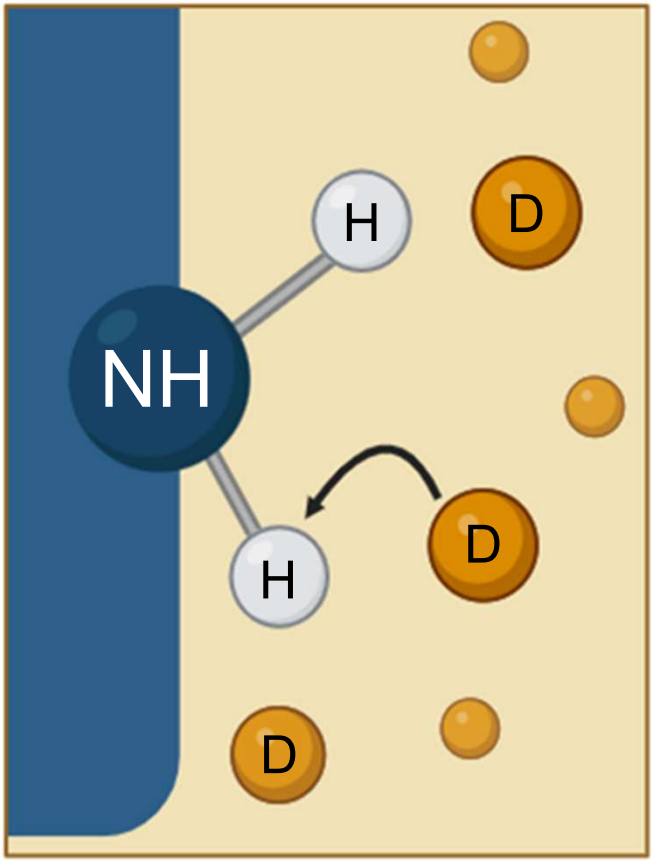


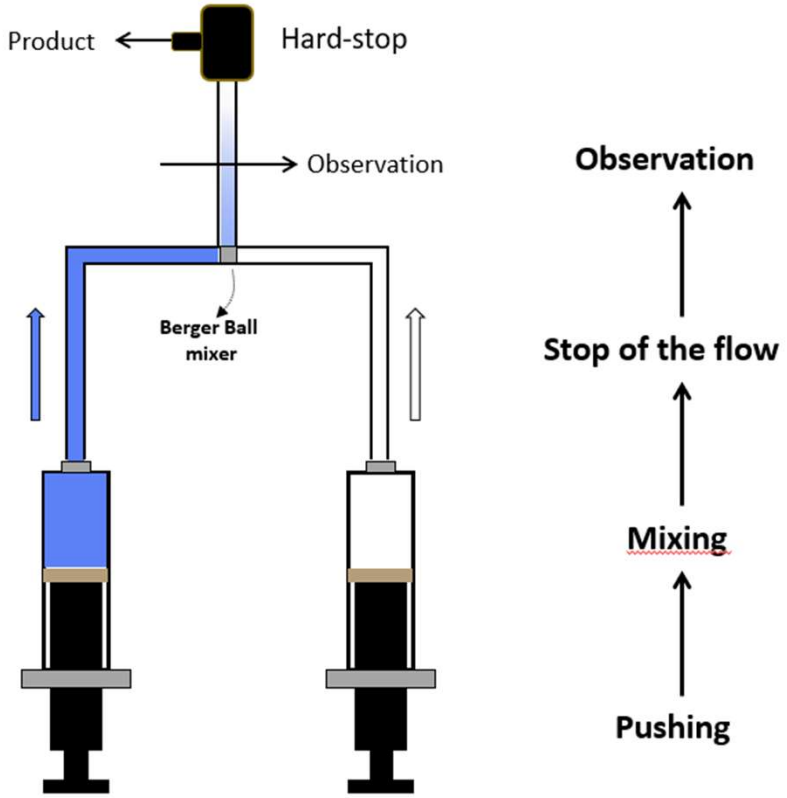
Stopped-Flow SANS Analysis for Early-Stage Hydrogen-Deuterium Exchange Kinetics

Amanda S. Rosario Rivera (UPRM)

Mentors: Chelsea Edwards, Ryan Murphy, Yun Liu

August 6, 2026

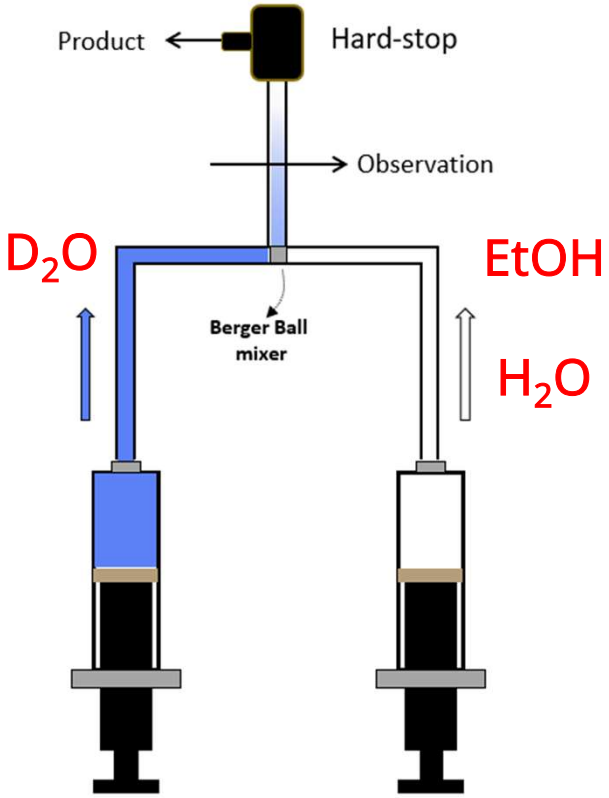
Stopped-flow enables fast and precise mixing



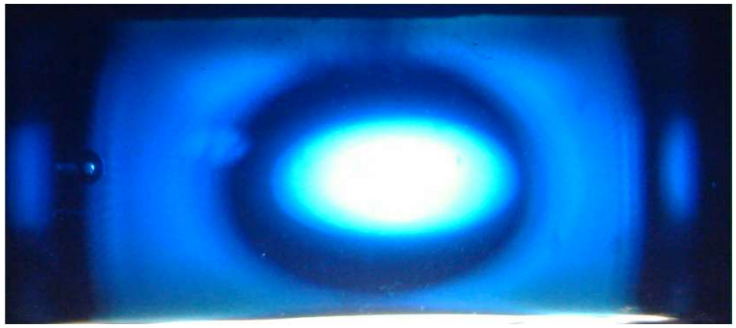
- Reduces time between mixing and measurement to the order of milliseconds
- Small sample sizes

BioLogic - Stopped Flows Diagram & System Architecture.

