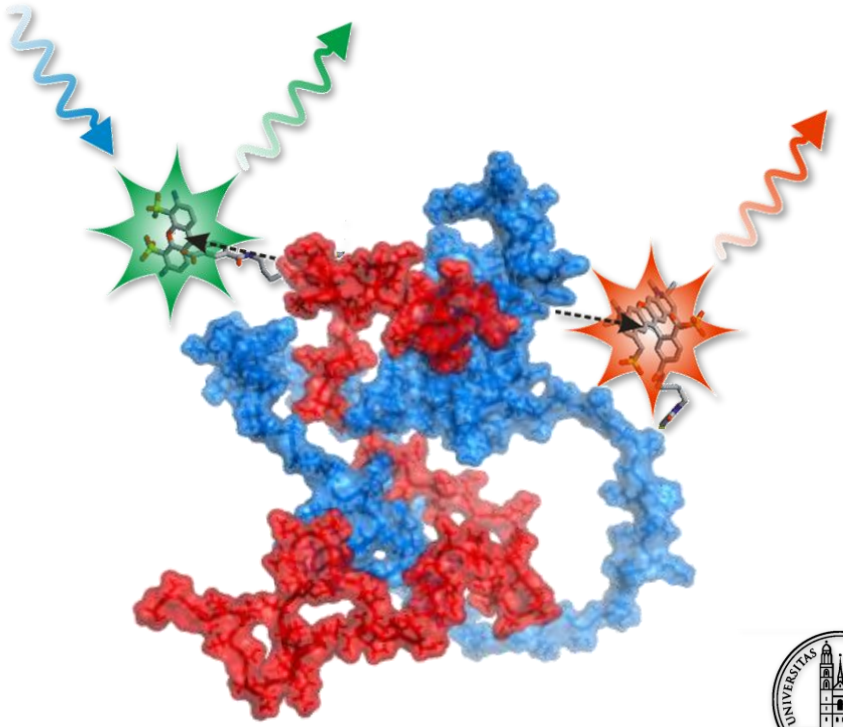
Probing the rapid chain dynamics of disordered proteins and nucleic acids with single-molecule spectroscopy

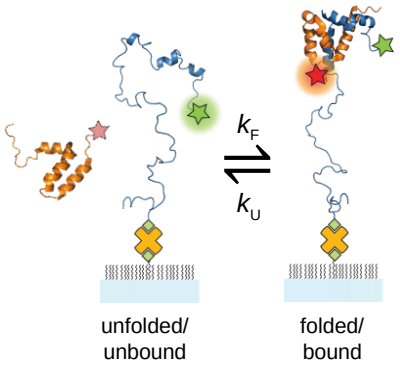
Ben Schuler



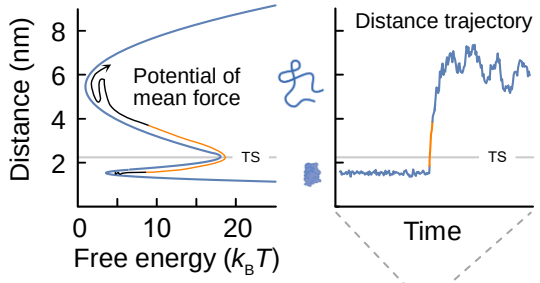
University of Zurich[UZH]

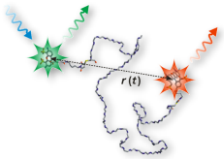
Dynamics from single-molecule FRET

- Kinetics of interconversion between states
- Transition path times
- Unfolded-state chain dynamics



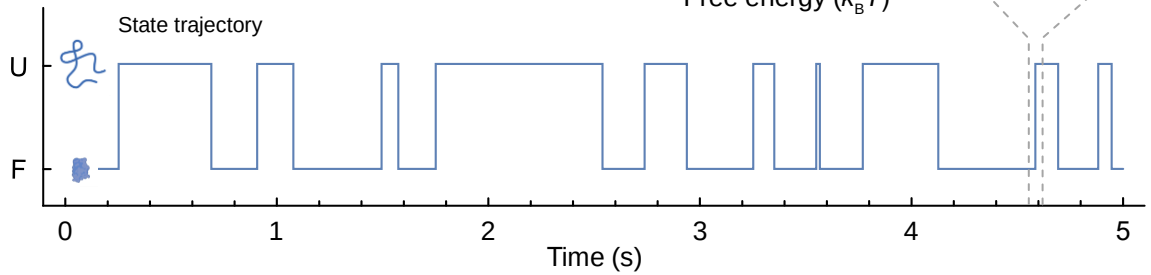
Zosel *et al. Nat Commun* 2018
Zosel *et al. PNAS* 2020

