Complementary approaches to obtaining thermodynamic parameters from protein ligand systems:
Challenges and opportunities and a case for neutrons

Mona Sarter

CCPBioSim Industry Talk

11th September 2024

40 Years of ISIS Neutron and Muon Source

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Rutherford Appleton Laboratory, UK

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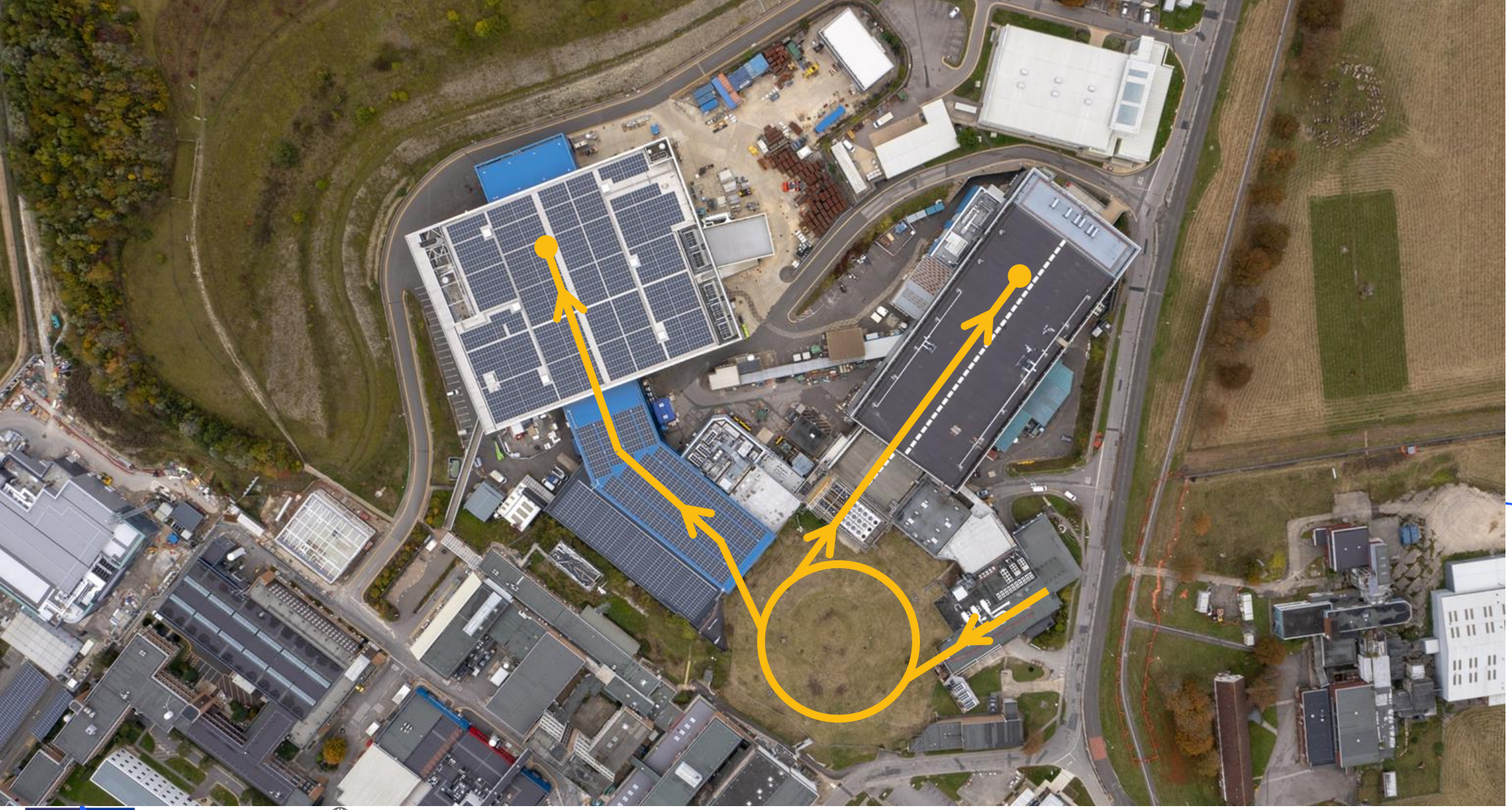

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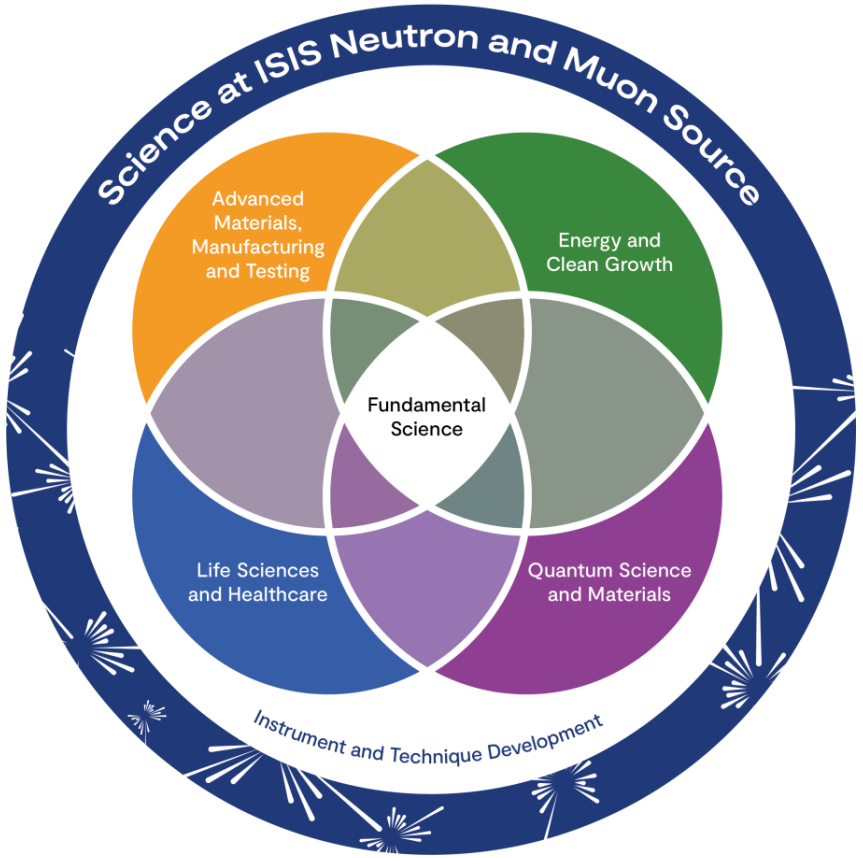
ISIS is a User Facility

35 instruments hosting **1200** experiments enabled by **600** staff for **3000** users from **30** countries generating **600** publications

Engaging Industry

In 2020, 85 companies were associated with ISIS proposals across all access routes. Some companies appear in more than one sector.



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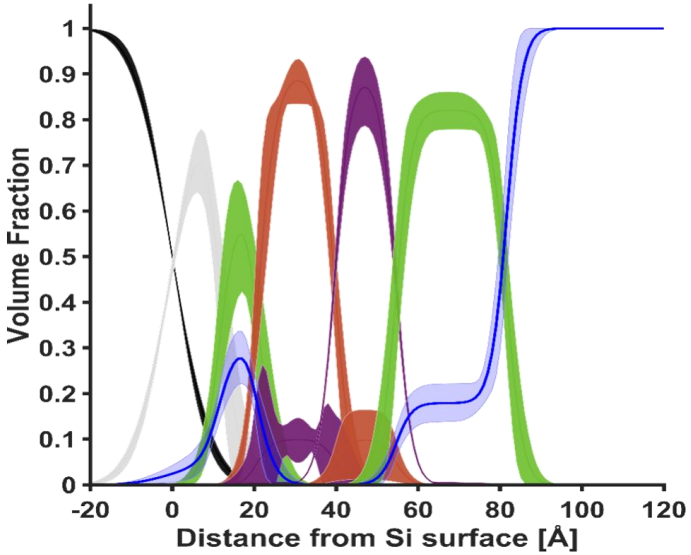
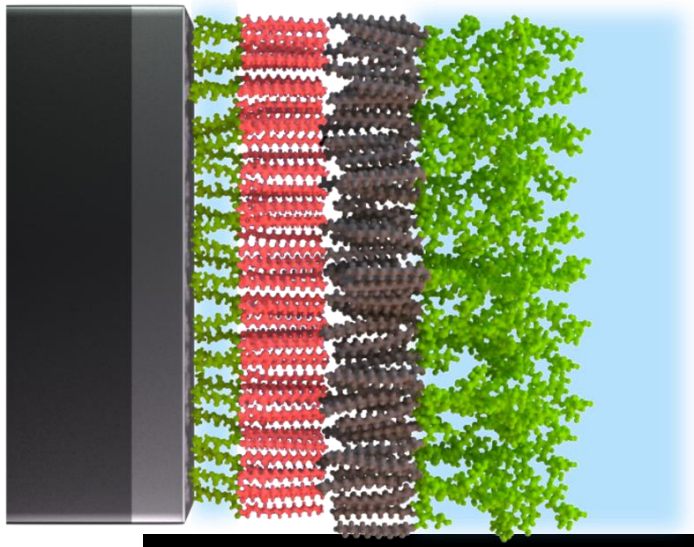
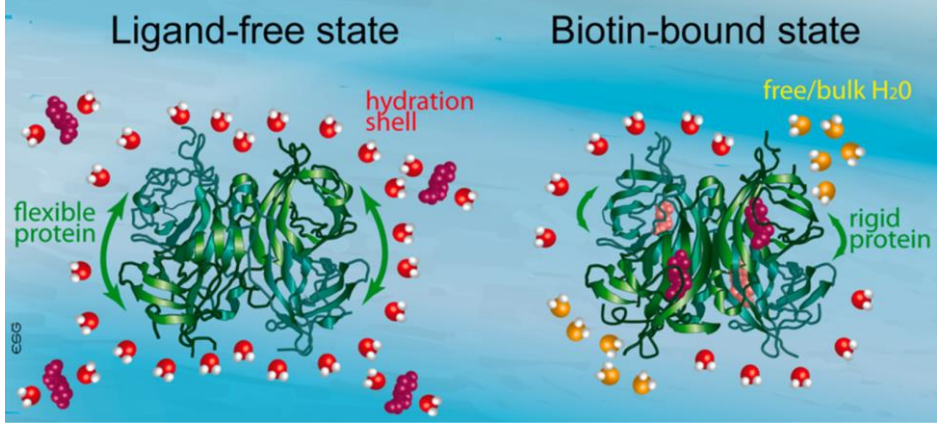
Life Science Examples

