Spectroscopy and Microscopy of Single Molecules and Single Nanoparticles

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Molecular Nano-Optics and Spins

Leiden University (Netherlands)

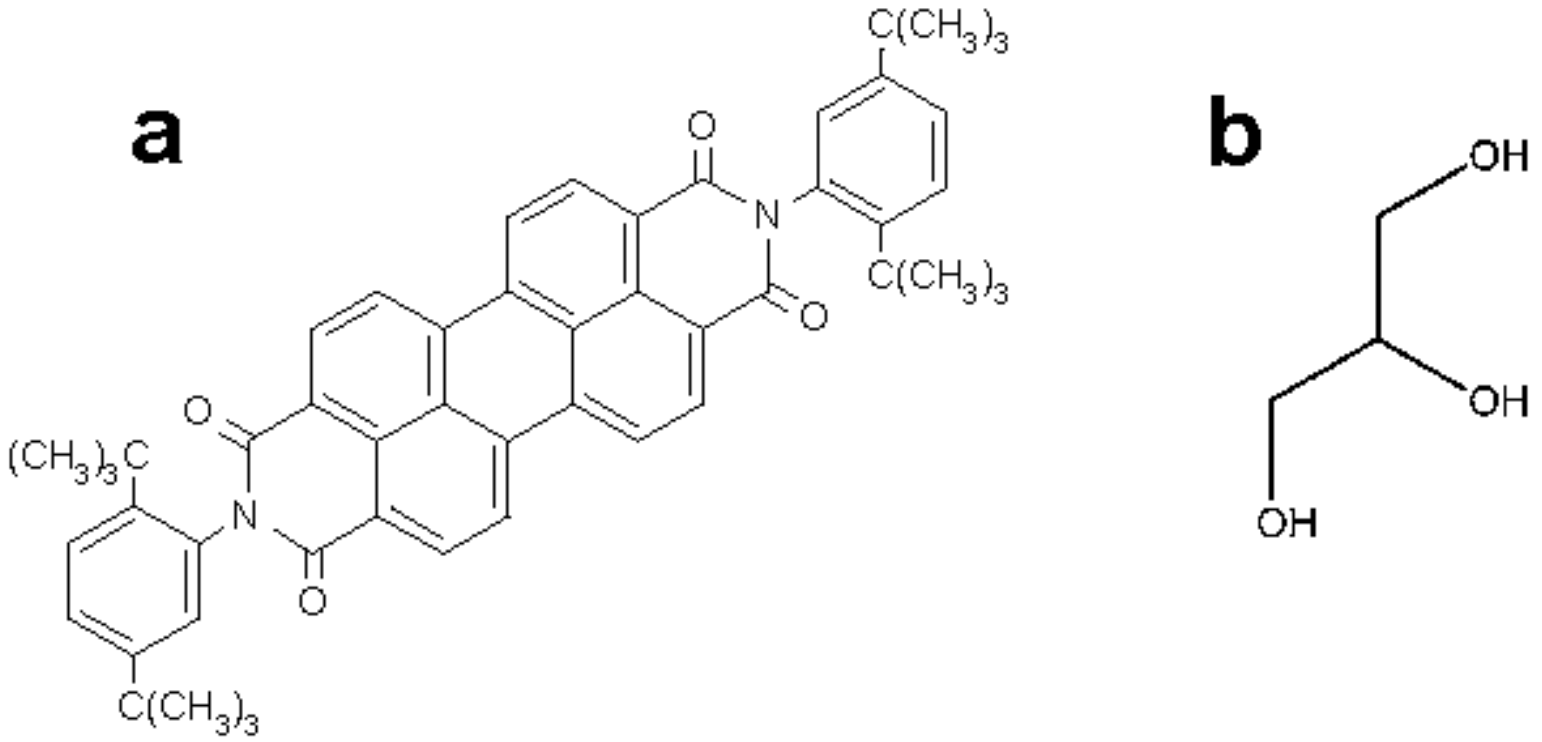
Han sur Lesse, 12 December 2013

Part II
Fluorescence and photothermal spectroscopy at room temperature

Outline (Part II)

- Supercooled liquids
- Temperature jumps
- Photothermal detection

Local probing of supercooled liquids

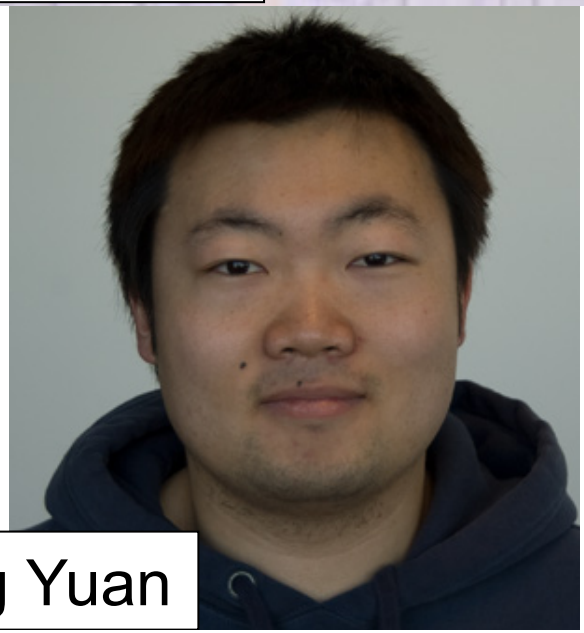


Perylene-di-imide (a) in glycerol (b)

Dr. Florian Kulzer

Dr. Rob Zondervan

Dr. Ted Xia



Haifeng Yuan

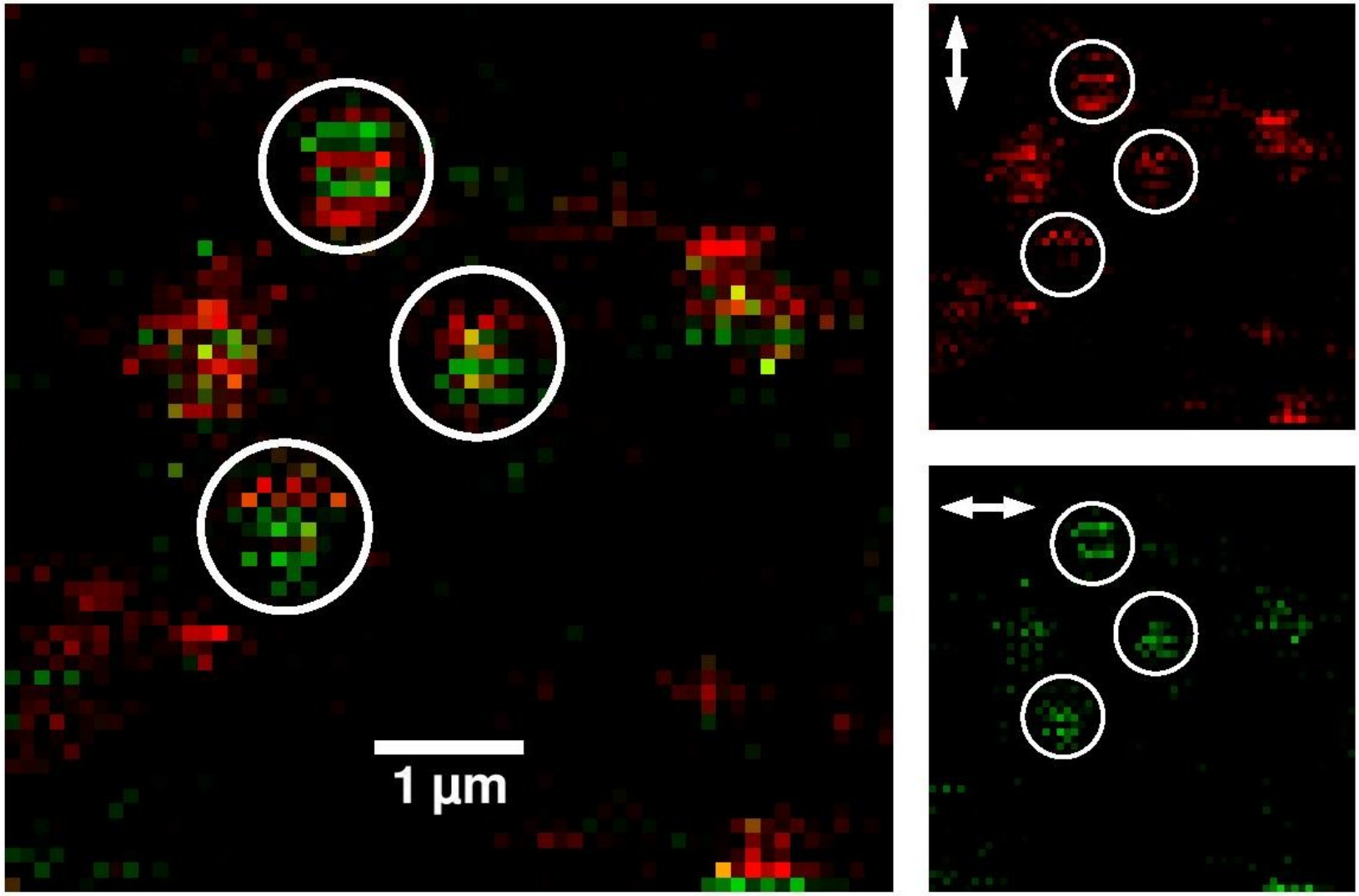
Probing viscosity with fluorescence

- Fluorescence Anisotropy (during emission)
- Polarization fluctuations (small ensembles)
- Single-molecule orientations

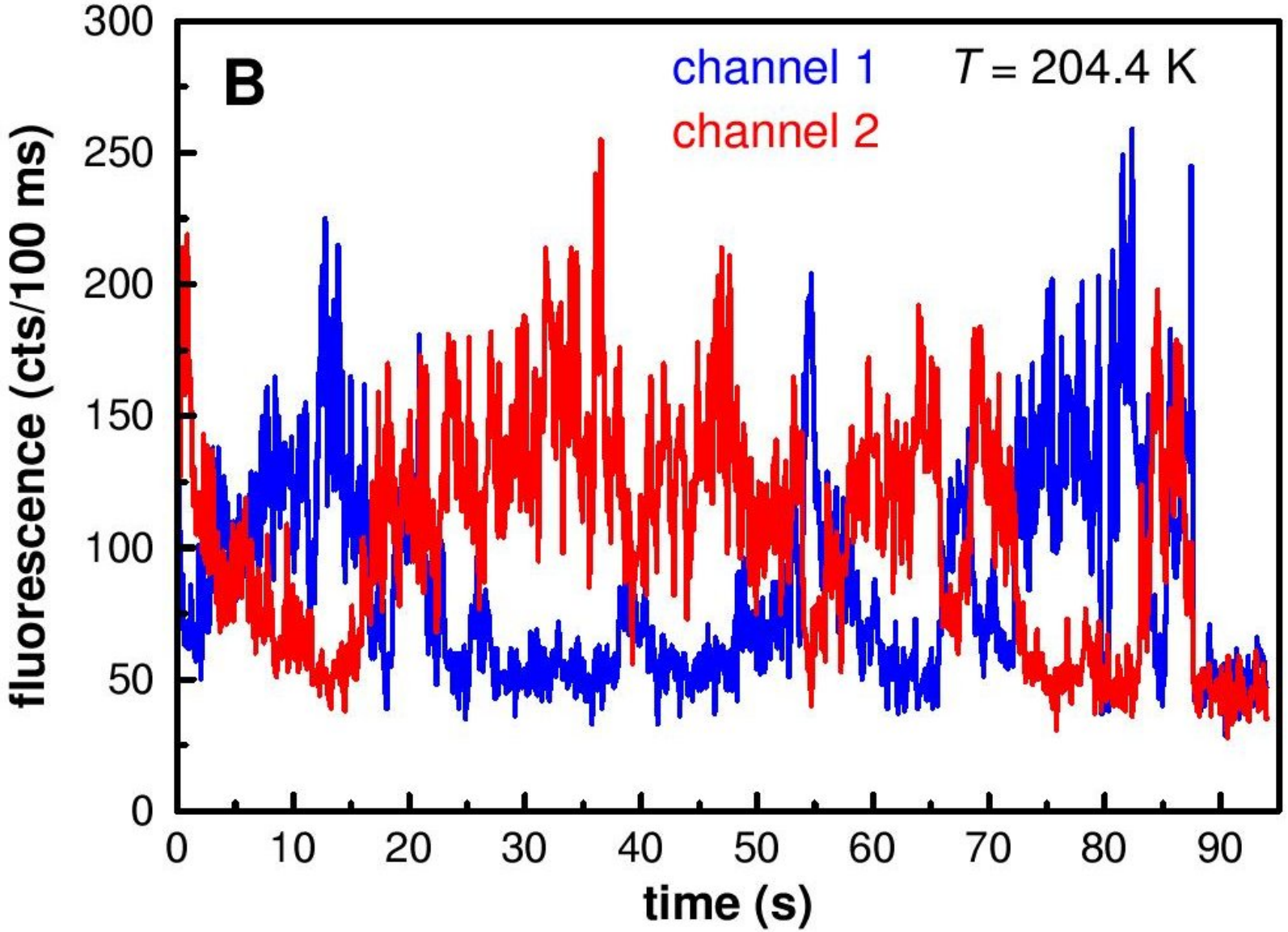
Rotational diffusion time: $$\tau = \frac{V\eta}{k_B T}$$