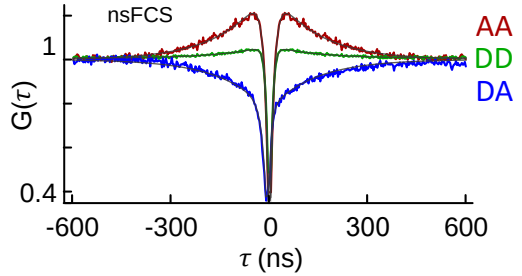


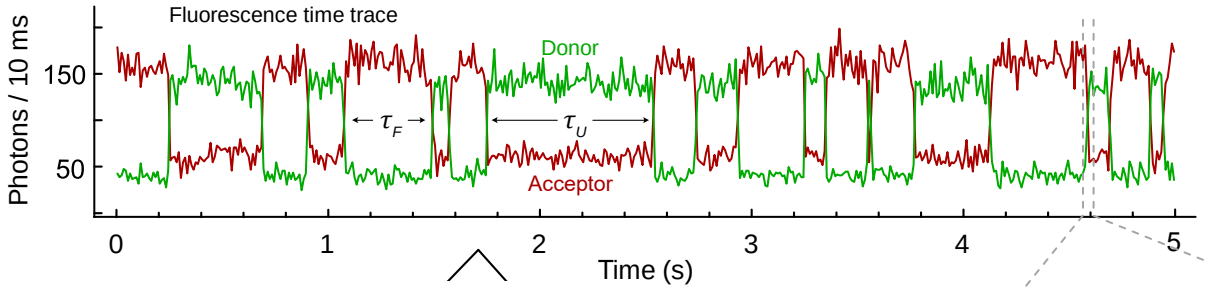


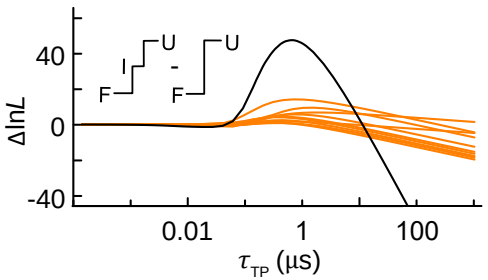
Unfolded-state
chain dynamics

Nettels *et al. PNAS* 2007
Soranno *et al. PNAS* 2012
Nüesch *et al. JACS* 2022



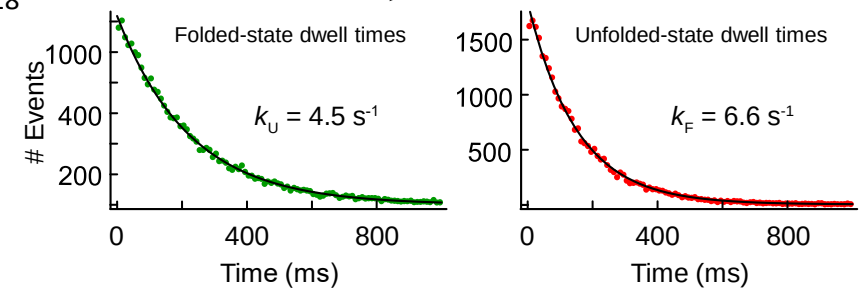




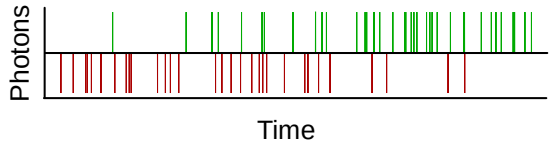


Transition path
time

Dwell time distributions



Single-photon time trace
during a transition

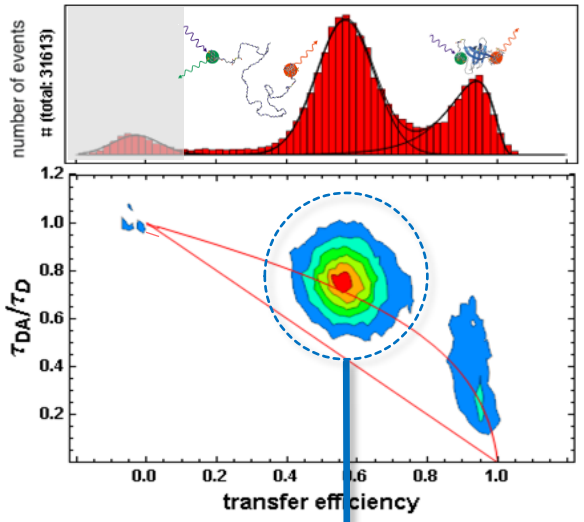


Sturzenegger *et al. Nat Commun* 2018
Grabenhorst ...Tinnefeld *JACS* 2024

Nettels *et al. Nat Phys Rev* 2024

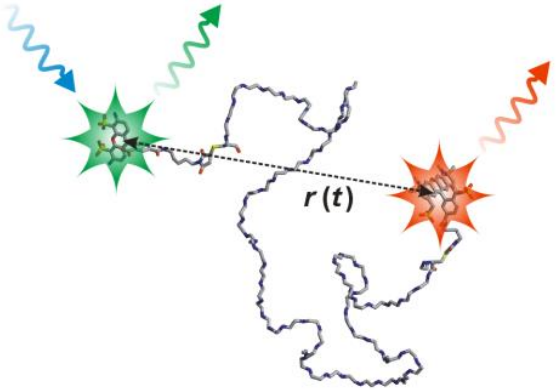
Combining FRET and nsFCS for quantifying chain dynamics

FRET efficiencies + fluorescence lifetimes

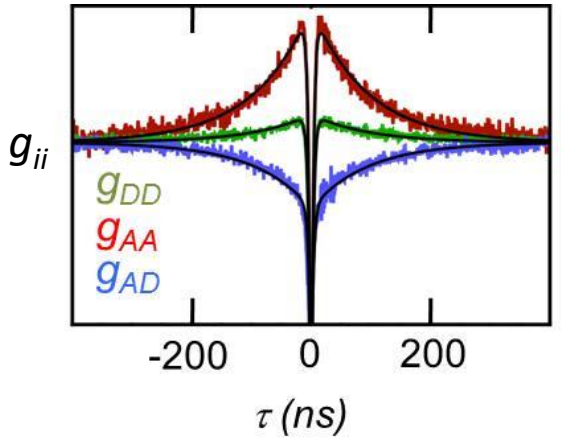


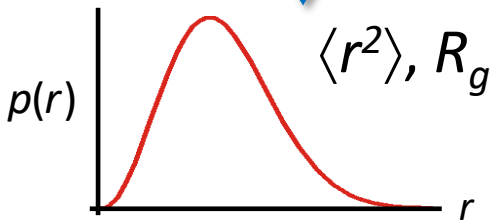
Signal not linearly related to distance

$$E = \frac{R_0^6}{R_0^6 + r^6}$$



Nanosecond fluorescence correlation spectroscopy (nsFCS)



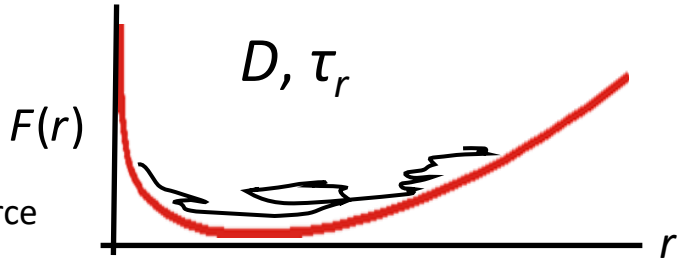


$\langle r^2 \rangle$, R_g

Equilibrium distributions and reconfiguration dynamics

$$F(r) = -k_B T \ln p(r)$$

Boltzmann inversion
→ Potential of mean force



$$g_{DA}(\tau) \propto \mathbf{1}^{\cdot}\ \mathbf{V}_A\, e^{\mathbf{K}\tau}\, \mathbf{V}_D\, \mathbf{p}_{ss}$$

Interpret dynamics in terms of diffusion in potential of mean force

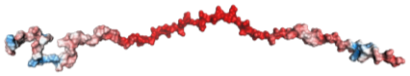
Holmstrom *et al*. (2018)
Meth Enzymol 611, 287-325

Nettels *et al*. (2007) *PNAS* 104, 2655-2660
Gopich & Szabo (2008) *In*: Barkai *et al*., *World Scientific*
Schuler *et al*. (2016) *Annu Rev Biophys* 45, 207-231

Histone H1 & ProTα: highly charged IDPs in the nucleus

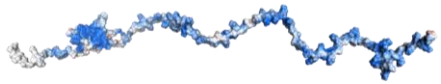
University of Zurich[UZH]

ProTα z = -44

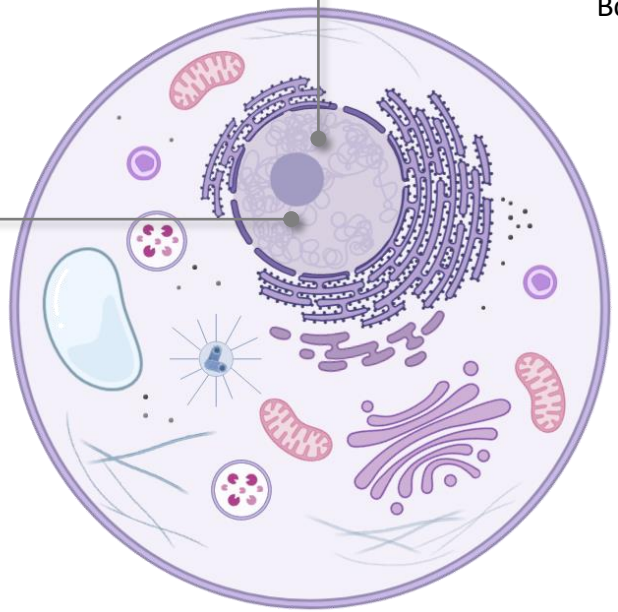


SDAAVDTSSEITTKDLKEKKEVVEEAENG
RDAPANGNAENEENGEQEADNEVDEEEEE
GGEEEEEEEEGDGEEEDGDEDEEAESATG
KRAAEDDEDDDVDTKKQKTDEDD

Histone H1 z = +53



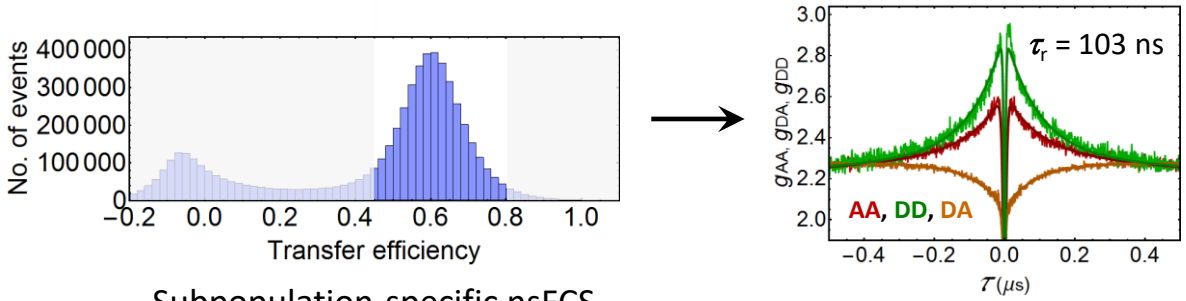
MTENSTSAPAAKPKRAKASKKSTDHPKYS
DMIVAAIQAEKNRAGSSRQSIQKYIKSHY
KVGENADSQIKLSIKRLVTTGVLKQTKGV
GASGSFRLAKSDEPKKSVAFKKTKKEIKK
VATPKKASKPKKAASKAPTKKPKATPVKK
AKKKLAATPKKAKKPKTVKAKPVKASKPK
KAKPVKPKAKSSAKRAGKKK



