Das **Institut für Biochemie** lädt gemeinsam mit dem Ortsverband der **Gesellschaft Deutscher Chemiker** zu einem

K o l l o q u i u m der GDCh

Großer Hörsaal des Instituts für Biochemie

Felix-Hausdorff-Str. 4, Greifswald

Montag, 17. Juni 2019, 16 Uhr c.t.

PD Dr. Hans-Heiner Gorris

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spricht zum Thema:

Single molecule techniques for investigating enzyme mechanisms and implementing digital immunoassays

Abstract:

(see following page)

Einladender
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Vorsitzende des Ortsverbandes der GDCh

Single molecule techniques for investigating enzyme mechanisms and implementing digital immunoassays

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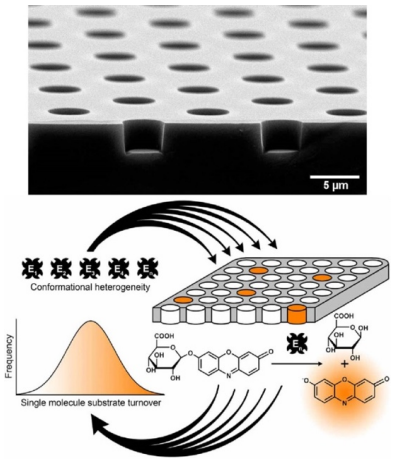

Single molecule experiments provide insights into the catalytic heterogeneity of an enzyme population that remain hidden in conventional ensemble measurements. We have designed large arrays of 250×250 (62 500) femtoliter chambers etched into fused silica to isolate single enzyme molecules and observe their individual catalytic rates in parallel (Figure, *JACS* 2014: TEM image and scheme of enzyme reaction). If a fluorogenic reaction is confined to a femtoliter volume, a single enzyme molecule generates a product concentration high enough for detection by wide-field fluorescence microscopy. Individual molecules of ß-galactosidase, ß-glucuronidase, horseradish peroxidase, and phosphatase display broadly distributed catalytic activities. Our single molecule approach provides new insights into the distribution of the free energy of activation ($\Delta G^{\ddagger}$) in an enzyme population, enzyme inhibition as well as molecular evolution.

Conventional fluorescence spectroscopy is limited by autofluorescence and light scattering of the surrounding medium. The optical background interference can be elegantly avoided by using photon-upconversion nanoparticles (UCNPs) that emit short-wavelength light under near-infrared (NIR, 980 nm) excitation (anti-Stokes emission). Thus, UCNPs enable new imaging applications in deep tissues. UCNPs are also an excellent alternative for enzymatic reporters in immunoassays that can even detect single molecules of an analyte in a highly reliable way. We determined the concentration of the cancer marker prostate-specific antigen (PSA) either (1) by using an upconversion microtiter plate reader (analog mode) or (2) by counting individual immune complexes under an upconversion wide-field microscope (digital mode). Counting single analyte molecules improves the limit of detection (LOD: 1.2 pg/mL) by more than one order of magnitude compared to the analog mode (Figure, *Anal. Chem.* 2017: detection scheme, calibration curve and microscope image of single UCNP labels). The upconversion detection mode has the potential to pave the way for a new generation of digital immunoassays.