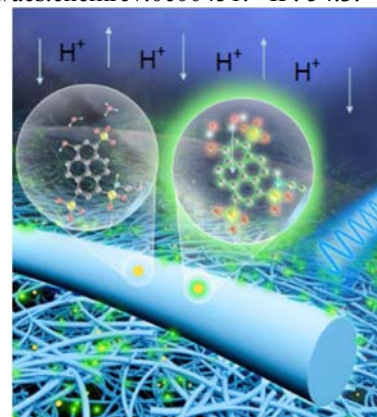


List of Papers on Fluorescent Nanosensors

vs. of 24-Jan-2021; edited by O. S. Wolfbeis

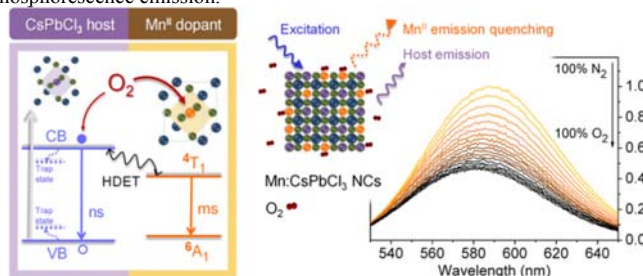
609. Review: Optical Sensing and Imaging of pH Values: Spectroscopies, Materials and Applications. A. Steinegger, O. S. Wolfbeis, S. M. Borisov; *Chem. Reviews* (2020) 120(22) 12357 - 12489. DOI: 10.1021/acs.chemrev.0c00451. IF: 54.3.

Abstract: This is the first comprehensive review on methods and materials for use in optical sensing of pH values, and on applications of such sensors. The Review starts with an introduction that contains subsections on the definition of the pH value, a brief look back on optical methods for sensing of pH, on the effects of ionic strength on pH values and pKa values, on the selectivity, sensitivity, precision, dynamic ranges and temperature dependence of such sensors. Commonly used optical sensing schemes are covered in a next main chapter, with subsections on methods based on absorptimetry, reflectometry, luminescence, refractive index, surface plasmon resonance, photonic crystals, turbidity, mechanical displacement, interferometry and solvatochromism. This is followed by sections on absorptimetric and luminescent molecular probes for use pH in sensors. Further large sections cover polymeric hosts and supports, and methods for immobilization of indicator dyes. Further and more specific sections summarize the state of the art in materials with dual functionality (indicator and host), nanomaterials, sensors based on upconversion and 2-photon absorption, multiparameter sensors, imaging, and sensors for extreme pH values. A chapter on the many sensing formats has subsections on planar, fiber optic, evanescent wave, refractive index, surface plasmon resonance and holography based sensor designs, and on distributed sensing. Another section summarizes selected applications in areas such as medicine, biology, oceanography, bioprocess monitoring, corrosion studies, on the use of pH sensors as transducers in biosensors and chemical sensors, and their integration into flow-injection analyzers, microfluidic devices and lab-on-a-chip systems. An extra section is devoted to current challenges, with subsections on challenges of general nature and those of specific nature. A concluding chapter gives an outlook on potential future trends and perspectives.



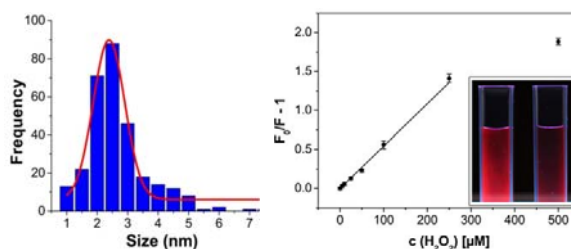
602. Mn(II)-Doped Cesium Lead Chloride Perovskite Nanocrystals: Demonstration of Oxygen Sensing Capability Based on Luminescent Dopants and Host-Dopant Energy Transfer. F. Lin, F. Li, Z. Lai, Z. Cai, Y. Wang, O. S. Wolfbeis, X. Chen. *ACS Appl. Mat. Interfaces* (2018), 10, 23335-23343. DOI: 10.1021/acsami.8b06329. IF: 8.5.

