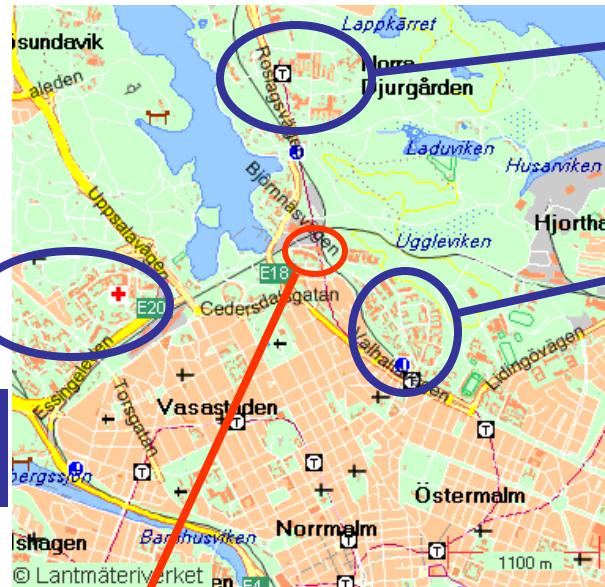
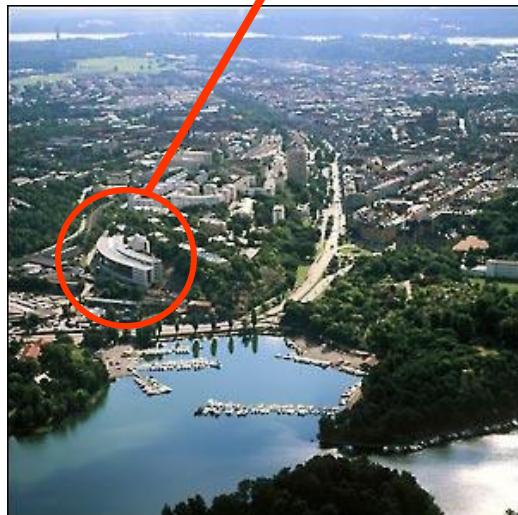




Karolinska  
Institutet

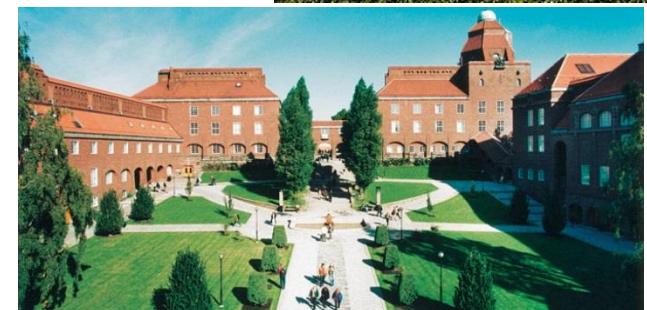


*Alanova University Center*



Stockholm  
University

KTH main  
campus



## *Experimental Biomolecular Physics, KTH*

11 researchers

1 professor

3 senior researchers

3 postdocs

4 PhD students

# Present members:



Niusha Bagheri  
(PhD stud)



Baris Demirbay  
(PhD stud)



Zhixue Du  
(postdoc)



Uliana Kostiv  
(postdoc)



Lucía Labrador Paéz  
(postdoc)



Haichun Liu  
(senior researcher)



Joachim Piguet  
(senior researcher)



Elin Sandberg  
(PhD stud)



Per Thyberg  
(senior researcher)



Chinmaya Venugopal  
Srambickal  
(PhD stud)



Jerker Widengren  
(professor)

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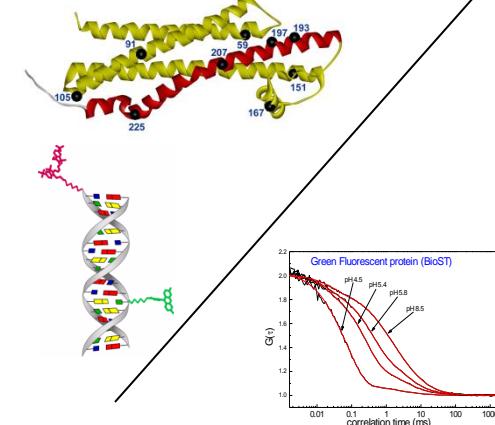
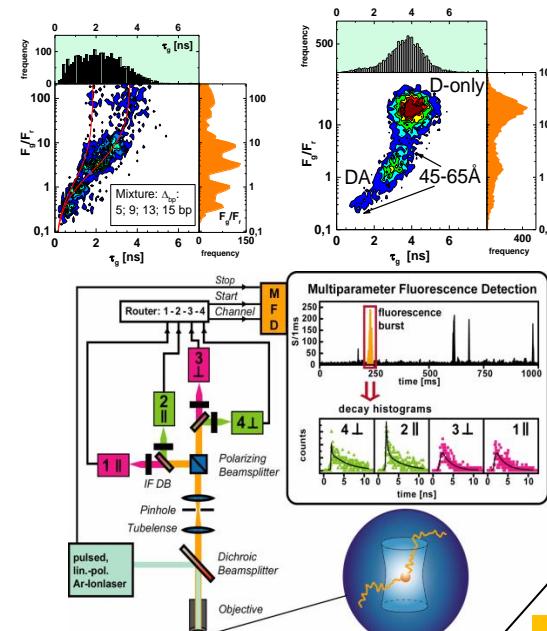
Jerker Widengren  
(professor)

