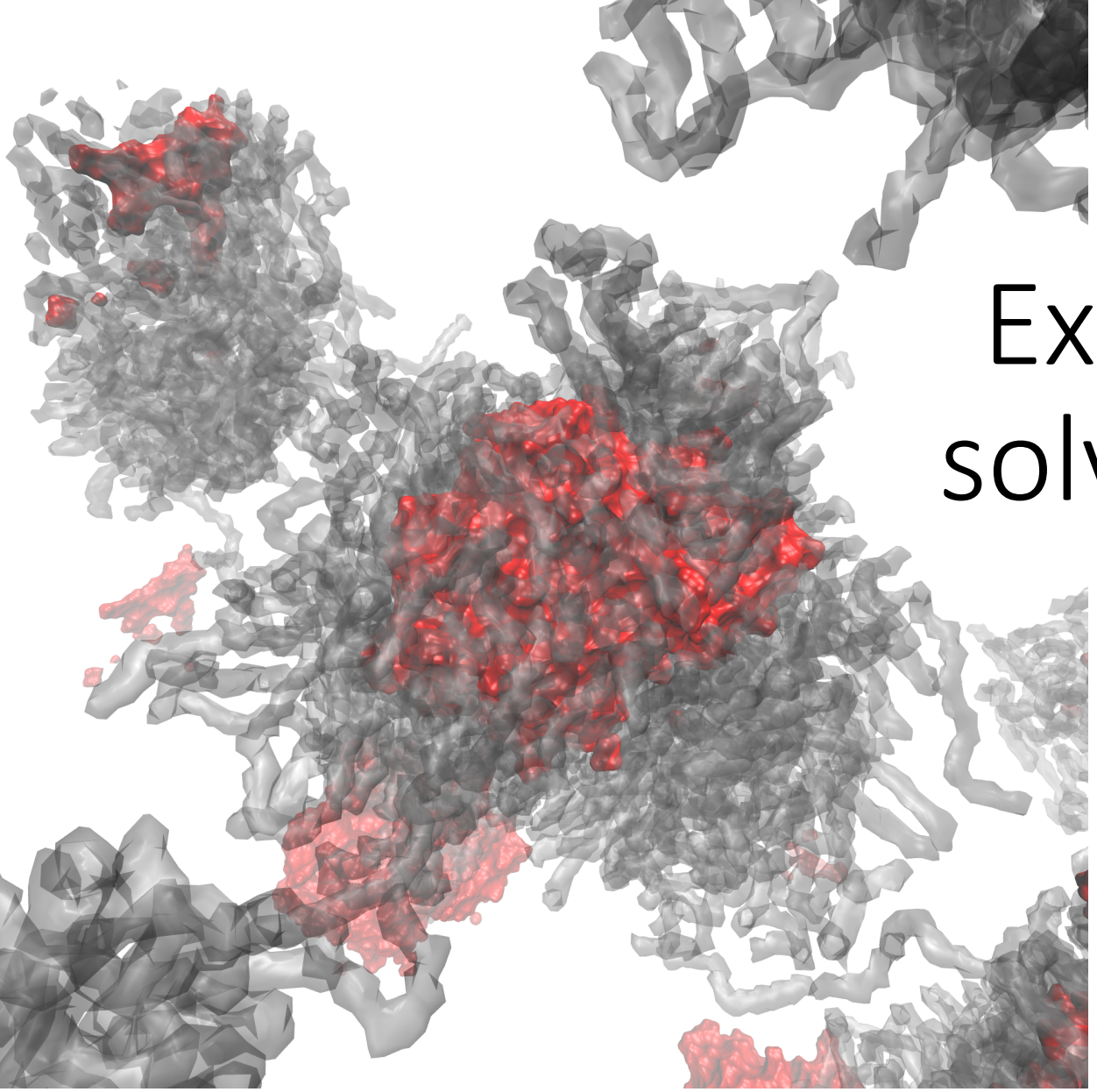


Exploring dynamics of solvent free myoglobin-polymer hybrid

Yann Fichou
IECB – Univ. Bordeaux

Collaborative work

Martin Weik
IBS (Grenoble)

Douglas Tobias
UC Irvine

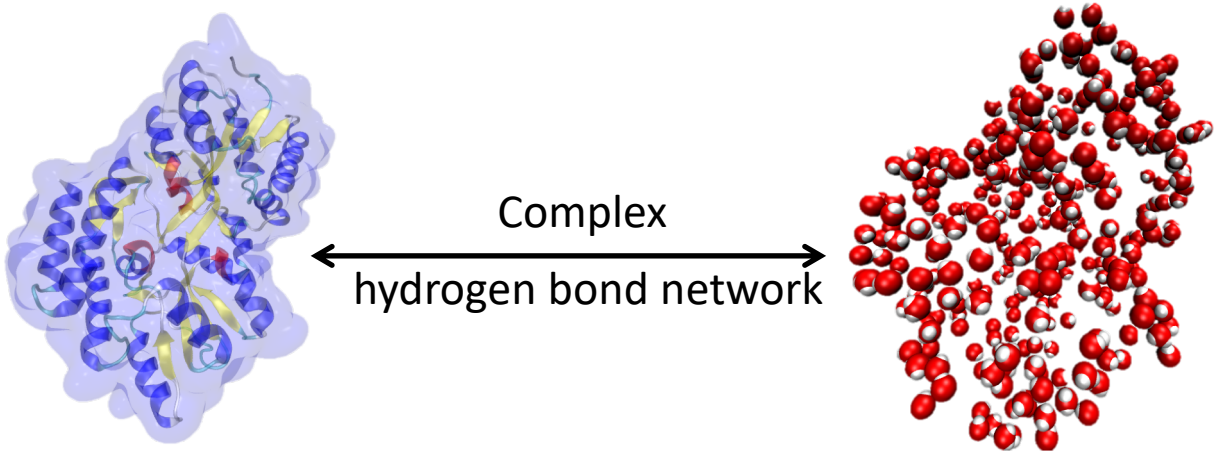
Giorgio Schirò
IBS (Grenoble)

Adam Perriman
University of Bristol

Alex P. S. Brogan
Richard Sessions
Wiebke Lohstroh
Michaela Zamponi
Gerald J. Schneider

François-Xavier Gallat
Alessandro Paciaroni
Douglas J. Tobias

Water and proteins, a love story



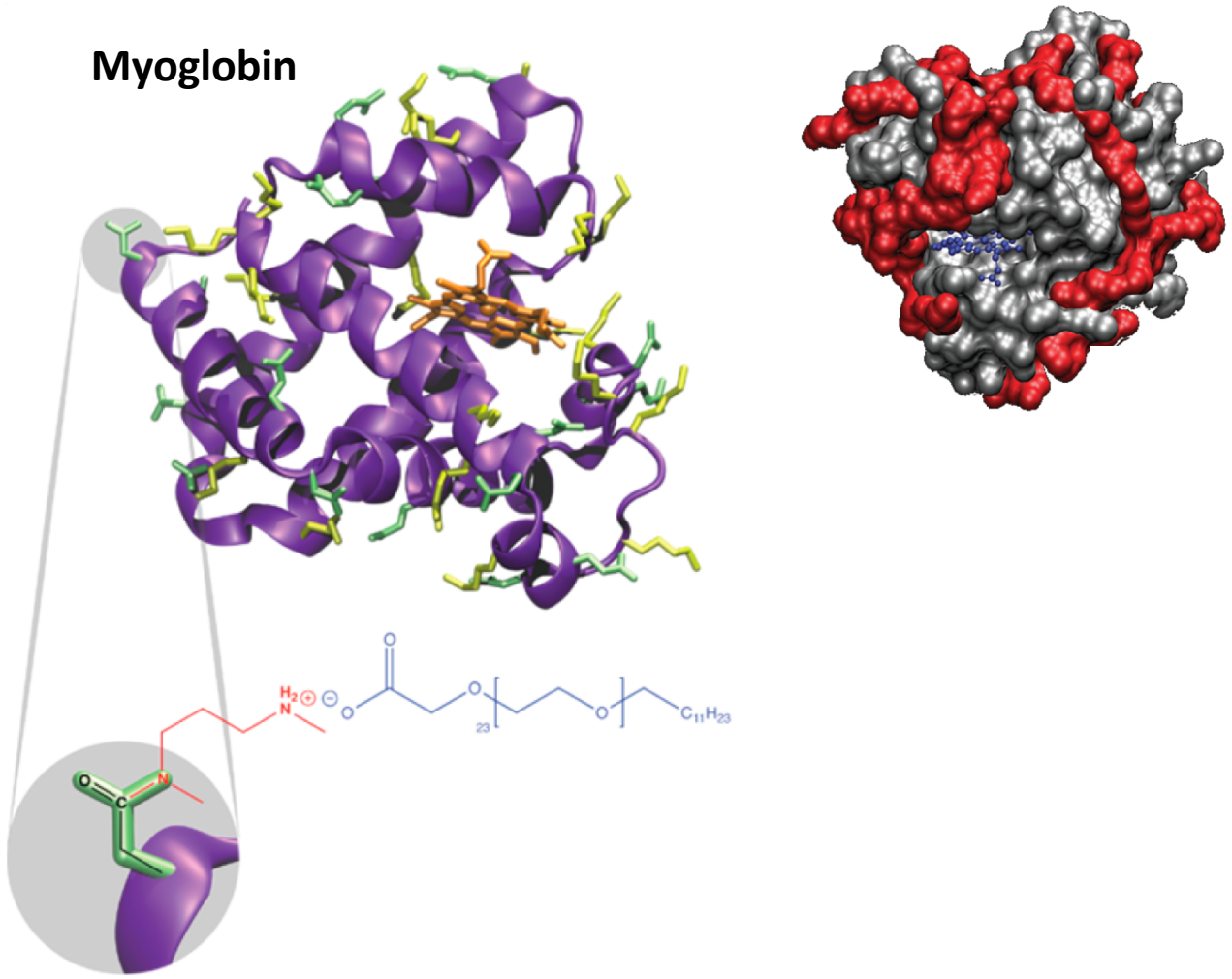
➔ Functional protein

Questions

- What are the water properties that enable protein function?
- Can a protein remain functional without water? 
- If yes, through what mechanisms?