Stopped-flow enables fast and precise mixing

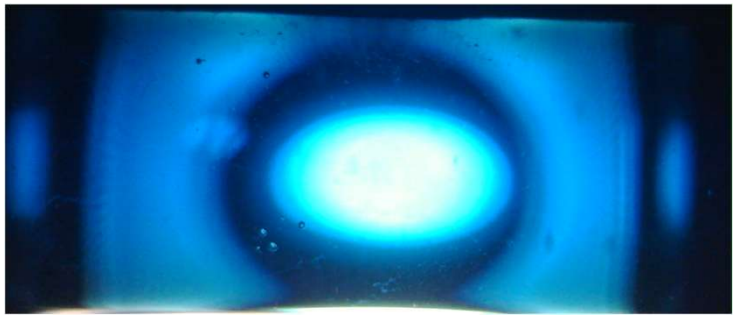


D_2O and EtOH



Density differences help showcase stop flow mechanism

D_2O and H_2O

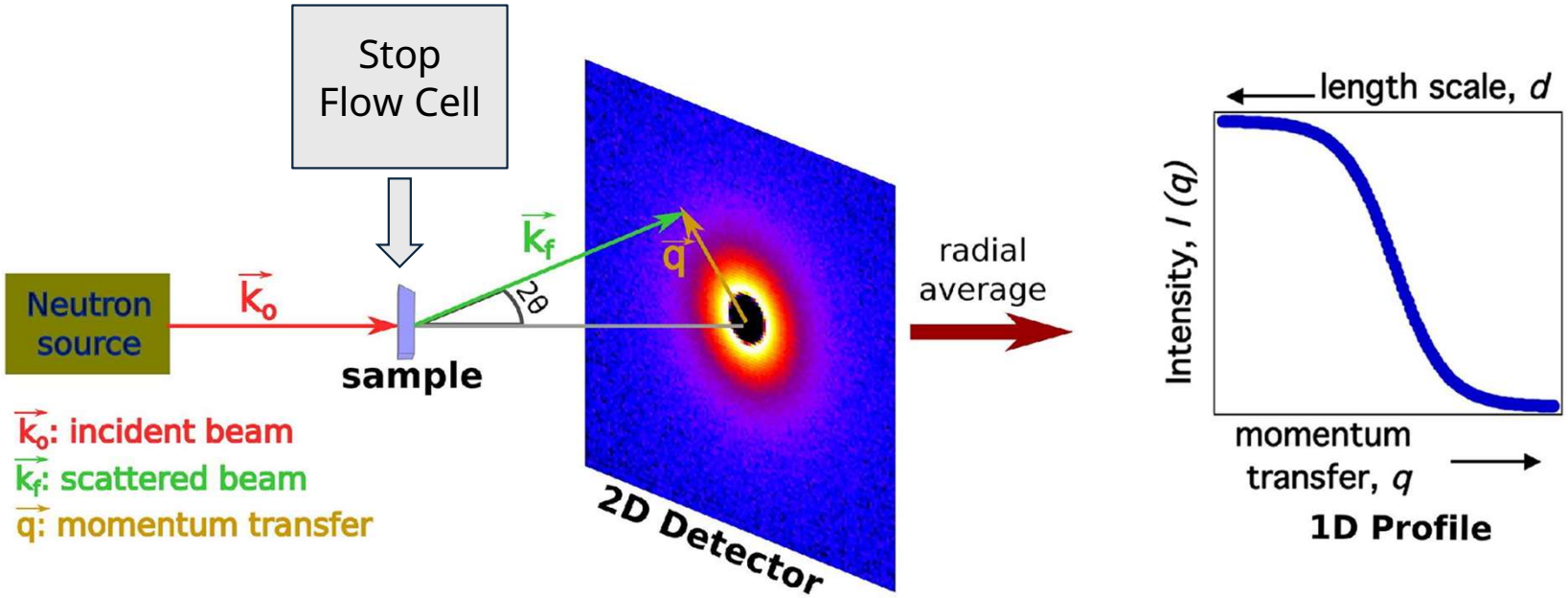


Fully homogenized mixing

BioLogic - Stopped Flows Diagram & System Architecture.

Small-angle neutron scattering (SANS)

Reveals **size, shape, distribution, interactions,** and **structural changes between particles**

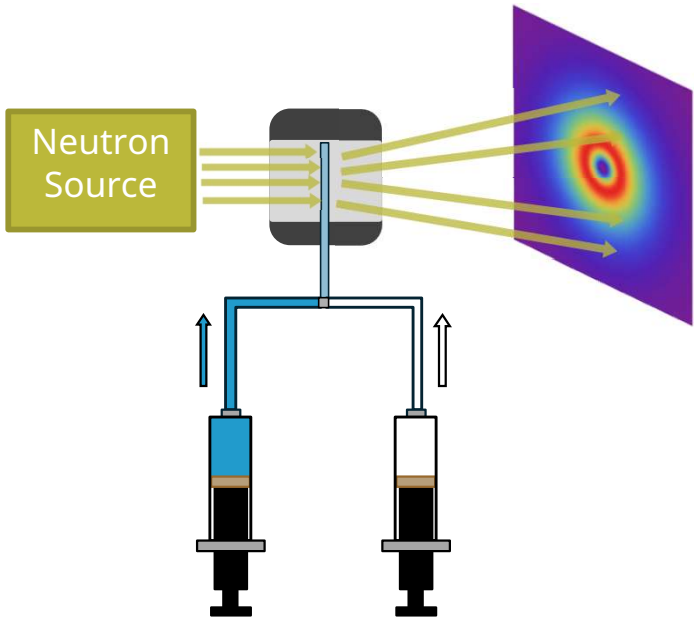


$$I(Q) = nV_p^2(\Delta\rho)^2 \mathrm{P}(Q)\mathrm{S}(\mathrm{Q})$$

Castellanos et al., *Comput Struct Biotechnol J.* 2017 (DOI: 10.1016/j.csbj.2016.12.004), CC BY 4.0.

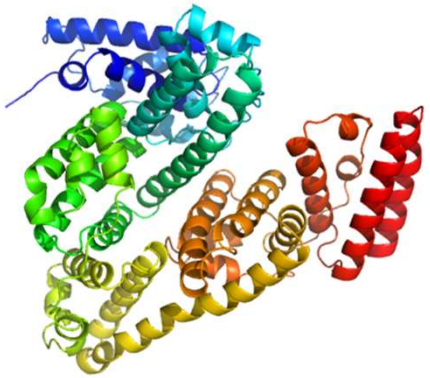
Stopped-flow SANS experiments can reveal time dependent information in soft systems

Stop-flow SANS



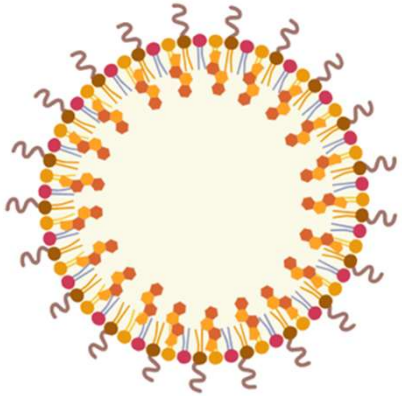
Pharmaceutically Relevant Systems

Bovine Serum Albumin (BSA) Hydrogen Deuterium Exchange (HDX)



Morishima et al., "Size-exclusion chromatography-small-angle neutron scattering", 2025.

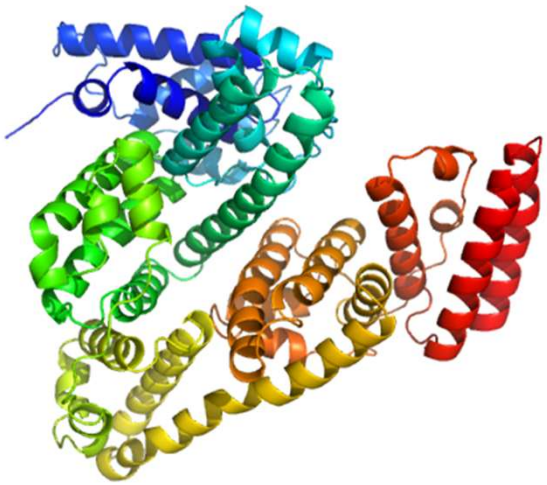
Lipid Nanoparticle (LNPs) Formation



From BioRender.com.

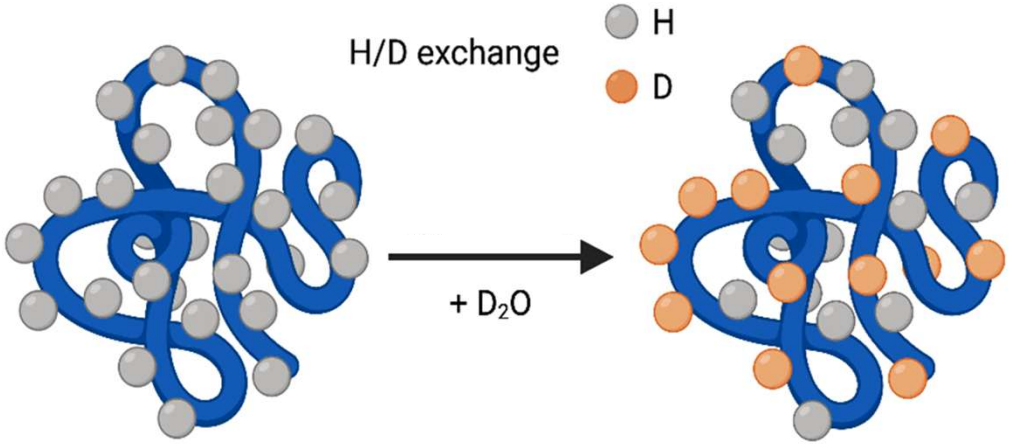
BSA as a model protein for measuring kinetics through hydrogen deuterium exchange (HDX)