Anna-Karin Ljung

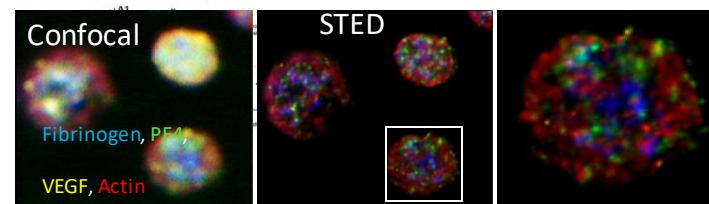
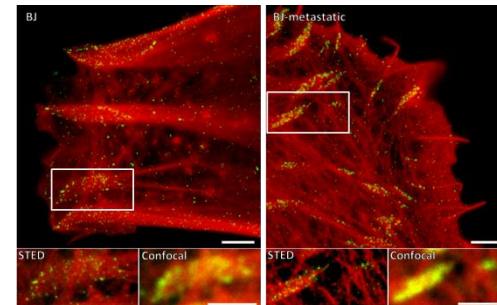
# Experimental Biomolecular Physics, KTH

Ultrasensitive / ultrahigh resolution fluorescence spectroscopy / imaging:  
Method development & applications (biomolecular research, diagnostics)

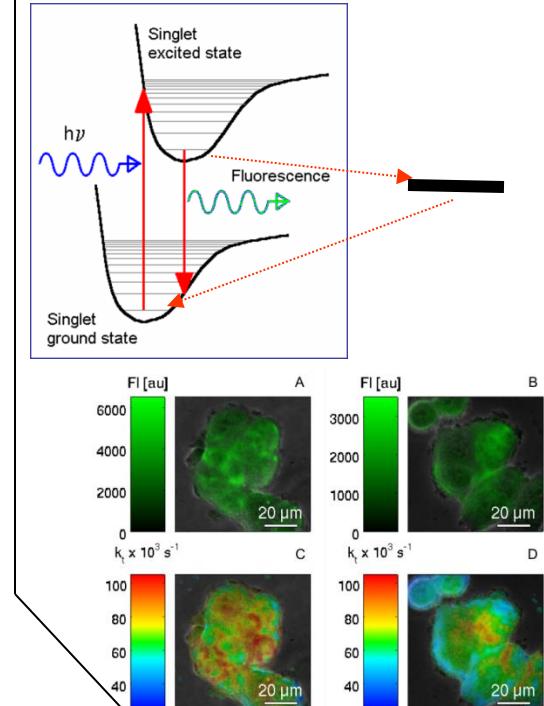
## Single molecule spectroscopy



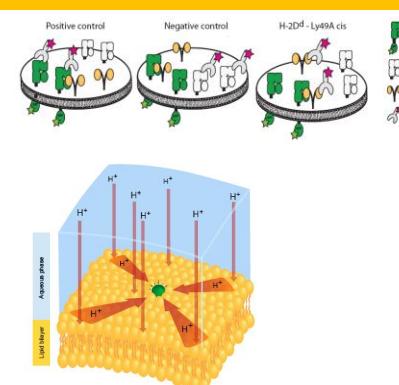
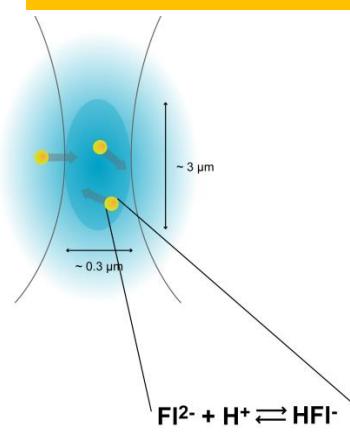
## STED microscopy



## TRAST microscopy



## Fluorescence Correlation Spectroscopy

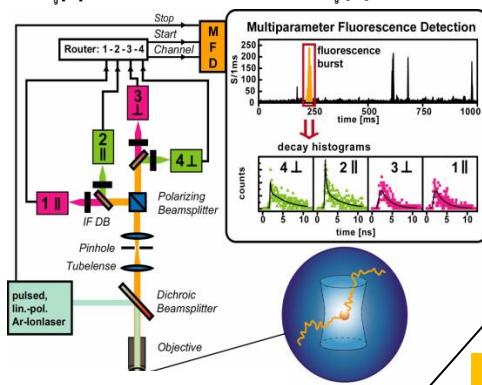
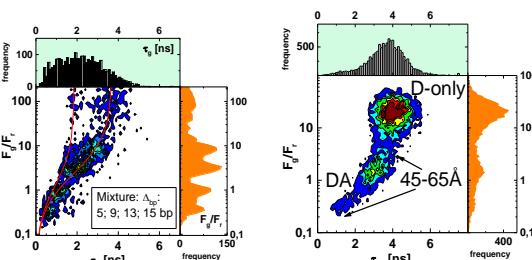