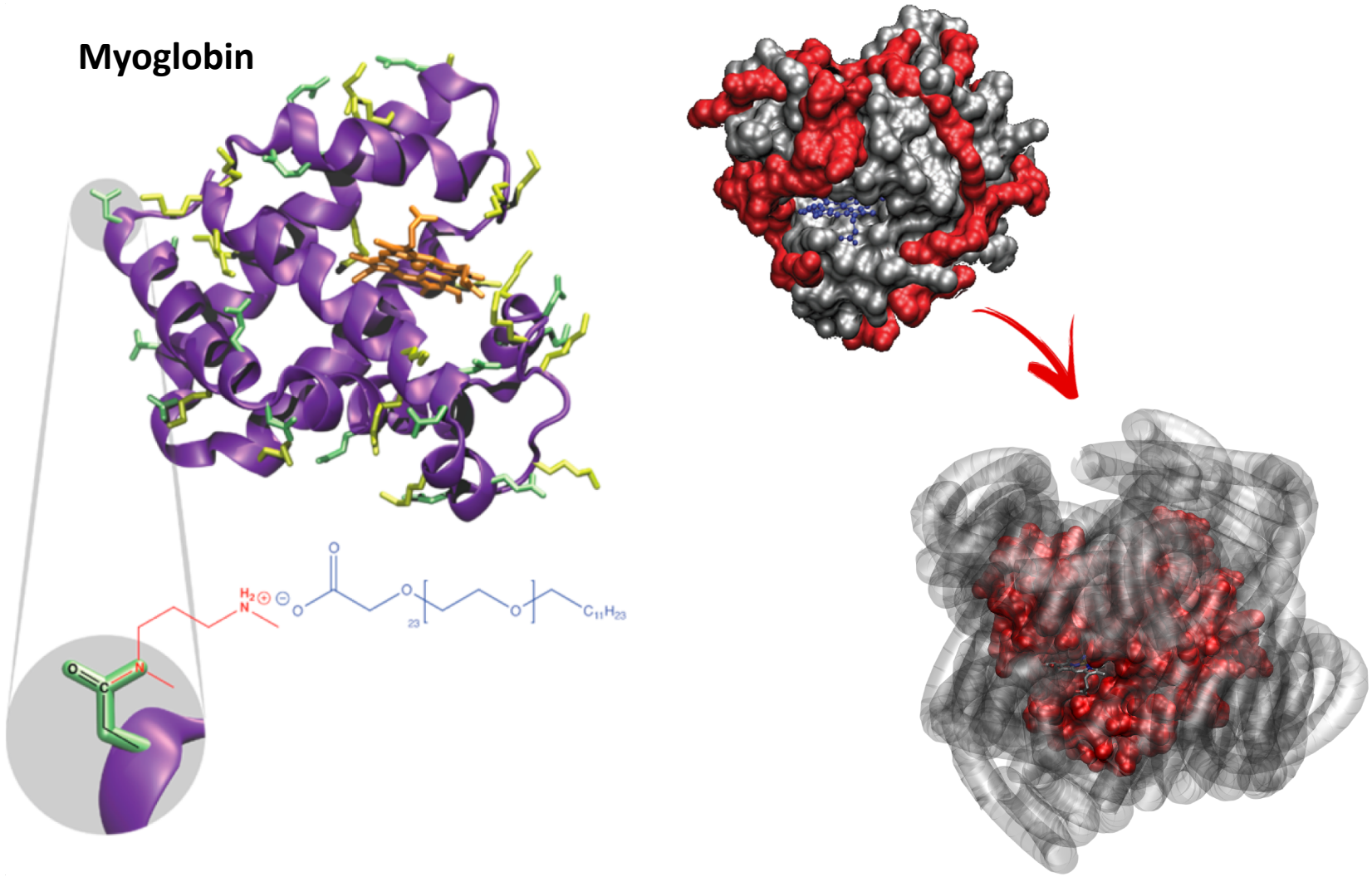
➔ Combining neutron scattering and MD simulations

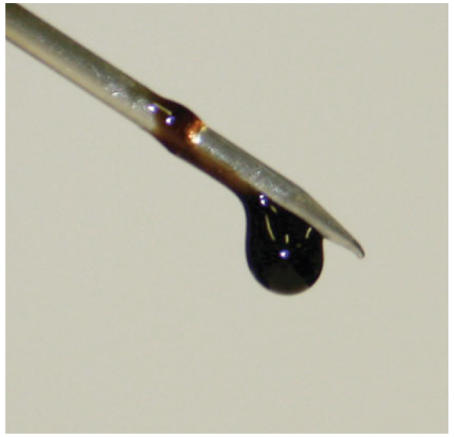
Protein function without water: protein-polymer hybrids

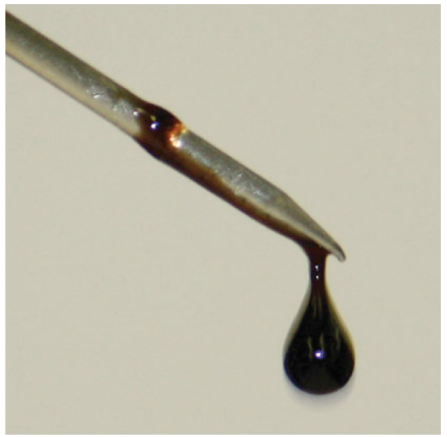


Perriman, Brogan, Coelfen,Tsoureas, Owen & Mann (2010) Nature Chemistry 2, 622

Protein function without water: protein-polymer hybrids







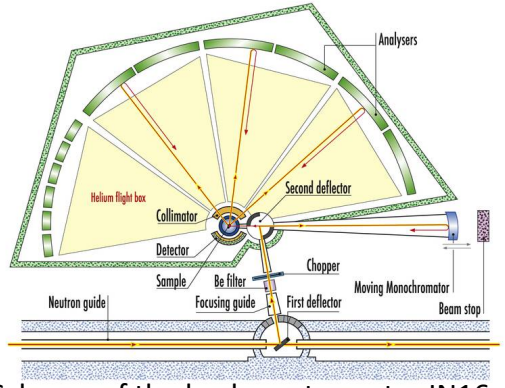
Perriman, Brogan, Coelfen,Tsoureas, Owen & Mann (2010) Nature Chemistry 2, 622

Neutron scattering to measure dynamics

- Backscattering spectrometers:
  - IN16, ILL, Grenoble
  - SPHERES, MLZ, Munich

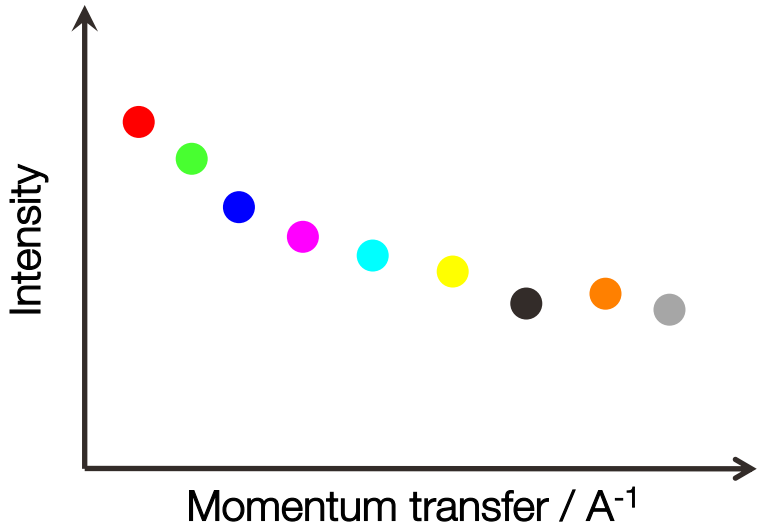
- About 1 µeV resolution

➔motions on the ps-ns timescale



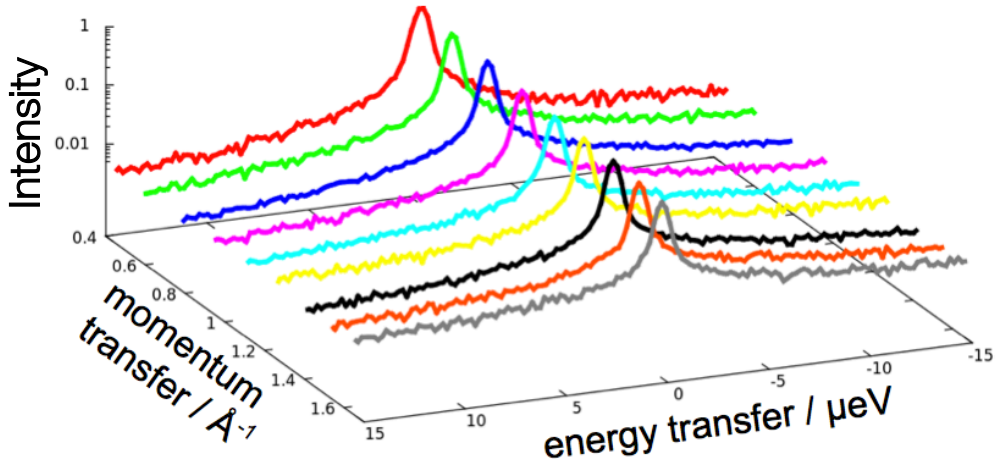
Scheme of the backspectrometer IN16

Elastic scattering



➔mean-squared displacements

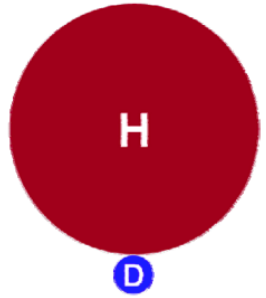
Quasi-elastic scattering

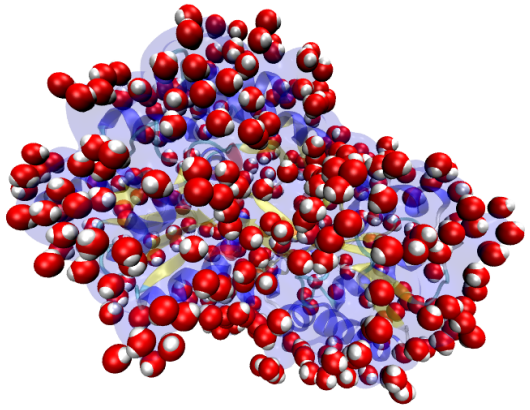


➔Type of motion (translation, rotation…)

