The Effects of High Intensity X-ray Laser Pulses in MHz-XPCS Measurements of Protein Solution Dynamics

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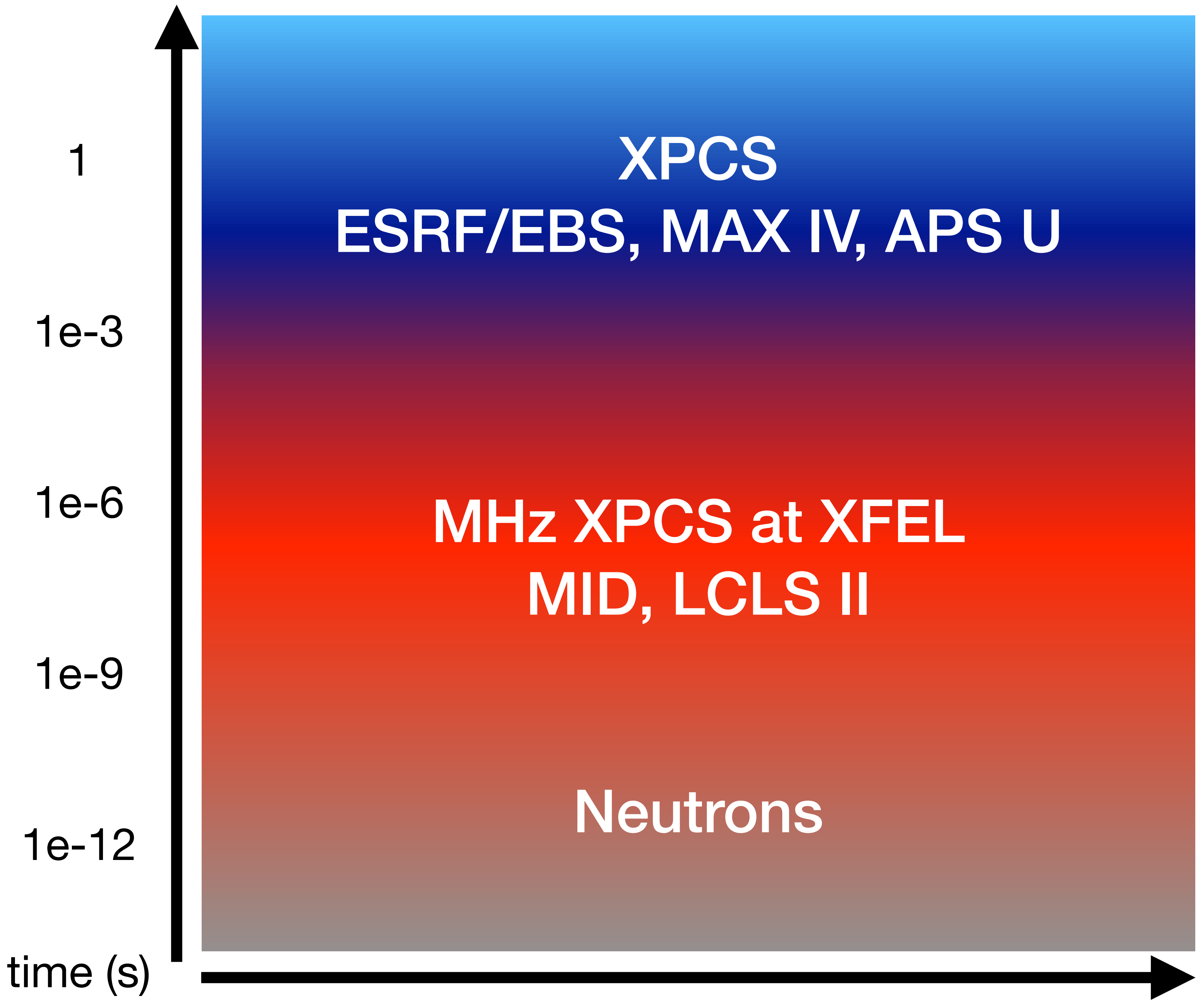
Mario Reiser, Jan 24 2022, XFEL MID Workshop

Time Scales Of Protein Diffusion



XPCS
ESRF/EBS, MAX IV, APS U

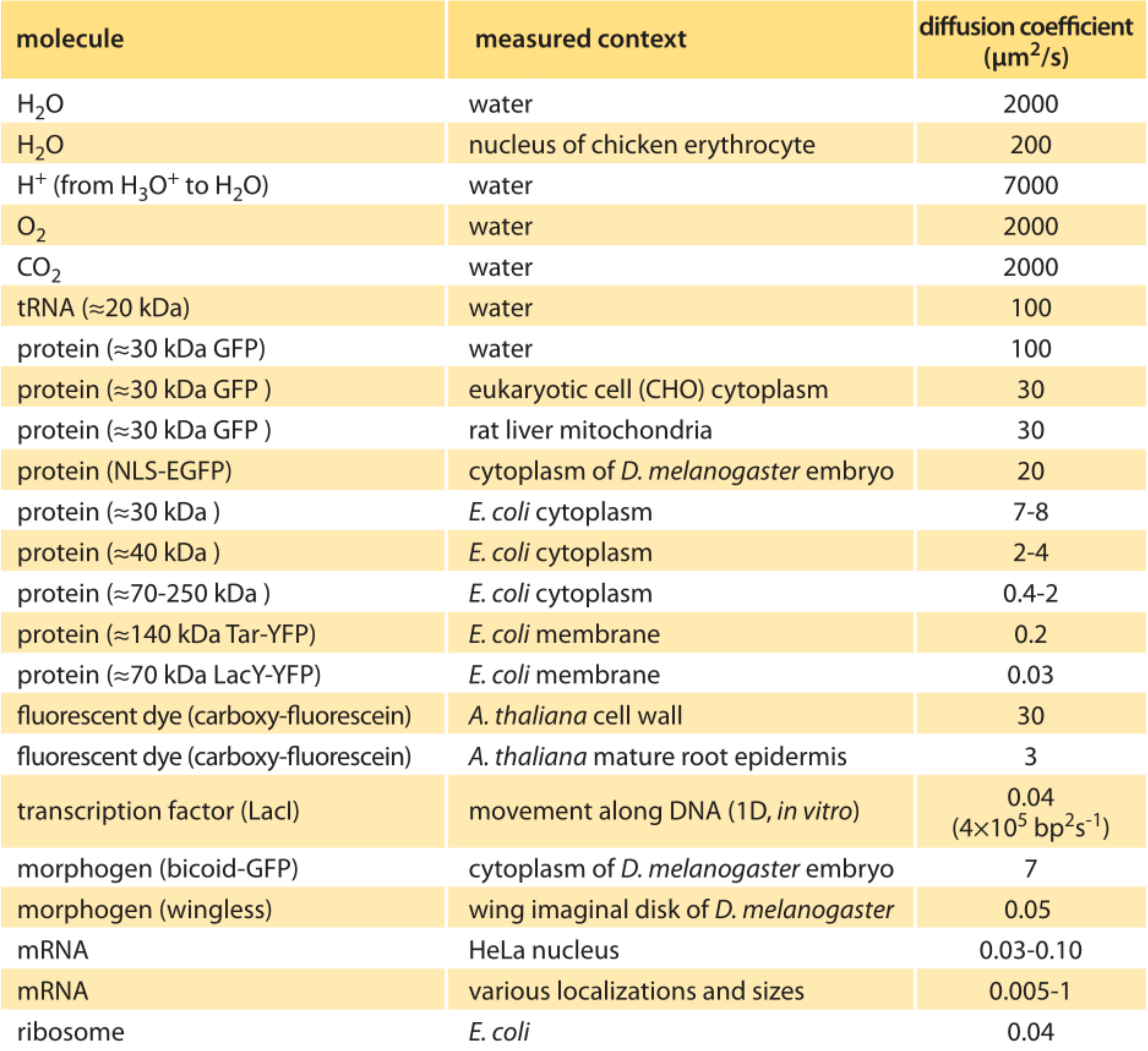
MHz XPCS at XFEL
MID, LCLS II

Neutrons

Length and time scales:

$$\tau_0(q) = 1/D_0 q^2 \Rightarrow \tau_0(1\mathrm{nm}^{-1}) \sim 1\,\mu\mathrm{s}$$