Applied Physics

# Experimental Biomolecular Physics, KTH

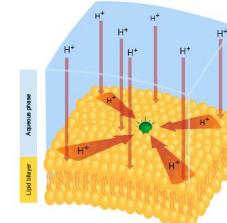
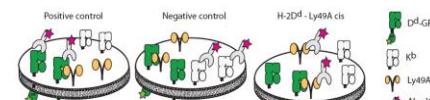
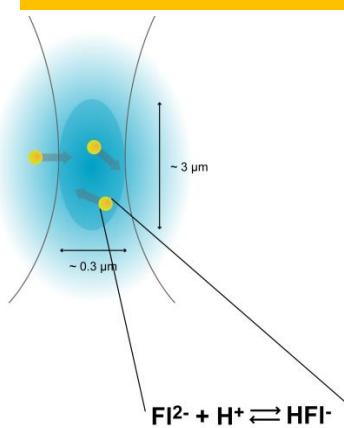
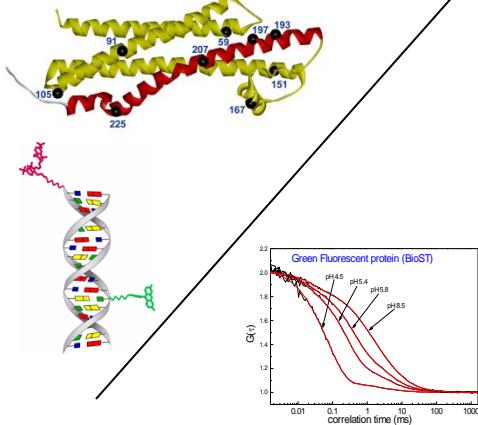
## Single molecule spectroscopy



## Limitations:

- Blinking
- Photophysics of dyes

## Fluorescence Correlation Spectroscopy

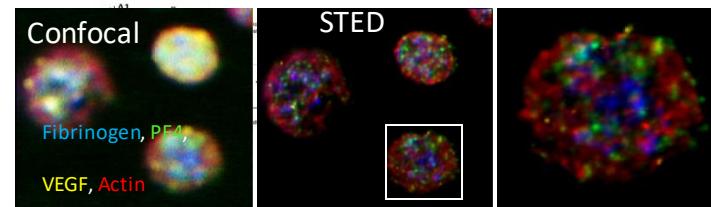
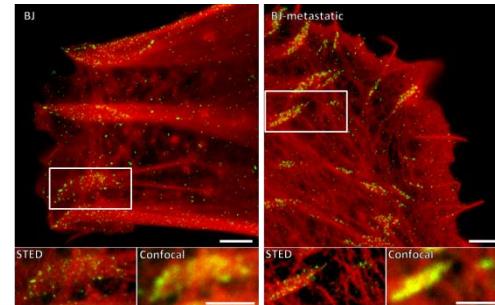