R. Zondervan et al., P. N. A. S. **104** (2007) 12628

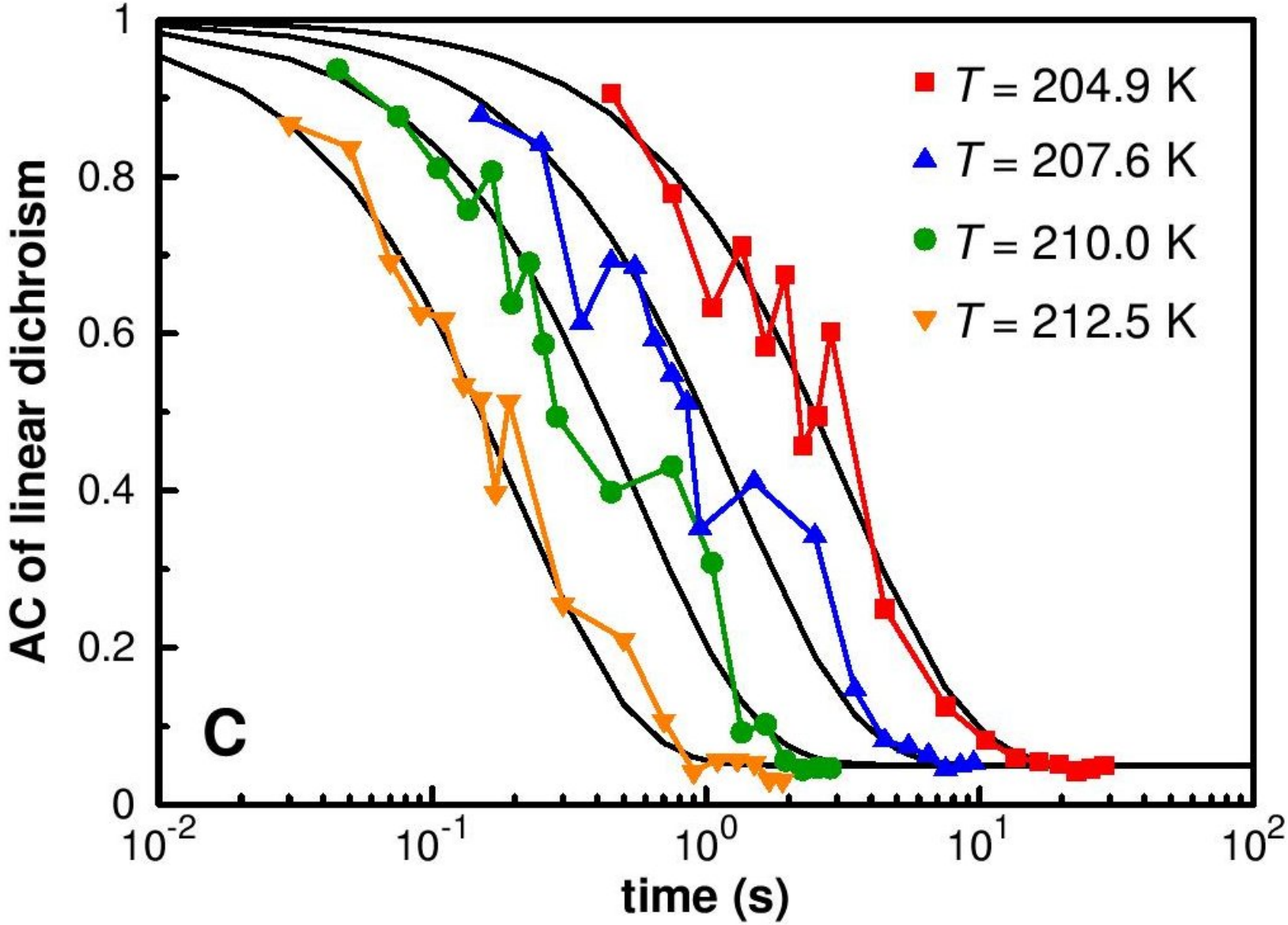
Polarized single-molecule fluorescence



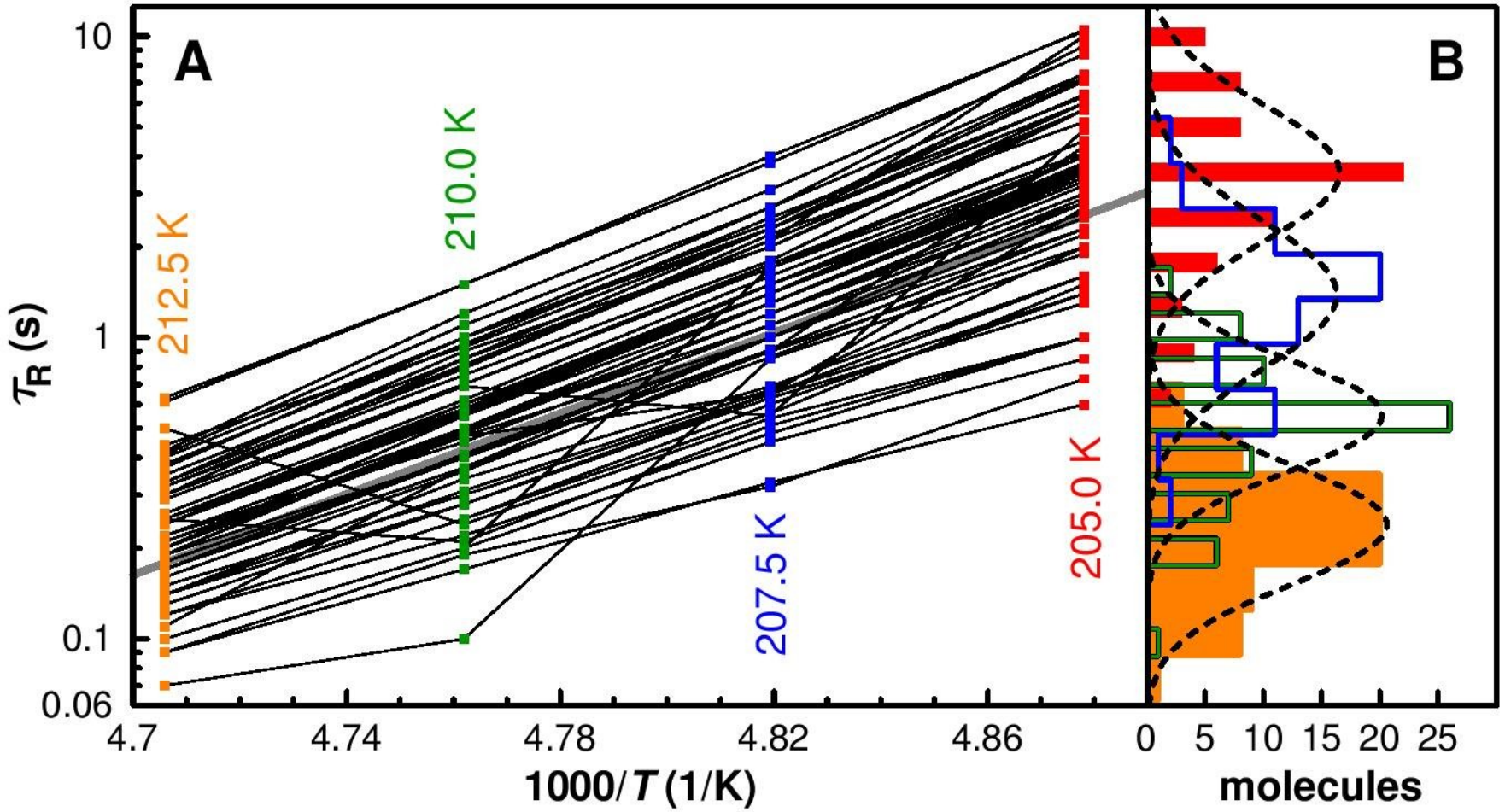
Anticorrelation of polarization channels



Single-molecule tumbling at variable T



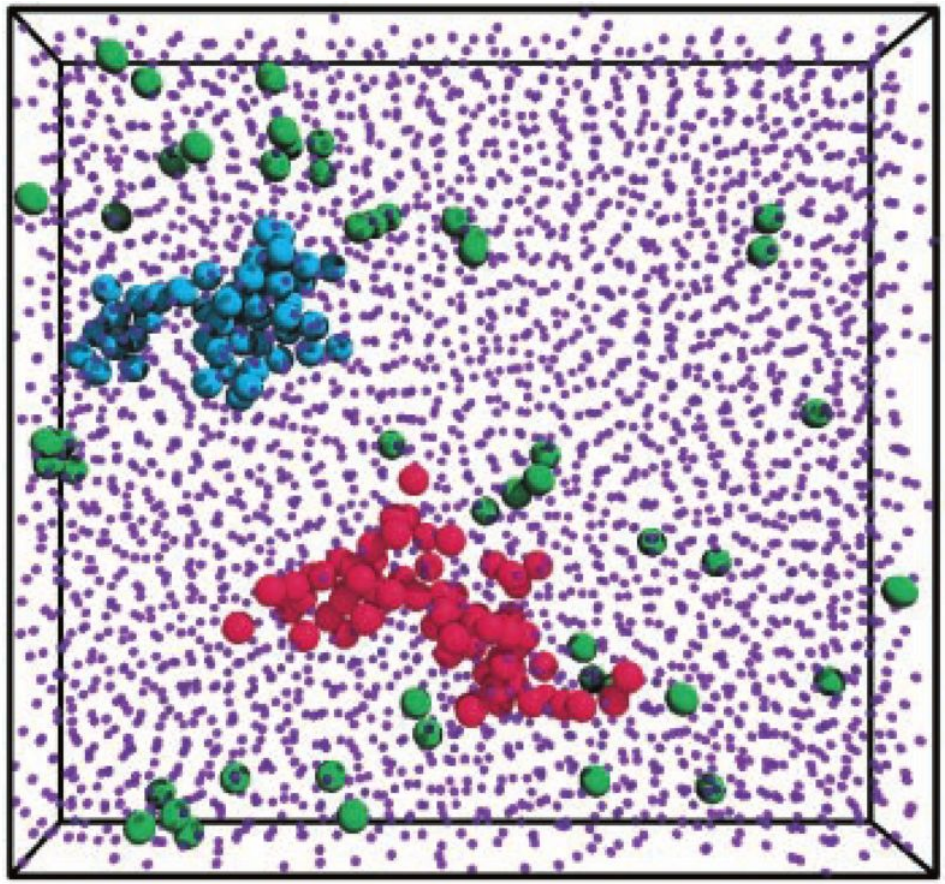
T-dep. of tumbling rates of 69 molecules



