single-molecule fluorescence resonance energy transfer II

(5) time resolution

michael börsch

19/11/2004

single-molecule FRET history

- Taekjip Ha: first experiments in 1996

Proc. Natl. Acad. Sci. USA
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Biophysics

Probing the interaction between two single molecules: Fluorescence resonance energy transfer between a single donor and a single acceptor

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Communicated by I. Tinoco, Jr., University of California, Berkeley, CA, March 5, 1996 (received for review January 17, 1996)

single-molecule FRET history

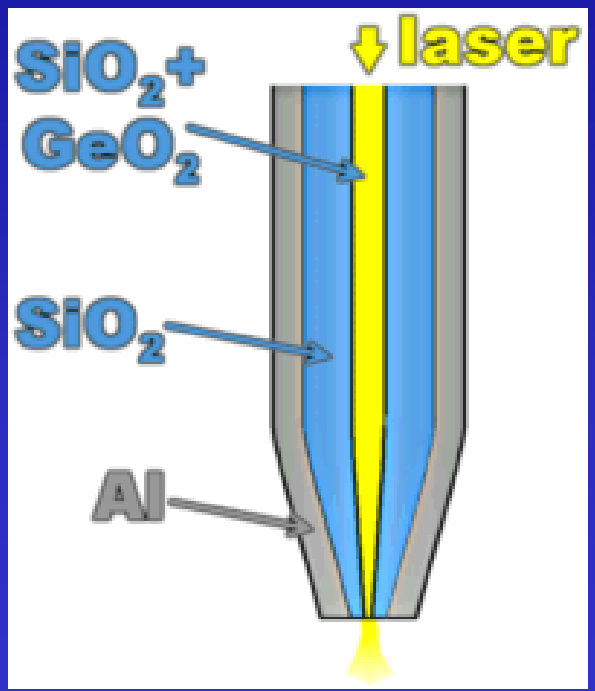
- Taekjip Ha: first experiments
- Tetramethylrhodamine TMR
- TexasRed TR
- DNA oligomer as spacer
  10mer E_{FRET}=0.65
  20mer E_{FRET}=0.32

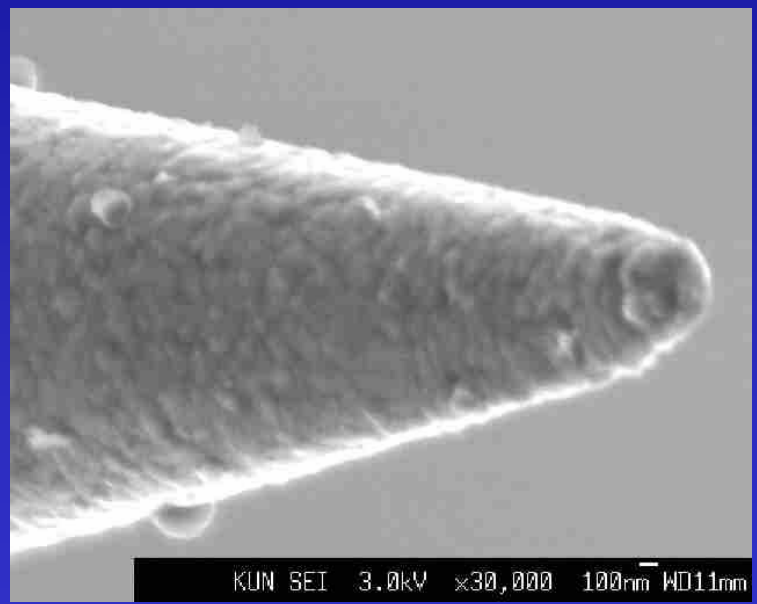
ABSTRACT We extend the sensitivity of fluorescence resonance energy transfer (FRET) to the single molecule level by measuring energy transfer between a single donor fluorophore and a single acceptor fluorophore. Near-field scanning optical microscopy (NSOM) is used to obtain simultaneous dual color images and emission spectra from donor and acceptor fluorophores linked by a short DNA molecule. Photodestruction dynamics of the donor or acceptor are used to determine the presence and efficiency of energy transfer. The classical equations used to measure energy transfer on ensembles of fluorophores are modified for single-molecule measurements. In contrast to ensemble measurements, dynamic events on a molecular scale are observable in single pair FRET measurements because they are not canceled out by random averaging. Monitoring conformational changes, such as rotations and distance changes on a nanometer scale, within single biological macromolecules, may be possible with single pair FRET.

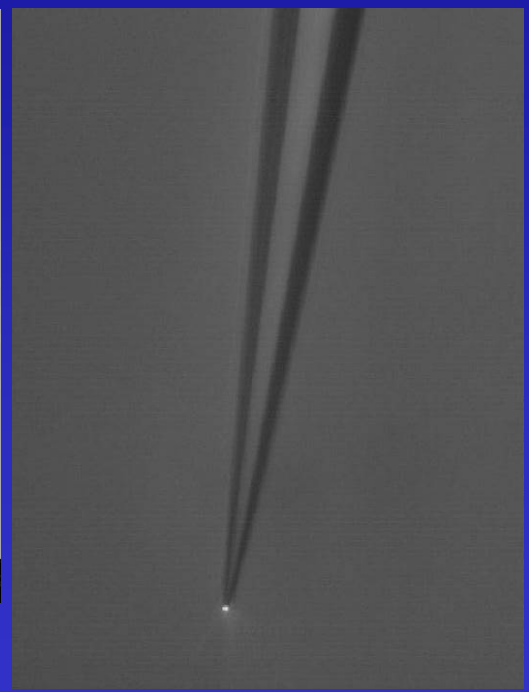
- anisotropy $r_{(TMR)}$ = 0.17
  $r_{(TR)}$ = 0.16
- immobilized on glass, moving the sample for scanning

single-molecule FRET history

- Nearfield Scanning Optical Microscope :
  5 nm distance between tip and sample



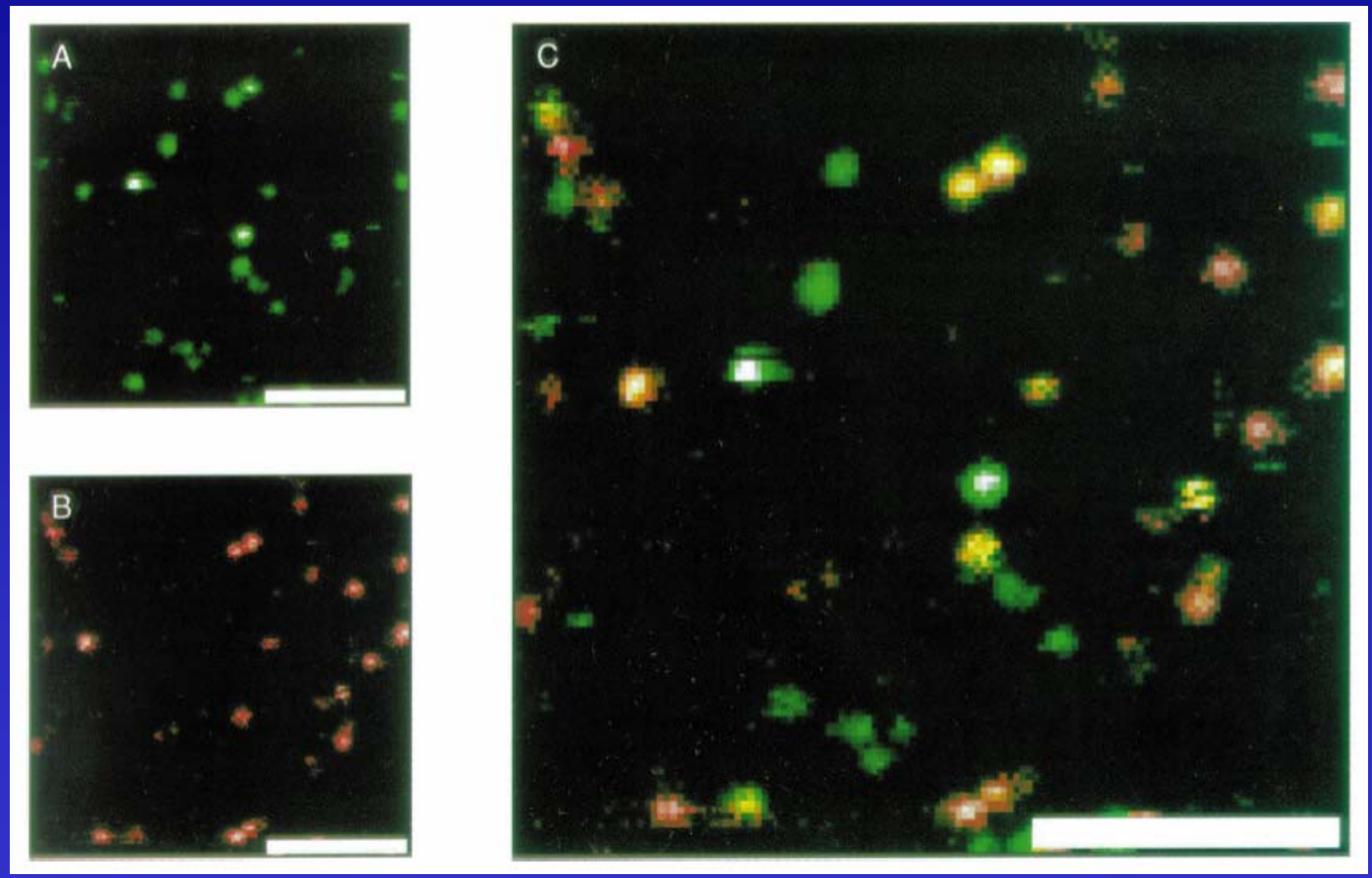




514.5 nm: 5 to 40 nW at the sample

single-molecule FRET history

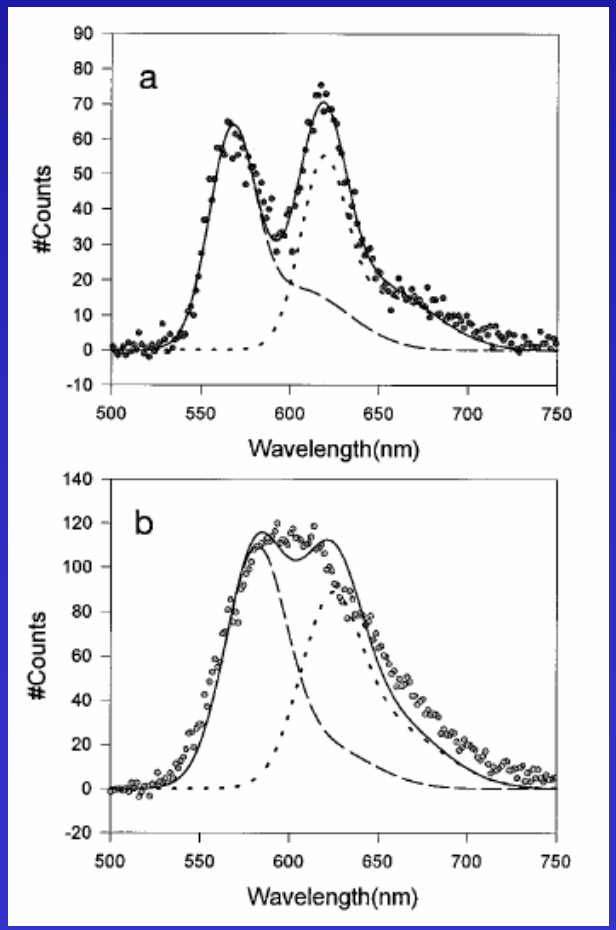
- NSOM
  5 nm distance between tip and sample
- 514.5 nm:
  5 to 40 nW
  at the sample
- interference filters: 50nm
- 2 APDs for FRET donor (green) and FRET acceptor (red), false colored E_{FRET}