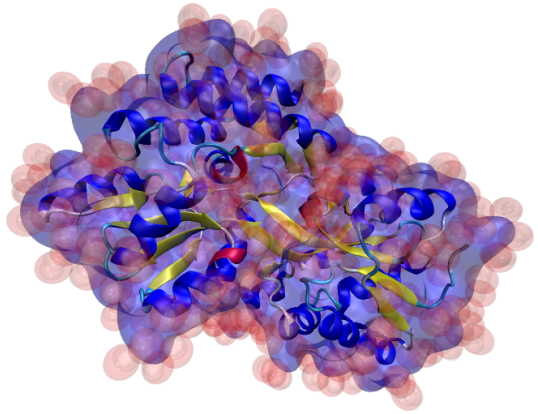
Neutron scattering and isotope labeling

- In biologicall sample : Mostly hydrogen dynamics
- Deuteration = masking



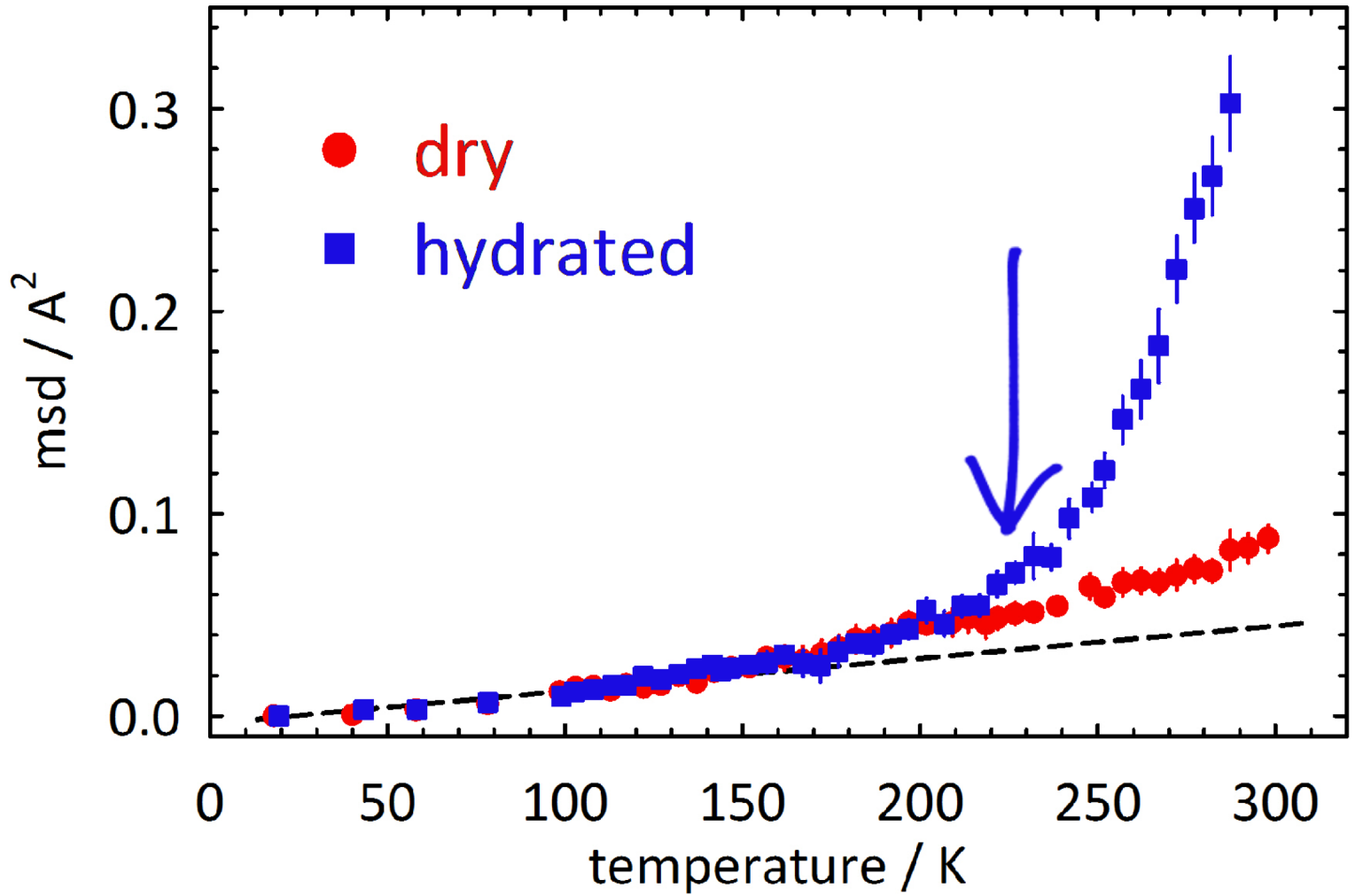


D-protein + H-solvent
Solvent dynamics



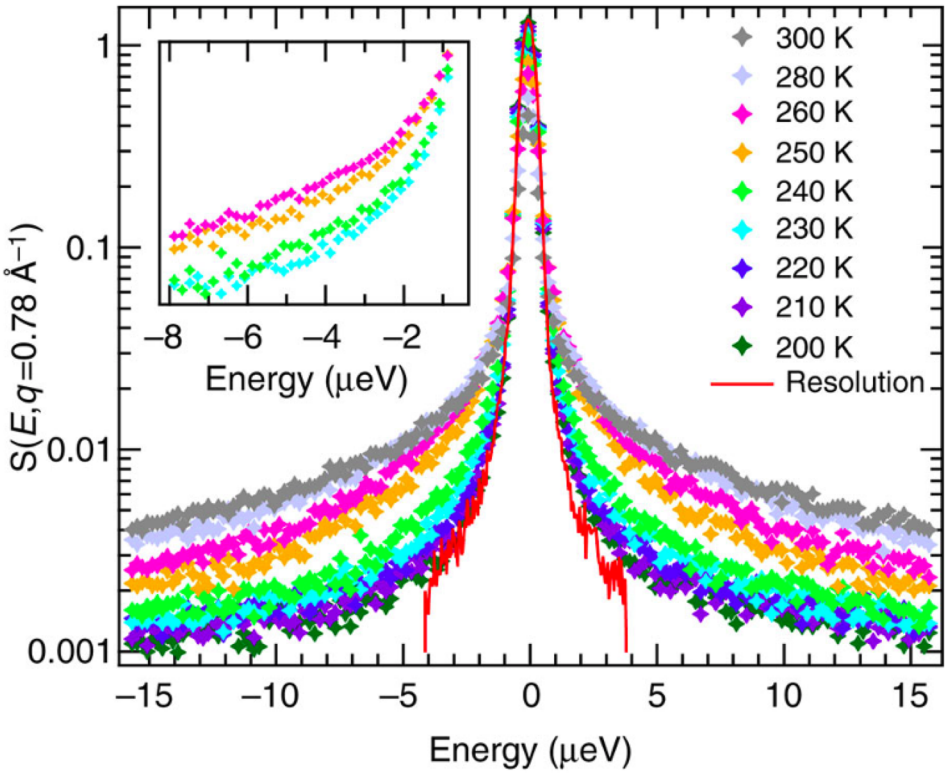
H-protein + D-solvent
Protein dynamics

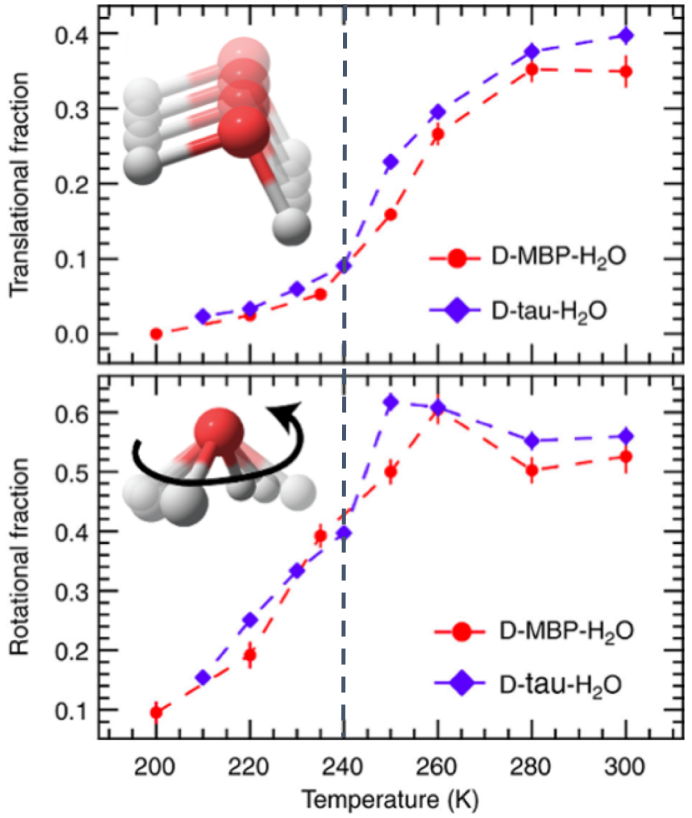
Neutrons reveal anharmonic onsets in proteins