Abstract: We demonstrate the O₂ sensing capability of Mn(II)-doped CsPbCl₃ nanocrystals (Mn:CsPbCl₃ NCs) and reveal the role of O₂ on the optical de-excitation of such perovskite nanocrystals (PNCs). By adjusting the amount and distribution of Mn(II) dopants, as well as the host-dopant energy transfer (HDET) process in PNCs, we highlight that O₂ can reversibly quench the Mn(II) emission due to their temporarily disturbance to the ligand field of near-surface dopants in PNCs. In the phosphorescence mode, the PL of the NCs is quenched by 53% on going from 0 to 100% of O₂. The Stern-Volmer plot is linear in the 0-12% O₂ concentration range. High sensing reversibility and rapid signal response are also achieved. In our perception, the mechanism study makes these PNCs well suited as optical probes for O₂, and it is enlightening to explore more possibilities of the inherent O₂ sensing based on semiconductor doped-NCs (not restricted to Mn-doped PNCs) with phosphorescence emission.



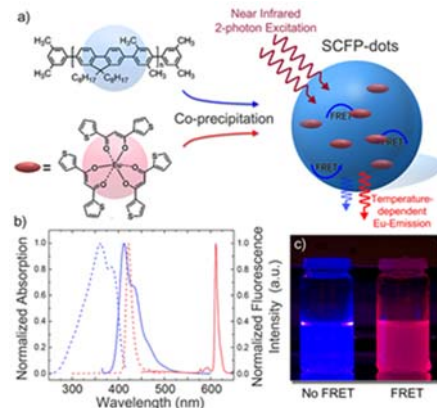
597. Europium-doped GdVO₄ Nanocrystals as a Luminescent Probe for Hydrogen Peroxide and for Enzymatic Sensing of Glucose. V. Muhr, M. Buchner, T. Hirsch, D. J. Jovanović, S. D. Dolić, M. D. Dramićanin, O. S. Wolfbeis; *Sensors Actuat., B: Chemical* (2017), 241, 349-356. DOI: 10.1016/j.snb.2016.10.090. IF: 6.4.

Abstract. The authors describe the preparation of Eu³⁺-doped GdVO₄ nanocrystals (NCs) by precipitation of the Gd³⁺(Eu³⁺)-citrate complex which was then converted to the respective vanadate by dialysis. The fractions of Eu³⁺ ranged from 5 to 100 mol%. The NCs were characterized by XRD, TEM, ICP-OES and dynamic light scattering which revealed that they possess superior colloidal stability in aqueous solutions in that no precipitation can be observed even after several months. The NCs display red and largely red-shifted fluorescence (peaking at 618 nm) on photoexcitation at around 300 nm. Fluorescence is strongly quenched by hydrogen peroxide. It is also shown that the fraction of doping with Eu³⁺ strongly affects quenchability. Most efficient quenching by H₂O₂ is observed if the NCs are doped with 50% of Eu³⁺. The findings were exploited to develop a fluorometric assay for H₂O₂ that works in the 5 to 250 μM concentration range, with a limit of detection as low as 1.6 μM (at a signal-to-noise ratio of 3). The probe was further employed to design a highly sensitive enzymatic assay for glucose via measurement of the quantity of H₂O₂ formed as a result of the catalytic action of glucose oxidase.