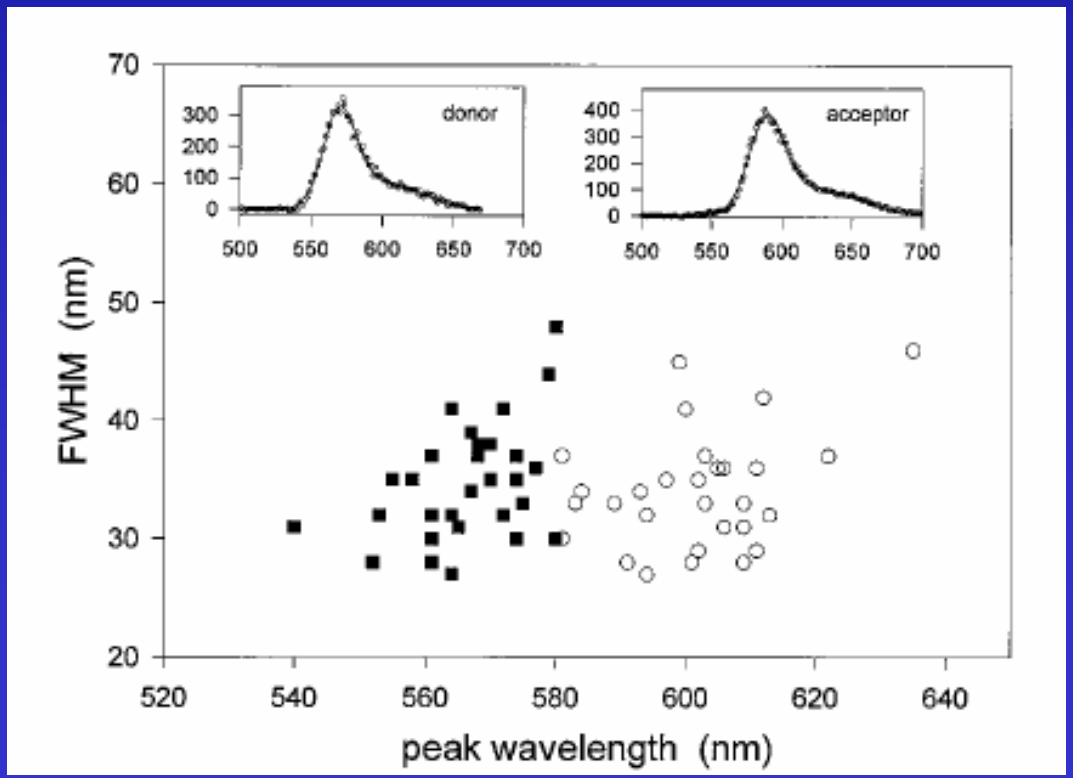
single-molecule FRET history

- Taekjip Ha: first expriments

FRET-labelled 20mer (a) and 10mer (b)



spectra of individual single-labelled DNA molecules
TMR: λ_{max} = 566.4 ± 9.1 nm, TR : λ_{max} = 601.6 ± 12.2 nm

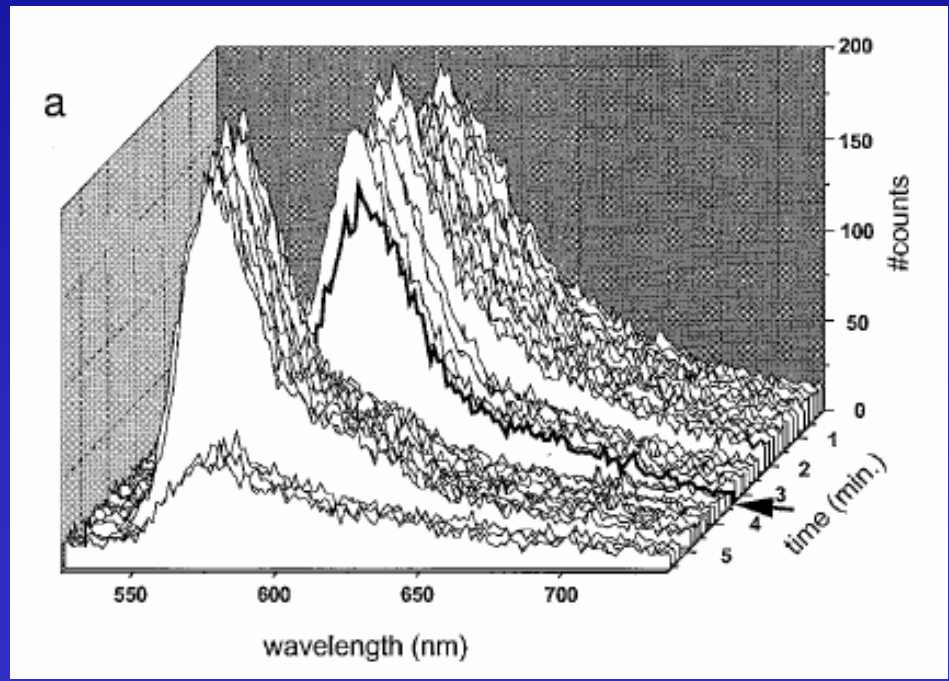


single-molecule FRET history

- Taekjip Ha: first expriments

- dynamics: photobleaching of the acceptor after 3.5 min

- time resolution:



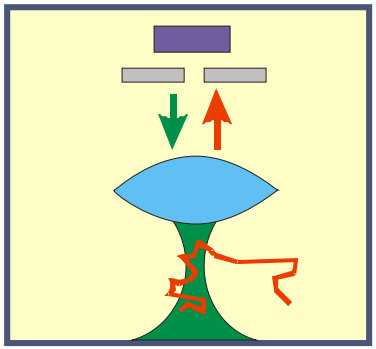
10 ms / pixel with APDs for single-molecule FRET imaging,

1 sec / spectrum with liquid nitrogen cooled CCD

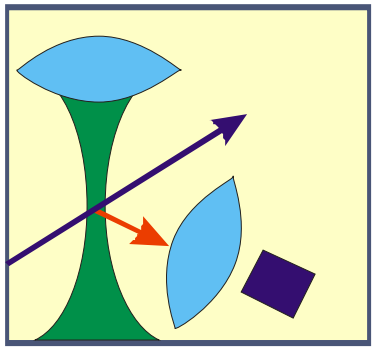
methods for single-molecule detection

in solution

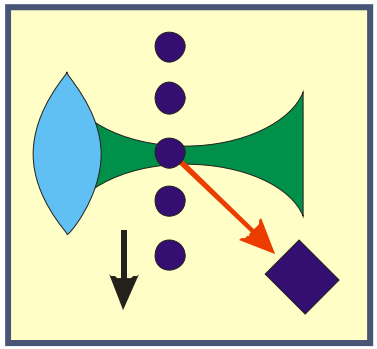
FCS



flow cell

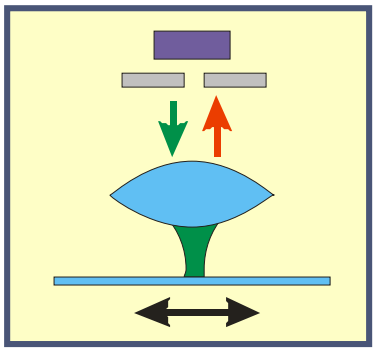


microdroplets

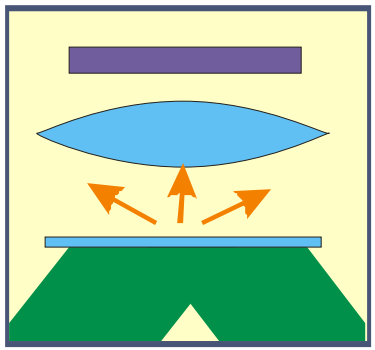


on surfaces

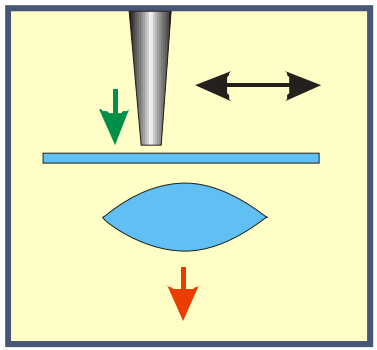
confocal microscope



widefield or TIRF plus CCD camera



near field microscope



methods for single-molecule detection

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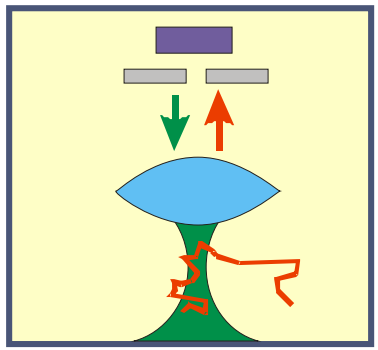

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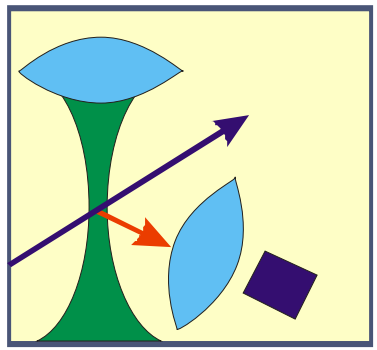

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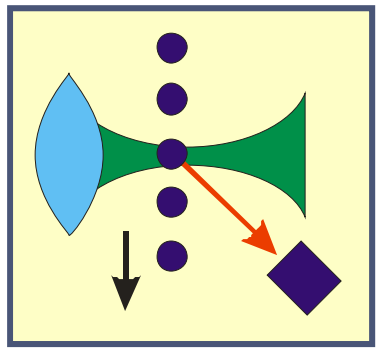
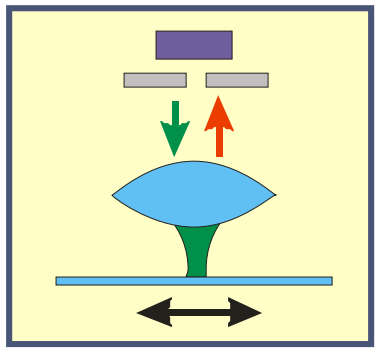
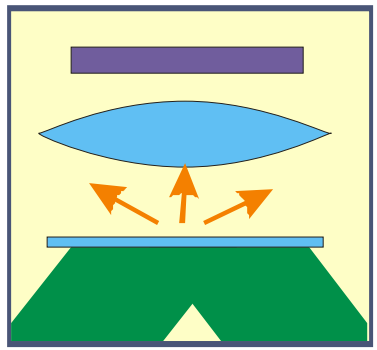


on surfaces

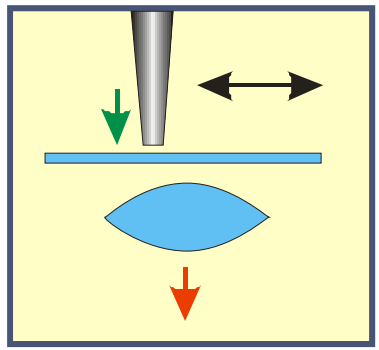
confocal microscope



widefield or TIRF plus CCD camera



near field microscope



single-molecule FRET history

- Taekjip Ha: enzyme dynamics in 1999

Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 893–898, February 1999
Biophysics

Single-molecule fluorescence spectroscopy of enzyme conformational dynamics and cleavage mechanism

