Improving single molecule fluorescence detection with zero-mode waveguide metal nanoapertures

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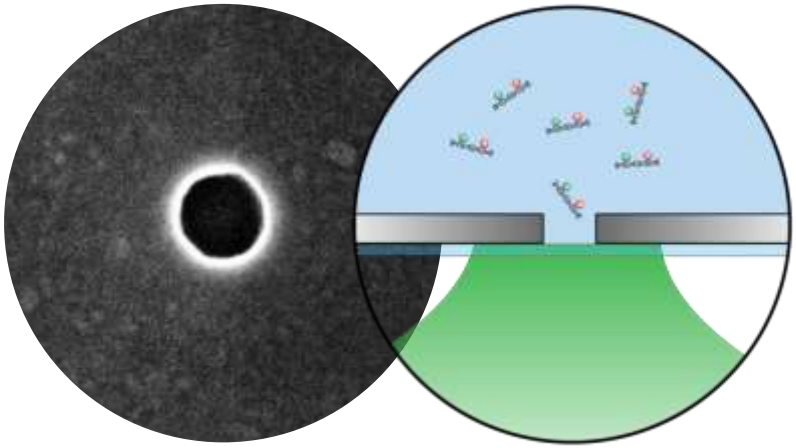
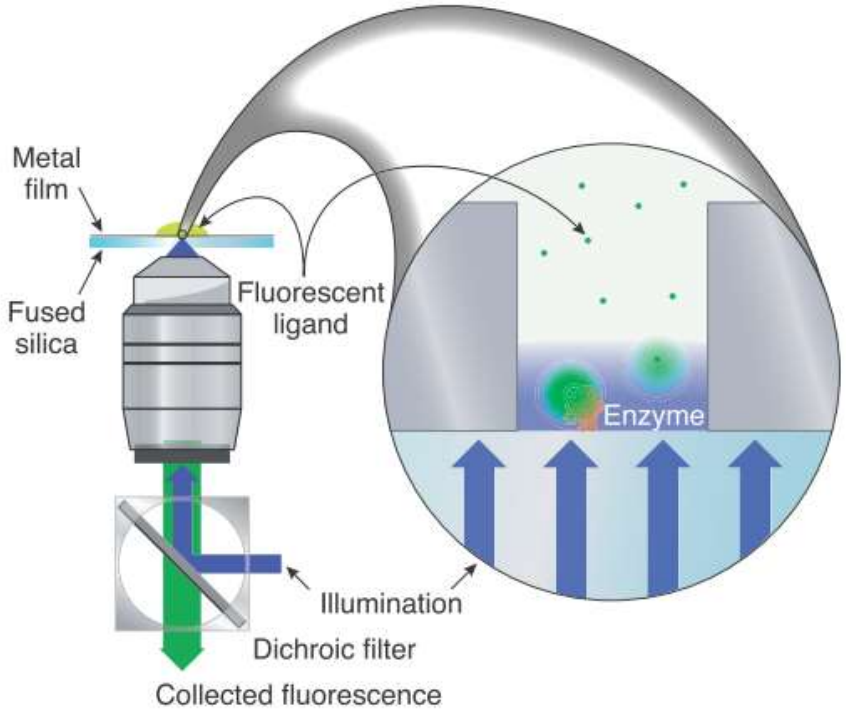
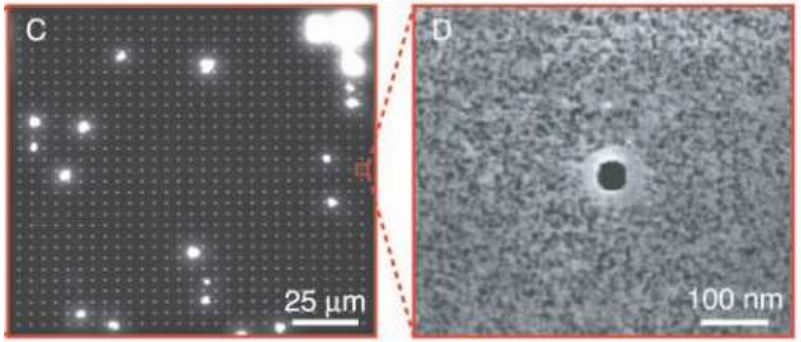


