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Optical spectroscopy and biosensors for investigation of biomolecules and their interactions

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Emerging Types of Optical Biosensors II - Single Molecule Interaction Studies

Content

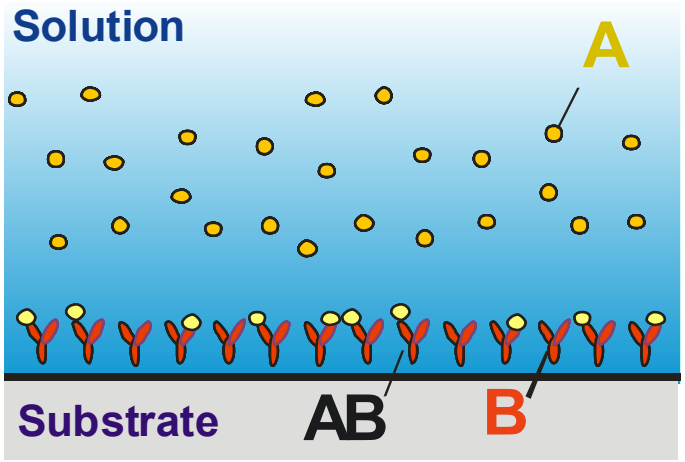
- General motivation for and increasing sensitivity in terms of LOD concentration
- Single molecule analysis vs ensembles of kinetics
- Implementations by using LSPR (wavelength interrogation and rotation).
- Implementations by using WGM.
- Implementations by using TIRF.

Aspects of Single Molecule Sensitivity of Biosensors

Langmuir Adsorption Isotherm

Equilibrium of a reaction:

$$A + B \underset{k_d}{\overset{k_a}{\rightleftarrows}} \qquad K = \frac{k_a}{k_d}$$



Kinetics of the reaction on a surface:

$$\frac{d\gamma}{dt} = k_a \alpha_0 (\beta - \gamma) - k_d \gamma$$

γ Concentration of [AB]

α_0 Concentration [A]

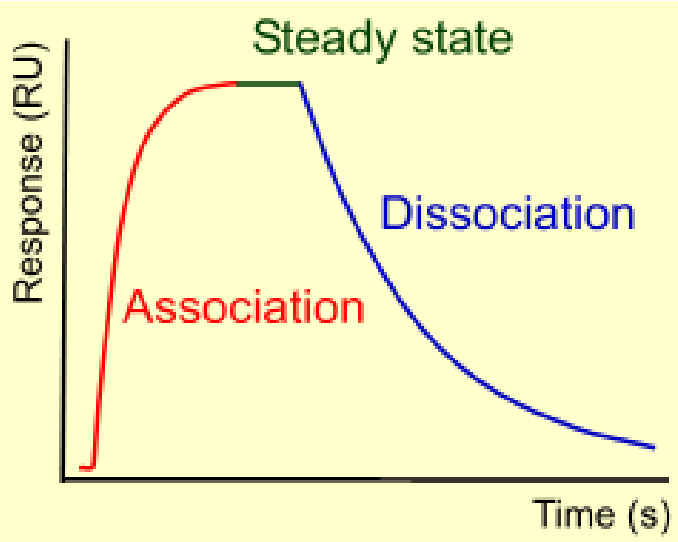
β Concentration [B]

Describes the interaction for:

a) Identical monovalent receptors B
b) Constant concentration of A in the solution ([A]>>[B])

(Possible to describe more complicated interactions e.g. multivalent receptors)

Binding Kinetics Sensorgram

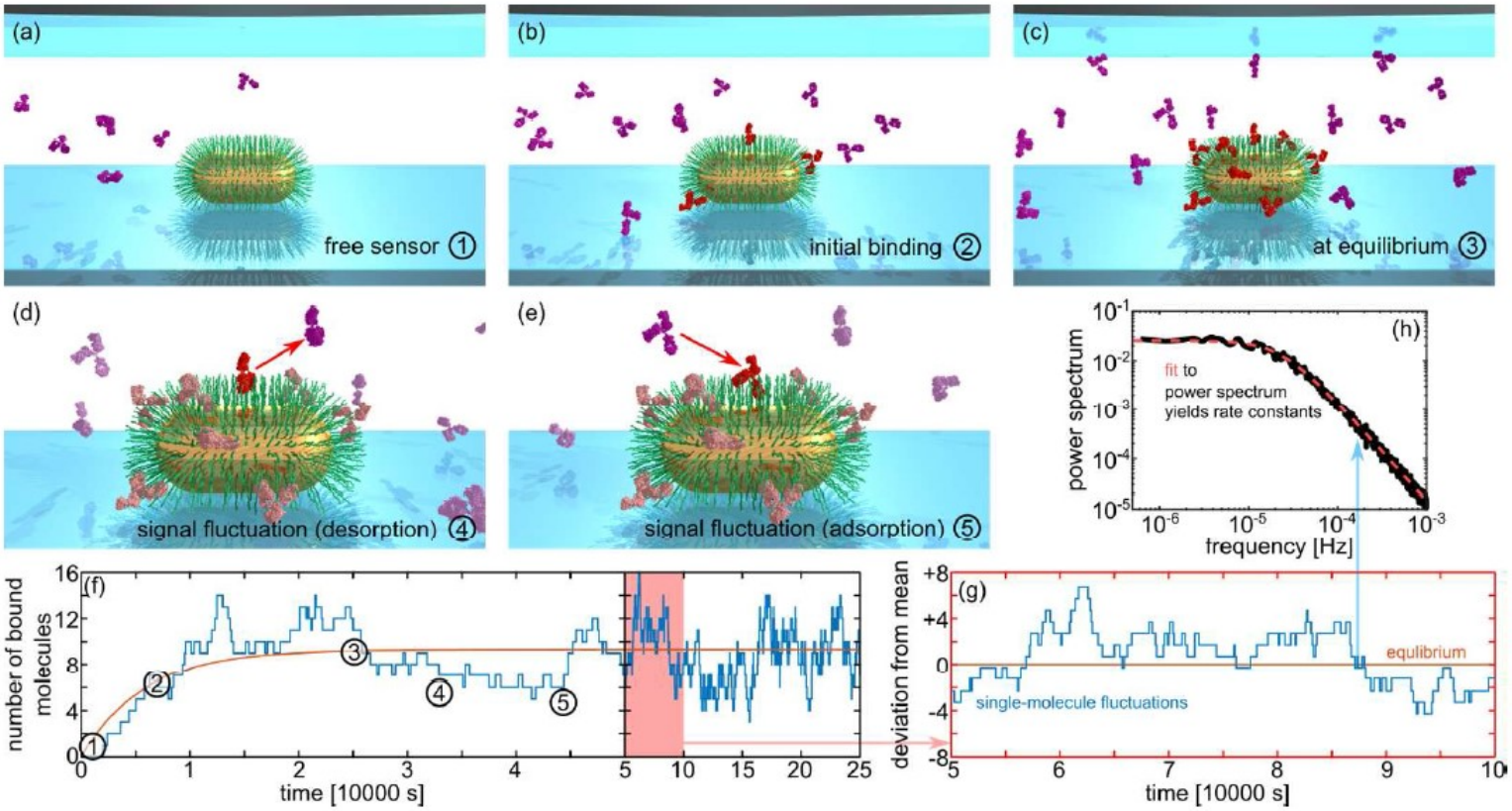


On ensembles represented by association and dissociation binding rates (k_a and k_d, respectively)

At single molecule level, are there some new phenomena hidden by averaging over many reactions monitored?

Probing of Small Volumes

10.1021/acsnano.8b04016

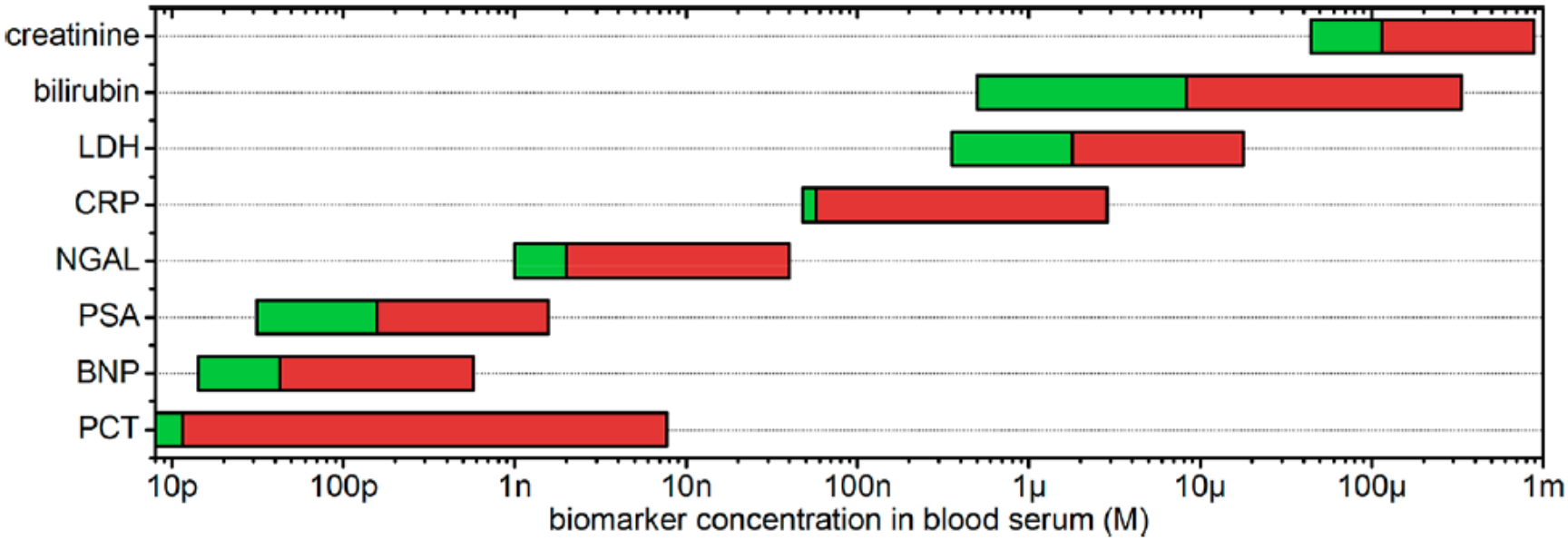


- Association and dissociation binding rates (k_a and k_d, respectively) are represented by the rate of molecules attachment and time until the molecule detaches.

Single Molecule Detection

- Ultimate sensitivity enabling counting of individual molecules (possible with arrays of miniature sensors – digital readout of assays)
- Monitoring of heterogeneities and fluctuations of biomolecules properties that are hidden in ensembles.
- Potentially better specificity as non specific interactions may be filtered out.