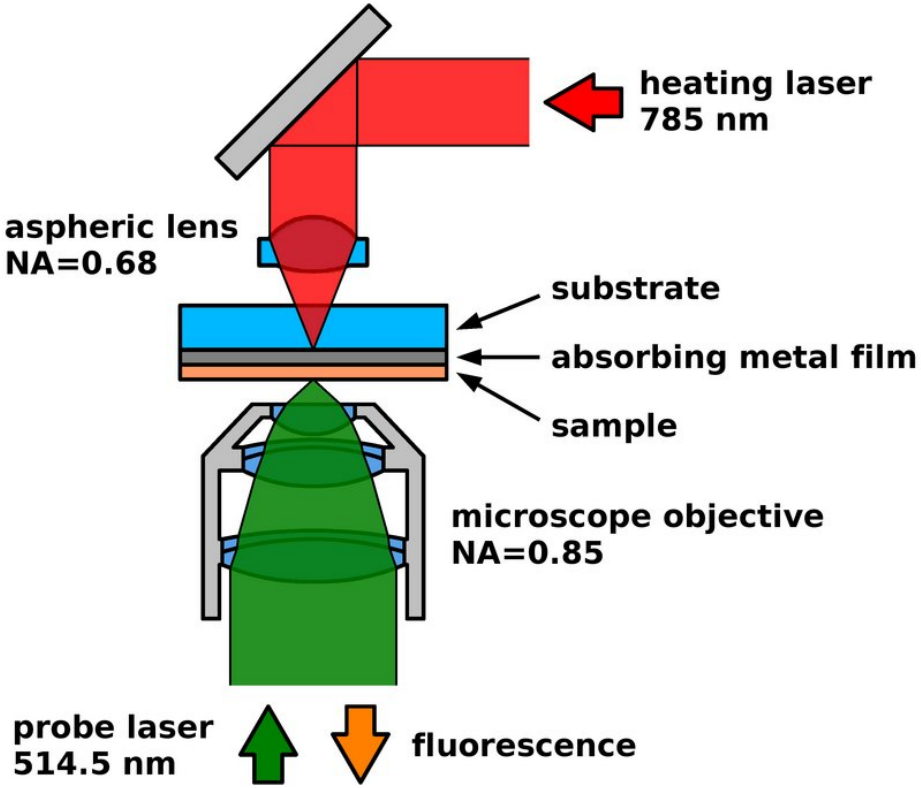
Extremely long memory of diffusion rate evidence for solid walls?

Seen already for colloidal suspensions in the glass phase:
E. R. Weeks et al.,
Science **287**(2000) 627

A solid matrix should be elastic : rheology

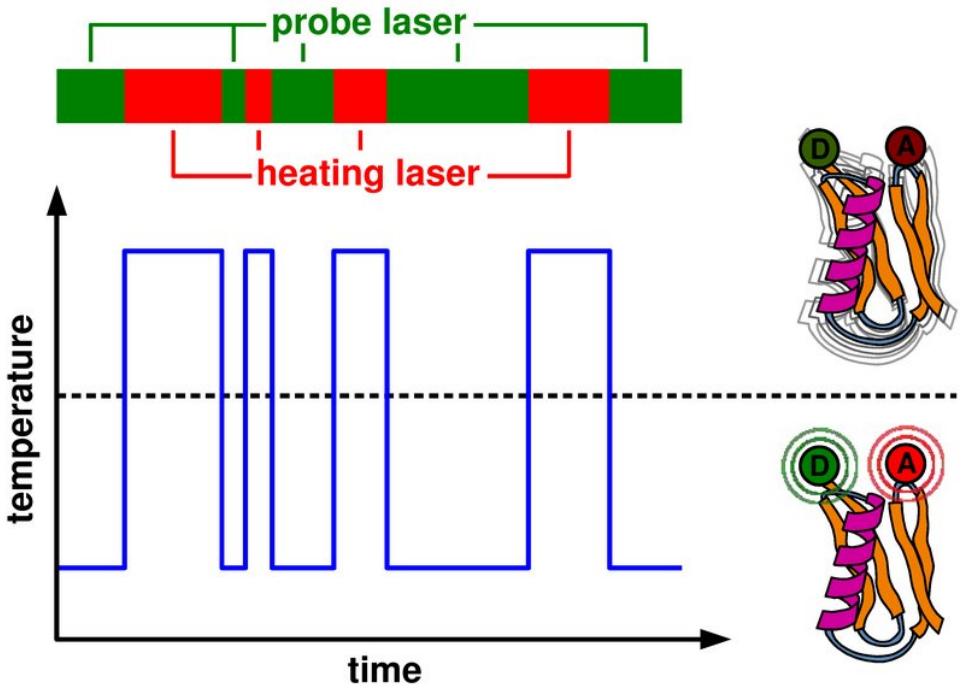


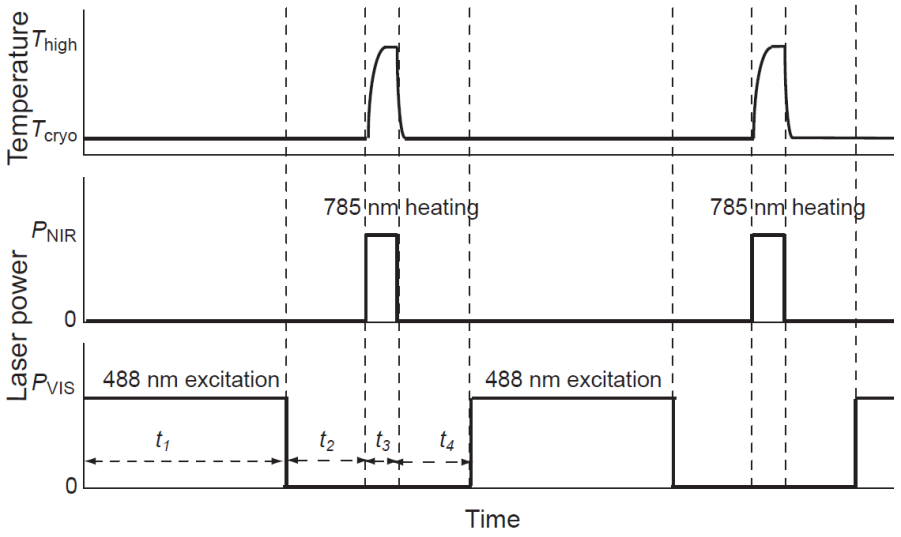
Temperature-cycle microscopy



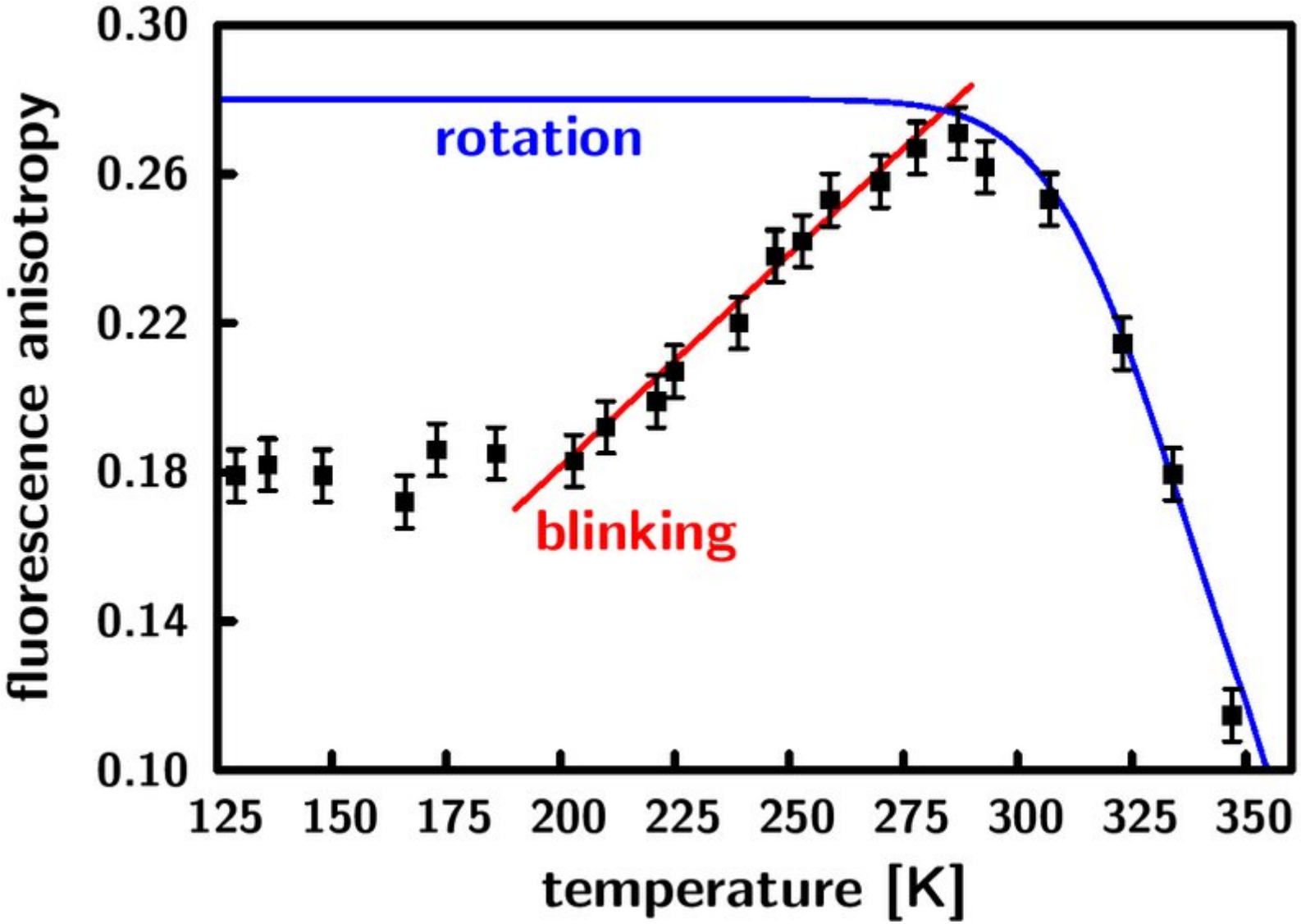
Zondervan et al., Biophys. J. **90** (2006) 2958