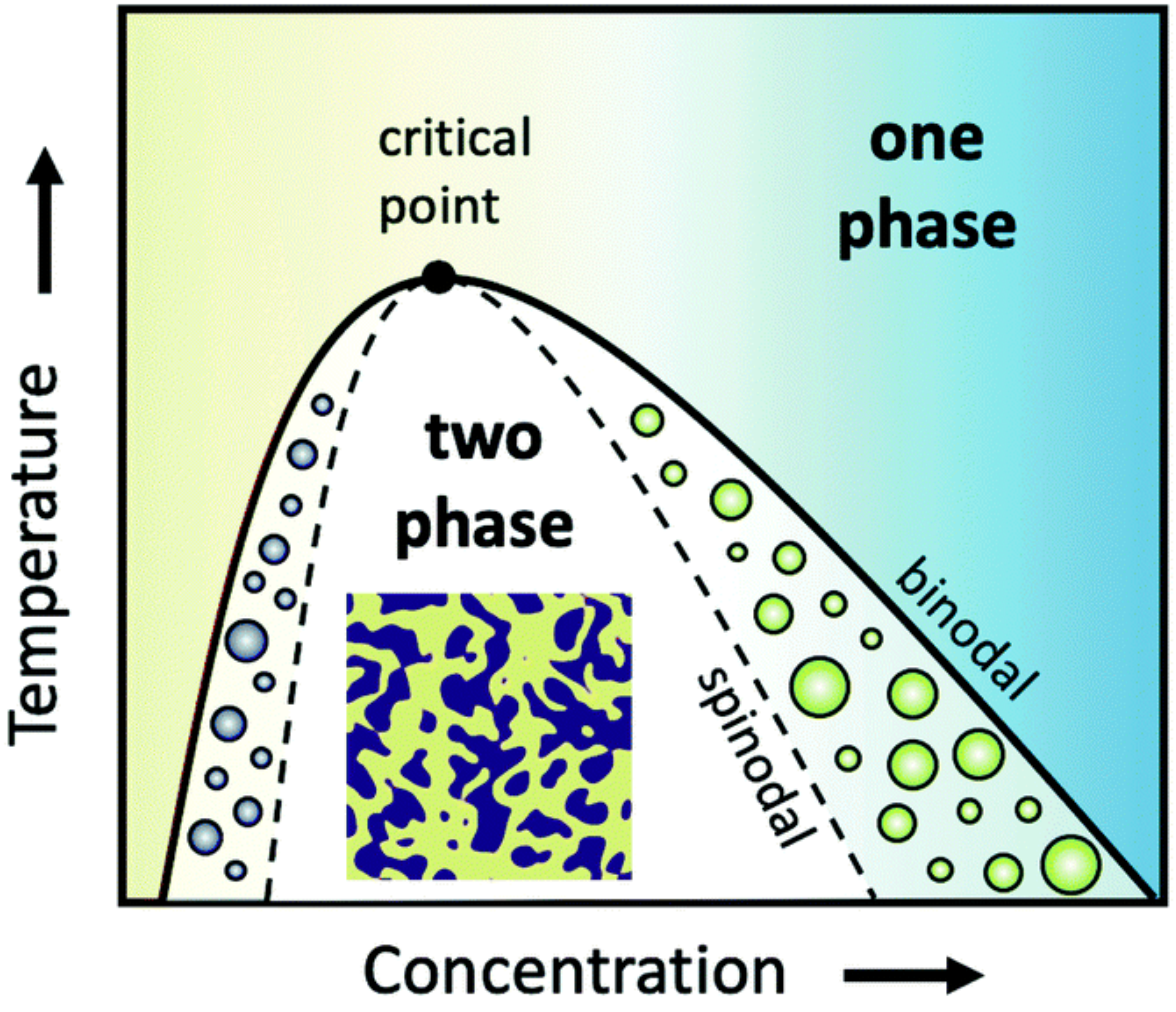
D_0



molecule	measured context	diffusion coefficient ($\mu m^2/s$)
H_2O	water	2000
H_2O	nucleus of chicken erythrocyte	200
H^+ (from H_3O^+ to H_2O)	water	7000
O_2	water	2000
CO_2	water	2000
tRNA (≈20 kDa)	water	100
protein (≈30 kDa GFP)	water	100
protein (≈30 kDa GFP)	eukaryotic cell (CHO) cytoplasm	30
protein (≈30 kDa GFP)	rat liver mitochondria	30
protein (NLS-EGFP)	cytoplasm of *D. melanogaster* embryo	20
protein (≈30 kDa)	*E. coli* cytoplasm	7-8
protein (≈40 kDa)	*E. coli* cytoplasm	2-4
protein (≈70-250 kDa)	*E. coli* cytoplasm	0.4-2
protein (≈140 kDa Tar-YFP)	*E. coli* membrane	0.2
protein (≈70 kDa LacY-YFP)	*E. coli* membrane	0.03
fluorescent dye (carboxy-fluorescein)	*A. thaliana* cell wall	30
fluorescent dye (carboxy-fluorescein)	*A. thaliana* mature root epidermis	3
transcription factor (LacI)	movement along DNA (1D, *in vitro*)	0.04 ($4\times10^5\ bp^2s^{-1}$)
morphogen (bicoid-GFP)	cytoplasm of *D. melanogaster* embryo	7
morphogen (wingless)	wing imaginal disk of *D. melanogaster*	0.05
mRNA	HeLa nucleus	0.03-0.10
mRNA	various localizations and sizes	0.005-1
ribosome	*E. coli*	0.04

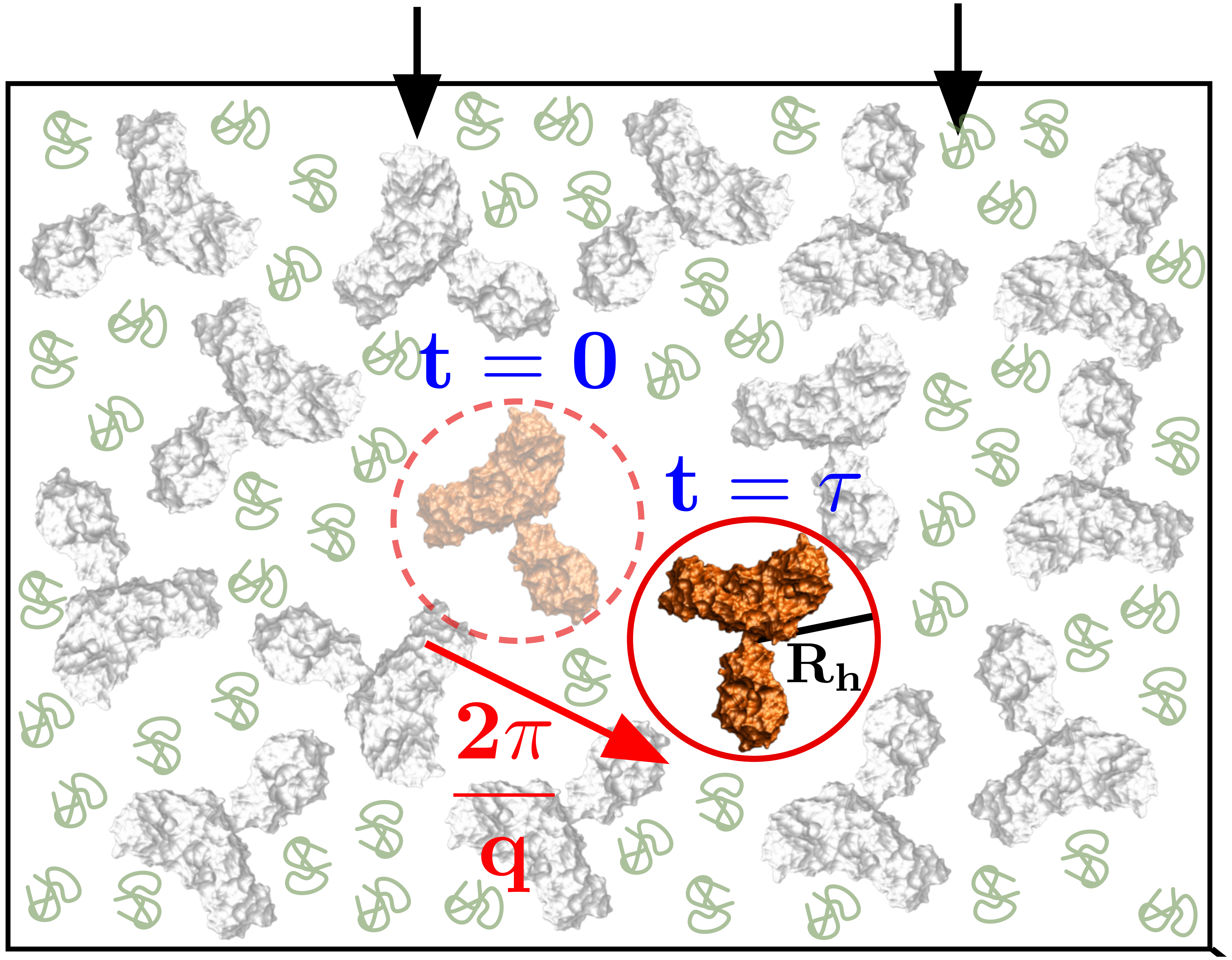
book.bionumbers.org

Antibody Solutions



Immunoglobulin (Ig)
Ig molecule

Polyethylene Glycol
PEG molecule



$T = 25\,^{\circ}\mathrm{C}$ concentration $\approx 250\,\mathrm{mg/mL}$ $R_h = 5.5\,\mathrm{nm}$ $\tau \approx 1 - 10\,\mu s$

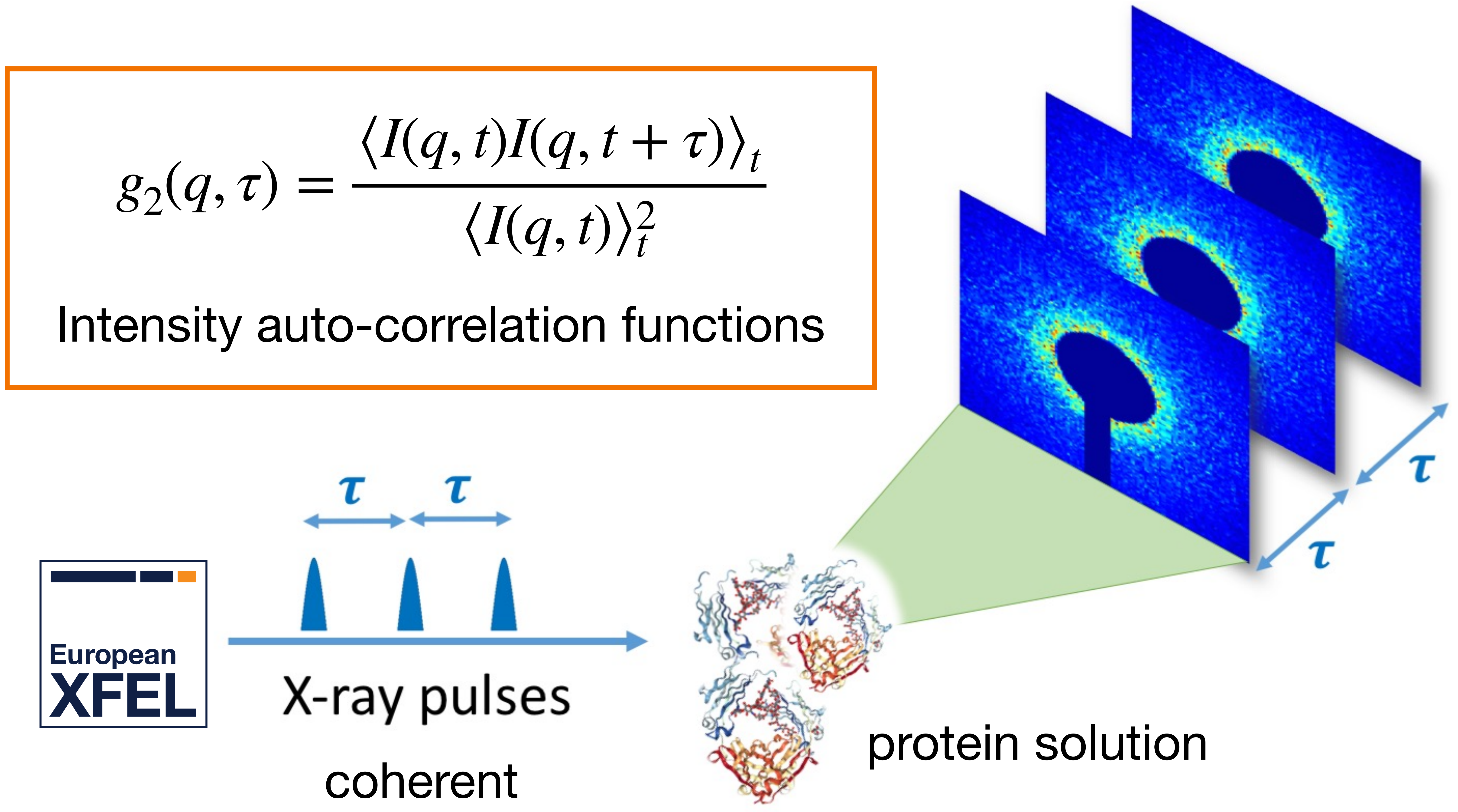
Ragulskaya, et al., *J. Phys. Chem. Lett.* **12** (2021): 7085.
Girelli, et al., et al. *Phys. Rev. Lett.* **126** (2021): 138004.
Begam, et al., *Phys. Rev. Lett.* **126** (2021): 098001.