Single-molecule FRET + nsFCS + circular dichroism + NMR (B. Kragelund) + simulations (R. Best): a highly disordered high-affinity complex

Borgia, Borgia, Bugge *et al.*, *Nature* 555, 61-66 (2018)

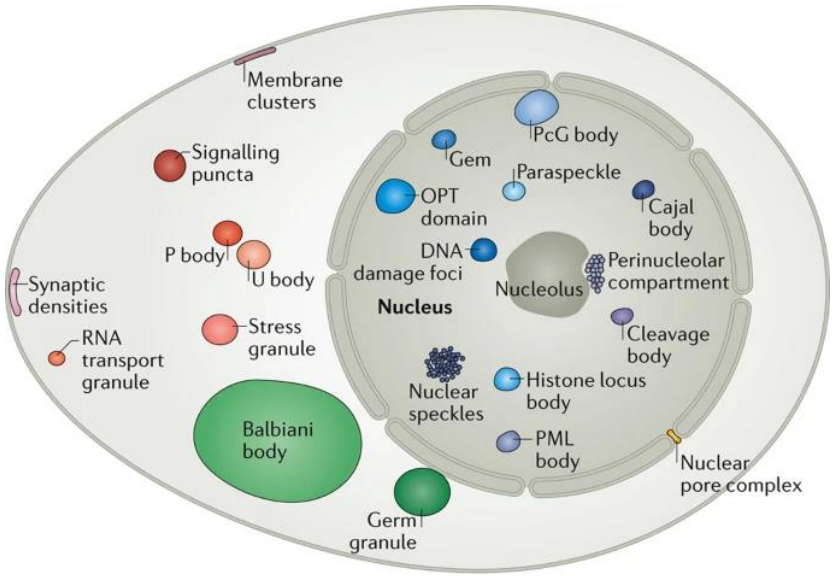
→ full-length human proteins
→ interact in the nucleus
→ biological polyelectrolytes



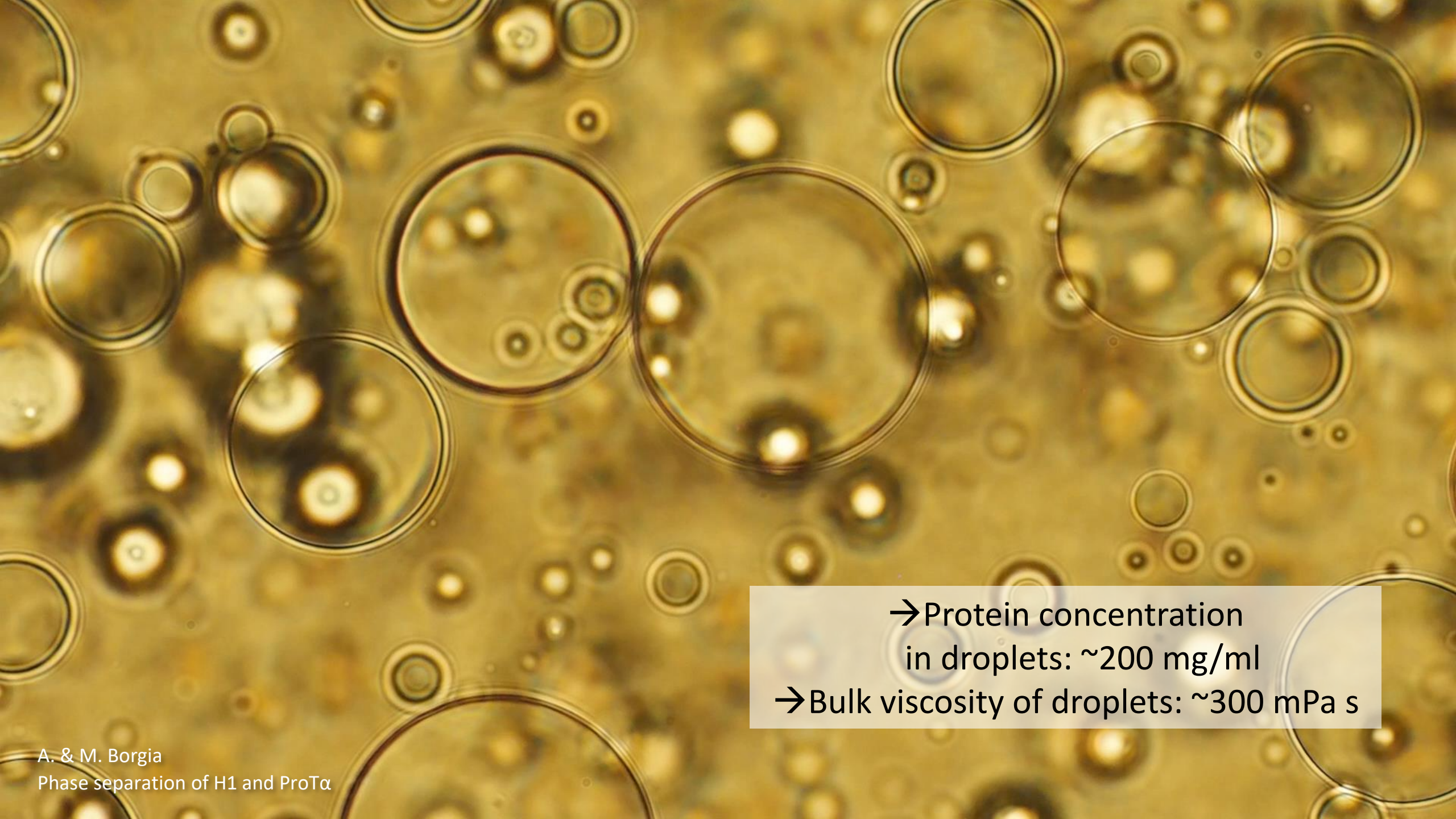
Subpopulation-specific nsFCS

University of Zurich[UZH]

Dynamics of H1 and ProTα upon **phase separation** by complex coacervation



Banani et al., Nat. Rev. Mol. Cell Biol. 18, 285–298 (2017)



→Protein concentration
in droplets: ~200 mg/ml
→Bulk viscosity of droplets: ~300 mPa s

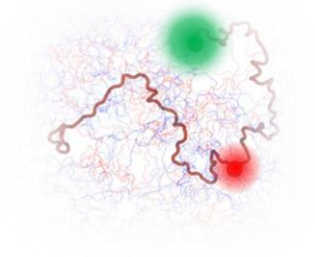
A. & M. Borgia
Phase separation of H1 and ProTα

