Dynamic cluster formation, viscosity and diffusion in monoclonal antibody solutions

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   - Pharmaceutical challenges
2. Materials and methods
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   - Small angle neutron scattering (SANS)
   - Molecular dynamics (MD) simulations
3. Data treatment and analysis
4. First results
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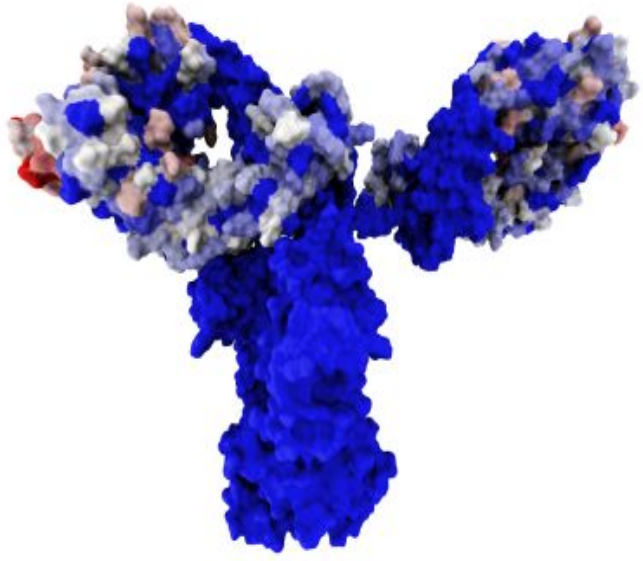
5. Conclusions and future developments



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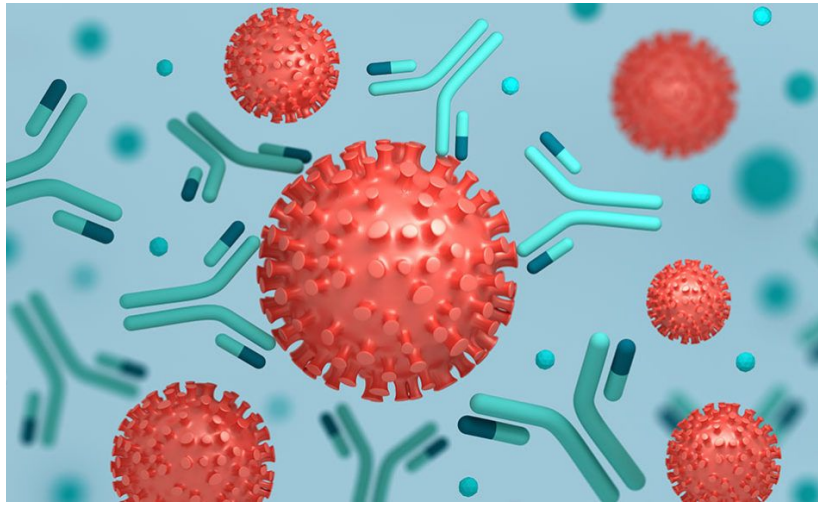
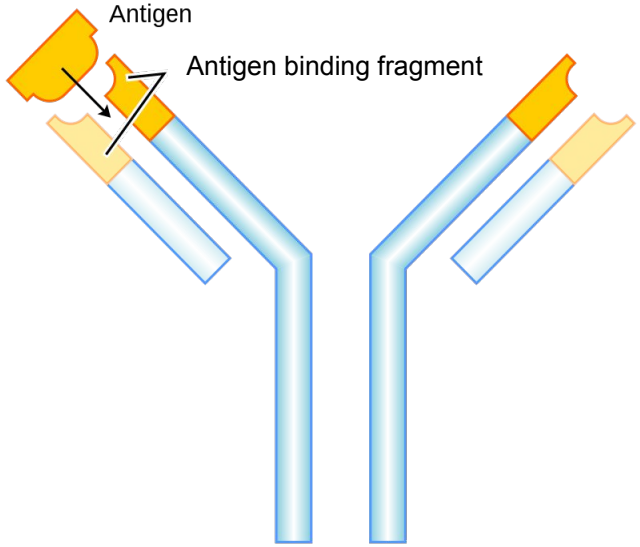
ILL-ESS UserMeeting; Lund, 06/10/2022

1. Introduction: Monoclonal antibodies (mAbs)

Antibodies: proteins, aka *immunoglobulins* (IgGs), secreted in response to pathogens

- 4 polypeptides, 150 kDa Y-shaped molecule
- Fab = antigen binding fragment → bivalent antigen binding





Monoclonal antibodies recognize only a single type of antigen!

→ high specificity and versatility

→ efficiency in **pharmaceutical targeting**

(1) Lipman *et al.*, ILAR Journal, vol. 46, 3, 258-268 (2005) **(2)** Johnson *et al.*, Mater Methods, 3:160 (2013)

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1. Introduction: Pharmaceutical challenges

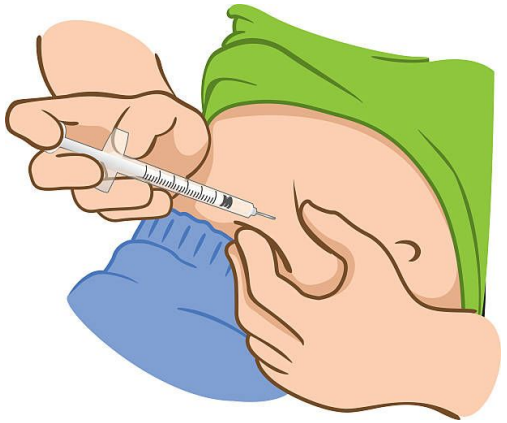
MAb solutions are employed in therapeutics for multiple diseases: cancers, psoriasis, viral infections, ...

Currently:
administration via *intravenous (IV) injection*

high administration frequency
skilled personnel required
less patient convenience

Market need:
administration via *subcutaneous (SC) injection*

low administration frequency
no skilled personnel required
more patient convenience



SC formulations:

- Low volumes (1-2 mL)
- High concentrations → high viscosity → difficulty to inject
- Viscosity reduction is possible via additives

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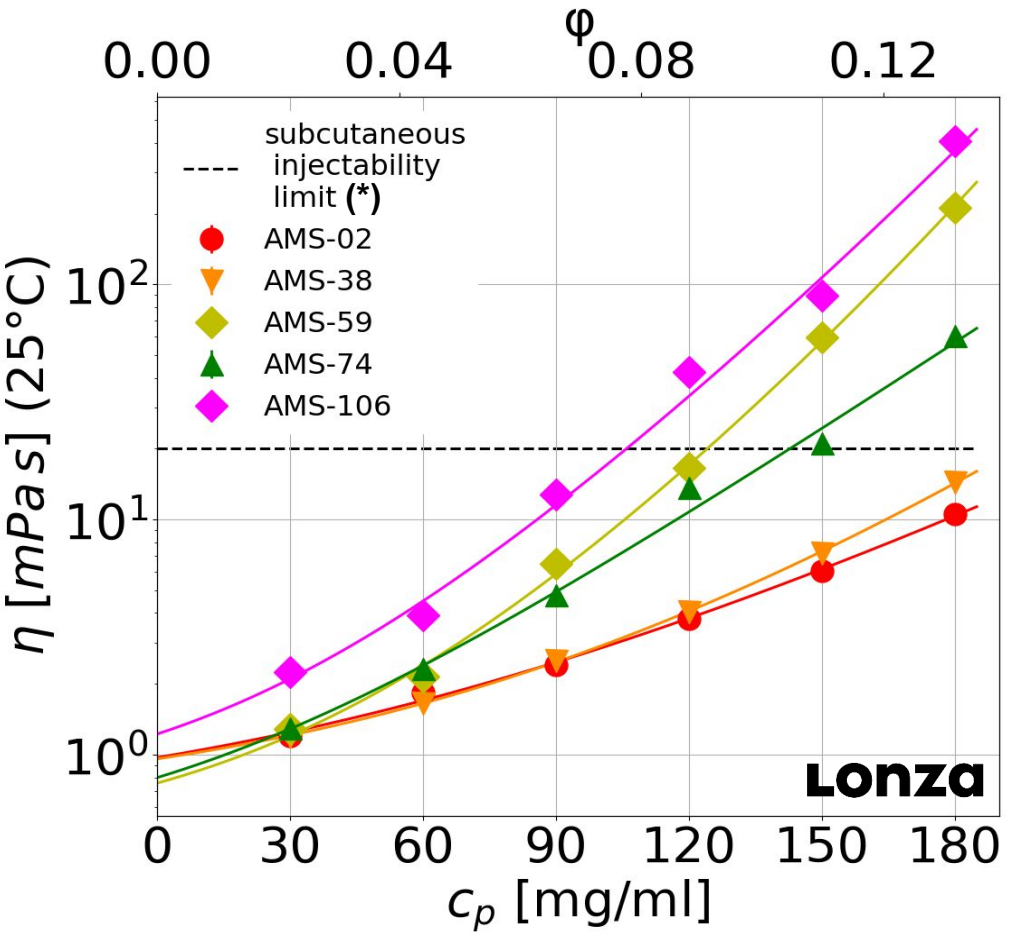
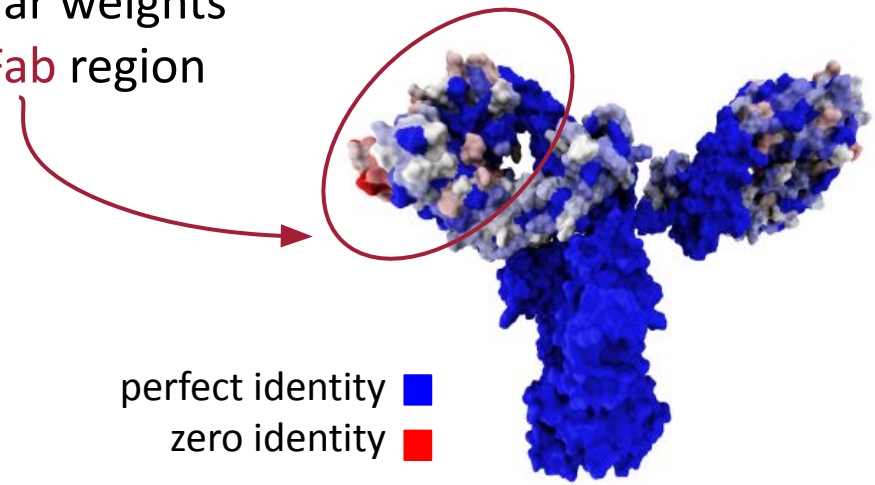
2. Materials and methods: Lonza mAbs



mAb type-dependent viscosity η
Similar molecular weights
Differences in Fab region