Zero-Mode Waveguides for Single-Molecule Analysis at High Concentrations

M. J. Levene,[1] J. Korlach,[1,2] S. W. Turner,[1]* M. Foquet,[1]
H. G. Craighead,[1] W. W. Webb[1]†

Science **2003**, *299*, 682-686





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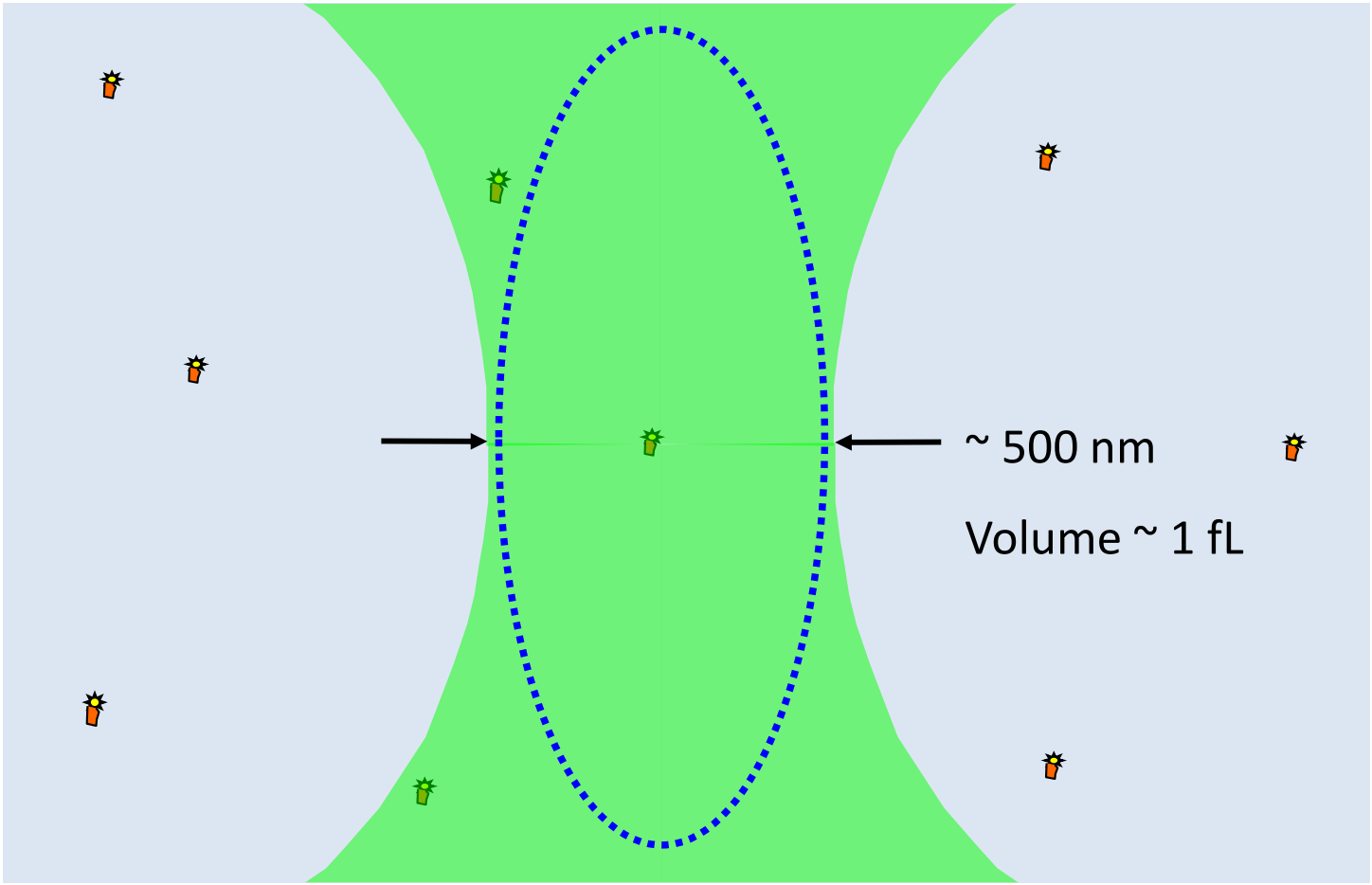
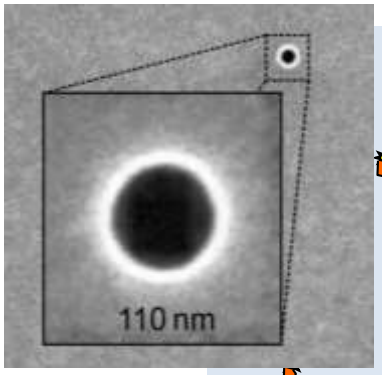
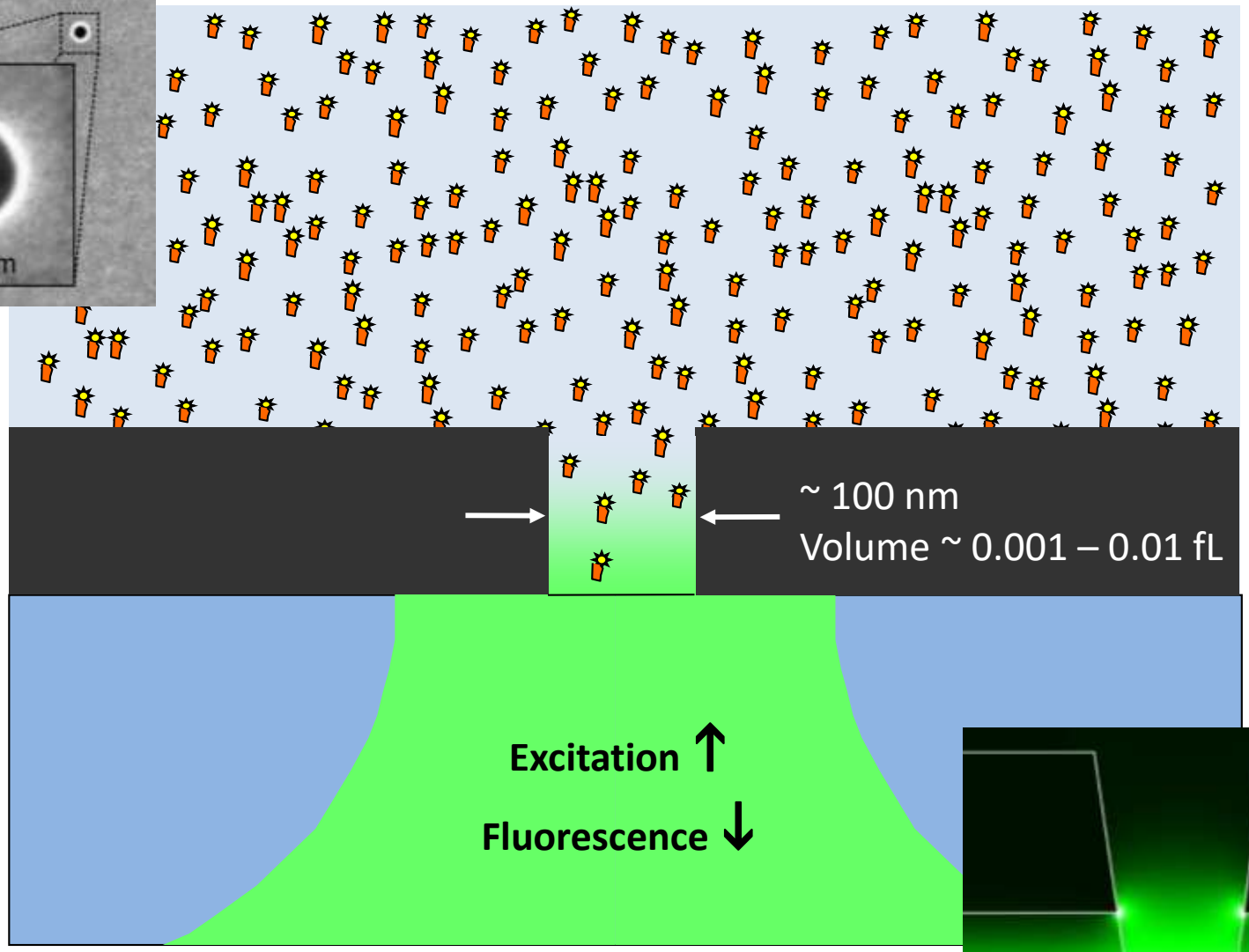
Real time single molecule sequencing