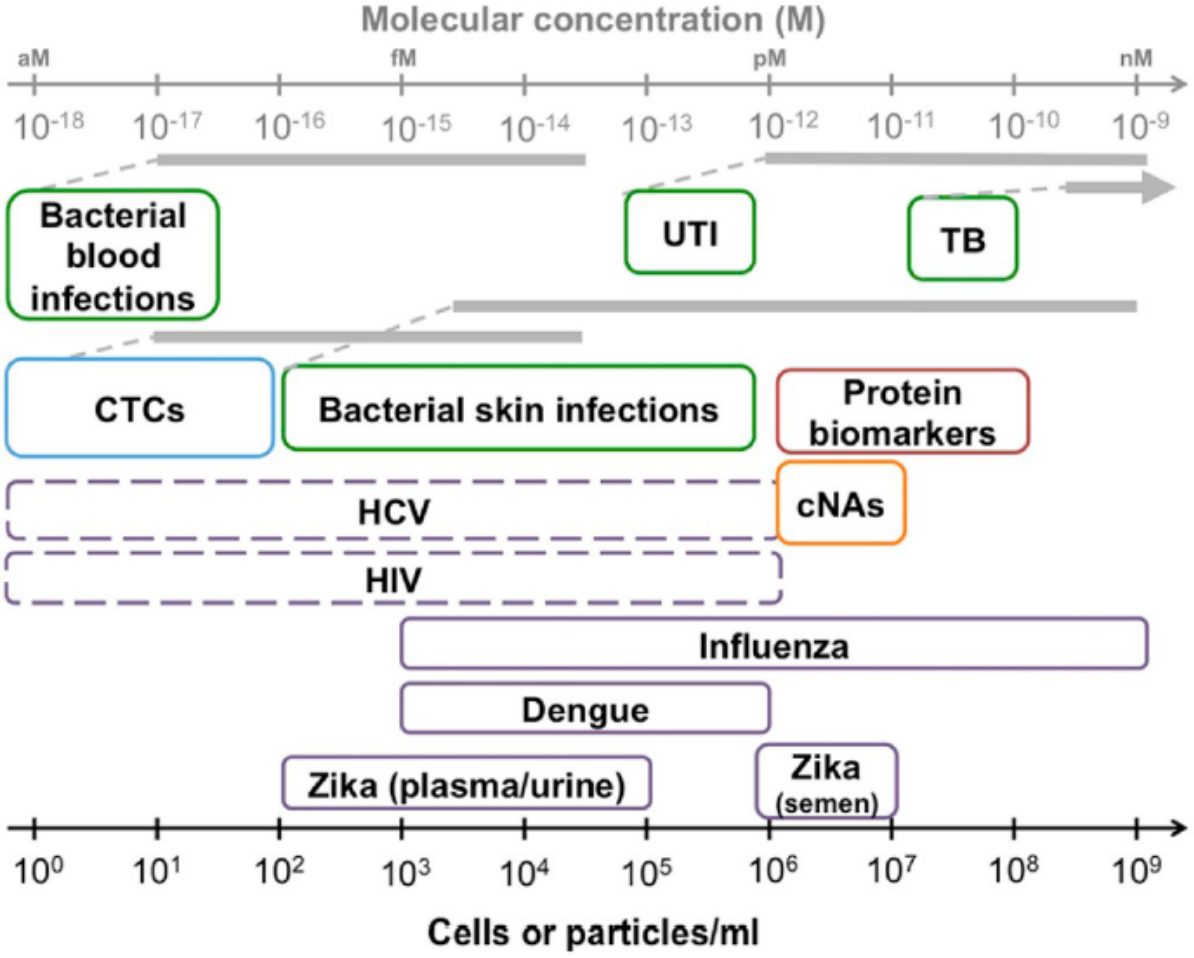
Concentration of Analytes in Clinical Samples



Typical concentration range of clinically relevant biomarkers in blood serum. The green bars indicate reference values for healthy persons, whereas the red extension to the right indicates elevated values associated with disease. LDH: lactate dehydrogenase, CRP: c-reactive protein, NGAL: neutrophil gelatinase-associated lipocalin, PSA: prostate specific antigen, BNP: B-type natriuretic peptide, PCT: pro-calcitonin

Mayo Medical Laboratories; www.mayomedicallaboratories.com, accessed 19-5-2017.

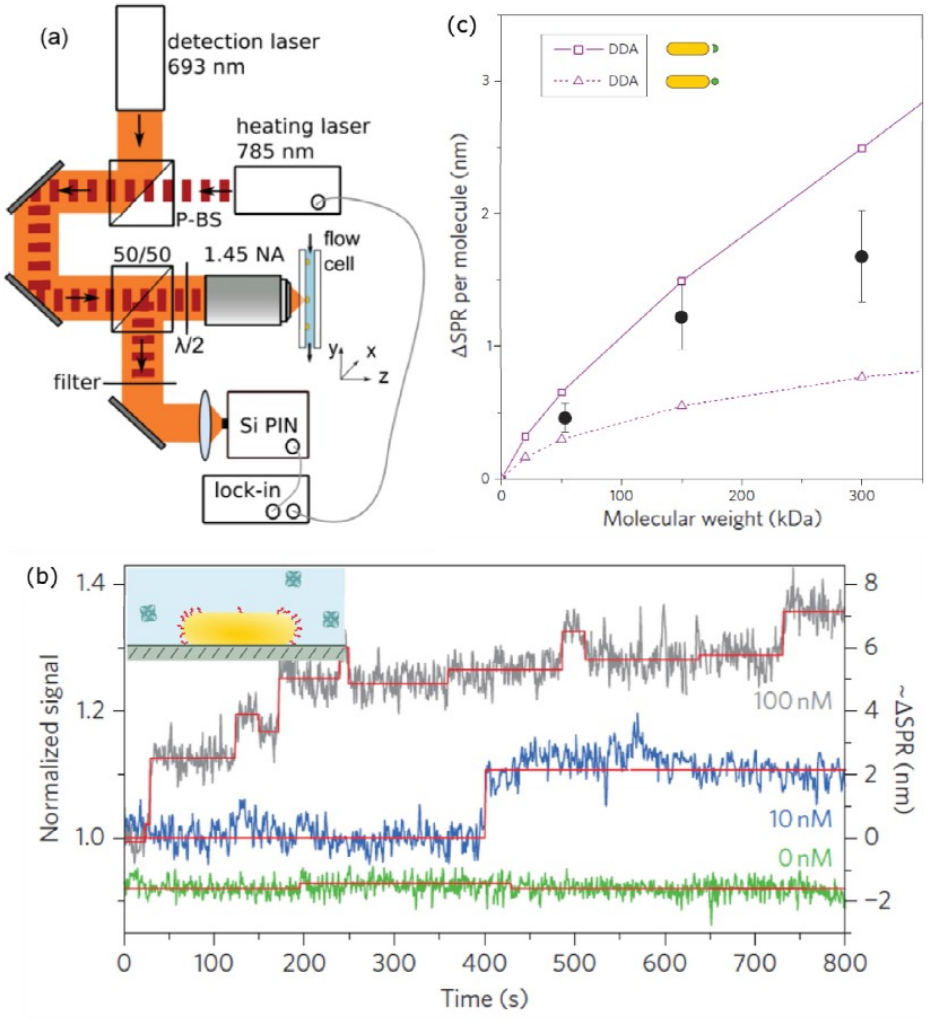
Concentration of Analytes in Clinical Samples



Kelley, S.O., 2017. What are clinically relevant levels of cellular and biomolecular analytes? ACS Sens. 2 (2), 193–197.

Localized Surface Plasmon Resonance – based Platforms (LSPR)

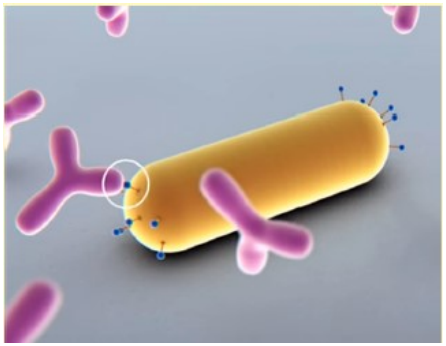
LSPR Observation of Single Molecule Binding Kinetics



- Tracking LSPR wavelength allows for monitoring of individual on and off binding events.
- First direct label-free detection of individual streptavidin molecules demonstrate in 2012.

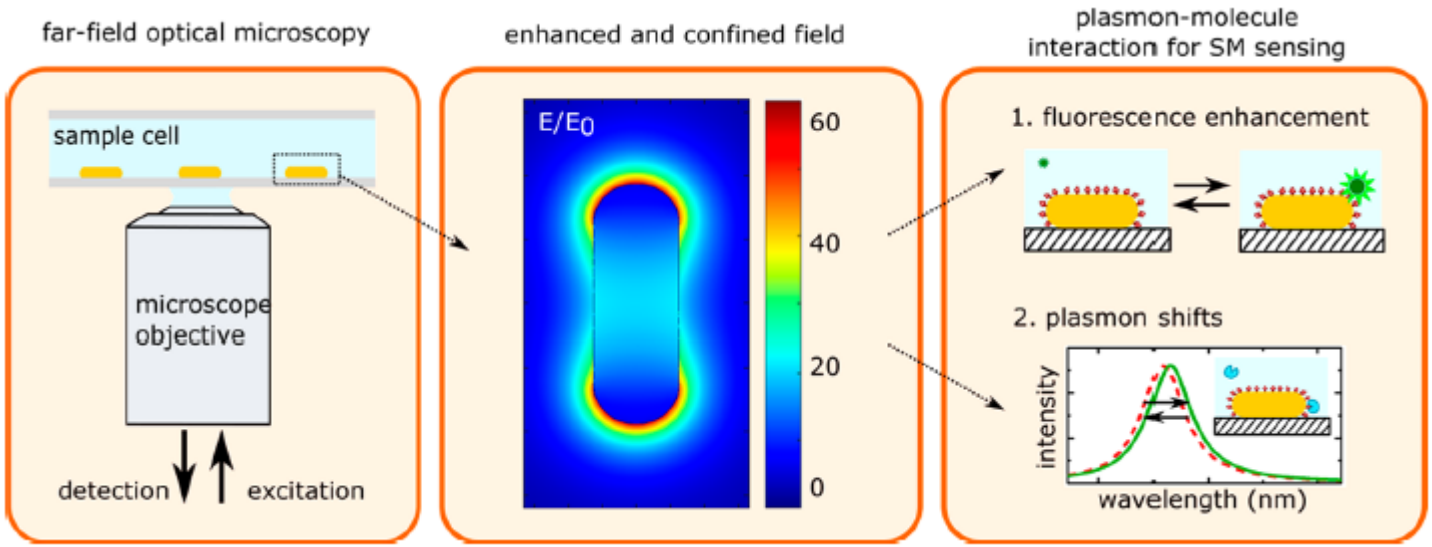
Zijlstra, P.; Paulo, P. M. R.; Orrit, M. Optical Detection of Single Non-Absorbing Molecules Using the Surface Plasmon Resonance of a Gold Nanorod. Nat. Nanotechnol. 2012, 7 (6), 379–382.