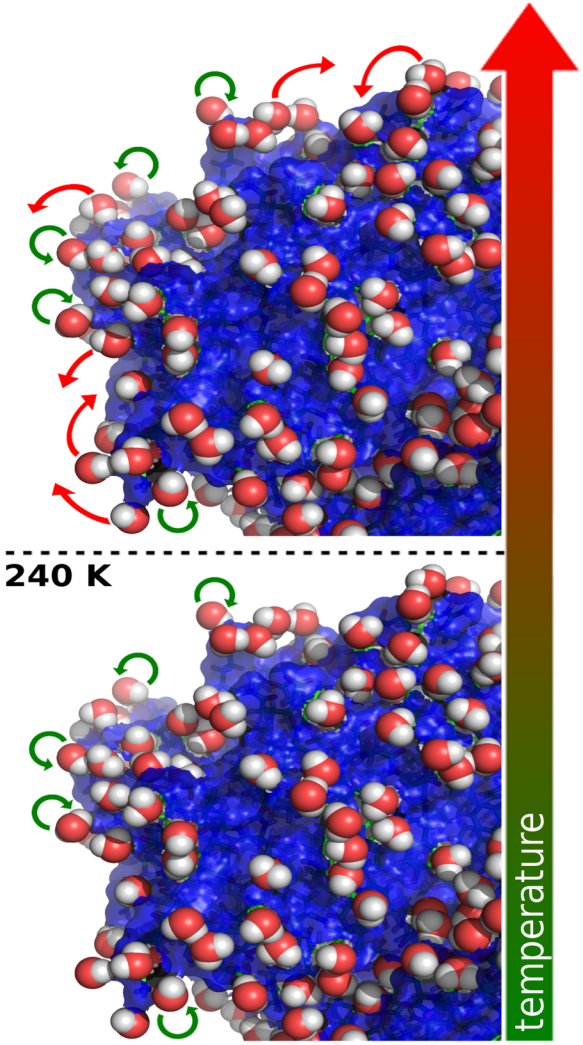


Doster et al. **Nature** 337, 754, 1989

Probing hydration water diffusion by QENS



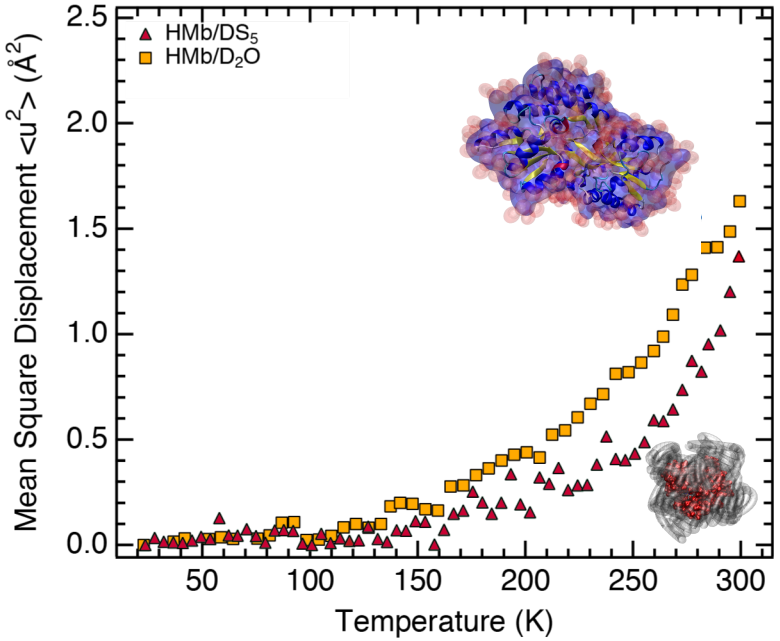




Schirò, Fichou, Gallat et al. **Nature Comm** 6 6490, 2015

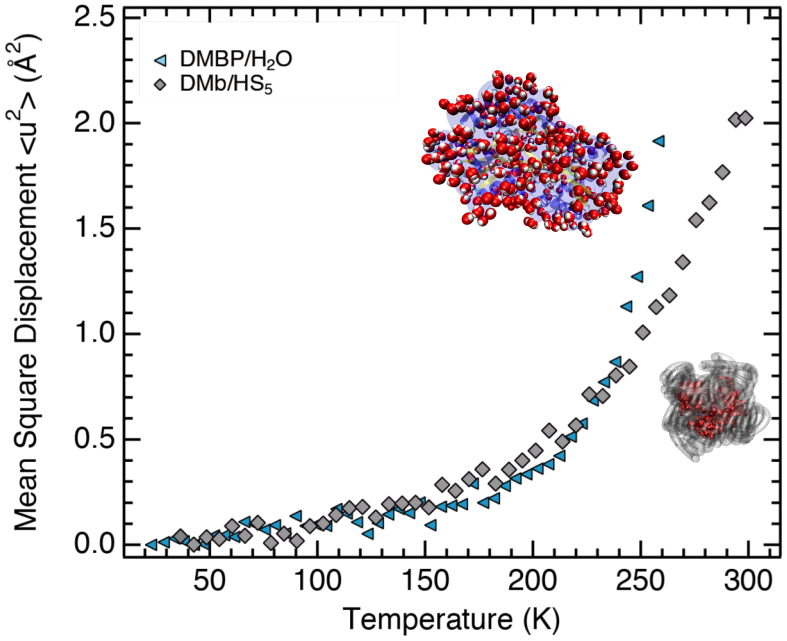
Anharmonic motions observed in myoglobin hybrid

myoglobin motions



… similar to motions of hydrated myoglobin

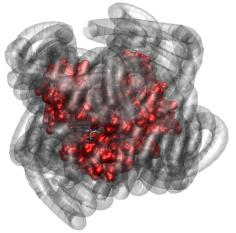
polymer motions

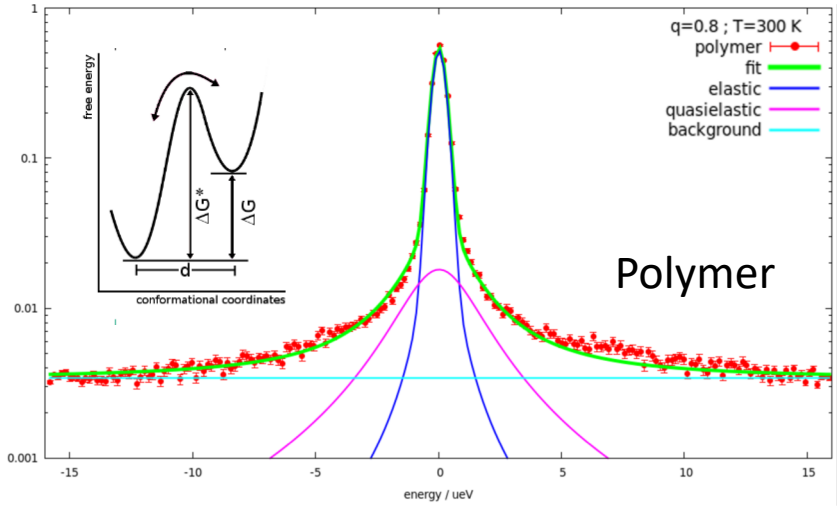


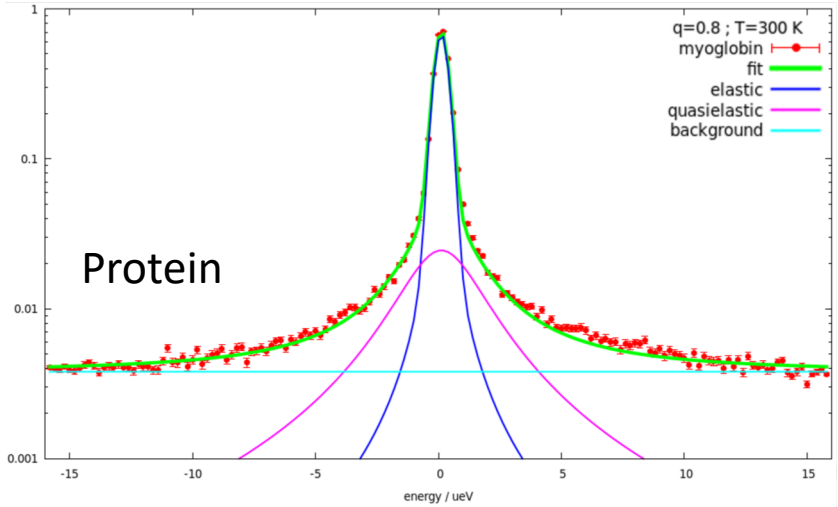
… similar to hydration water motions

Gallat, Brogan, Fichou, McGrath, Moulin,Härtlein, Combet, Wuttke, Mann, Zaccai, Jackson, Perriman, Weik (2012) JACS 134, 13168

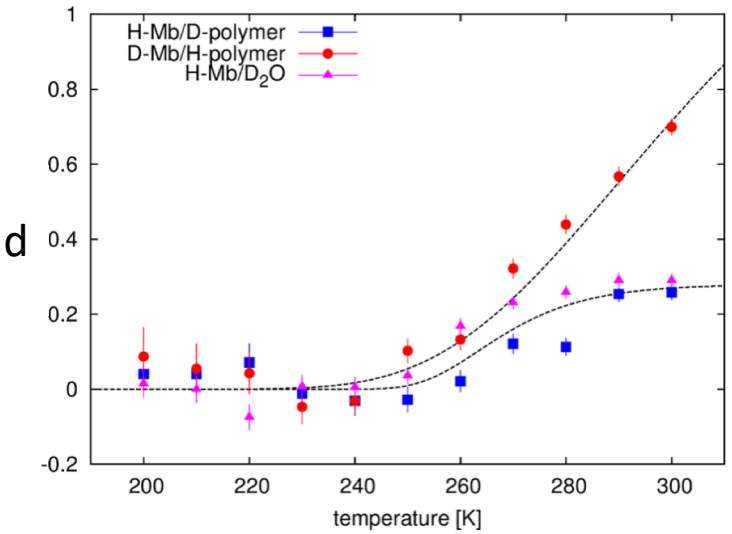
QENS of myoglobin-polymer hybrid

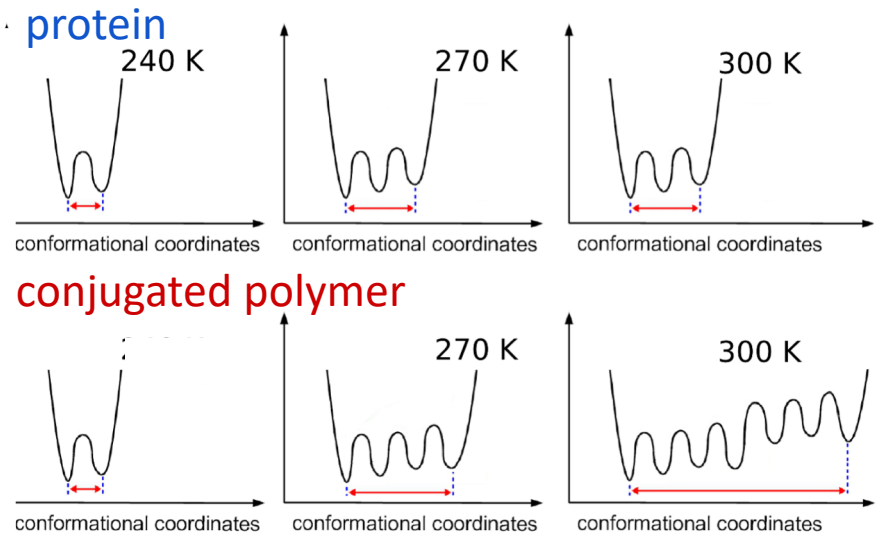






Protein and polymer behave similarly





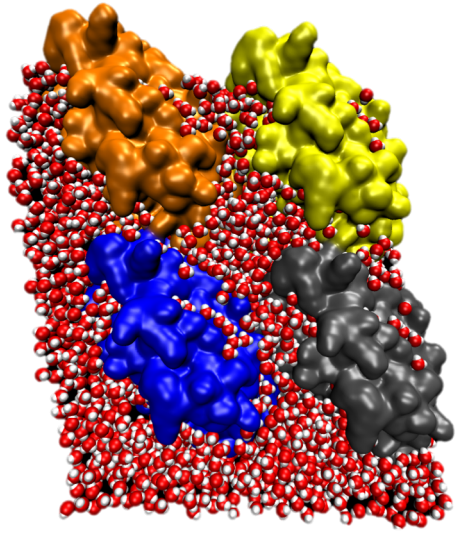
But

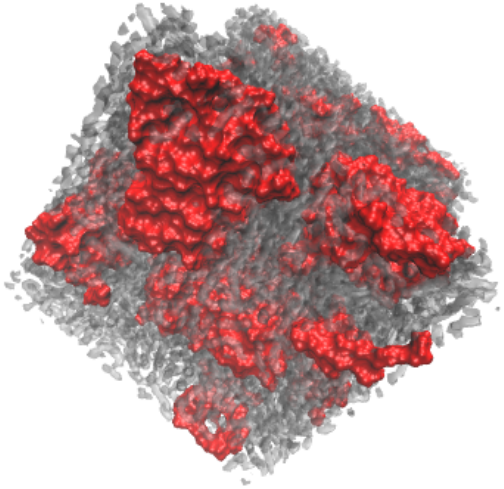
Jump distances of polymer increase with temperature