Principle of temperature cycle microscopy

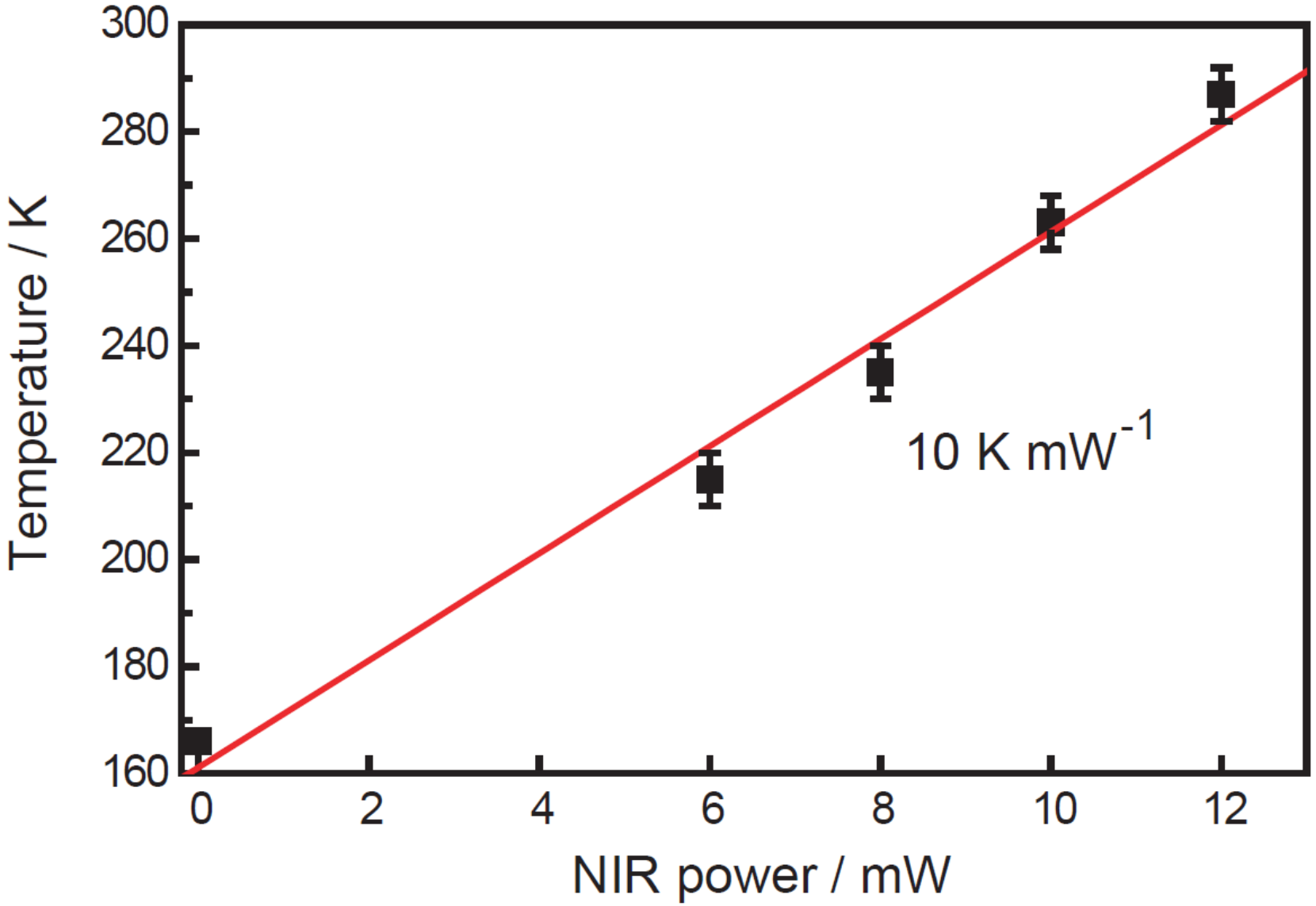




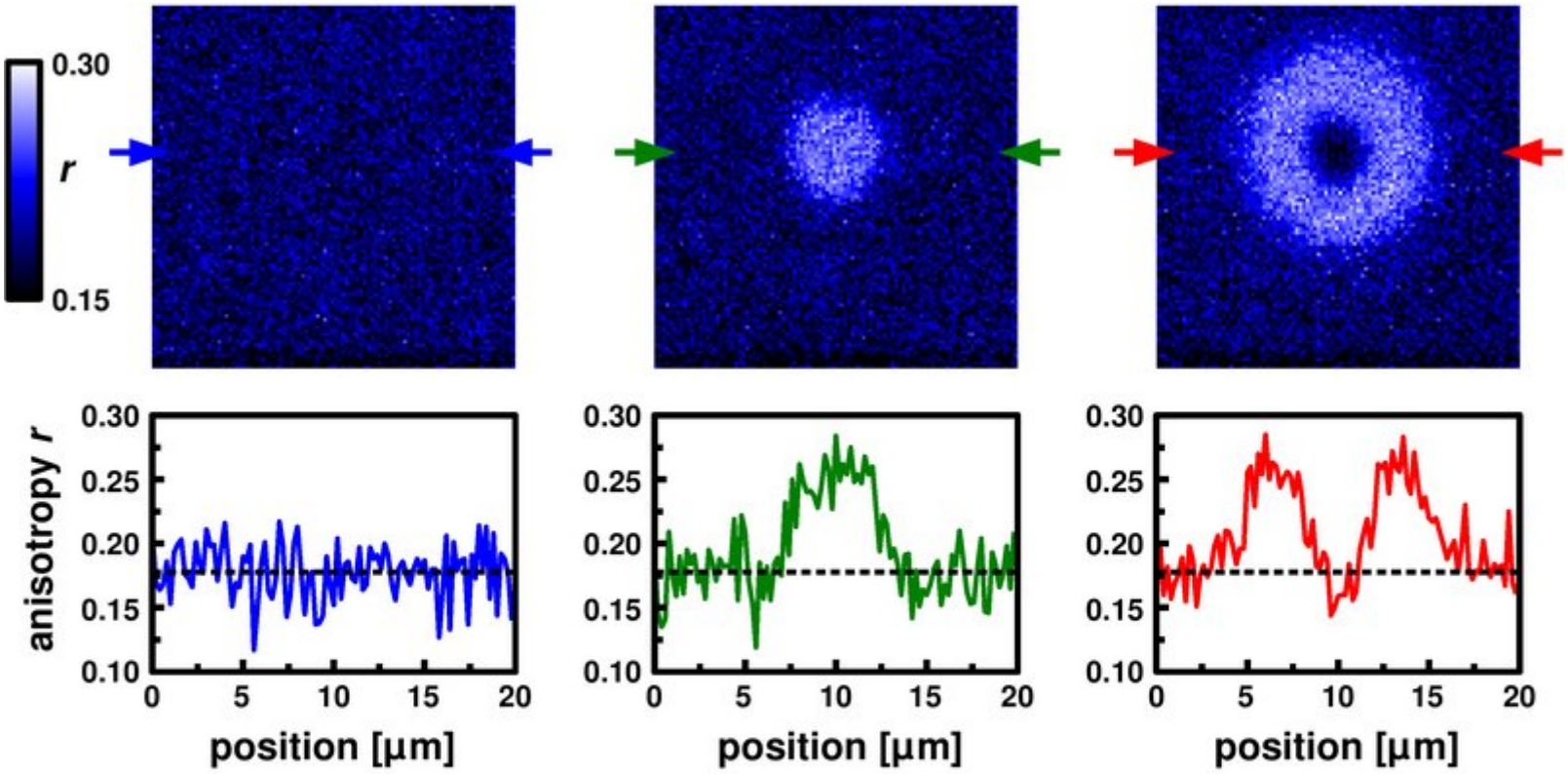
Calibration with fluorescence anisotropy