MHz X-Ray Photon Correlation Spectroscopy

(sequential) XPCS

$$g_2(q,\tau) = \frac{\langle I(q,t)I(q,t+\tau)\rangle_t}{\langle I(q,t)\rangle_t^2}$$

Intensity auto-correlation functions



X-ray pulses

coherent

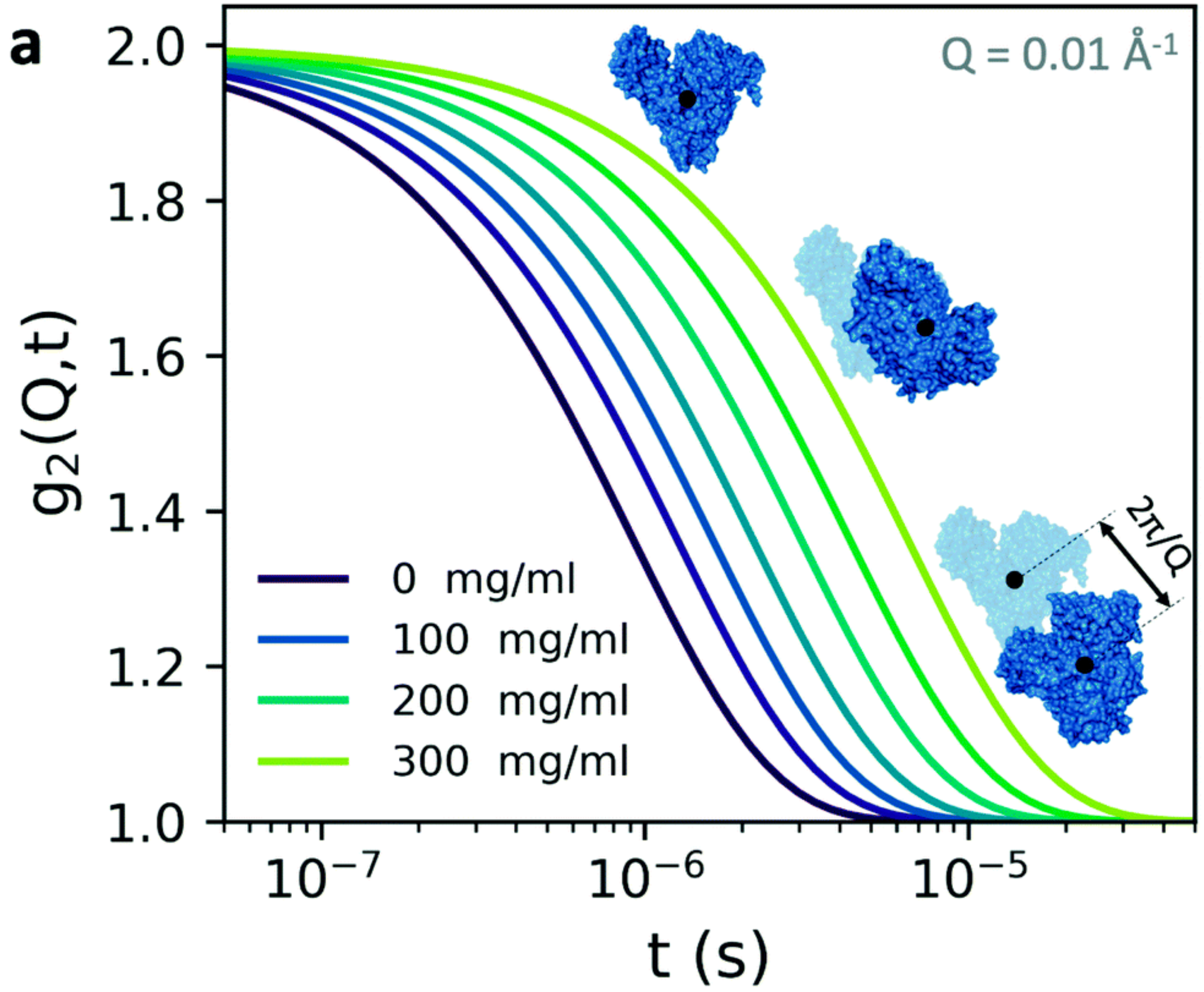
protein solution

XFEL Freq. (MHz)	temporal resolution τ (ns)
4.5	220
2.2	440
1.1	880
…	…

Measuring Protein Dynamics with MHz XPCS

Correlation Functions

a



$$g_2(Q,t) = 1 + e^{-2D(Q)Q^2 t}$$

interactions

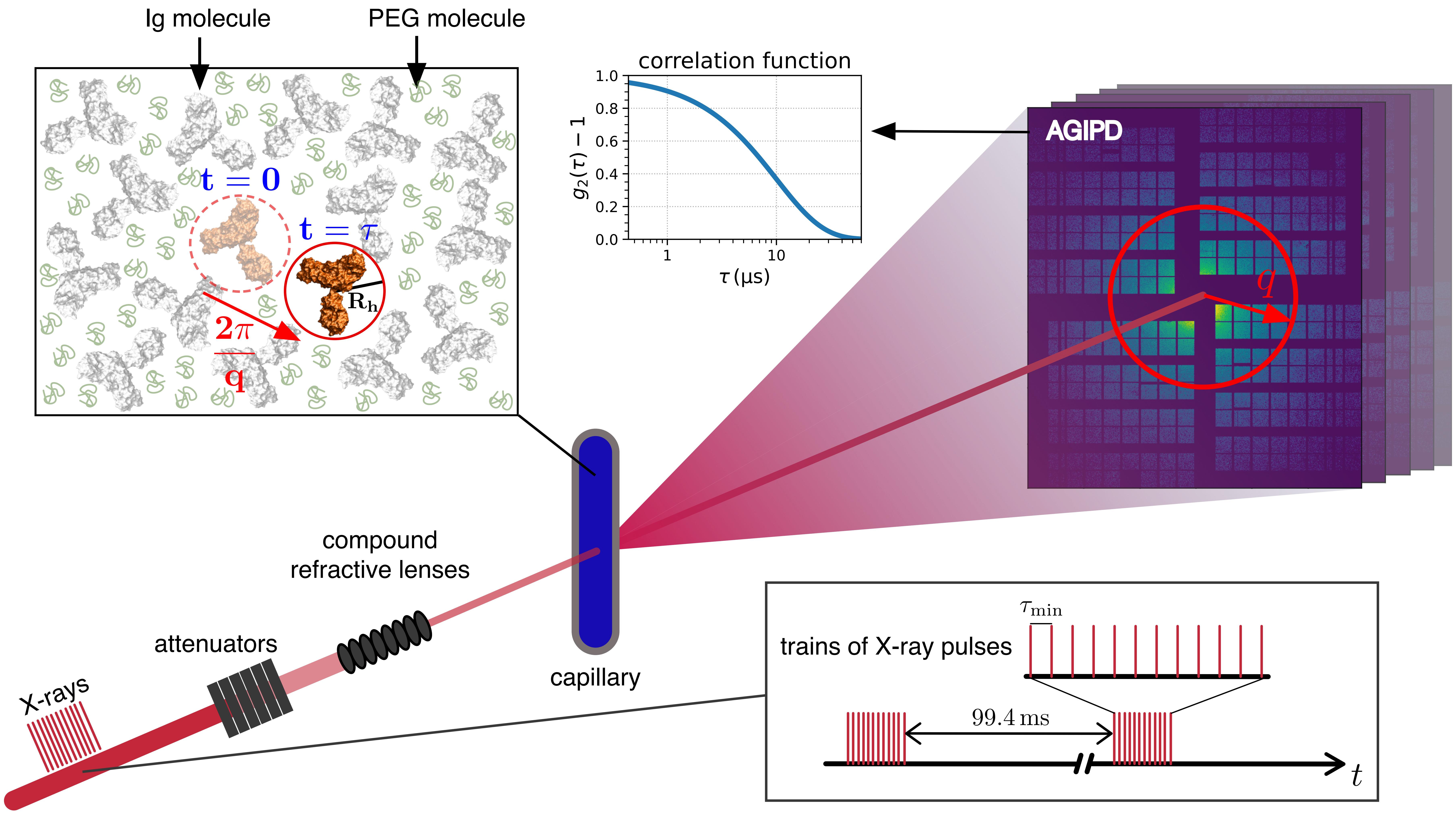
$$D(q) = \Gamma(q)/q^2 = D_0 \frac{H(q)}{S(q)}$$

Perakis and Gutt, *Phys. Chem. Chem. Phys.* **22** (2020): 19443.

SAXS MHz-XPCS Setup at MID

Setup Parameters

beam size	10µm x 10µm
attenuation	> 99 %
flux (attenuated)	1e9 photons / pulse
signal	~0.01 to 0.1 photons / pixel
sample detector distance	8m
photon energy	9keV
beam mode	pink beam
data volume	800TB
pulses per train	up to 200



Ig molecule

PEG molecule

correlation function

compound refractive lenses

attenuators

X-rays

capillary

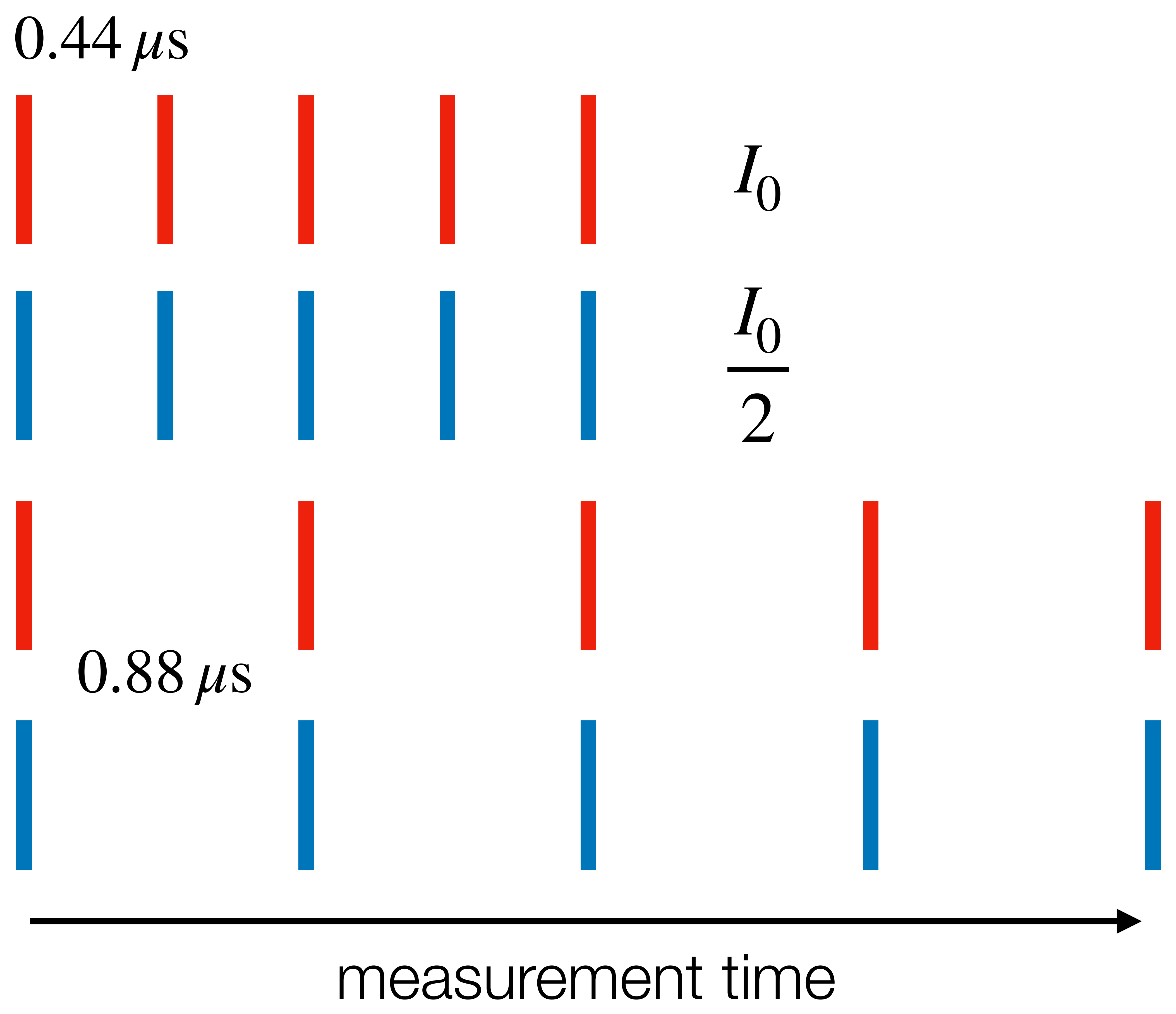
trains of X-ray pulses

Madsen, et al., *J Synchrotron Radiat* 28 (2021): 637.