Binding at Plasmonic Hotspot



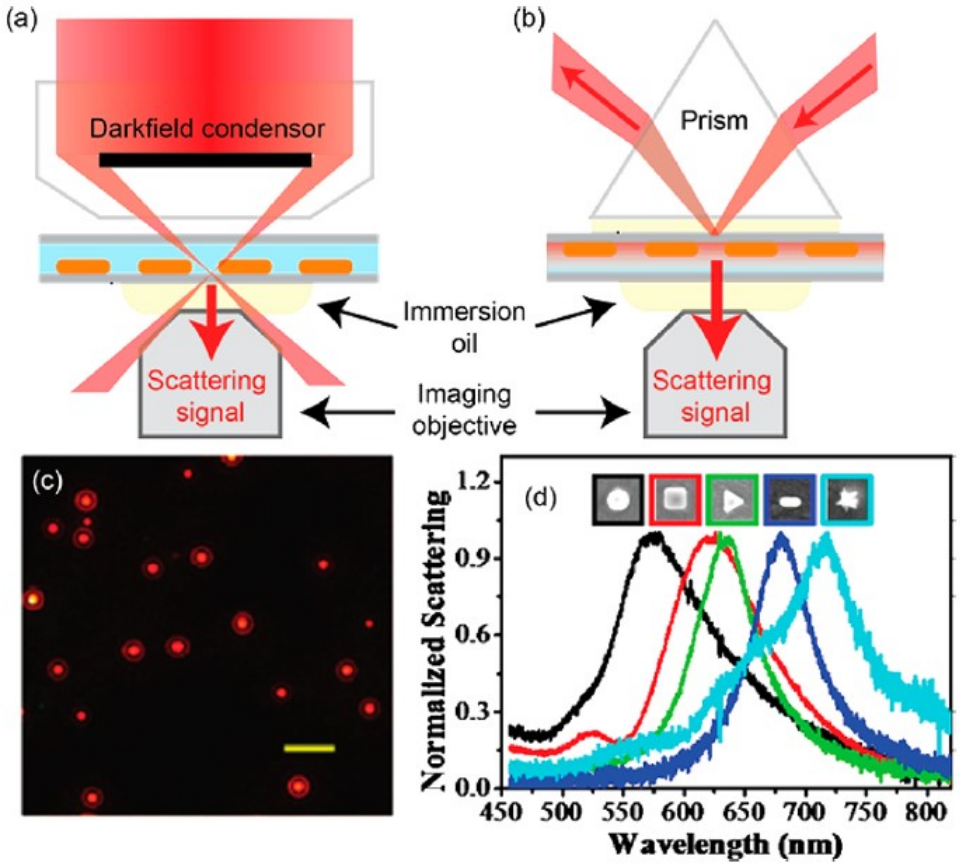
- Only at plasmonic hotspot the light energy is confined and LSPR is sensitive to changes due to binding events.

DOI: 10.1021/acssensors.7b00382
ACS Sens. 2017, 2, 1103−1122



LSPR Probing on Individual Nanoparticles

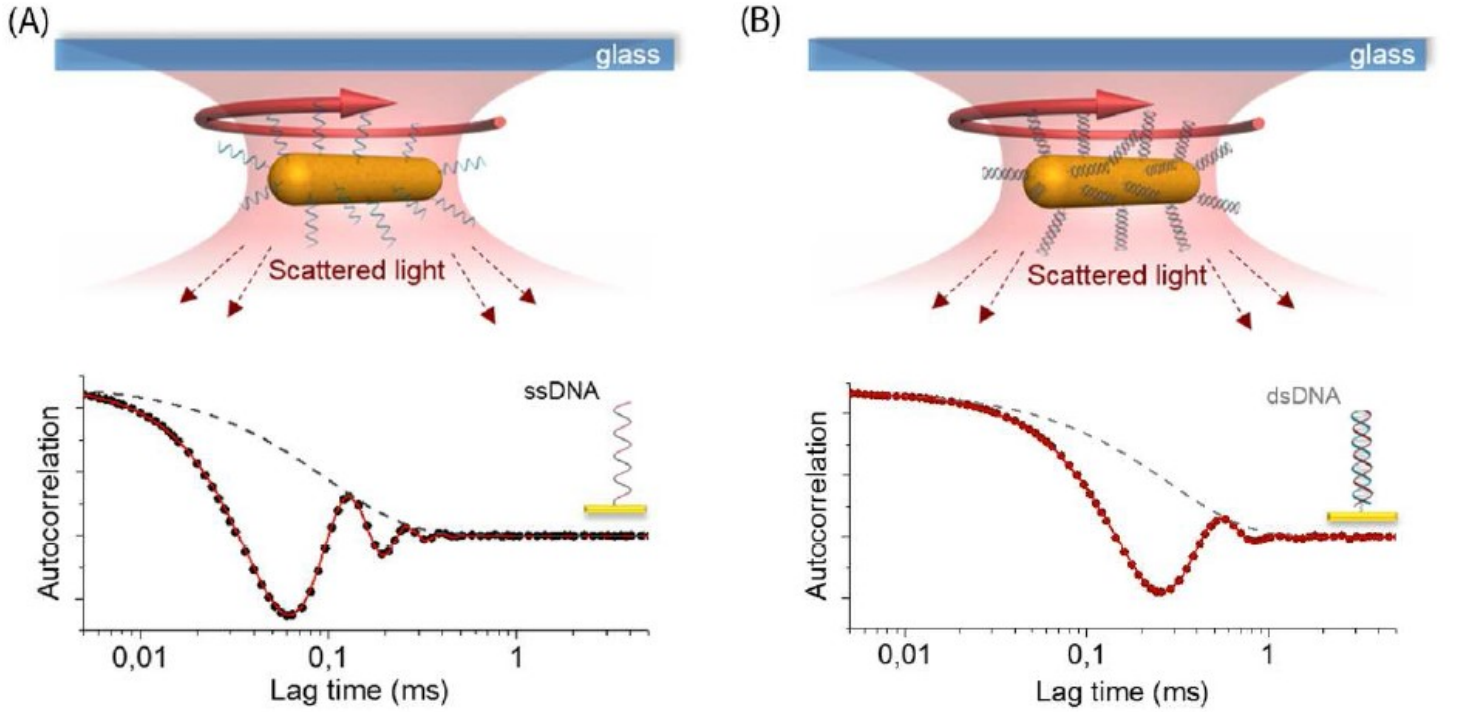
DOI: 10.1021/acssensors.7b00382
ACS Sens. 2017, 2, 1103−1122



- Dark field microscopy combined with wavelength spectroscopy of LSPR-scattered light

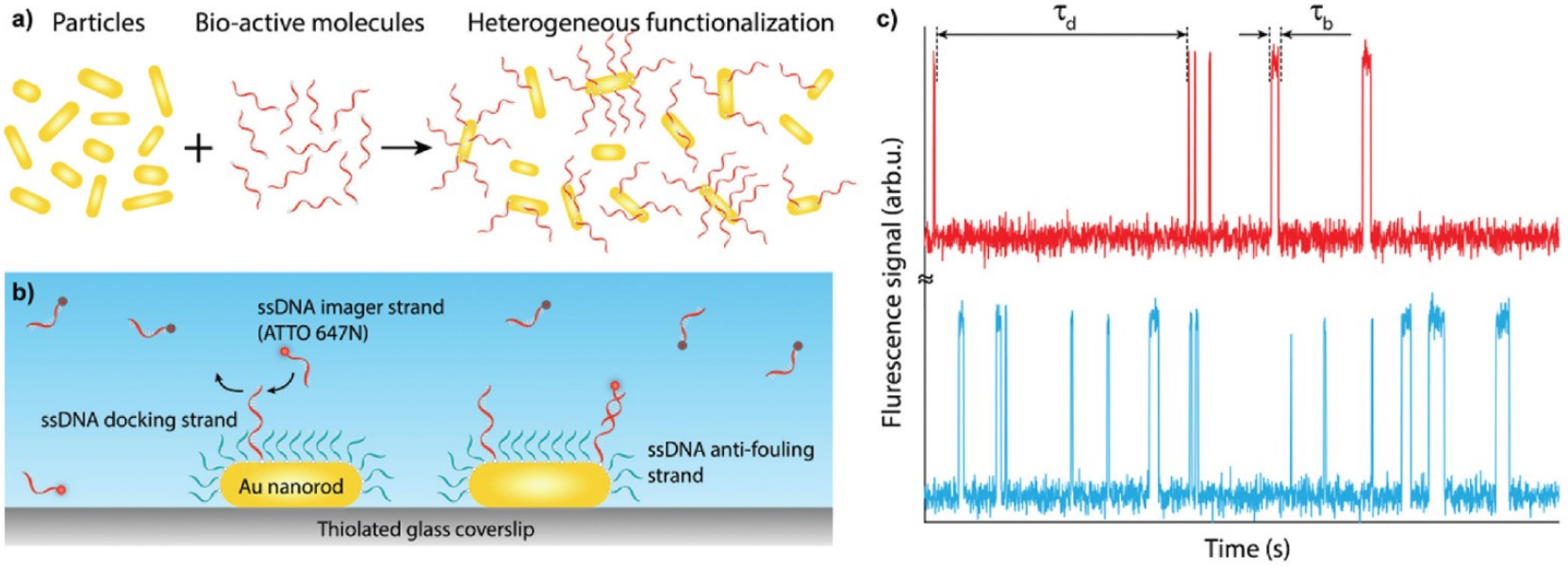
LSPR Probing on Individual Nanoparticles

10.1021/acsphotonics.8b00034



- Optical tweezer can be used for tapping and spectroscopy on individual LSPR nanoparticles.
- Employed for monitoring of rotation and detection of a lag between riving force and rotation (see autocorrelation function).

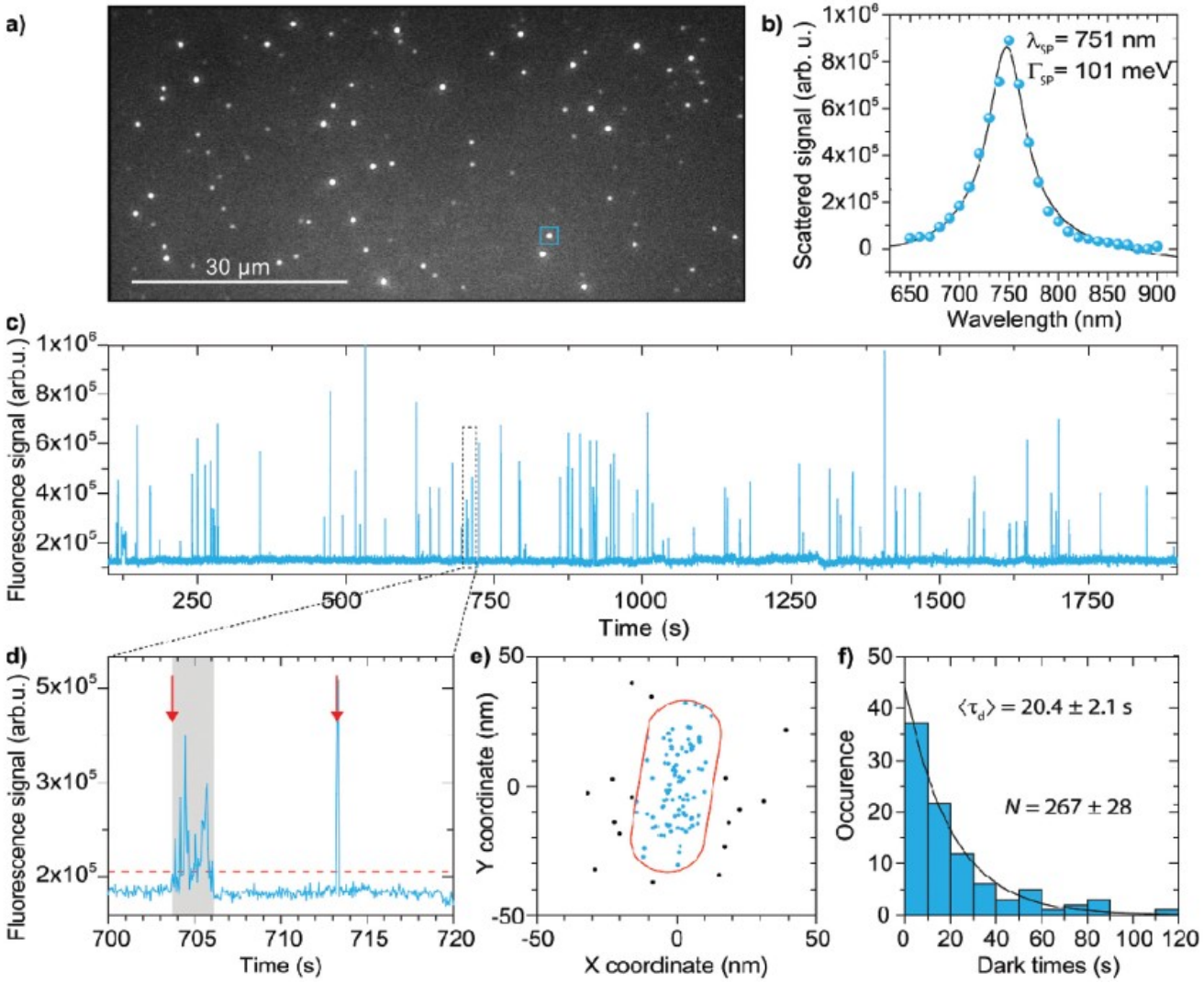
Fluorescence Mapping of Functionalization Homogeneity



DOI: 10.1039/c9nr10218c

- Arrival of fluorophore labeled molecules observed as series of fluorescence signal bursts.