→ differences in surface charge and protein-protein interactions
→ aggregation / reversible self-association [1,2]

(*) Jolles S., Sleasman J. W., Adv. Ther. 28, 521 (2011) **(1)** Liu, J., et al., J. Pharm. Sci. 94:1928–40 (2005) **(2)** Yearley E. et al., Biophys. J. 140:1763-70 (2014)

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2. Materials and methods

Neutron backscattering spectroscopy
+ small angle neutron scattering (SANS)

D11 @ILL:

three Lonza mAbs in D2O solution
at c_p = 80 mg/mL, T = 18, 21, 37 °C

IN16B @ILL:

five Lonza mAbs + polyclonal IgG (reference) in
D2O solution, 20mM His-HCl buffer
at different c_p and T (280, 295, 310 K)

https://www.ill.eu/users/instruments/instruments-list/in16b, d11

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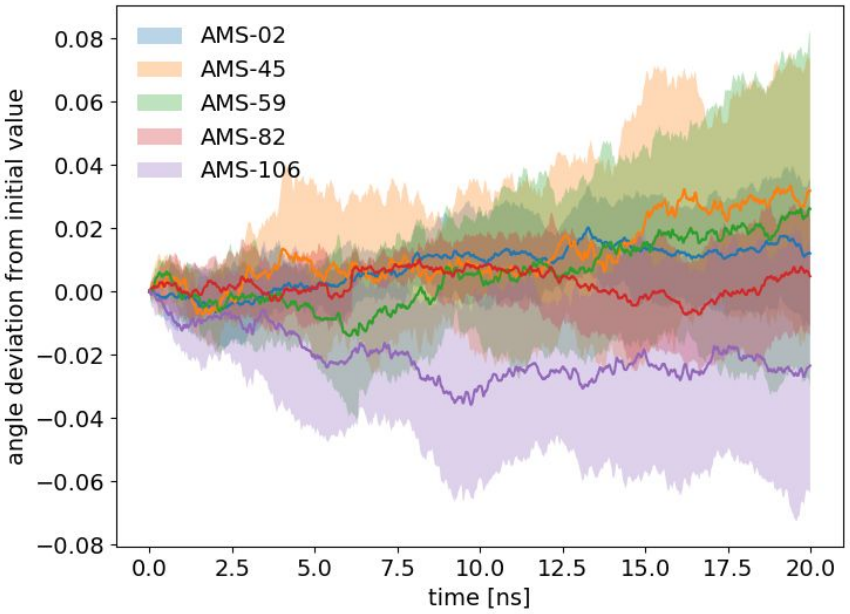
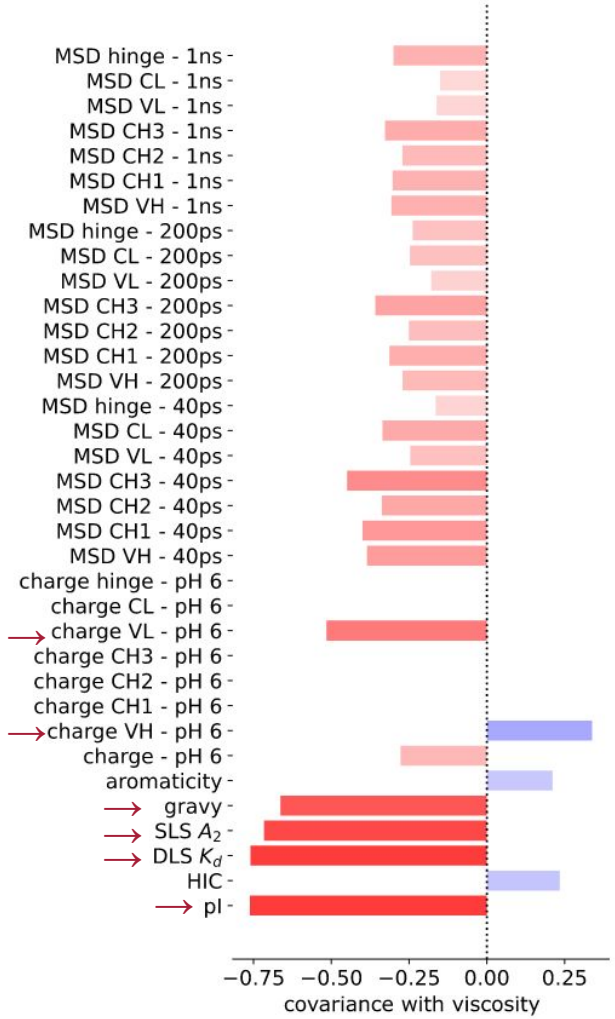
2. Materials and methods

MD simulations (*K. Pounot*)

- Single-molecule simulations via *NAMD* using CHARMM36 force field [1,2]
- Calculation of MSDs, angles between the lobes, internal diffusion
- Determination of the charge of different domains (*pdb2pqr*) [3]
- Calculation of isoelectric point, hydrophobicity, aromaticity via *BioPython*

⟶ internal dynamics does not strongly depend on mAb type
⟶ V domains charge and PPI parameters correlate with viscosity





(1) Huang J. *et al.,* J. Comput. Chem., 34:2135-45 (2013) **(2)** Huang J., *et al.,* Nature Methods, 14, 1:71-73 (2017) **(3)** Jurrus E. *et al.*, Prot. Sci., 27:112-128 (2018)

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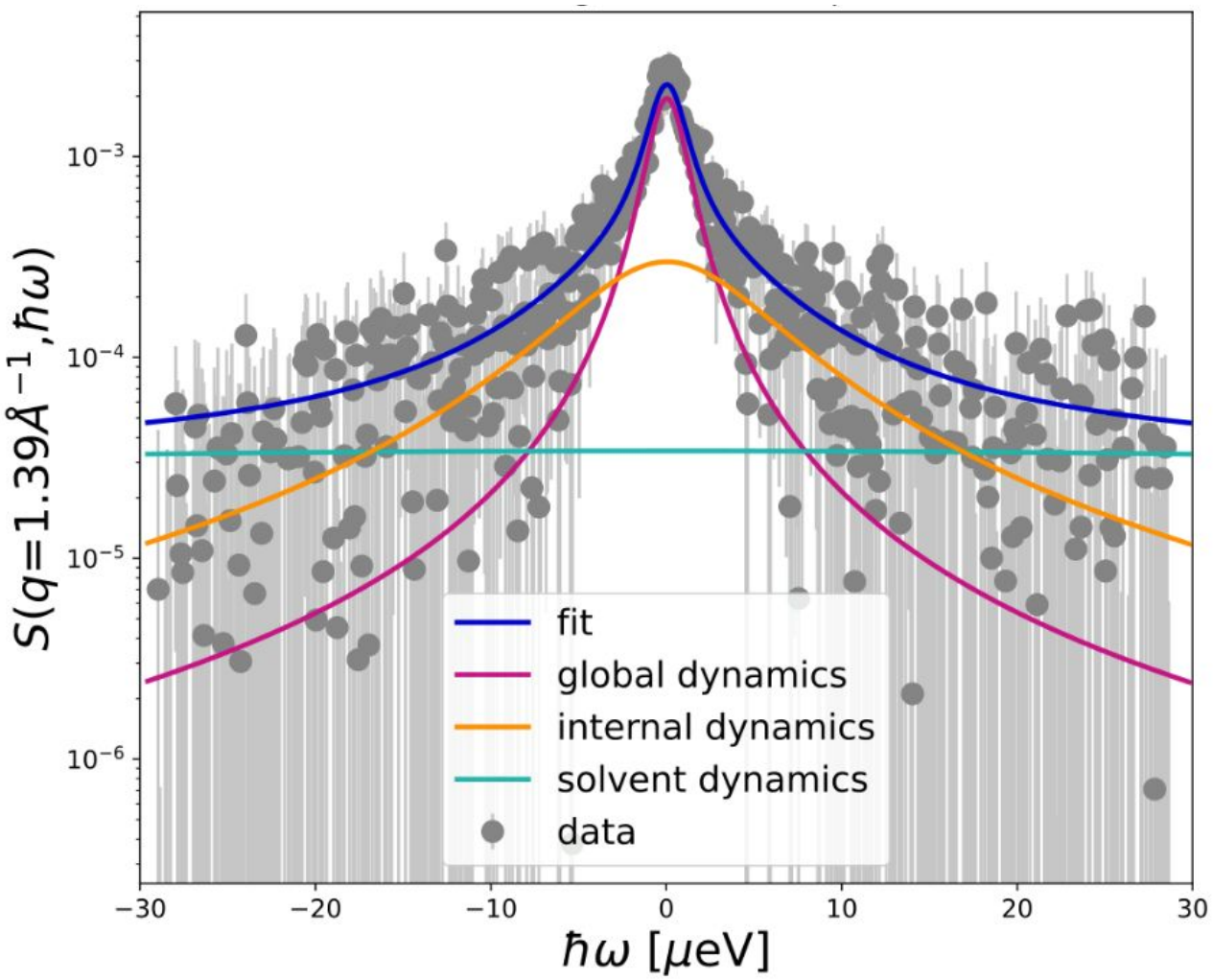
3. Data treatment and analysis



Sample: mAb **AMS-59**, by **Lonza**

Buffer: 20mM His-HCl in D2O

c_p = 105 mg/mL

T = 280 K

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3. Data treatment and analysis