Applied Physics

## Experimental Biomolecular Physics, KTH

### STED microscopy



2005-2007



FLUODIAMON

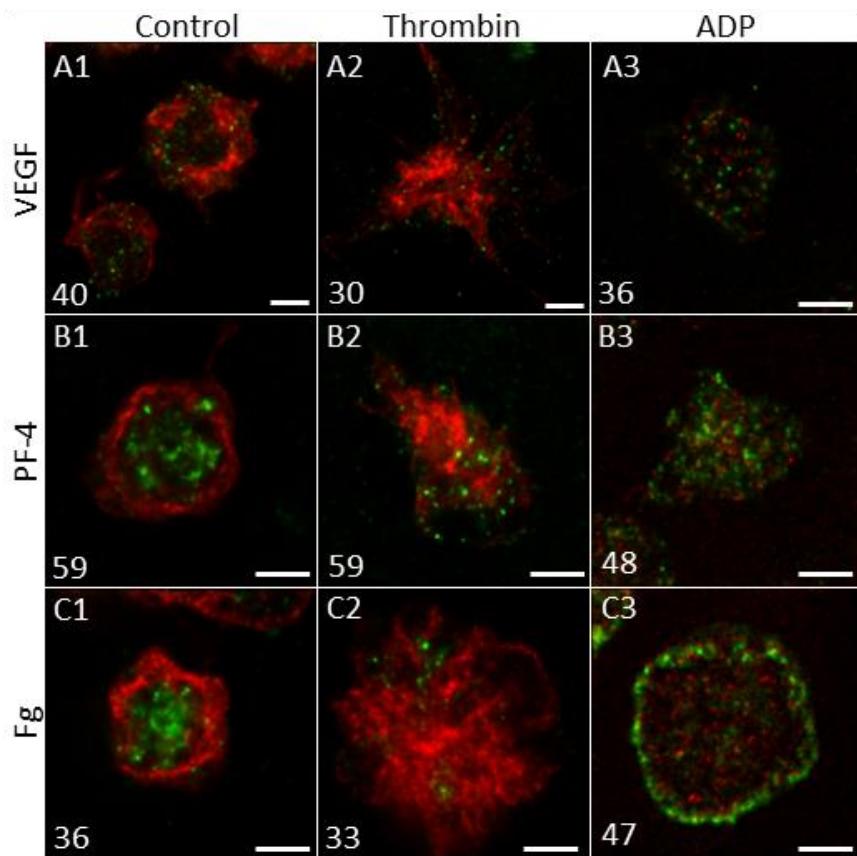
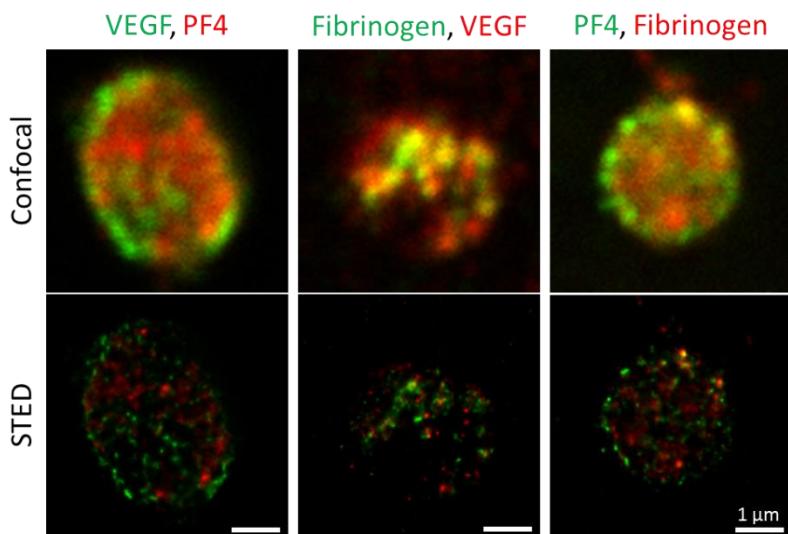


Spin-off  
research

2011-

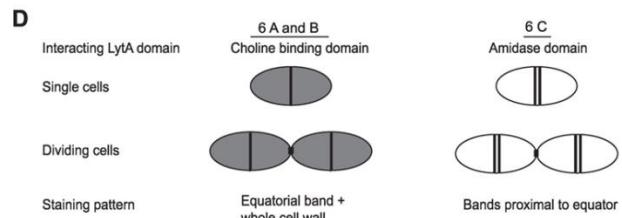
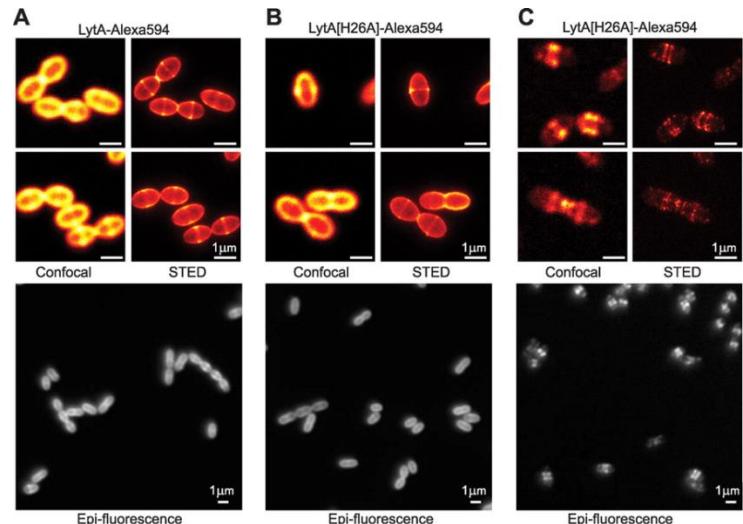
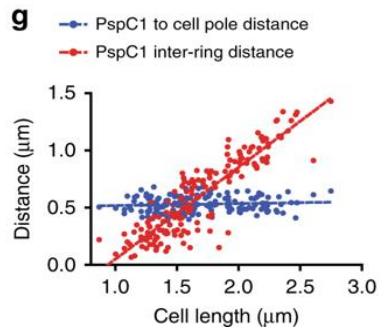
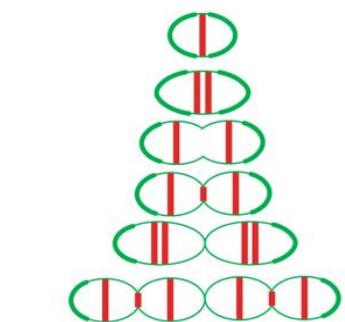
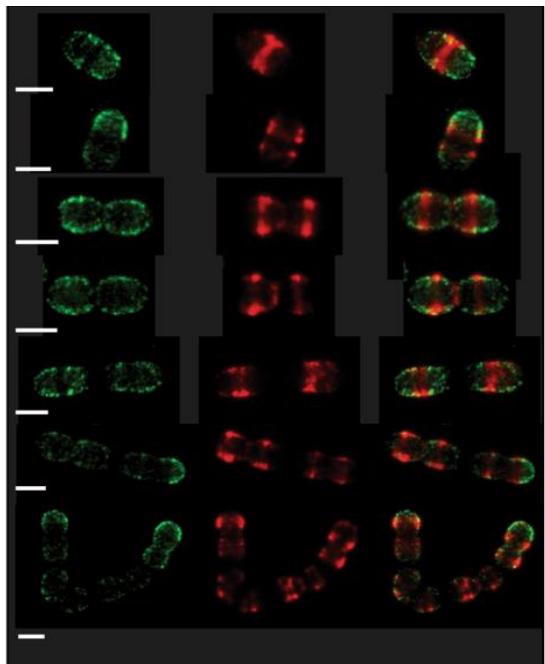
# Platelet studies:

(collab. Gert Auer, Karolinska inst)



# Pneumococci

Collab. Birgitta Henriques-Normark, KI



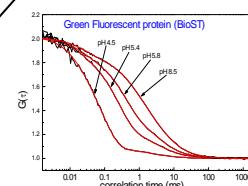
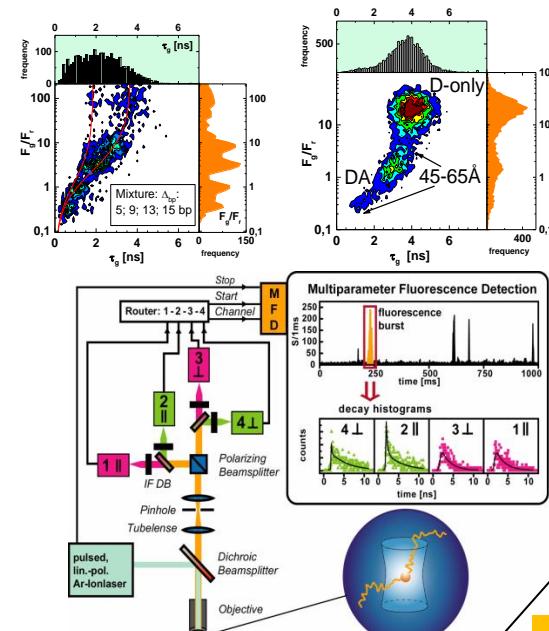
P Mellroth *et al*, *J Biol Chem*, 2012

Pathak *et al*, *Nat Comm*, 2017

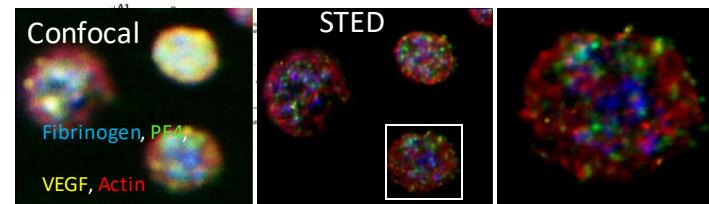
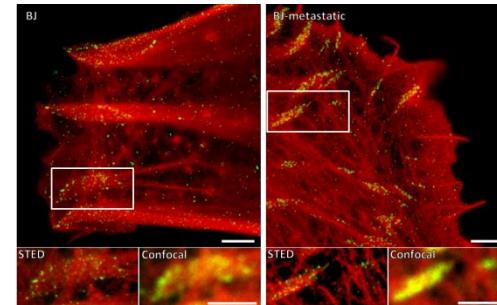
# Experimental Biomolecular Physics, KTH

Ultrasensitive / ultrahigh resolution fluorescence spectroscopy / imaging:  
Method development & applications (biomolecular research, diagnostics)