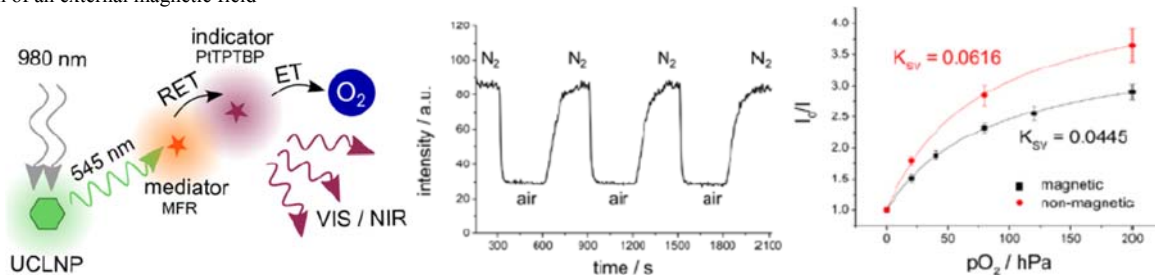
596. Two-Photon Excitation Temperature Nanosensors Based on a Conjugated Fluorescent Polymer Doped with a Europium Probe. X.-D. Wang, R. J. Meier, M. Schaeferling, S. Bange, J. M. Lupton, M. Sperber, J. Wegener, V. Ondrus, U. Beifuss, U. Henne, C. Klein, O. S. Wolfbeis; *Adv. Opt. Mat.* (2016), 4, 1854-1859. DOI: 10.1002/adom.201600601. IF: 6.8.

Abstract. A strongly fluorescent organic semiconducting polymer doped with a highly temperature dependent fluorescent europium(III) complex was converted into a nanosized material that is capable of optically sensing temperature (T) in the range from 0 to 50 °C via two-photon excitation at 720 nm. The nanosensors were prepared from a blue-fluorescent polyfluorene that acts as both a light-harvesting antenna (to capture two-photon energy) and an energy donor in a FRET system. The photonic energy absorbed by the polymer is transferred to the T-sensitive red-luminescent europium complex contained in the nanoparticles. The close spatial proximity of the donor and the acceptor warrants efficient FRET. A poly(ethylene glycol)-co-poly(propylene oxide) block copolymer was also added to render the particles biocompatible. We show that T can be calculated from (a) the intensity of the luminescence of the europium complex, (b) the ratio of the intensities of the red and blue luminescence, or (c) the T-dependent luminescence lifetime of the Eu(III) complex.



592. Composite Particles with Magnetic Properties, Near-Infrared Excitation, and Far-Red Emission for Luminescence-Based Oxygen Sensing. E. Scheucher, S. Wilhelm, O. S. Wolfbeis, T. Hirsch, T. Mayr, *Microsyst. Nanoeng.* (2016), vol. 1, 15026. DOI: 10.1038/micronano.2015.26. Open access.

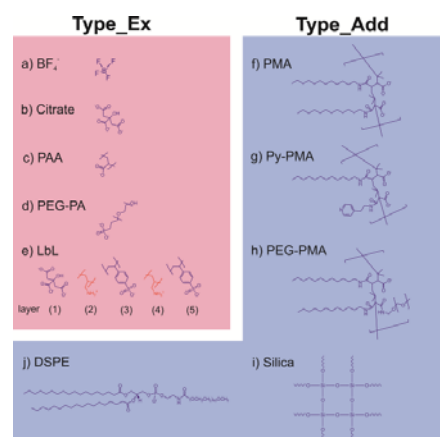
Abstract: Oxygen sensing, magnetic and upconversion luminescence properties are combined in multi-functional composite particles prepared herein by a simple mixing, baking and grinding procedure. Upconverting nanocrystals are used as an excitation source and an oxygen indicator with far-red emission. The composite particles are excited with near infrared laser light (980 nm). The visible upconversion emission is converted into an oxygen concentration-dependent far-red emission (< 750 nm) using an inert mediator dye and a platinum benzoporphyrin dye. This concept combines the advantages of NIR excitation and far red emissive indicator dyes, offering minimized auto-fluorescence and enhanced membrane permeability. Additional functionality is obtained by incorporating magnetic nanoparticles into the composite particles, which enables easy manipulation and separation of the particles by the application of an external magnetic field