Data reduction using Mantid, empty cell subtraction
Fitting and analysis using Python (*q*-wise and global fits)

$$S(q,\omega) = R(q,\omega) \otimes \left\{ \beta \left[A_0(q)\mathcal{L}_{\Gamma_{glob}} + (1 - A_0(q))\mathcal{L}_{\Gamma_{int}} \right] + \mathcal{L}_{\Gamma_{D_2O}} \right\}$$

1. Fickian global + free internal dynamics + parametrized EISF:

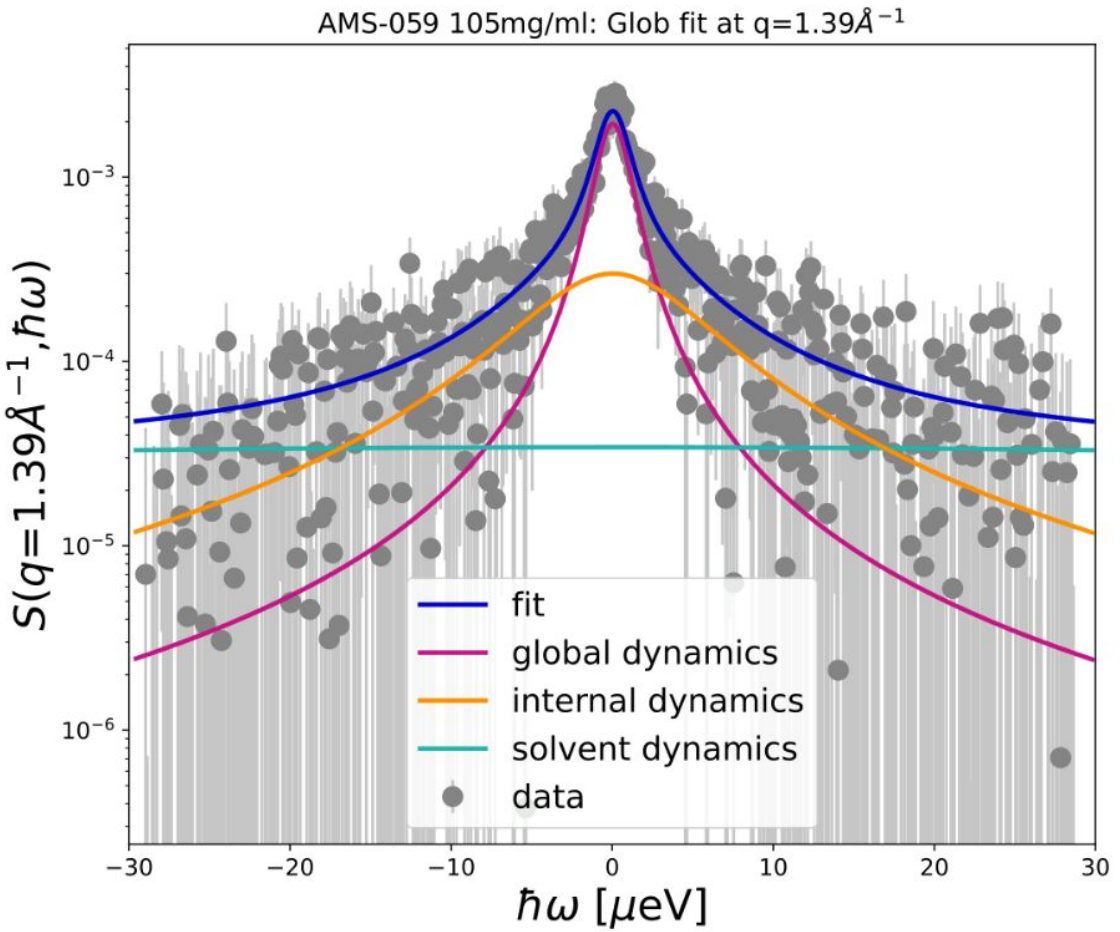
$$\Gamma_{glob} = D\,q^2$$

$$A_0(q) = p + (1-p)\left[p_1\, A_{3jump}(q) + (1-p_1)\, A_{sphere}(q) \right]$$

$$A_{3\text{-jump}}(q) = \frac{1}{3}\left[1 + 2j_0(qa)\right] \qquad A_{\text{sphere}}(q) = \left| \frac{3\,j_1(qR)}{qR} \right|^2$$

2. Fickian global + jump-like internal dynamics:

$$\Gamma_{glob} = D\,q^2 \qquad \Gamma_{int} = \frac{D_1 q^2}{1 + \tau\, D_1 q^2}$$



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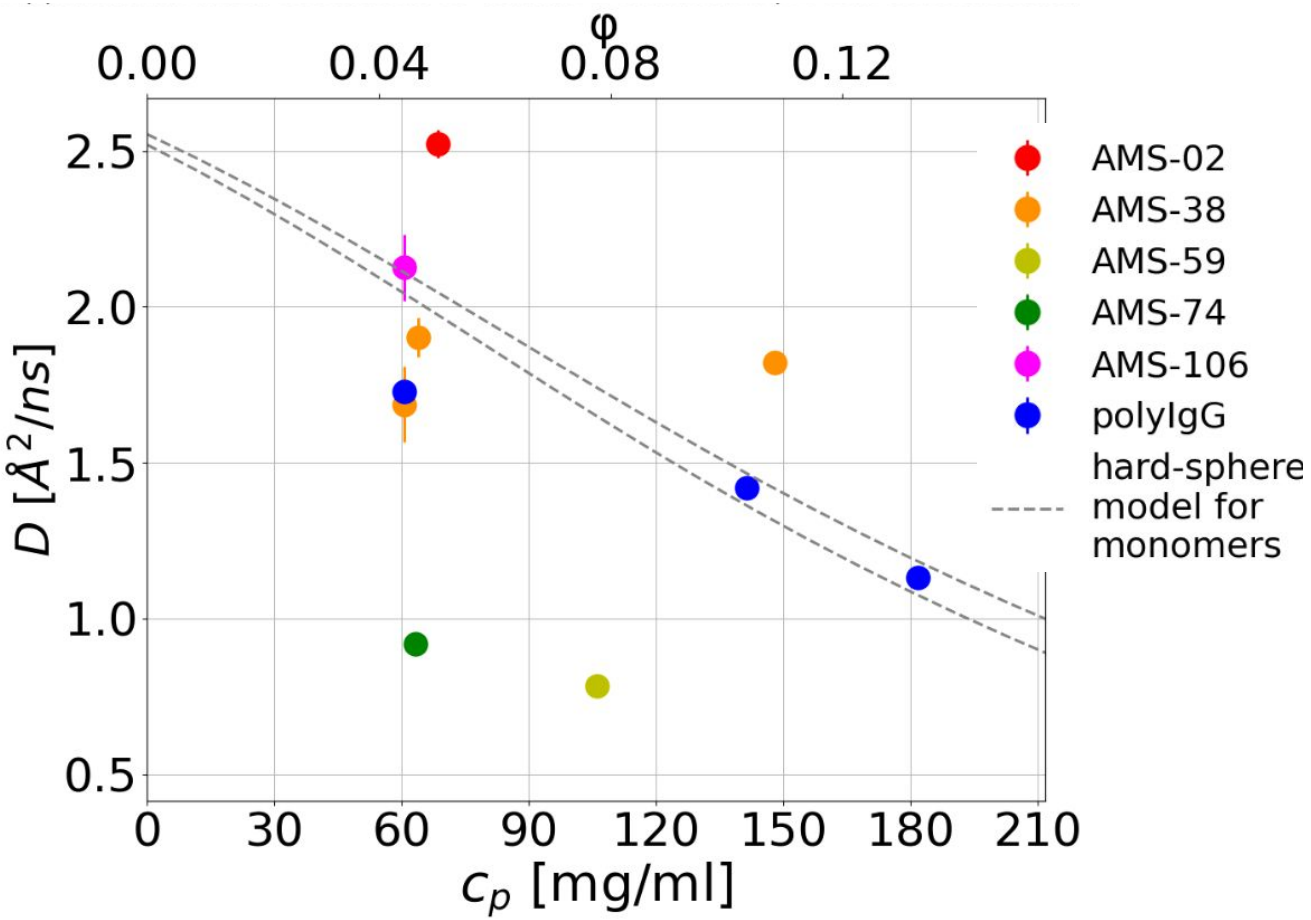
4. First results: QENS

Center of mass self-diffusion (at 280 K)



Theoretical prediction of short-time diffusion coeff. ***D = D (Dt(φ) , Dr(φ))*** using hard-sphere model from colloid physics:

$$D_t(\varphi) = \frac{D_{t0}}{1 + L(\varphi)} \quad (1)$$

$$D_r(\varphi) = D_{r0}(1 - 1.3\varphi^2) \quad (2)$$

Effective volume fraction φ_{eff}

$$\varphi_{eff} = \varphi \left(\frac{R_h}{R_{dry}}\right)^3 \quad (3)$$

Dt_0, Dr_0, R_{dry} obtained via HYDROPRO10 (4)

(1) Tokuyama M, Oppenheim I., Physica A 216, 85-119 (1995)
(2) Banchio A., Nägele G., J. Chem. Phys. 128, 104903 (2008)
(3) Roosen-Runge F., *et al.*, PNAS 108, 29, 11815-11820 (2011)
(4) Ortega A., *et al.*, Biophys. J. 101, 892-898 (2011)