Fluorescence Mapping of Functionalization Homogeneity



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Fluorescence Mapping of Functionalization Homogeneity

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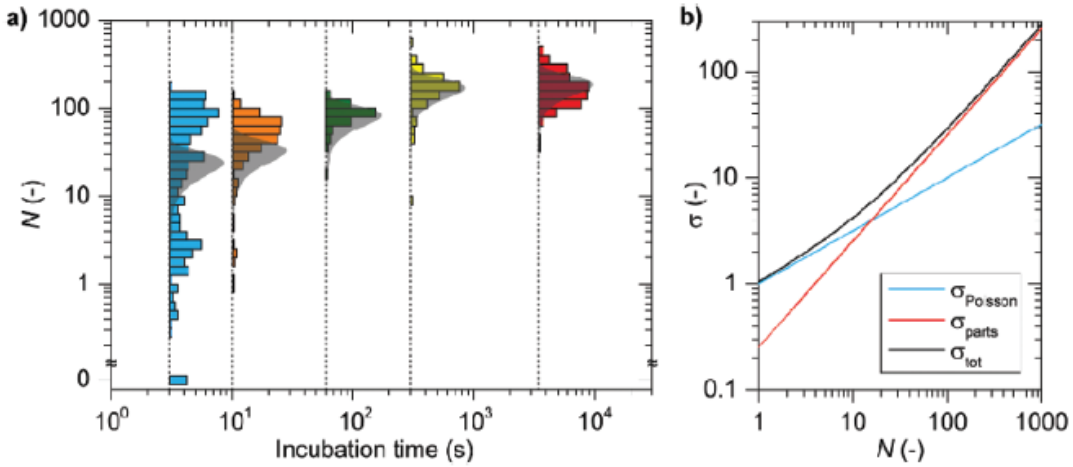
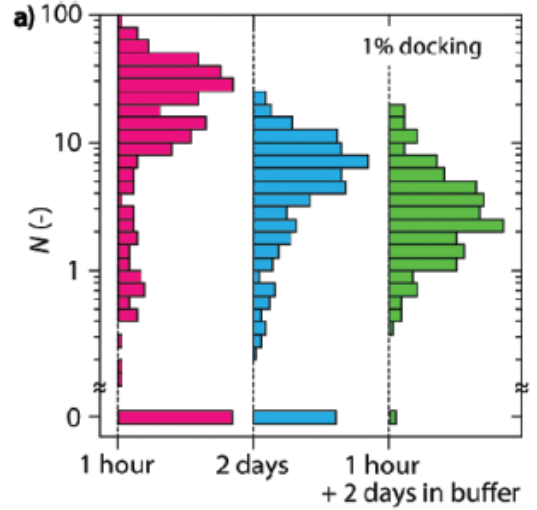
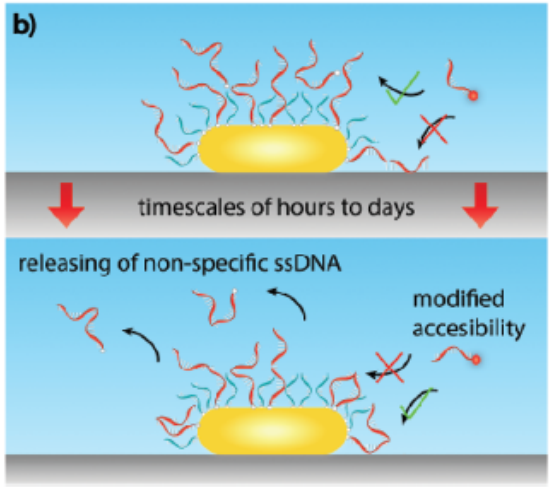


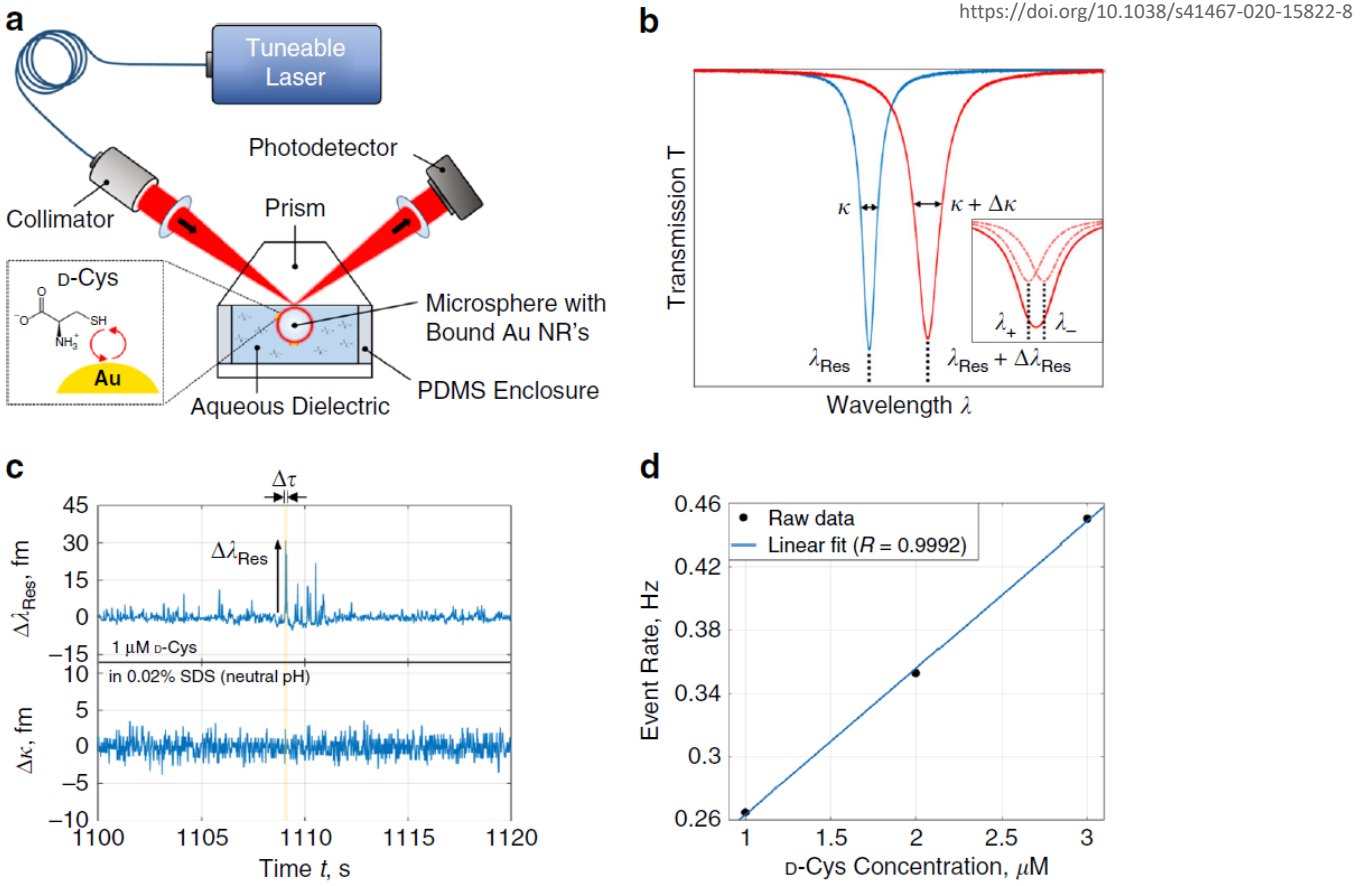




- Single molecule counting enables determining statistics of inter-particle differences and changes in affinity binding of target complementary strands.

Whispering Gallery – based Platforms (WGM)

WGM coupled with LSPs

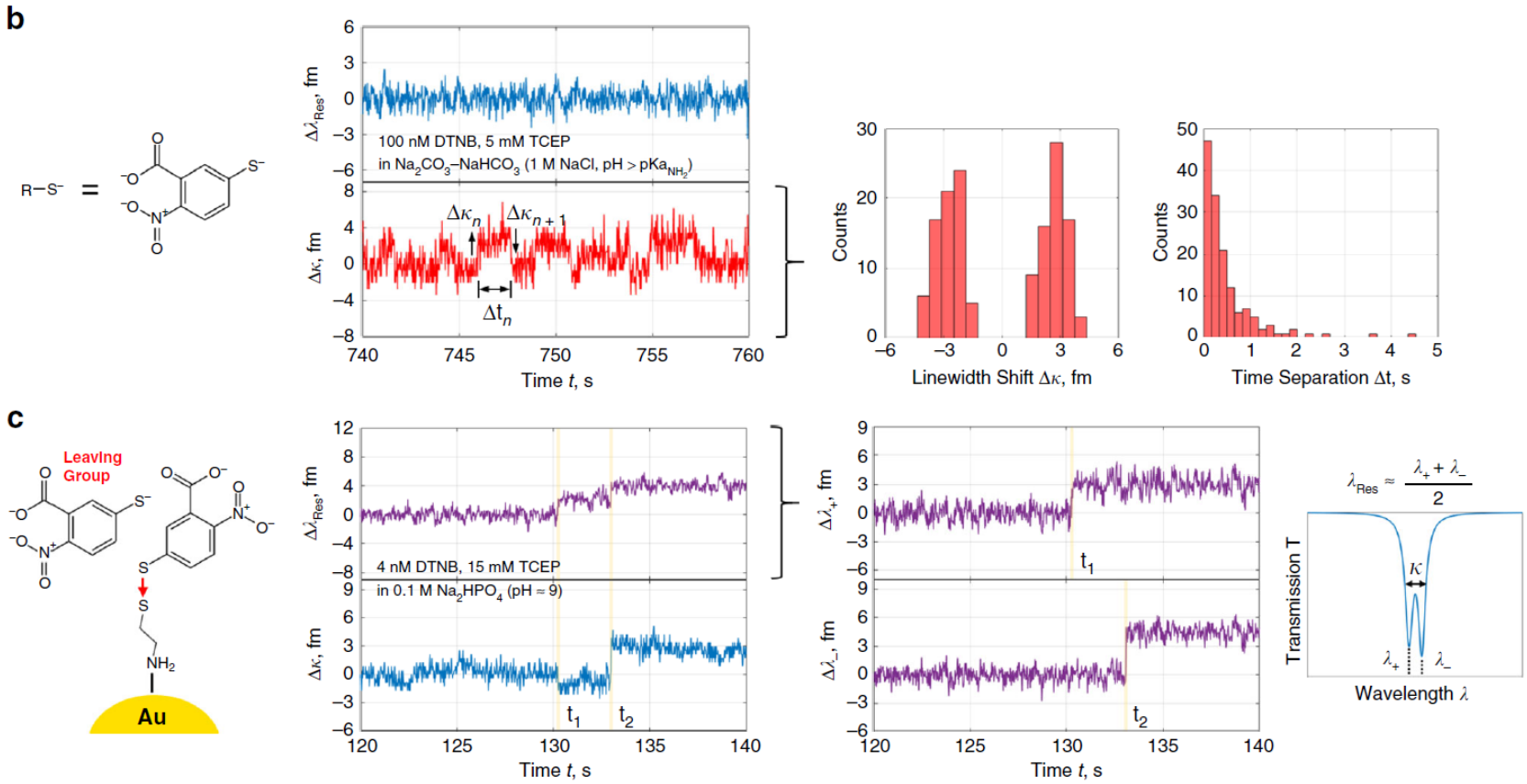


https://doi.org/10.1038/s41467-020-15822-8

- Confinement of the probing field provided by attaching metallic nanoparticles with glass beads supporting WGM.