Dose and Dose Rate

MHz-XPCS with a pulsed source

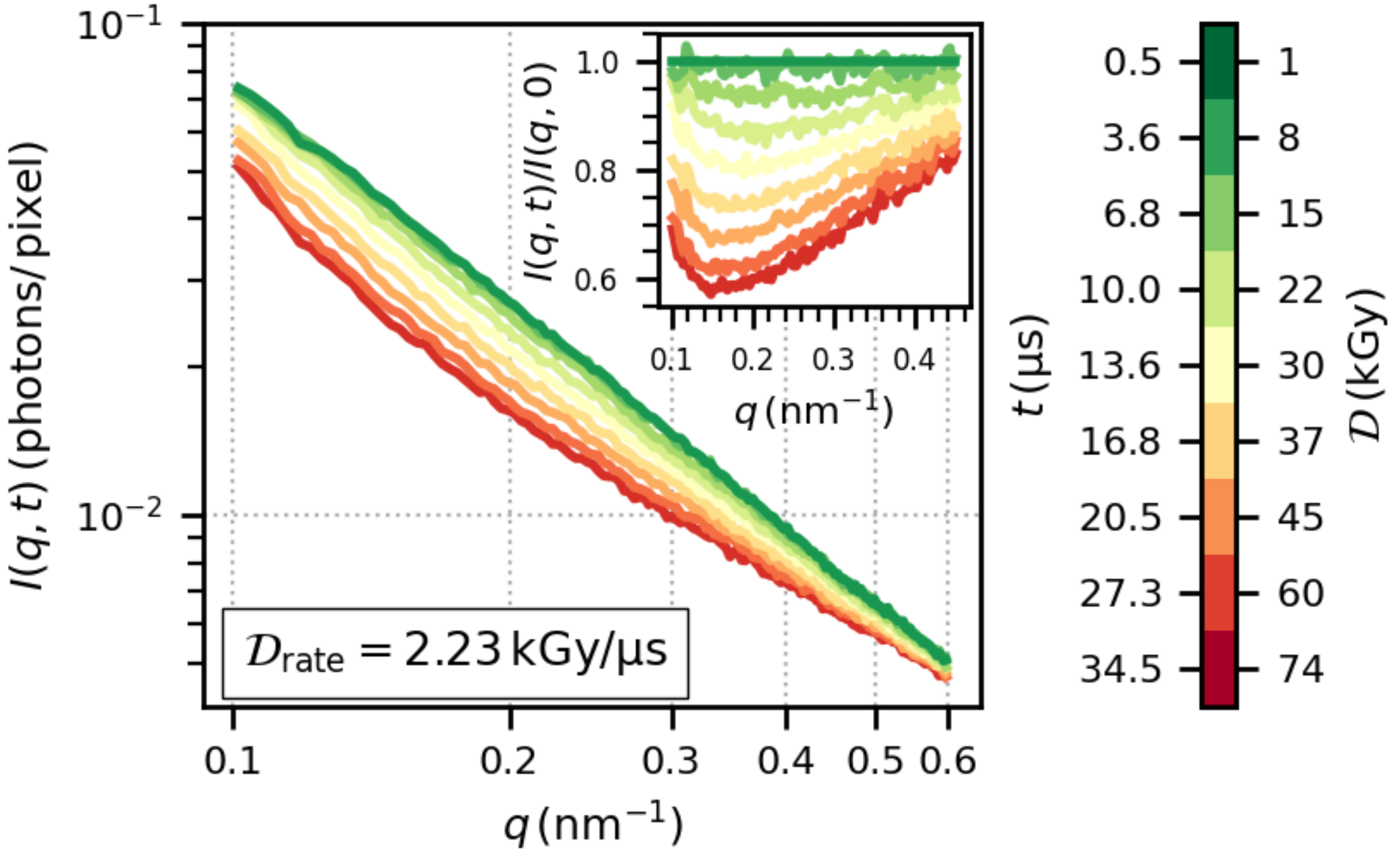
Dose: 1kGy - 1kJ/kg
Dose rate: 1kGyMHz - 1kGy/μs

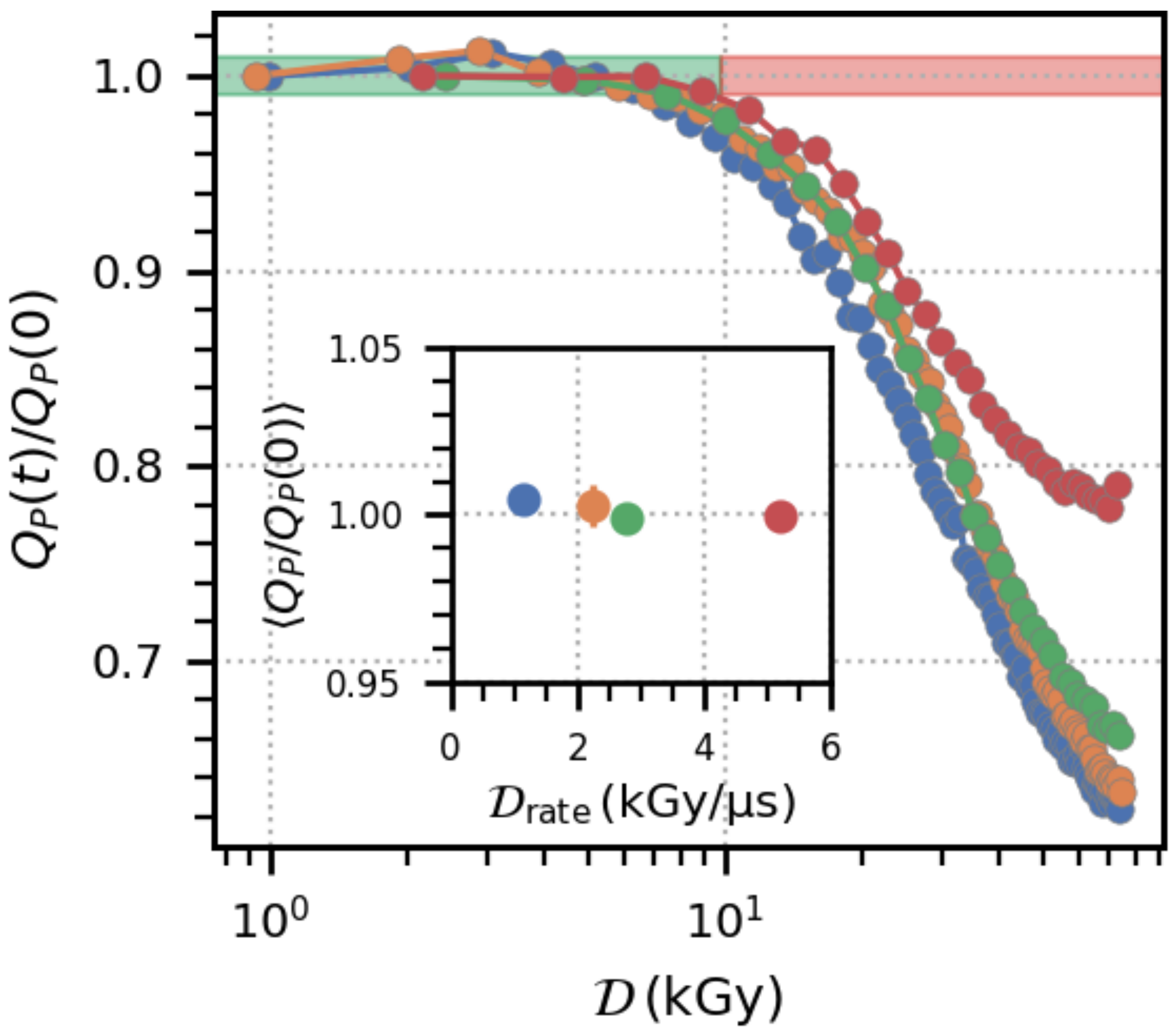


	dose $\mathscr{D}$	**dose rate** $\mathscr{D}_{\text{rate}}$
$2.2\,\text{MHz}$	$10\,\text{kGy}$	$4.5\,\text{kGy}/\mu s$
$2.2\,\text{MHz}$	$5\,\text{kGy}$	$2.25\,\text{kGy}/\mu s$
$1.1\,\text{MHz}$	$10\,\text{kGy}$	$2.25\,\text{kGy}/\mu s$
$1.1\,\text{MHz}$	$5\,\text{kGy}$	$1.23\,\text{kGy}/\mu s$

Structural Changes

Azimuthally Integrated Intensity





Porod invariant $Q_P(t) = \int_{q_{\min}}^{q_{\max}} q^2 I(q,t)\, dq$

Time Resolved Dynamics

Two-Time Correlation Functions

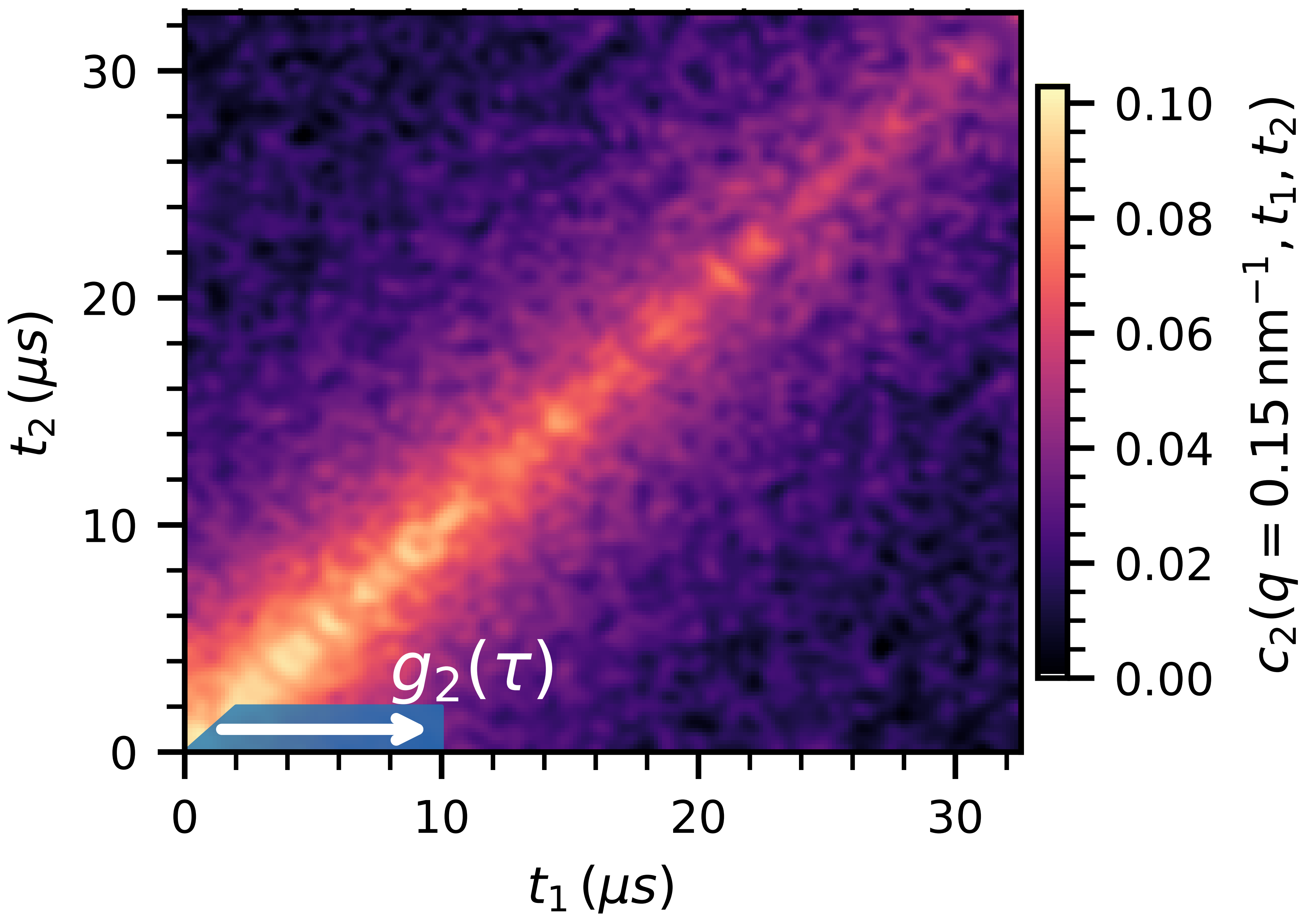
$$g_2(q, \tau) = \frac{\langle I(q,t) I(q,t+\tau)\rangle_t}{\langle I(q,t)\rangle_t^2}$$