588. Water Dispersible Upconverting Nanoparticles: Effects of Surface Modification on Luminescence and Colloidal Stability. S. Wilhelm, M. Kaiser, C. Würth, J. Heiland, C. Carrillo-Carrion, V. Muhr, O. S. Wolfbeis, W. J. Parak, U. Resch-Genger, T. Hirsch, *Nanoscale* (2015), 7, 1403-1410. DOI: 10.1039/c4nr05954a. IF: 7.4

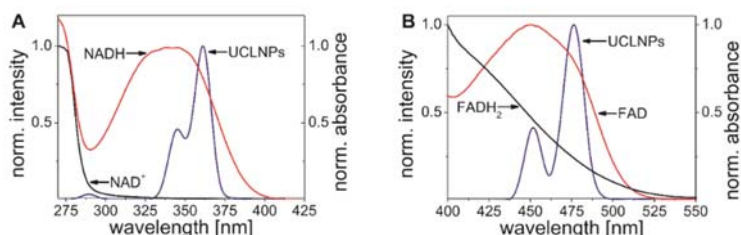
We present a systematic study on the effect of surface ligands on the luminescence properties and colloidal stability of β -NaYF₄:Yb,Er upconversion nanoparticles (UCNPs), comparing nine different surface coatings to render these UCNPs water-dispersible and bioconjugatable. A prerequisite for this study was a large-scale synthetic method that yields ~2 g per batch of monodisperse oleate-capped UCNPs providing identical core particles. These ~23-nm sized UCNPs display an upconversion quantum yield of ~0.35% when dispersed in cyclohexane and excited with a power density of 150 W cm⁻². A comparison of the colloidal stability and luminescence properties of these UCNPs, subsequently surface-modified with ligand exchange or encapsulation protocols, revealed that the ratio of the green (545 nm) and red (658 nm) emission bands determined at a constant excitation power density depends on the surface chemistry. We demonstrate that the brightness of the upconverted luminescence is strongly affected by the type of surface modification, i.e., ligand exchange or encapsulation, yet hardly by the chemical nature of the ligand.

The Scheme at the right gives an overview of strategies for surface modification of oleate-coated UCNPs. The modifications can be classified into two categories: (a) - (e) ligand exchange methods (type_Ex); (f) - (j): addition of an amphiphilic layer or silica coating (type_Add).



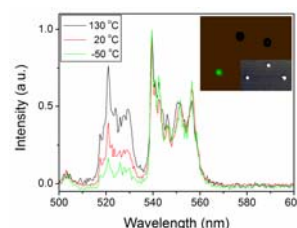
583. Spectrally Matched Upconverting Luminescent Nanoparticles for Monitoring Enzymatic Reactions. S. Wilhelm, M. del Barrio, J. Heiland, S. F. Himmelstoss, J. Galbán, O. S. Wolfbeis, T. Hirsch; *ACS Appl. Mat. Interf.* (2014), 6, 15427-15433. DOI: 10.1021/am5038643. IF: 5.0.

Abstract: We report on upconverting luminescent nanoparticles (UCLNPs) that are spectrally tuned such that their emission matches the absorption bands of the two most important species associated with enzymatic redox reactions. The core-shell UCLNPs consist of a hexagonal NaYF₄ core doped with Yb(III) and Tm(III) ions, and a shell of undoped hexagonal NaYF₄. Upon 980-nm excitation, they display emission bands peaking at 360 nm and 475 nm which is a perfect match to the absorption bands of the enzyme cosubstrate NADH and the coenzyme FAD, respectively. By exploiting these spectral overlaps, we have designed fluorescent detection schemes for NADH and FAD that are based on the modulation of the emission intensities of UCLNPs by FAD and NADH via an inner filter effect. The Figures below show the normalized upconversion luminescence spectra of hydrophilic hexagonal NaYF₄:Yb,Tm@NaYF₄ core-shell particles dispersed in MES buffer, and the overlap with the absorption bands of NADH (left) and FAD (right)



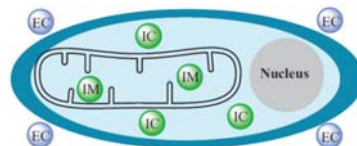
582. Size Dependence of the Upconverted Luminescence of NaYF₄:Er,Yb Microspheres for Use in Ratiometric Thermometry. B. Dong, R. N. Hua, B. S. Cao, Z. P. Li, Y. Y. He, O. S. Wolfbeis; *PhysChemChemPhys* (2014) 16, 20009-20012. DOI: 10.1039/C4CP01966K. IF: 4.2.