Low Molecular Weight Analyte Reactions

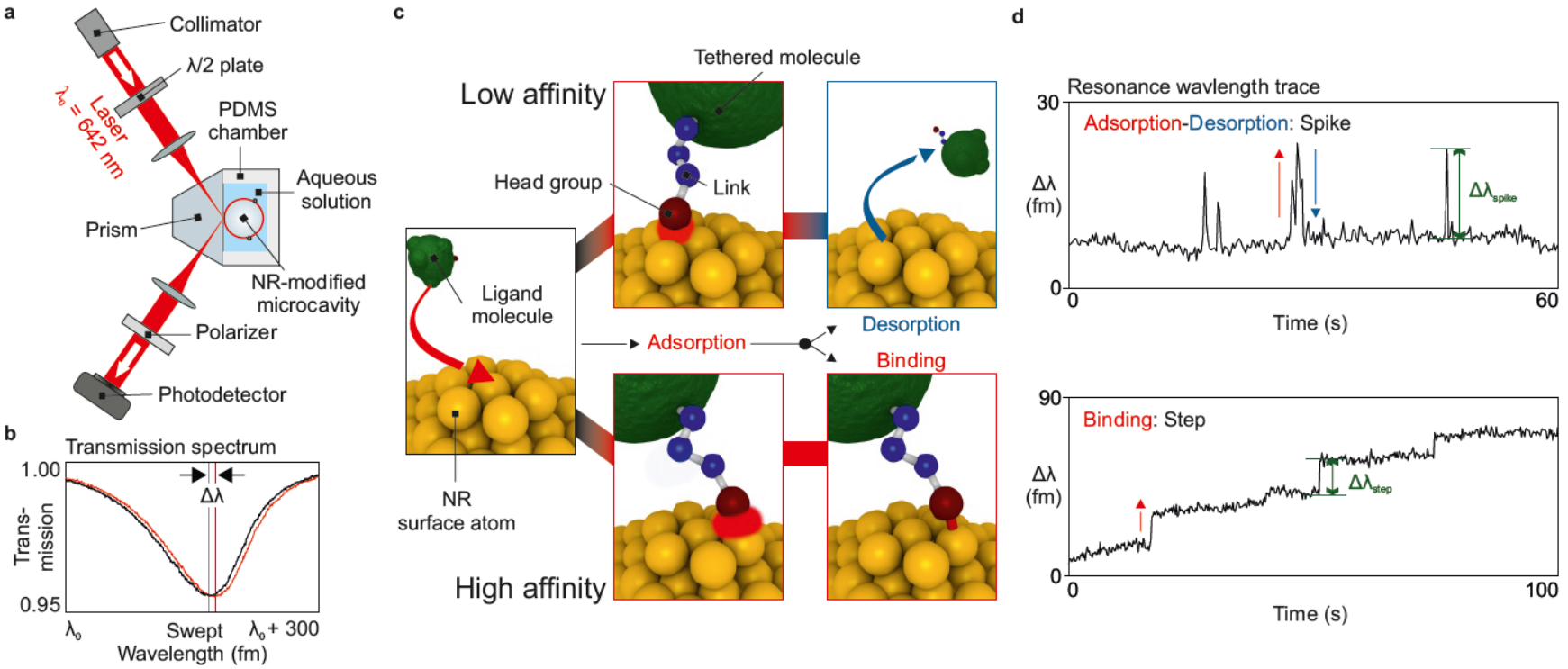
https://doi.org/10.1038/s41467-020-15822-8



- Tracking of changes in resonant wavelength and width upon individual molecule reaction steps.

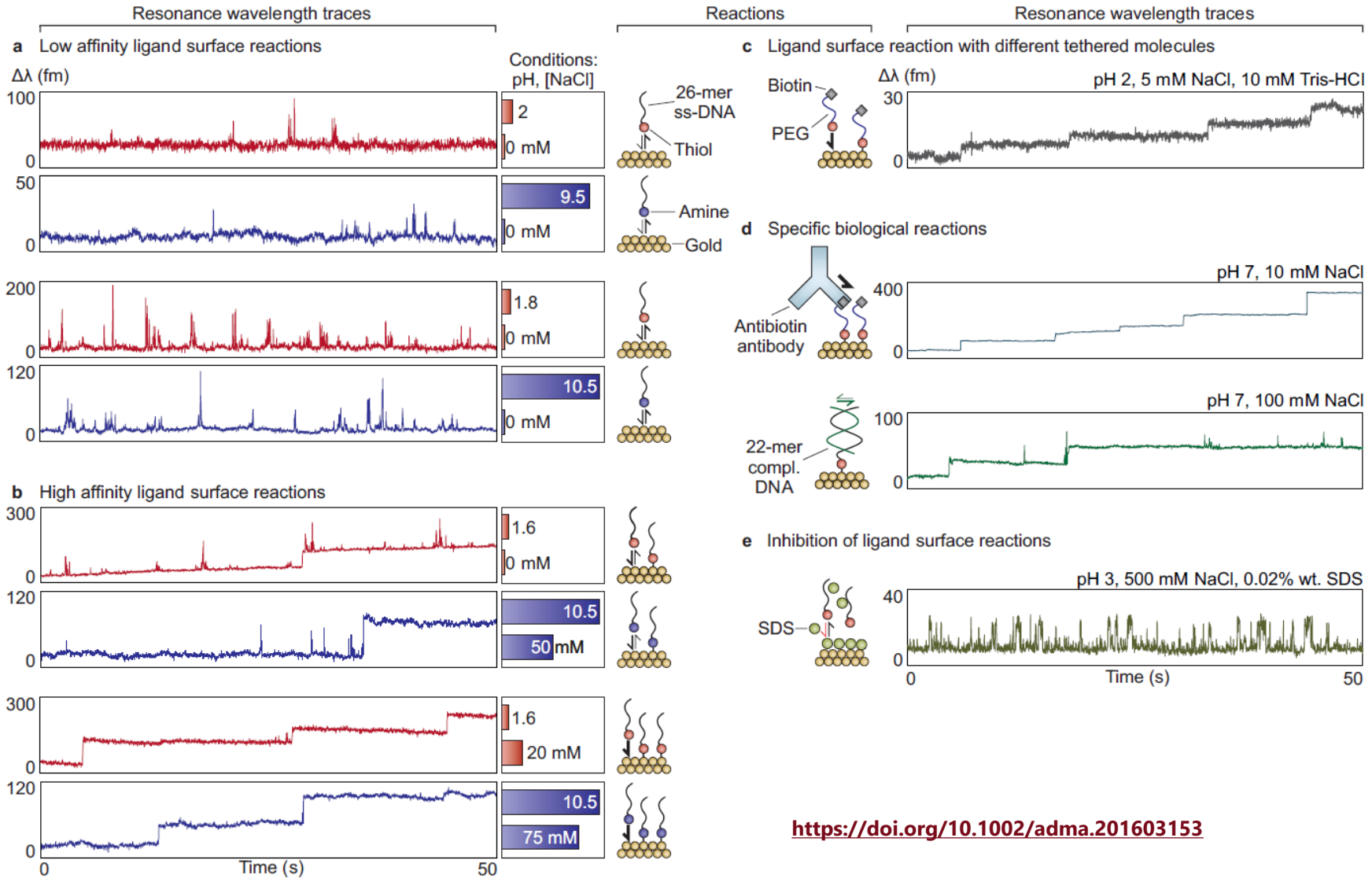
Effect of Affinity

https://doi.org/10.1002/adma.201603153



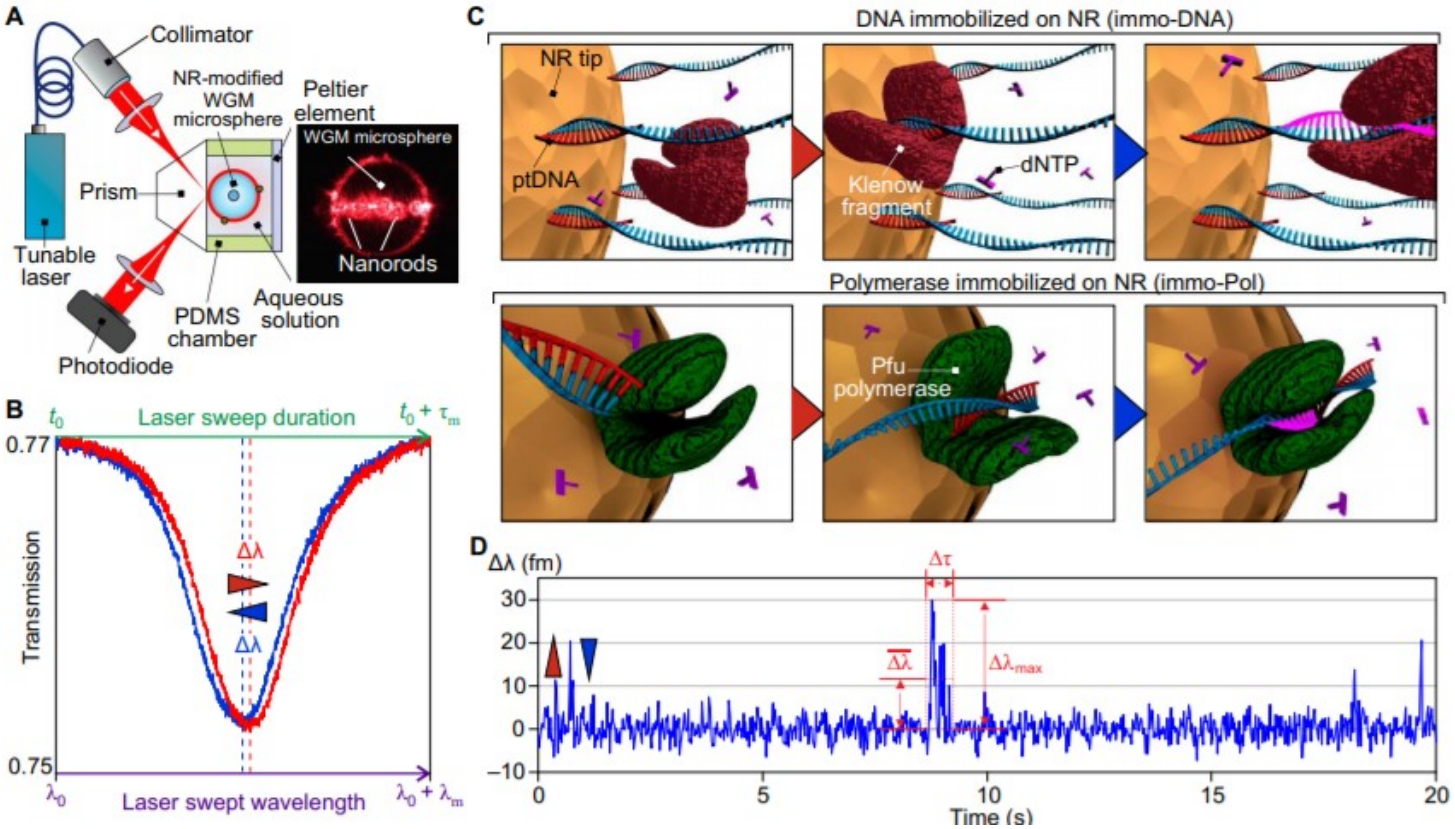
- Low affinity interaction are seen as spikes with short residence time ($1/k_d$) while high affinity interactions manifest themselves as steps.