BSA



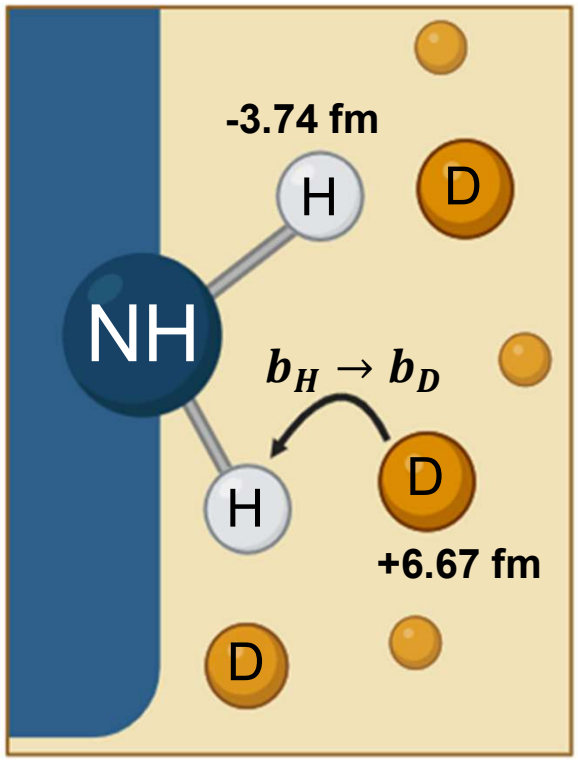
Morishima et al., "Size-exclusion chromatography-small-angle neutron scattering", 2025.

Proteins exchange labile hydrogens with their solvent



Rapid Novor (n.d.)

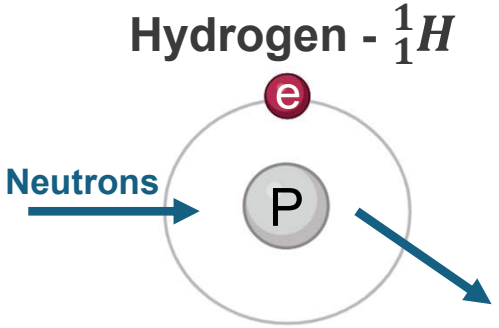
Using SANS to track HDX

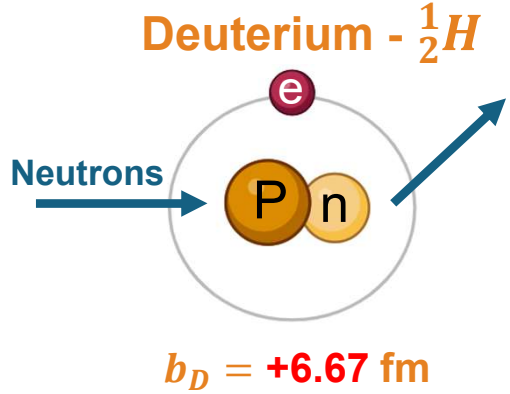


Scattering Length Density (SLD)

$$\rho = \frac{\sum b_i}{V_m}$$

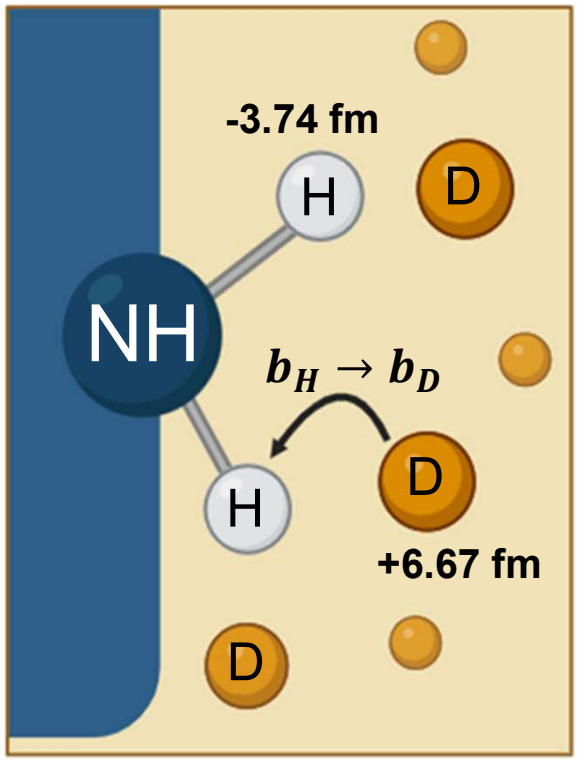
Isotopes scatter differently





All figures were created with BioRender.com; adapted from Rapid Novor (n.d.) & R.B. Donnelly et al., *Mol. Pharmaceutics* (2023).

Using SANS to track HDX



Scattering Length Density (SLD)

$$\rho = \frac{\sum b_i}{V_m}$$

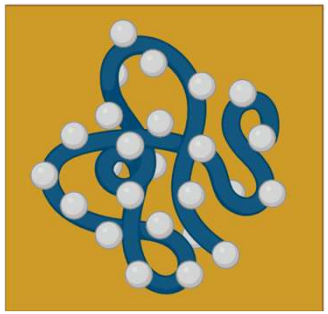
Intensity Impact

$$I(Q) \propto (\rho_{protein} - \rho_{solvent})^2$$

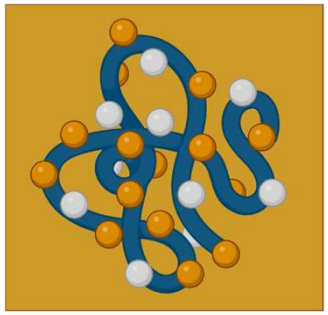
$$\boldsymbol{I(Q) \propto \Delta\rho^2}$$

Protein becomes more “invisible” to neutrons

t = 0

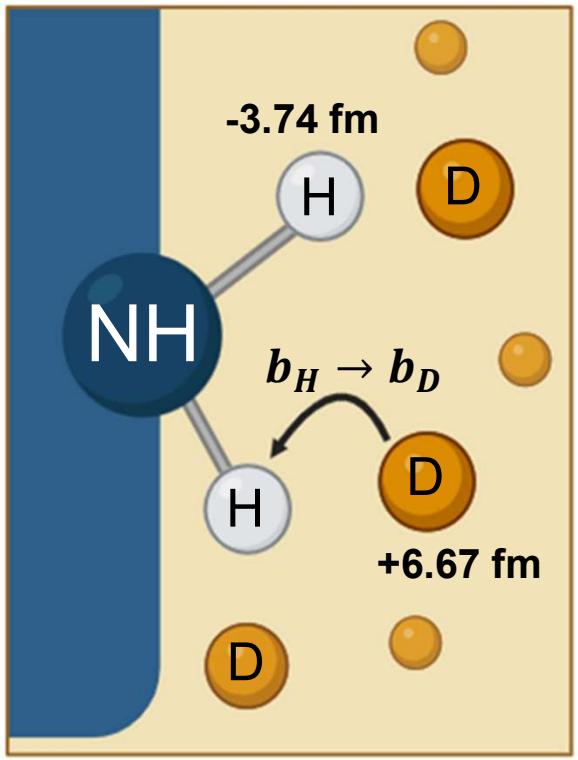


t = n



All figures were created with BioRender.com; adapted from Rapid Novor (n.d.) & R.B. Donnelly et al., *Mol. Pharmaceutics* (2023).

Using SANS to track HDX



Scattering Length Density (SLD)

$$\rho = \frac{\sum b_i}{V_m}$$

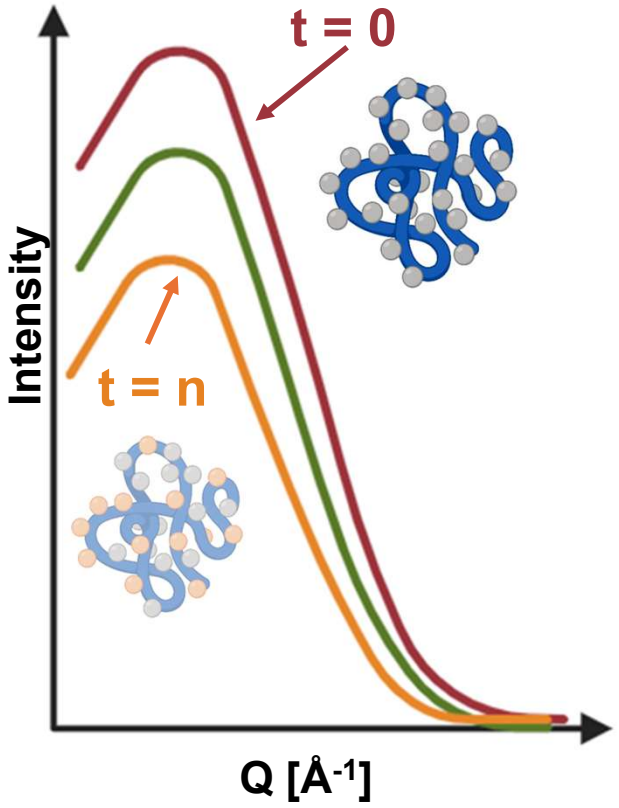
Intensity Impact

$$I(Q) \propto (\rho_{protein} - \rho_{solvent})^2$$

$$\boldsymbol{I(Q) \propto \Delta\rho^2}$$

I(Q) vs Q graphs relate to exchange time rates and number of exchanged deuterons.