Temperature calibration



Spatial temperature profile

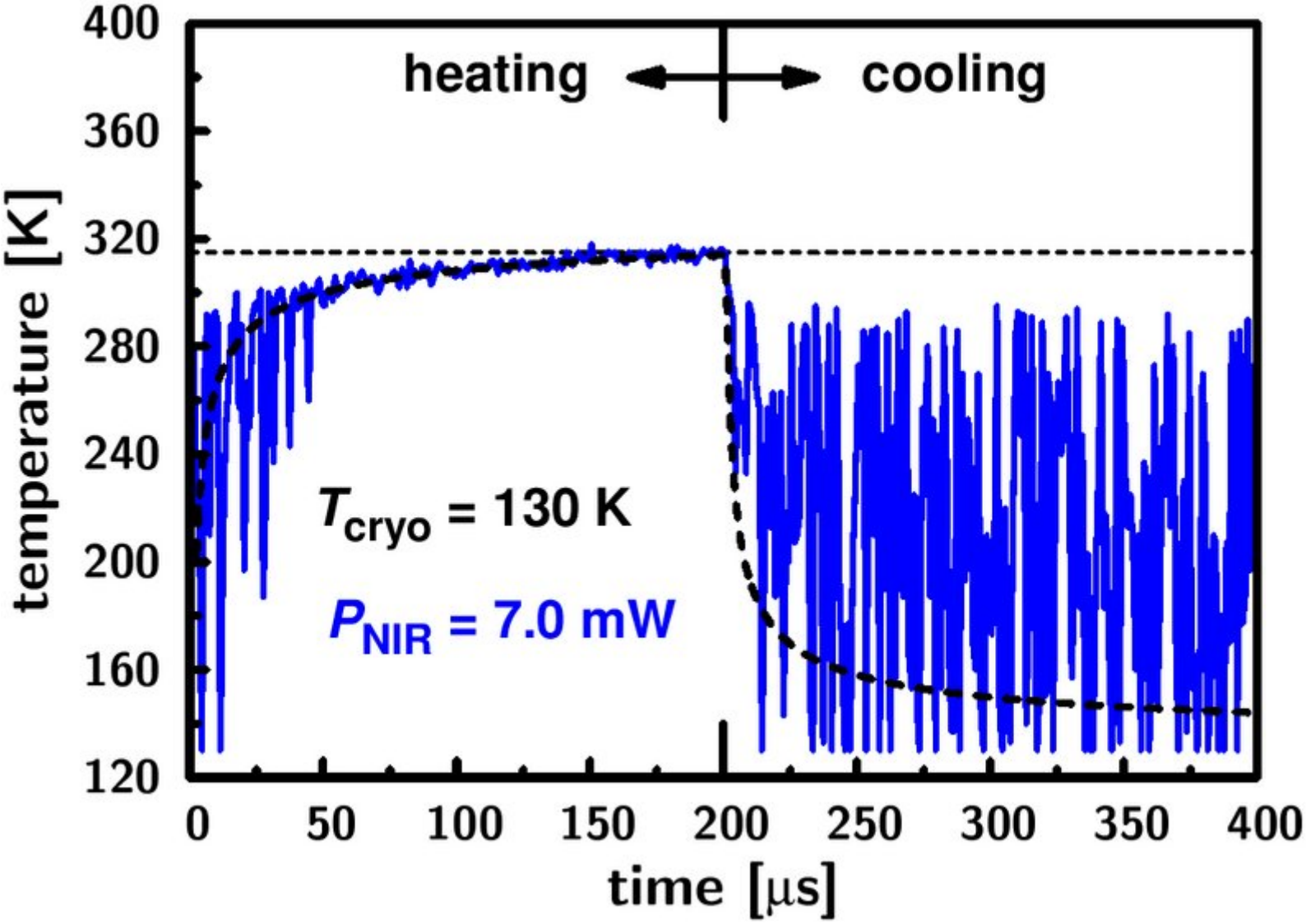


P_{NIR} = 0 mW
T = 130 K

P_{NIR} = 5.0 mW
T_{max} ≈ 280 K

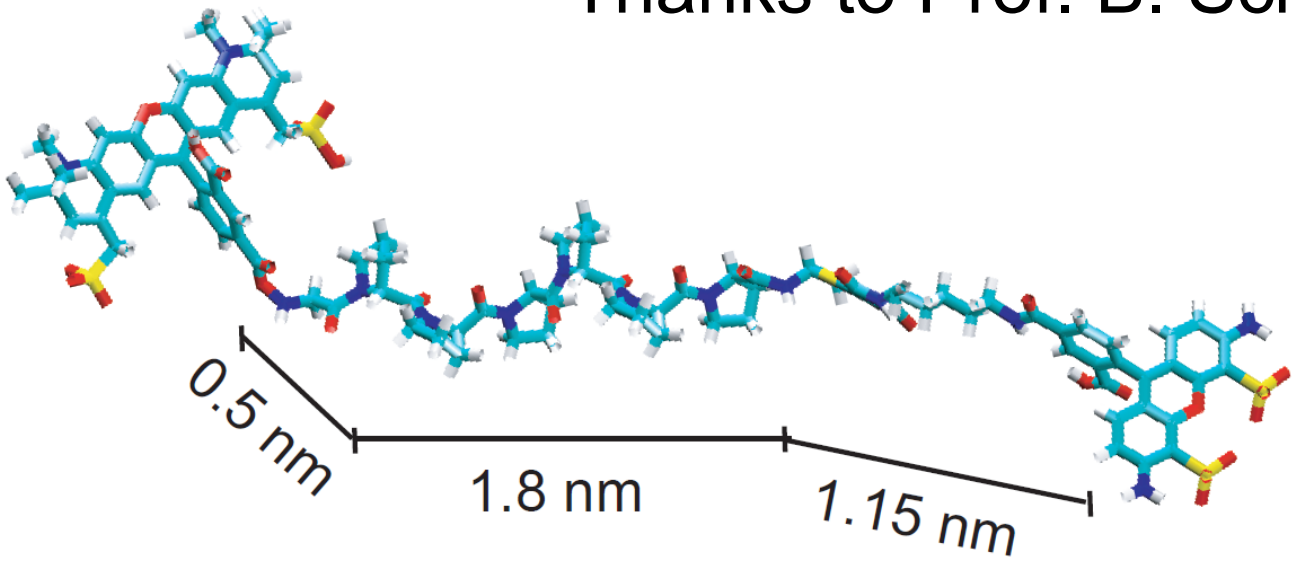
P_{NIR} = 8.5 mW
T_{max} ≈ 335 K

Temperature kinetics

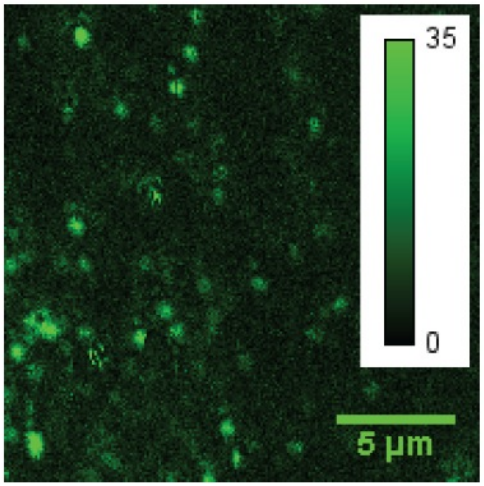


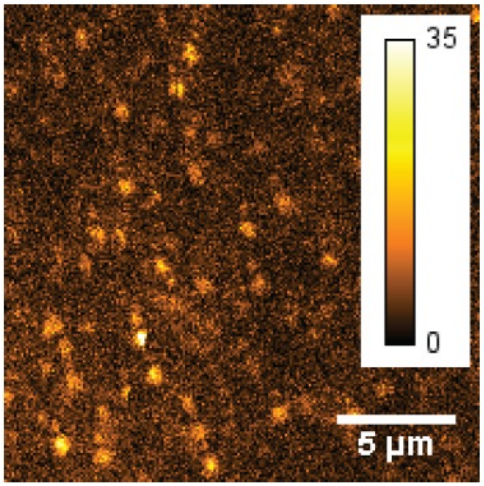
FRET-labeled Oligopeptide (polyproline)

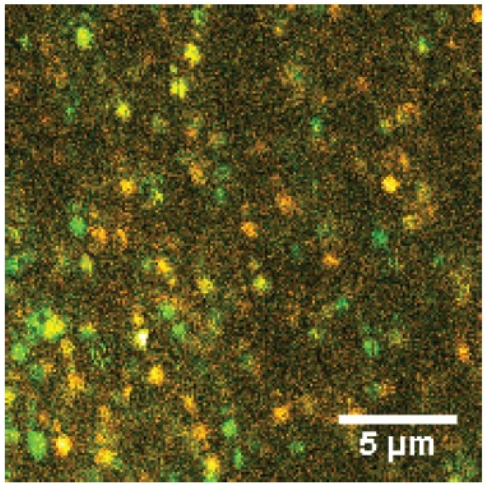
Thanks to Prof. B. Schuler



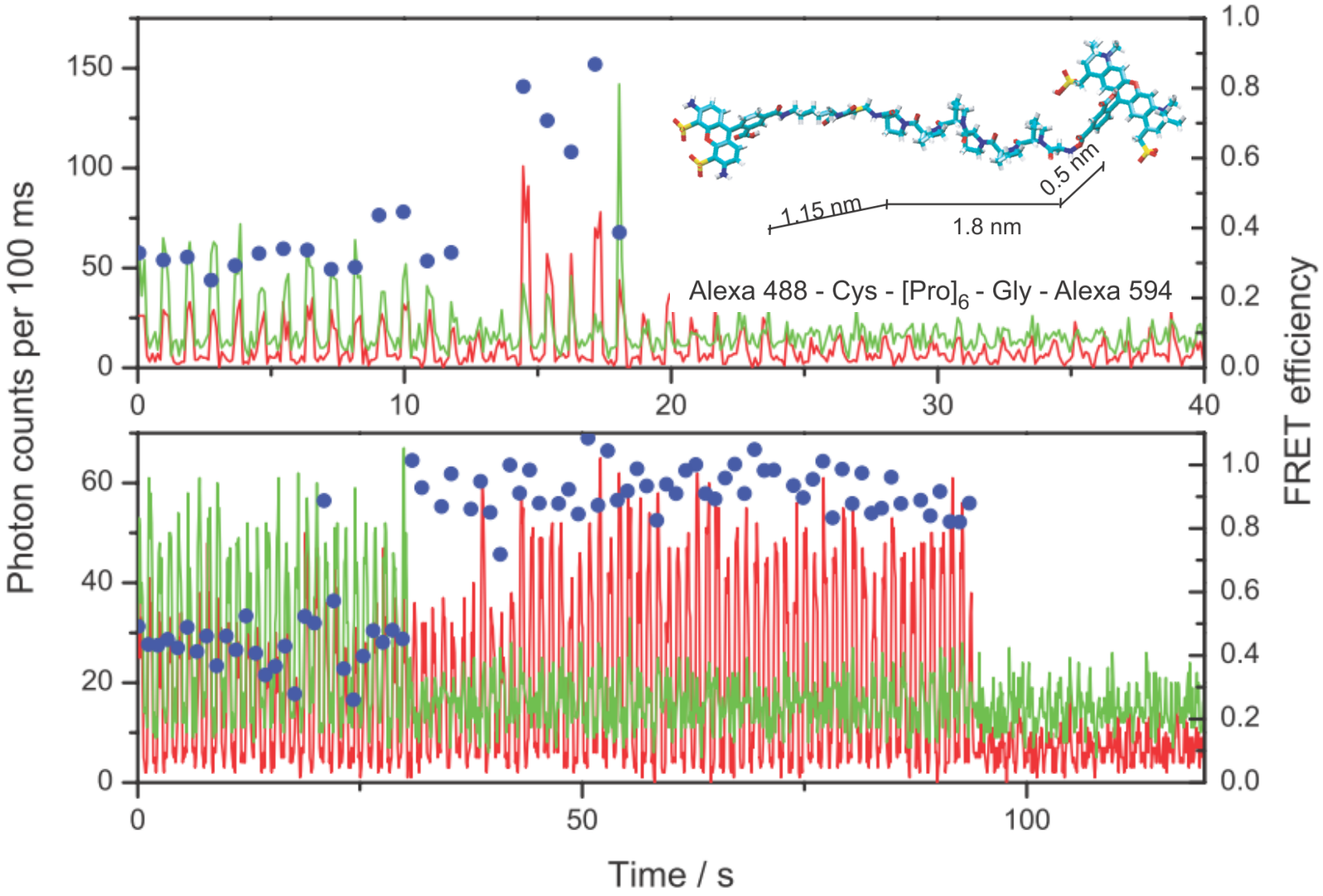
Alexa594 - Gly - $[Pro]_6$ - Cys - Alexa488





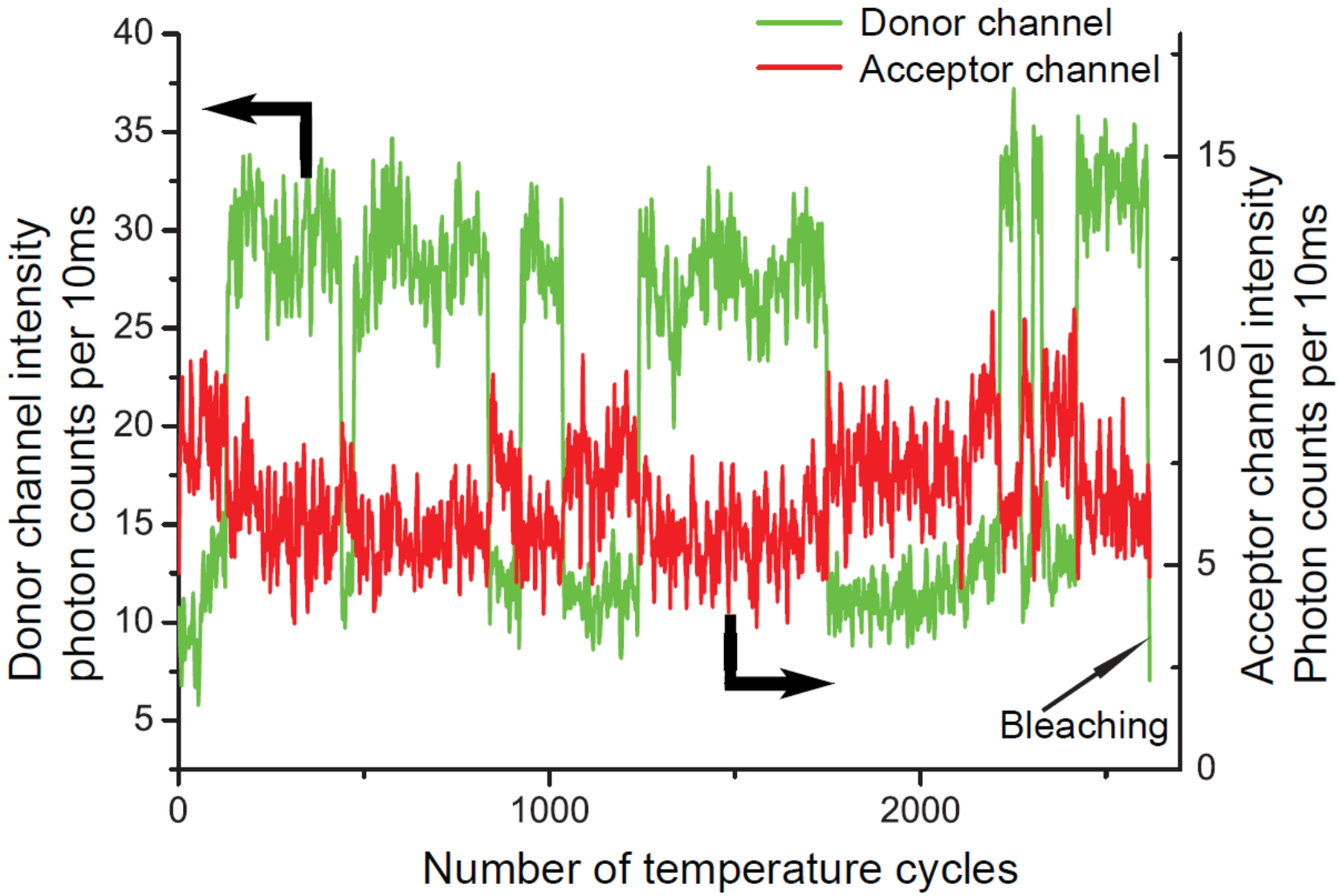


Examples of temperature cycles (polyproline)