Effect of Affinity and Molecular Weight



https://doi.org/10.1002/adma.201603153

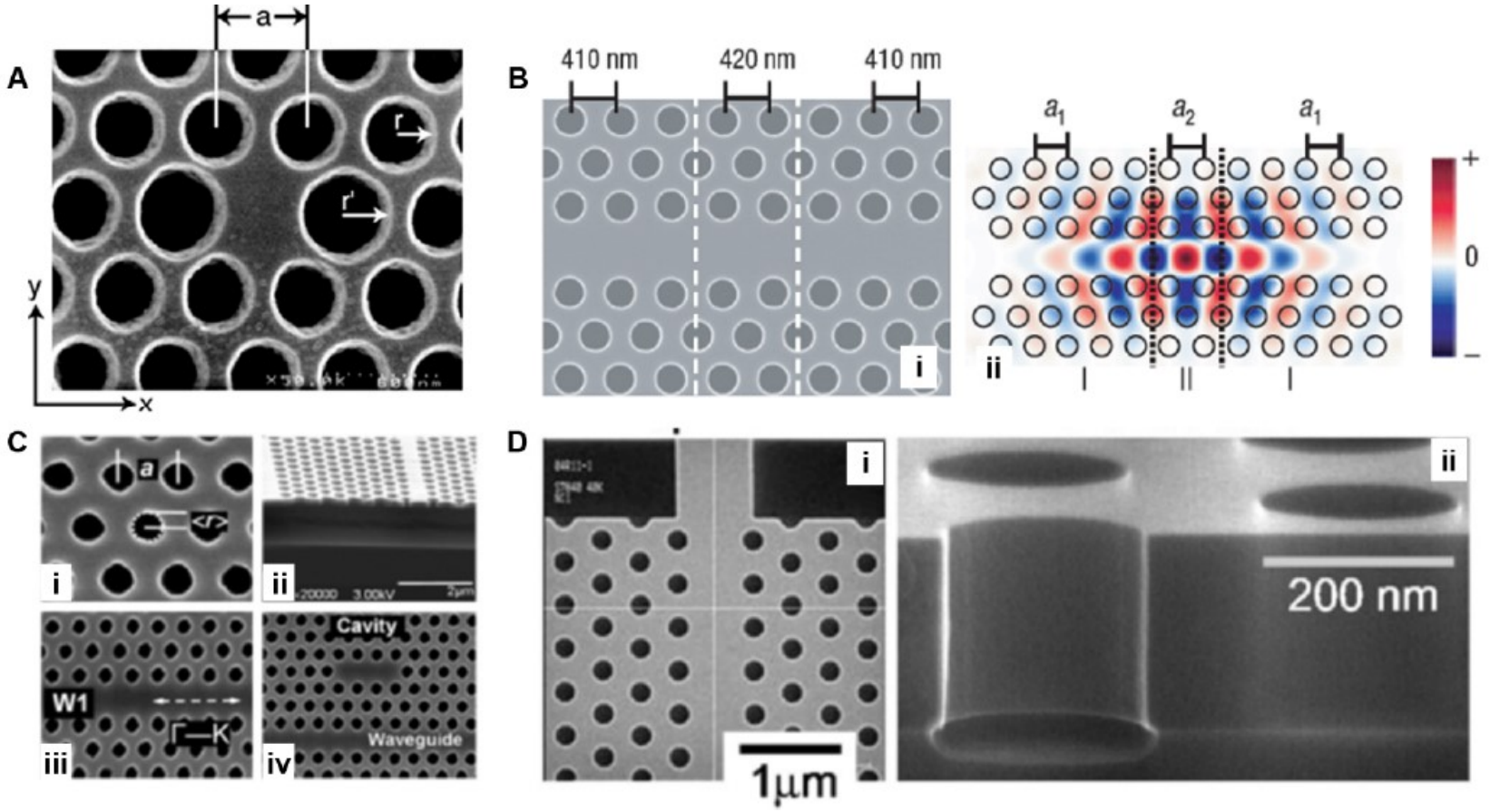
Conformation Changes



- Observation of conformational changes of polymerase enzyme was claimed at single molecule level.

DOI: 10.1126/sciadv.1603044

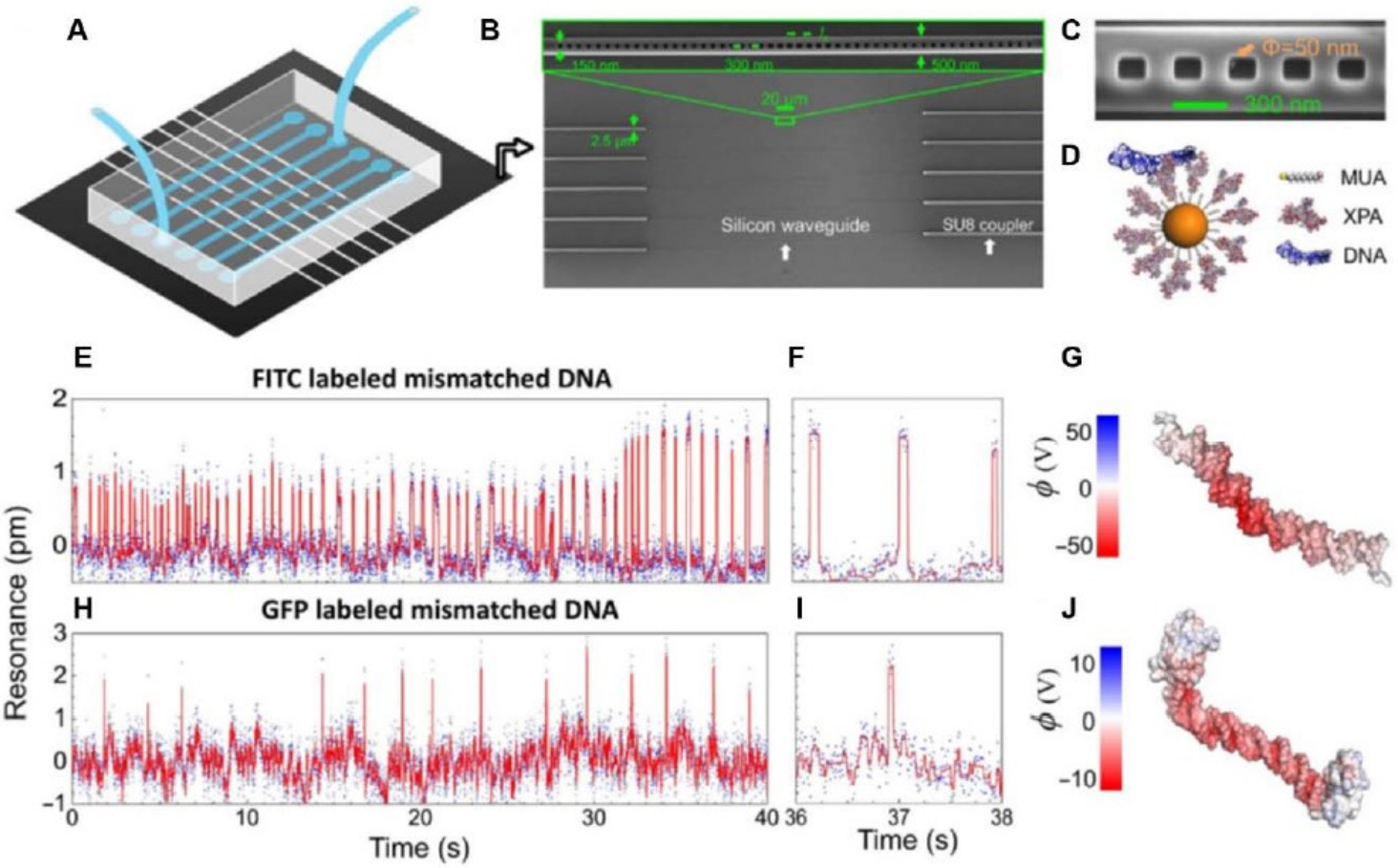
Photonics Crystal - Alternative to WGM



- Lithography-based preparation of photonic crystals with defects enables forming low loss cavity modes.

https://doi.org/10.1002/adma.201603153

Photonics Crystal - Alternative to WGM



- Individual binding events observed by using metallic nanoparticle labels.

https://doi.org/10.1002/adma.201603153

Other Label – based Platforms

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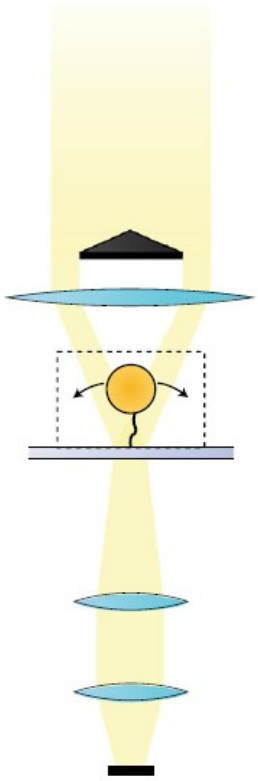
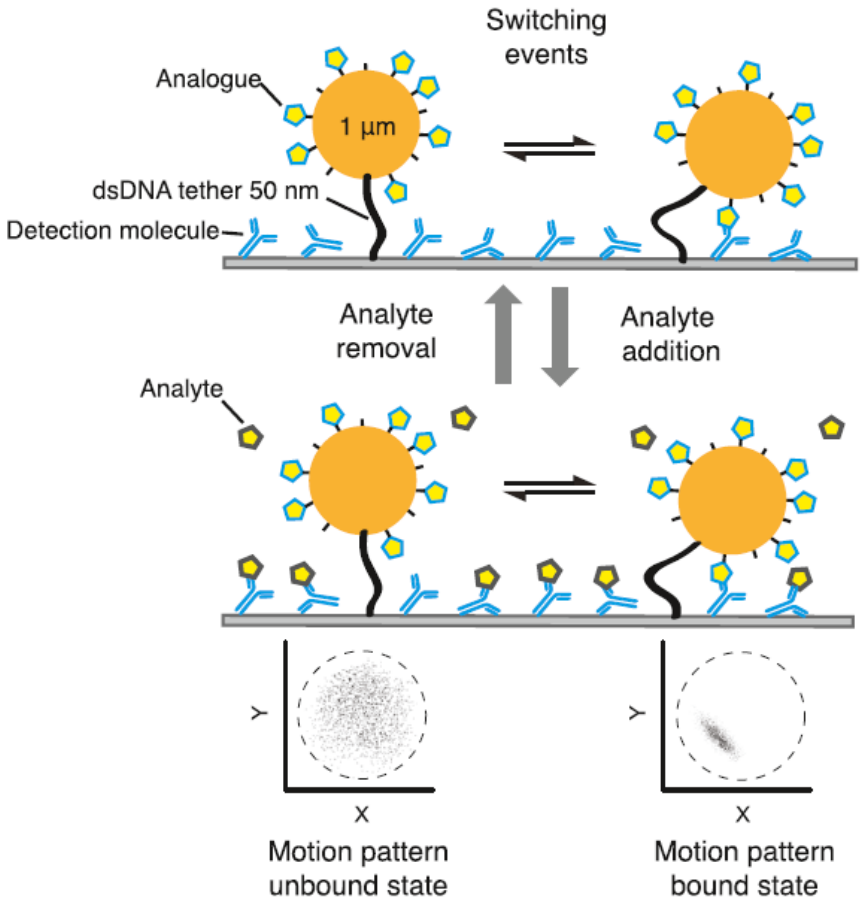
Scattering-based Continuous Detection of Low Molecular Weight Analyte





- Monitoring of Brownian motion of microparticles attached via flexible polymer chain.
- Affinity interaction with fast k_{off} used for reversible capturing and releasing the particle.

