Sensing and Spectroscopy of Single Molecules on Optoplasmonic Microcavities: A New Thermo-Plasmonic Detection Mechanism to Measure the Absorption Cross Section of a Single Molecule

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Abstract: Single-molecule sensors based on whispering-gallery mode coupled to plasmonic resonant systems are studied. We reveal changes of the reactive sensing mechanisms under different intensities of modes – anomalous decrease of the resonant wavelength.

1. Optoplasmonic Sensors Optical sensors based on whispering-gallery mode (WGM) microresonators are exceptional tools for the detection of molecules at the single level. Their impressive sensitivity is related to their high Q-factors, which can reach the order of 10^7, even when not in clean-room conditions. The limiting factor of their sensitivity and figure of merit was their relatively big mode volume, which has order of 100s of micrometers. To overcome this problem, the hybridization of WGM resonators with metal nanoparticles supporting plasmonic oscillations has been proposed. Adding plasmonic nanoparticles reduces Q-factor, within an order of magnitude, but the probing light is tightly localized, reducing the mode volume by 2-3 orders of magnitude. Such systems, so-called optoplasmonic sensors, have already proven their ability to detect single molecules and even single ions [1]. They have been used for sensing as conventional instruments based on the reactive mechanism, i.e. tracking their WGM red-shifting and resonance broadening upon attaching objects under test. In this contribution, we reveal a new mechanism of WGM sensing of single molecules implemented with slightly increased values of WGM excitation powers.

2. Experimental Details As objects of investigation, enzymatic molecules of 3-phosphoglycerate kinase (3PGK) were chosen. WGM resonators with sizes of 80–100 μm were made by melting standard telecommunication fibers with CO_2 lasers. A tunable diode laser with 780 nm emission was used for modes excitation via a prism coupler. The power of the incident beam was randomly varied in the range of 0.02 mW to 5.48 mW. All experiment on molecules binding and modes tracking were made in chambers made of standard polymer PDMS. WGM spherical microresonators were covered with CTAB-capped gold nanorods, with plasmon resonances at 780 nm, from their colloidal solutions in a one-by-one attachment regime. For enzyme immobilization, the optoplasmonic sensor was preliminarily treated with linkers based on NTA and PEG, with the former being charged with nickel ions as a specific agent to bind 3PGK molecules. A solution of 3PGK was injected in the chamber filled with HEPES buffer (pH 7.4) to reach the final concentration of enzymes of ~1–100 nM.

3. Power-Dependent Sensing Fig. 1a represents an example of WGM resonant wavelength shift ($\Delta\lambda$) denoting the binding event of 3PGK to the sensor. Alongside with changes of $\Delta\lambda$, the resonances full width at half maxima (FWHM, $\Delta\kappa$) changes were registered. Typically, WGM microsphere resonators support many modes; in these experiments, a mode with higher coupling efficiency in the spectral range was selected. For this particular mode, the center wavelength and FWHM were recorded to define orbital mode number as well as the rate of losses in the resonator. These values, sizes of resonators and plasmon enhancement factor, put equal to 800, were used to define light intensity around a nanorod. At relatively low intensities, 0.5–10 MW/cm^2, binding events shows positive changes of λ, $\Delta\lambda$ = 6 fm on average (Fig. 2), and do not show changes of FWHM. Increased WGM power and electric field around nanorods led to observations of single-molecule detection as close to zero changes of λ accompanied by changes of FWHM, 3 fm in average (Fig. 1b). Further increase of the intensity gives negative changes of λ, for 164 MW/cm^2 they achieved -10 fm on average. These results are summarized in Fig. 2.