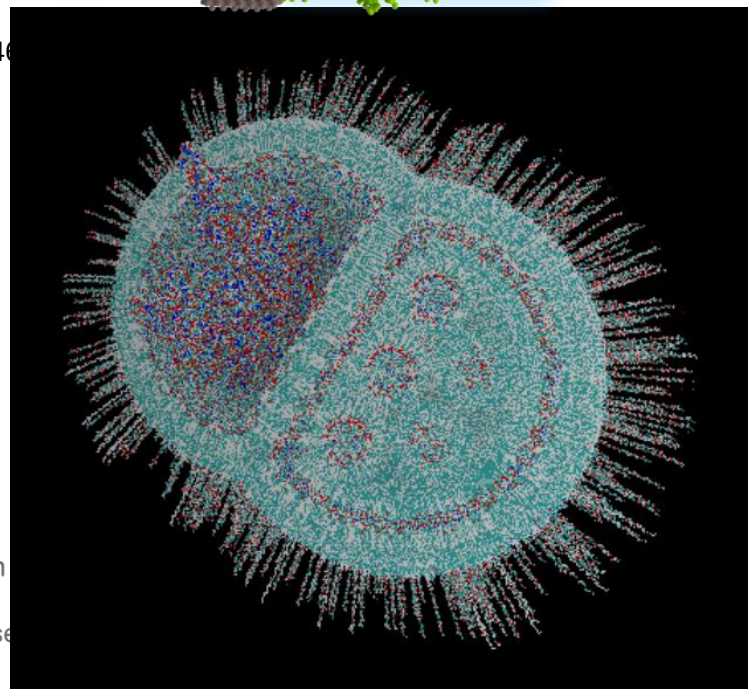


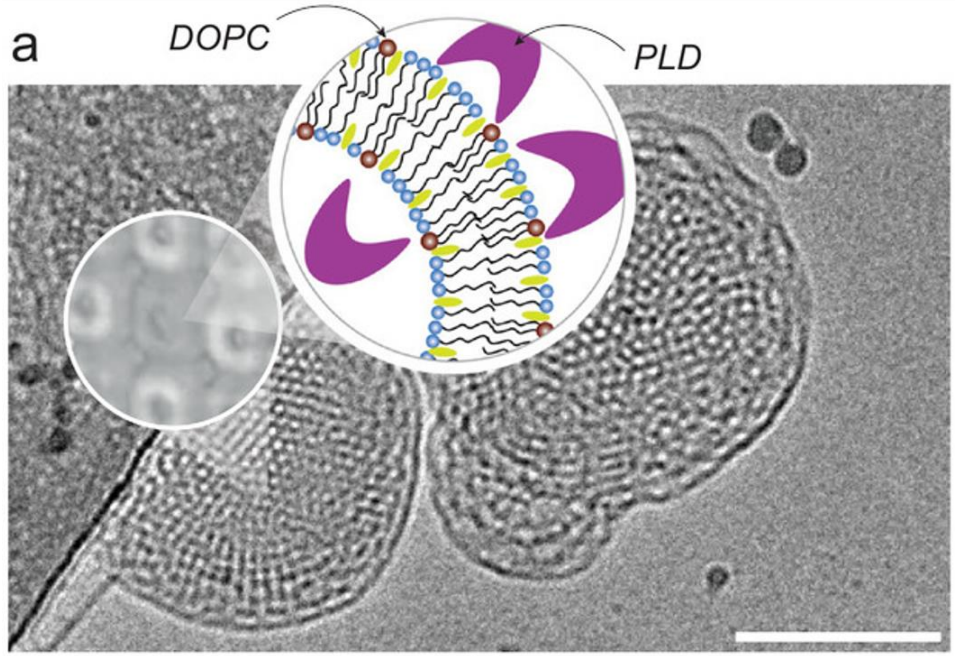


Clifton, Biochemical Society Transactions, 2021, 49 (4), 1537-154



Sarter et al, *J. Phys. Chem. B* 2020, 124, 2, 324–335





Barriga et al, https://doi.org/10.1002/adma.202200839

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Structure

- A bit of physics
- Introduction to our model system
- Isothermal Titration Calorimetry (ITC)
- thermal diffusion forced Rayleigh scattering (TDFRS)
- Quasi-elastic neutron scattering (QENS)
- Conclusion

Just a bit of physics and thermodynamics

Spontaneous binding

$$\Delta G = G_{complex} - G_{free} < 0$$

$$\Delta G = \Delta H - T\Delta S$$

Gibb's free Energy Enthalpy Entropy

$$\Delta G = -RTln(\frac{1}{K_d})$$

Just a bit of physics and thermodynamics

$$\Delta G = \Delta H - T\Delta S \qquad \Delta G < 0$$

$$\Delta S = \Delta S_{protein} + \Delta S_{hydr} + \Delta S_{ligand}$$

$$\Delta S_{protein} = \Delta S_{conf,prot} + \Delta S_{trans,prot}$$

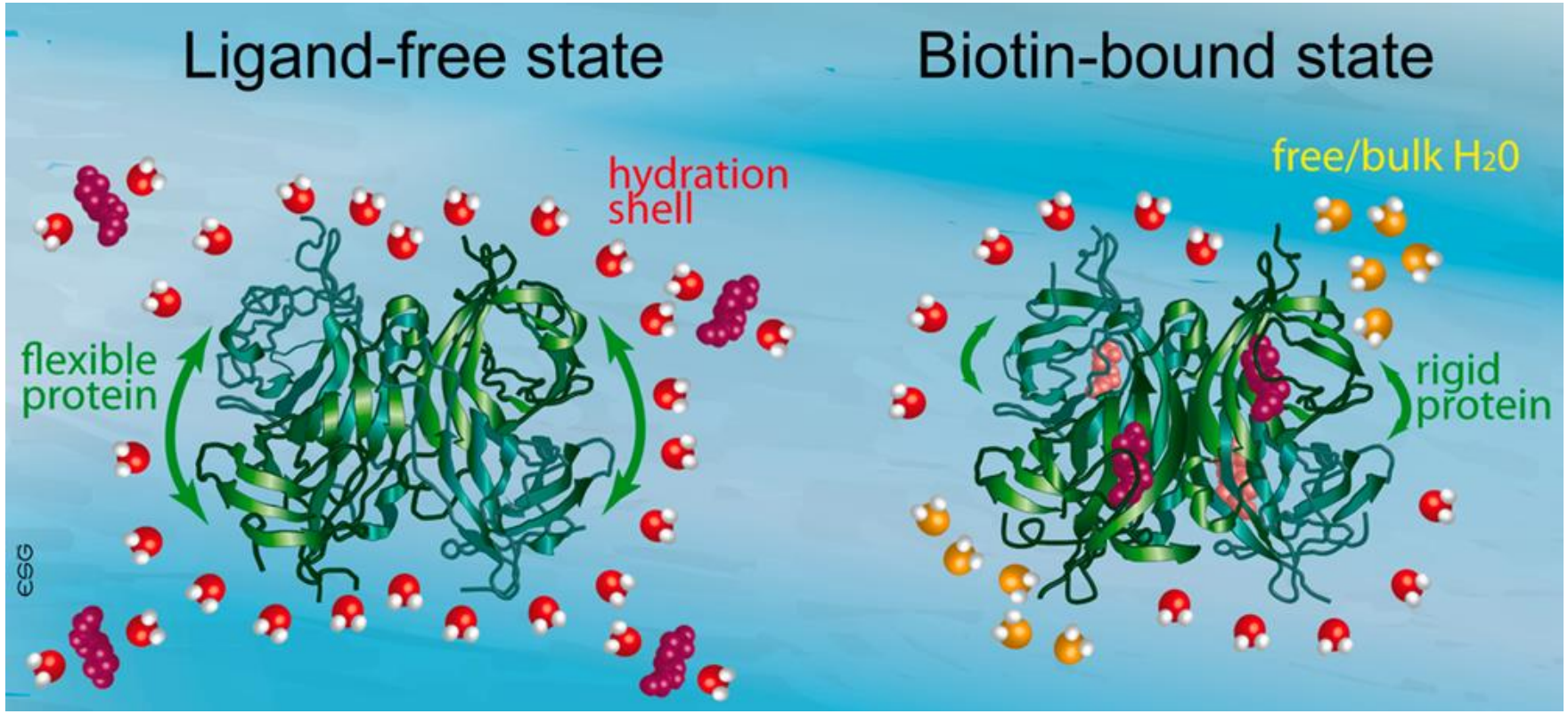
$$\Delta S_{hydr} = \Delta S_{displaced,hydr} + \Delta S_{surrounding,hydr}$$

$$\Delta S_{ligand} = \Delta S_{conf,ligand} + \Delta S_{trans,rot,ligand}$$

Just a bit of physics and thermodynamics

- Gibb's free Energy is minimised for spontaneous binding
- It is affected by affinity, enthalpy, and entropy
- There are multiple entropic components

Meet our model system



- Streptavidin is a homo-tetramer 53.1kDa
- 4:1 Biotin binding stoichiometry
- $K_d \approx 1 \cdot 10^{-14}$ M from literature

Sarter et al, *J. Phys. Chem. B* 2020, 124, 2, 324–335

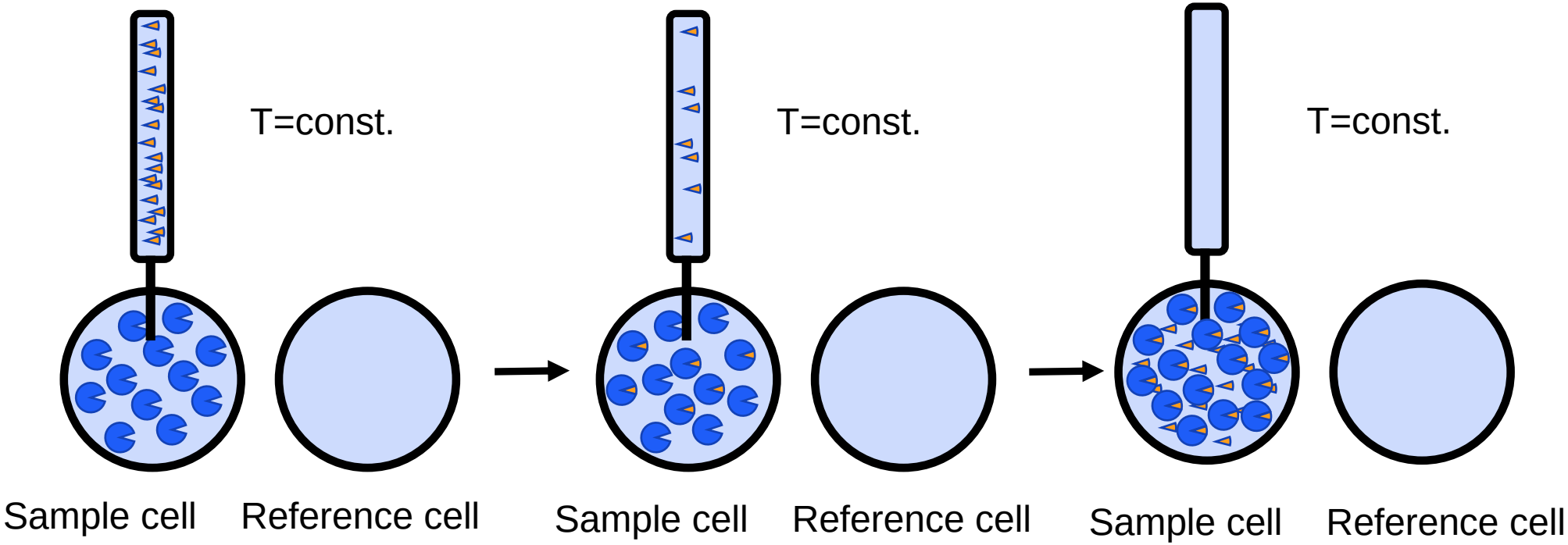
Isothermal Titration Calorimetry (ITC)

- How does the technique work
- Some results for streptavidin and biotin
- How to choose the ideal system for this technique