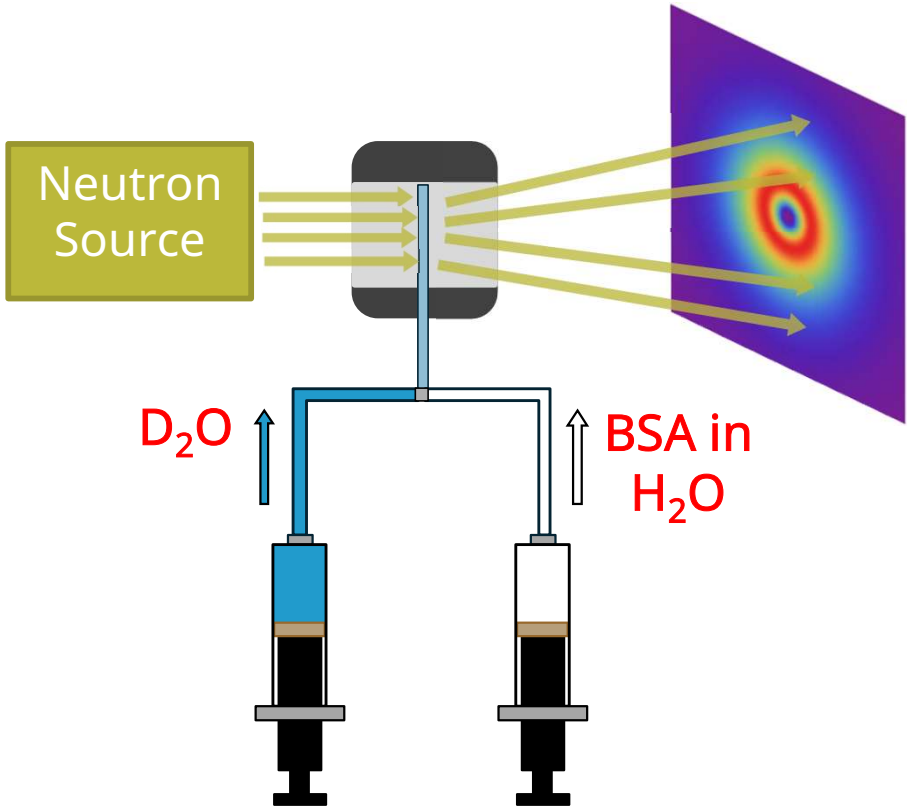
Can we measure HDX in the first 5 minutes after mixing?



All figures were created with BioRender.com; adapted from Rapid Novor (n.d.) & R.B. Donnelly et al., *Mol. Pharmaceutics* (2023).

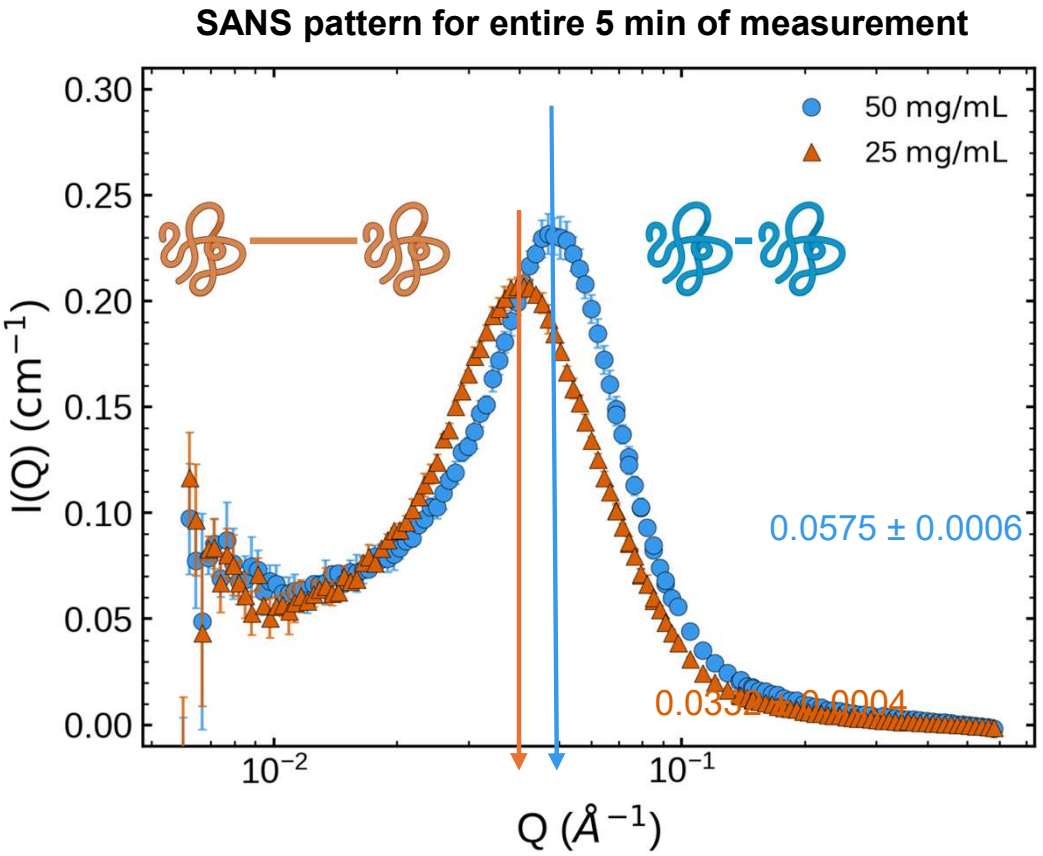
Sample information and initial data processing

Mixing Ratio	4:1	9:1
BSA volume rate $\left(\frac{mL}{s}\right)$	2	0.75
D_2O volume rate $\left(\frac{mL}{s}\right)$	8	6.75
Total Volume Rate $\left(\frac{mL}{s}\right)$	10.00	7.50
BSA in solution $\left(\frac{mg}{mL}\right)$	50.00	25.00
Replicates	4	4