Schiro, Fichou et al, PRL, *in press*

MD simulations to decipher atomic motions

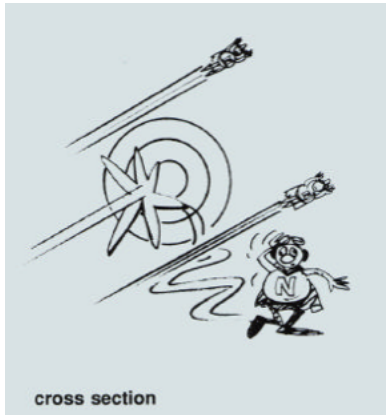
- Models of hydrated protein powders and protein-polymer hybrids

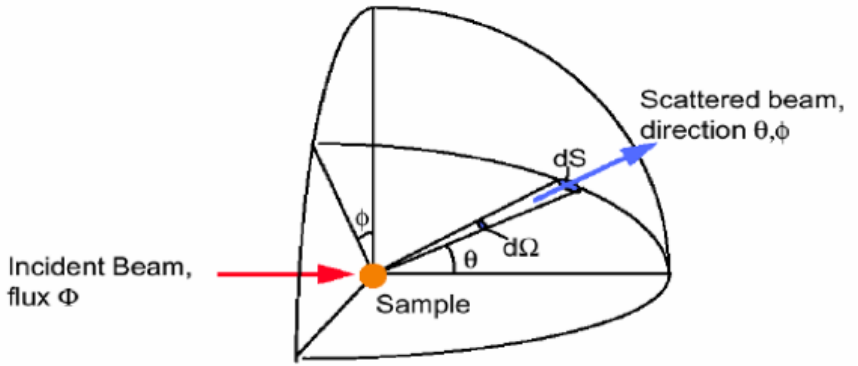




- Simulations run between 20K and 300K
- Atomic motions on the fs to μs timescale for systems of 10^5 atoms

Principle of the “virtual” neutron experiment





$$\frac{\partial^2\sigma}{\partial\Omega\partial\omega} \Leftrightarrow \frac{\text{Number of neutrons scattered into } d\Omega \text{ with the energy } d\omega}{\text{incident flux}}$$

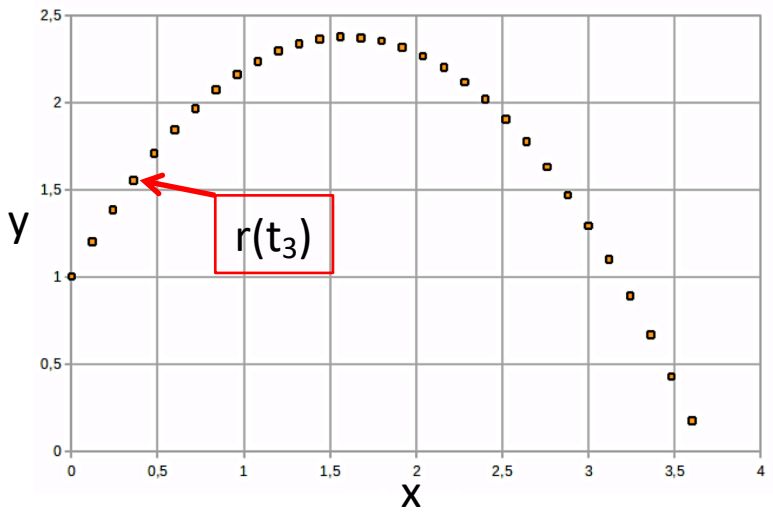
$$\frac{\partial^2\sigma}{\partial\Omega\partial E} = N\frac{k_{out}}{k_{inc}}b^2 \times S(Q,\omega) \qquad S(Q,\omega) \text{ Scattering function}$$

$$S(Q,\omega) = \frac{1}{2\pi}\int I(Q,t)e^{-i\omega t}dt = FT[I(Q,t)] \qquad I(Q,t) \text{ Intermediate Scattering function}$$

$$I(Q,t) = \frac{1}{N}\sum_{jk} < e^{iQr_k(t)} \times e^{iQr_j(t)} > \qquad \text{I(q,t) is correlation function in time and space!}$$

Principle of the “virtual” neutron experiment

$$I(Q,t) = \frac{1}{N}\sum_{jk} < e^{iQr_k(t)} \times e^{iQr_j(t)} > = \frac{1}{N}[\underbrace{\sum_{j \neq k} < e^{iQr_k(t)} \times e^{iQr_j(t)} >}_{\text{Cross-correlation = coherent Scat.}} + \underbrace{\sum_{k} < e^{iQr_k(t)} \times e^{iQr_k(t)} >}_{\text{Auto-correlation = incoherent Scat.}}]$$

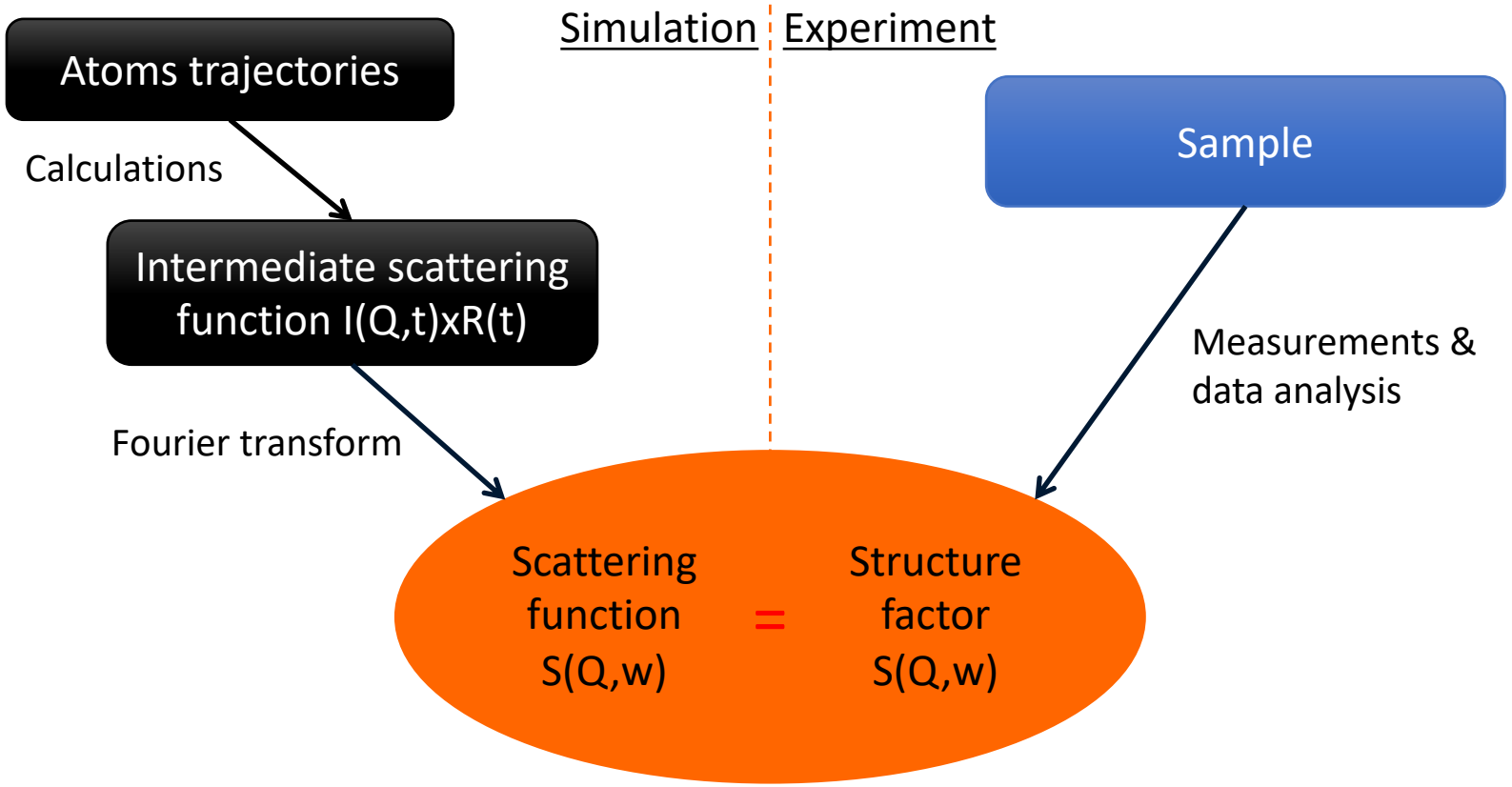


➔ **Can be calculated from the atom positions**

Principle of the “virtual” neutron experiment

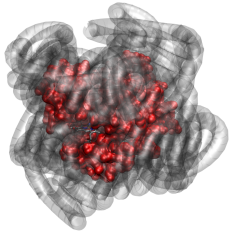
In practice, the instrument has a finite resolution R(ω)

$$S(Q,\omega)_{meas} = S(Q,\omega) \otimes R(\omega) \quad \Leftrightarrow \quad I(Q,t)_{meas} = I(Q,t) \times R(t)$$

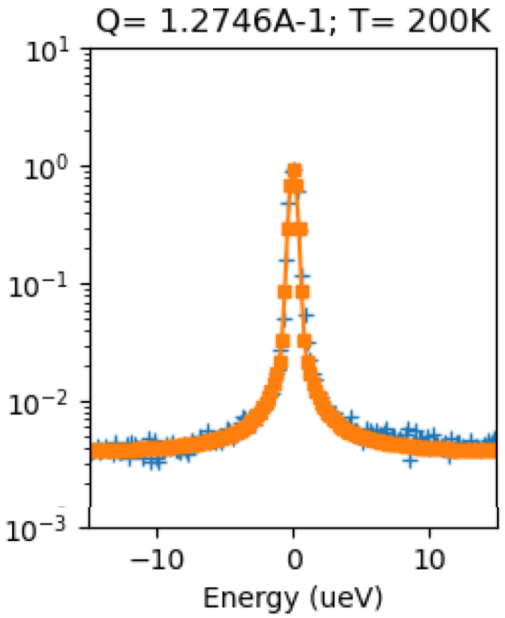


Same Quantity !