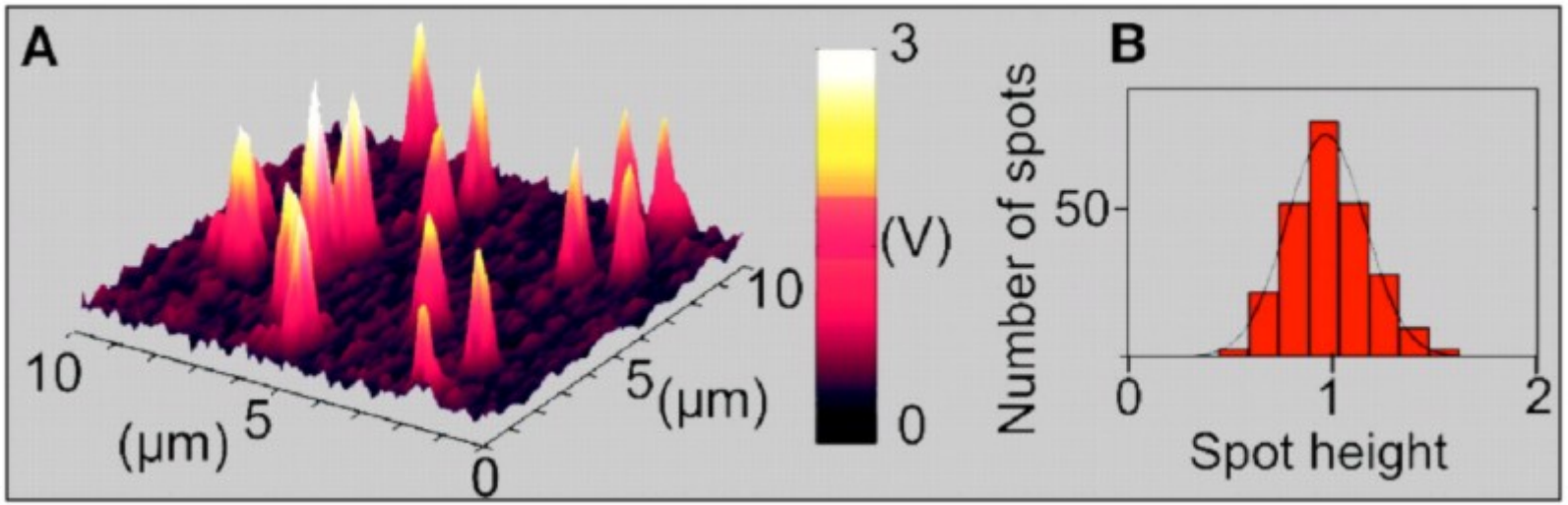
Changes in FRET due to dye reorientations;
Yuan et al., PCCP (2011).

Long series of temperature cycles (170-250 K)



Imaging Absorption by Photothermal Contrast

Interferometric detection of the temperature rise due to absorption

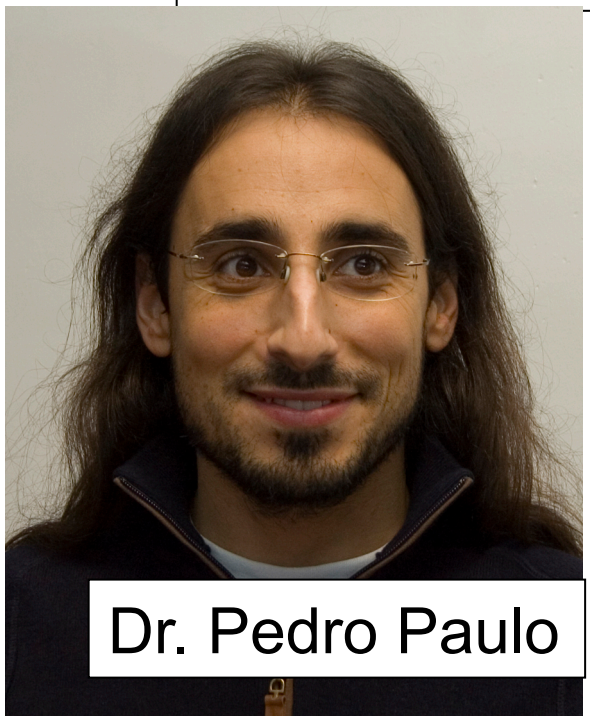


Au colloids, diameter 5 nm

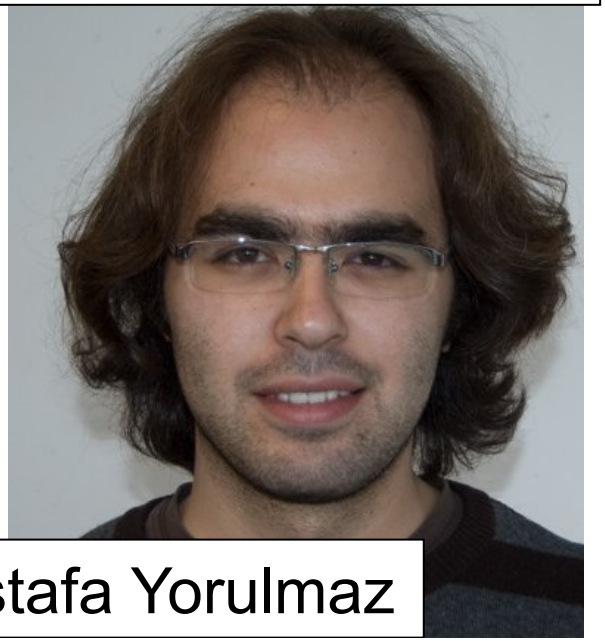
from D. Boyer et al., Science **297** (2002) 1160

Dr. Florian Kulzer

Dr. Alexander Gaiduk

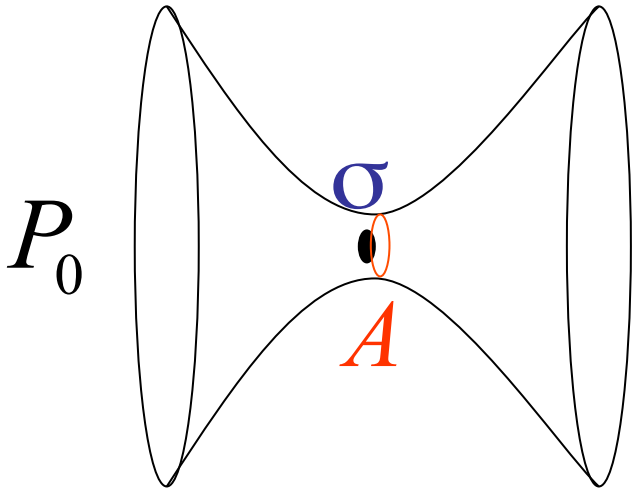


Dr. Pedro Paulo



Mustafa Yorulmaz

Signal-to-Noise Ratio in direct absorption



- signal $\frac{\sigma}{A}\frac{P_0}{h\nu}\cdot\Delta t$

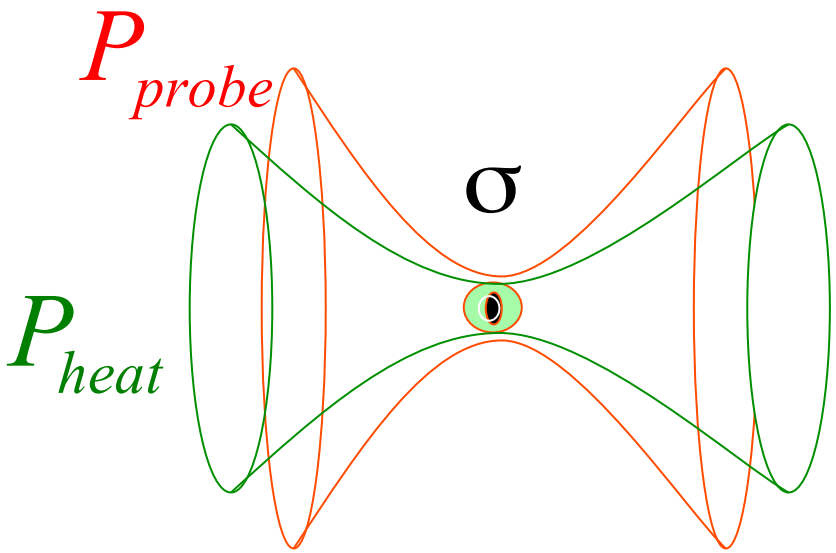
- noise $\sqrt{\frac{P_0\cdot\Delta t}{h\nu}}$

$$SNR=\frac{\sigma}{A}\sqrt{\frac{P_0\cdot\Delta t}{h\nu}}$$

is limited by the saturation power

$$P_0 < P_{sat}$$

Signal/Noise in photothermal detection



- signal $\frac{P_{probe}}{h\nu} \cdot \frac{1}{VC_P} \frac{\partial n}{\partial T} P_{heat} \cdot \tau$

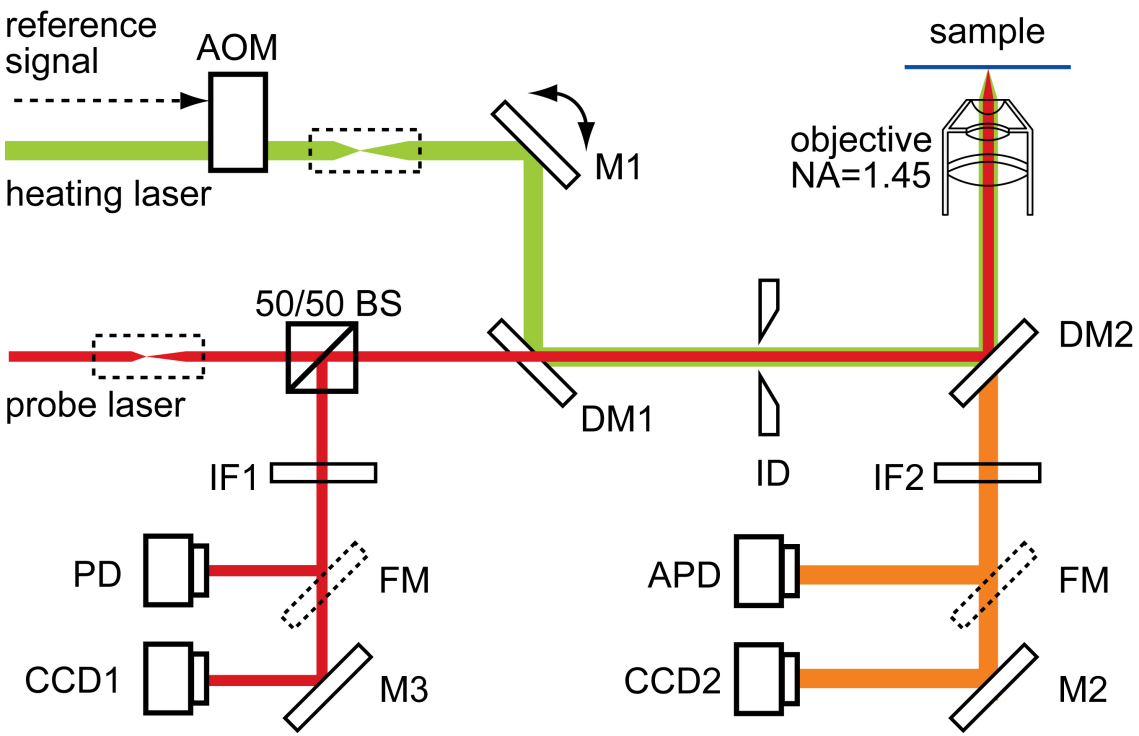
- noise $\sqrt{\frac{P_{probe} \cdot \Delta t}{h\nu}}$

$$(SNR)_{phototh.} = \Delta n_{sat} \sqrt{\frac{P_{probe} \cdot \Delta t}{h\nu}}$$