Junhong Yan, Laura van Smeden, Maarten Merkx, Peter Zijlstra, and Menno W. J. Prins, Continuous Small-Molecule Monitoring with a Digital Single-Particle Switch, ACS Sens. 2020, 5, 1168−1176.
Emiel W.A. Visser, Junhong Yan, Leo J. van Ijzendoorn, Menno W.J. Prins, Continuous biomarker monitoring by particle mobility sensing with single molecule resolution, DOI: 10.1038/s41467-018-04802-8 |www.nature.com/naturecommunications

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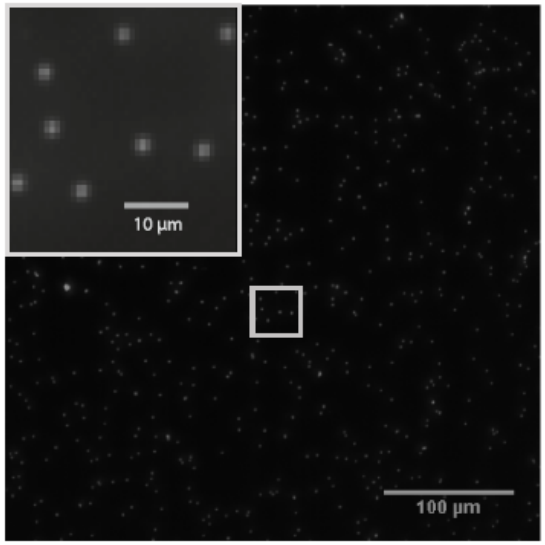
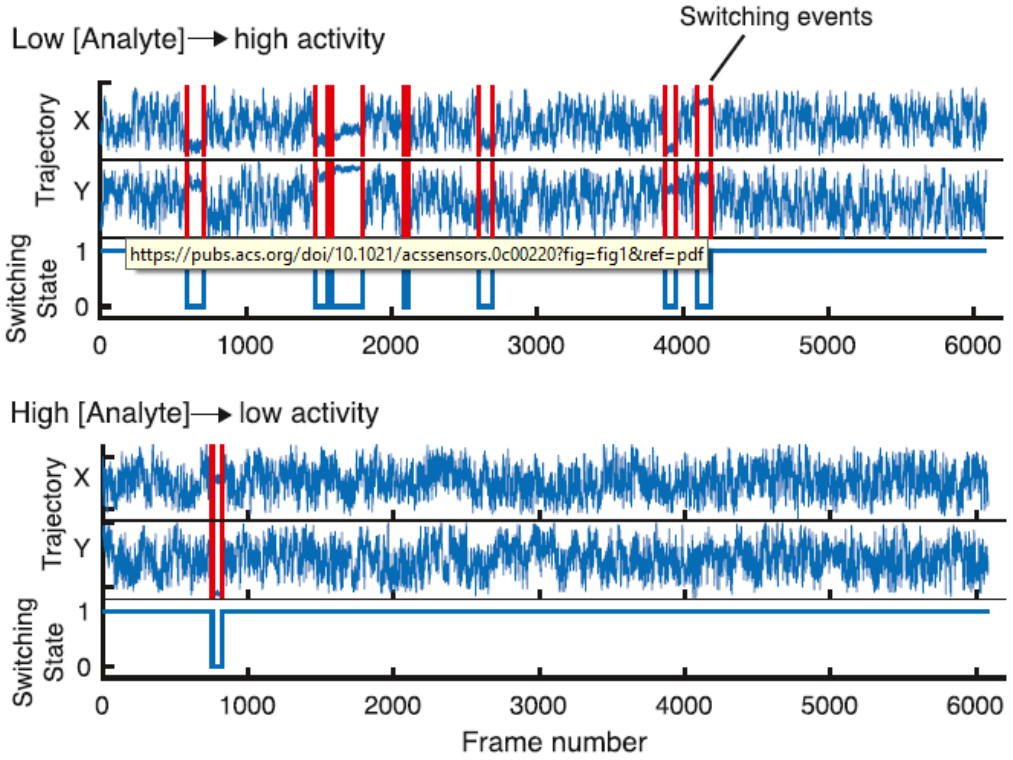


$$Activity = \frac{N_{events}}{N_{particles}}$$



- Monitoring of particle trajectory with series of affinity binding events

Junhong Yan, Laura van Smeden, Maarten Merkx, Peter Zijlstra, and Menno W. J. Prins, Continuous Small-Molecule Monitoring with a Digital Single-Particle Switch, ACS Sens. 2020, 5, 1168−1176.

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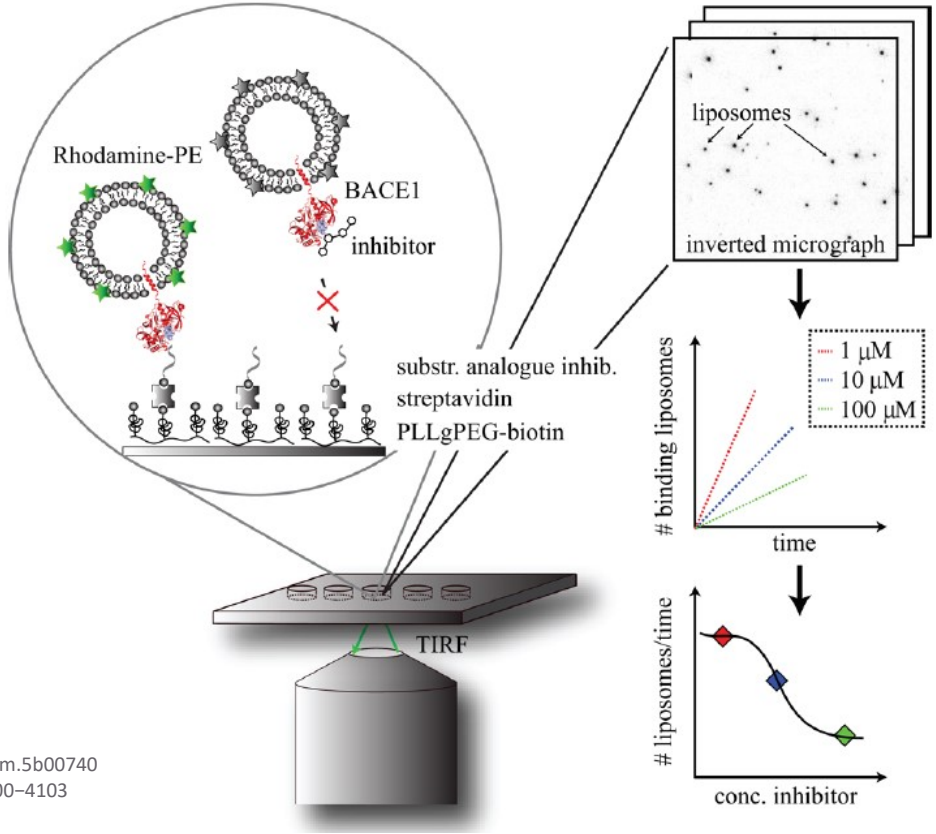
Fluorescence-based Inhibition in Solution Assay



DOI: 10.1021/acs.analchem.5b00740
Anal. Chem. 2015, 87, 4100−4103

- Single binding events observation with fluorescence microscopy

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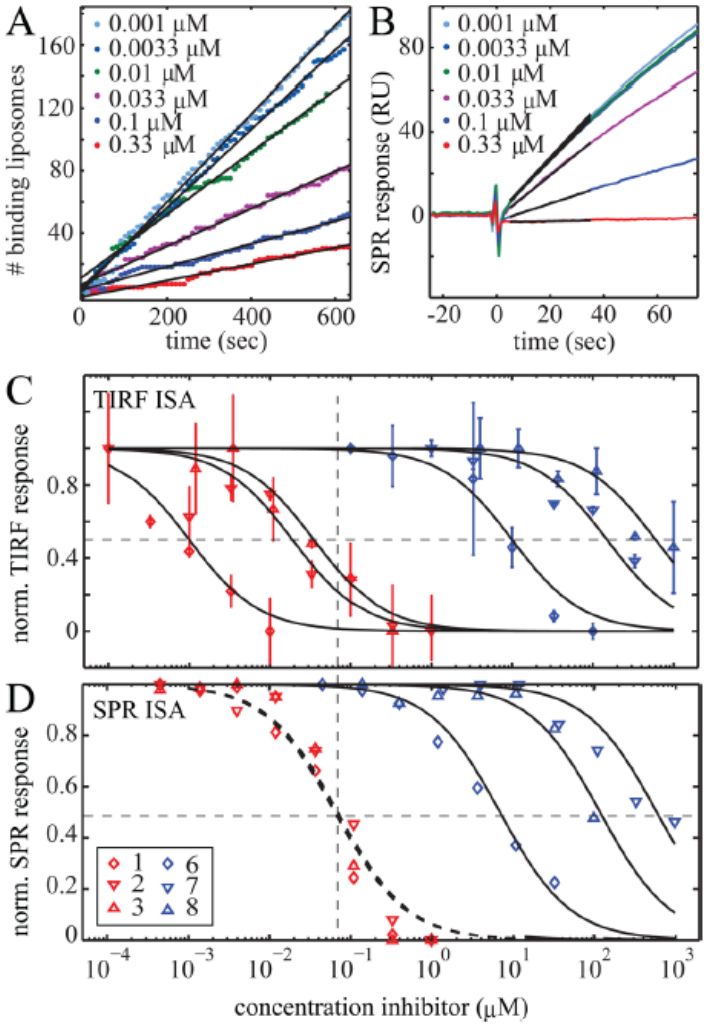
Fluorescence-based Inhibition in Solution Assay

- Enhanced sensitivity demonstrated with respect to classing SPR readout with ATR configuration.
- Employed for screening of a set of small molecule inhibitors against β-secretase 1 (BACE1).