Abstract: We report on the size dependence of the upconversion luminescence of temperature (*T*)-sensitive particles of the type NaYF₄:Er,Yb with a size between 0.7 and 2 μm that have been prepared by a poly(acrylic acid)-assisted hydrothermal process. It is found that the fluorescence intensity ratio of their green upconversion emissions (with peaks at 521 and 539 nm) is strongly size-dependent at *T*'s between 223 and 403 K. If the size of the spheres is increased from 0.7 to 1.6 μm, the slope of the sensitivity to *T* strongly decreases. This effect is mainly attributed to the larger specific surface area of the smaller spheres where relatively more Er(III) ions are located at the surface. It also shows that sensing *T* by this method is prone to error unless particles sizes are well controlled. The Figure shows the upconversion emission spectra at 223 K, 293 K and 403 K.



580. Targetable Phosphorescent Oxygen Nanosensors for the Assessment of Tumor Mitochondrial Dysfunction by Monitoring Respiratory Activity. X. Wang, H. Peng, L. Yang, F. You, F. Teng, L. Hou, O. S. Wolfbeis; *Angew. Chem. Intl. Ed.*, (2014) 53, 12471-12475. DOI: 10.1002/anie.201405048. IF: 11.3.

Abstract: Most oxygen sensing strategies merely report extracellular (*ec*-) or intracellular (*ic*-) oxygen rather than intra-mitochondrial (*im*-) oxygen. The latter is much desired however, particularly in diagnosis of subtle mitochondrial dysfunction. We are presenting a method to assess tumor mitochondrial dysfunction (by comparison to healthy cells) by using three kinds of luminescent nanosensors for oxygen. Targeted sensing is accomplished by proper modification of the surface of the polystyrene nanoparticles with either silica (for *ec*-oxygen), polylysine (*ic*-oxygen), or triphenylphosphonium groups (for *im*-oxygen). This strategy enables targeted placement of the nanoprobes so that they can respond to *ec*-, *ic*-, and *im*-oxygen, respectively. Time-resolved luminescence is applied to determine the respective oxygen consumption rates (OCRs) under varying respiratory conditions. The results demonstrate that mitochondria in tumor cells are distinctly less active than those of healthy cells, which is interpreted in terms of both restrained glucose utilization and physically impaired mitochondria in tumor cells. The figure shows a schematic of the placement of the 3 targetable nanosensors inside a cell.



574. Direct Formation of Mesoporous Upconverting Nanoparticles for Bioimaging of Living Cells. T. Liu, L. Sun, Y. Qiu, J. Liu, F. Li, L. Shi, O. S. Wolfbeis; *Microchim. Acta* (2014), 181, 775-782. DOI: 10.1007/s00604-013-1073-9. IF: 3.7.

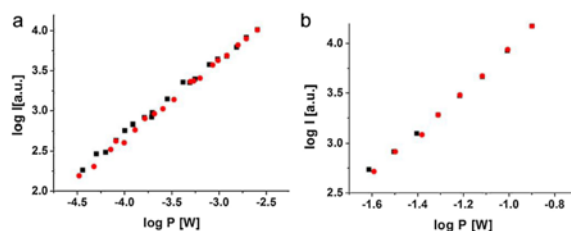
Abstract: We describe a single-step method for the synthesis of mesoporous upconverting nanoprobe (MUCNs) of the type NaYF₄:Yb,Er@mSiO₂, with the mesoporous and assisted by CTAB which serve as both phase transfer assistant agents and pore-generating templates. With effective emission upon 980-nm light excitation and low cytotoxicity according to the thiazolyltetrazolium assay, the MUCNs can be applied to image human nasopharyngeal epidermal carcinoma cells in-vitro via laser scanning upconversion luminescence microscopy. silica directly encapsulating the hydrophobic upconversion nanoparticles