Provides

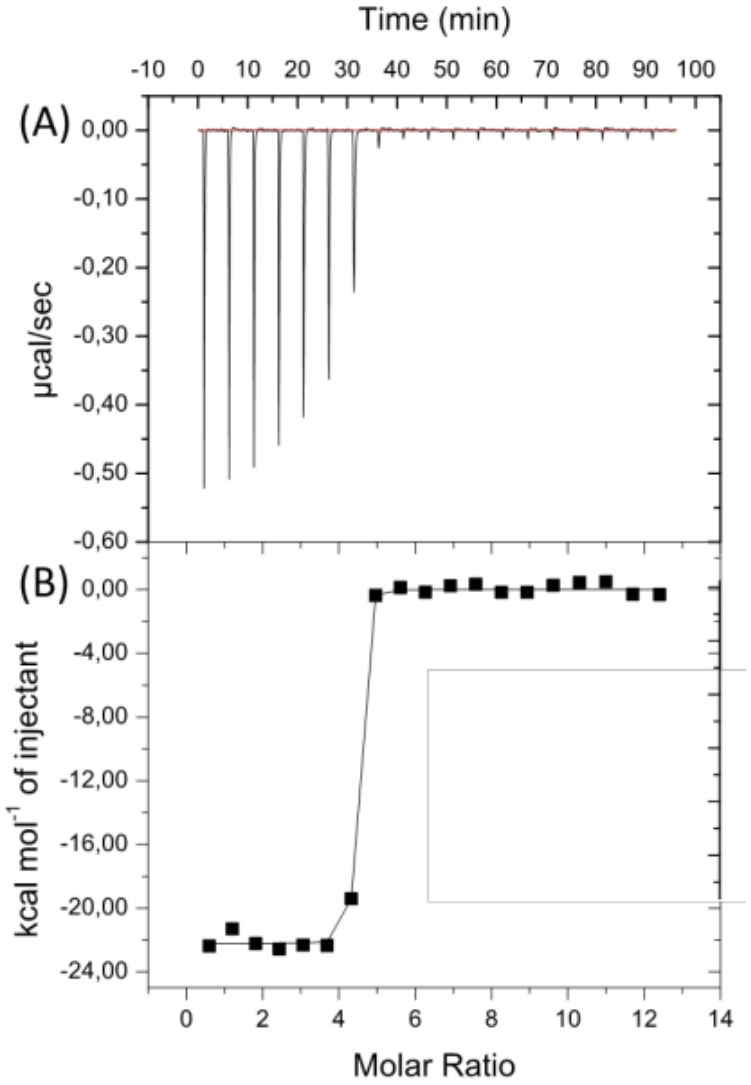
- ΔH
- K_d
- ΔG
- ΔS

How does ITC work



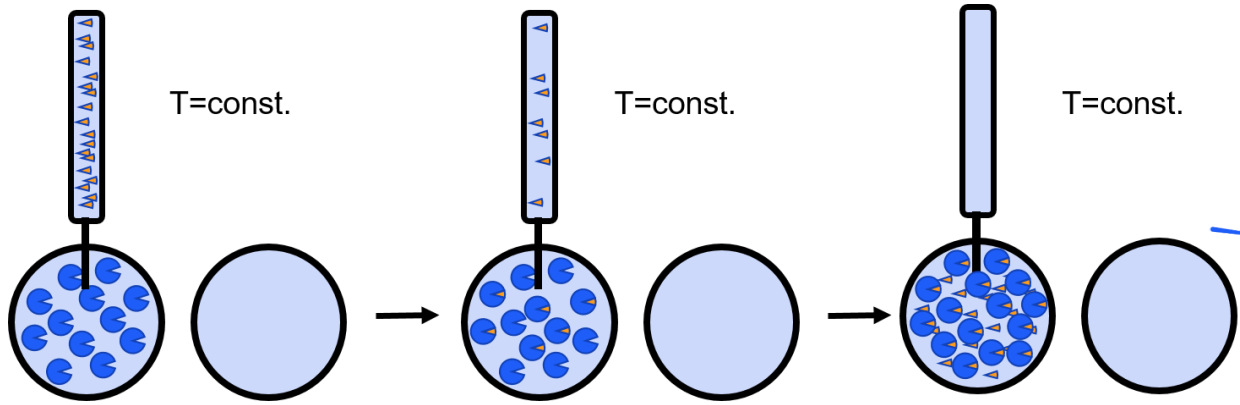
The energy required to heat the sample cell to the same temperature as the reference cell is constantly measured.

Results from ITC



$$\Delta\bar{H} = -472.1 \pm 47.2 \frac{kJ}{mol}$$

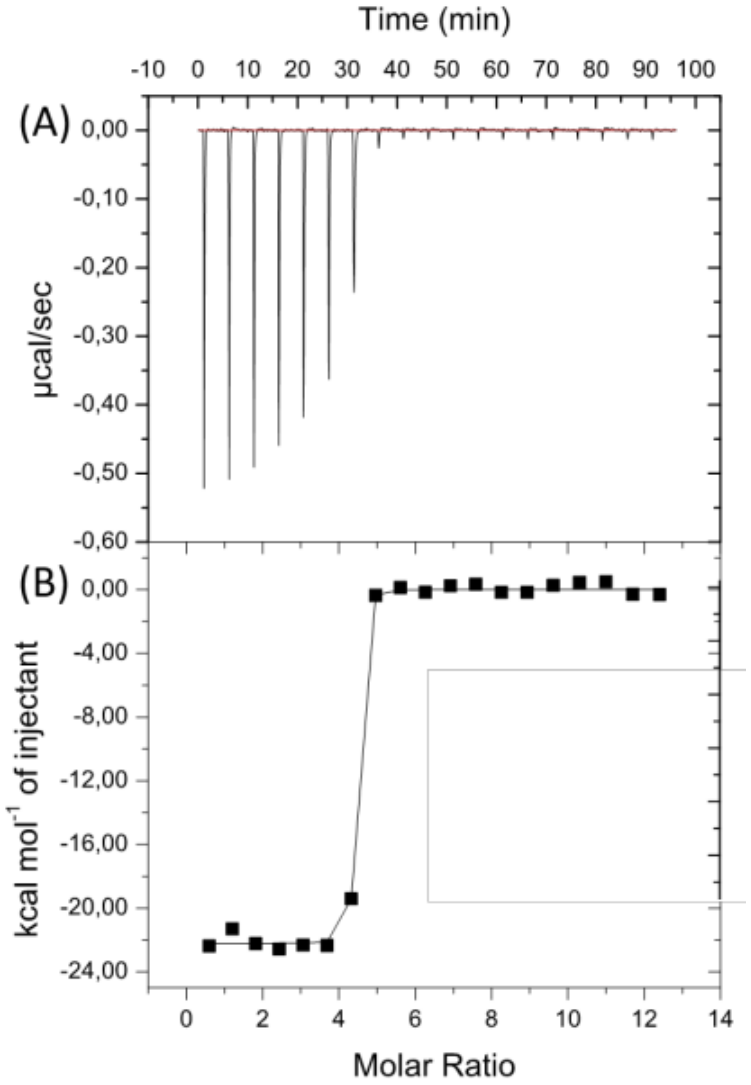
$$\Delta G = -RTln(\frac{1}{K_d})$$



$$\Delta S = \frac{\Delta G - \Delta H}{T}$$

Sarter, *Dissertation, RWTH Aachen University*, 2020

Results from ITC



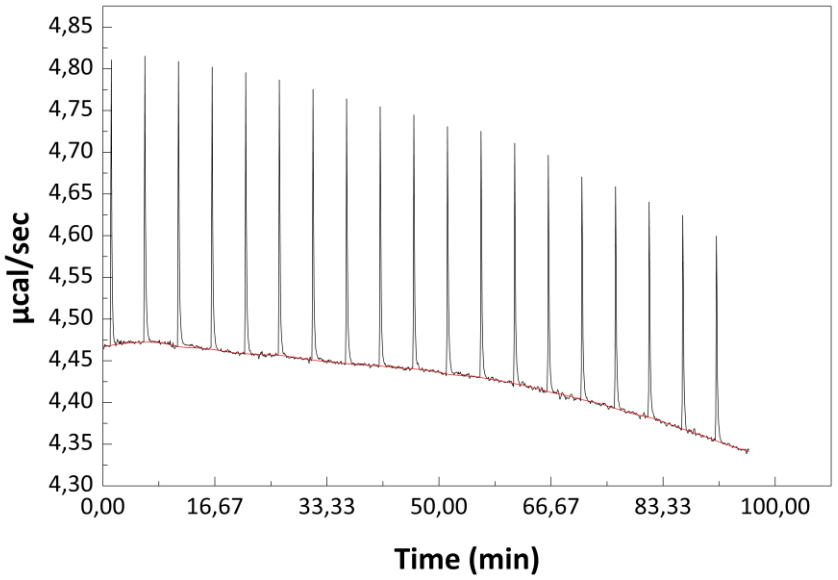
$$\Delta\bar{H} = -472.1 \pm 47.2 \frac{kJ}{mol}$$

Sample number	Stoichiometry N	$\Delta H \left[\frac{kJ}{mol}\right]$
1	4.3	-367.2
2	4.7	-371.5
3	4.2	-446.9
4	4.6	-367.2
5	4.5	-404.0
6	3.8	-455.1
7	3.7	-462.9
8	3.9	-462.8
9	3.5	-506.3

Sarter, *Dissertation, RWTH Aachen University*, 2020

ITC: Challenges and the ideal system

- The ligand must be soluble at high concentrations
- The binding model must be known
- The binding must be of a suitable strength
- For neutron scattering the reaction must occur in H_2O and D_2O based solvents
- Very sensitive to temperature changes in the lab
- Potential sensitivity to dilution effects
- Experiments should be repeated for statistics



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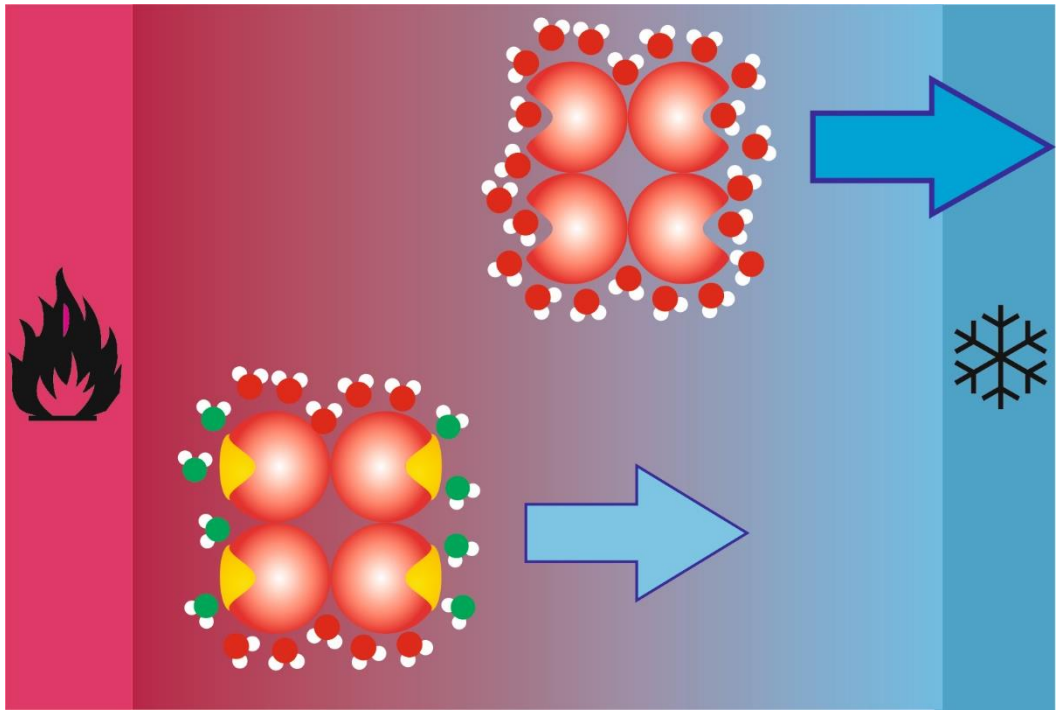
thermal diffusion forced Rayleigh scattering (TDFRS)