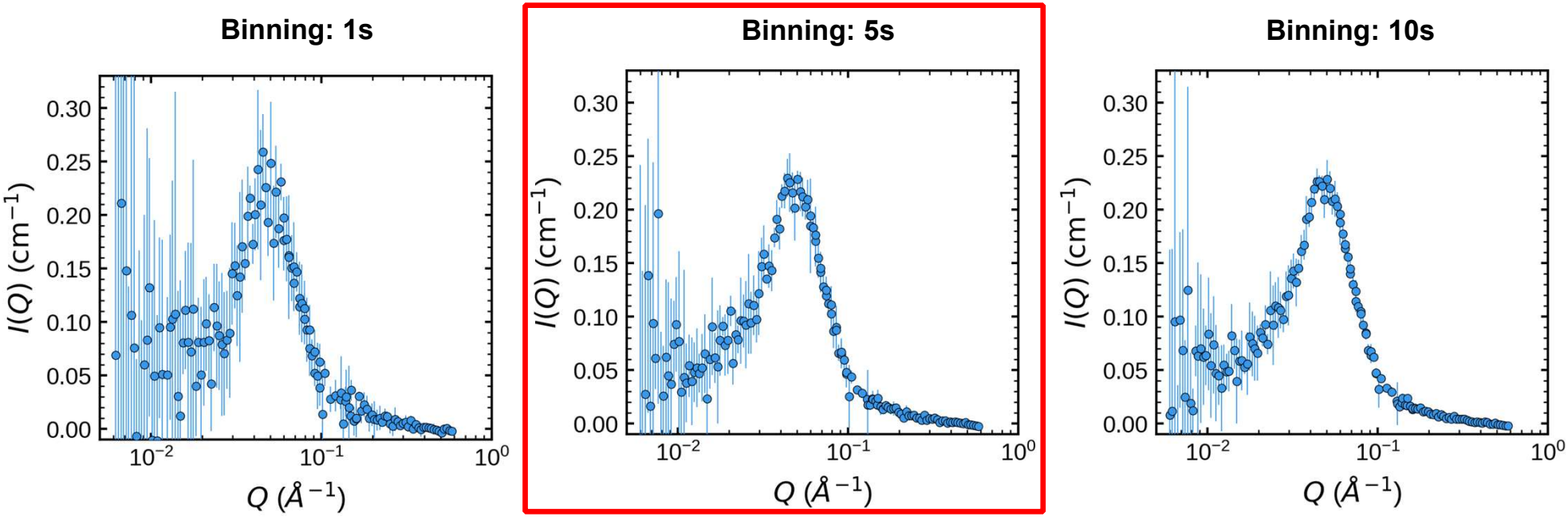


Sample information and initial data processing

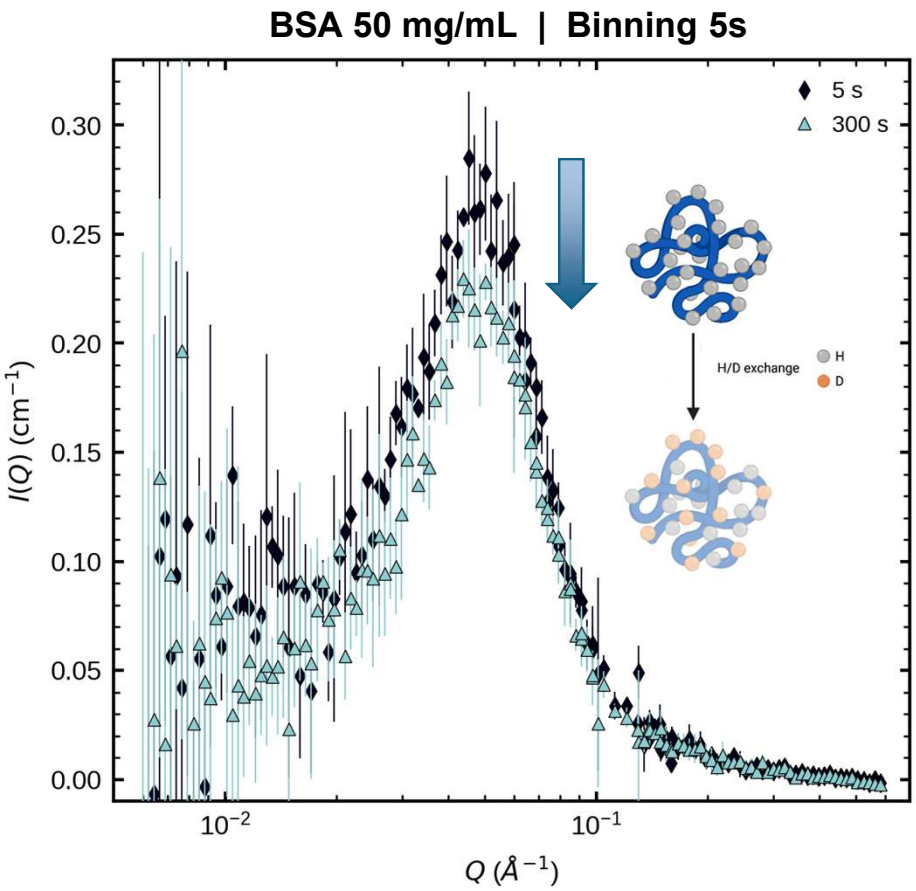
Mixing Ratio	4:1	9:1
BSA volume rate $\left(\frac{mL}{s}\right)$	2	0.75
D_2O volume rate $\left(\frac{mL}{s}\right)$	8	6.75
Total Volume Rate $\left(\frac{mL}{s}\right)$	10.00	7.50
BSA in solution $\left(\frac{mg}{mL}\right)$	50.00	25.00
Replicates	4	4