Confocal microscopy single molecule sub-nM concentration



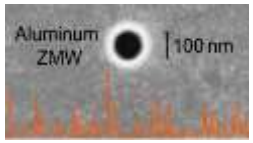
Nanoaperture >100nM concentration single molecule detection





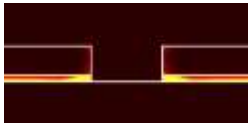
Fluorescence & metal nanoapertures: what you can do

- **Low volume:** single molecule detection at micromolar concentration



- **High signal:** monitor fast dynamics and reduce acquisition time

- **Enhanced FRET:** detect energy transfer at distances > 10 nm

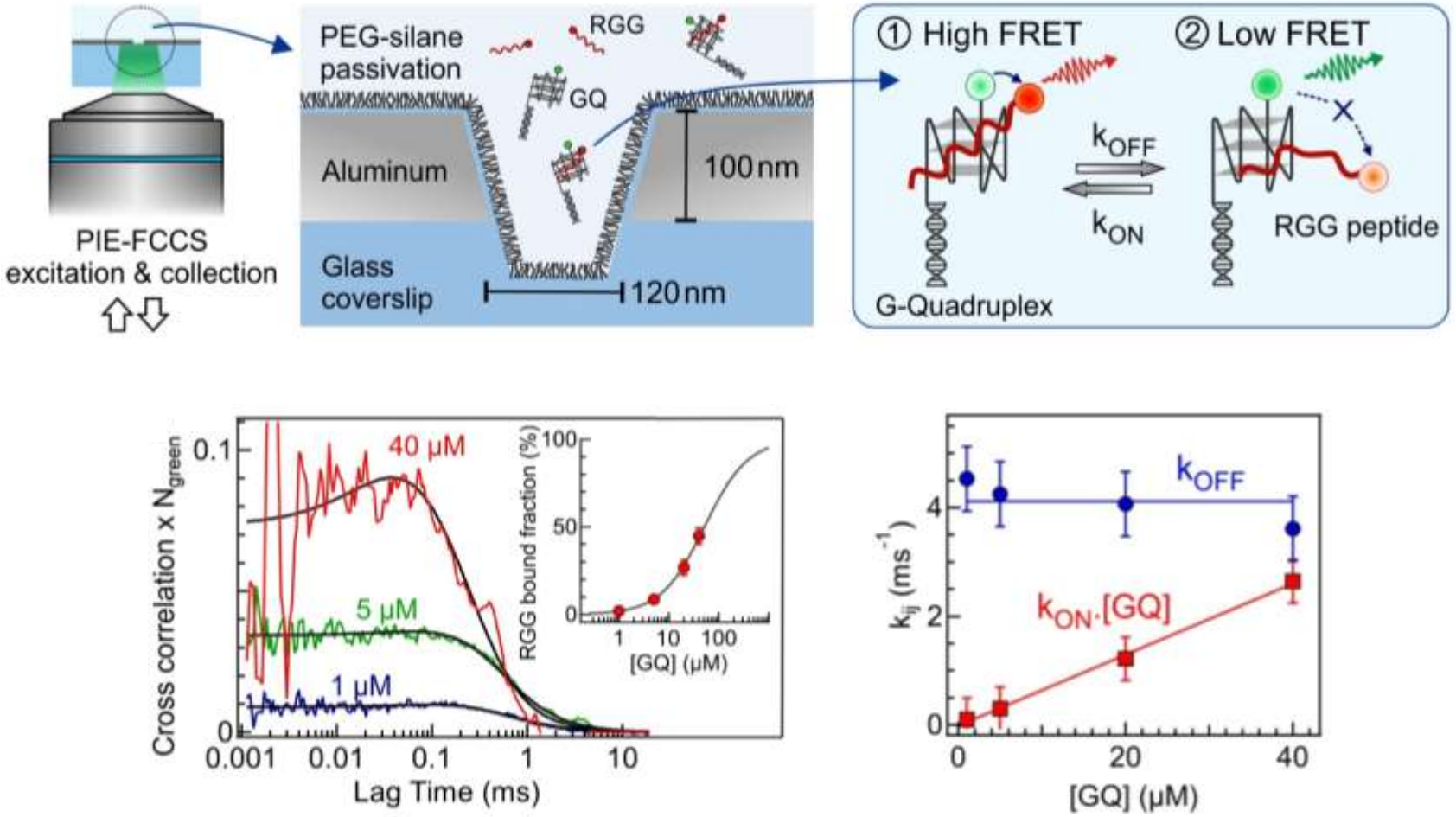