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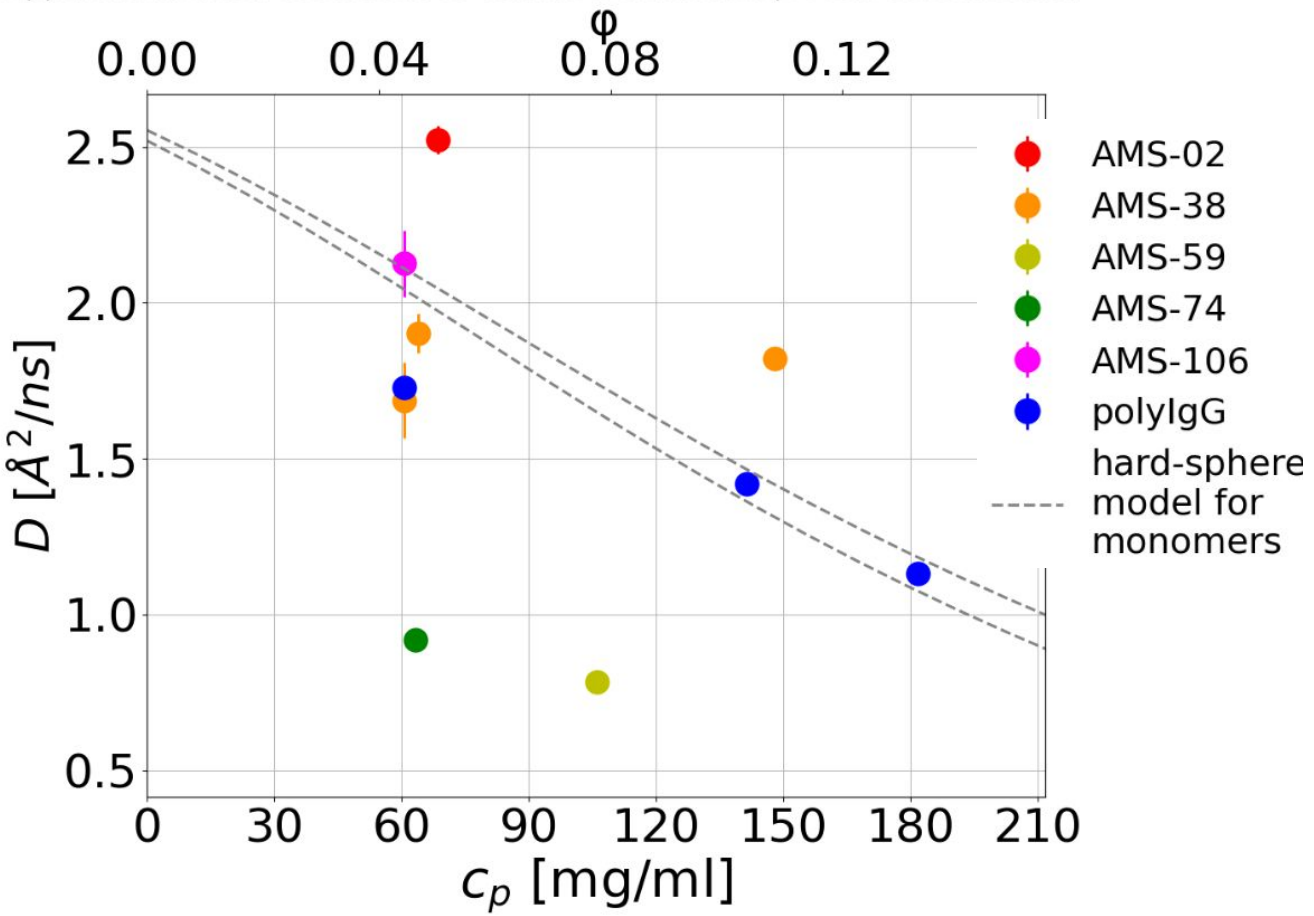

- Points on the lines: monomers
- Points below: clusters
- Points above: possibly heterogeneous samples or phase separation

Possibilities:

1. Incomplete model for heterogeneous situations
2. Cross-talking between global and internal motions
3. Discrepancies between nominal and real protein concentration

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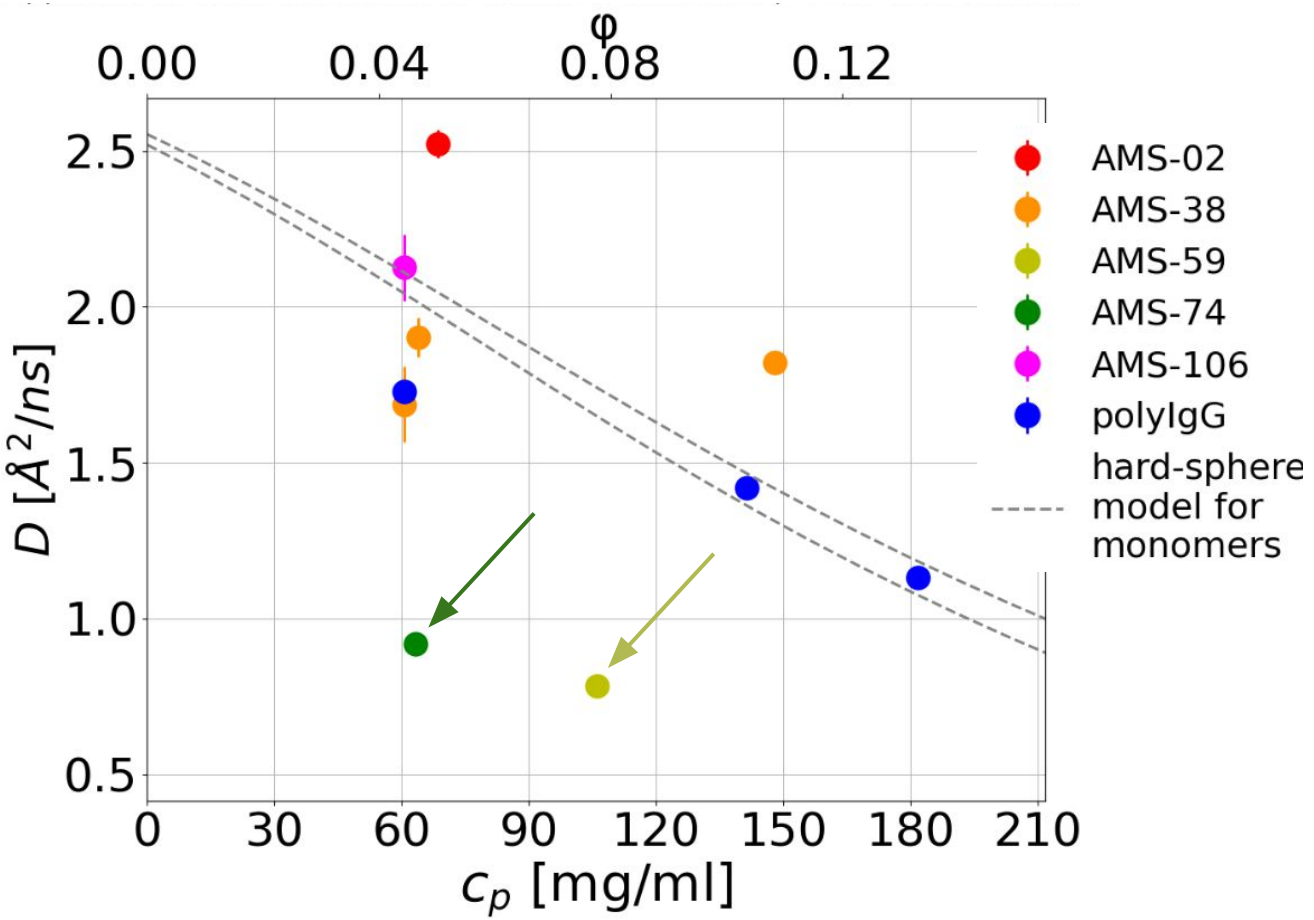
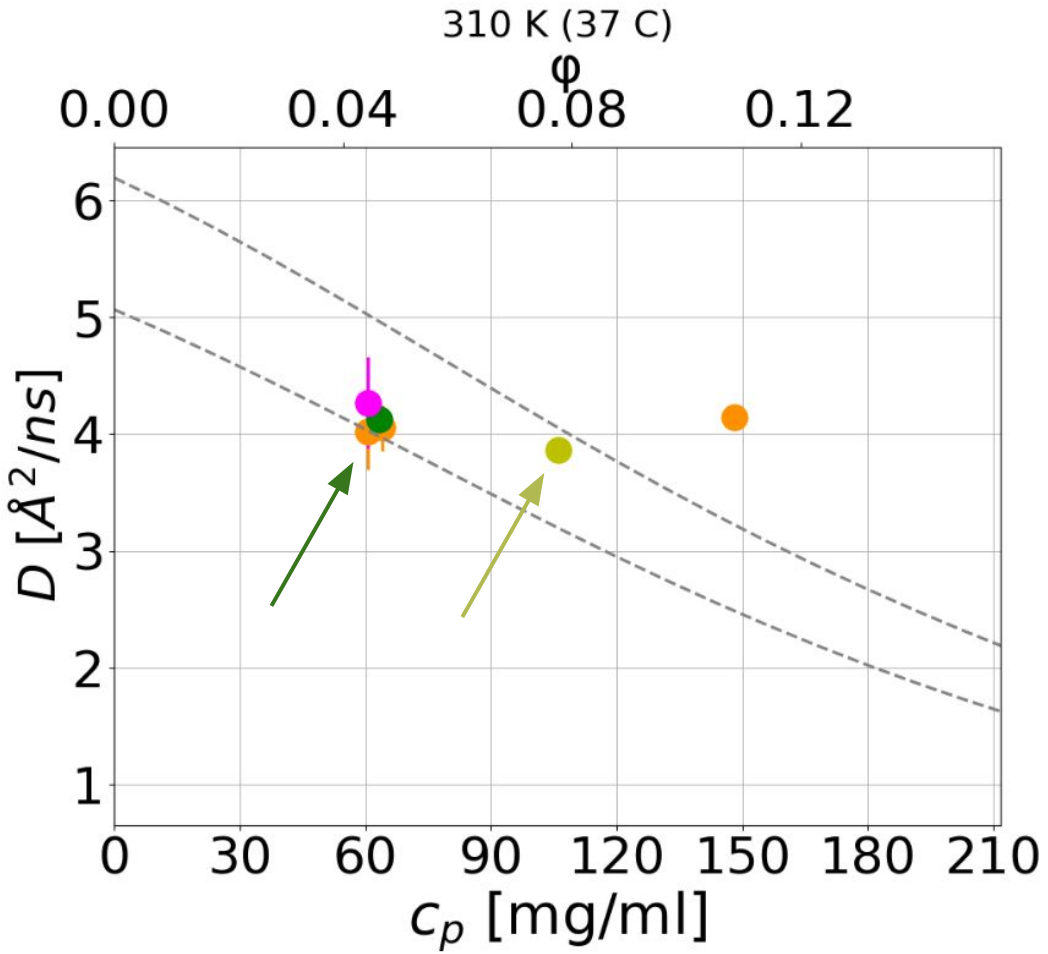
4. First results: QENS