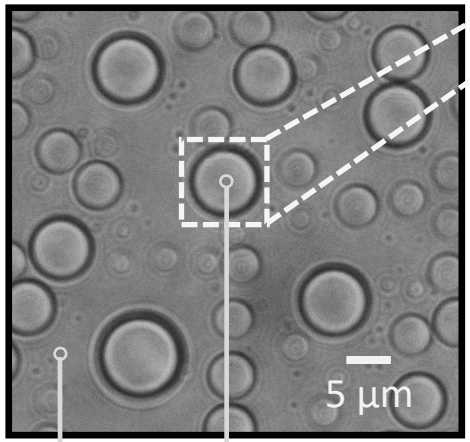
Single-molecule FRET in biomolecular condensates

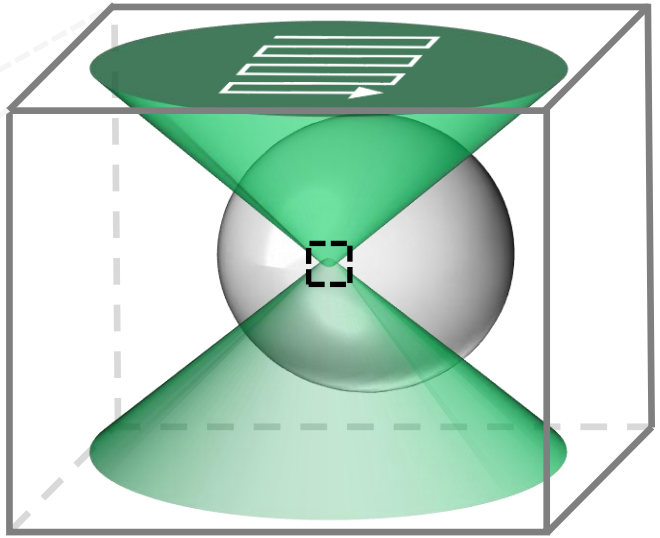
Nicola Galvanetto

Large concentrations of unlabeled proteins with picomolar labeled ProTα

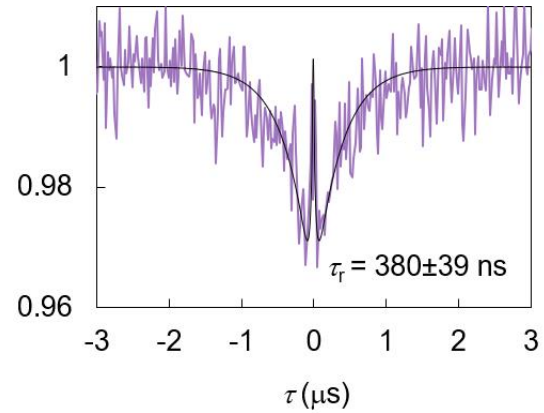
ProTα-Cy3B/CF660R



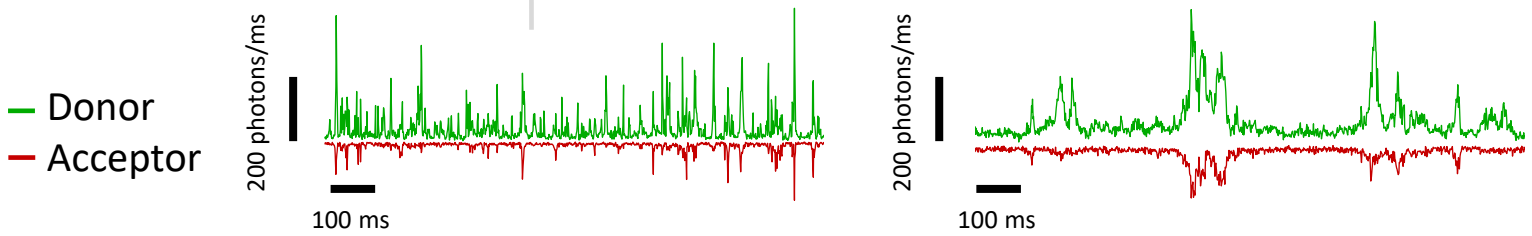




nsFCS



→ Chain dynamics in the droplets surprisingly rapid



Galvanetto, Ivanović *et al.*, *Nature* 619, 876-883 (2023)

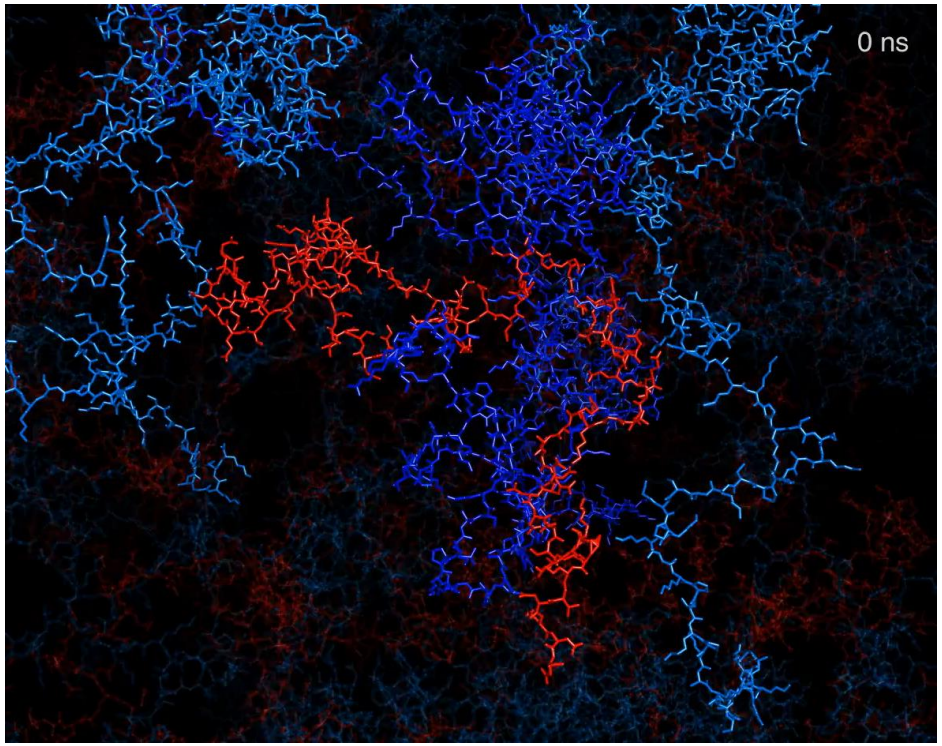
Rapid chain dynamics despite high viscosity

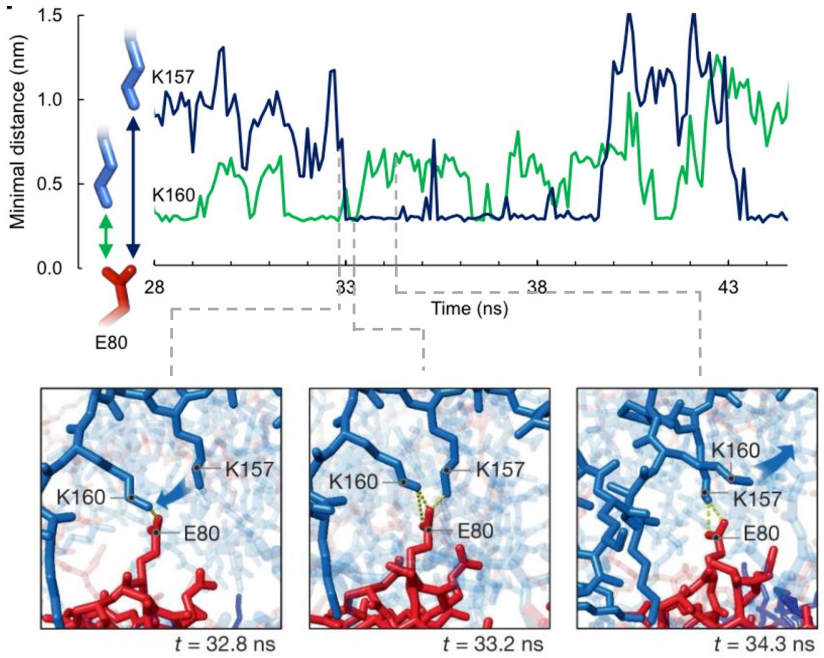
Bulk viscosity
~**300×** higher in the droplets
than in dilute solution