TAEKJIP HA*†‡, ALICE Y. TING‡§, JOY LIANG*, W. BRETT CALDWELL§, ASHOK A. DENIZ§, DANIEL S. CHEMLA*¶, PETER G. SCHULTZ§‖, AND SHIMON WEISS*‖**

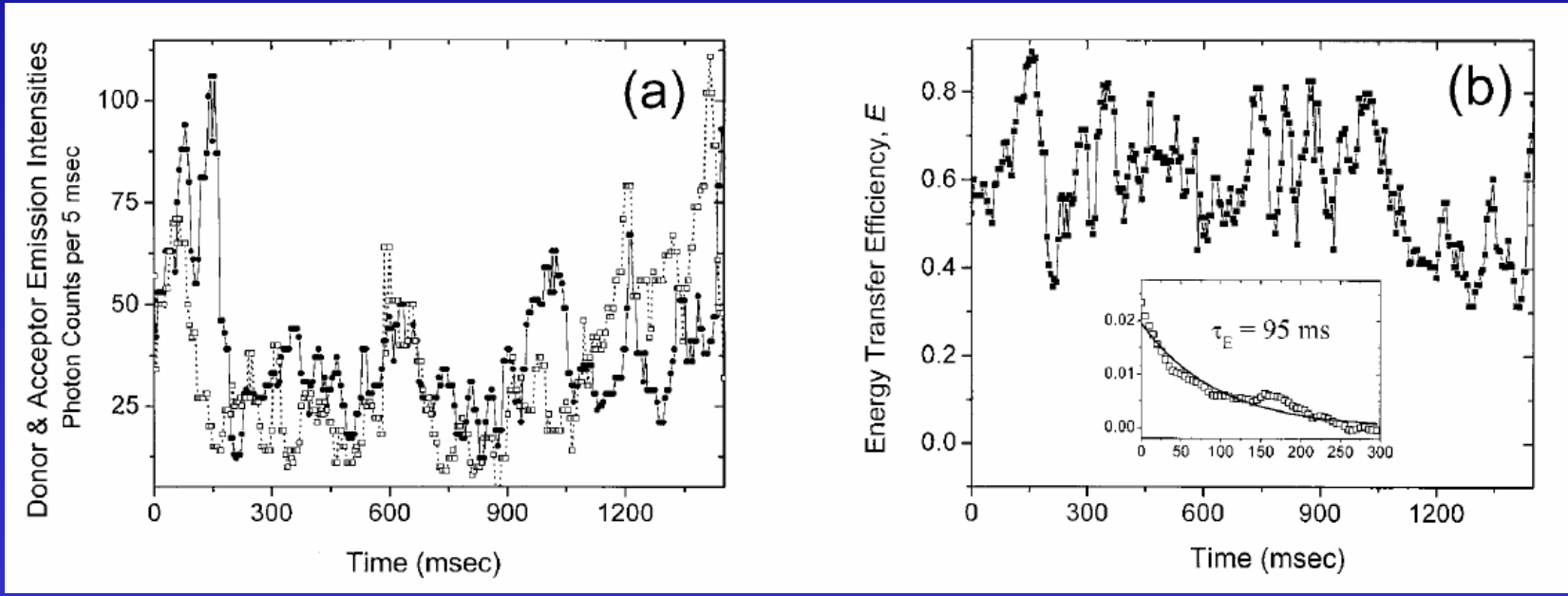
*Materials Sciences Division and **Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720; and §Howard Hughes Medical Institute, Department of Chemistry and ¶Department of Physics, University of California, Berkeley, CA 94720

Contributed by Peter G. Schultz, November 12, 1998

- staphylococcal nuclease immobilized on glass
- hydrolysis of DNA or RNA

single-molecule FRET history

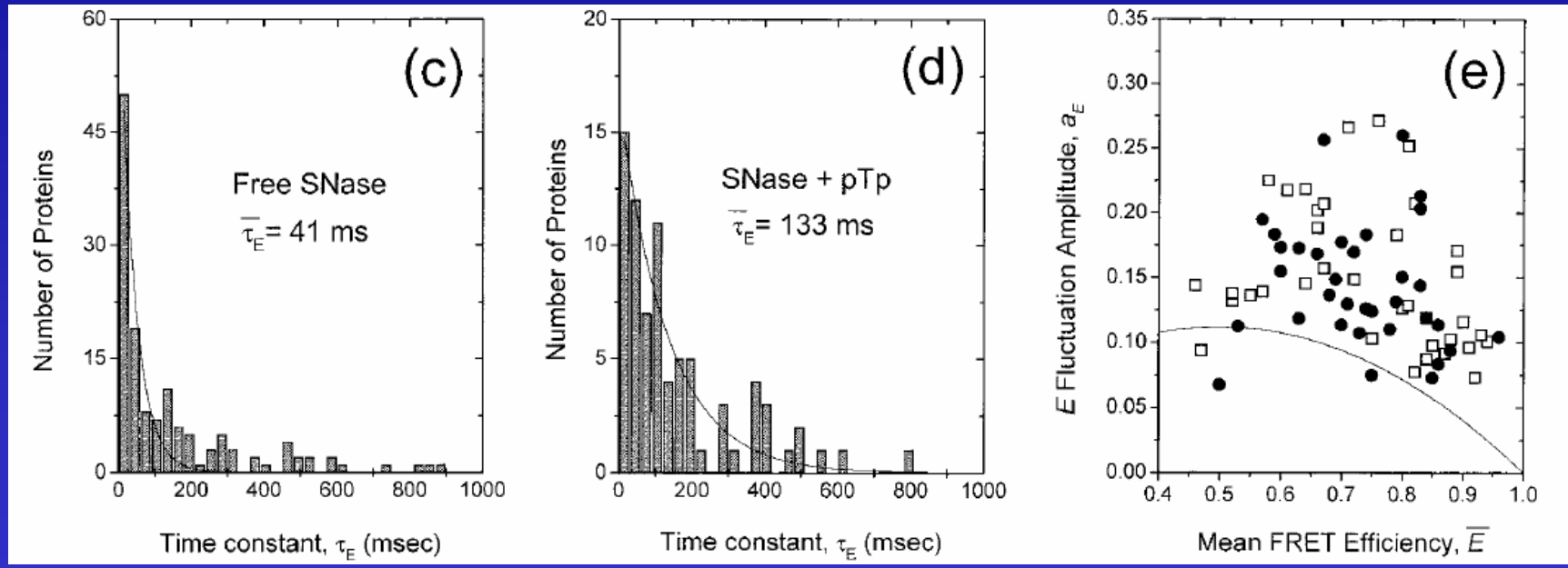
- Taekjip Ha: enzyme dynamics in 1999



staphylococcal nuclease immobilized on glass, with two labels (TMR and Cy5),

single-molecule FRET history

- Taekjip Ha: dynamics in enzymes 1999

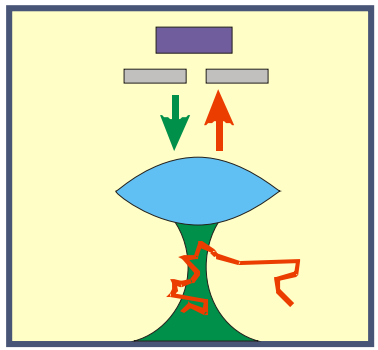


- dwell time analyis of different states,
- inhibition with pTp on the active site
- 5 ms binning

methods for single-molecule detection

in solution

FCS



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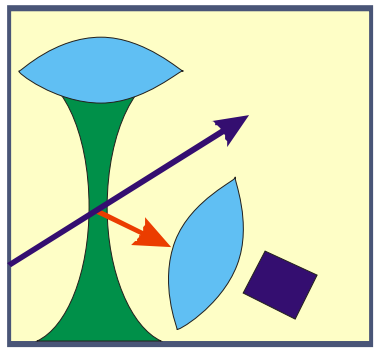
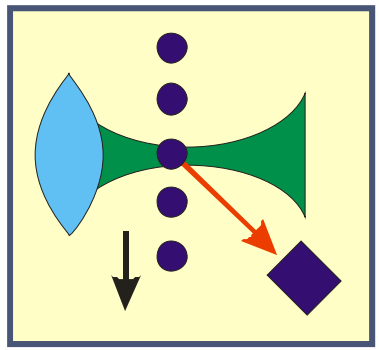
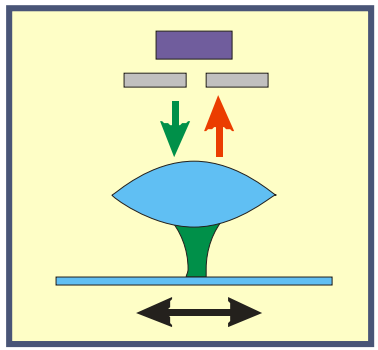


microdroplets

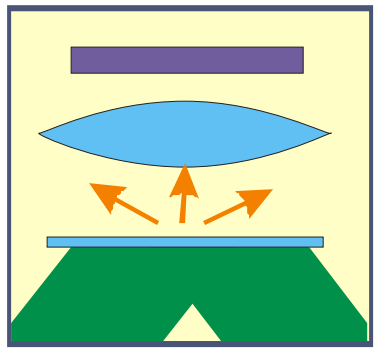


on surfaces

confocal microscope



widefield or TIRF plus CCD camera



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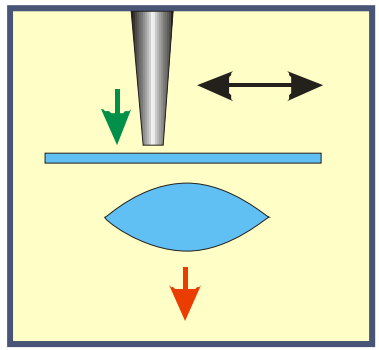
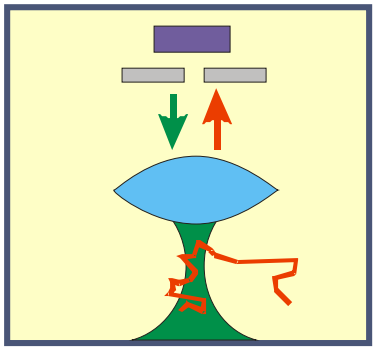