569. Imaging of Cellular Oxygen via Two-Photon Excitation of Fluorescent Sensor Nanoparticles. X. Wang, D. E. Achatz, C. Hupf, Sperber, J. Wegener, S. Bange, J. M. Lupton, O. S. Wolfbeis; *Sensors & Actuators, B (Chem.)* (2013), 188, 257-262. DOI: 10.1016/j.snb.2013.06.087. IF: 3.8.

Abstract: Polystyrene nanoparticles (PSNPs) with an average size of 85 nm and loaded with an oxygen-quenchable luminescent ruthenium complex were used to image oxygen inside cells following 2-photon excitation (2-PE). The ruthenium probe possesses a large 2-photon absorption cross-section, and 2-PE is achieved by irradiation in the near infrared with fs-pulsed laser systems.

The luminescence of the dye-loaded PSNPs is strongly quenched by oxygen, and Stern-Volmer plots are linear for both conventional 1-PE and for 2-PE. The particles are readily taken up by mammalian cells (MCF-7), presumably via membrane mediated pathways. The 2-PE is considered to be advantageous over conventional imaging techniques because it works in the near-infrared where background absorption and luminescence of biomatter is much weaker than at excitation wavelengths of <600 nm. The Fig. shows double-logarithmic plots of laser power versus emission intensity of the oxygen probe Ru(dpp). Plot (a): excitation via single-photon absorption; the slope is 0.95. Plot (b): excitation via 2-PE (slope 2.04).



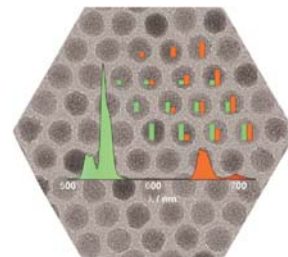
567. Review: Luminescent Probes and Sensors for Temperature. X. Wang, R. J. Meier, O. S. Wolfbeis; *Chem. Soc. Rev.* (2013), 42, 7834–7869. DOI: 10.1039/c3cs60102a. Open access. IF: 24.9.

Abstract: Temperature (T) is the most fundamental parameter in science. Respective sensors also are widely used in daily life. Besides conventional thermometers, optical sensors are considered to be attractive alternatives in sensing and on-line monitoring of T . This review focuses on all kinds of luminescent probes and sensors for measurement of T and summarizes the recent progress in their design and application formats. In the introduction, we cover the significance of optical probes for T , the origin of their T -dependent spectra, and the various detection modes. This is followed by a survey on (a) molecular probes, (b) nanomaterials, and (c) bulk materials for sensing T , a discussion of polymeric matrices for immobilizing probes and an overview on the various application formats of T -sensors. The review closes with a discussion on the prospects, challenges, and new directions in the design of new probes and sensors. 248 references.



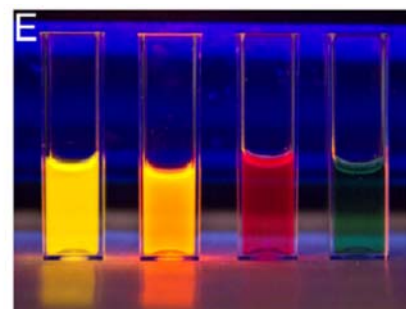
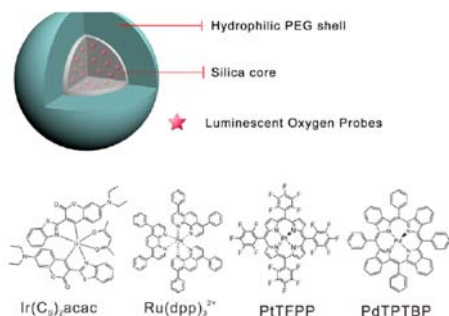
565. Review: Photon-Upconverting Nanoparticles for Optical Encoding and Multiplexing of Cells, Biomolecules and Microspheres. H.-H. Gorris, O. S. Wolfbeis; *Angew. Chem. Int. Ed.* (2013), 52, 3584–3600. DOI: 10.1002/anie.201208196. IF: 13.4.