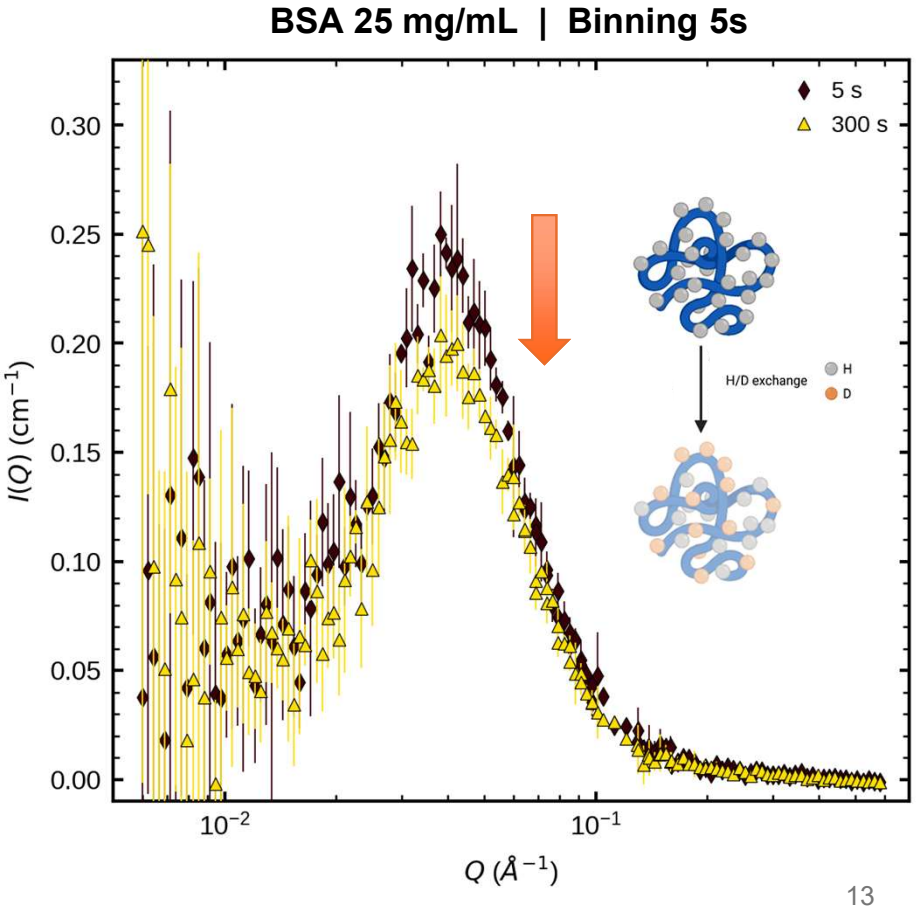


5s bin balanced signal-to-noise with time resolution

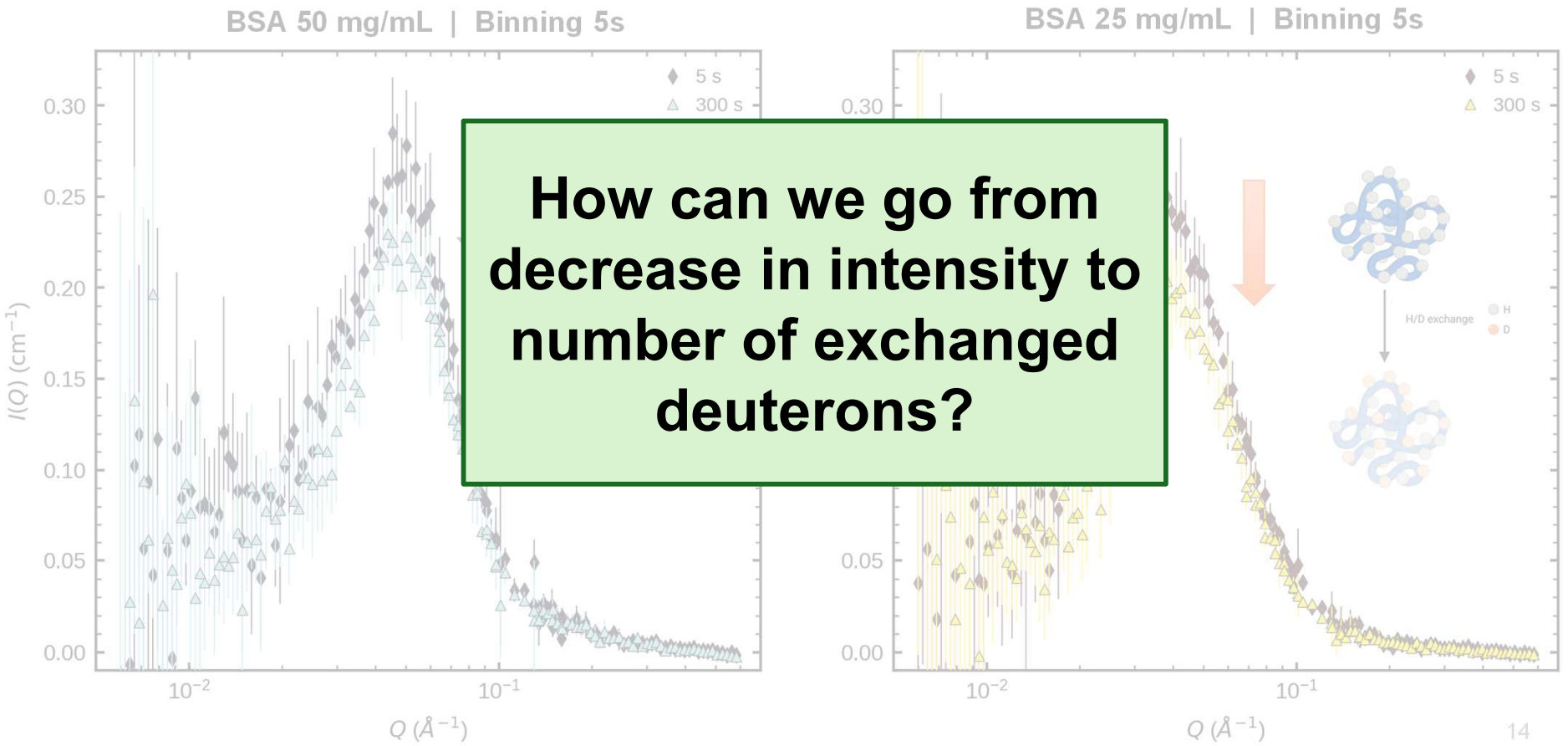


Intensity is diminishing through time





Intensity is diminishing through time



How can we go from decrease in intensity to number of exchanged deuterons?

Constant R(Q) indicates no structural change

$$I(Q) = nV_p^2(\Delta\rho)^2 \mathrm{P}(Q)\mathrm{S}(\mathrm{Q})$$

Scattering Intensity ratio

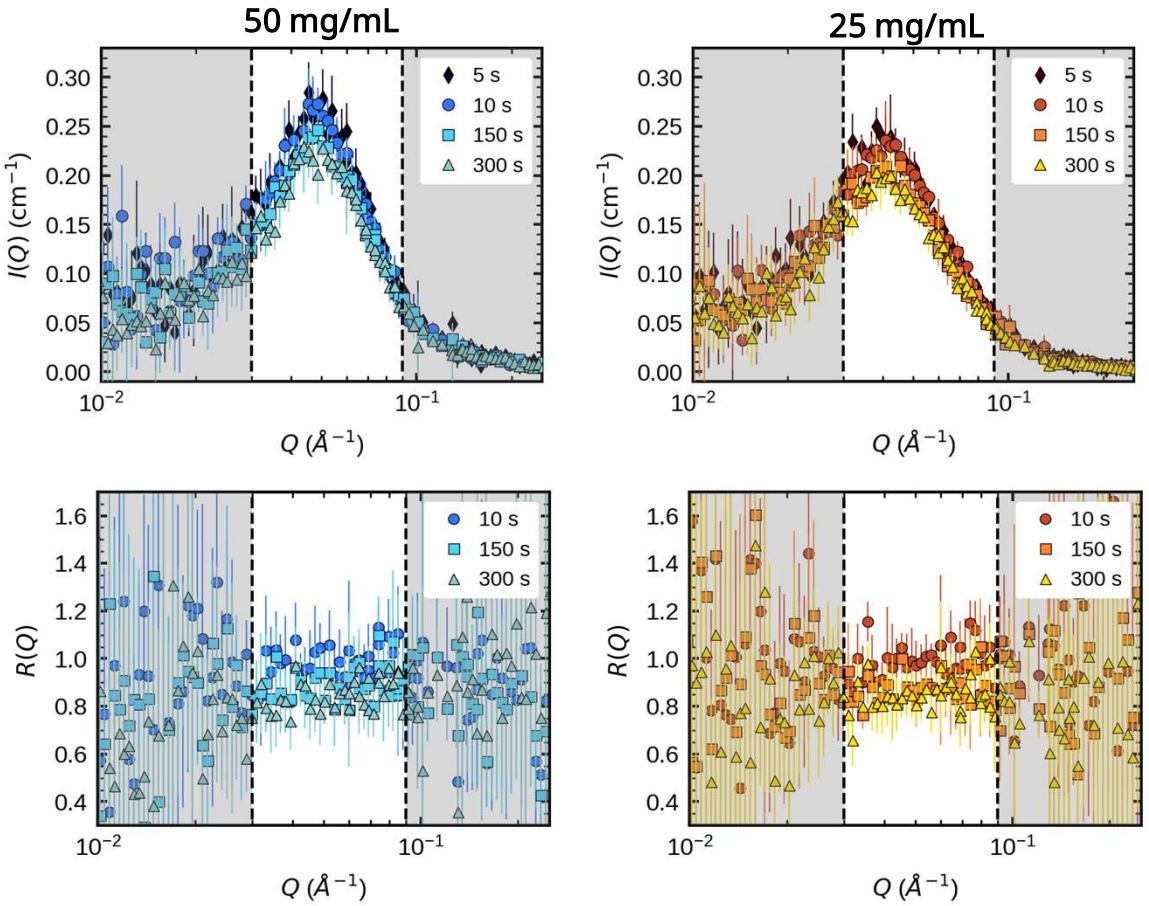
$$R(Q) = \frac{I(Q)_t}{I(Q)_{t=0}}$$

Assuming constant:

- Particle number density
- Protein volume
- Form factor
- Structure factor

$$R(Q) = \frac{(\Delta\rho_t)^2}{(\Delta\rho_0)^2}$$

Selected Q-range
0.03 – 0.09 Å^{-1}



Number of exchanged deuterons

Integrate intensity

$$I(t) = \int_{0.03}^{0.09} I(Q)dQ$$

Take the square root

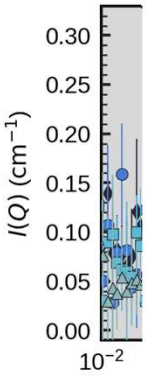
$$\sqrt{I(t)} \propto \Delta\rho_t$$

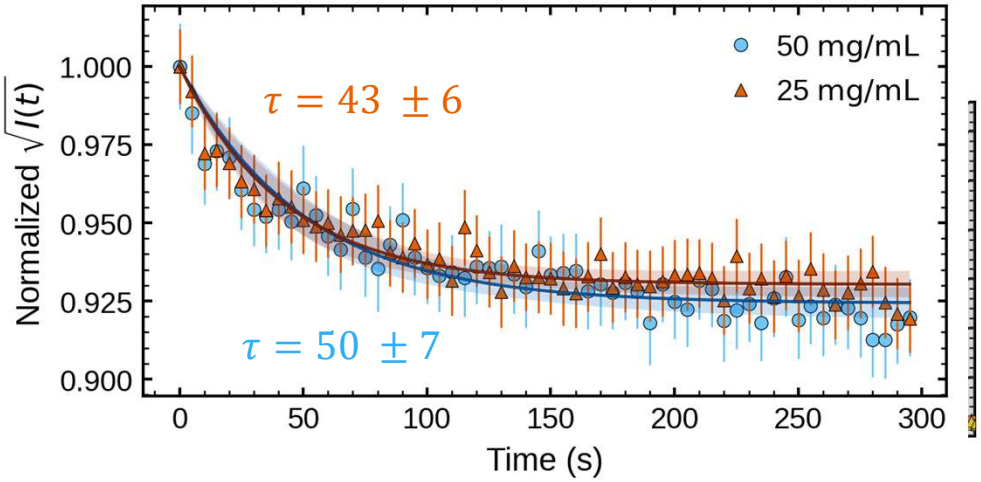
Normalize to the initial state

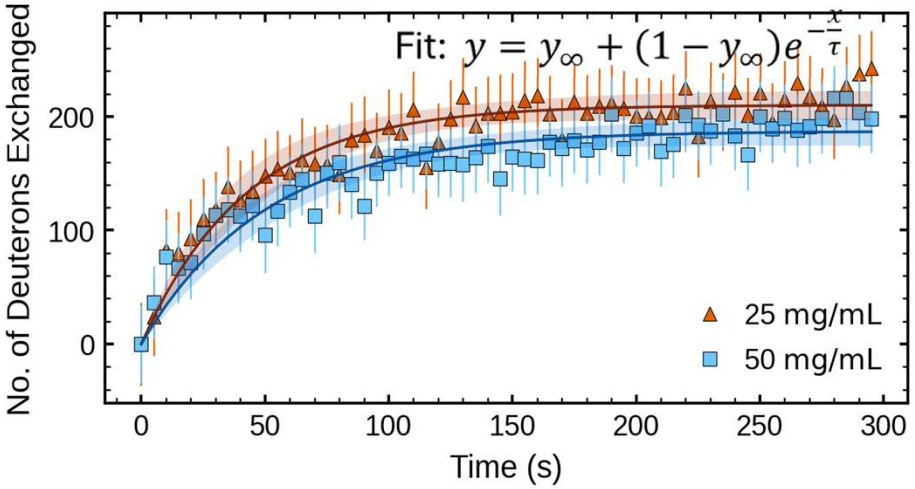
$$\frac{\sqrt{I(t)}}{\sqrt{I(0)}} = \frac{\Delta\rho_t}{\Delta\rho_0}$$