Fig. 1 below (a) *Optoplasmonic single-molecule sensor scheme. A 780 nm incident cw laser beam excites whispering-gallery modes (WGMs) in a 90 μm-diameter silica microsphere placed in a prism. The microsphere is immersed in a polydimethylsiloxane (PDMS) chamber filled with buffer and protein solutions. Resonance wavelength shift (Δλ) and full width at half maximum (FWHM) of WGMs are tracked from the reflected beam with a photodetector, connected to a data acquisition card (DAQ) and a feedback loop set up*

with the laser controller. Gold nanorods are attached to the microsphere surface. Protein samples (Adk or 3PGK; conjugated or not with AlexaFluor 790) bind to these nanorods at the tips, within an enhanced electric field that can detect perturbations of polarizability and hence the presence of protein molecules ***(b)*** *Traces of resonance peak changes, Δλ (top), and full width at half maximum, Δκ (bottom) vs time. Two binding events of >2 fm can be observed.*

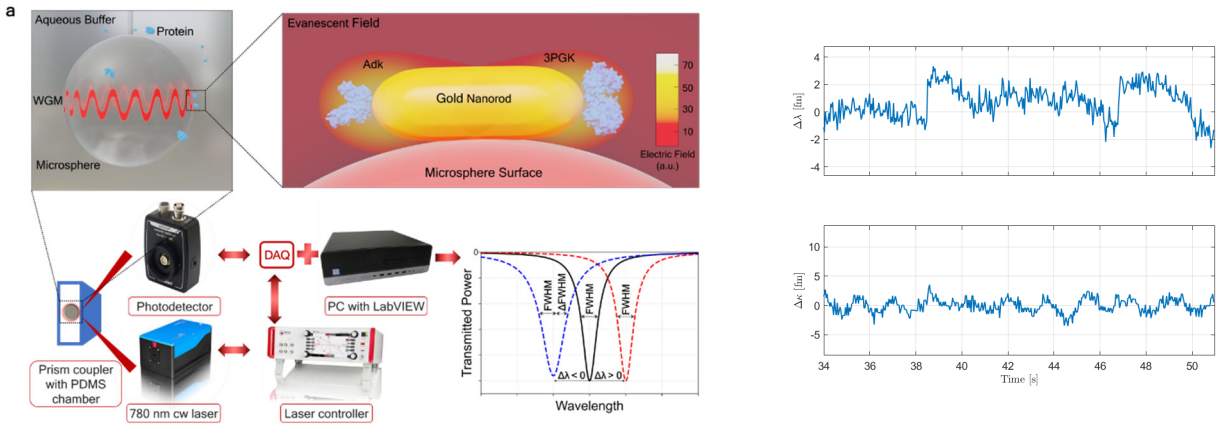
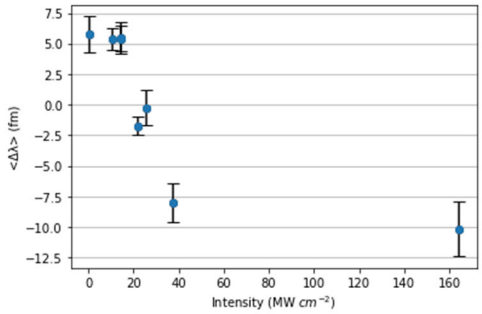


Fig. 2 below *Average resonance wavelength shift upon 3PGK binding to gold-nanorod-microresonators during WGM experiments at changing intensity. Positive wavelength shifts occur at lower laser intensities, while greater intensities produce negative wavelength shifts, with an intermediary intensity where both positive and negative wavelength shifts are observed around 20 MW/cm^2.*



4. Discussion and Conclusion

The negative changes of resonant wavelength observed at higher intensities of WGM were confirmed with the same methodology but using molecules of a different enzyme, adenylate kinase. The dependence of changes on intensities of WGM reveals a new mechanism of single-molecule detection with optoplasmonic sensors. We consider that this mechanism is related to strong mutual influence of WGM modes and plasmon nanorods where nanorods form virtual hot spots due to plasmon-enhanced fields. At the same time, they generate increased temperature due to Ohmic losses originated from plasmonic oscillations. Binding events of 3PGK molecules to the nanorod contribute partially to absorption of energy by different parts of molecules and partially to absorption of the excessive heat energy. In turn, absorbed energy and temperature make the refractive index smaller in the hot spots, resulting in a negative resonance wavelength shift. Thus, we demonstrated a new sensing mechanism which is caused by thermal changes in optoplasmonic sensors, or thermooptoplasmonic (TOP) sensing.

[1] M. Baaske and F. Vollmer, "Optical observation of single atomic ions interacting with plasmonic nanorods in aqueous solution," Nature Photon **10,** 733-739 (2016).