- How does the technique work
- Some results for streptavidin and biotin
- How to choose the ideal system for this technique

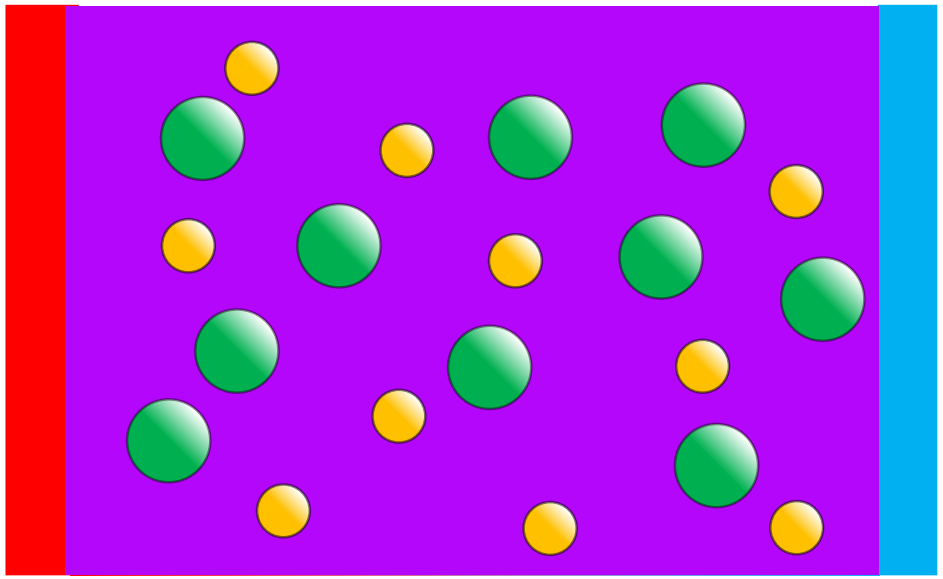
Provides

- $\Delta S_{surrounding}$
- Qualitative information



Sarter et al, *EPJ Web of Conferences* 272, 01016 (2022) QENS/WINS 2022

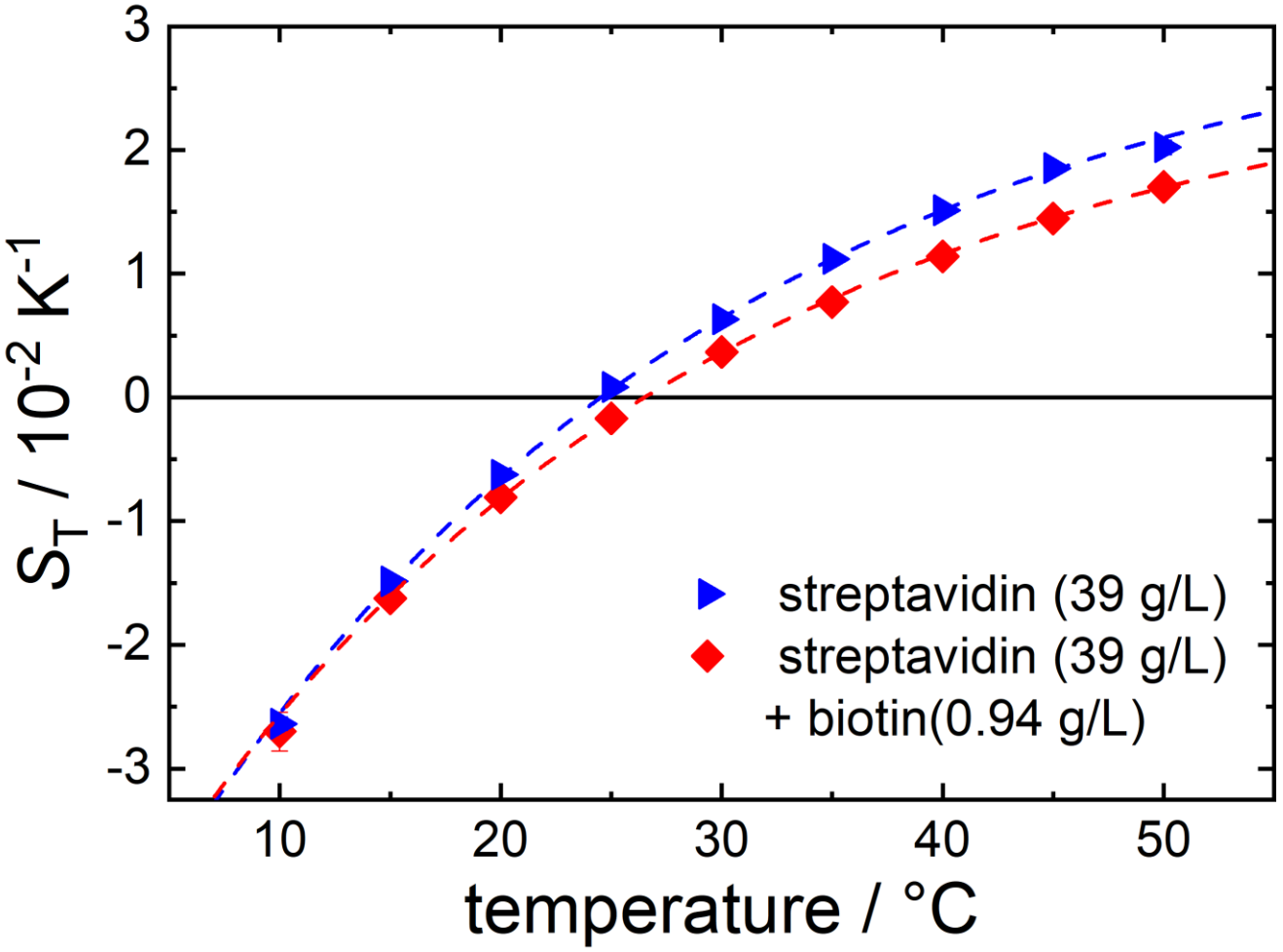
How does TDFRS work



- Highly sensitive to number and strength of H-bonds
- Measured at different temperatures
- Temperature grid caused by interferometry $\lambda = 980\ \mathrm{nm}$

Results from TDFRS

Experiments by Dr. Doreen Niether and Prof. Simone Wiegand



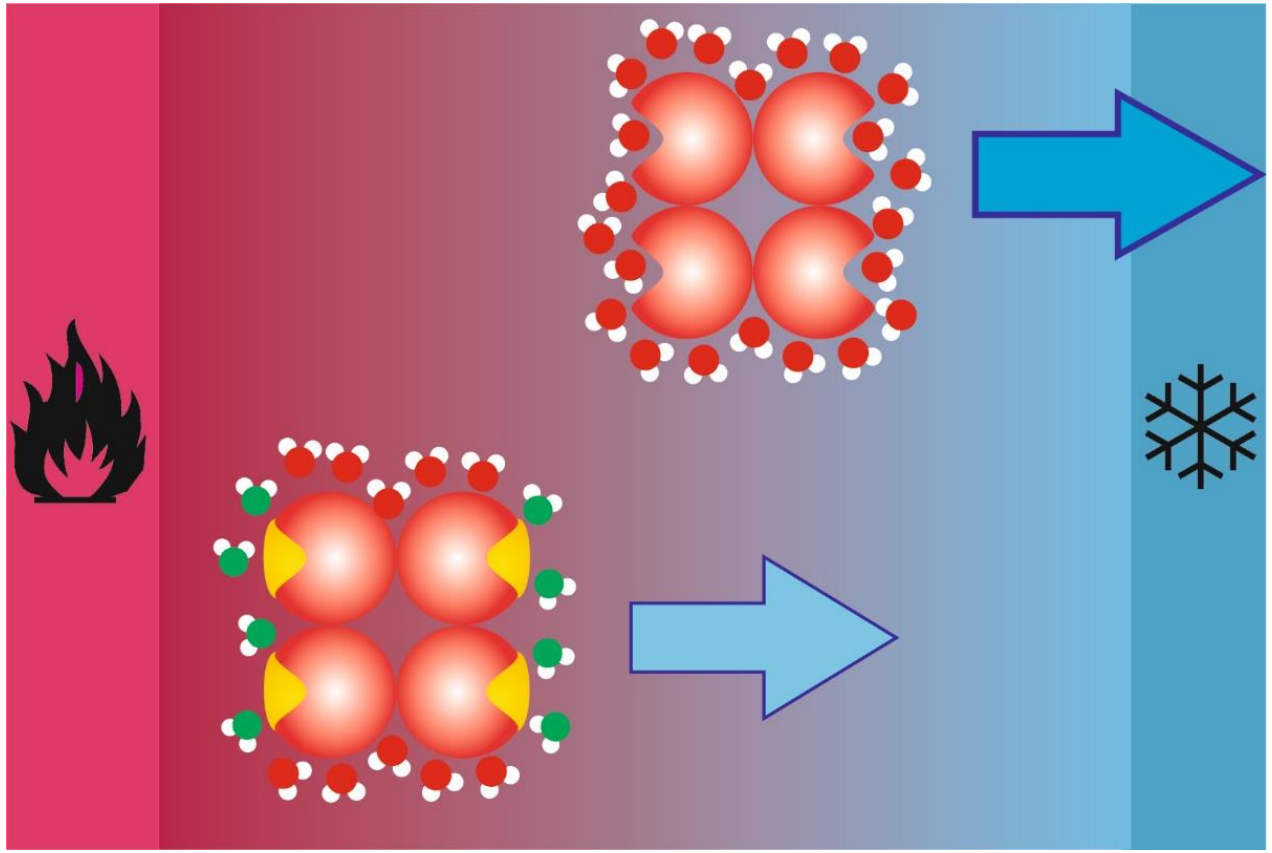
$$S_T = \frac{D_T}{D}$$

$$A = \left(\frac{\partial n}{\partial c}\right)_{p,T} \left(\frac{\partial n}{\partial T}\right)_{p,c}^{-1} S_T c(1-c)$$

Sarter et al, *EPJ Web of Conferences* 272, 01016 (2022) QENS/WINS 2022

Results from TDFRS

Experiments by Dr. Doreen Niether and Prof. Simone Wiegand



$$S_T = \frac{D_T}{D}$$

$$A = \left(\frac{\partial n}{\partial c}\right)_{p,T} \left(\frac{\partial n}{\partial T}\right)_{p,c}^{-1} S_T c(1-c)$$

- ΔS_{hydr} has increased
- Less H-bonds between complex than free
- Reduced order and therefore higher entropy

