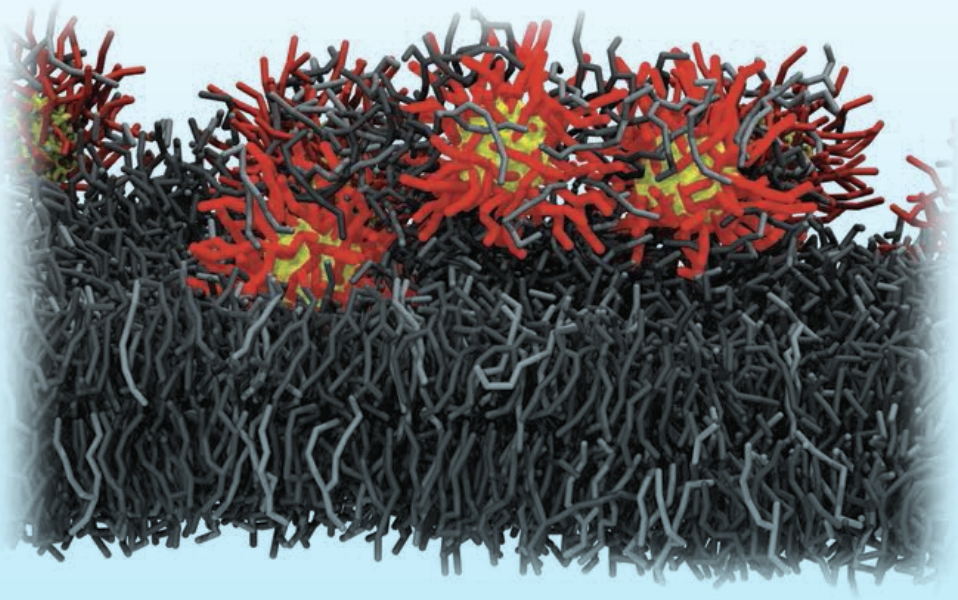


AuNP interacting with the DSPC membrane surface taken after 200 ns of cooling down the simulation to 300 K (gel phase)



coarse-grained simulation at 343 K after 21 μs showing the lipid extraction effect

membrane destabilization



AuNP trimer formation at 4 μs for negative bilayers containing 25 mol% DSPG

case study: intake/uptake of cationic AuNPs into lipid bylayers

FULL PAPER

Nanoparticle–Membrane Interactions

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The Role of Temperature and Lipid Charge on Intake/ Uptake of Cationic Gold Nanoparticles into Lipid Bilayers

Fabio Lolicato, Loic Joly, Hector Martinez-Seara, Giovanna Fragneto, Ernesto Scoppola, Francesca Baldelli Bombelli, Ilpo Vattulainen, Jaakko Akola, and Marco Maccarini**

Understanding the molecular mechanisms governing nanoparticle–membrane interactions is of prime importance for drug delivery and biomedical applications. Neutron reflectometry (NR) experiments are combined with atomistic and coarse-grained molecular dynamics (MD) simulations to study the interaction between cationic gold nanoparticles (AuNPs) and model lipid membranes composed of a mixture of zwitterionic di-stearoyl-phosphatidylcholine (DSPC) and anionic di-stearoyl-phosphatidylglycerol (DSPG). MD simulations show that the interaction between AuNPs and a pure DSPC lipid bilayer is modulated by a free energy barrier. This can be overcome by increasing temperature, which promotes an irreversible AuNP incorporation into the lipid bilayer. NR experiments confirm the encapsulation of the AuNPs within the lipid bilayer at temperatures around 55 °C. In contrast, the AuNP adsorption is weak and impaired by heating for a DSPC–DSPG (3:1) lipid bilayer. These results demonstrate that both the lipid charge and the temperature play pivotal roles in AuNP–membrane interactions. Furthermore, NR experiments indicate that the (negative) DSPG lipids are associated with lipid extraction upon AuNP

1. Introduction

The enhanced use of nanoengineered materials exposes humans, animals, and the environment to their potential risks.[1] Therefore, it is imperative to gain fundamental understanding of the undesired effects of nanoparticles on living systems that go beyond their primary function. The toxicity of nanoparticles and, more generally, their interaction with plasma membranes depends in a complex manner on several factors such as the size,[2] shape,[3,4] charge,[5,6] concentration, and functionalization of the nanoparticle.[7] The complexity of nanotoxicity studies is further increased by the fact that in vitro observations of toxicity are often not representative nor directly transferable to in vivo studies.[8]

Since the first contact between a nano-

03 March 2023 — dr Oleksandr Tomchuk's talk on SANS

15 February 2023 — deadline for proposal submission @ ILL

Thank you for your attention