Intensity auto-correlation functions

$$c_2(q, t_1, t_2) = \frac{\langle I(q,t_1) I(q,t_2)\rangle}{\langle I(q,t_1)\rangle \langle I(q,t_2)\rangle}$$

two-time correlation function

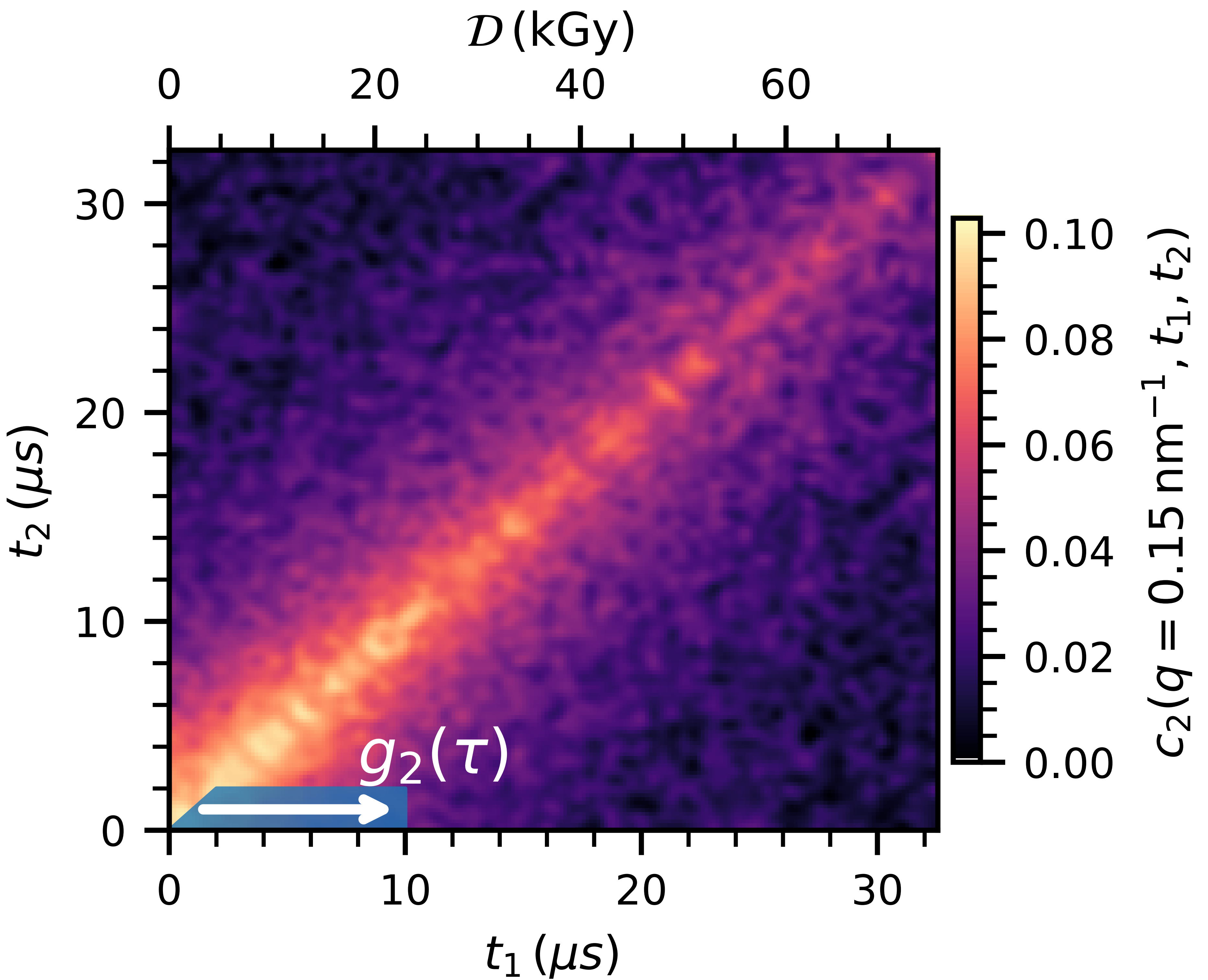
average over 5000 trains

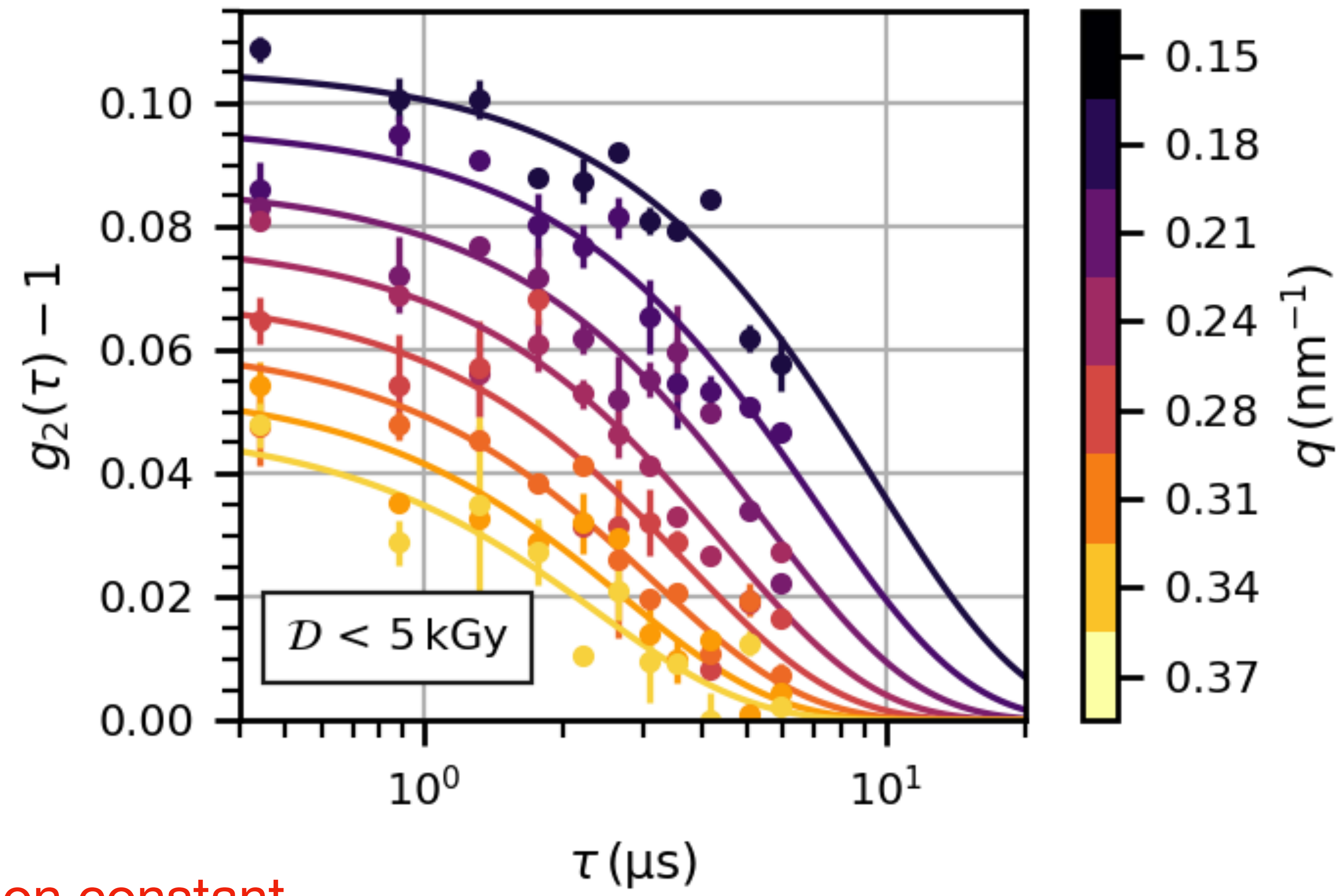


Dallari, Reiser, et al., *Appl. Sci.* 11 (2021): 8037.