Convert to exchanged deuterons

$$n_{HDX} = \frac{V_p\left(\frac{\Delta\rho_t}{\Delta\rho_0} - 1\right)\left(\rho_{protein,0} - \rho_{solvent}\right)}{b_D - b_H}$$



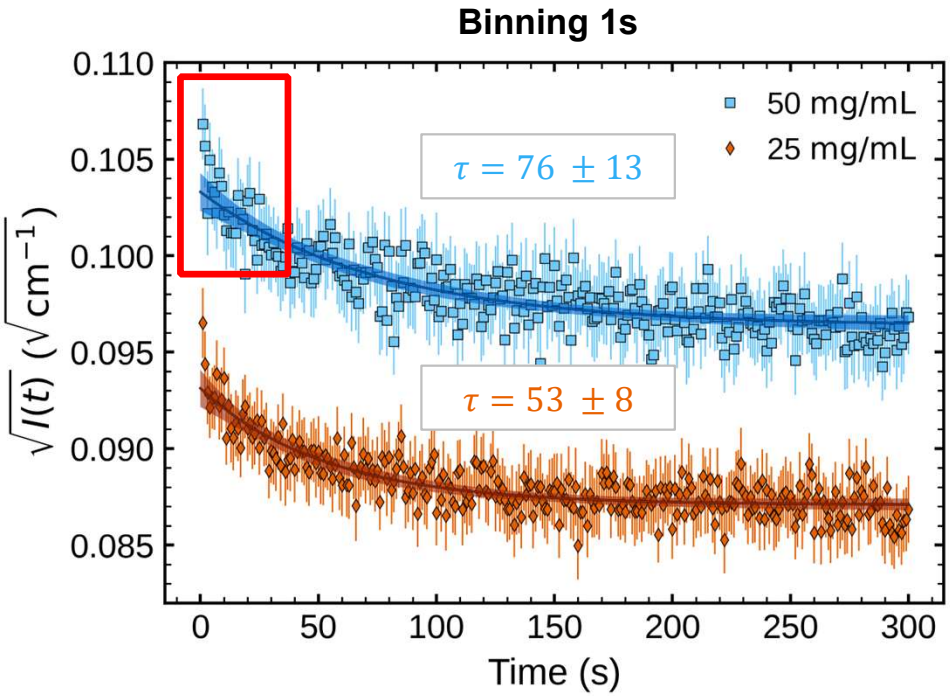


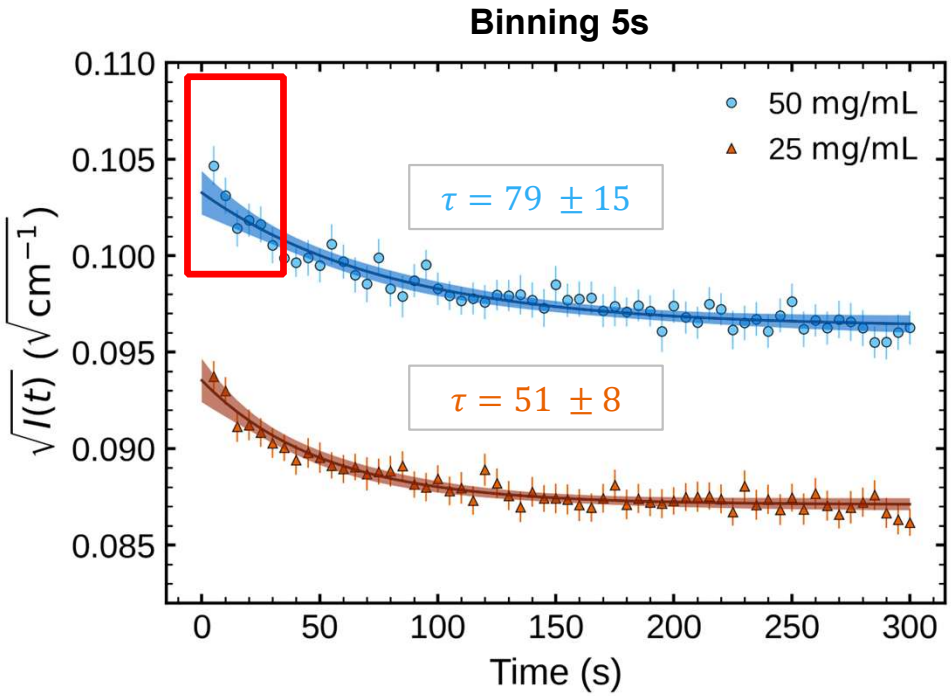


R.B. Donnelly et al., *Mol. Pharmaceutics* (2023).

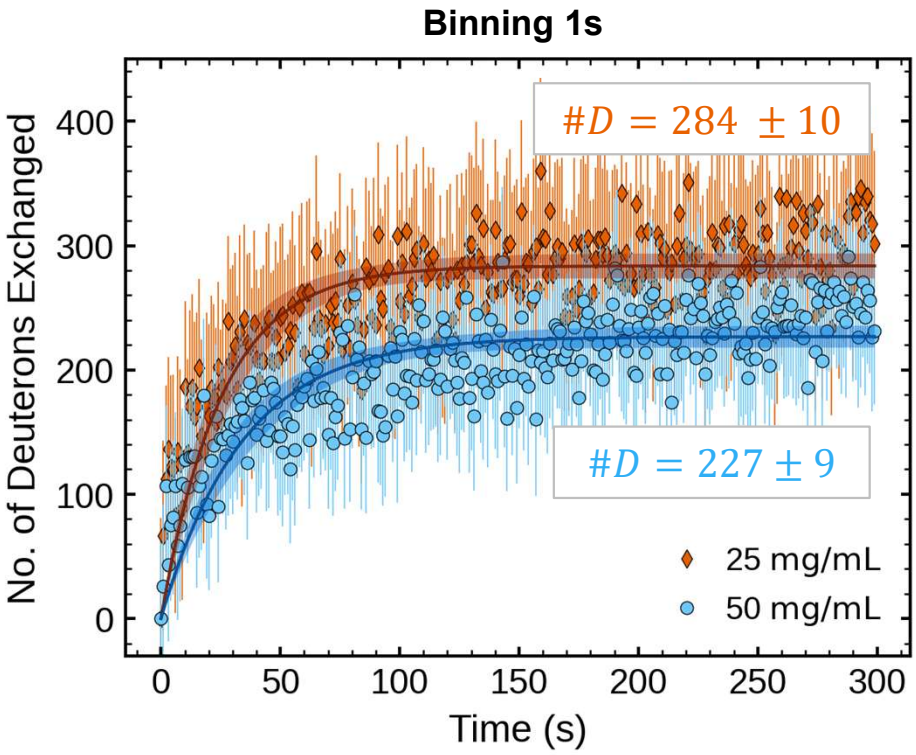
Smaller binning hint toward a second exchange rate