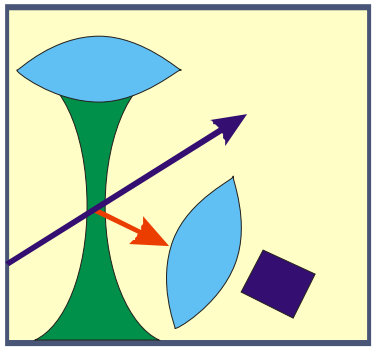
methods for single-molecule detection

in solution

FCS



flow cell



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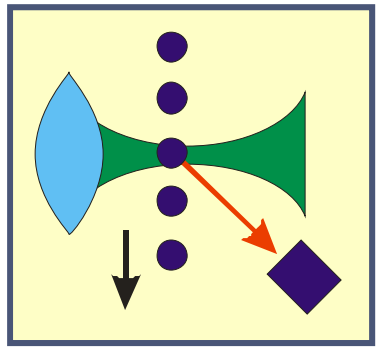

on surfaces

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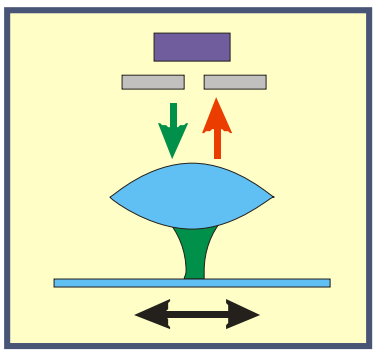
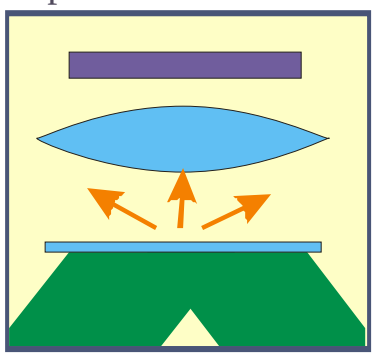
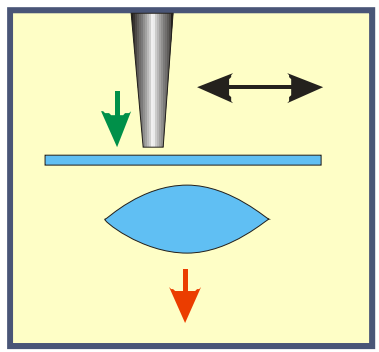


widefield or TIRF plus CCD camera

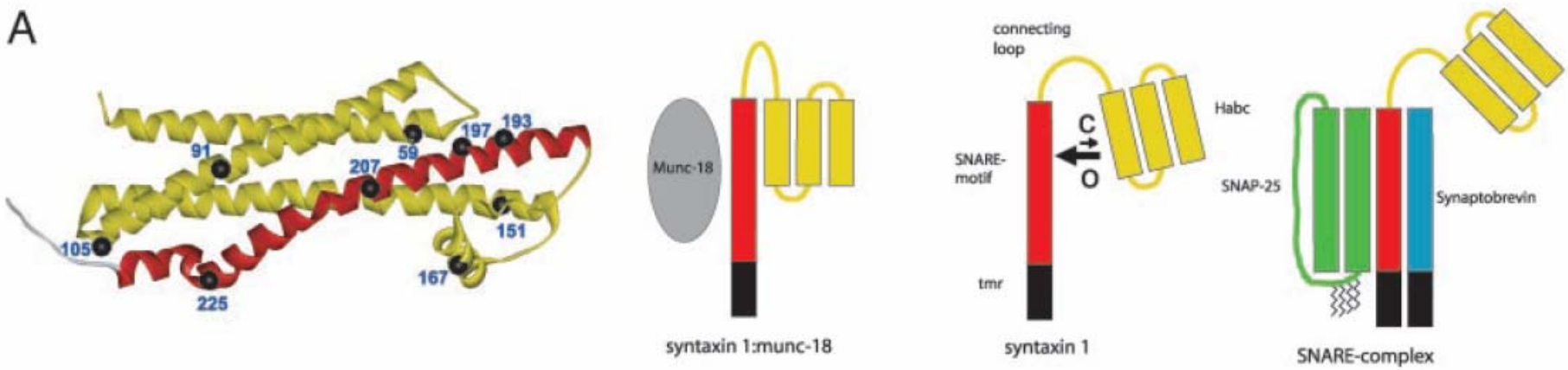


near field microscope



single-molecule FRET history

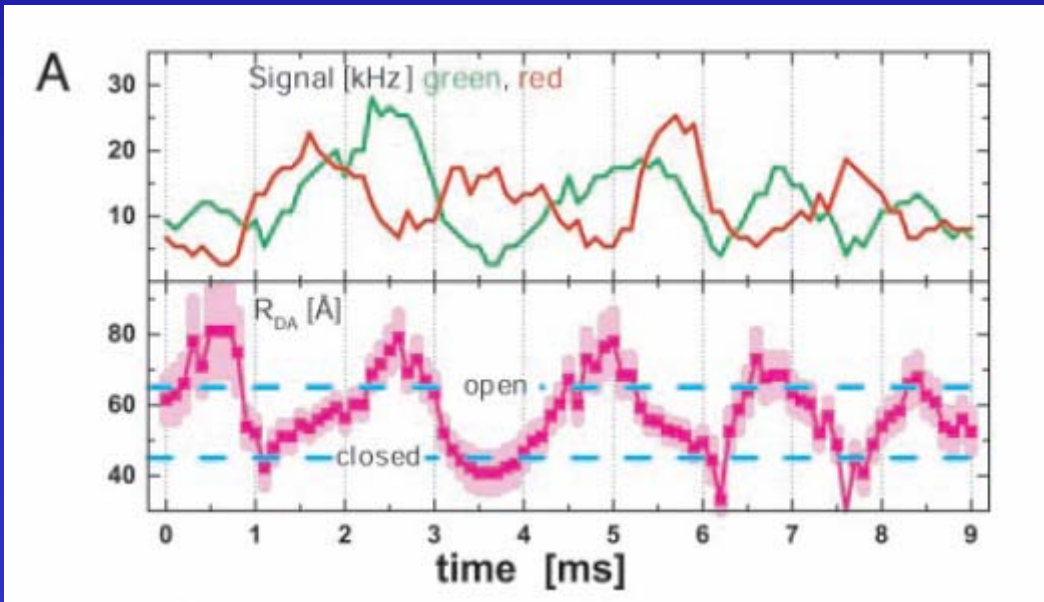
- Claus seidel: dynamic experiments in 2004 (PNAS) conformational dynamics of synthaxin



- intramolecular FRET between pairs of labelled cysteines
- Alexa488 and Alexa594, statistically labelled,

single-molecule FRET history

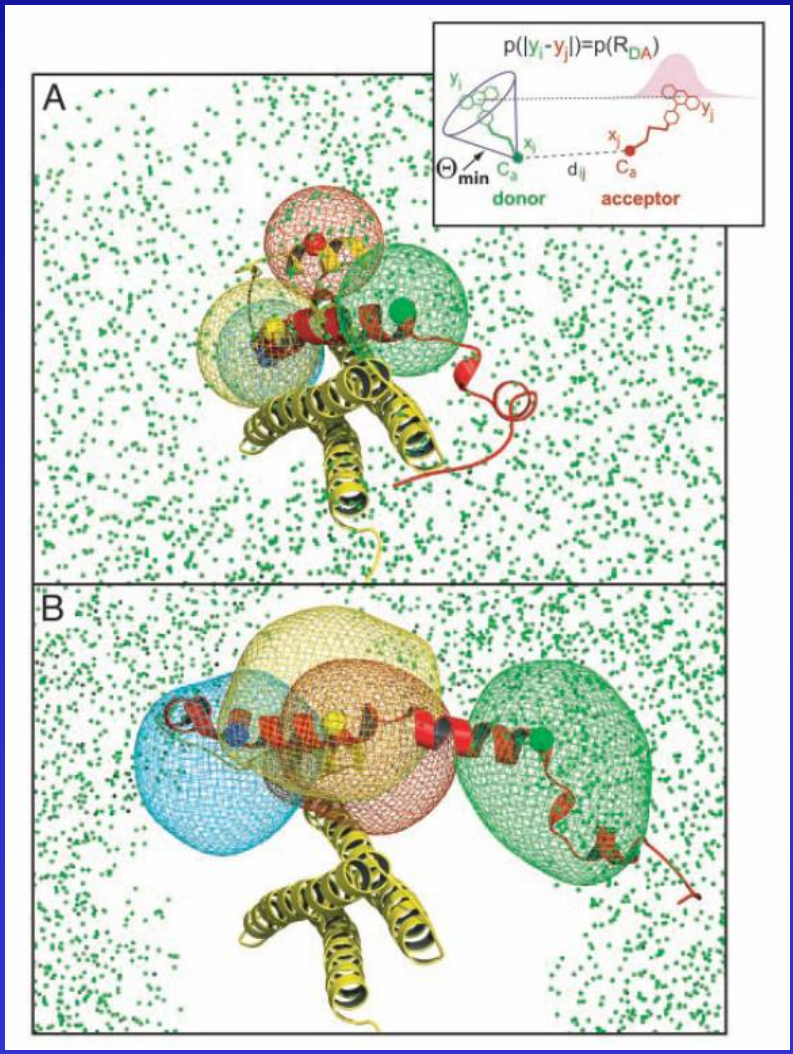
- Claus seidel: dynamic experiments in 2004 (PNAS) conformational dynamics of synthaxin



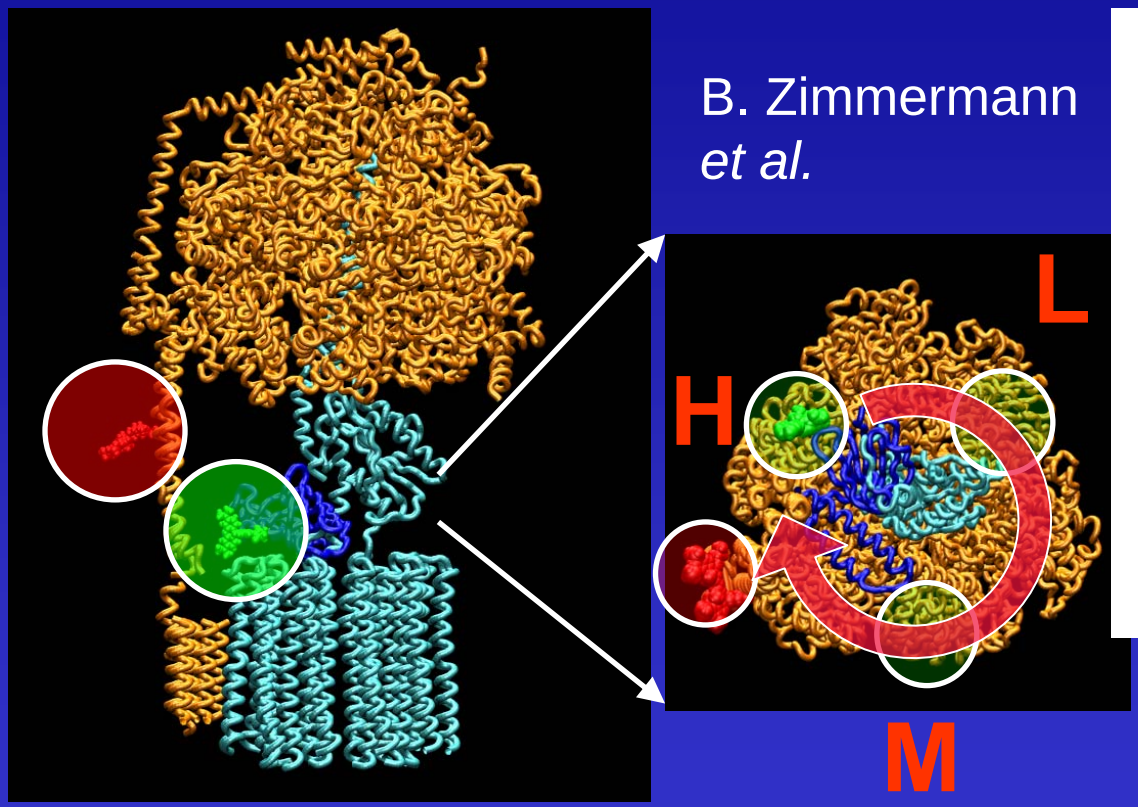
- submillisecond time resolution with intensity-based FRET analysis