Sarter et al, *EPJ Web of Conferences* 272, 01016 (2022) QENS/WINS 2022

TDFRS: Challenges and the ideal system

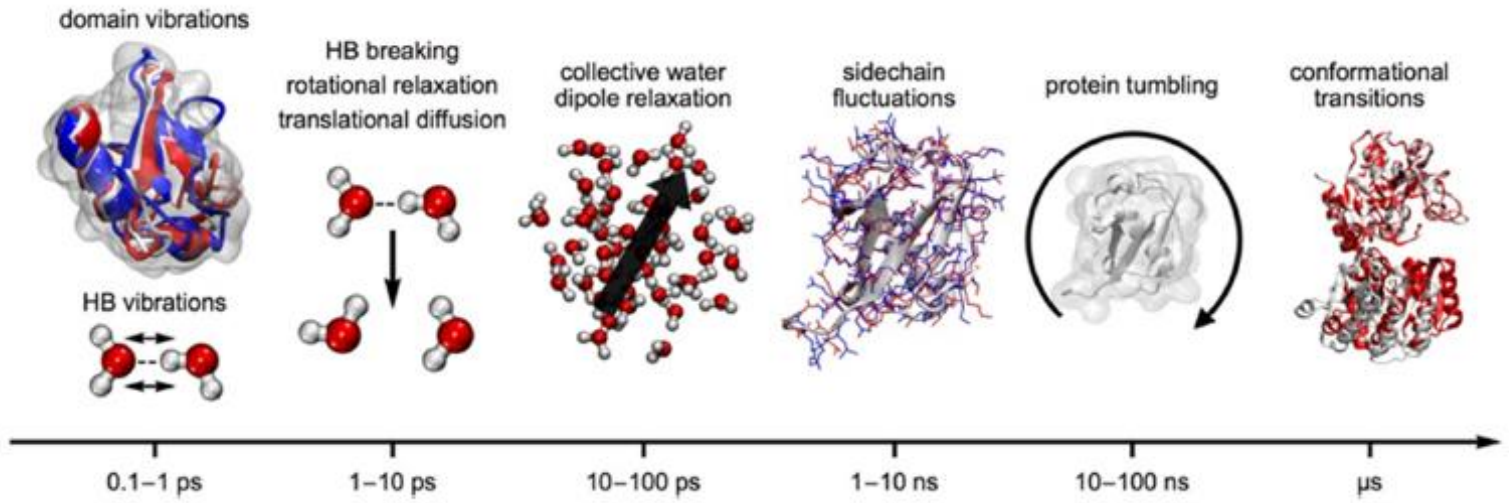
- Requires the diffusion coefficient D
- Gives qualitative information on the hydration layer
- Cannot be performed in pure D_2O but measuring different H_2O D_2O ratios allows for extrapolation to pure D_2O
- Can be used to check if change in solvent affects sample

Quasi-elastic neutron scattering (QENS)

- How does the technique work
- Some results for streptavidin and biotin
- How to choose the ideal system for this technique

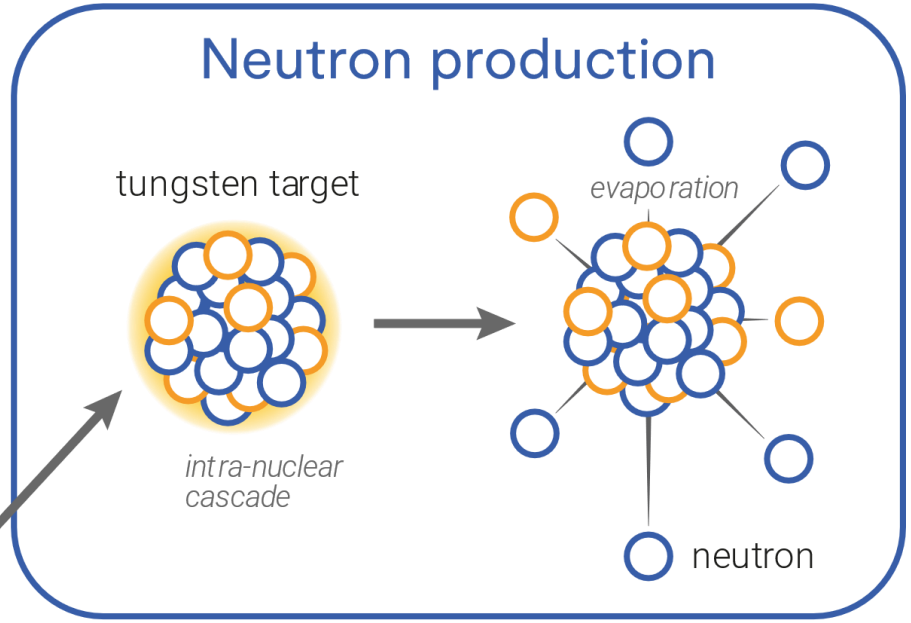
Provides

- ΔS_{conf}
- D_{eff}
- Dynamic information
- Average flexibility



Y. Xu et al, J. Chem. Phys.143, 170901 (2015), DOI: 10.1063/1.4934504

How does QENS work – How do we get neutrons?



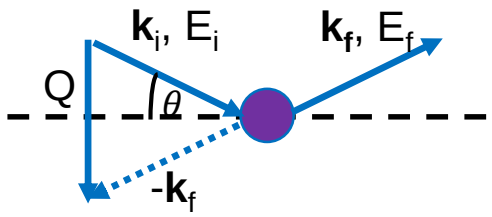
From the accelerator

high-energy protons

- Neutrons are neutrally charged
- $\text{spin} = \frac{1}{2}$
- Interact with nucleus opposed to electron shell

	σ_{coh} $[b]$	σ_{inc} $[b]$
Hydrogen	1.76	80.2
Deuterium	5.59	2.05
Carbon	5.56	0.00
Nitrogen	11.0	0.50
Oxygen	4.23	0.00

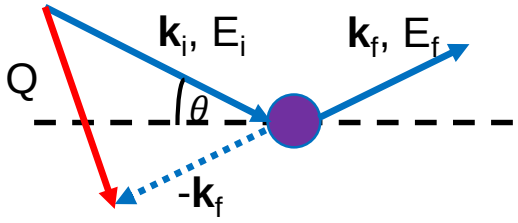
How does QENS work – Quasi-elastic?



So where is quasi-elastic?

Quasi means:

- Appears to be something but is not really so
- Partly, almost



Elastic scattering

$$|\boldsymbol{k}_i| = |\boldsymbol{k}_f| = \frac{2\pi}{\lambda}$$

$$\Delta E = 0$$

$$Q = 2|\boldsymbol{k}|\sin(\theta) = \frac{4\pi}{\lambda}\sin(\theta)$$

Here: Almost elastic scattering
Almost no energy transfer

Quasi elastic scattering

$$|\boldsymbol{k}_i| \neq |\boldsymbol{k}_f|$$

ΔE small and centred around 0

$$\Delta E = \hbar\omega = \frac{h}{\tau} = E_i - E_f = \frac{\hbar^2}{2m_n}\left(\boldsymbol{k}_i - \boldsymbol{k}_f\right)^2$$

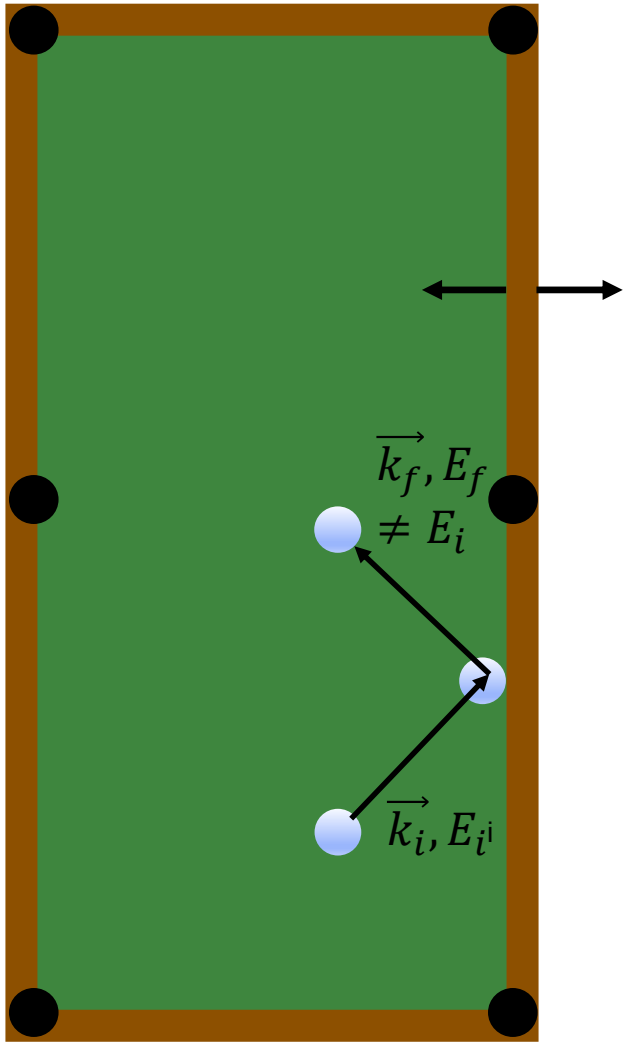
Inelastic scattering

$$|\boldsymbol{k}_i| \neq |\boldsymbol{k}_f|$$

$$\Delta E \neq 0$$

$$\Delta E = \hbar\omega = E_i - E_f = \frac{\hbar^2}{2m_n}\left(\boldsymbol{k}_i - \boldsymbol{k}_f\right)^2$$

How does QENS work – What do we observe?



Elastic scattering

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$$\Delta E = 0$$

$$Q = 2|\boldsymbol{k}|\sin(\theta) = \frac{4\pi}{\lambda}\sin(\theta)$$

Quasi elastic scattering

$$|\boldsymbol{k}_i| \neq |\boldsymbol{k}_f|$$

ΔE small and centred around 0

$$\Delta E = \hbar\omega = \frac{h}{\tau} = E_i - E_f$$

$$= \frac{\hbar^2}{2m_n}\left(\boldsymbol{k}_i - \boldsymbol{k}_f\right)^2$$

What do we observed from the second ball?

- Change in energy
- Incident angle not necessarily exit angle

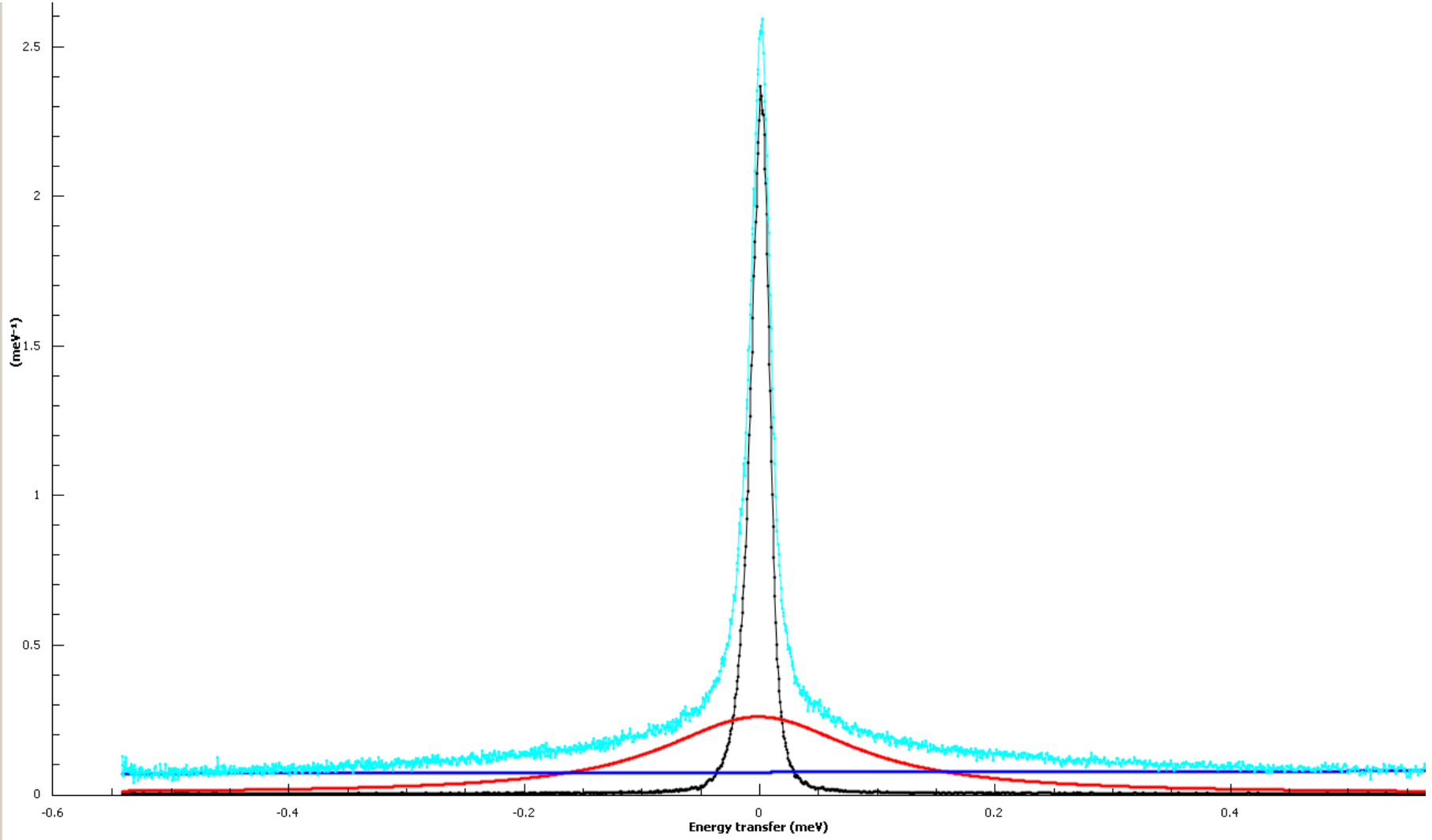
What can we deduce for our samples?