Center of mass diffusion (280K and 310K)





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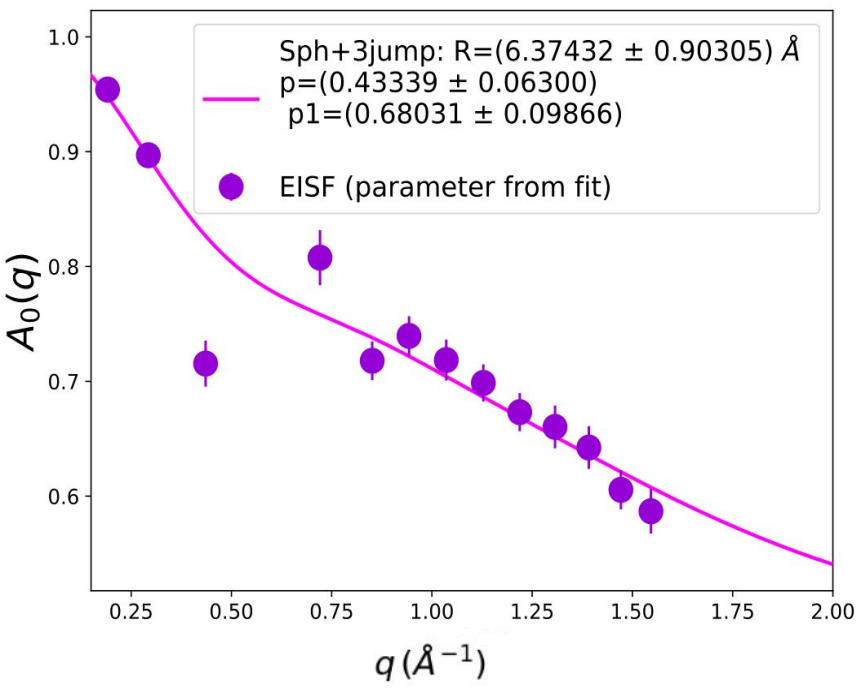
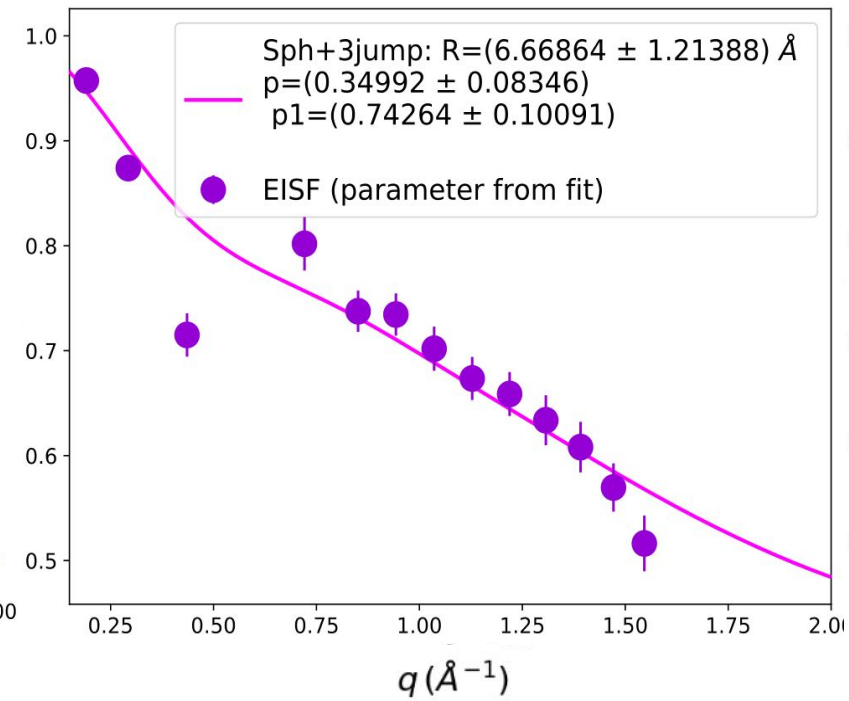
4. First results: QENS

Fitted elastic incoherent structure factor (EISF) $A_0(q)$

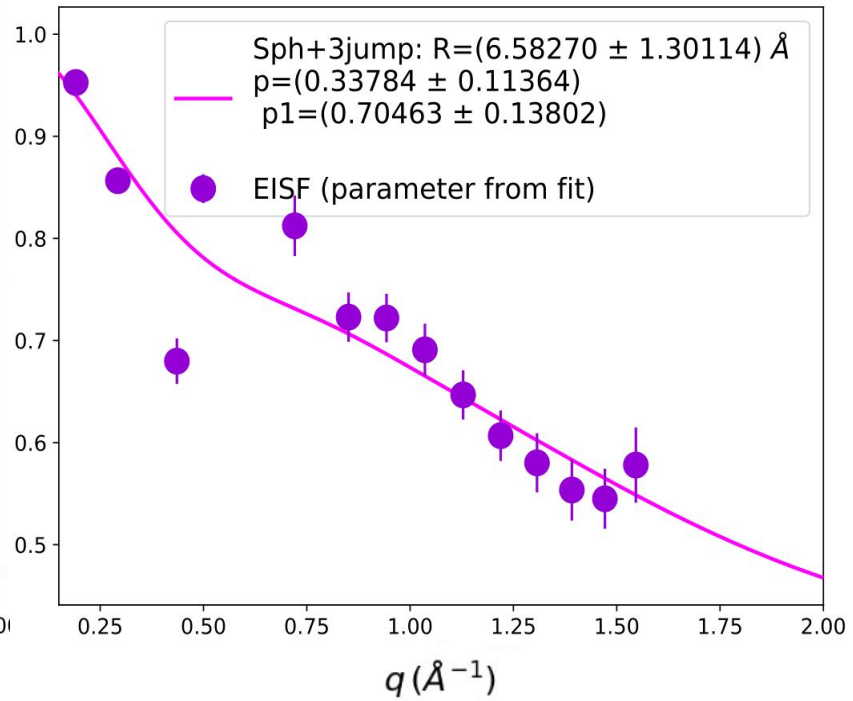
AMS-38 146 mg/mL, 280 K



295 K



310 K



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4. First results: QENS

Parameters R, p, p_1 from the EISF

$$A_0(q) = p + (1-p)\left[p_1\, A_{3jump}(q) + (1-p_1)\, A_{sphere}(q)\right]$$

$$A_{3\text{-jump}}(q) = \frac{1}{3}\left[1 + 2j_0(qa)\right] \qquad A_{\text{sphere}}(q) = \left|\frac{3j_1(qR)}{qR}\right|^2$$

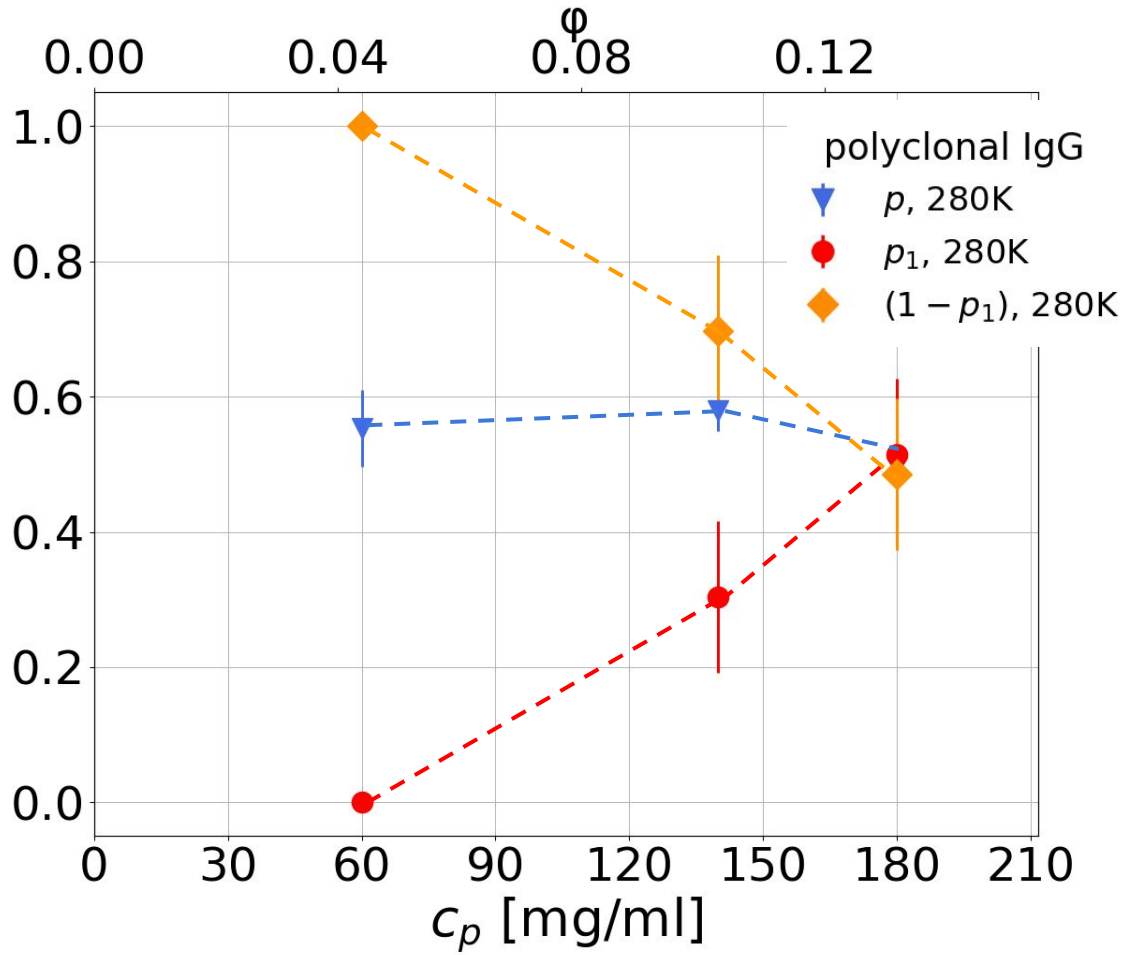
a ≃ 1.7155 Å

p independent from c_p

p_1 increases with increasing c_p

(1-p_1) decreases at increasing c_p (1)