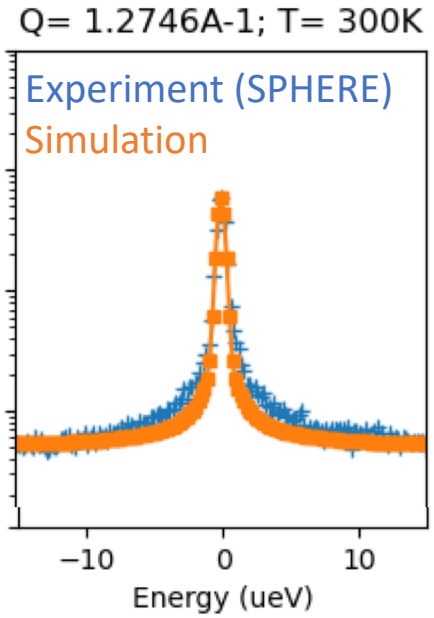
Validation of the sample modelling



Protein

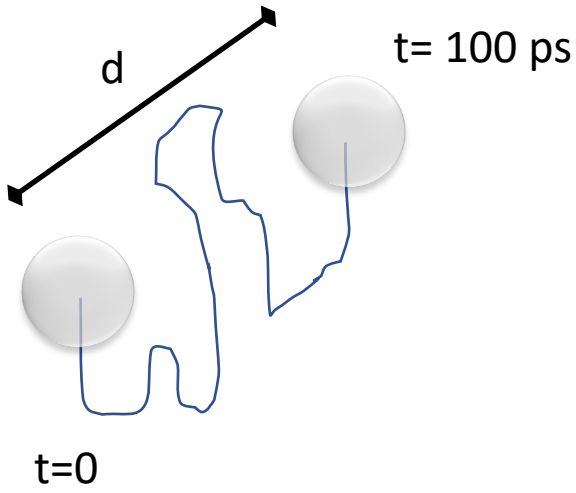


Protein



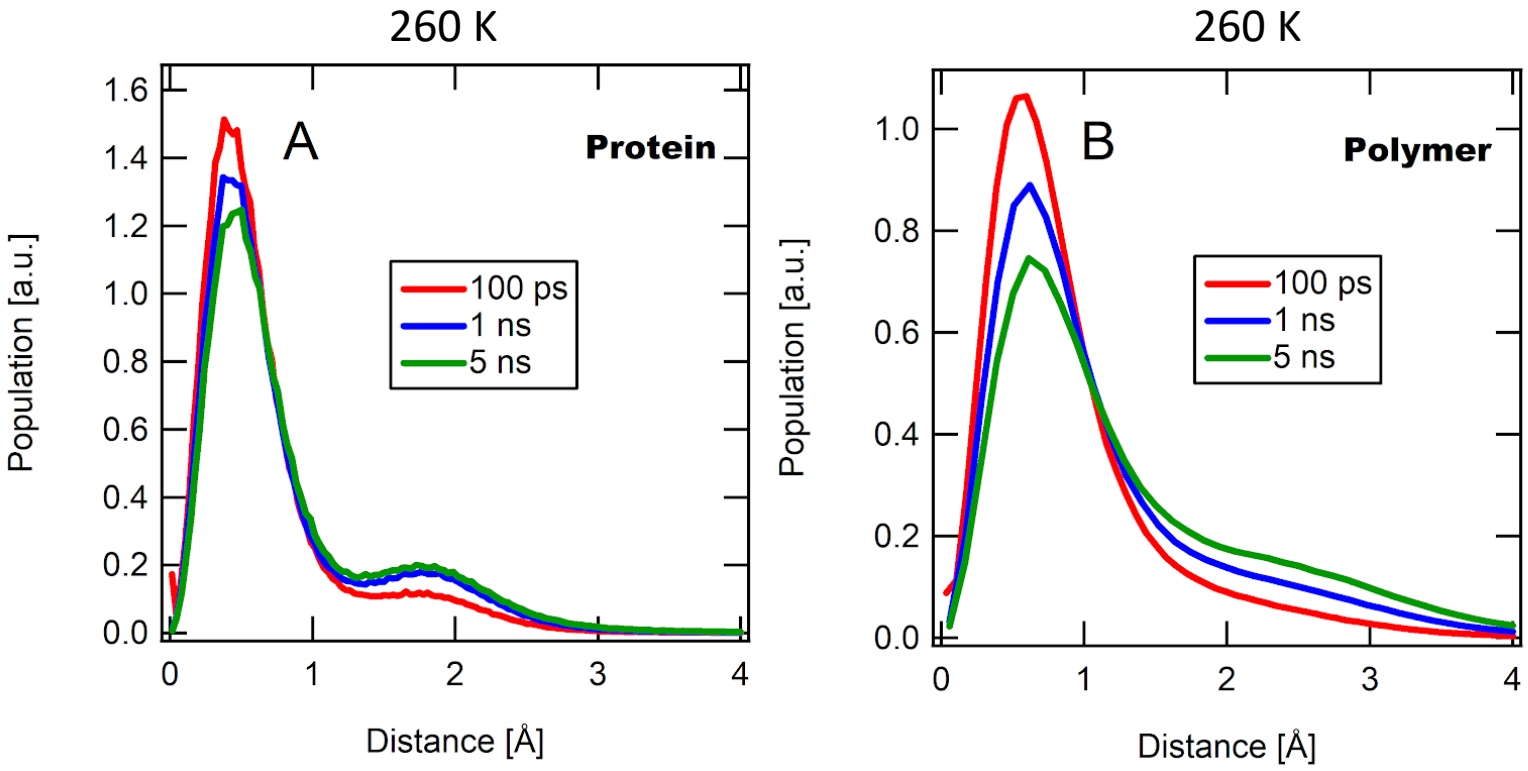
	Protein hydrogens	Polymer hydrogens
200K	All Q ok	All Q ok
220K	All Q ok	All Q ok
240K	All Q ok	All Q ok
260K	All Q acceptable	All Q ok
280K	Acceptable Q above 1.27 A^{-1} (6 detectors)	All Q acceptable
300K	Acceptable Q above 1.33 A^{-1} (3 detectors)	All Q acceptable