- Diffusion at molecular scale
- Aggregate state
- Able to differentiate diffusion and confined dynamics
- Time- and length-scale comparable to MD simulations
- Contrast can be adjusted/ matched

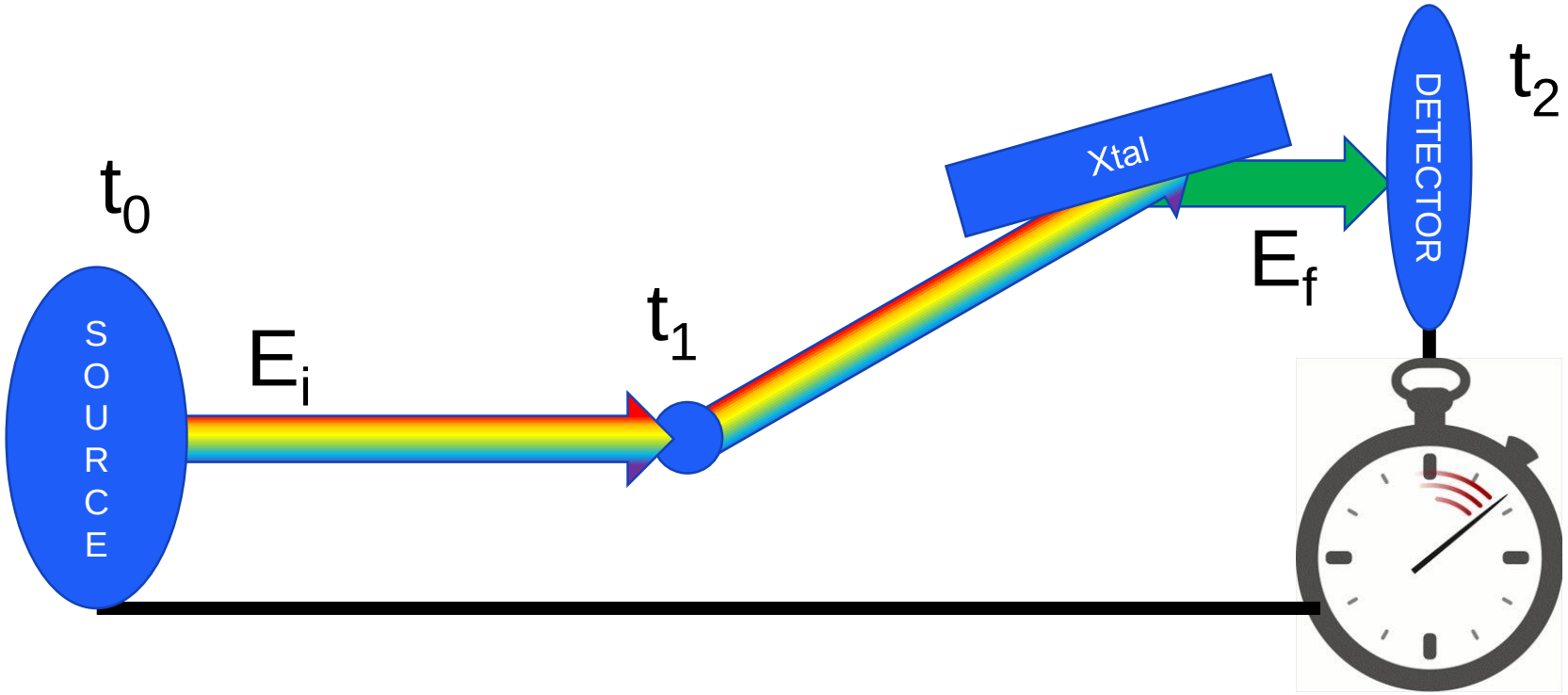
Examples

- Diffusion in MOFs & zeolites, fuel cells, polymers, clays, ionic liquids, …
- Protein dynamics, drug delivery, degree of hydration, water dynamics in cells
- Confinement, glasses

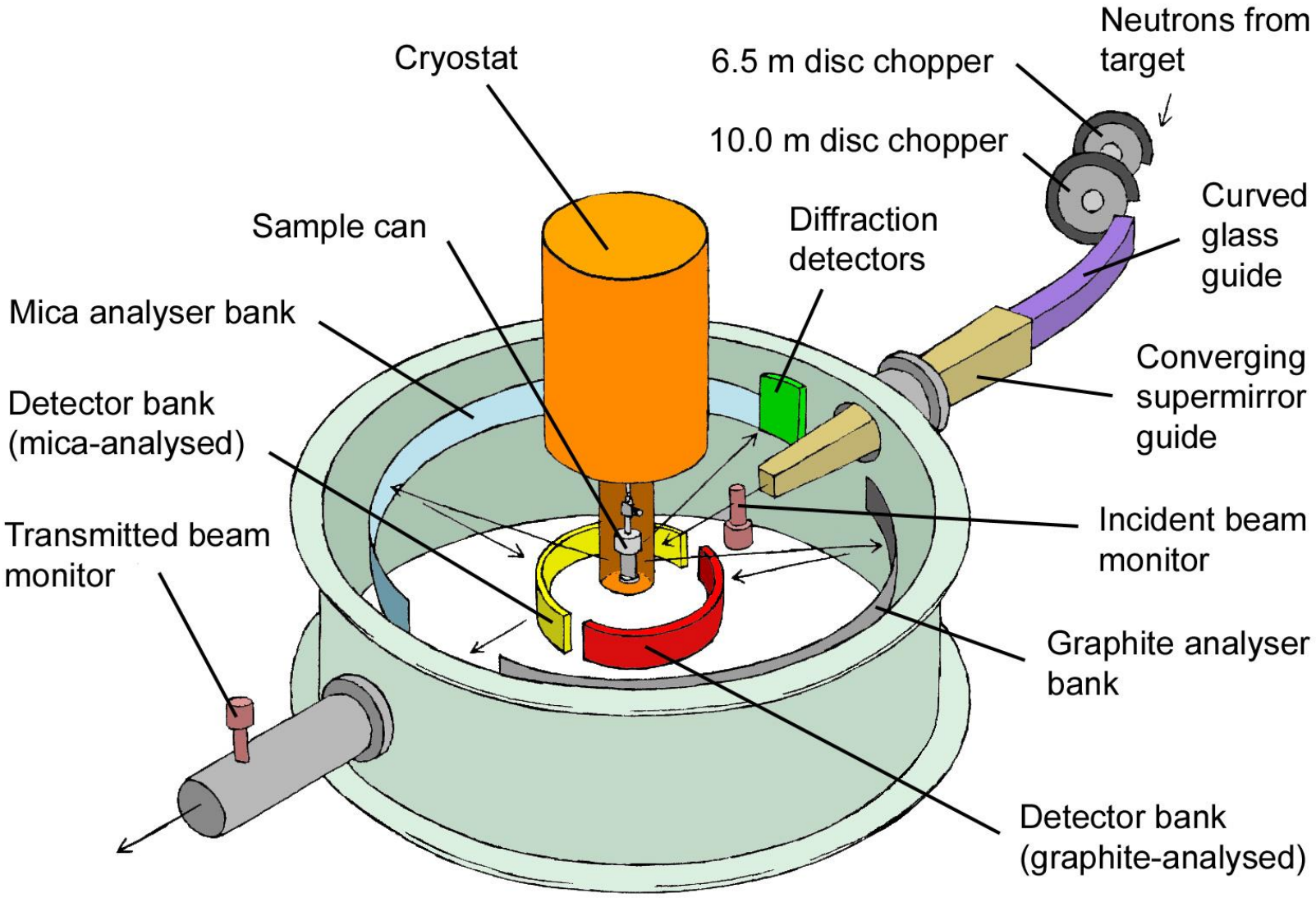
How does QENS work – What do we observe?



How does QENS work – How does the instrument work?



How does QENS work – How does the instrum

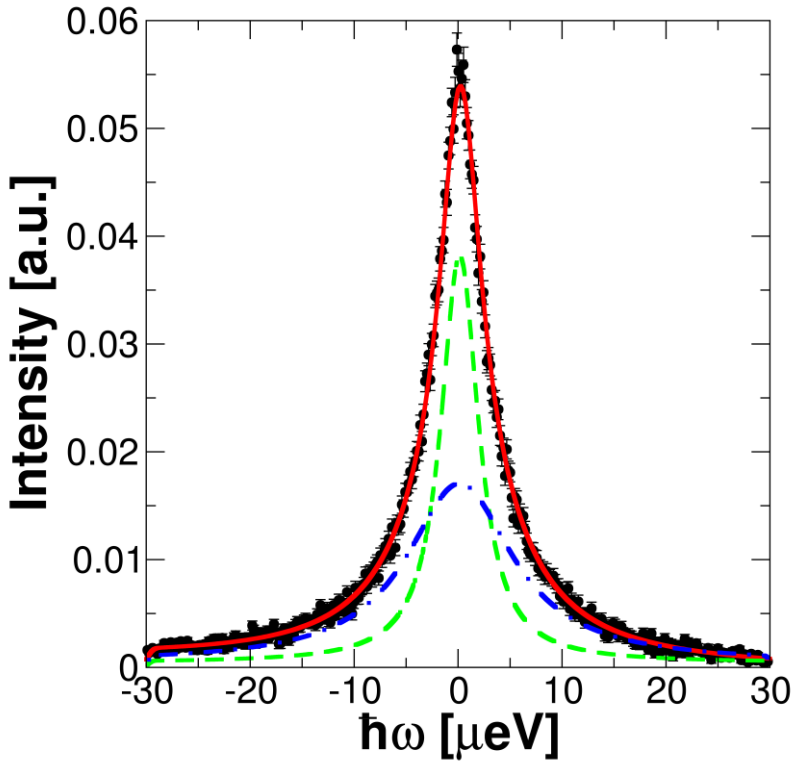


Results from QENS

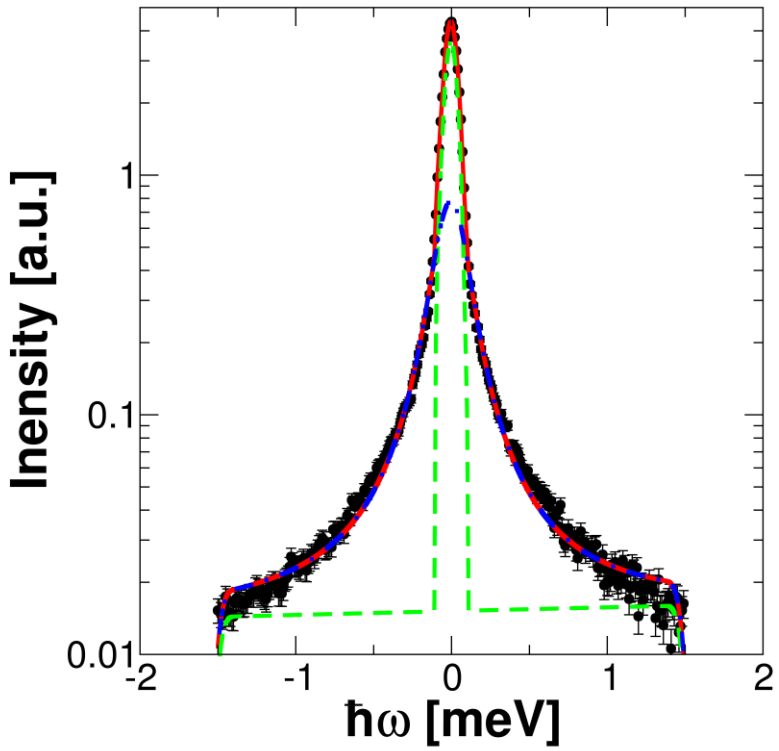
$$S_I = A_0(q) \cdot \delta(\omega) + \left(1 - A_0(q)\right) \cdot L(q,\omega)$$