- **Temperature control:** perform fast submillisecond heating cycles

- **Label-free:** detect the intrinsic UV autofluorescence of a protein

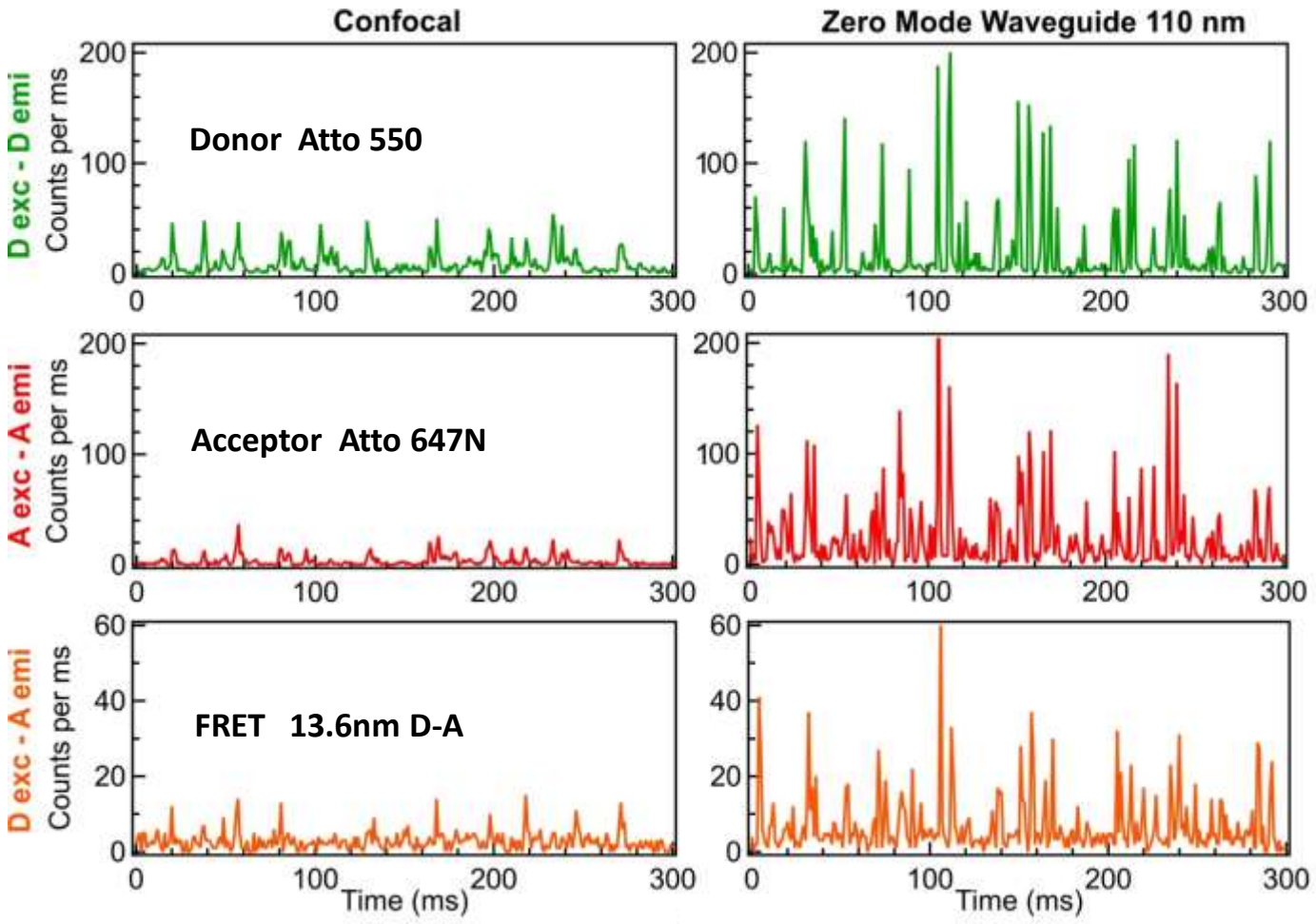
Publications, details, contact → www.jeromewenger.com jerome.wenger@fresnel.fr

Low volume: single molecule at micromolar concentration



Patra et al, Nucleic Acids Res. **2021**, *49*, 12348-12357

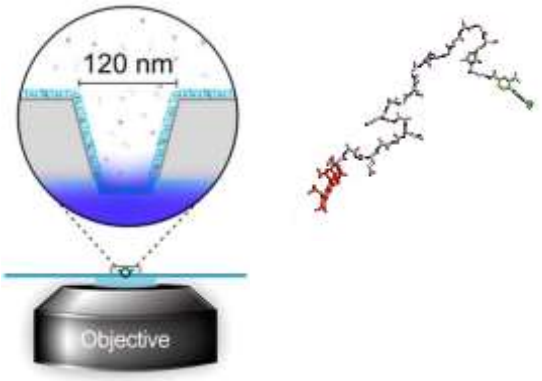
High signal: monitor fast dynamics and reduce acquisition time