single-molecule FRET history

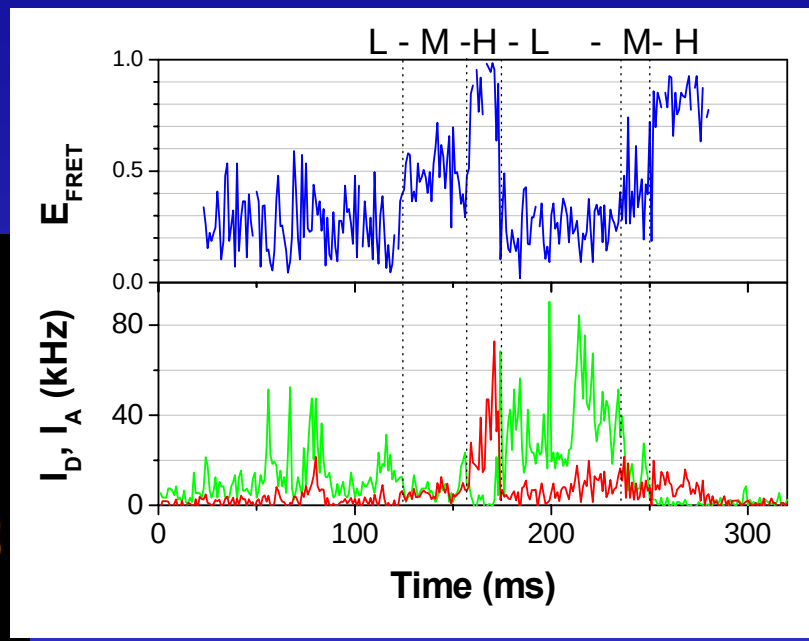
- Claus Seidel (2004) conformational dynamics of synthaxin
- Helmut Grubmüller simulations of fluorophore dynamics for FRET 'wobble in cone' model
- conformational (protein) dynamics impaired by ligands or other proteins



single-molecule FRET: ε-subunit rotation in F_0F_1-ATP synthase



B. Zimmermann *et al.*



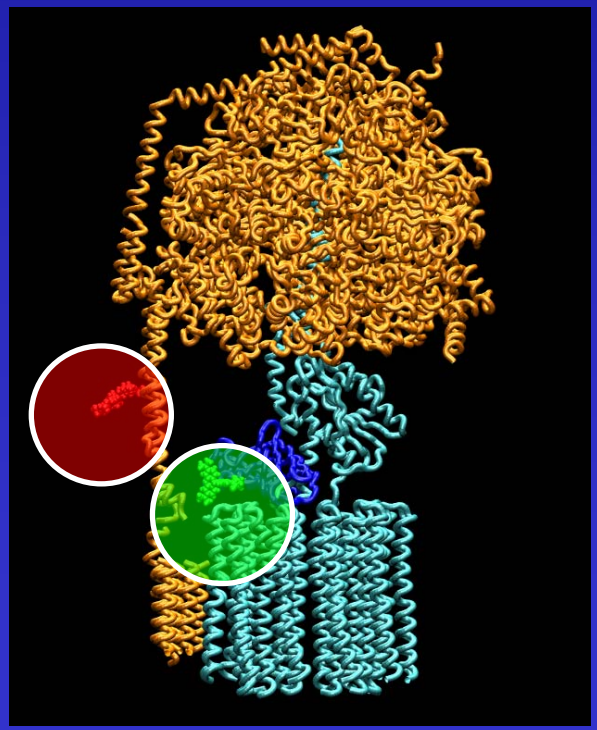
FRET donor tetramethylrhodamine @ ε (rotating)
FRET acceptor cyanine-5 @ b (static)

$$E_{FRET} = \frac{R_0^6}{R_0^6 + r^6}$$

→ detecting rotation by cyclic distance changes

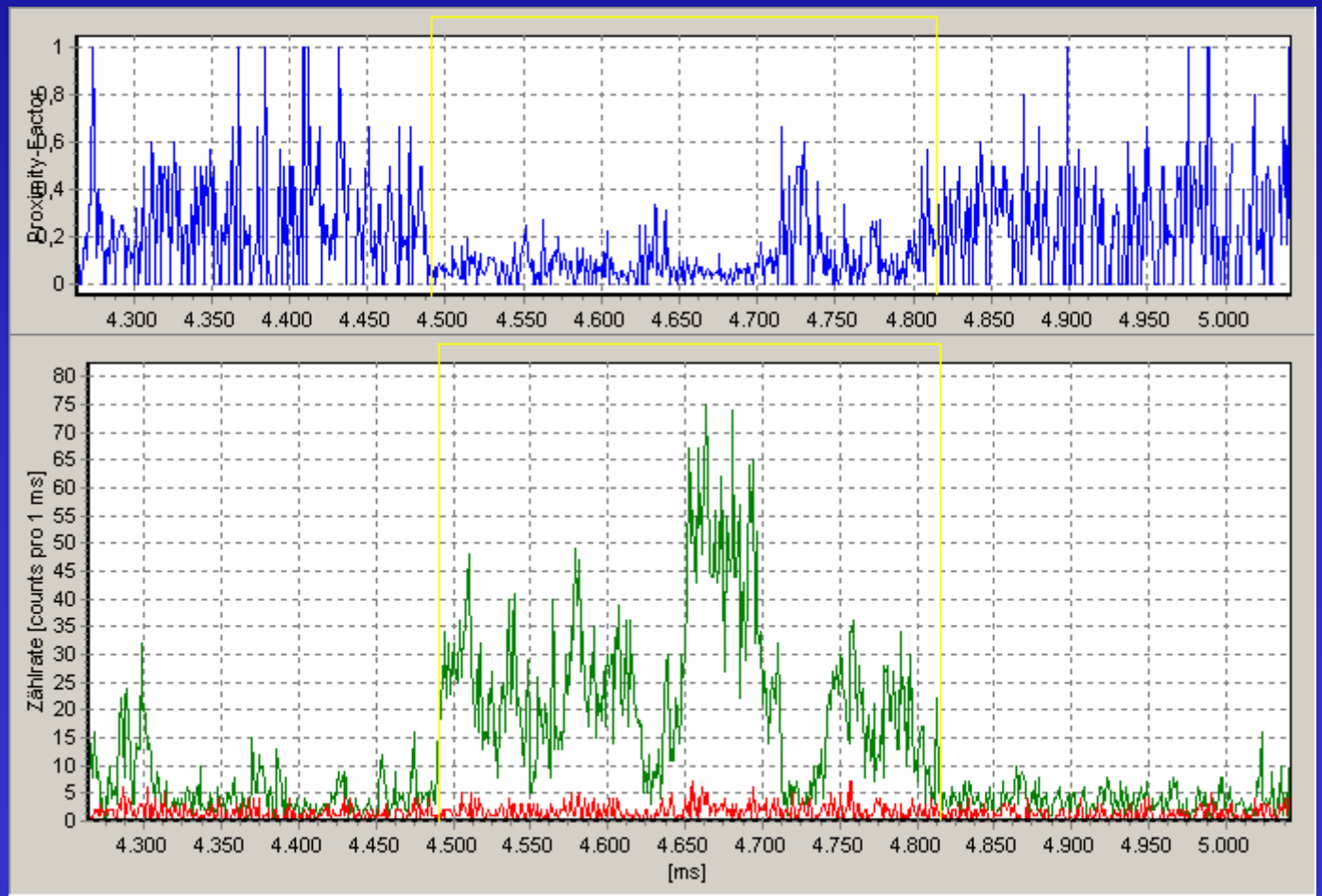
single-molecule FRET: local news

- F_0F_1-ATP synthase trapped with AMPPNP
- ε56 labelled with Alexa546, b64 with bis-Cy5
- lifetime traces: laser@532nm with 50 ps, 40 MHz, PicoTA



single-molecule FRET news

- F_0F_1-ATP synthase with AMPPNP : Donor only burst



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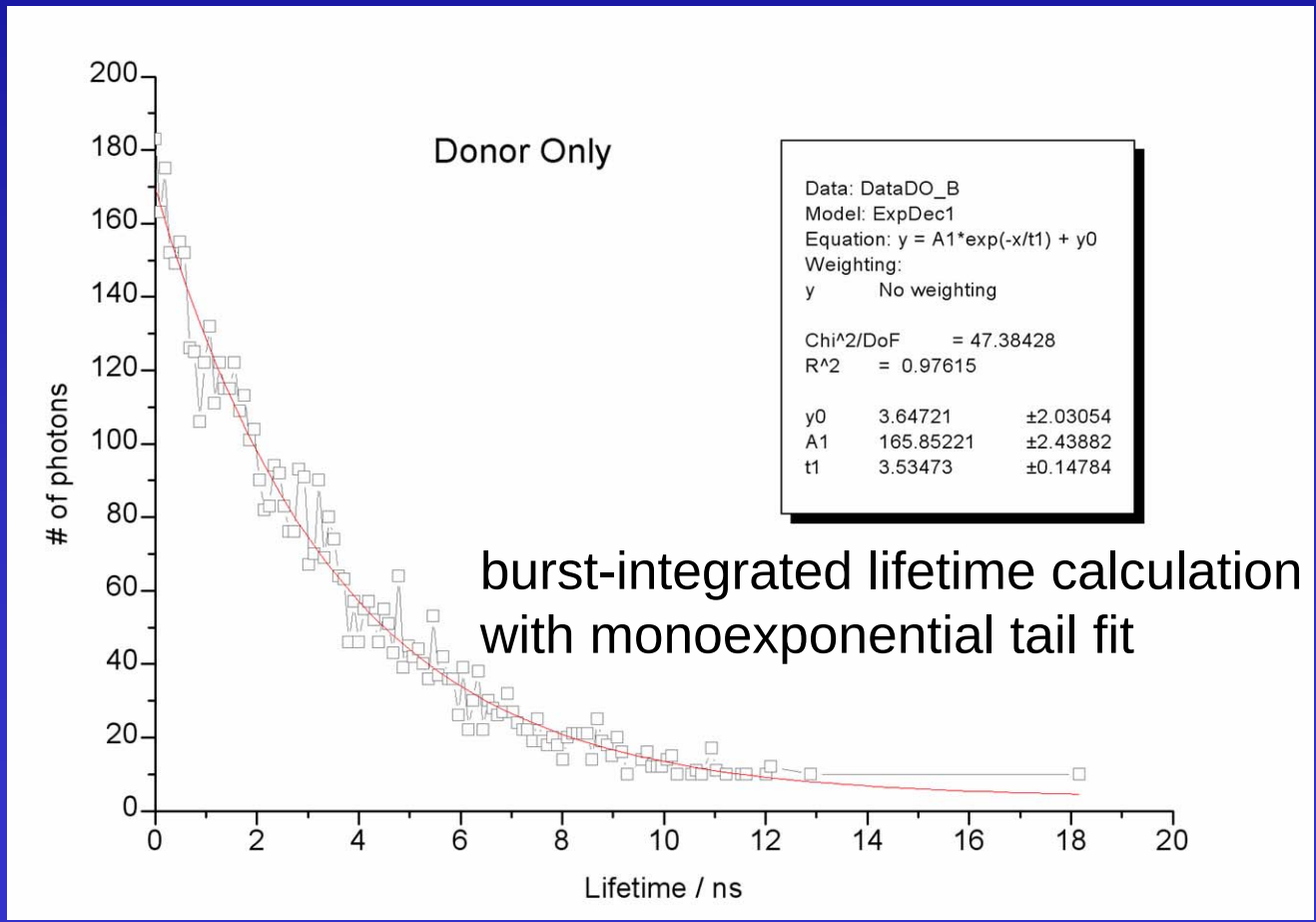
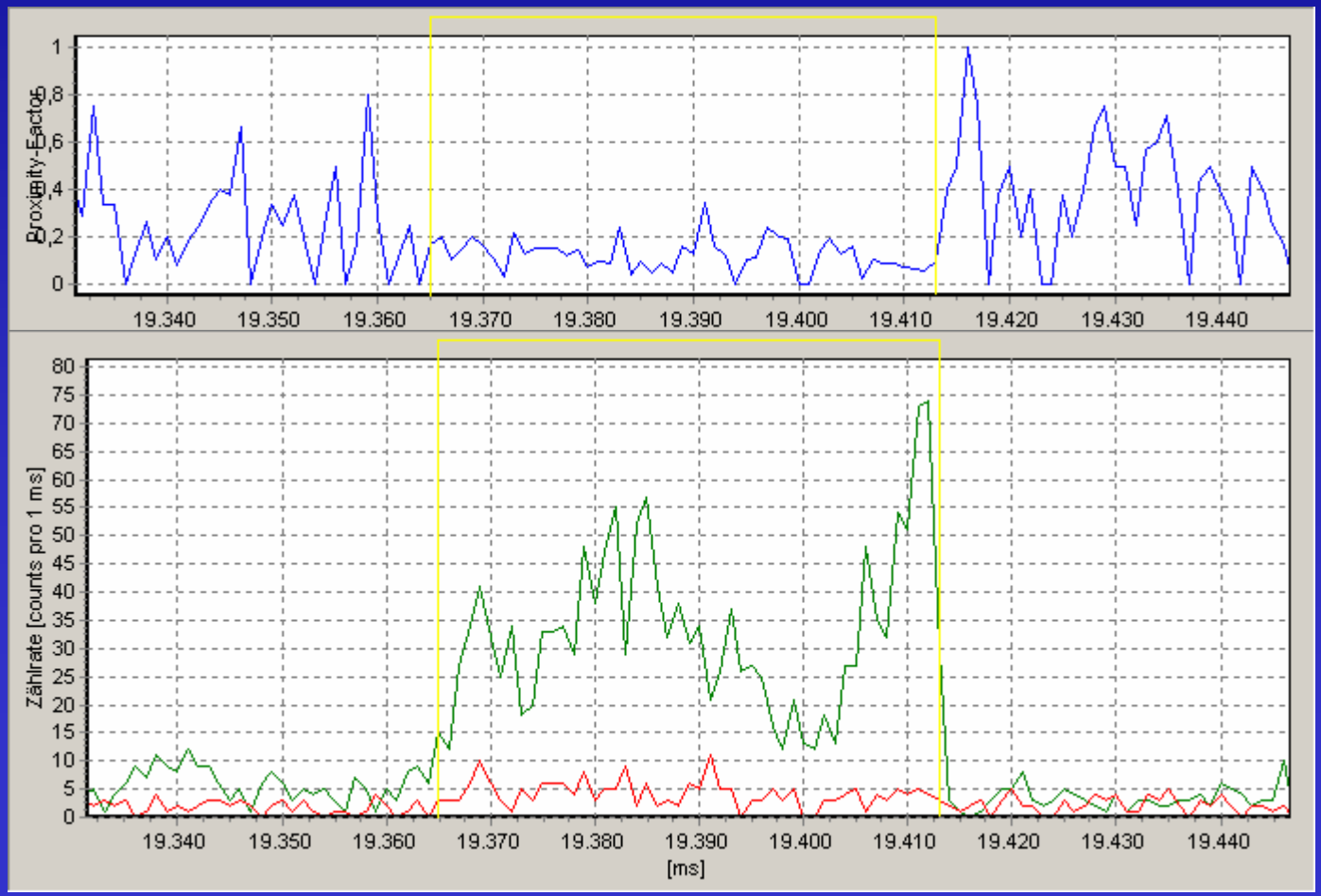