Abstract: Photon upconverting nanoparticles (UCNPs) can emit visible light under near-infrared excitation (anti-Stokes emission). This unique optical property precludes background fluorescence and light scattering by biological materials. The emission of multiple and narrow emission lines is an additional hallmark of UCNPs that opens new avenues for optical encoding. Distinct emission signatures can be obtained if the multiple emission of UCNPs is tuned by their dopant composition or by surface modification with dyes. Tuning only one of the multiple emission lines and using another one as a constant reference signal enables the design of ratiometric codes that are resistant to fluctuations in absolute signal intensities. Combining several UCNPs, each displaying a distinct set of emission lines, expands the coding capacity exponentially and lays the foundation for highly multiplexed analyte detection. The review highlights the potential of UCNPs for labeling and encoding biomolecules, microspheres, and of whole cells.



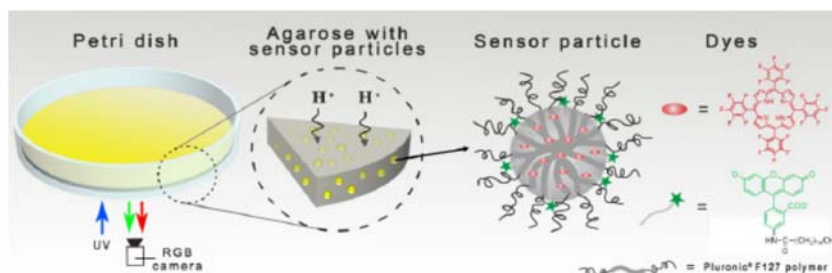
566. Ultra-Small, Highly Stable, and Membrane-Impermeable Fluorescent Nanosensors for Oxygen. X. Wang, J. A. Stolwijk, M. Sperber, R. J. Meier, J. Wegener, O. S. Wolfbeis; *Meth. Appl. Fluoresc. (IOP Publ.; London)* (2013), 1, 035002 (7 pp). DOI: 10.1088/2050-6120/1/3/035002.

Abstract: We report on the preparation of ultra-small fluorescent nanosensors for oxygen via a one-pot approach. The nanoparticles have a hydrophobic core capable of firmly hosting hydrophobic luminescent oxygen probes. Their surface is composed of a dense and long-chain poly(ethylene glycol) shell, which renders them cell-membrane impermeable but yet highly sensitive to oxygen, and also highly stable in aqueous solutions and cell culture media. These features make them potentially suitable for sensing oxygen in extracellular fluids such as blood, interstitial and brain fluid, in (micro) bioreactors and micro- or nanoscale fluidic devices. Four kinds of nanosensors are presented, whose excitation spectra cover a wide spectral range (395 – 630 nm), thus matching many common laser lines, and with emission maxima ranging from 565 to 800 nm, thereby minimizing interference from background luminescence of biomatter. The unquenched lifetimes are on the order of 5.8 – 234 μ s, which – in turn – enables lifetime imaging and background separation via time-gated methods. The Figure below shows (on the left) a schematic of the structure of the ultra-small oxygen-sensitive nanoparticles (NPs) and the chemical structures of the oxygen indicators. (D) shows photographic pictures of oxygen nanosensors in aqueous solutions under ambient light; (E) shows the same cuvettes under UV light. From left to right: Ir-NPs, Ru-NPs, Pt-NPs and Pd-NPs, respectively.