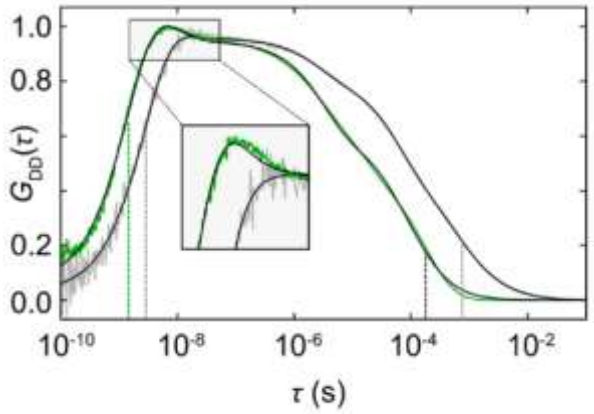


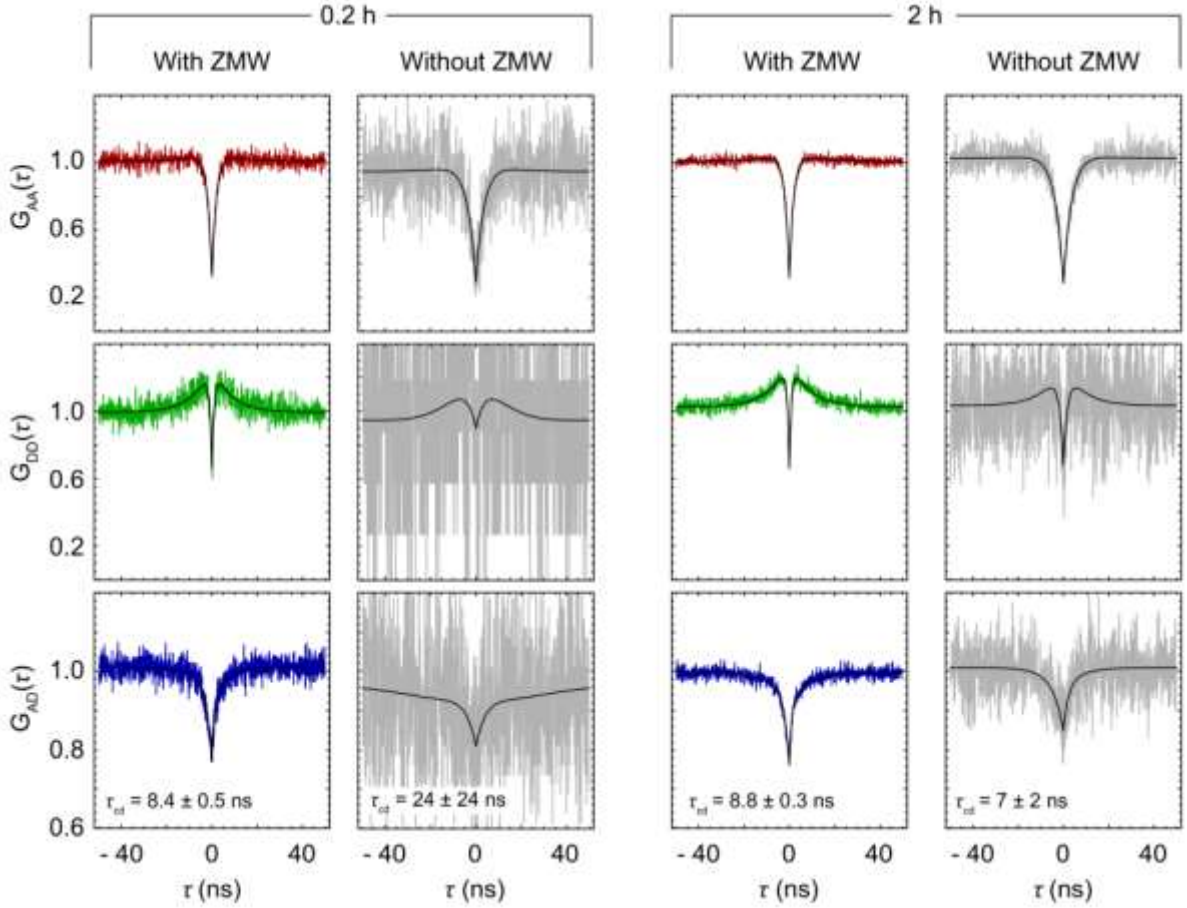
8x Atto550 donor brightness enhancement

12x Atto647N acceptor brightness enhancement

High signal: monitor fast dynamics and reduce acquisition time



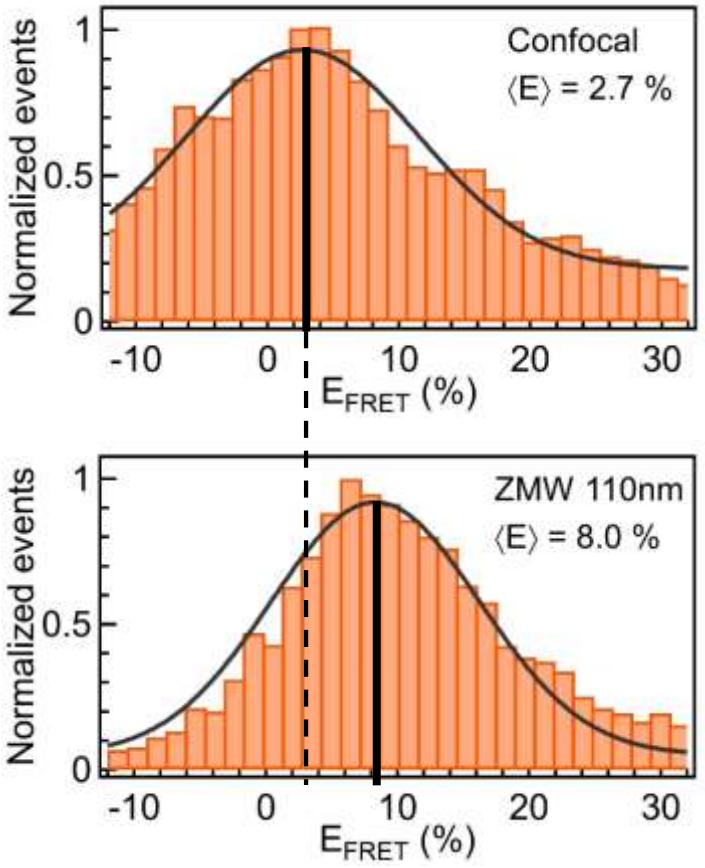


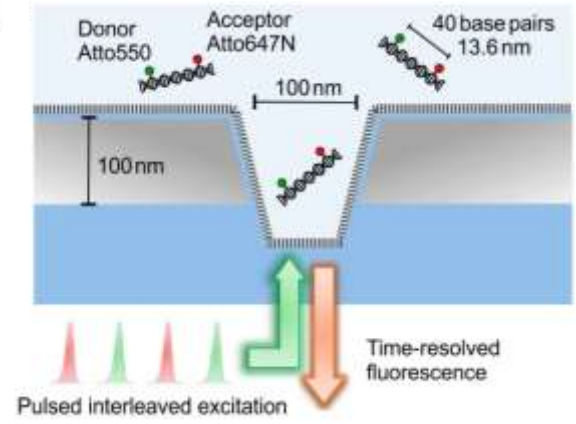


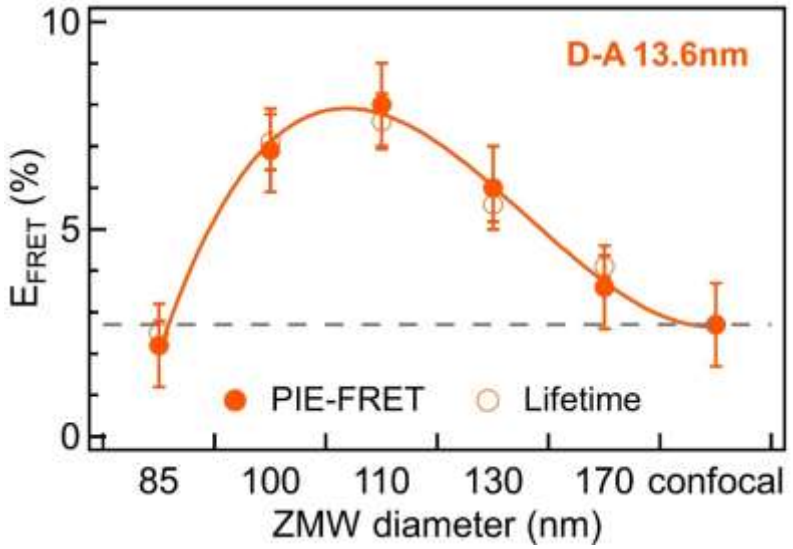
Collab Ben Schuler
Zurich Univ.