R in agreement with previous work on γ-globulin (1,2)



(1) Grimaldo M. *et al.*, J. Phys. Chem. B 2014, 118, 7203−7209

(2) Stagg L. *et al.*, PNAS USA 104:18976–18981 (2007)

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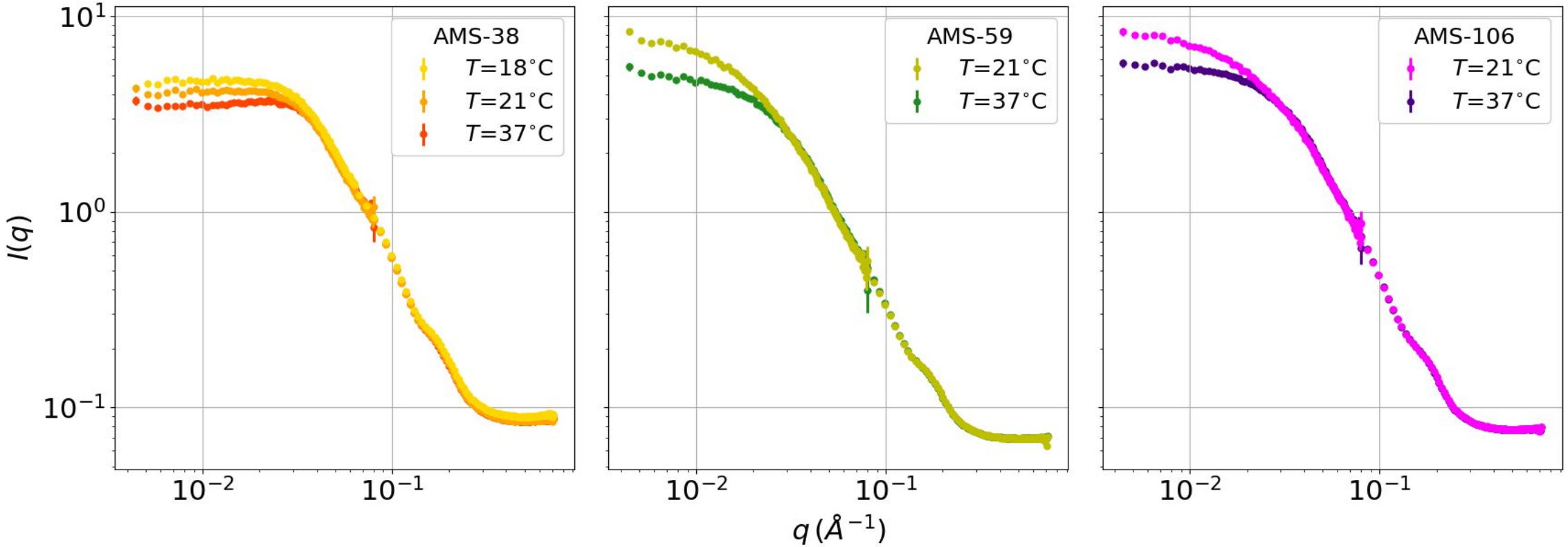
4. First results: SANS

SANS curves, temperature dependence

Subset of mAbs: AMS-38, AMS-59, AMS-106; c_p = 80 mg/ml, buffered in 20mM His-HCl in D_2O



Rosenbaum, D., *et al.*, Phys. Rev. Lett. 76, 1, 150 (1996)

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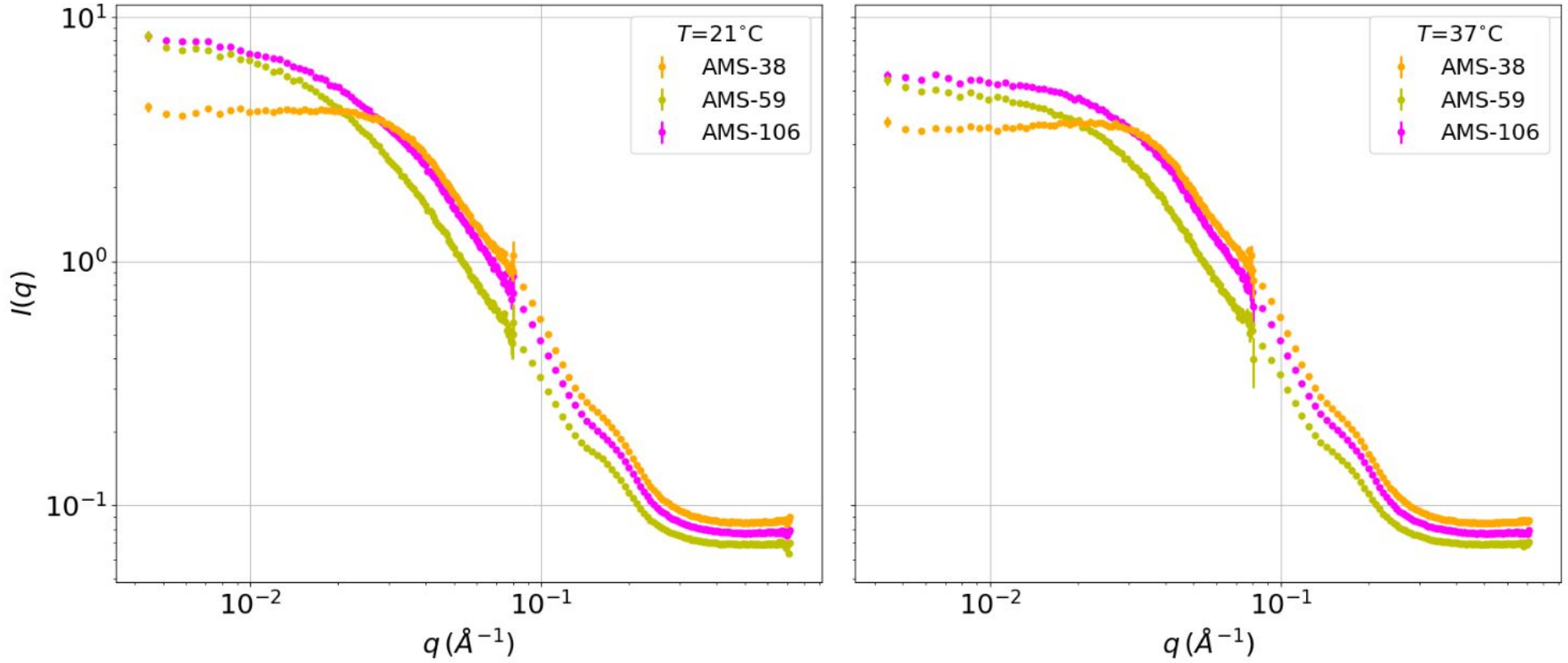
4. First results: SANS

SANS curves, mAb-type dependence

Subset of mAbs: AMS-38, AMS-59, AMS-106; c_p = 80 mg/ml, buffered in 20mM His-HCl in D_2O



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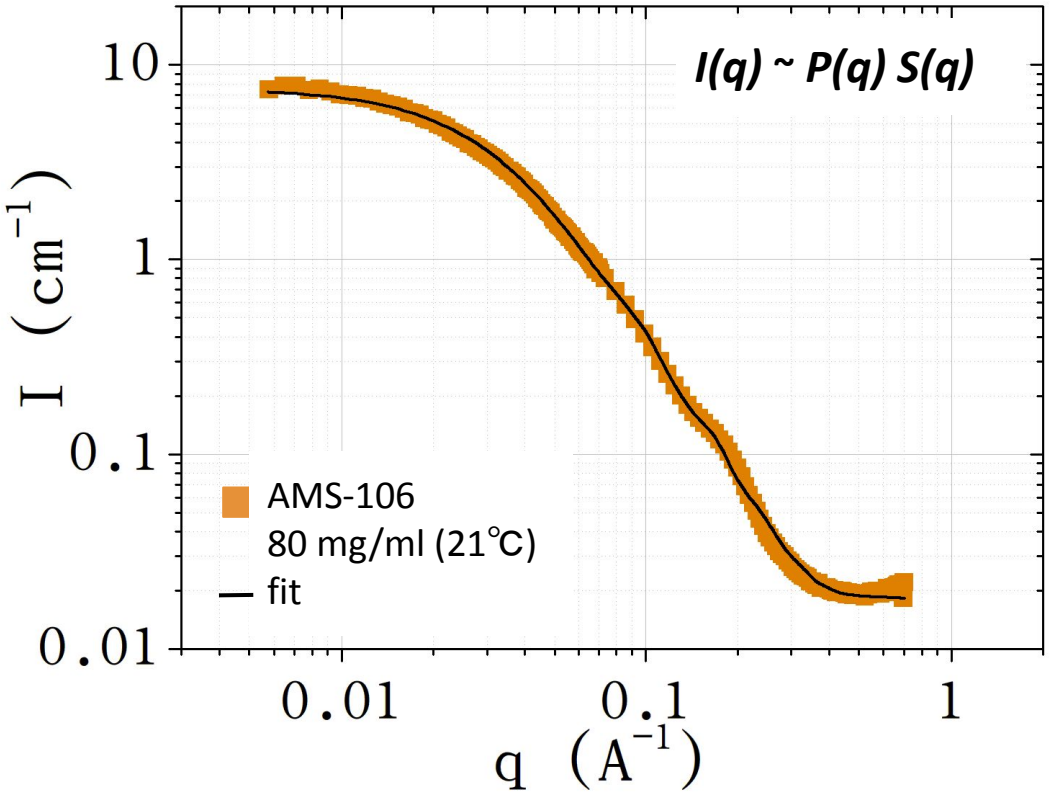
4. First results: SANS