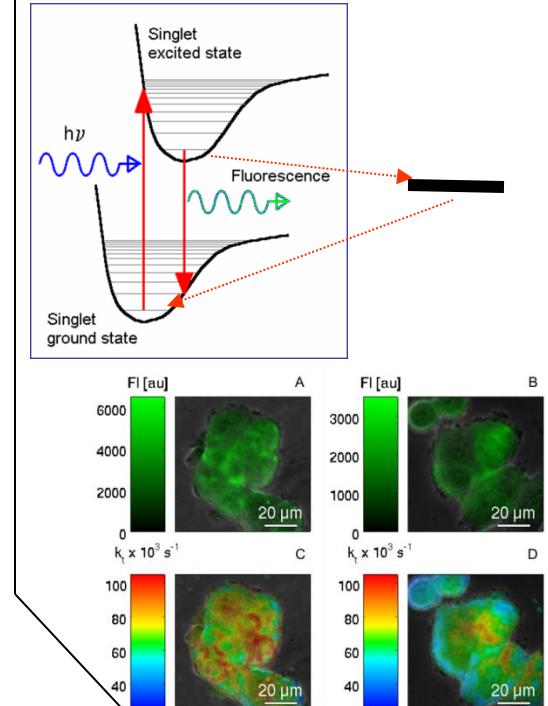
## Single molecule spectroscopy



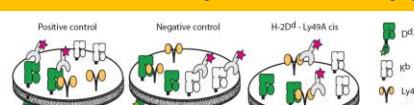
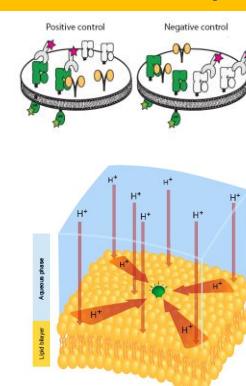
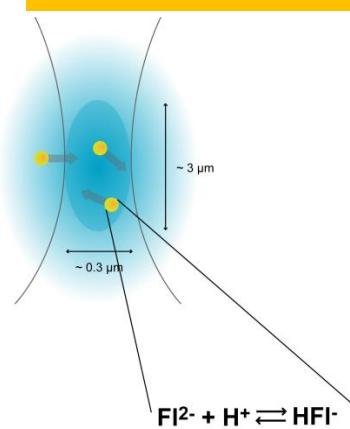
## STED microscopy



## TRAST microscopy

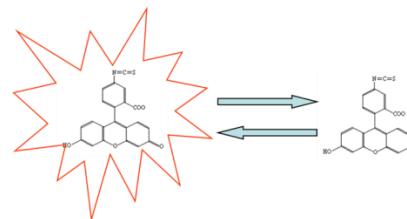
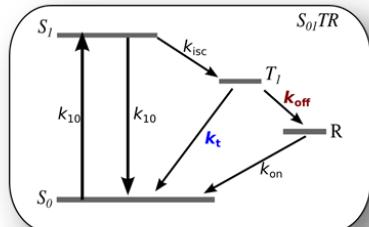


## Fluorescence Correlation Spectroscopy

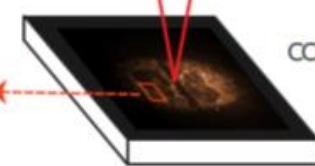
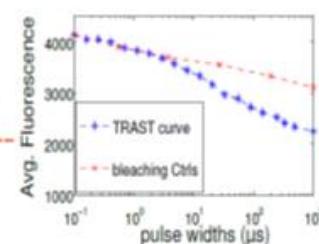
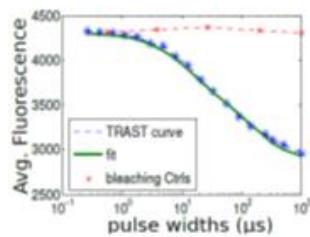
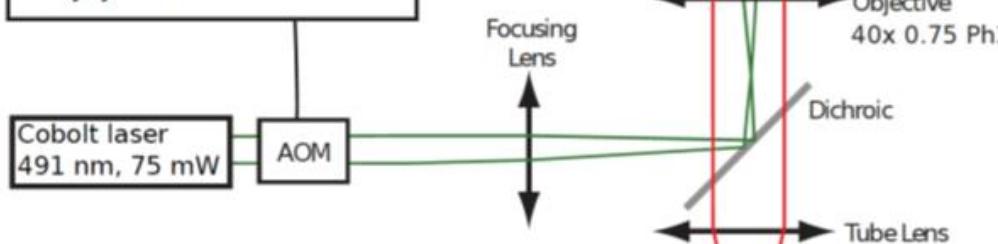


# Experimental Biomolecular Physics, KTH

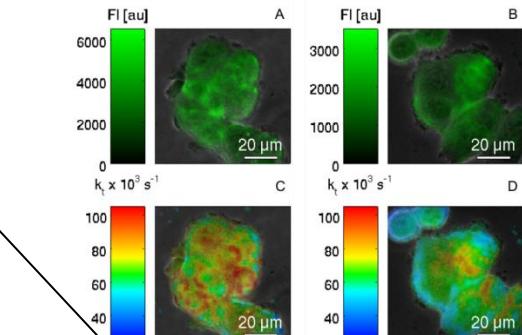
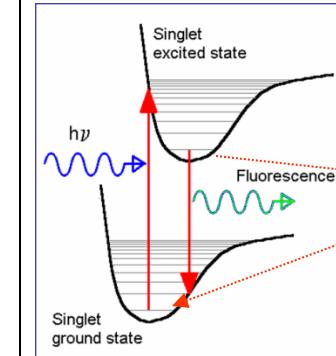
Blinking/switching: source of information