Chain dynamics only
~**3×** slower in the droplets
than in ProTα-H1 dimer

Rapid exchange of contacts in the dense phase

→ Very high concentration of charged side chains in the dense phase (~1 M)

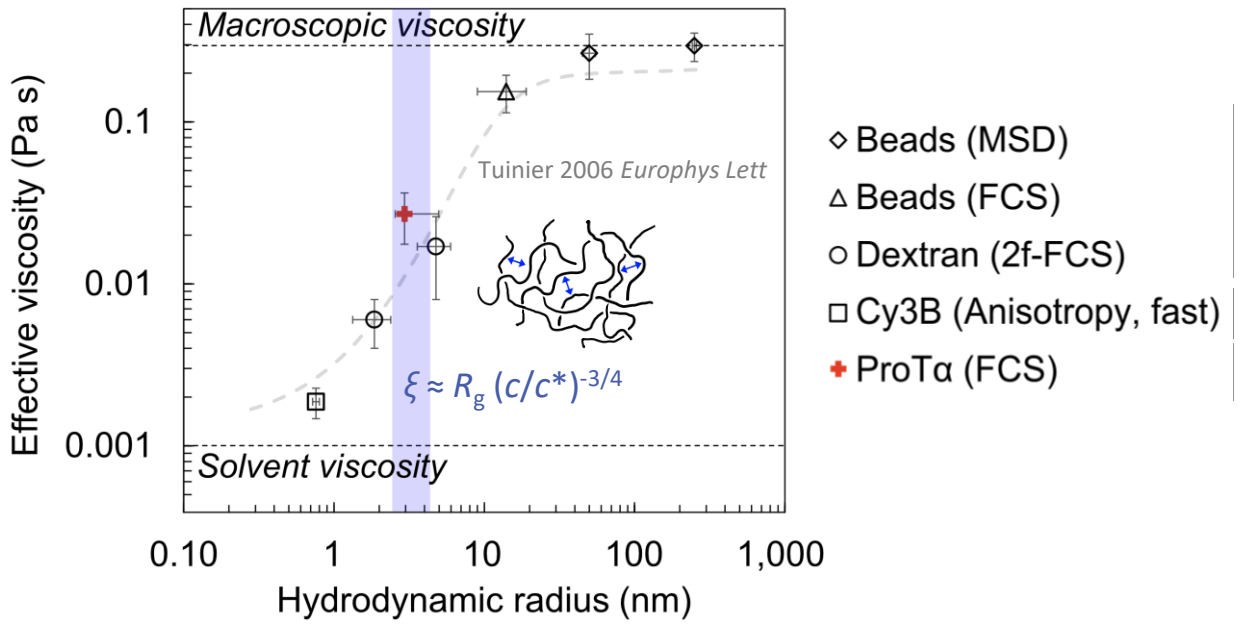




→ Rapid exchange/dynamic shuffling between contacts
enables extremely rapid local dynamics despite large bulk viscosity

Galvanetto, Ivanović *et al.*, *Nature* 619, 876-883 (2023)

Length scale-dependent effective viscosity in the dense phase



→ Effective viscosity from translational diffusion (Stokes-Einstein) depends on length scale
→ Motion on length scales < correlation length facilitated
→ Described quantitatively based on depletion interactions (Tuinier 2006)
→ ProTα dimensions ≈ correlation length ξ
→ ProTα part of the network,
but explains part of the discrepancy between chain dynamics and bulk viscosity

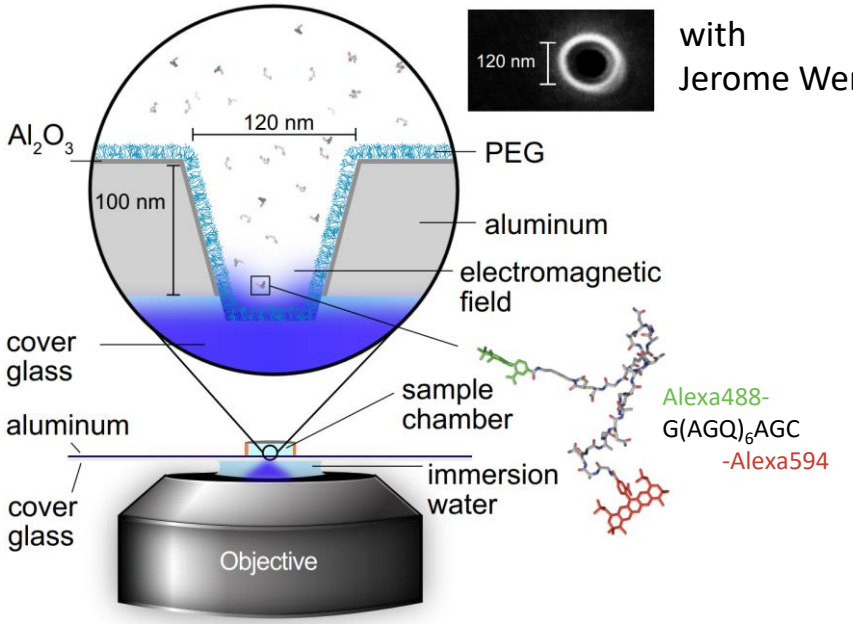
Enhancing nsFCS with nanophotonics

Limitations of nsFCS:

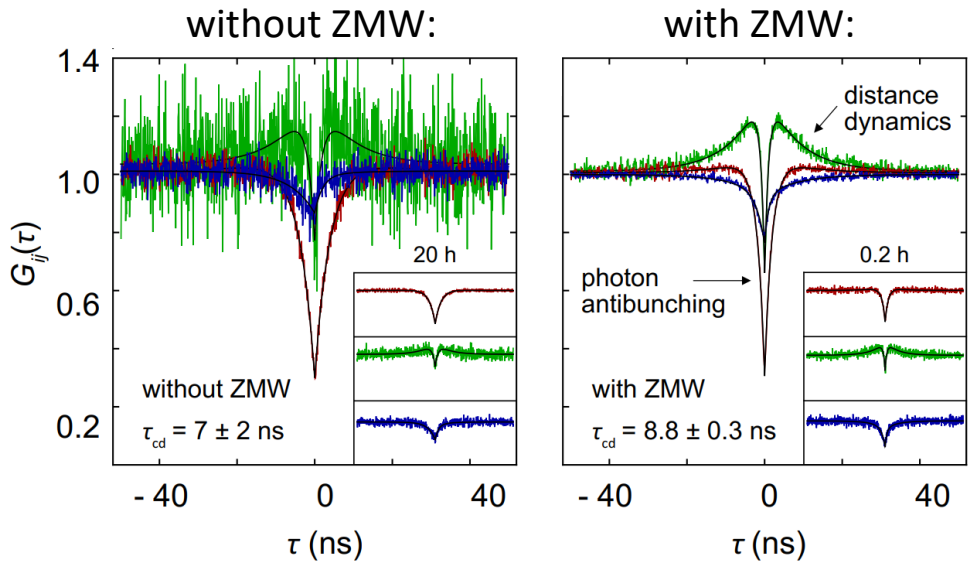
- dynamics ≲10 ns cannot be resolved (photon antibunching)
- long data acquisition times (typically ~10h)