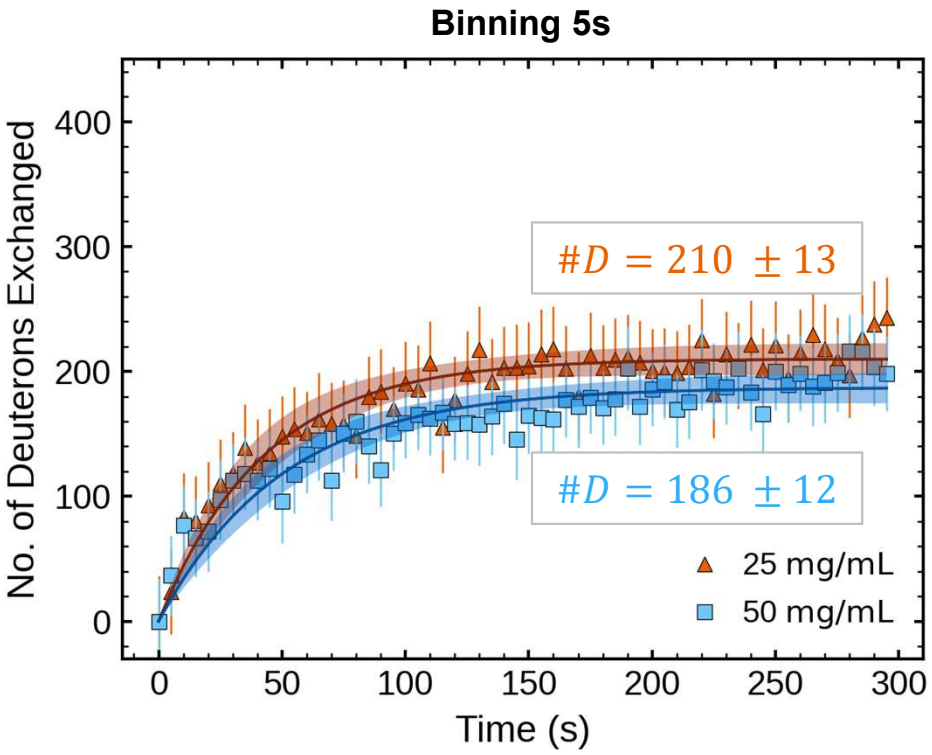
Fit: $y = Ae^{-\frac{x}{\tau}} + c$





Binning affects reported number of deuterons exchanged



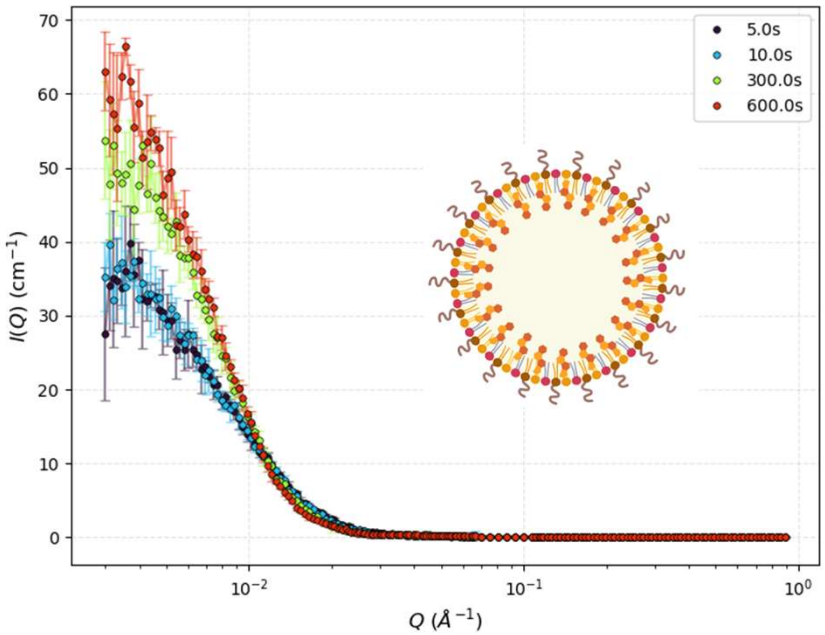


Summary

- Stopped-flow HDX SANS data reduced and analyzed to reveal early-stage kinetics.
- Extracted time constants and number of deuterons exchanged from BSA data.
- Paper on this method and BSA dataset is in progress!

Future Work

- BSA stopped-flow HDX SANS experiments at low temperatures (second time constant?)
- Ongoing analysis of lipid nanoparticle formation.



Acknowledgments

Dr. Chelsea Edwards

Dr. Ryan Murphy

Dr. Yun Liu

SURF Organizers:

Cara O'Malley

Dr. Susana Marujo

Dr. Julie Borchers

Dr. Kelsi Rehmann

SURF NIST

NIST Center for Neutron Research

CHRNS NSF CENTER FOR HIGH RESOLUTION NEUTRON SCATTERING NIST

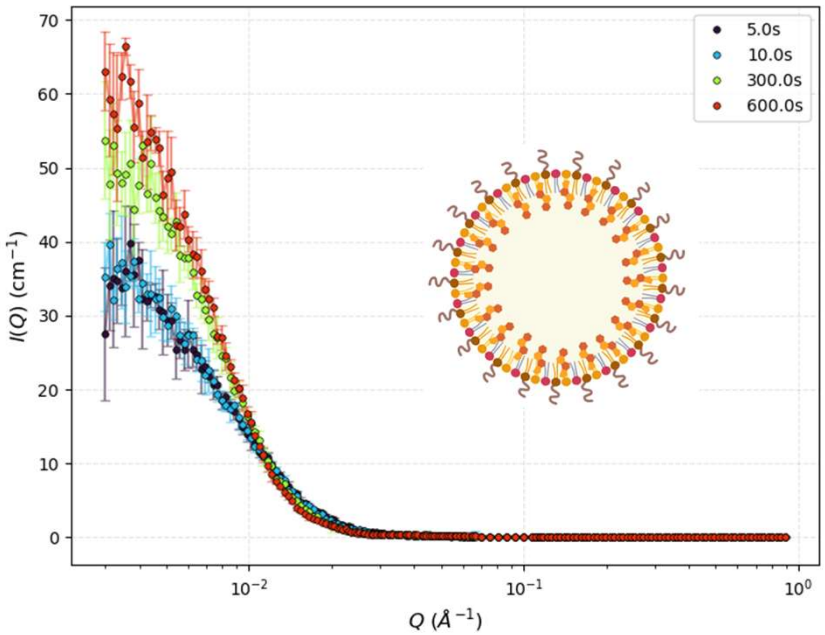
NIST National Institute of Standards and Technology U.S. Department of Commerce

Summary

- Stopped-flow HDX SANS data reduced and analyzed to reveal early-stage kinetics.
- Extracted time constants and number of deuterons exchanged from BSA data.
- Paper on this method and BSA dataset is in progress!

Future Work

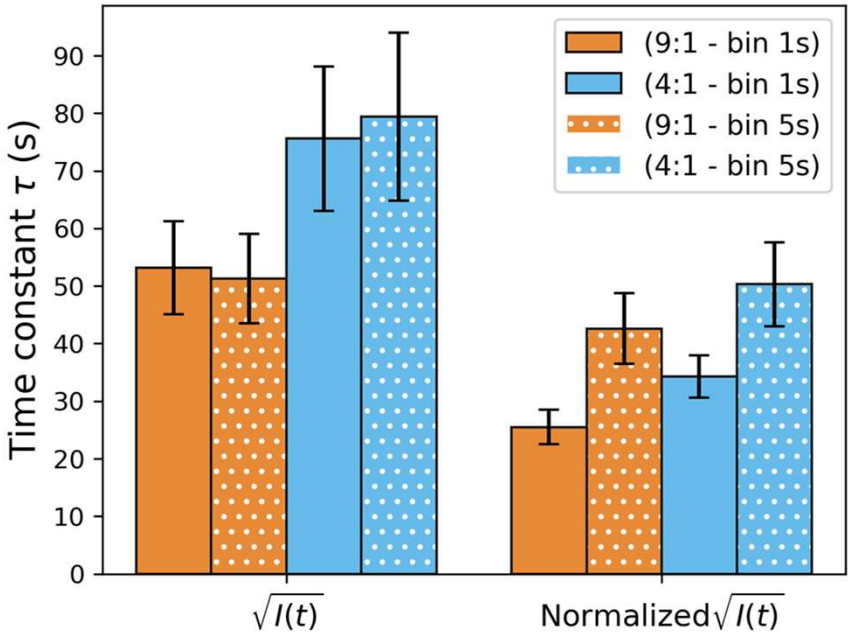
- BSA stopped-flow HDX SANS experiments at low temperatures (second time constant?)
- Ongoing analysis of lipid nanoparticle formation.



Extra Slides

Smaller binning hint toward a second exchange rate

- 1s binning appears to capture a second, faster exchange constant



Fit: $y = Ae^{-\frac{x}{\tau}} + c$