DOI: 10.1021/acs.analchem.5b00740
Anal. Chem. 2015, 87, 4100−4103

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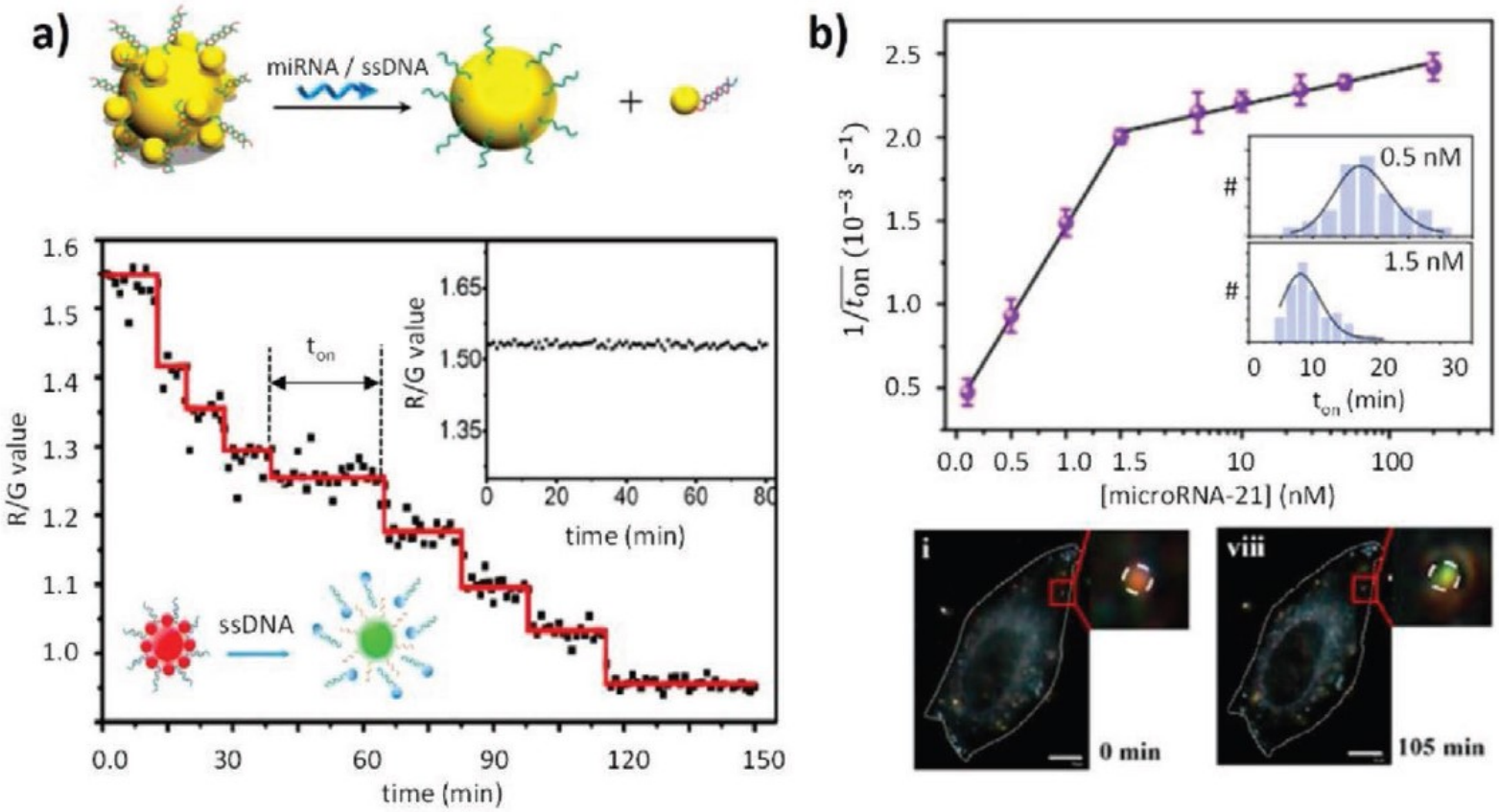
LSPR Assay with Metallic NP Labels



- Quantitative concentration of mRNA monitoring in living cells

https://doi.org/10.1021/acsnano.7b08673

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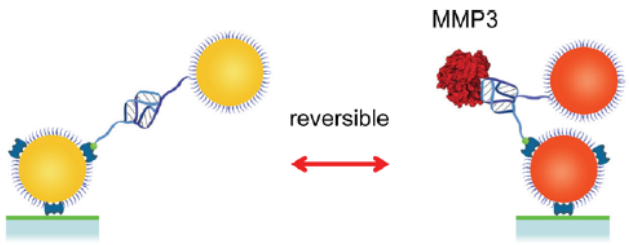
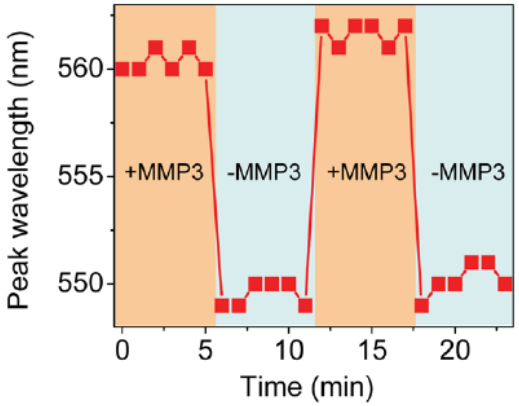
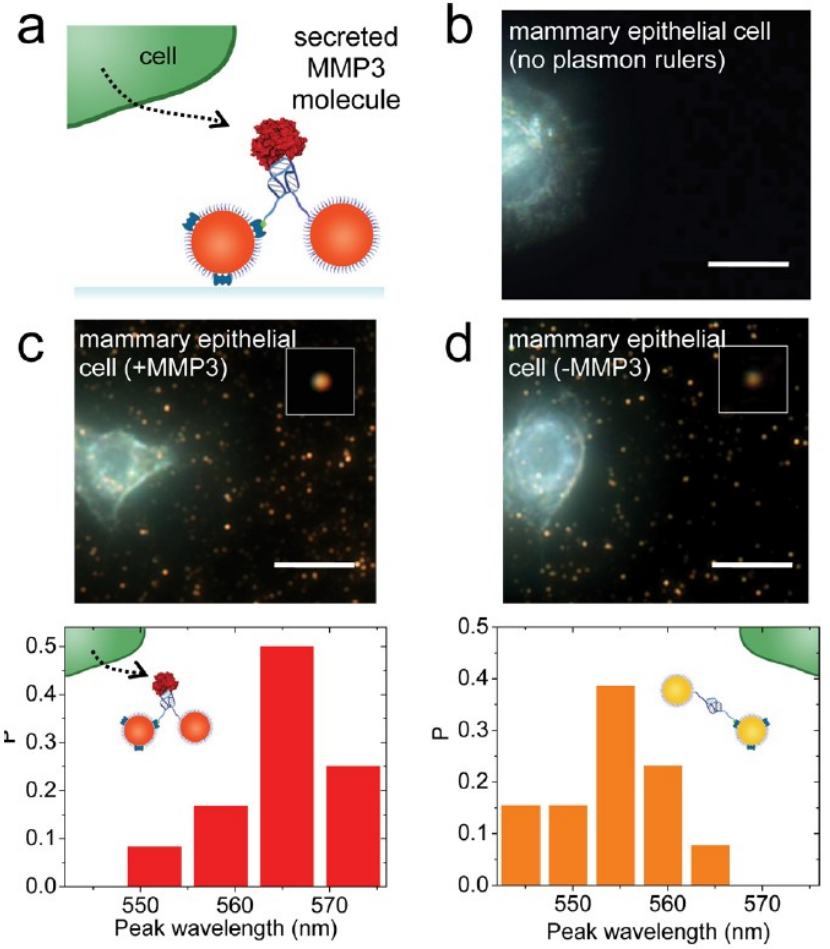
Plasmonic Ruler – based Monitoring





Demonstrated for detection of metalloproteinase (MMP3) secreted by epithelial cells.



Alivisatos lab: http://pubs.acs.org/doi/abs/10.1021/acs.nanolett.5b01161

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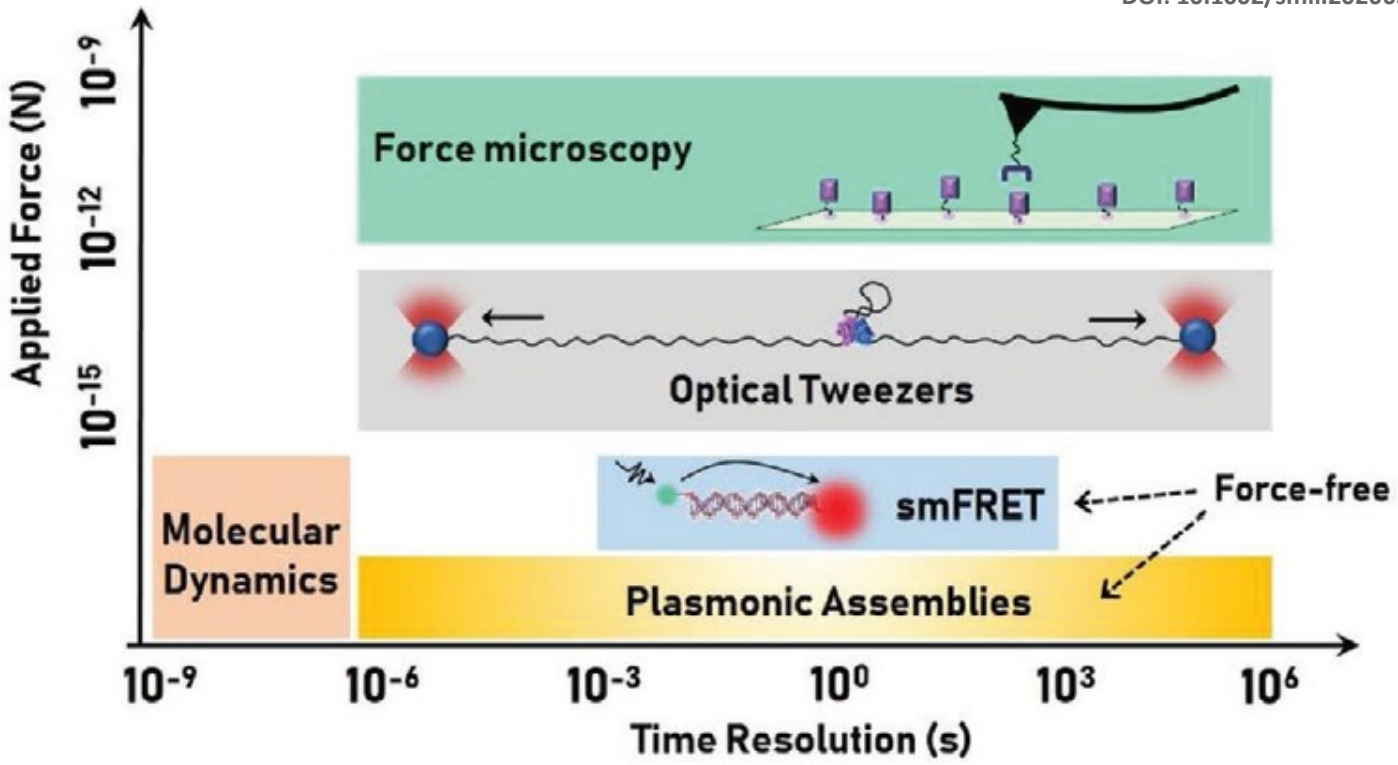
Plasmonic Ruler – based Monitoring

DOI: 10.1002/smll.202003934



- Common single-molecule techniques, shown as function of applied force versus time resolution. Nanoparticle assemblies fill the timescale gap between molecular dynamics and single-molecule FRET (smFRET) while still a force-free biophysical method