Zero-mode waveguides (ZMW):

- ~7× photon rate increase
  → reduced data acquisition time
- ~2× lifetime decrease
  → faster dynamics accessible



Mark Nüesch



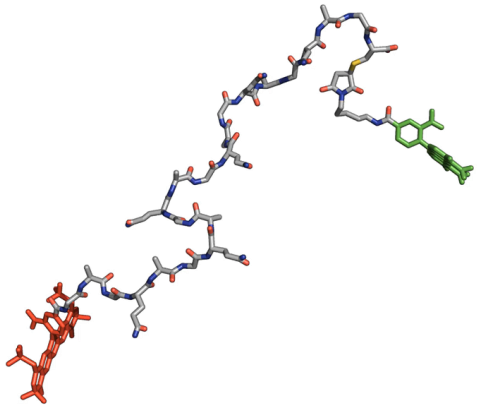
Nüesch, Ivanović et al. *JACS* 2022

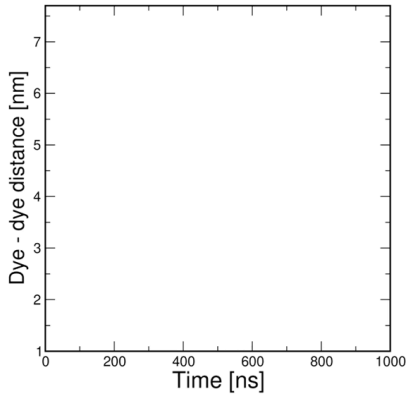
Comparing nsFCS and FRET with all-atom MD simulations

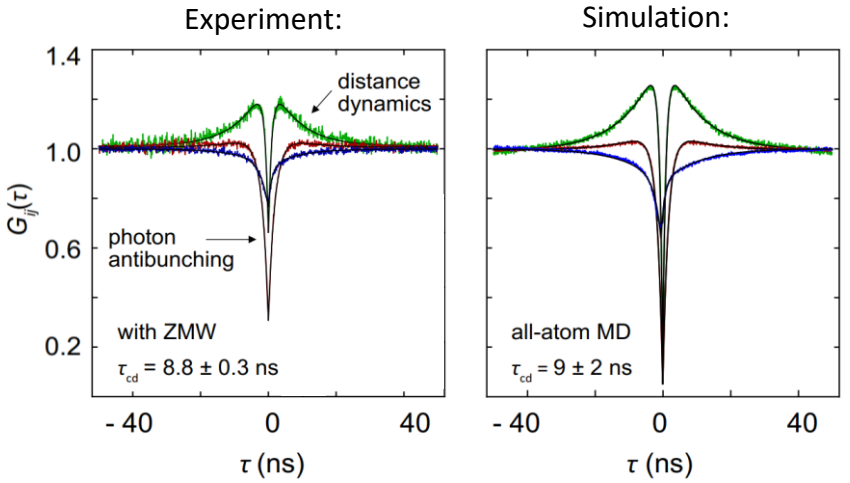
Miloš Ivanović
with R. Best

All-atom explicit-solvent MD:

- Amber99SBws/TIP4P2005s (Best *et al.* (2014) *J Chem Theory Comput* 10, 5113)
- Explicit fluorophores (Best *et al*. (2015) *Biophys J* 108, 2721)
- with and without urea (Zheng *et al.* (2015) *J Chem Theory Comput* 11, 5543)
- Total simulation time 16 µs each condition



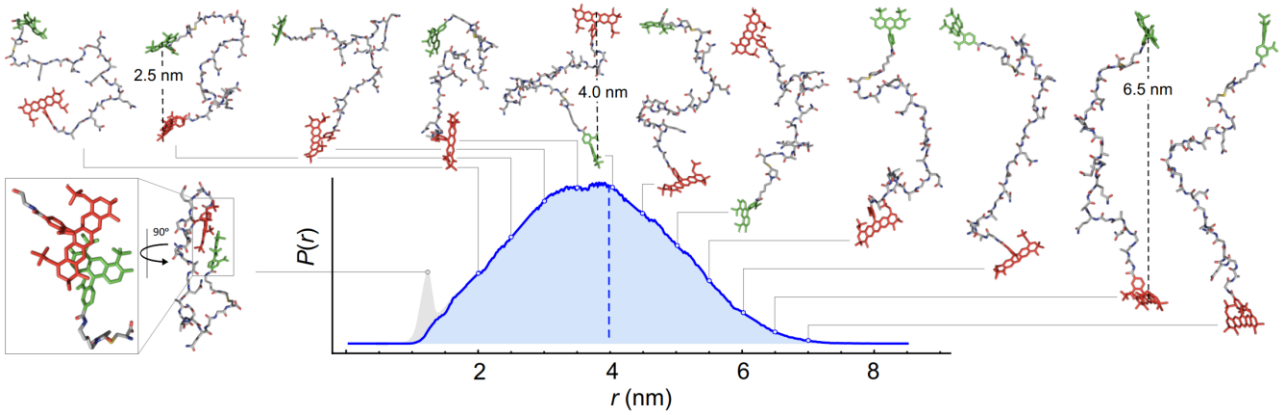




Transfer efficiencies:

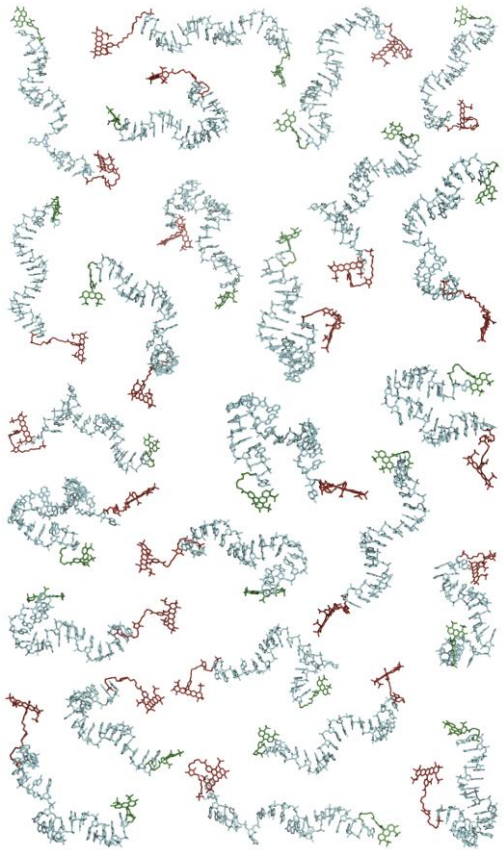
with urea:
exp.: E = 0.82±0.03
sim.: E = 0.80±0.01

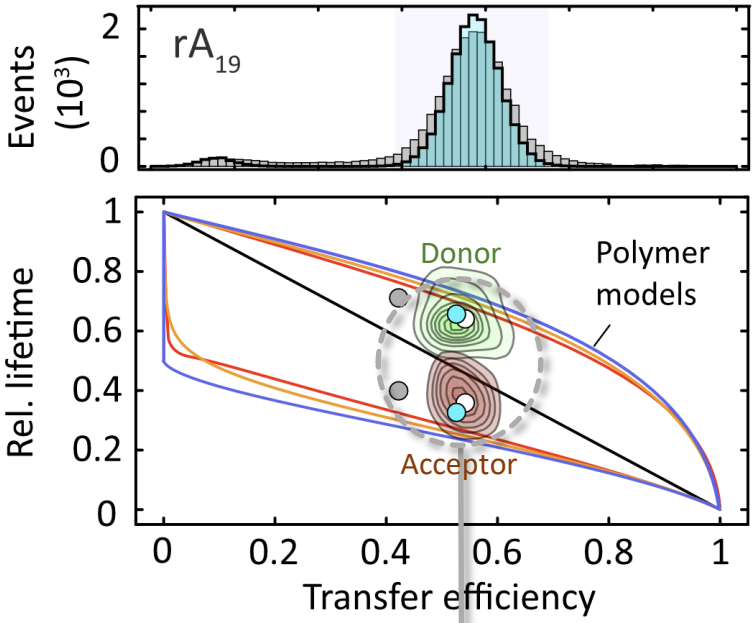
without urea:
exp.: E = 0.94±0.03
sim.: E = 0.93±0.01