Function generator:  
pulse widths: 100 ns ... 1 ms  
dutycycle: 1 %

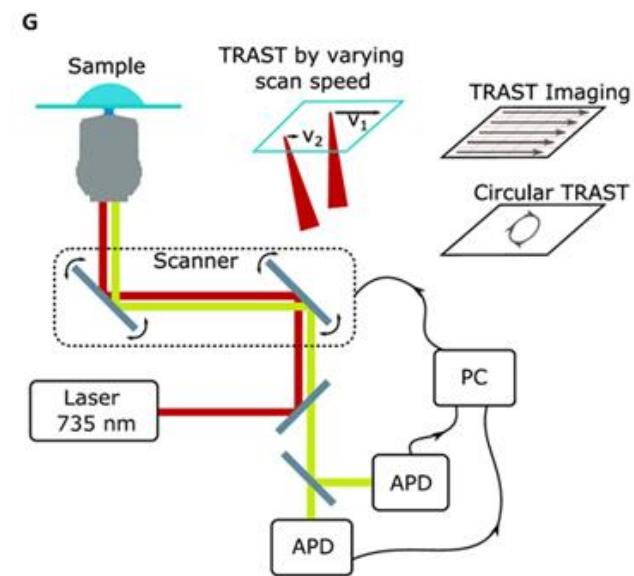
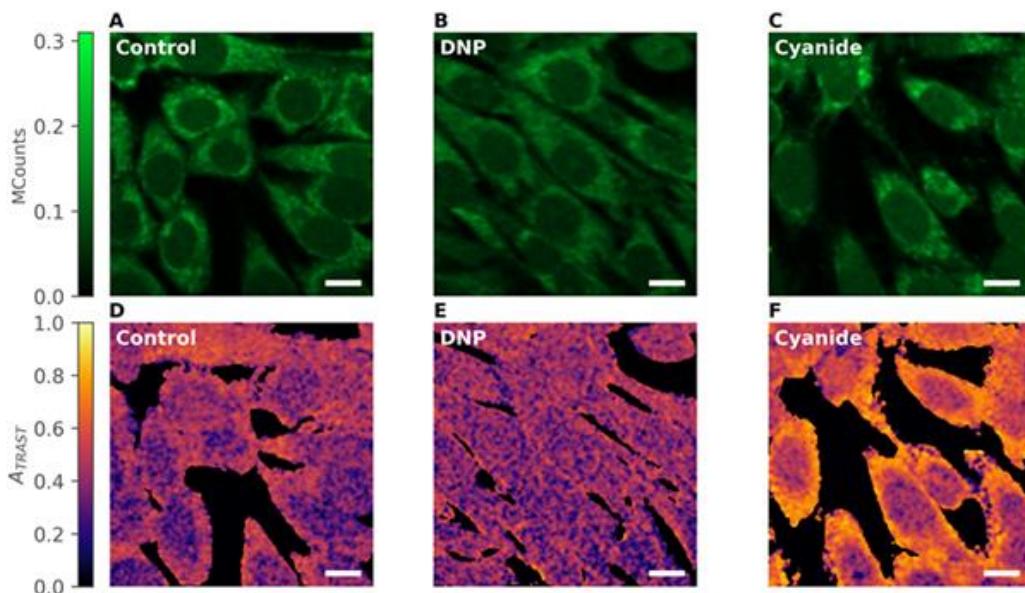


TRAST microscopy



## NanoVIB:

- Additional imaging parameter(s)
- FCS/TRAST for fluorophore characterization



# MMs of KTH:

WP1: 4

WP2: 14

WP3: 1

WP4: 1

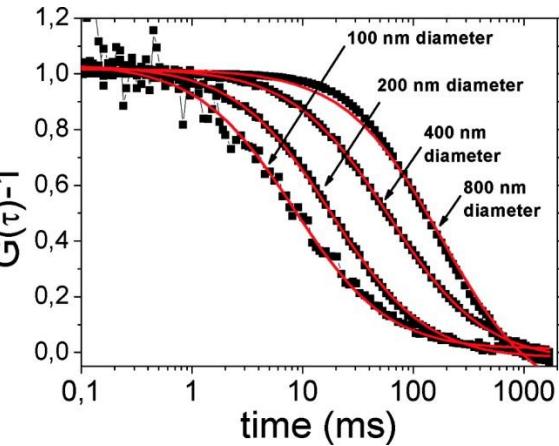
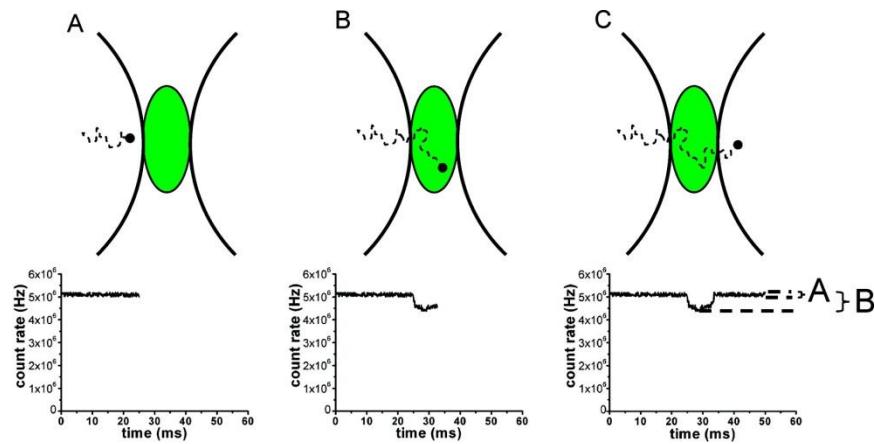
WP5: 72