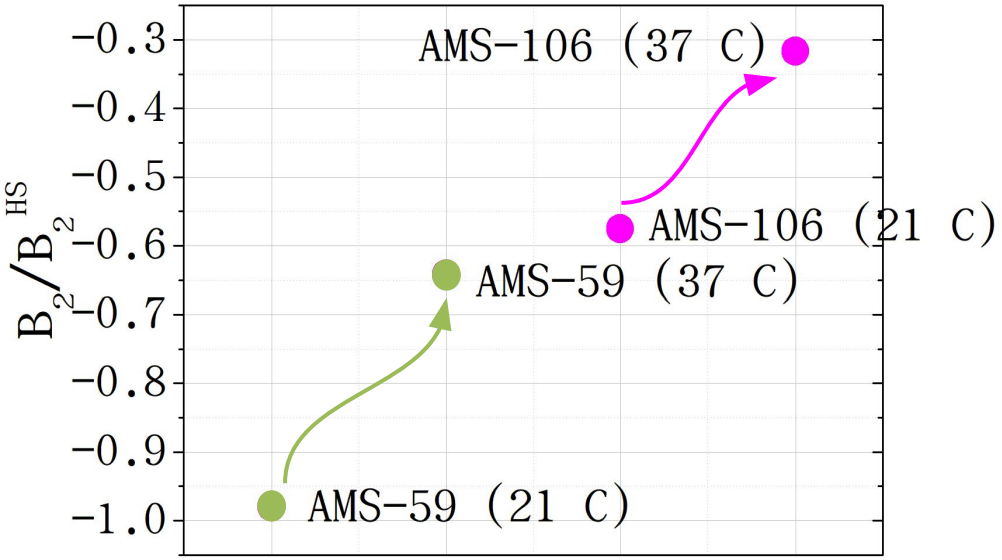
Fit: ellipsoid form factor ***P(q)***, sticky hard sphere (SHS) structure factor ***S(q)*** [1,2]



Calculation of 2nd virial coefficient B_2

$$B_2 = 2\pi \int_0^\infty dr\ r^2 [1 - e^{\frac{-u(r)}{k_B T}}]$$

→ attraction decreases with increasing temperature



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(2) G. A. Vliegenthart , H. N. W. Lekkerkerker, J. Chem. Phys. 112 (2000)

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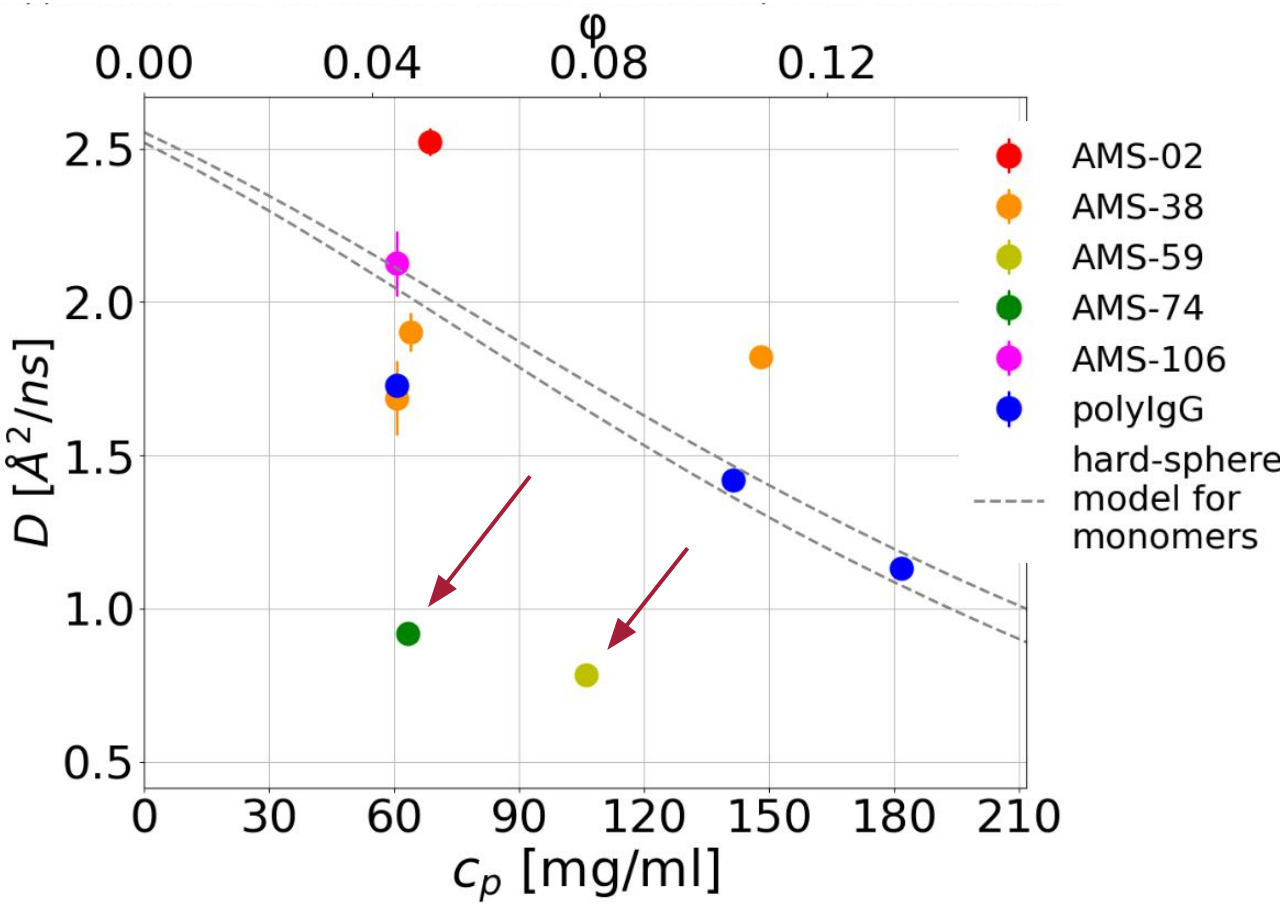
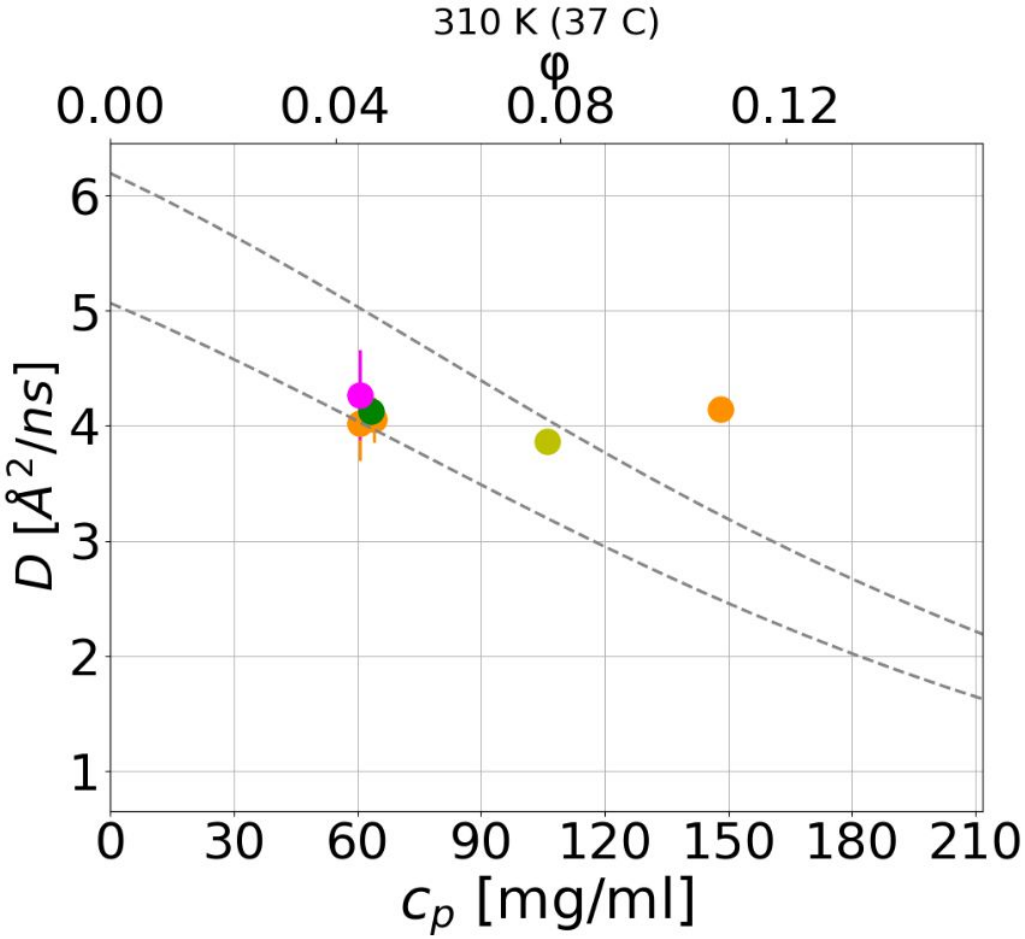
4. First results: QENS

Center of mass diffusion (280K and 310K)