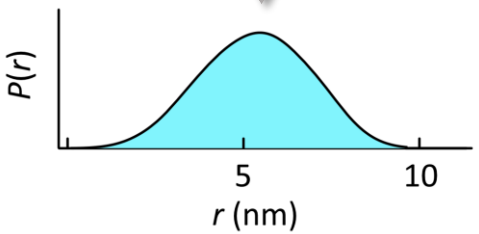


Nüesch, Ivanović et al. *JACS* 2022

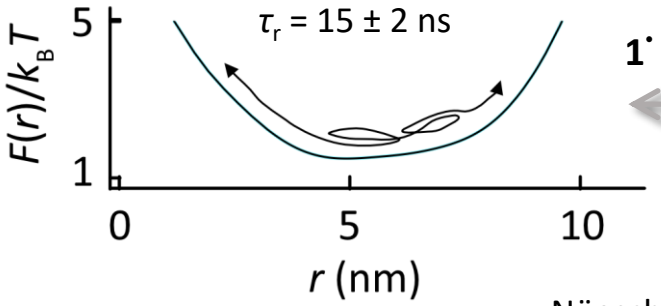
Nanosecond chain dynamics of single-stranded nucleic acids







Boltzmann inversion
→ Potential of mean force

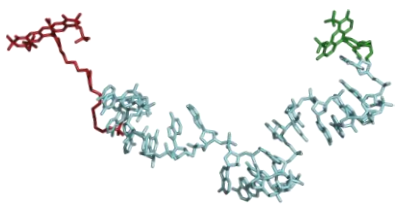


Equilibrium distributions
and
chain reconfiguration times

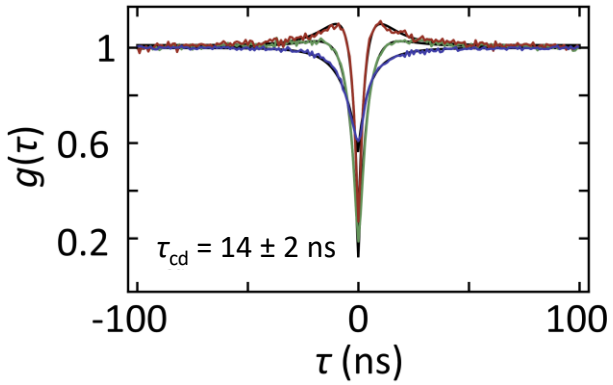
$$g_{DA}(\tau) \propto \mathbf{1}^{\cdot}\, \mathbf{V}_A\, e^{[\Lambda+\mathbf{K}_0]\,\tau}\, \mathbf{V}_D\, \mathbf{p}_{ss}$$

Describe dynamics as diffusion in potential of mean force

ssRNA, ssDNA
polyA, polyC, polyT, polyU



Nanosecond FCS

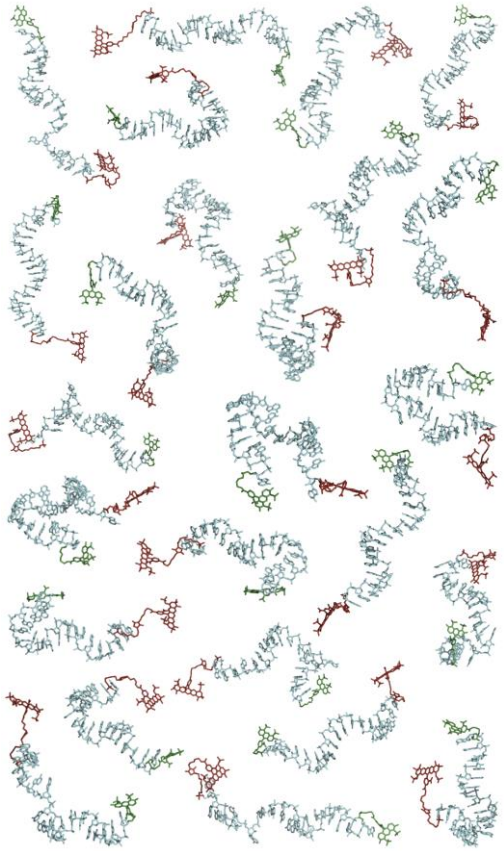


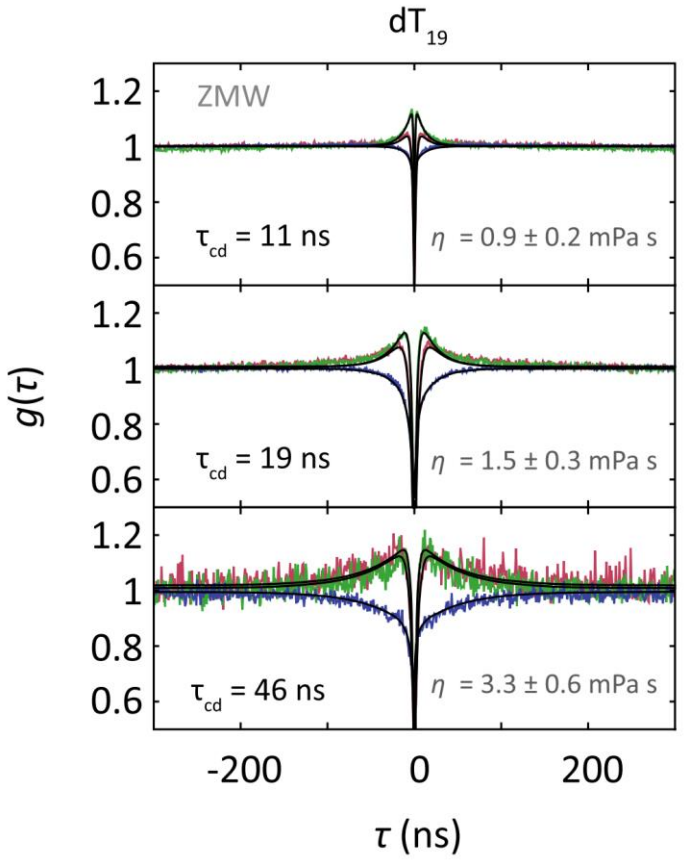
Hierarchical chain growth
and Bayesian esensemble reweighting
(with L. Pietrek & G. Hummer)