burst-integrated lifetime calculation with monoexponential tail fit

single-molecule FRET news

- F_0F_1-ATP synthase with AMPPNP: the low FRET state



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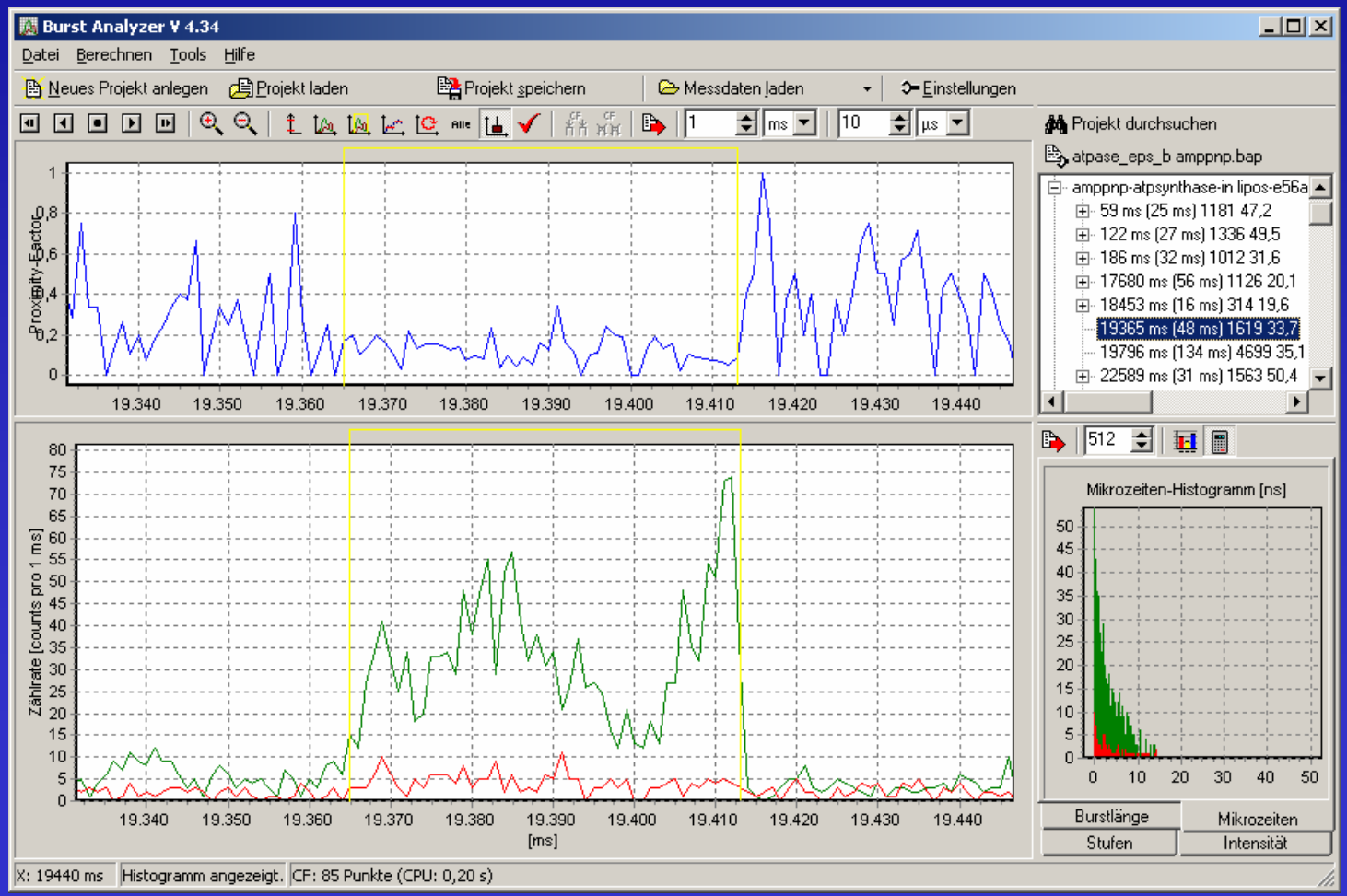

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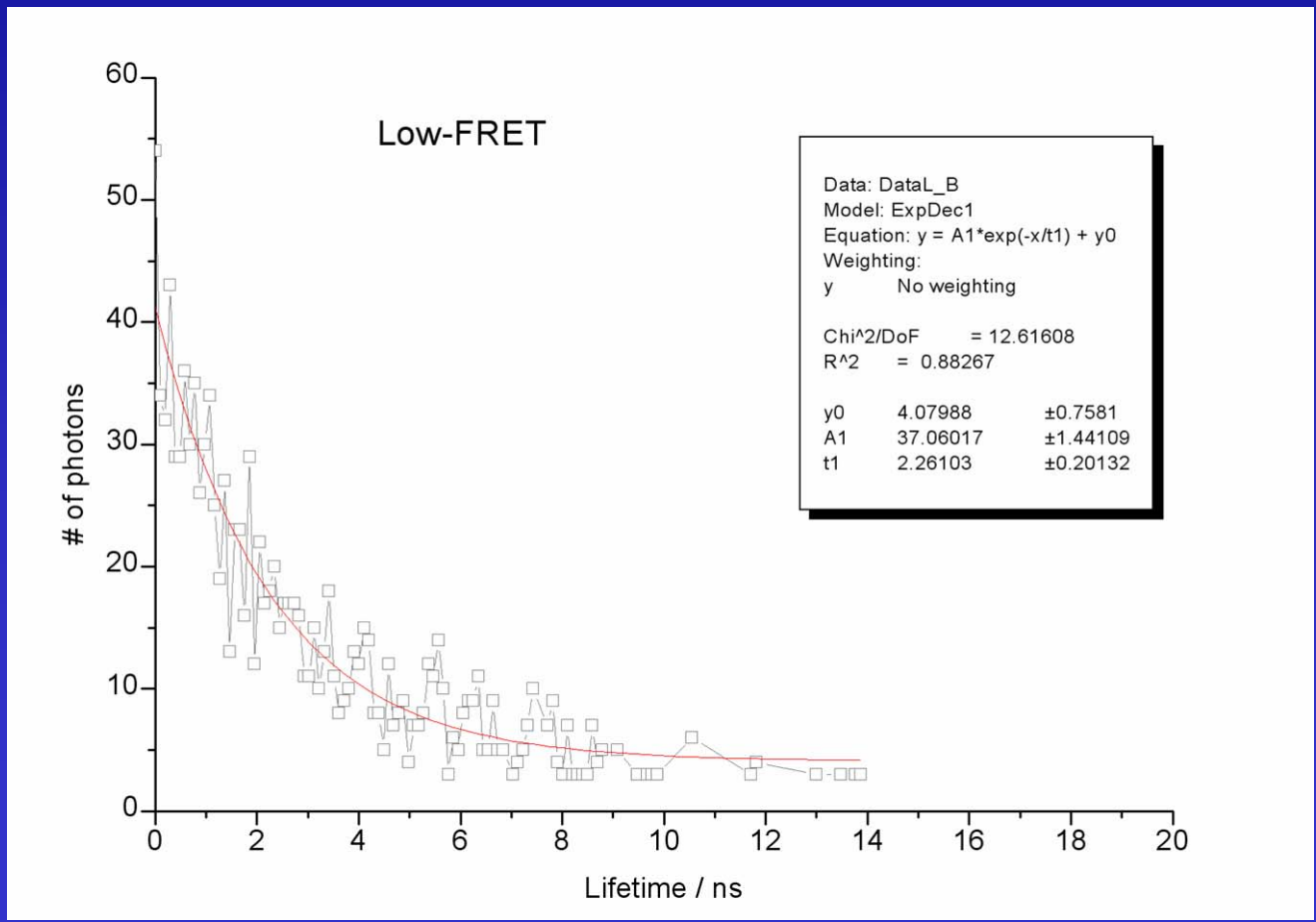
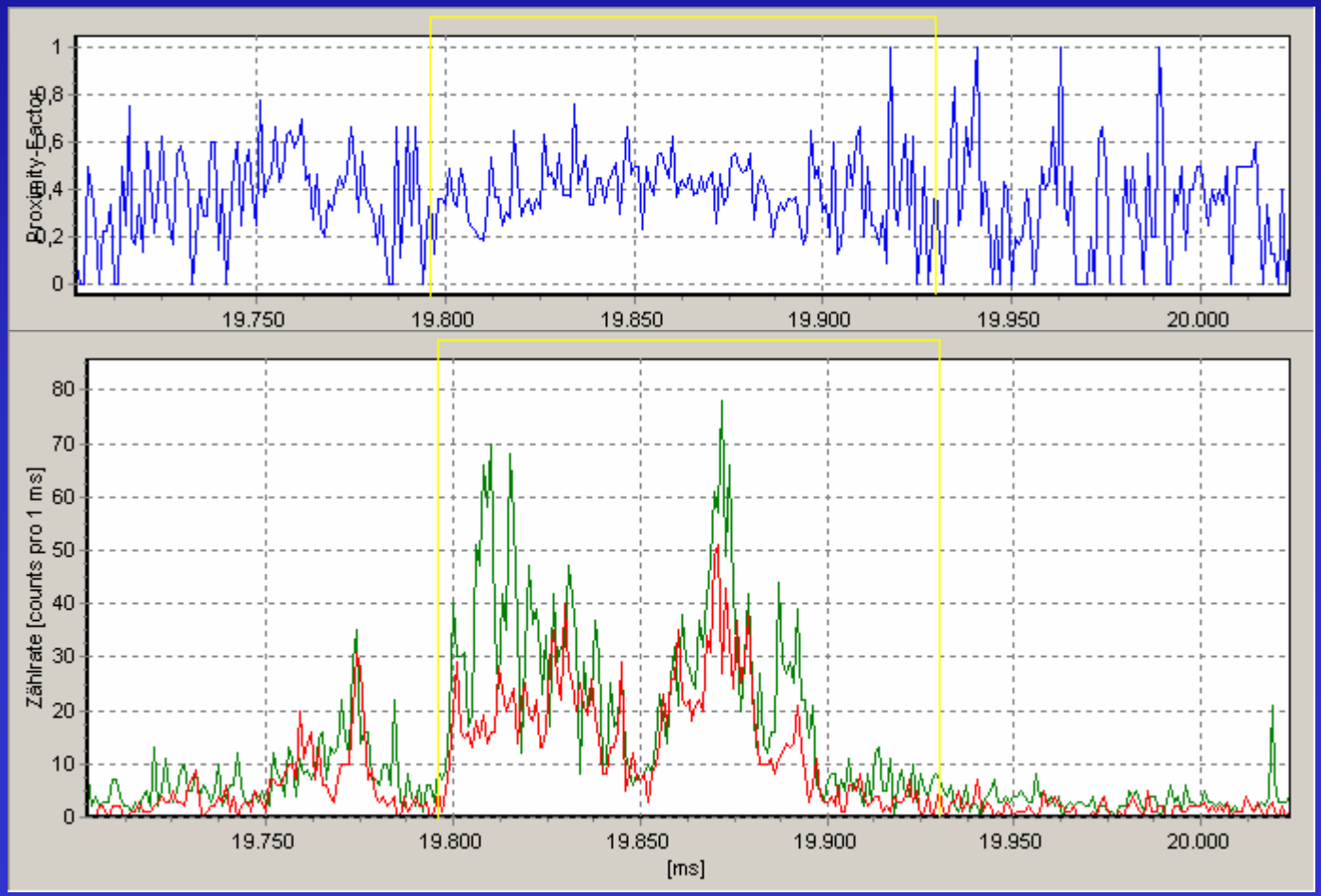