MD simulations reveal polymer diffusive motions



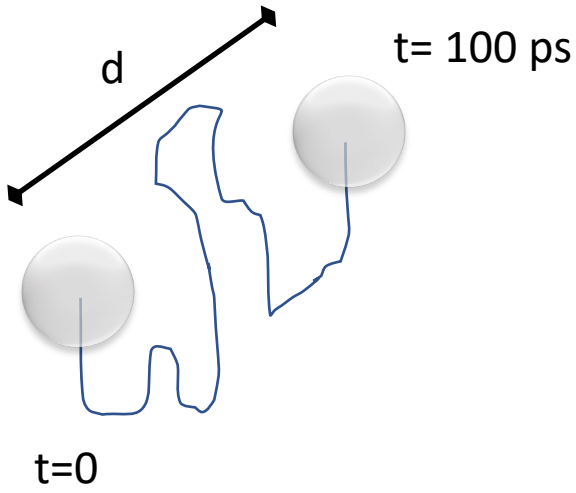
Displacement distribution function

$4\pi r^2 G(r,t)$



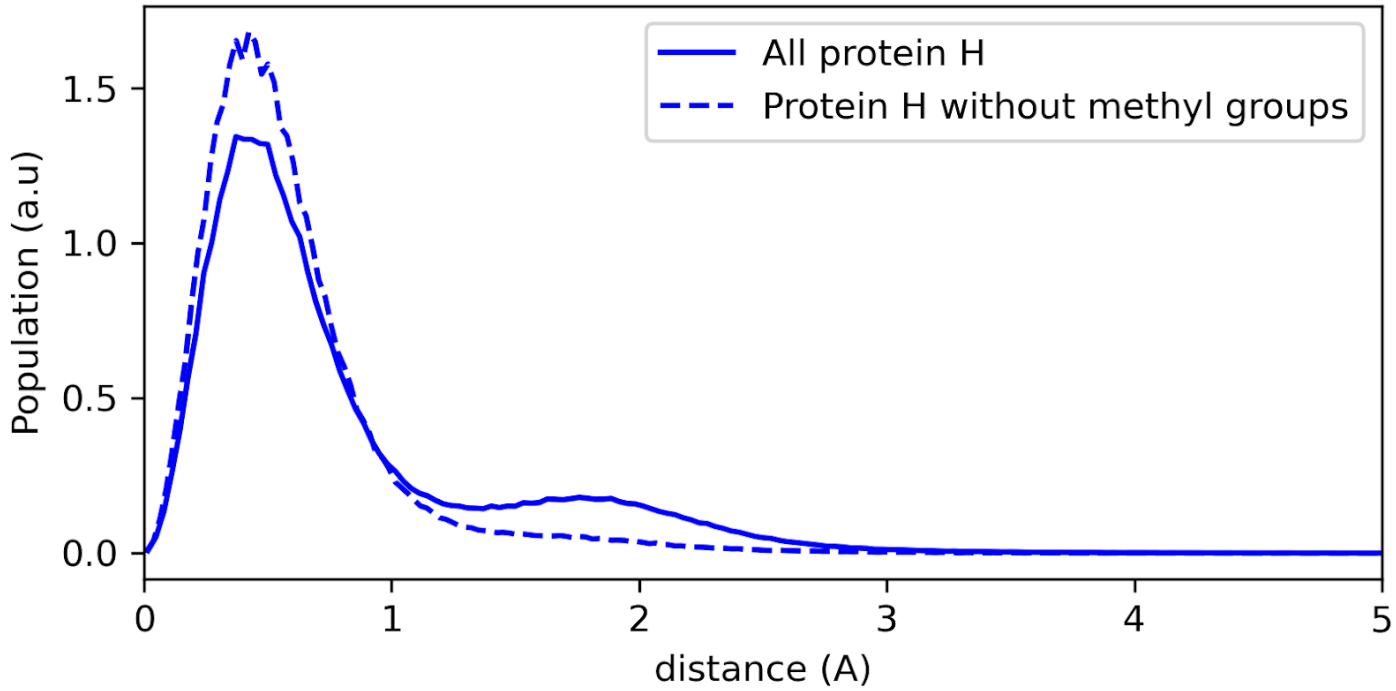
Schiro, Fichou et al, PRL, *in press*

MD simulations reveal polymer diffusive motions



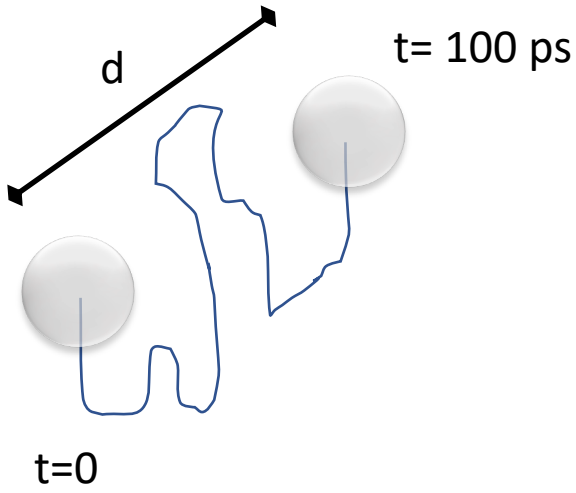
Displacement distribution function

$4\pi r^2 G(r,t)$



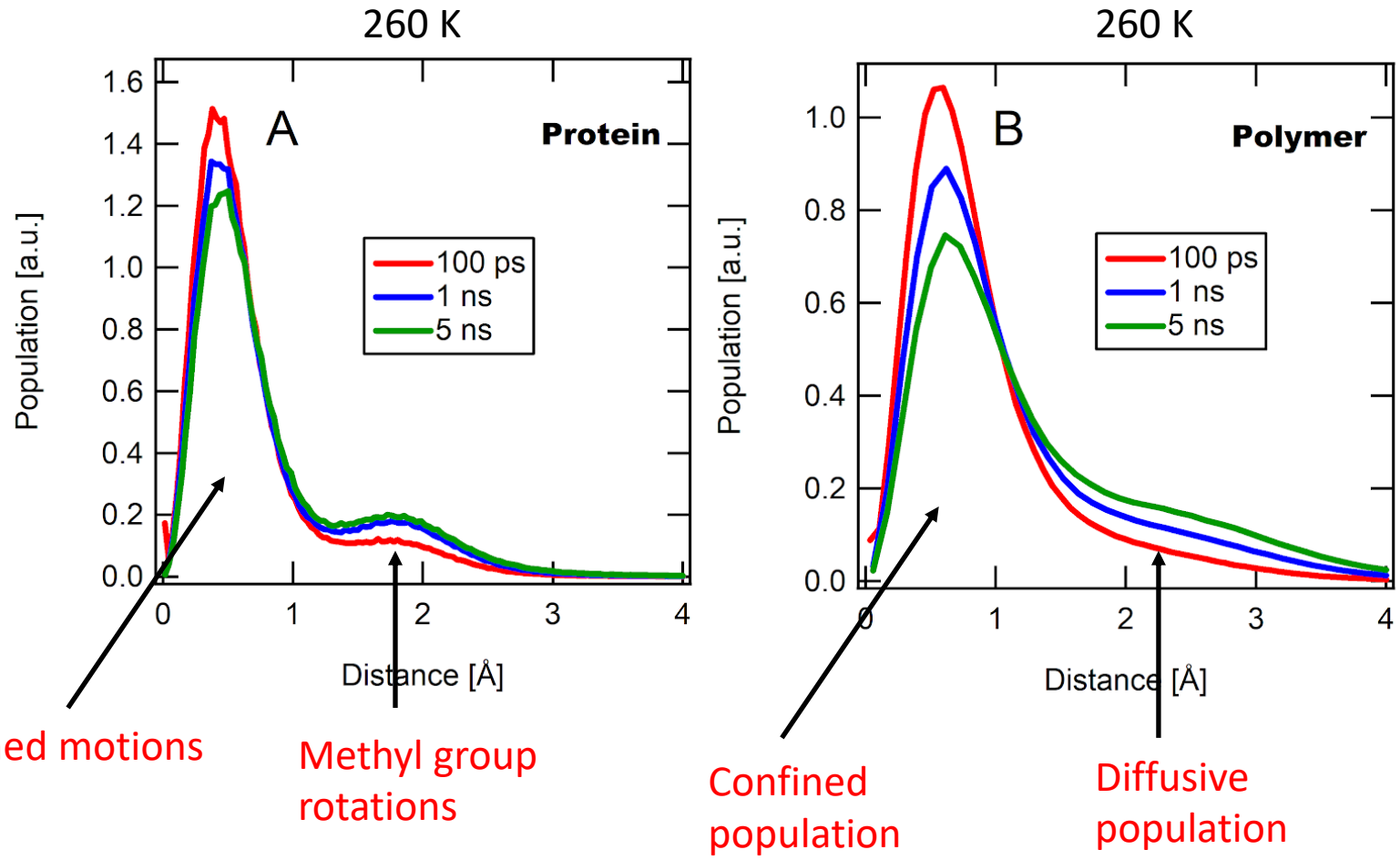
Schiro, Fichou et al, PRL, *in press*

MD simulations reveal polymer diffusive motions



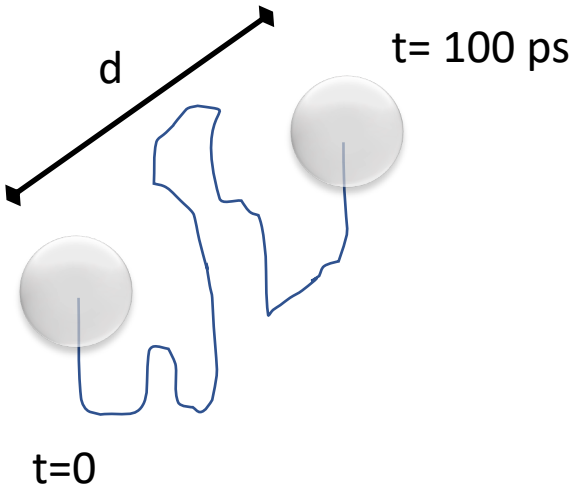
Displacement distribution function

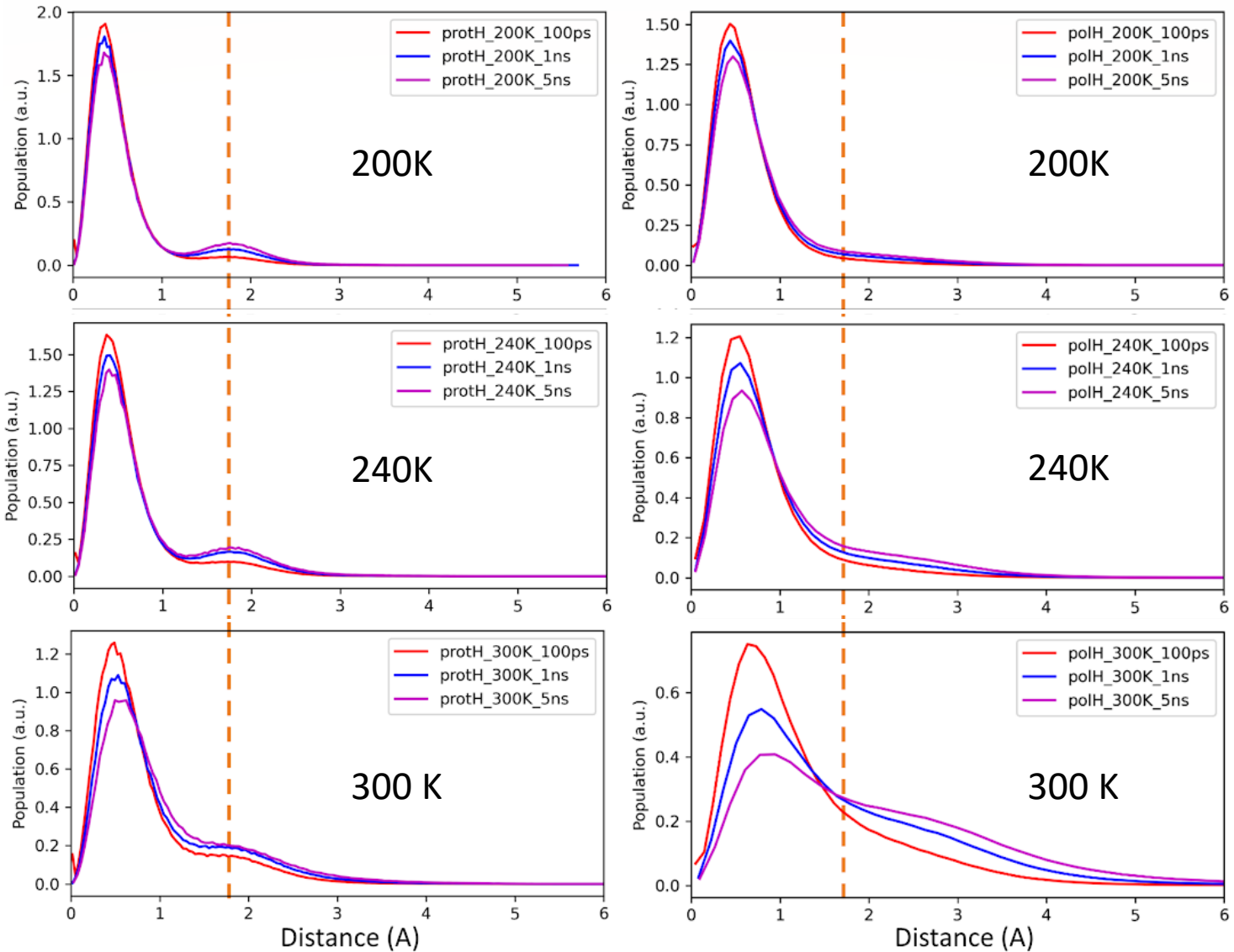
$4\pi r^2 G(r,t)$



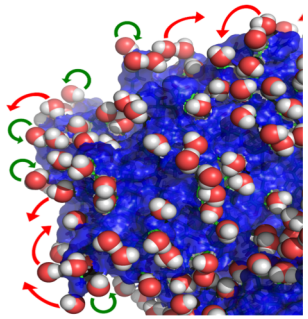
Schiro, Fichou et al, PRL, *in press*

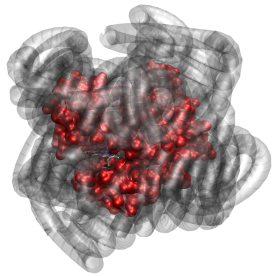
MD simulations reveal polymer diffusive motions