$\Delta n_{sat} = \frac{1}{VC_P} \frac{\partial n}{\partial T} P_{sat} \cdot \tau$ is limited by saturation,

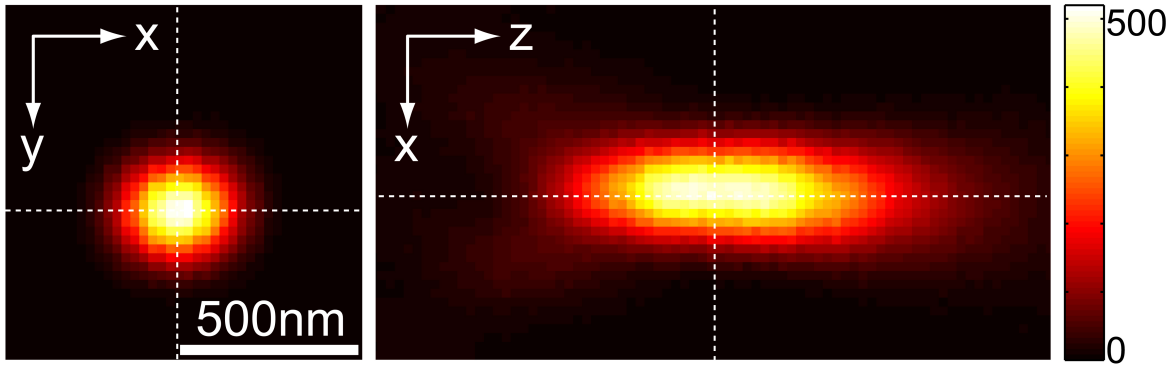
but SNR is not (in principle).

Optical setup



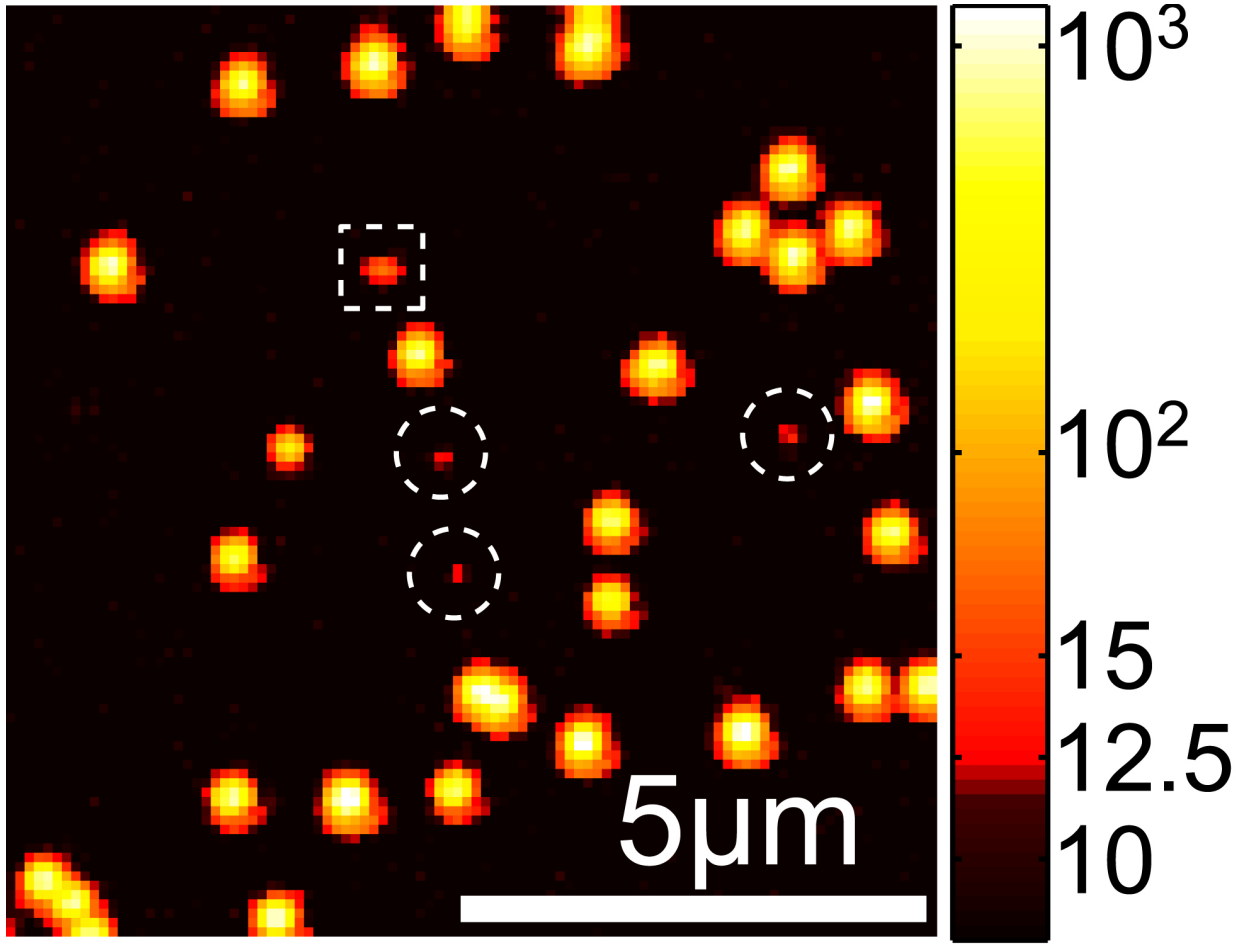
Similar to:
Berciaud et al., PRB **73** (2006) 045424

Fluorescence and photothermal



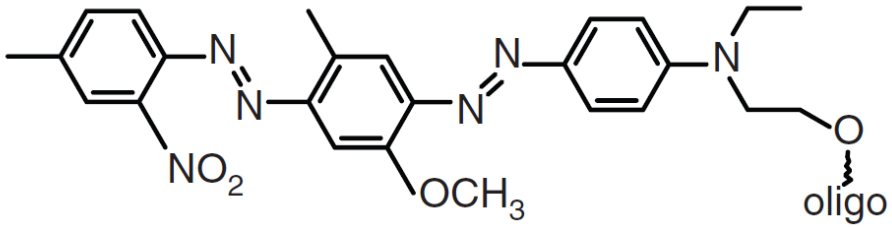
Point spread function:
about 200 nm FWHM

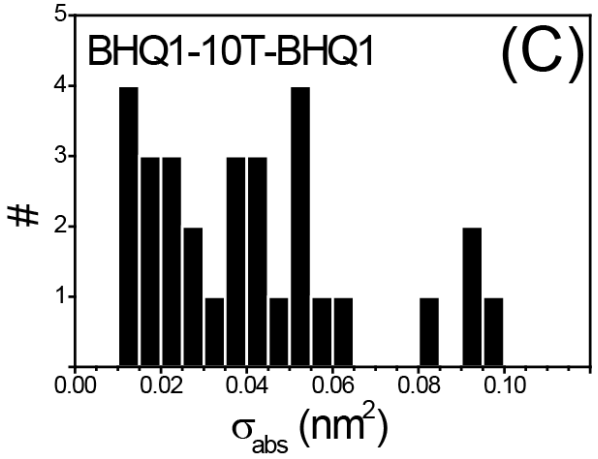
Simultaneous imaging of 5 nm and 20 nm Au NP's

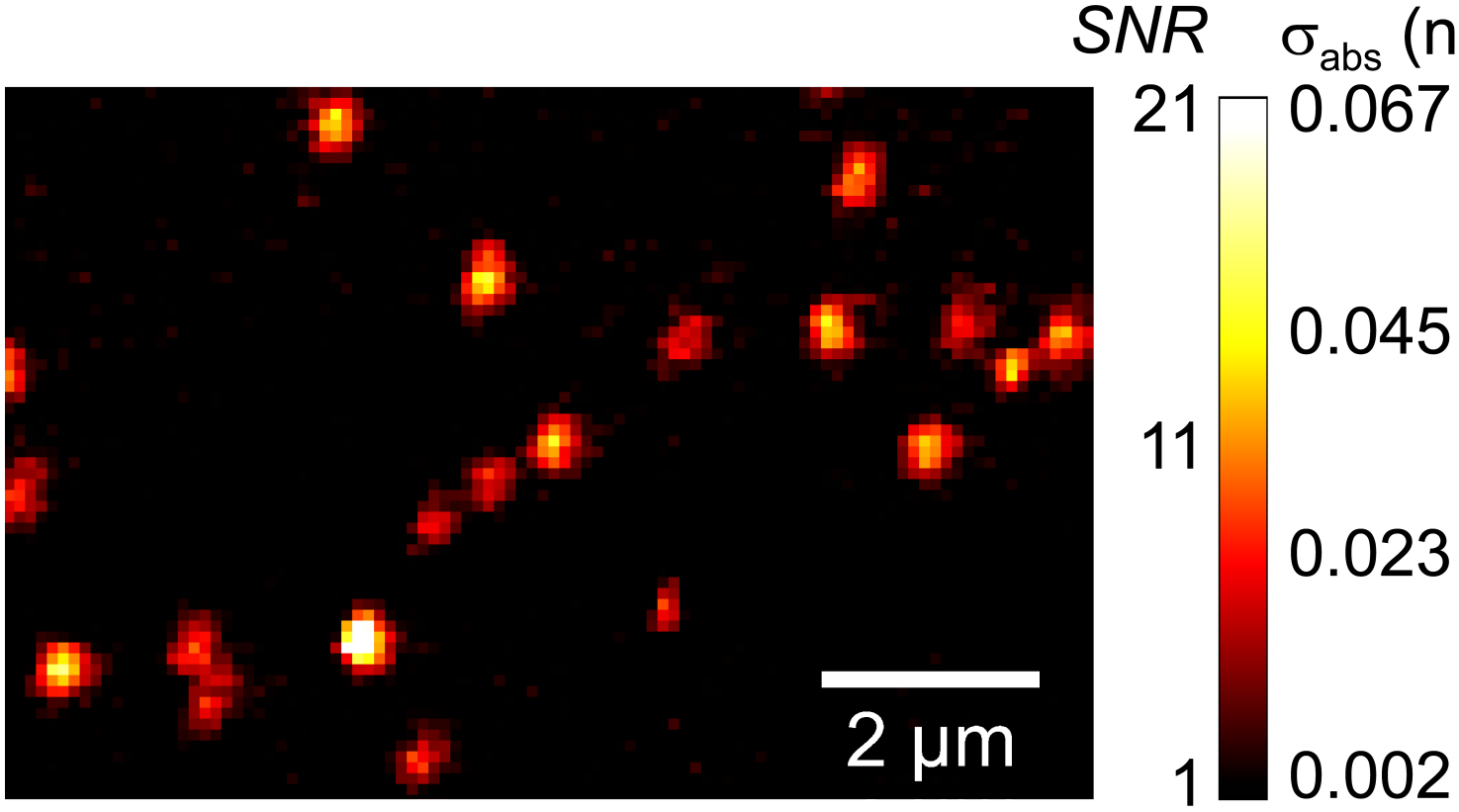


Gaiduk et al., Chemical Science 1 (2010) 343.