WP6: 16

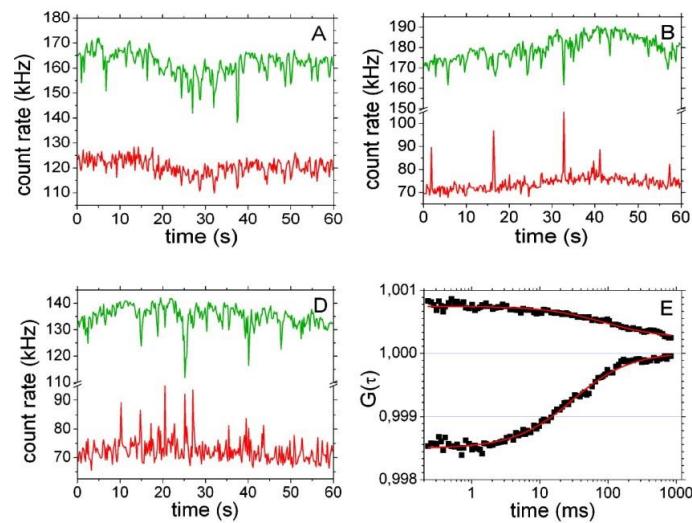
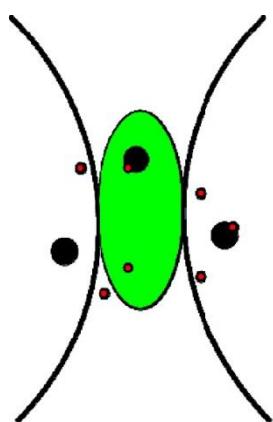
WP7: 20

X Deliverables, ★ Milestones	Months	Year 1	Year 2	Year 3	Year 4
		03 06 09 12	15 18 21 24	27 30 33 36	39 42 45 48
<b>WP1 (AI) Platform development</b>					
T1.1: Construction of two modular MINFLUX platforms		X			
T1.2: Plan modified Gen I SPAD array detection electronics and interface with them		X			
T1.3: Develop acquisition electronics interfacing with Gen II SPAD array electronics				X ★	
T1.4: Design/test integrated VIS-NIR-MINFLUX microscope with array detection				★ X	X
<b>WP2 (LLG) Optical integration</b>					
T2.1: Expand MINFLUX platform from WP1 to the NIR, point detection		X ★		★	
T2.2: Integrate SRS components, implement SRS-MINFLUX acquisition schemes			X		
T2.3: Integrate Gen I SPAD array, prototype acquisition algorithms			X		
T2.4: Implement two photon activation and TPE TRAST-MINFLUX imaging				X	
T2.5: Optimize and stabilize optical setup and provide critical feedback				X	X
<b>WP3 (PII) Detector development</b>					
T3.1: Adapt hardware platform to MINFLUX platform (Gen I electronics)		X			
T3.2: Develop new hardware platform & communication protocol (Gen II electron.)				X	
T3.3: Develop enhanced red and NIR sensitivity CMOS SPAD				★ X	
T3.4: Develop 10×10 CMOS SPAD array with integrated time-gating				X	
<b>WP4 (APE) Laser for MINFLUX and SRS operation</b>					
T4.1: Develop ultra-fast targeting of arbitrary wavelengths for ps SRS-lasers		X	X	★	
T4.2: Development of pulse-length switching between ps and fs regimes			X	X	
<b>WP5 (KTH) Labels, acquisition and protocols</b>					
T5.1: Identify fluorophore suitable for NIR-MINFLUX		★ X			
T5.2: Define acquisition schemes for all imaging modes for fixed and live cells				X	
T5.3: Establish VIS-NIR MINFLUX protein labeling/sample preparation protocols				X	
T5.4: Verify SRS and TPE TRAST imaging on bacteria and host cells				X	
T5.5: Establish combined use of MINFLUX with SRS and/or TPE TRAST imaging				★ X	
<b>WP6 (KI) Lead application and dissemination</b>					
T6.1: Study pneumococcal surface proteins					
T6.2: Study co-localization of pneumococcal surface/pilus with receptor proteins					
T6.3: Study nanoscale localization of protein virulence factors					★
T6.4: Study distribution patterns of pneumococcal proteins					X
T6.5: Facility open to potential end-users				X	★
<b>WP7 (KTH) Project management and communication</b>					
T7.1: Kick-off meeting, establishment of PMC, AB and I <sup>2</sup> EMG.		X X			
T7.2: Communication activities		X X			X X
T7.3: Monitor progress through supervision of deliverables & milestones					
T7.4: Prepare EC interim & final project reports					

# inverse-FCS



# inverse-FCCS



Wennmalm S et al, *Anal. Chem.* 81(22), 9209-9215, 2009

Wennmalm S and Widengren J, *Anal. Chem.* 82(13), 5646-5651, 2010

Wennmalm S and Widengren J, *Frontiers in Bioscience* (2011)

# Inverse CARS-based Correlation Spectroscopy (iCARS-CS)