$$S_{total} = A_0(q) \cdot L_G(q,\omega) + \left(1 - A_0(q)\right) \cdot L_{G+I}(q,\omega)$$

(A) ns



(C) ps



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Sarter et al, *J. Phys. Chem. B* 2020, 124, 2, 324–335

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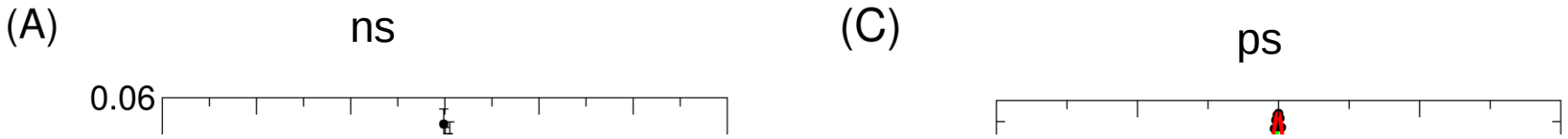
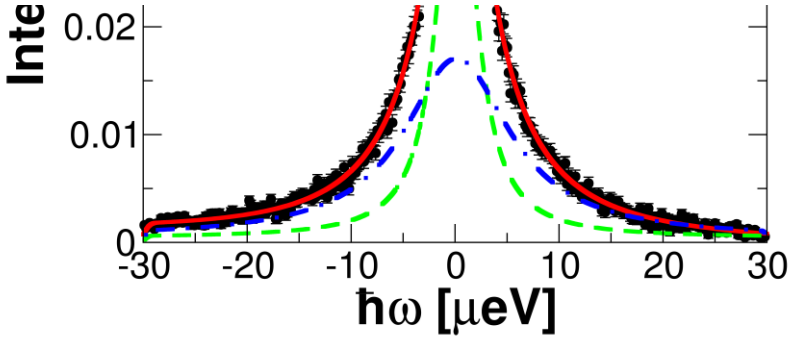
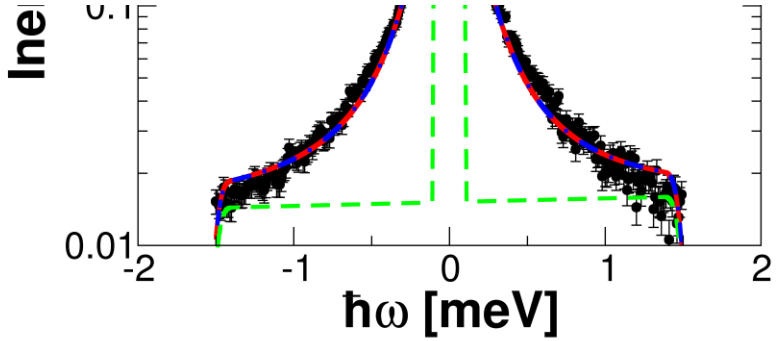


This amounts to we can separate different dynamics.

Just assign one Lorentzian per dynamic.

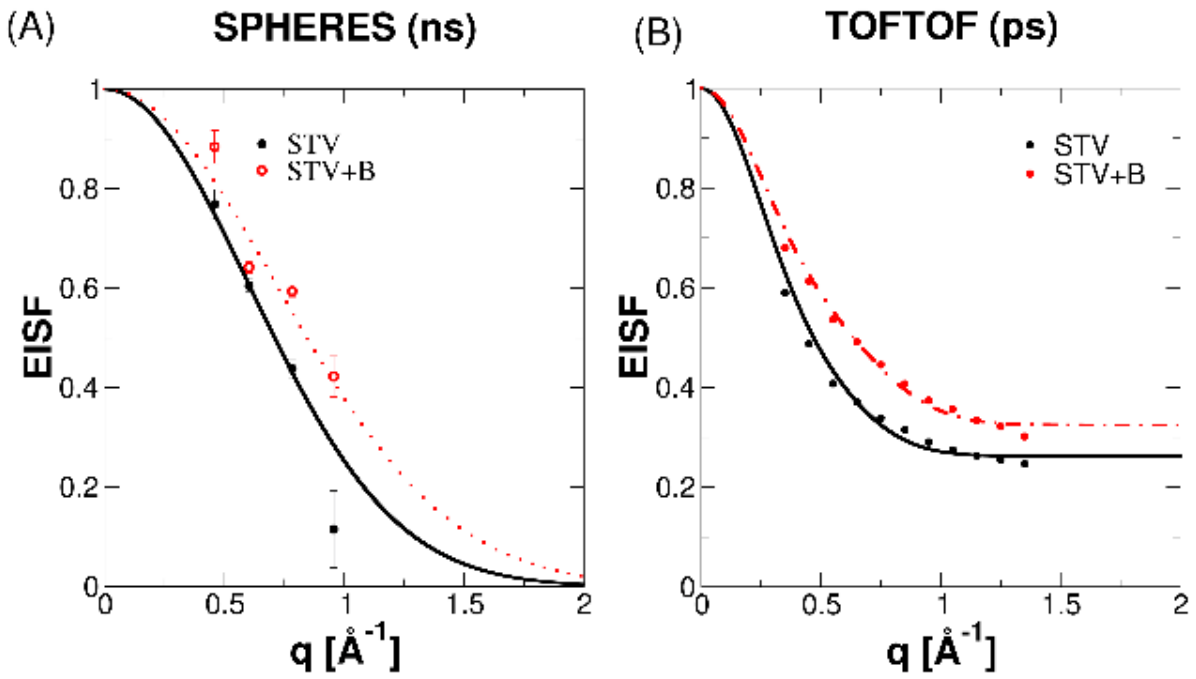




Sarter et al, *J. Phys. Chem. B* 2020, 124, 2, 324–335

Results from QENS– Conformational entropy change

$A_0(q) = e^{-<u^2>\cdot q^2}(1-p) + p$, $\langle u^2 \rangle$ mean-square displacement MSD



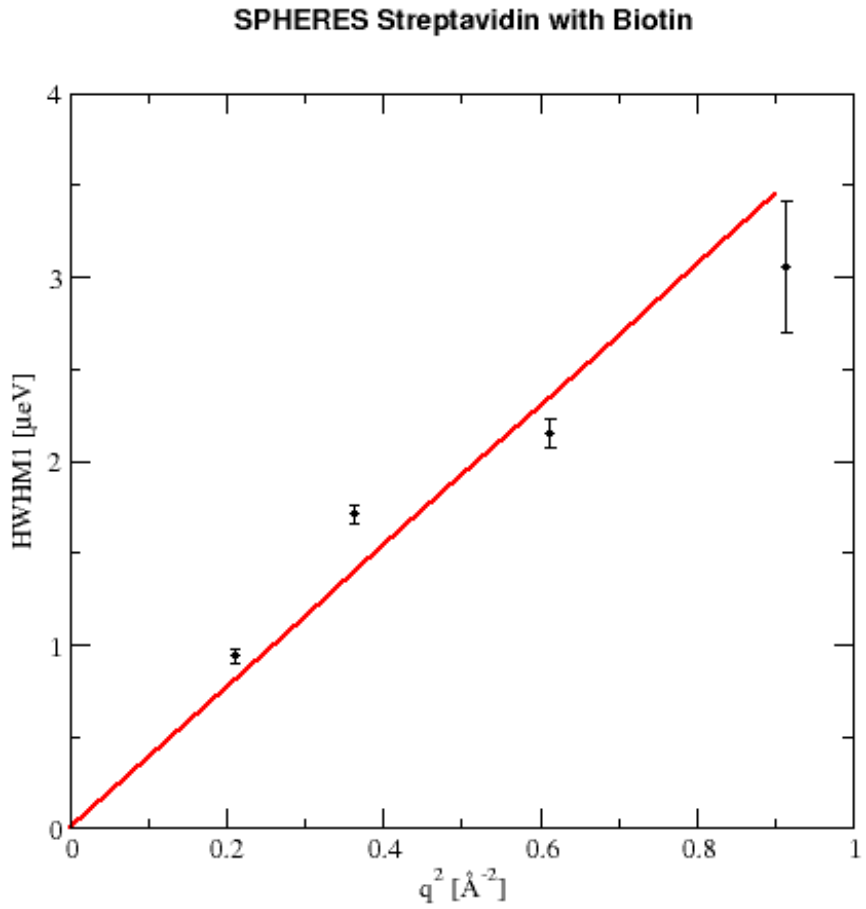
$$\Delta S_{conf,ns} = 3R \ln \left(\sqrt{\frac{MSD_{complex}}{MSD_{free}}} \right)$$

$$= -2.2 \pm 0.3 \frac{kJ}{molK}$$

Sarter et al, *J. Phys. Chem. B* 2020, 124, 2, 324–335

Results from QENS– Apart from entropy

- Diffusion of particles in solution
- Internal dynamics
- Domain vibrations
- Side chain fluctuations
- Conformational transitions
- Broadening, EISF, MSD