Nüesch et al, JACS **2022**, *144*, 52-56

Enhanced FRET: detect energy transfer at distances > 10 nm







Baibakov et al, ACS Nano **2019**, *13*, 8469-8480

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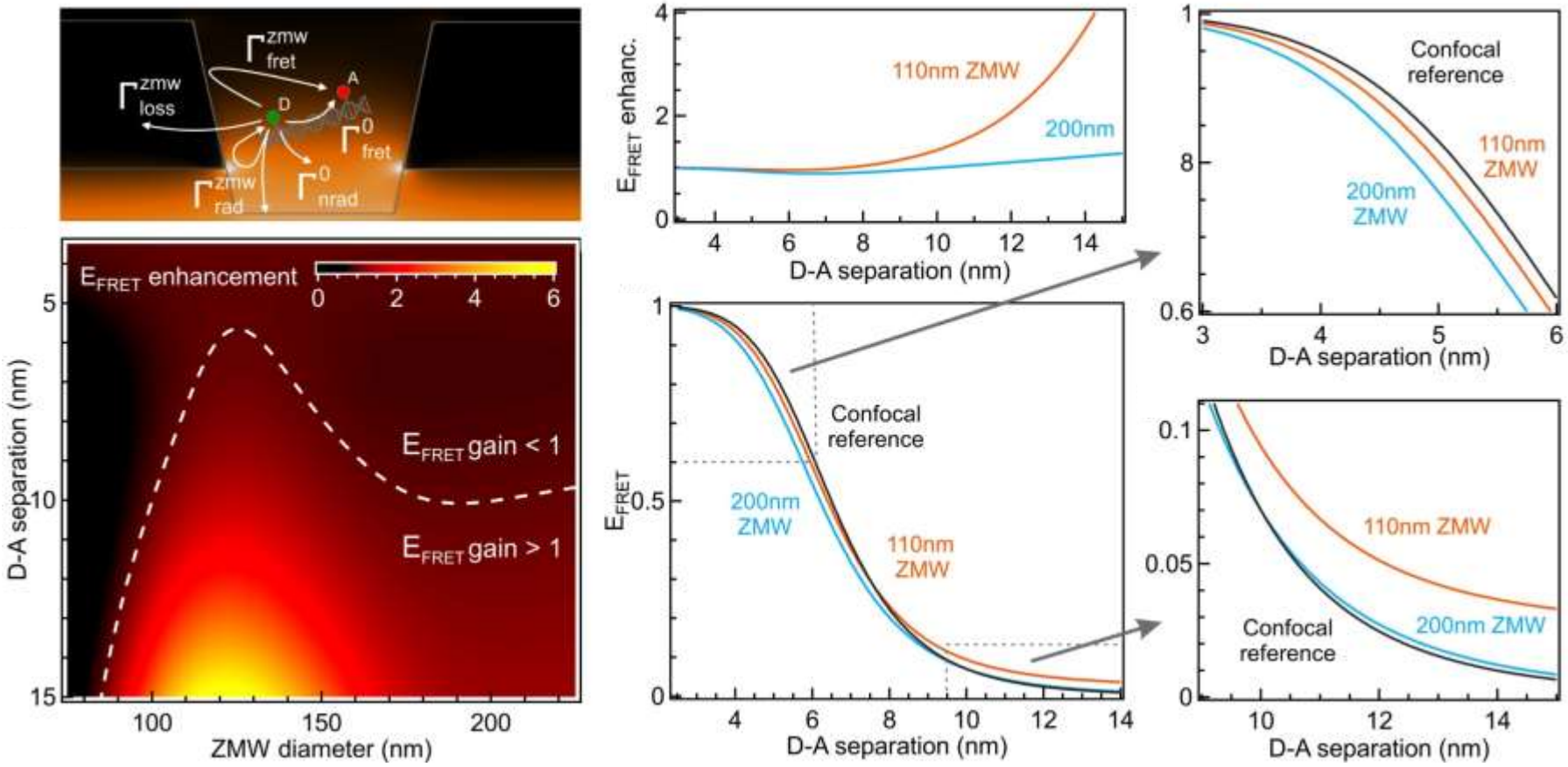
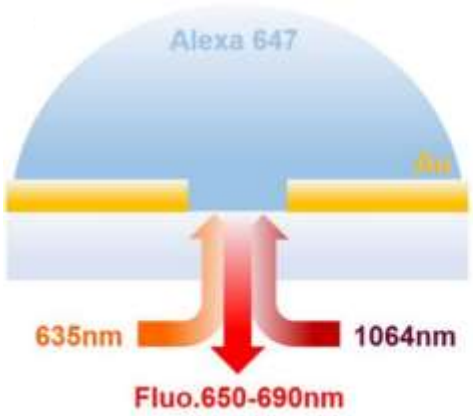
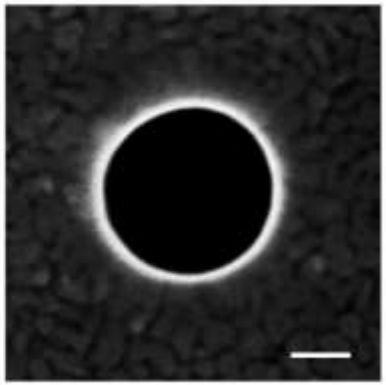
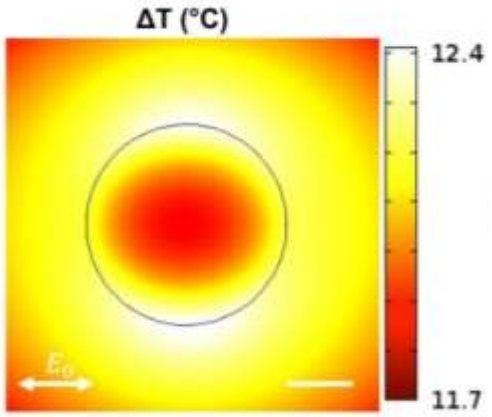