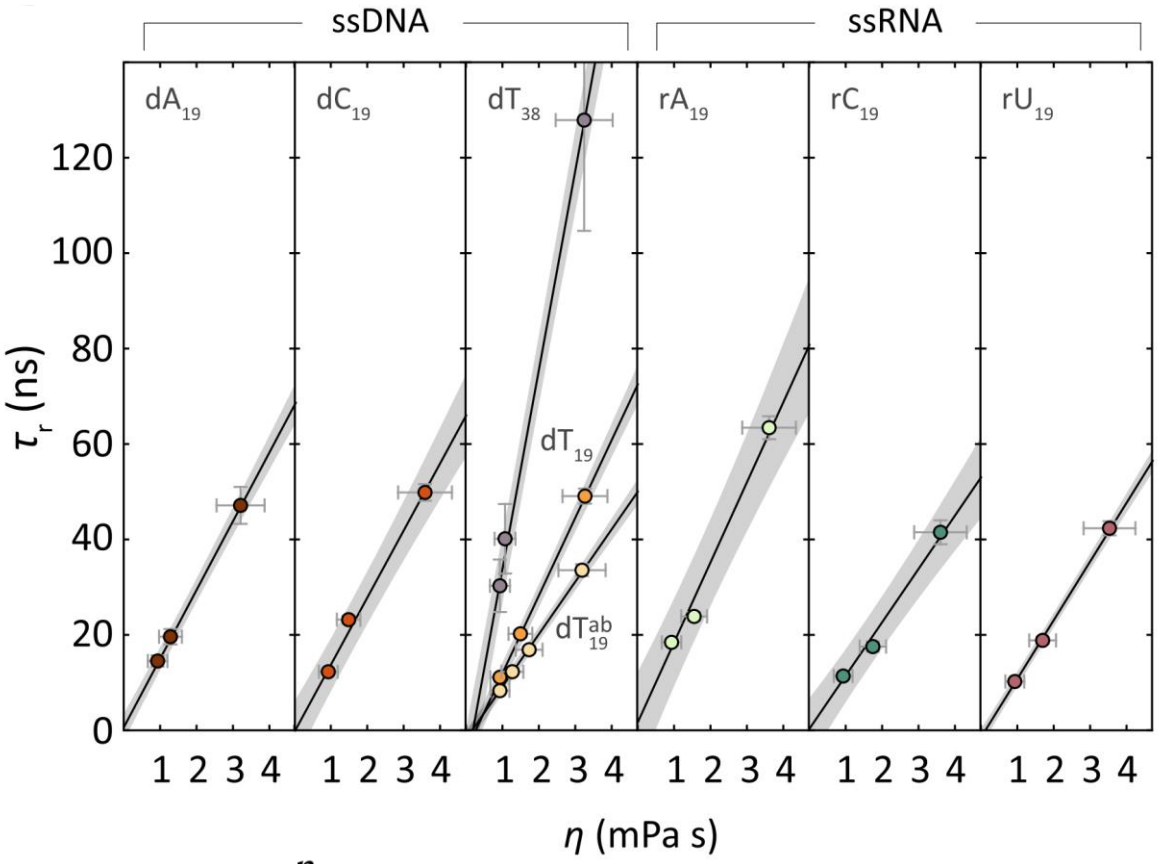
Pietrek *et al*. (2024) *J Chem Theory Comput* 20, 2246
Hummer & Köfinger (2015) *J Chem Phys* 143, 243150

Nüesch *et al. (2024) Nat Commun* 15, 6010

Absence of internal friction in ssRNA and ssDNA dynamics







$$\tau_r \approx \tau_i + \frac{\eta}{\eta_o}\tau_s(\eta_o)$$ Rouse model with internal friction

Hierarchical chain growth
and Bayesian esensemble reweighting
(with L. Pietrek & G. Hummer)

Pietrek *et al*. (2024) *J Chem Theory Comput* 20, 2246
Hummer & Köfinger (2015) *J Chem Phys* 143, 243150

→ No detectable internal friction for homopolymeric ssRNA and ssDNA

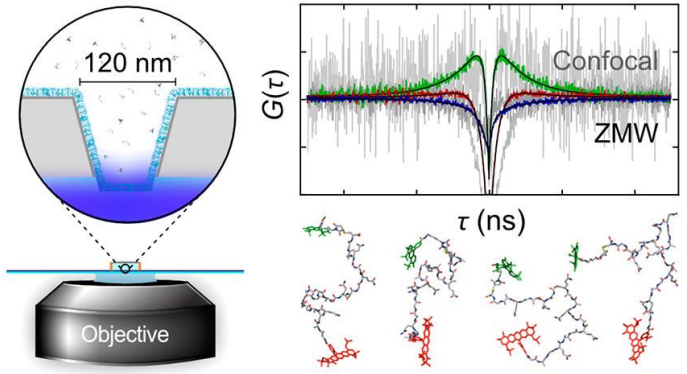
→ Hinge-like motion of stacked segments?

Nüesch *et al. (2024) Nat Commun* 15, 6010

Summary

nsFCS provides access to rapid dynamics of unfolded and disordered proteins and nucleic acids, including complex environments, such as crowding, phase separation, live cells

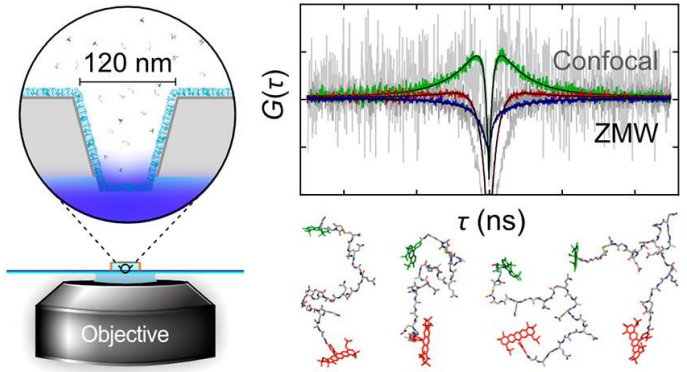
nsFCS can be enhanced by nanophotonics in zero mode waveguides to probe dynamics in the low nanosecond range with 100x shorter data acquisition times



Rapid interaction dynamics of disordered proteins

nsFCS provides access to rapid dynamics of unfolded and disordered proteins, including complex environments, such as crowding, phase separation, live cells

nsFCS can be enhanced by nanophotonics in zero mode waveguides to probe dynamics in the low nanosecond range with 100x shorter data acquisition times



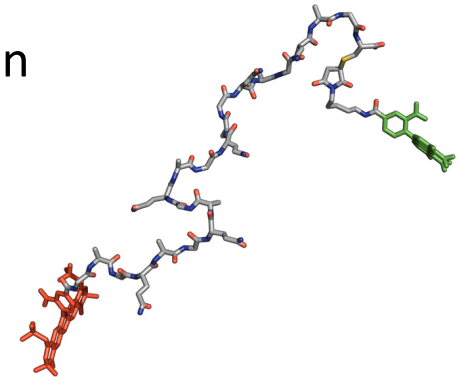
Molecular simulations ideally complement single-molecule spectroscopy

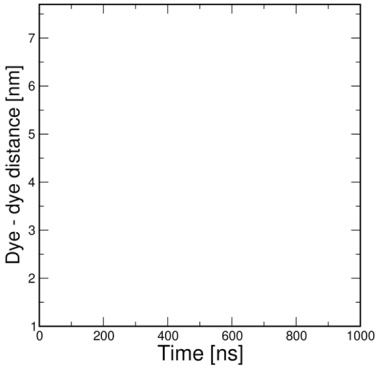
→ Increasing quality of force fields for IDPs

→ Increasing overlap between timescales in experiment and simulation

→ Enable interpretation of data in terms of molecular mechanisms

→ Single-molecule data provide useful benchmarks





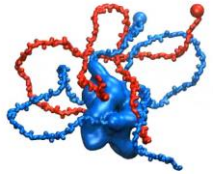
University of Zurich[UZH]

Miloš Ivanović
Nicola Galvanetto
Mark Nüesch
Alessandro Borgia
Madeleine Borgia

Aritra Chowdhury
Andrea Sottini
Pétur Heiðarsson
Davide Mercadante
Daniel Nettels

National Institutes of Health

Robert Best



INSTITUT FRESNEL

Jérôme Wenger
Jean-Benoît Claude

MAX-PLANCK-INSTITUT FÜR BIOPHYSIK

Gerhard Hummer
Lisa Pietrek

University of Copenhagen

Birthe Kragelund
Katrine Bugge
Catarina Fernandes

Carlsberg Foundation

novo nordisk fonden

CSCS
Centro Svizzero di Calcolo Scientifico
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