Correlation Functions

Parameter Estimation





average diffusion constant

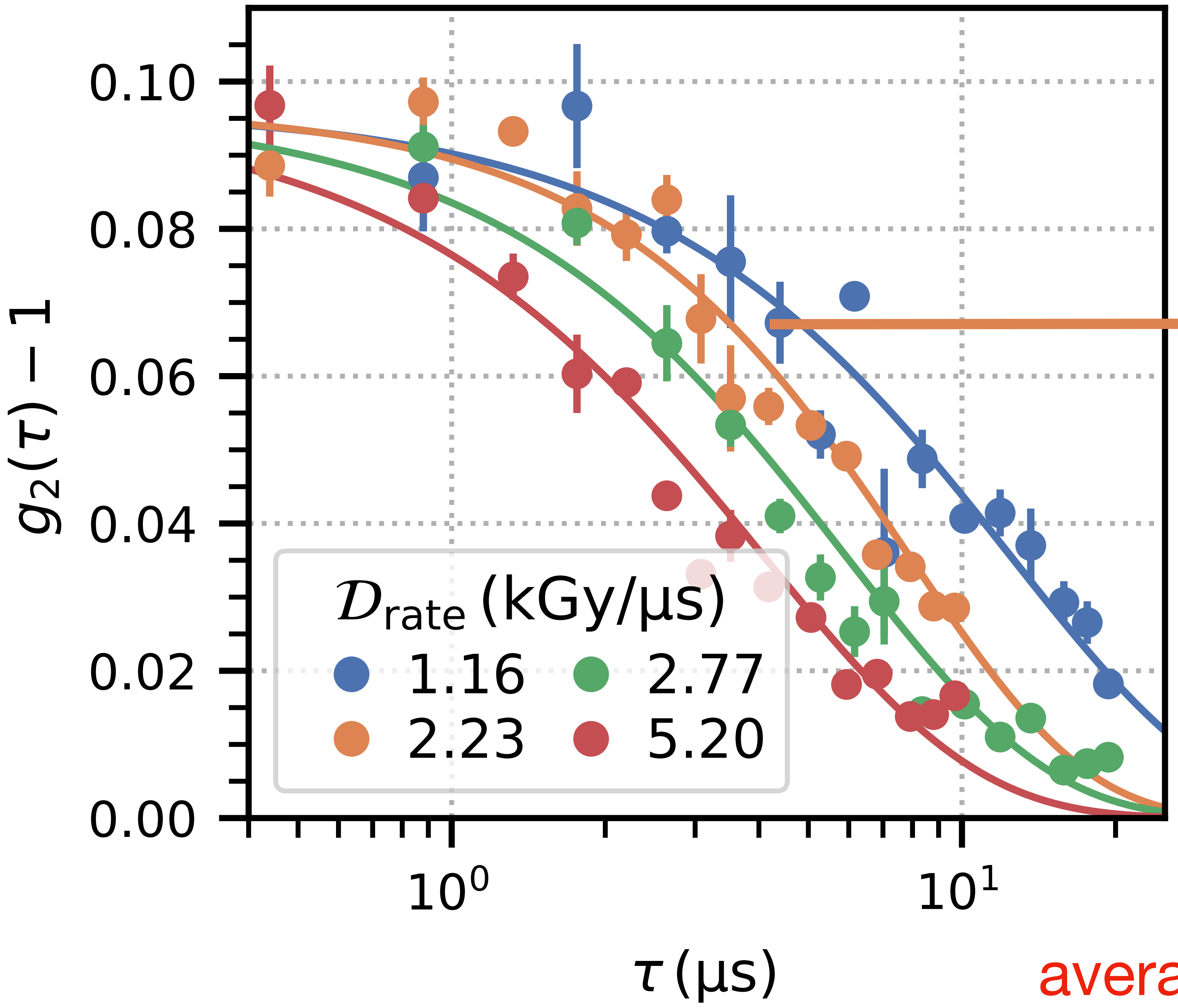
$$g_2(q,\tau) - 1 = \beta(q)\, e^{-2(D_0\, q^2 \tau)^{\alpha}}$$

Kohlrausch-Williams-Watts exponent (KWW)