single-molecule FRET news

- F_0F_1-ATP synthase with AMPPNP: the medium FRET state



single-molecule FRET news

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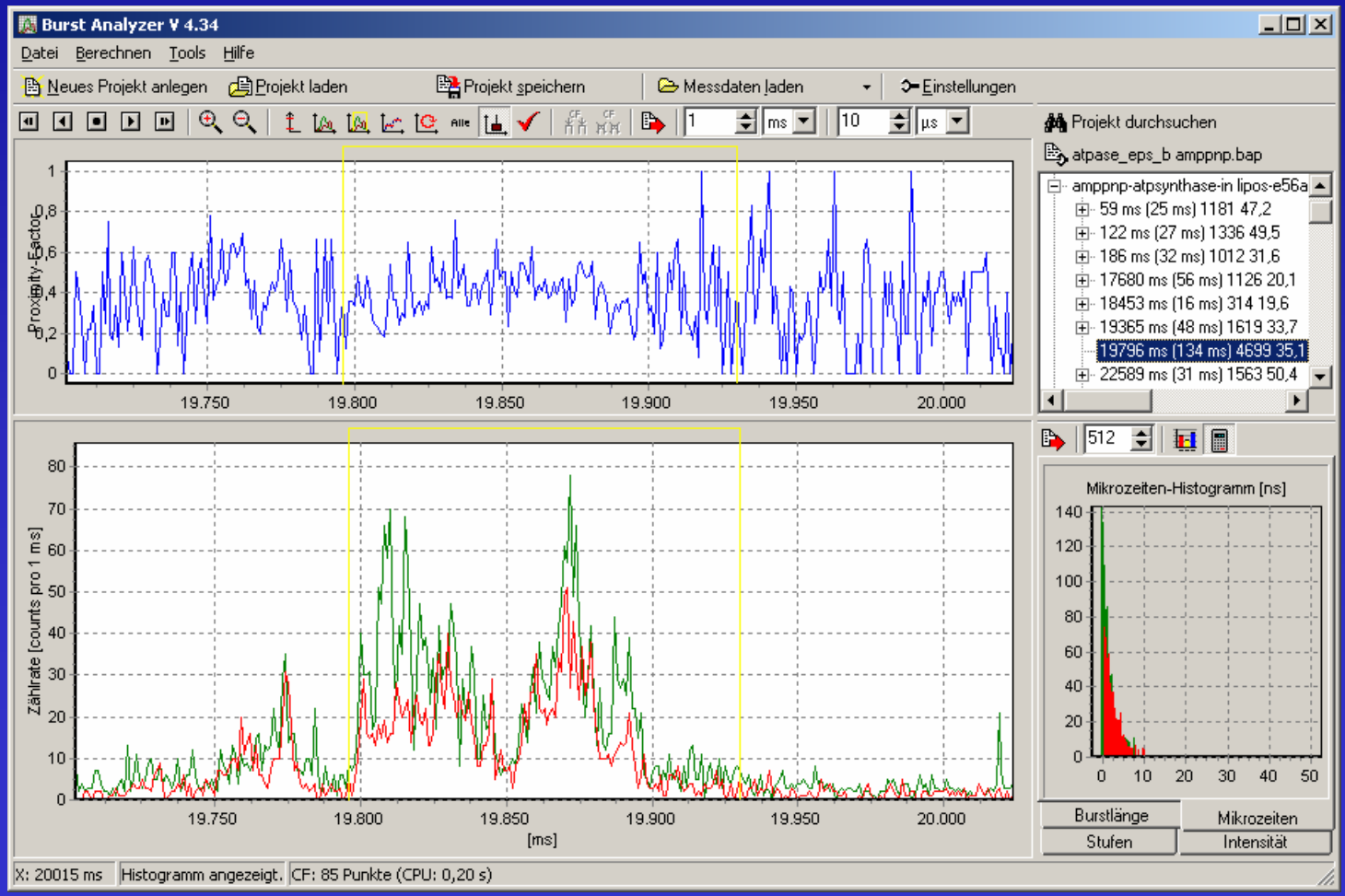

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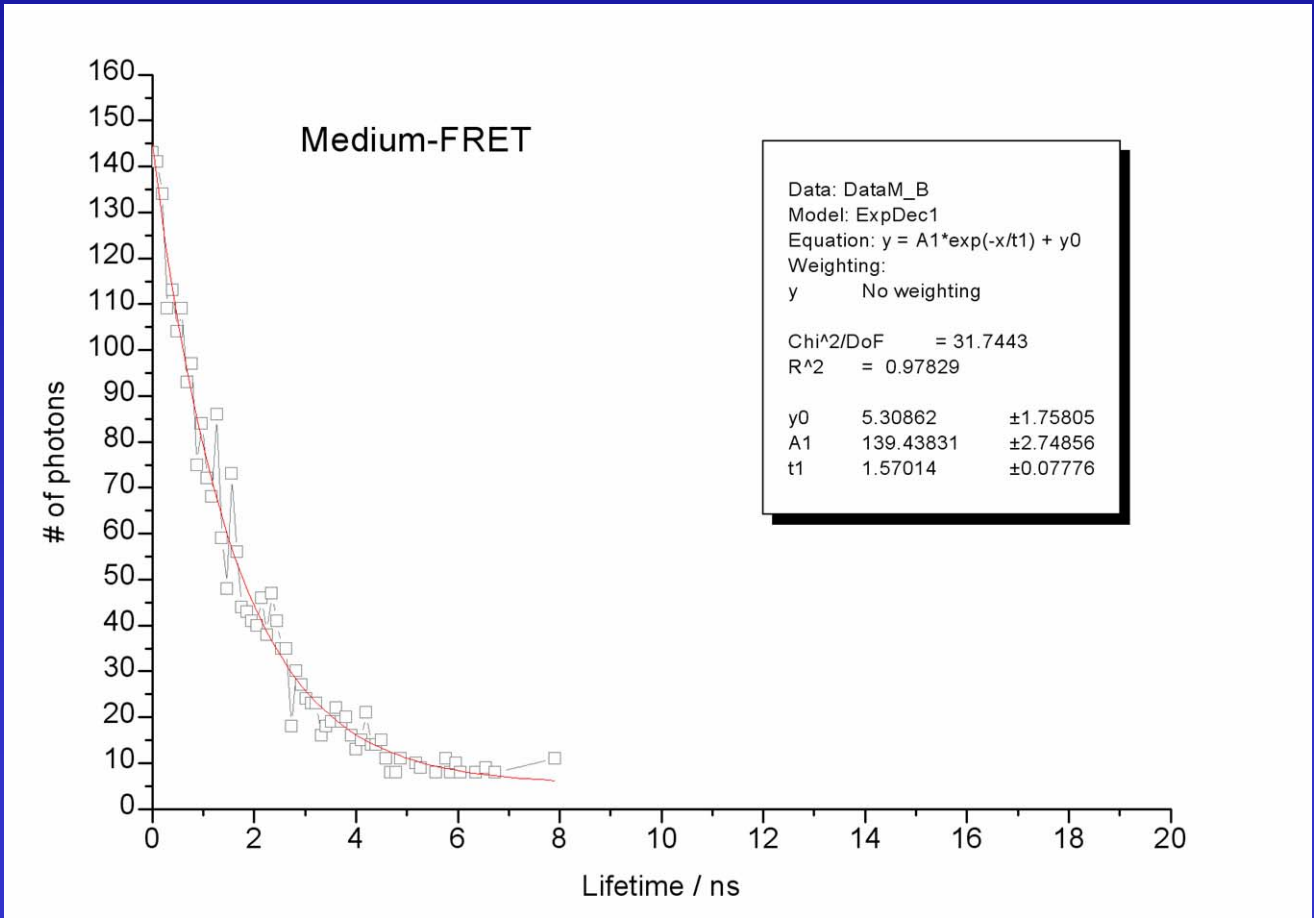
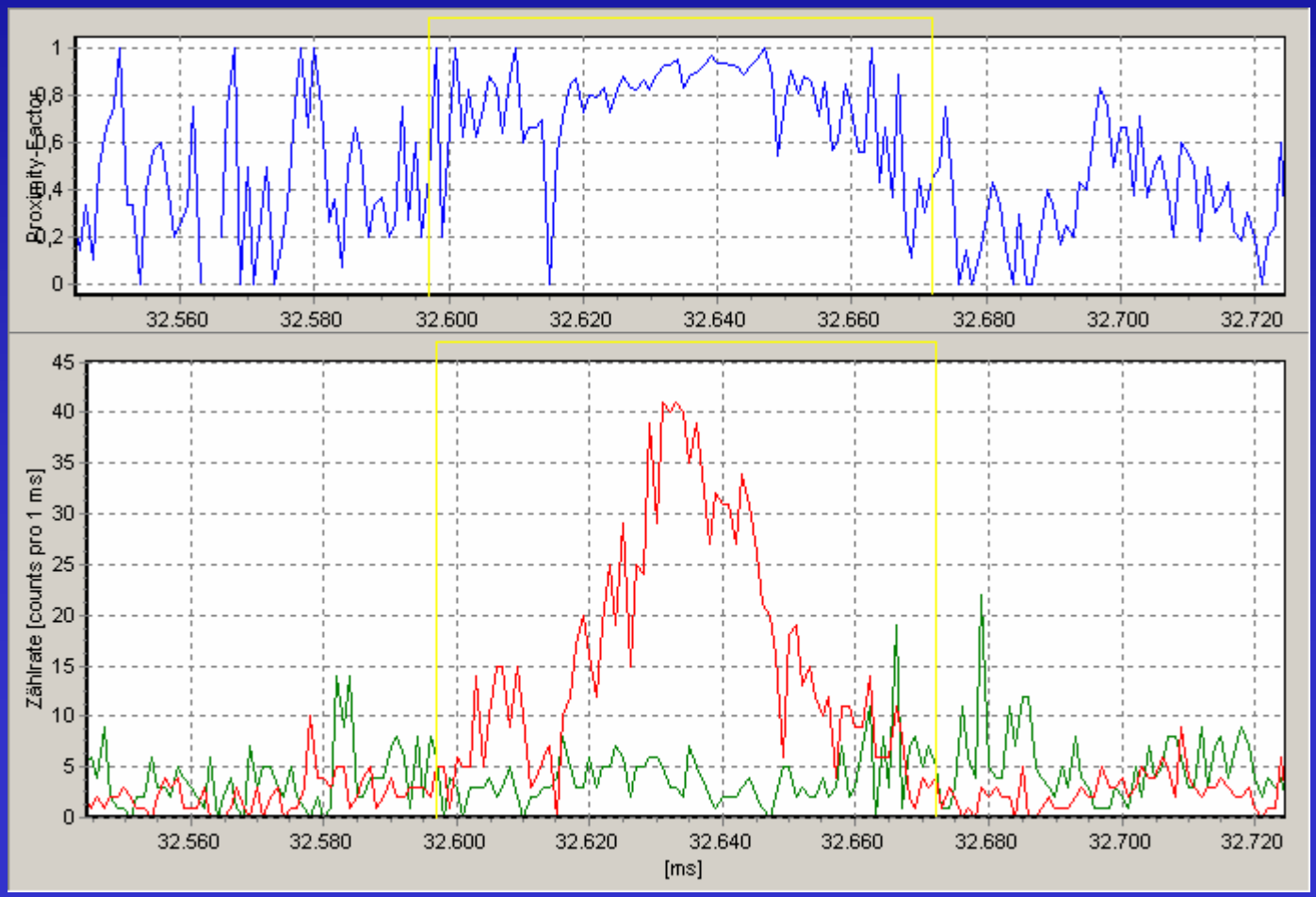