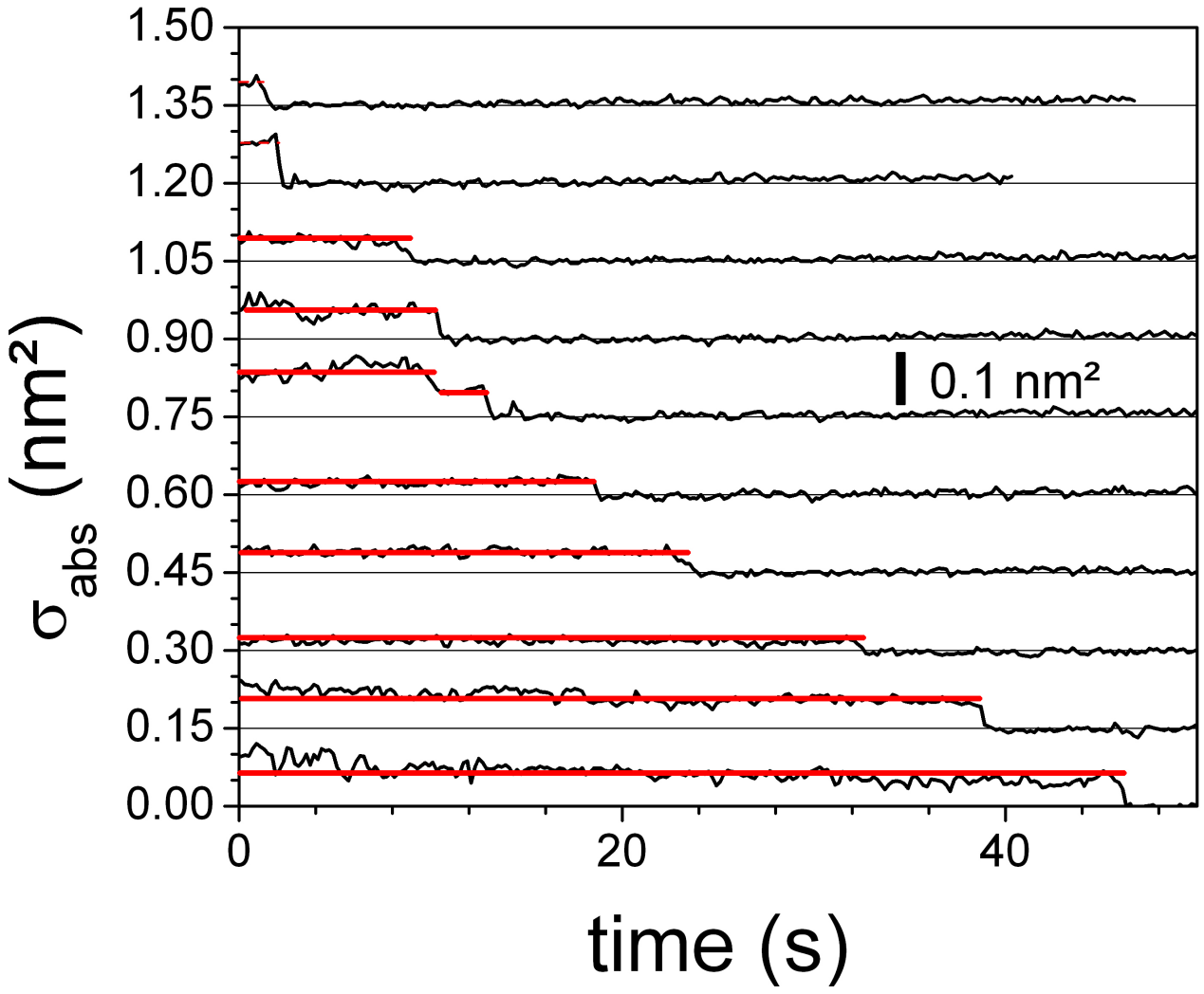
Single-molecule absorption





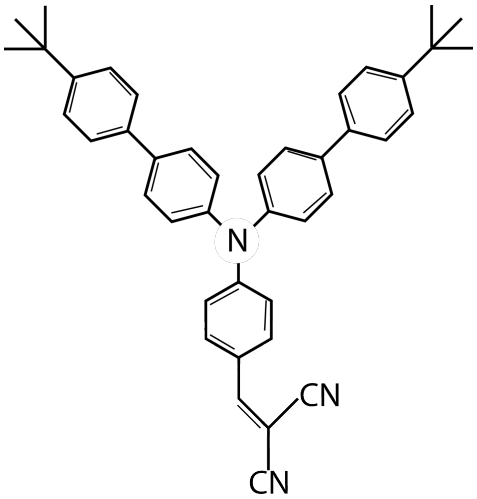


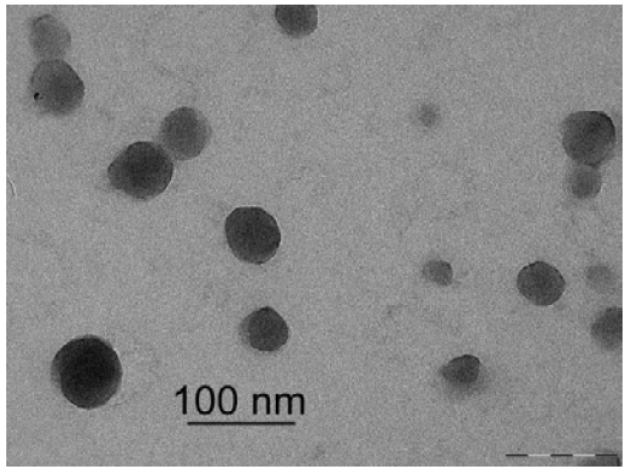
Single-step photobleaching

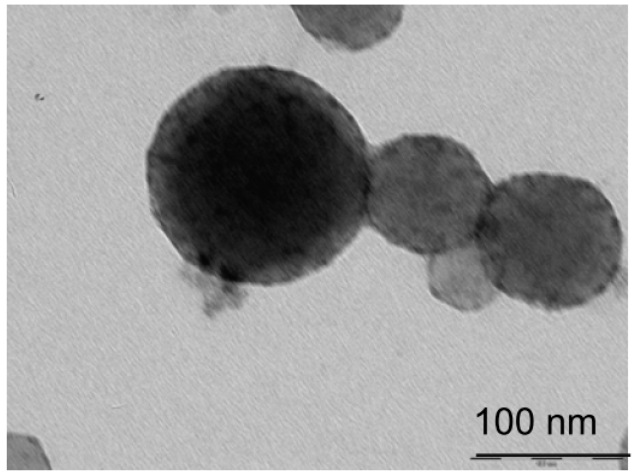


Gaiduk et al., Science 330 (2010) 353

Determination of luminescence quantum yields

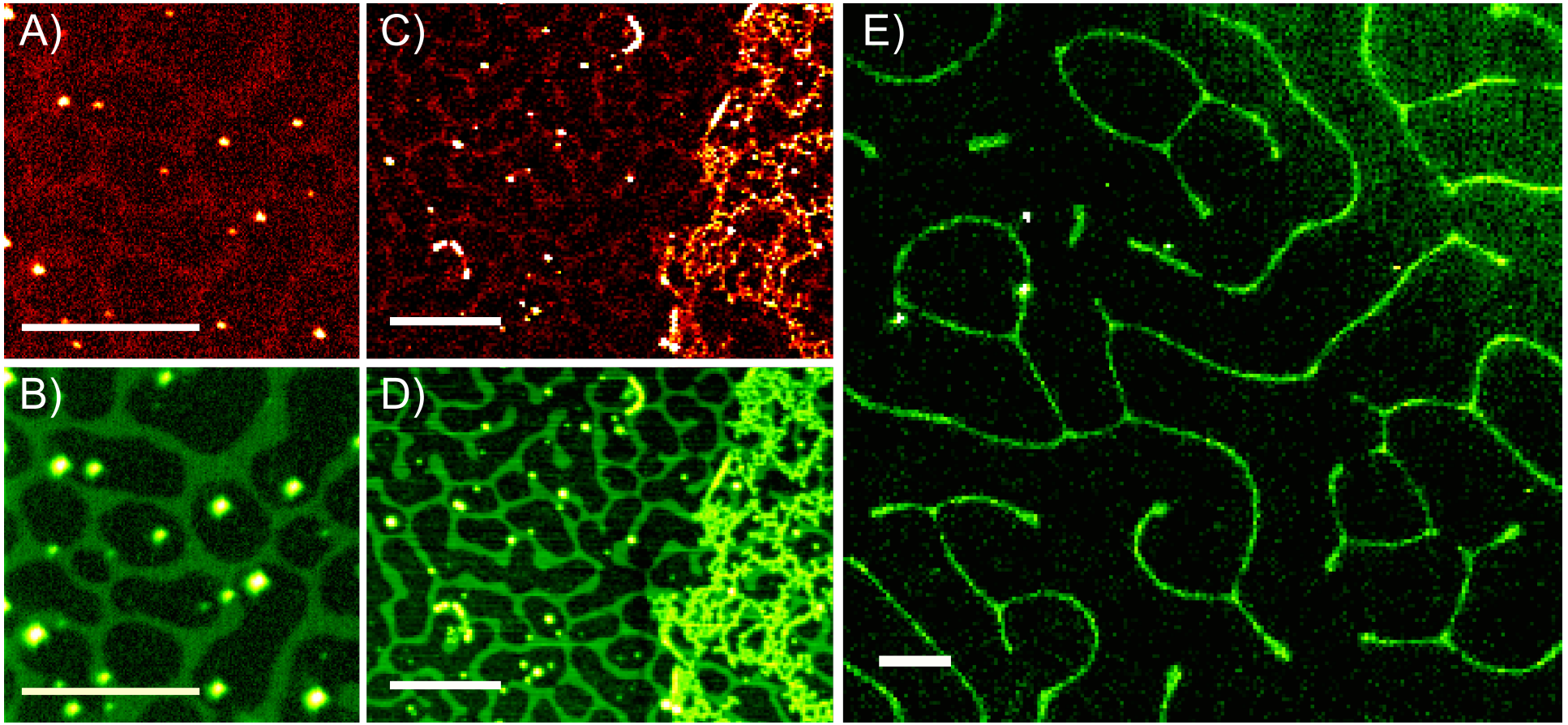






Gaiduk et al., ChemPhysChem **13** (2011) 946; collab. E. Ishow, Nantes

Labyrinth pattern formation upon spin-coating



Correlation between photothermal and fluorescence