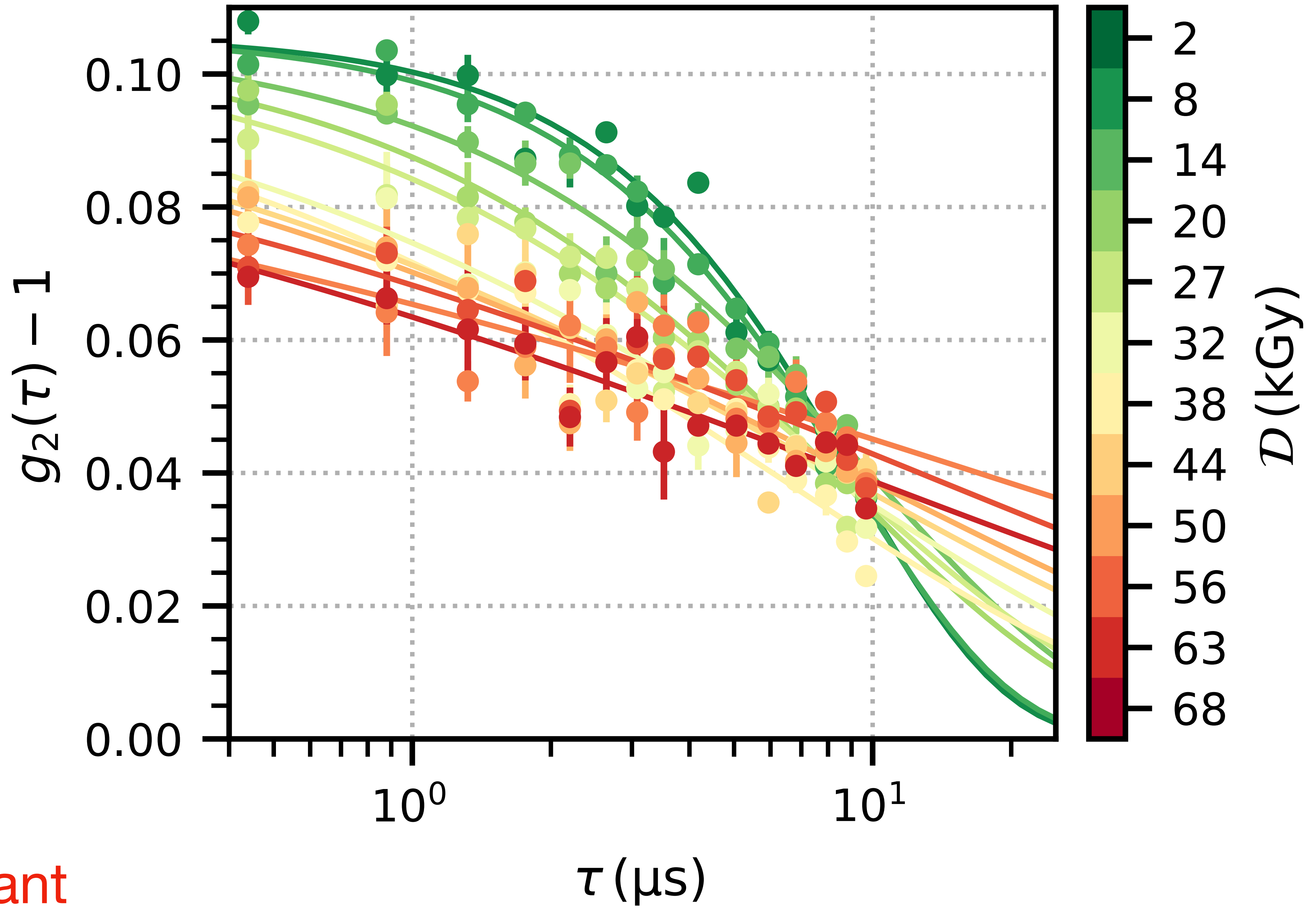
Understanding Non-Stationary Dynamics

Correlation Functions of Different Doses and Dose Rates

Dose Rate



Dose



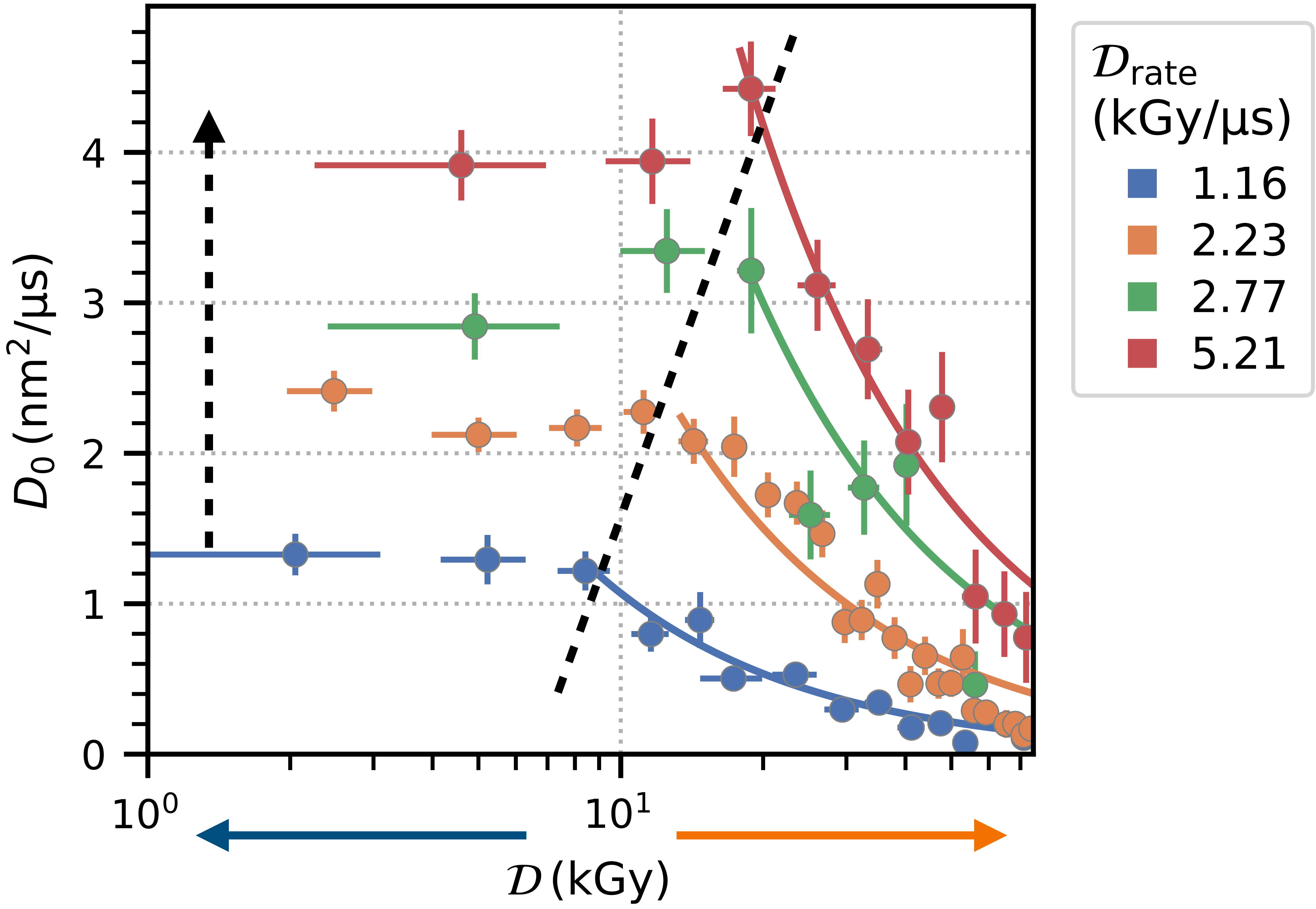
average diffusion constant

$$g_2(q, \tau) - 1 = \beta(q)\, e^{-2(D_0\, q^2 \tau)^\alpha}$$

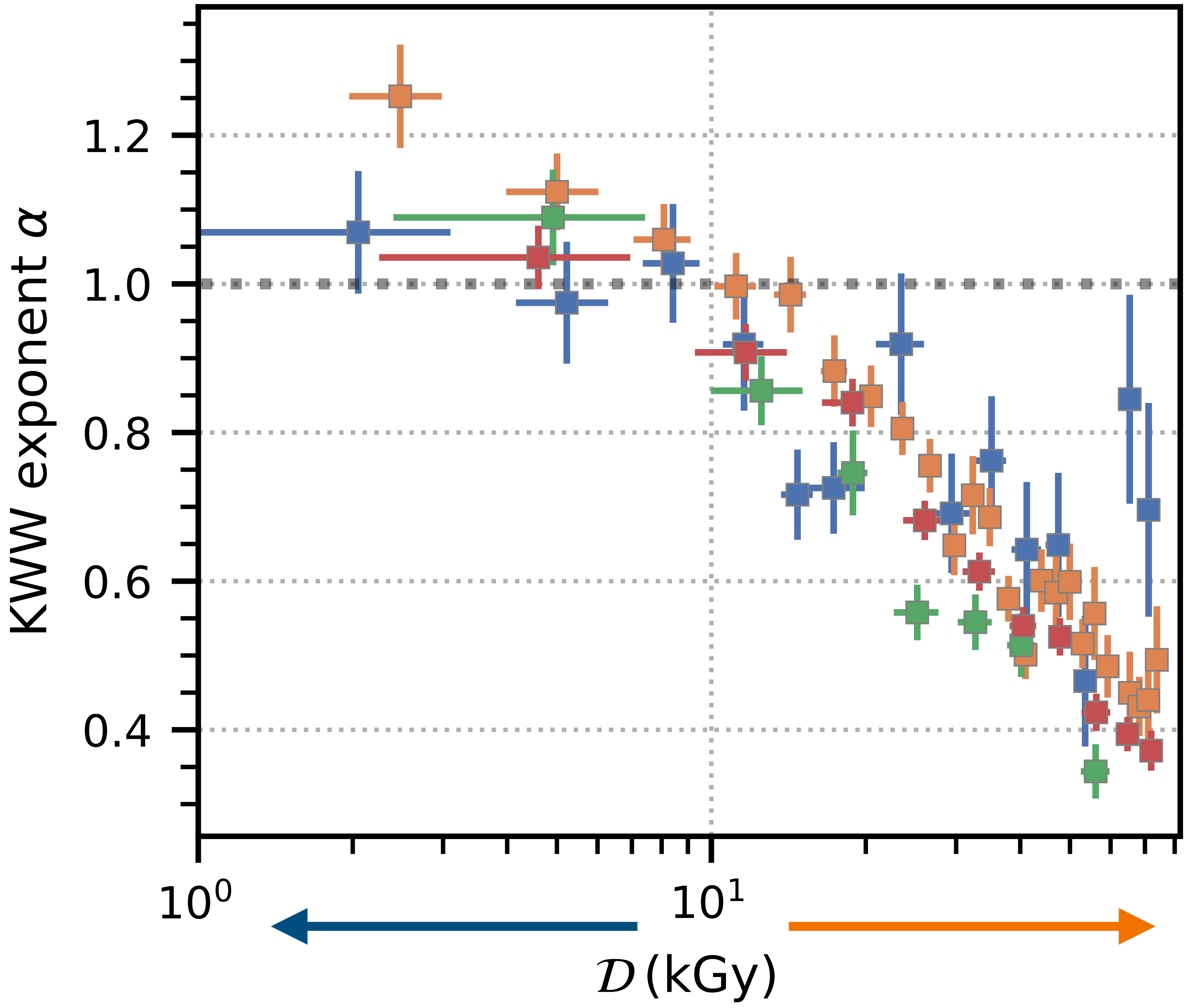
Kohlrausch-Williams-Watts exponent (KWW)

Dynamic Parameters

Diffusion Coefficient D_0



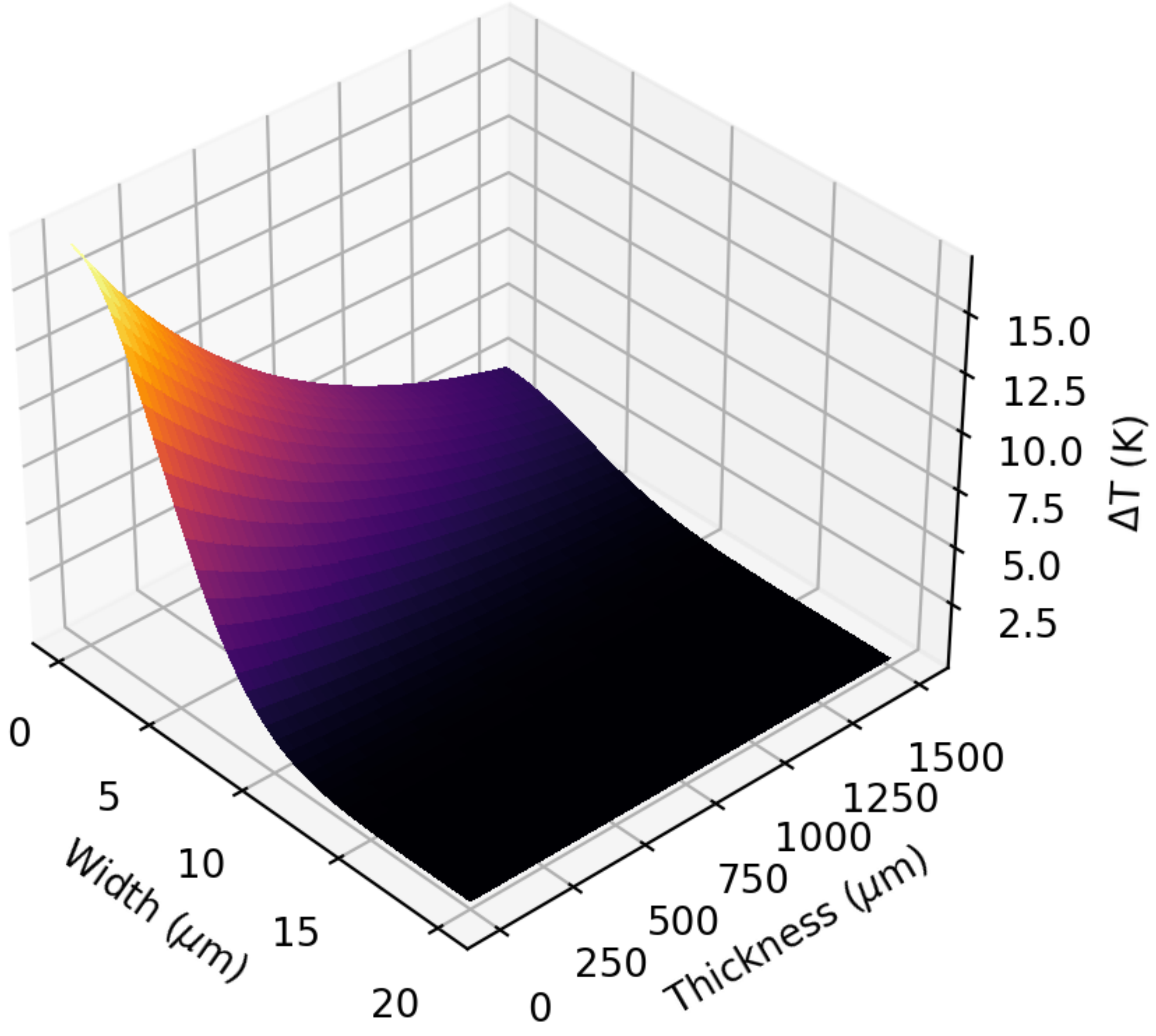
KWW exponent α



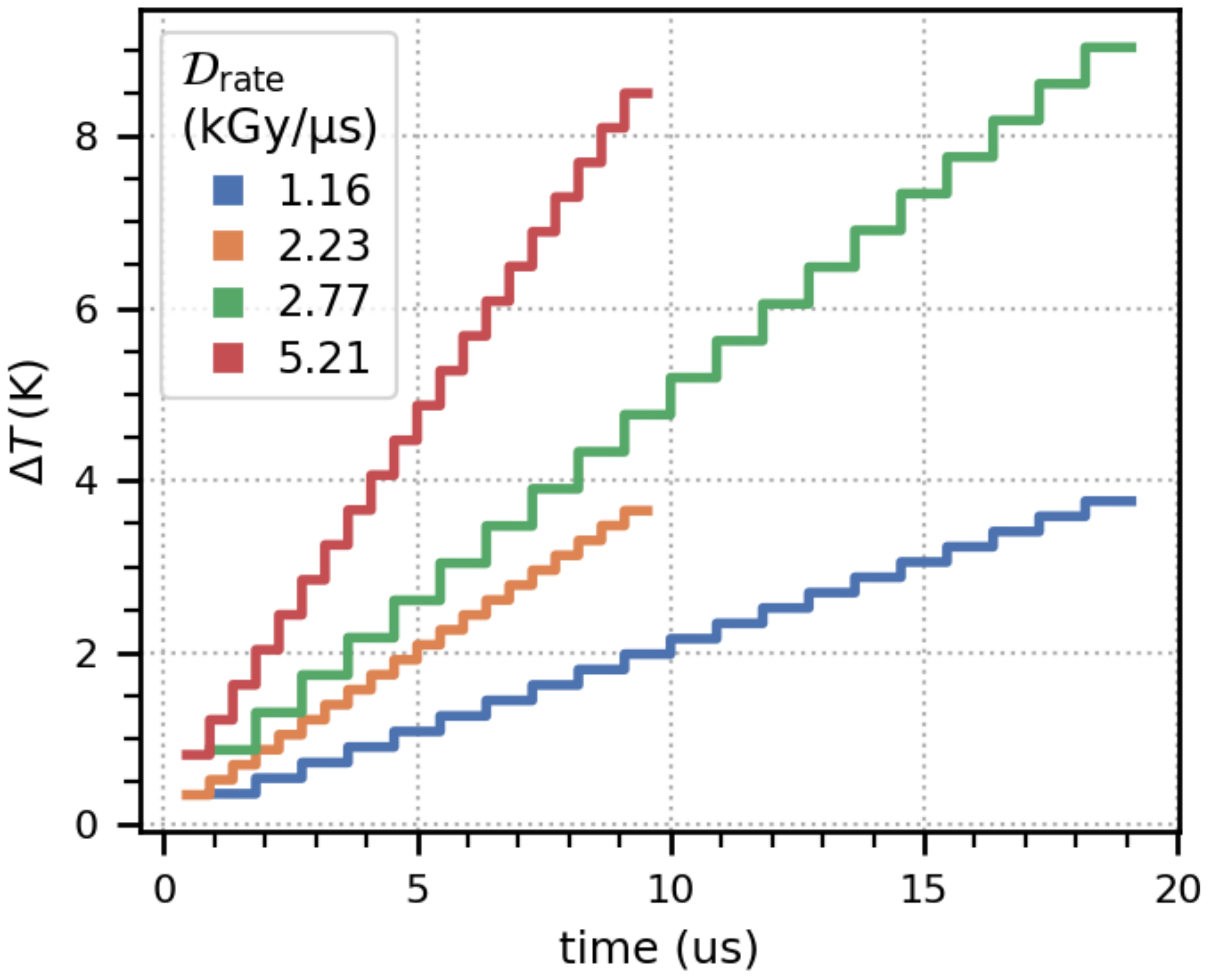
- **aggregation:** decreasing D_0 and increasing heterogeneity ($\alpha < 1$).
- **dose rate dependence:** D_0 increases with dose rate simple exponential behavior ($\alpha \approx 1$).