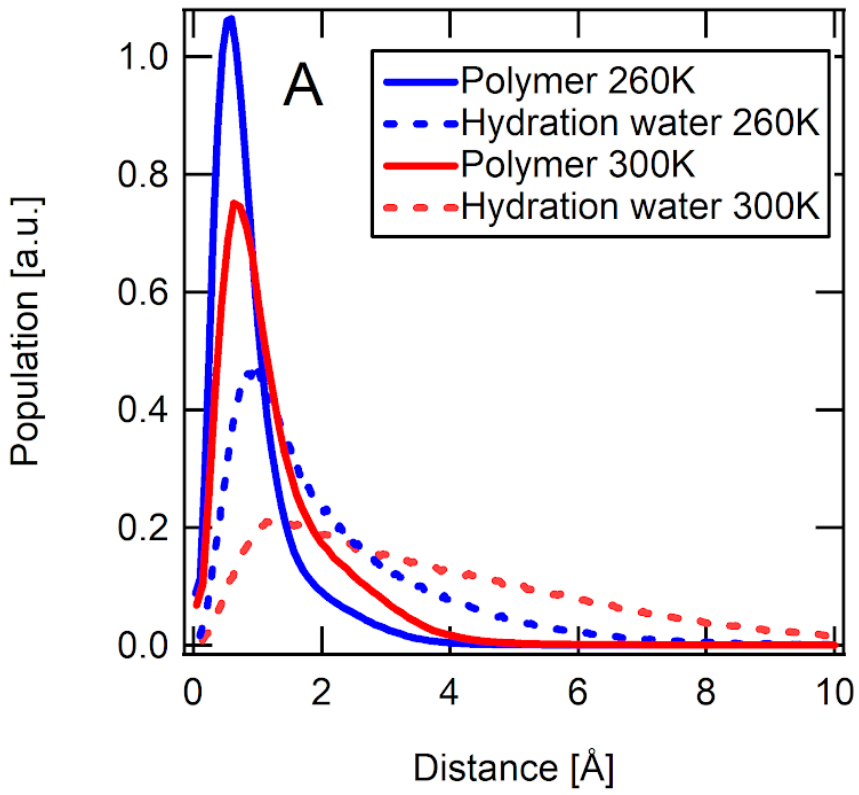




Quantitative differences with hydration water





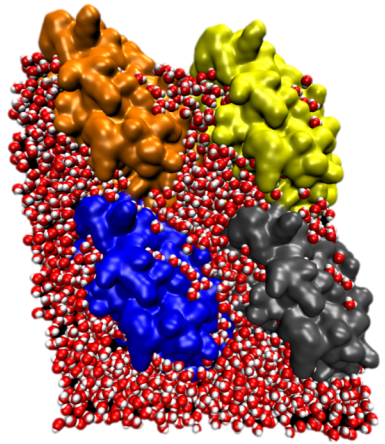


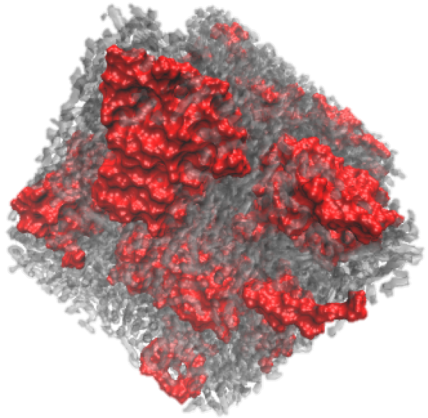
➢ Hydration water is more mobile than polymers

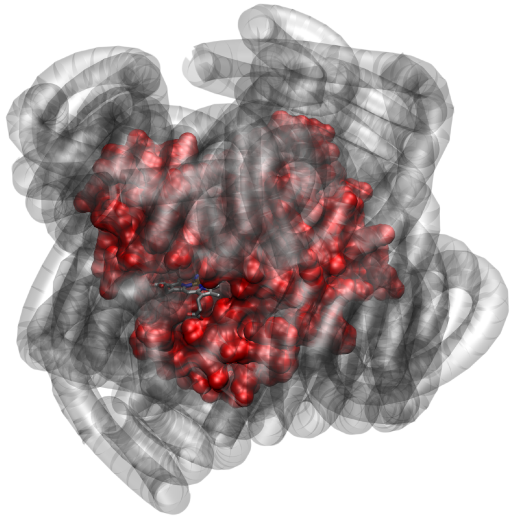
Schiro, Fichou et al, PRL, *in press*

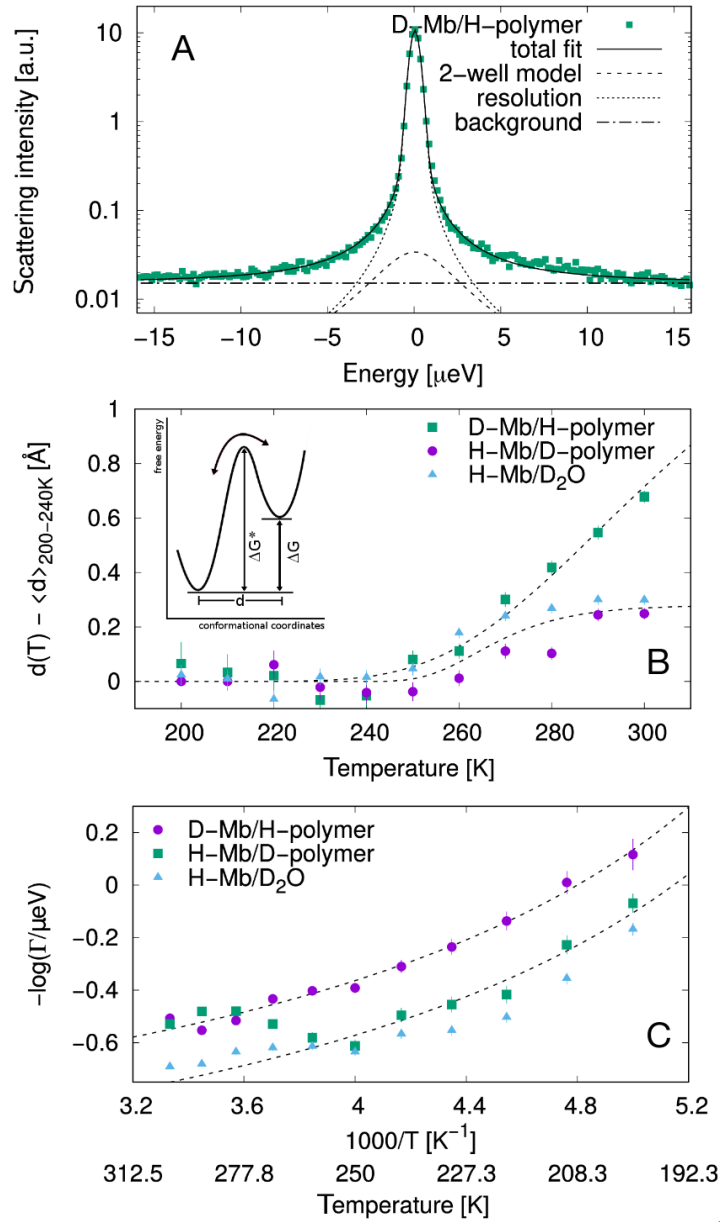
Conclusions

- Neutron scattering observables can be directly computed
- MD simulations allow to decipher the nature of molecular motions
- Water-free polymer-protein hybrid can be dynamic and active
- Polymer diffusive-like motions « plasticize » myoglobin and provide functional dynamics

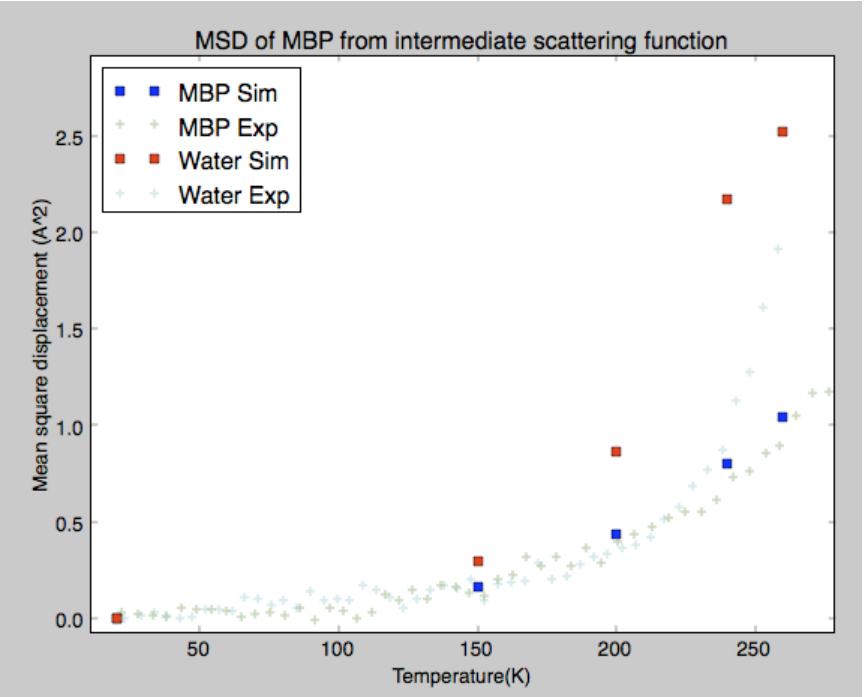







A
D–Mb/H–polymer
total fit
2–well model
resolution
background
Scattering intensity [a.u.]
Energy [μeV]
B
D–Mb/H–polymer
H–Mb/D–polymer
H–Mb/D2O
free energy
ΔG*
ΔG
d
conformational coordinates
d(T) – <d>200–240K [Å]
Temperature [K]
C
D–Mb/H–polymer
H–Mb/D–polymer
H–Mb/D2O
–log(Γ/μeV)
1000/T [K–1]
Temperature [K]

- Mean square displacement of water hydrogen atoms



➔ **No quantitative agreement.**