Baibakov et al, ACS Nano **2019**, *13*, 8469-8480

Temperature control: perform fast submillisecond heating cycles

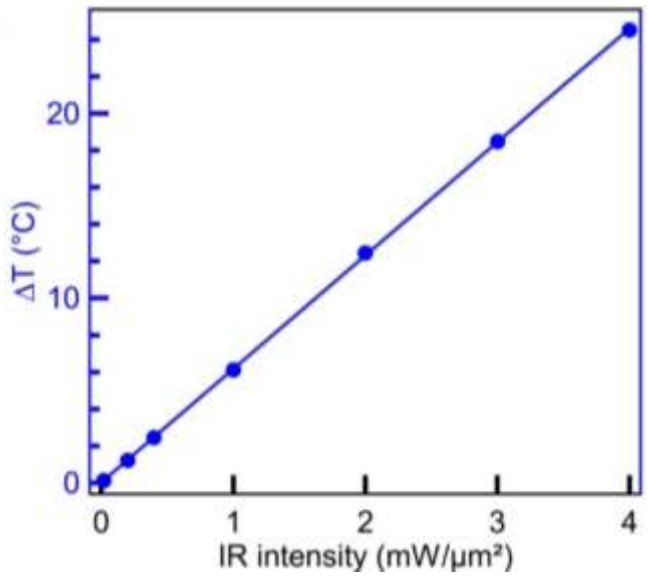


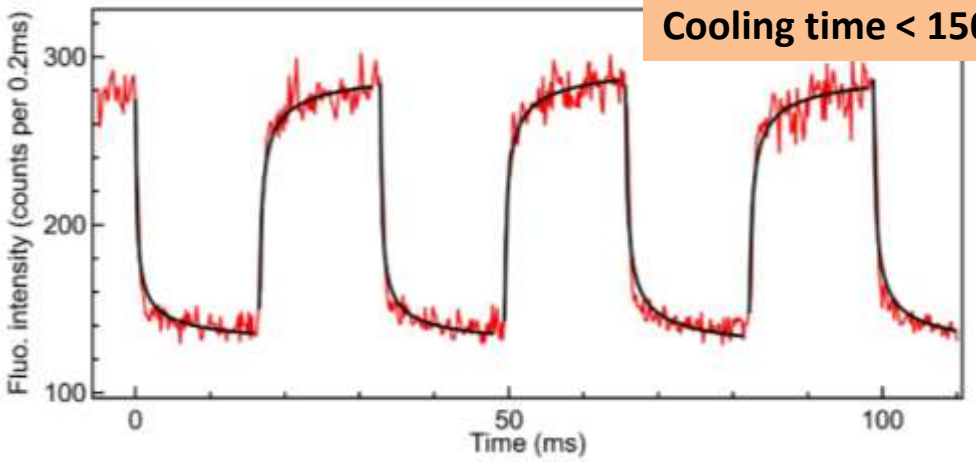




Heating time < 90 µs

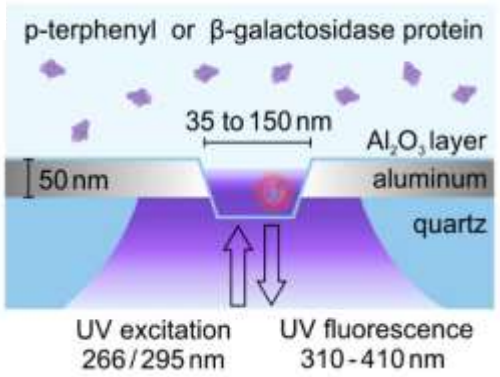
Cooling time < 150 µs

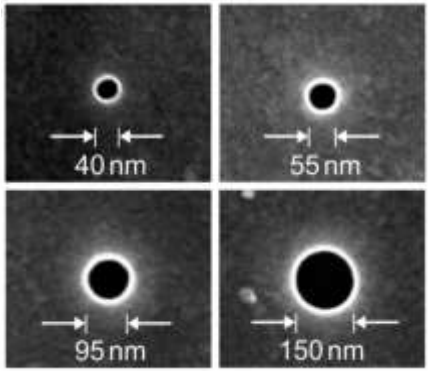


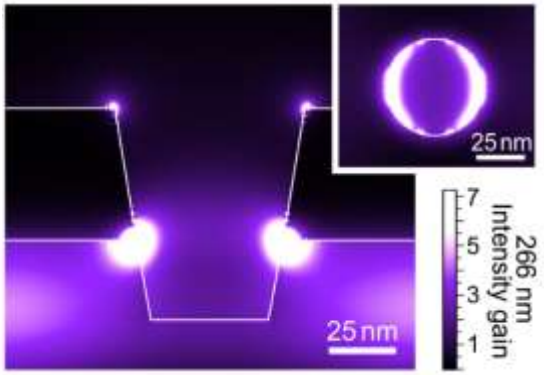


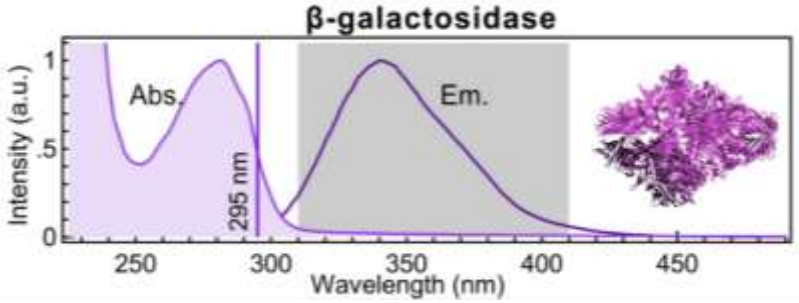
Jiang et al, ACS Photonics **2019**, *6*, 1763-1773

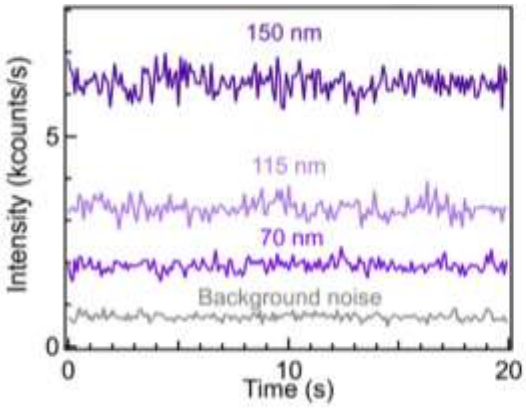
Label-free: detect the intrinsic UV autofluorescence of a protein