The Effect of X-Ray Induced Heating

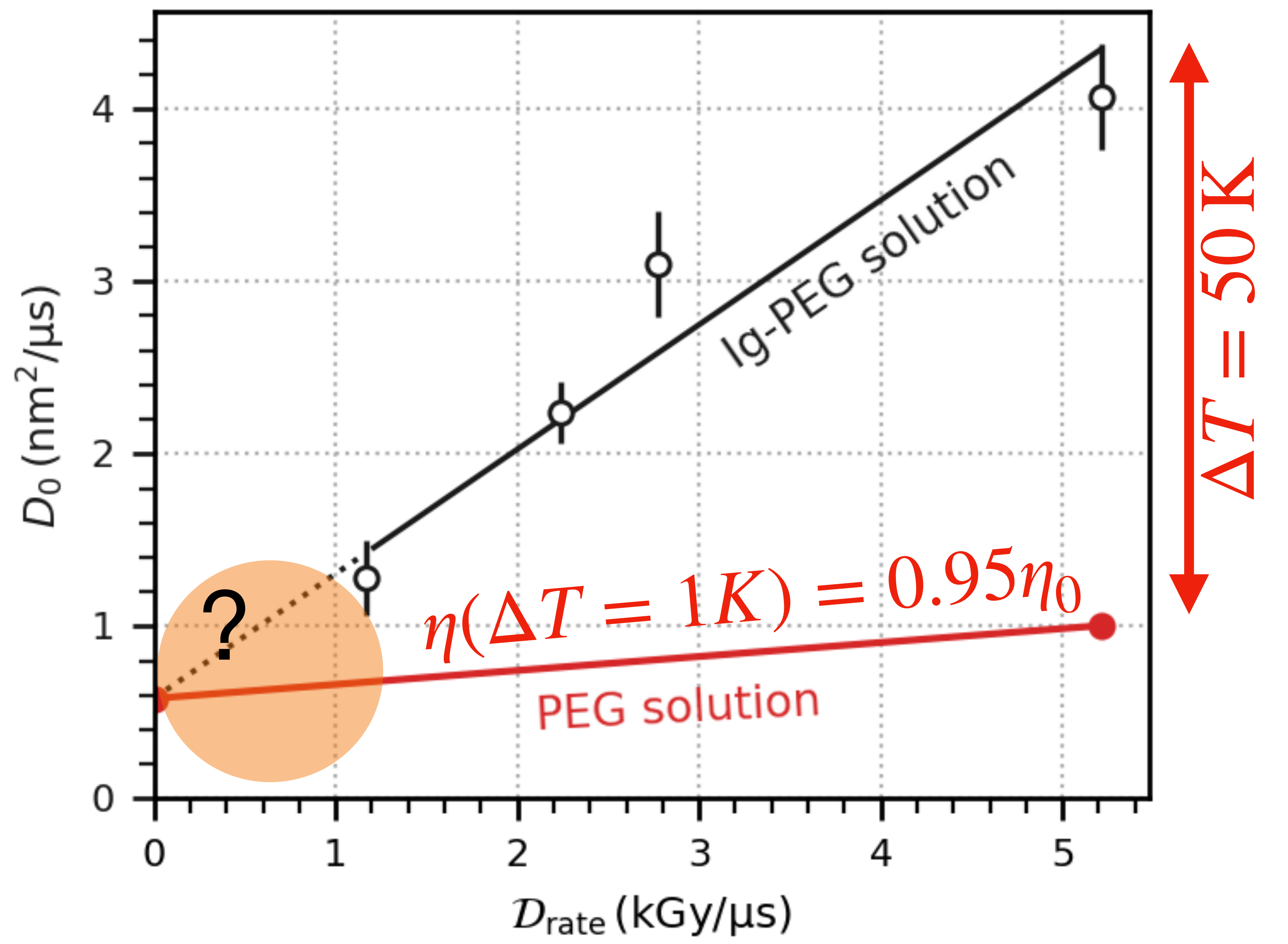
temperature profile after 20 pulses



average temperature increase



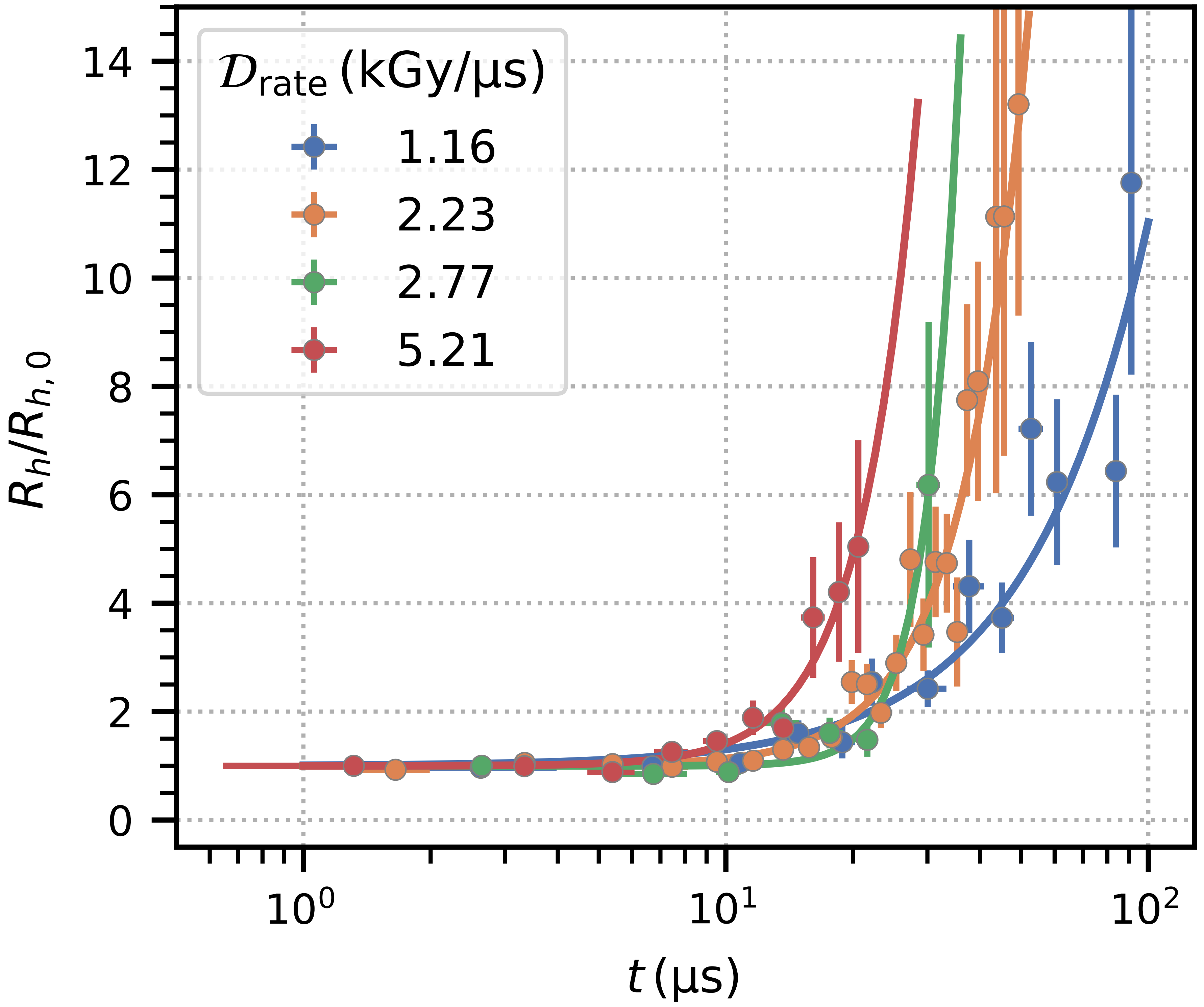
dose rate dependence of D_0



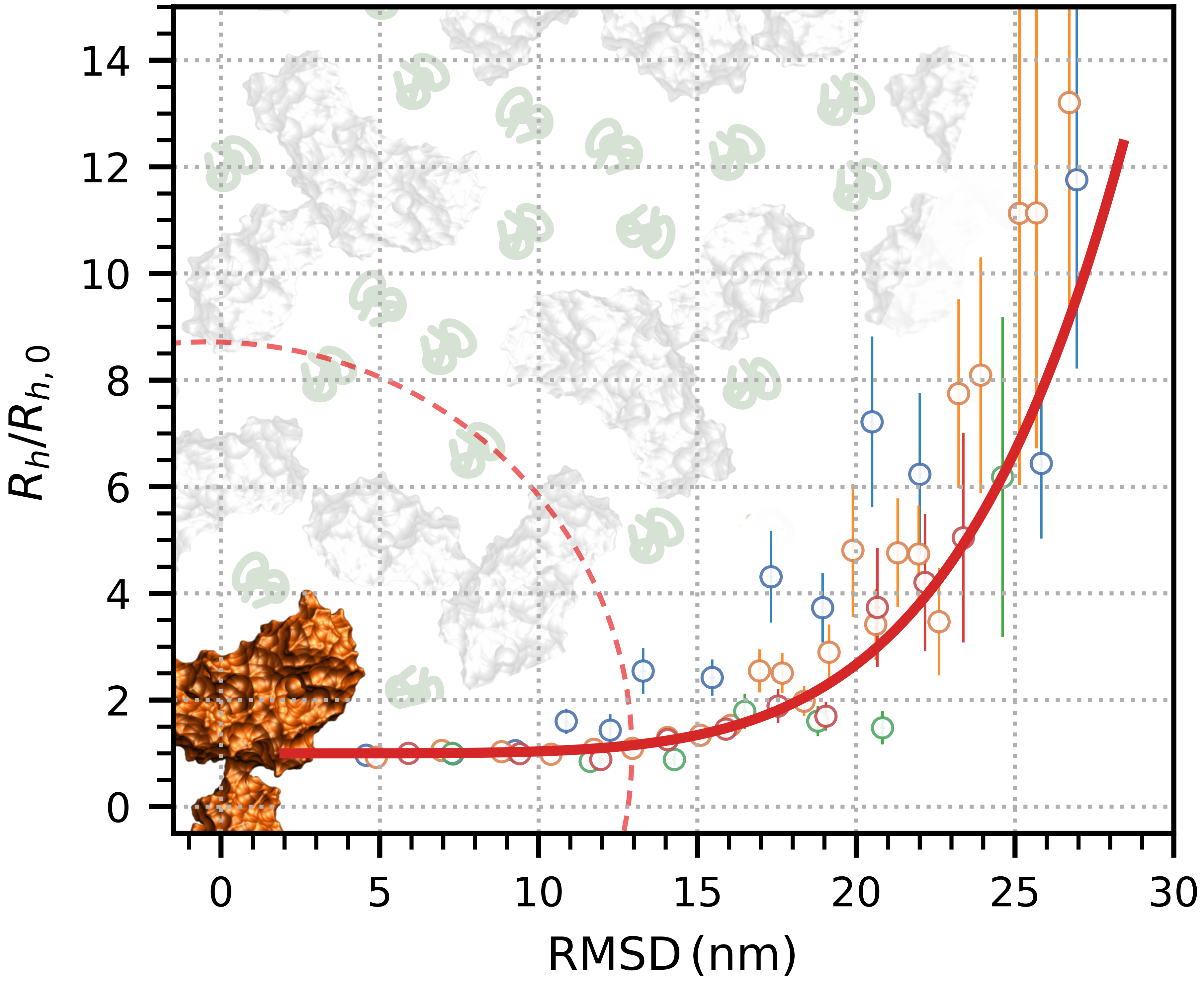
$$D_0 = \frac{k_B T}{6\pi\eta(T)R_h}$$

Lehmkühler, et al. *PNAS* 117 (2020): 24110.
Dallari et al. *IUCrJ* 8 (2021)

Correlation Before Aggregation



$$R_h = \frac{k_B T}{6\pi\eta D_0}$$



number of collisions before aggregation

$$N = \frac{\mathrm{RMSD}^2}{\text{mean free path}^2} \approx 15$$

MHz XPCS With Antibody Solutions

First Step: The Effect of Dose and Dose Rate

- We demonstrated the feasibility of MHz-XPCS with IgG-PEG solutions.
- Distinguish between X-ray induced aggregation and speed-up.
- Dose rate dependence implies X-ray induced dynamics beyond heating.

- MHz-XPCS at XFEL would benefit from higher degree of longitudinal coherence (monochromator, seeding), faster repetition rates (4.5 MHz), longer trains (more pulses), and smaller pixels.

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Thank You.

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