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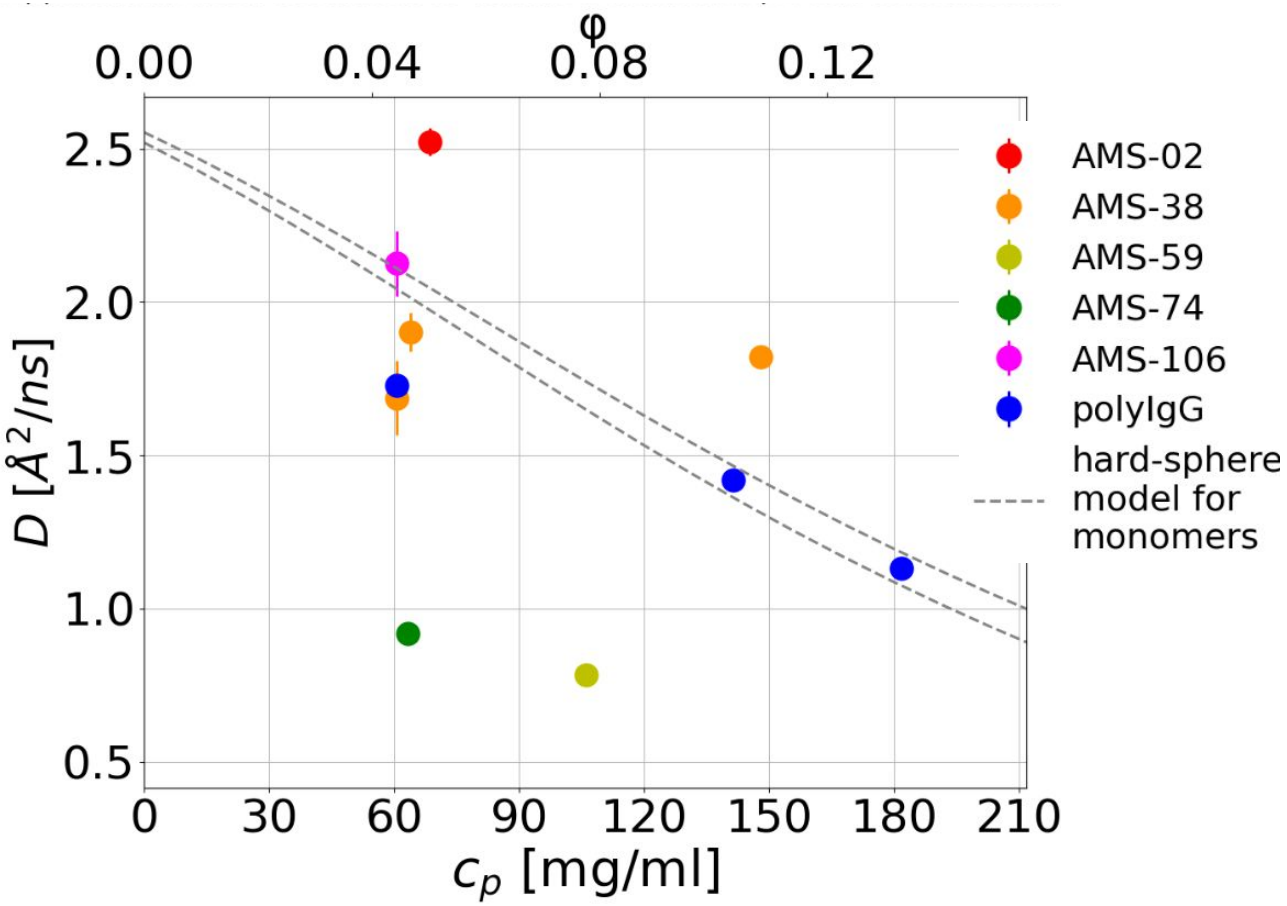

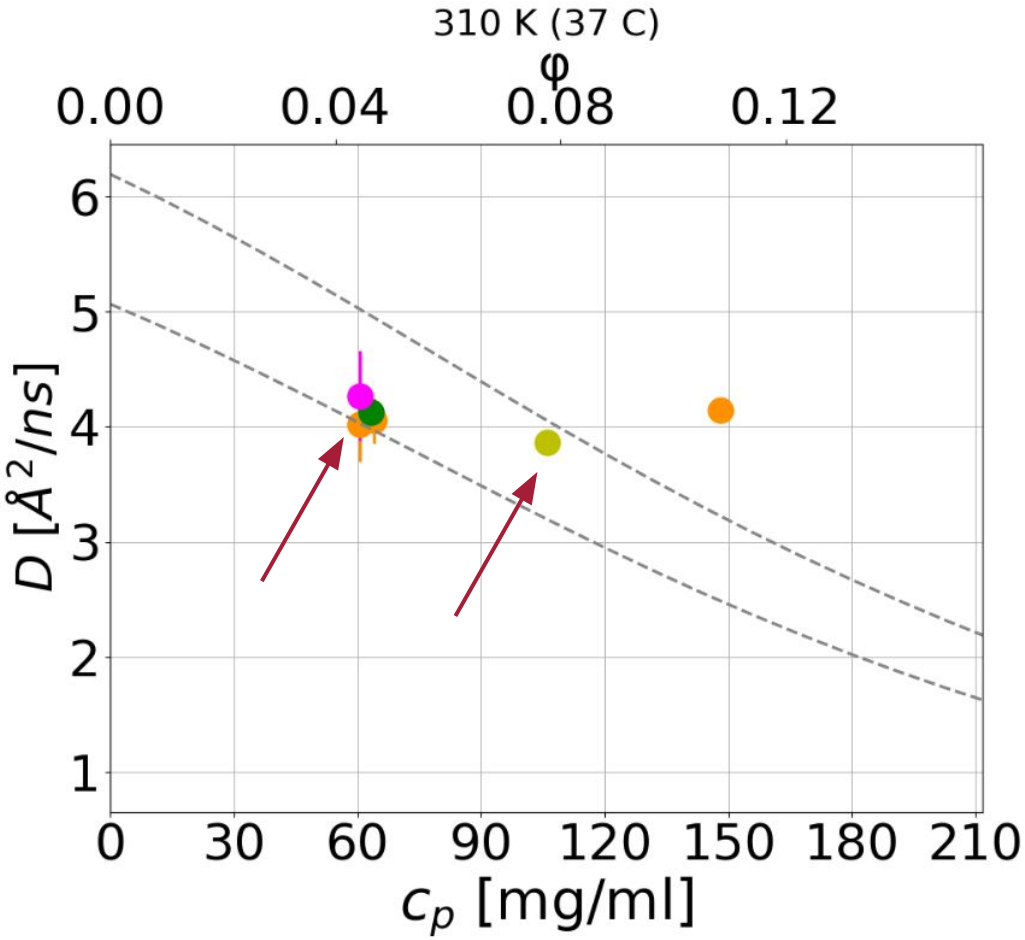

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5. Conclusions and future developments

Conclusions

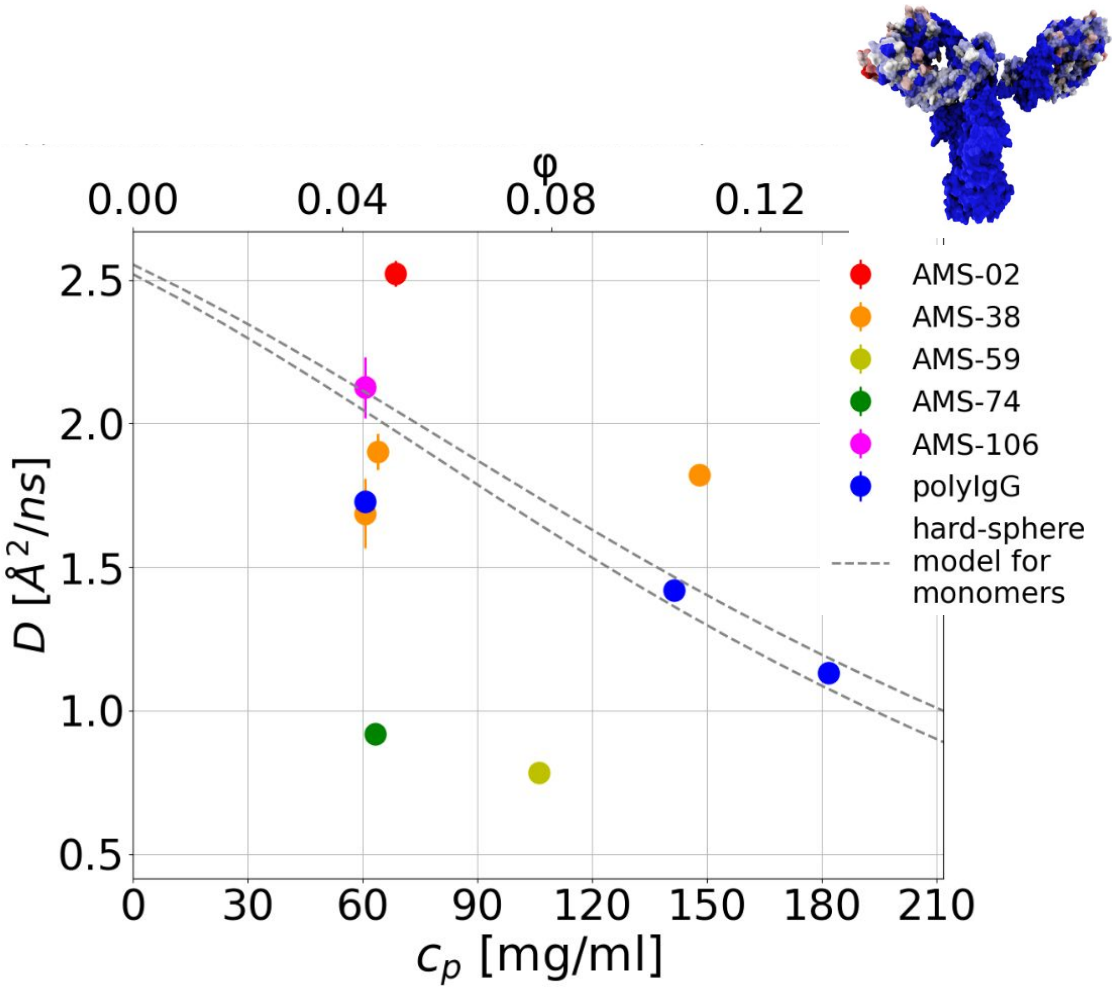
- Different rheology behavior of the mAb variants
- Most of them form clusters at 280K which may dissociate at higher *T*
- MD simulations indicate correlation between viscosity and surface charge and PPI parameters

Future developments

Analysis of data from:

- QENS @BATS (accurate info on internal dynamics)
- QENS @IN16B & SANS @D11, ILL (mAbs + trehalose)

Upcoming SAXS beamtime @ID02, ESRF: studies of mAb concentration, temperature and additives



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