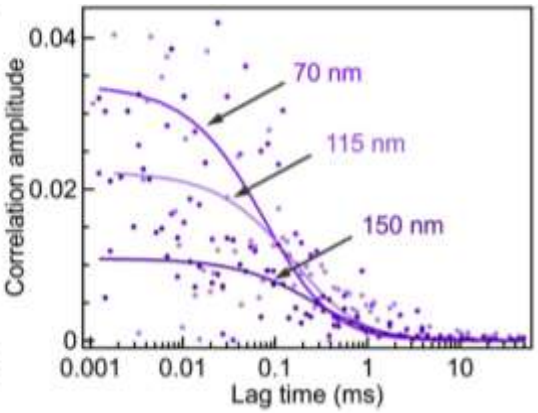












Barulin et al, Nano Lett **2019**, *19*, 7434-7442

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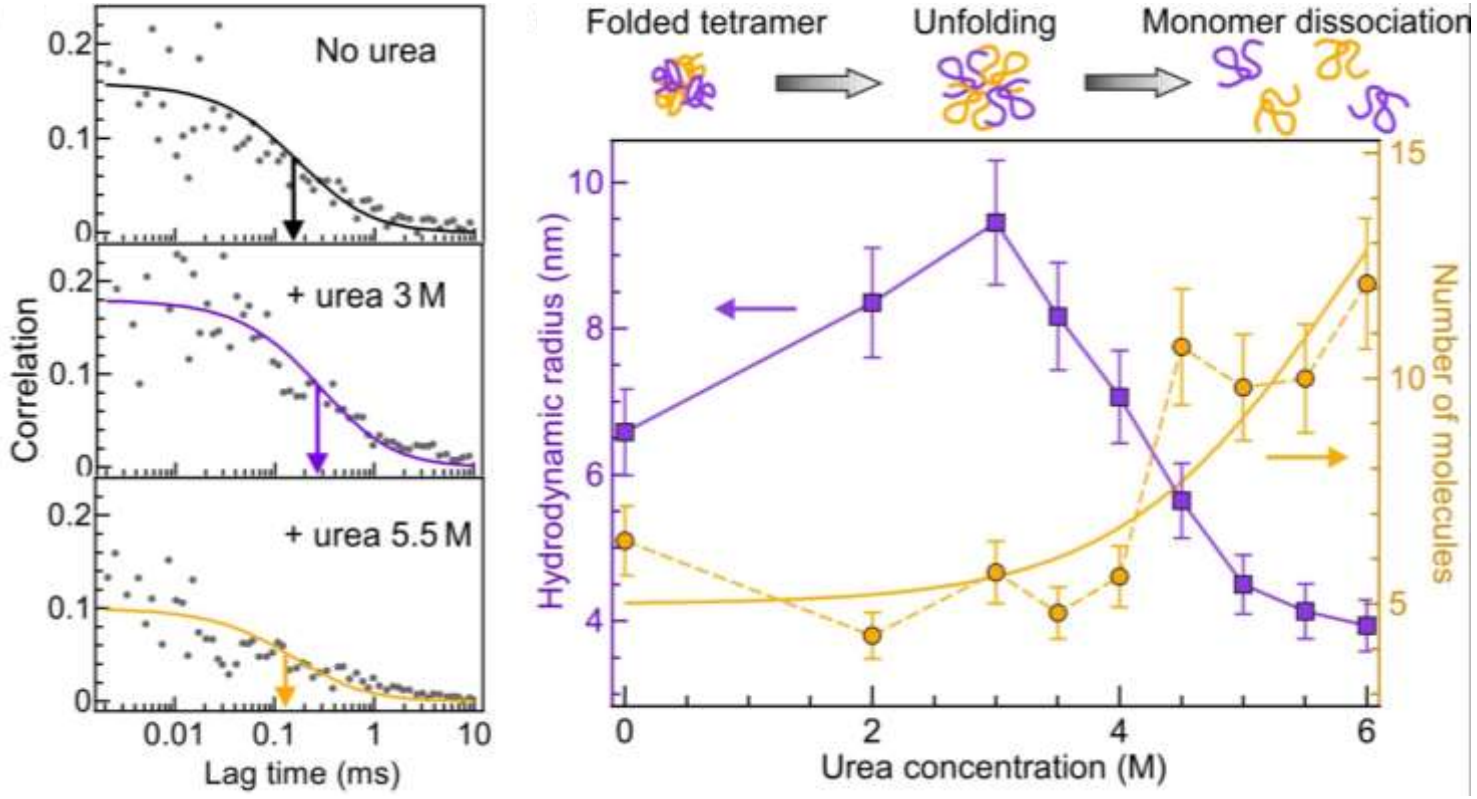
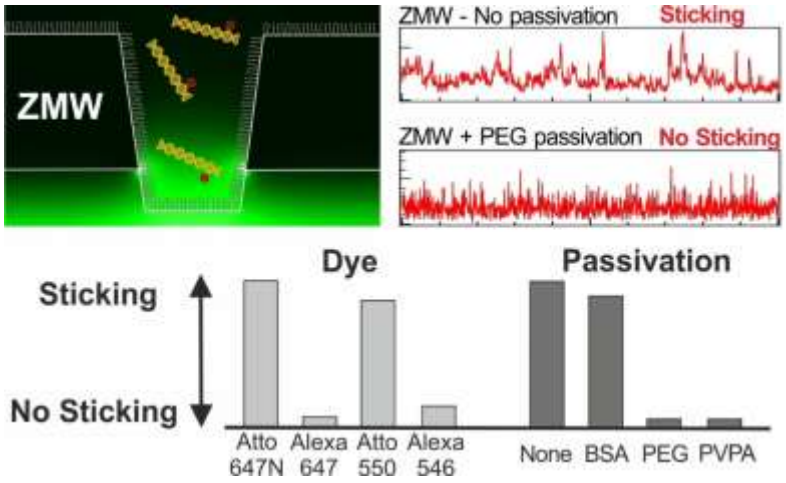


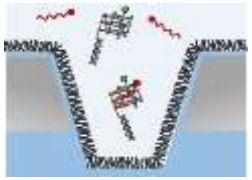
Troubleshooting tips

- **Fabrication:** focused ion beam, ebeam lithography, nanosphere litho.
- **Metal choice:** aluminum for broadband visible, gold for red and near-IR
- **Biomolecules sticking:** minimize with PEG surface passivation, depends on fluorophore
- **Reusability:** several weeks OK after UV-ozone or low-power plasma cleaning



Patra et al, Sci. Rep. **2020**, 10:5235

Conclusions: what nanoapertures ZMWs can do for you



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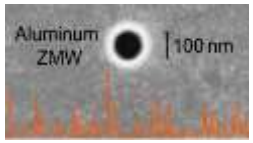

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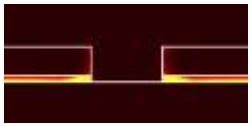

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Aleksandr Barulin, Mikhail Baibakov, Jean-Benoit Claude, Prithu Roy, Benoit Rogez, Quanbo Jiang, Satyajit Patra, Guillaume Baffou, Mark Nüesch, Miloš Ivanović, Daniel Nettels, Robert Best, Ben Schuler, Eloïse Devaux, José Dintinger, Oussama Mahboub, Hervé Rigneault, Thomas Ebbesen