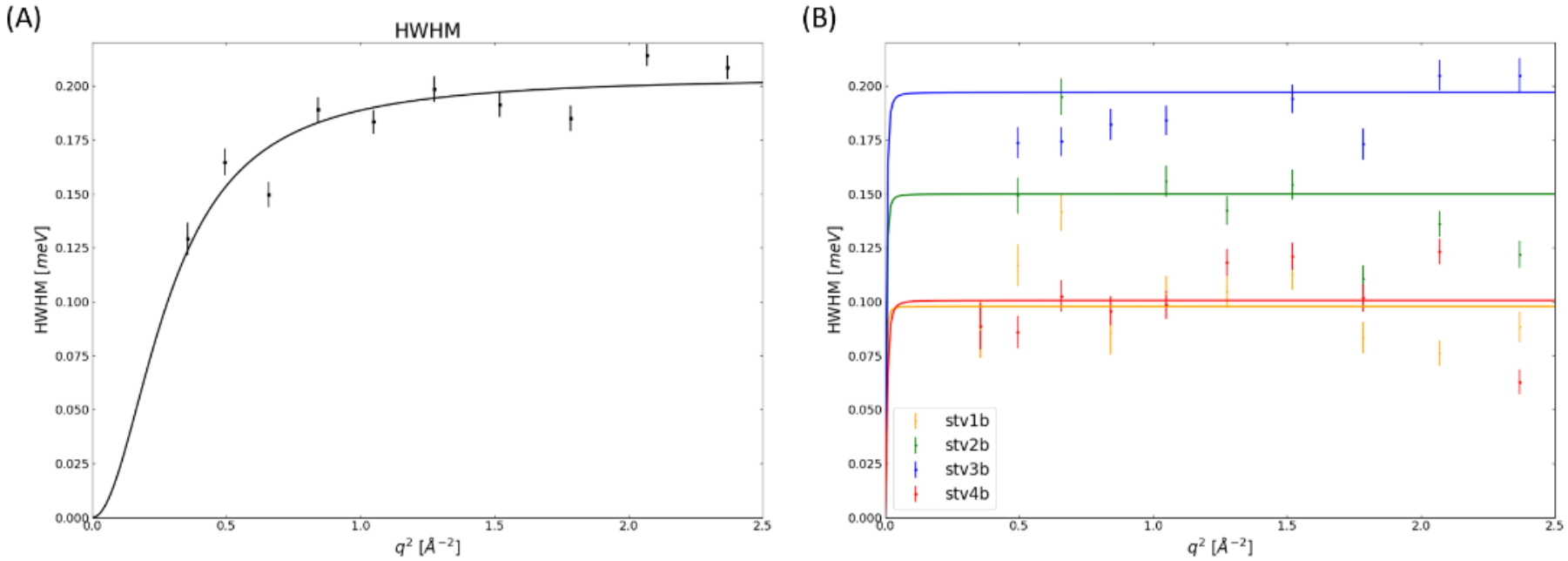
Sarter et al, *J. Phys. Chem. B* 2020, 124, 2, 324–335

Sarter, *J. Phys. Chem. B* 2023 *127* (14), 3241-3247

Results from QENS– Apart from entropy

This indicates cooperative binding!

- Diffusion of particles in solution
- Internal dynamics
- Domain vibrations
- Side chain fluctuations
- Conformational transitions
- Broadening, EISF, MSD

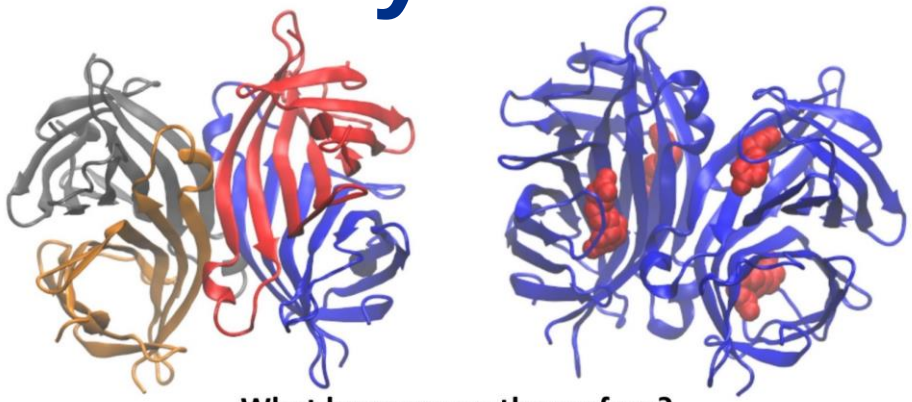


Sarter et al, *J. Phys. Chem. B* 2020, 124, 2, 324–335

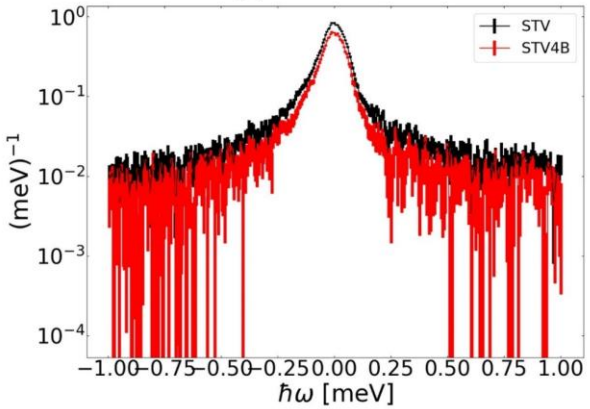
Sarter, *J. Phys. Chem. B* 2023 *127* (14), 3241-3247

QENS: Challenges and the ideal system

- The reaction must take place in D_2O
- The stronger the binding the better
- High concentrations and sample volumes of $\approx 1 - 2$ ml
- For neutron scattering the reaction must occur in H_2O and D_2O based solvents



What happens on the surface?



	σ_{coh} [b]	σ_{inc} [b]
Hydrogen	1.76	80.2
Deuterium	5.59	2.05
Carbon	5.56	0.00
Nitrogen	11.0	0.50
Oxygen	4.23	0.00

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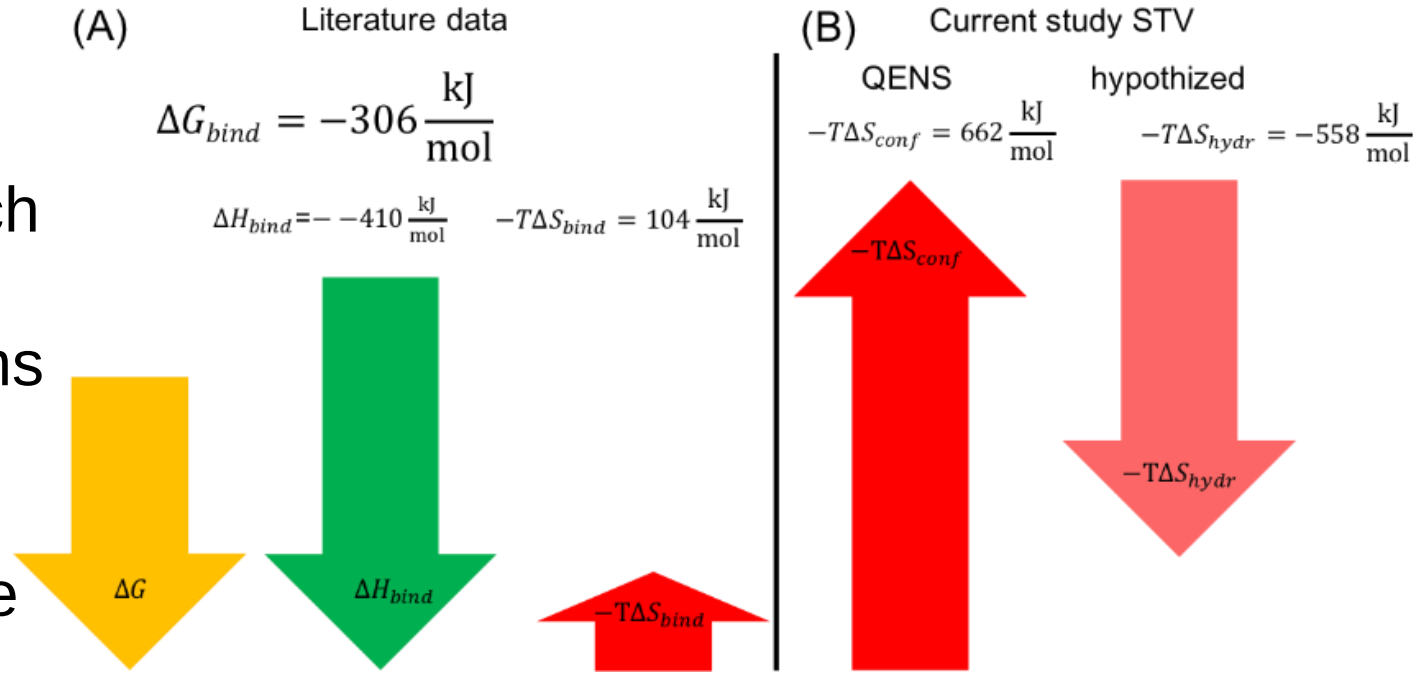
Conclusion

- A complementary approach can vastly increase our understanding of a systems components



- Samples need to be stable during experiment
- Samples need to be appropriate for each method chosen
- No one method can answer all these questions
- Molecular Dynamics simulations in addition to the experiments would be great

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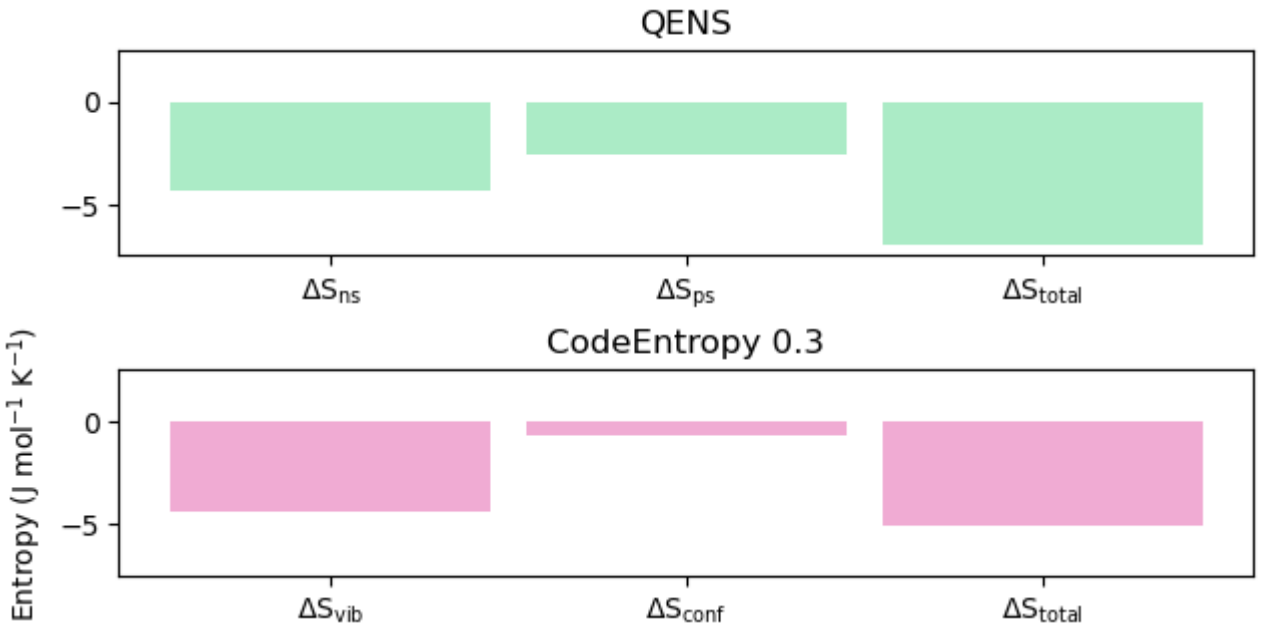
Conclusion

- Simulations were done using code entropy
- The experimental and simulated results match very well

Simulations by Ioana Papa during her Master Thesis (supervised by Sarah Fegan)

Term	Value (J mol^{-1} K^{-1})
$\Delta S_{ns,QENS}$/residue	-4.37 ± 0.59
$\Delta S_{ps,QENS}$/residue	-2.58 ± 0.43
$\Delta S_{total,QENS}$/residue	-6.95 ± 0.73
$\Delta S_{conf,CodeEntropy\ 0.3}$/residue	-0.67 ± 0.00
$\Delta S_{vib,CodeEntropy\ 0.3}$/residue	-7.32 ± 0.91
$\Delta S_{total,CodeEntropy\ 0.3}$/residue	-7.99 ± 0.91



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