single-molecule FRET news

- F_0F_1-ATP synthase with AMPPNP: the high FRET state



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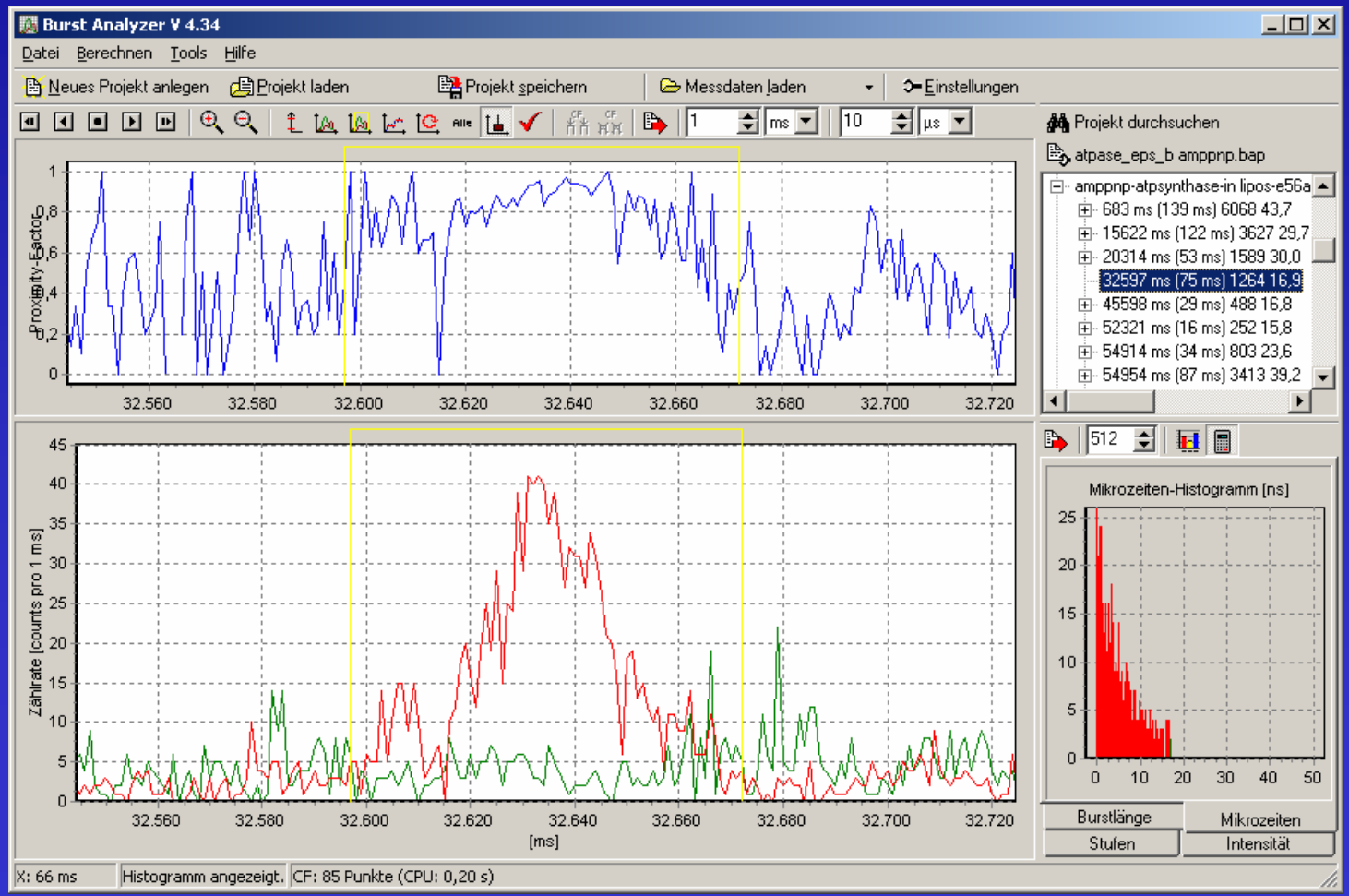

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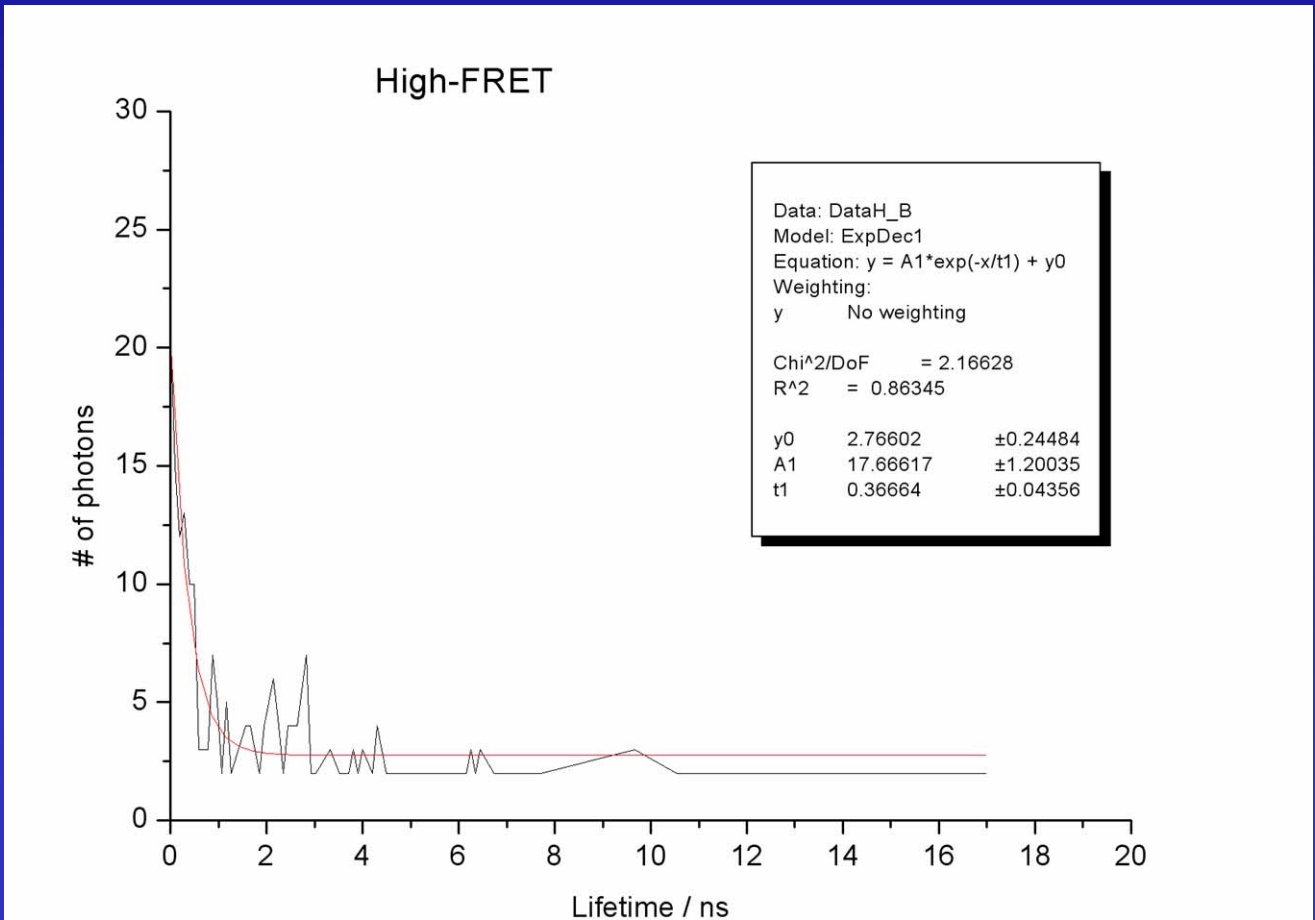

single-molecule FRET news

- F_0F_1-ATP synthase with AMPPNP (18-11-2004)
- ε56 labelled with Alexa546, b64 with Cy5bis
- Donor only: τ_{DO} = 3.53 ns within a single photon burst
- low FRET : τ_{low} = 2.26 ns
- medium FRET τ_{low} = 1.57 ns
- high FRET τ_{low} = 0.37 ns

- similar results (ε56-TMR, b64-Cy5bis) in press :
  N. Zarrabi, B. Zimmermann, M. Diez, P. Gräber, J. Wrachtrup & M. Börsch., *Proc. SPIE*, invited paper **5699A-25** (2005)