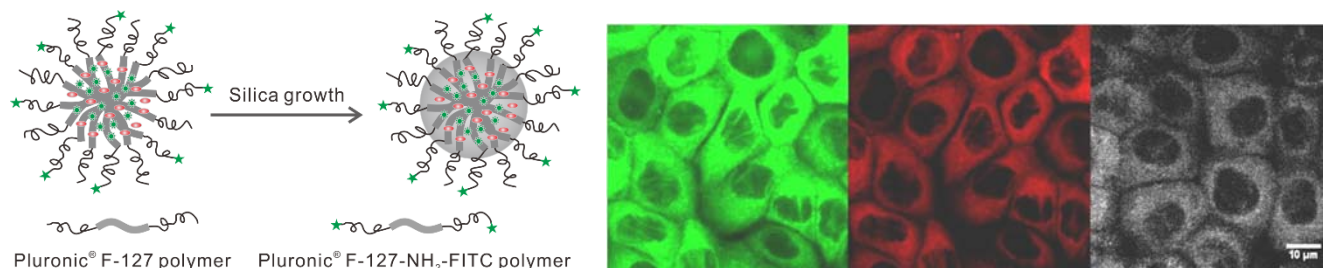


561. Fluorescent pH-Sensitive Nanoparticles in an Agarose Matrix for Imaging of Bacterial Growth and Metabolism. X. Wang, R. J. Meier, O. S. Wolfbeis; *Angew. Chem. Int. Ed.* (2013), 52, 406–409. DOI: 10.1002/anie.201205715. IF: 13.4.

Abstract: We report on novel nanosensors for fluorescent imaging of physiological pH values. Features include (a) very small diameters (12 nm); (b) biocompatibility due to the use of a hydrogel kind of material [a commercial poly(ethylene glycol)-co-poly(ethyleneoxide)], non-covalent immobilization (based on strong hydrophobic interactions), and (c) lack of toxicity. Such nanosensors, if incorporated into an agar film, enable continuous monitoring of the pH value of bacterial cultures, and thus of their growth.

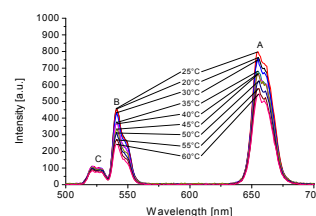


556. Ultra-Small, Highly Stable and Sensitive Dual Nanosensors for Imaging Intracellular Oxygen and pH in Cytosol. X. Wang, J. A. Stolwijk, T. Lang, M. Sperber, R. J. Meier, J. Wegener, O. S. Wolfbeis; *J. Am. Chem. Soc.* (2012), 134, 17011-17014. DOI: 10.1021/ja308830e. IF: 9.9. Article featured in *JACS Spotlights* (*J. Am. Chem. Soc.* (2012), 134, 18151-18152). **Abstract:** We report on the first dual nanosensors for imaging of pH values and oxygen partial pressure in cells. The sensors have a unique nanostructure in that a soft core structure is rigidized with a silane reagent, while poly(ethylene glycol) chains form an outer shell. Lipophilic oxygen-sensitive probes and reference dyes are encapsulated inside the hydrophobic core, while a pH-sensitive probe is covalently attached to the poly(ethylene glycol) end-group on the shell. The core/shell structure renders the nanosensors well dispersed and highly stable in various kinds of aqueous media. Their average size is 12 nm, and they respond to both pH values and oxygen in the physiological range. They do not pass cell-membranes, but can be internalized into the cellular cytosol by electroporation, upon which they enable sensing and imaging of pH values and oxygen with high spatial resolution. The *Figure* shows confocal laser scanning microscopy images of the nanosensors internalized into normal rat kidney cells via electroporation. (A) The green luminescence of the pH-dependent signal of the nanosensors as seen with a 520-nm bandpass filter; (B) The red luminescence as seen with a 650-nm longpass filter; (C) The oxygen-dependent NIR luminescence (black/white) of the nanoparticles.



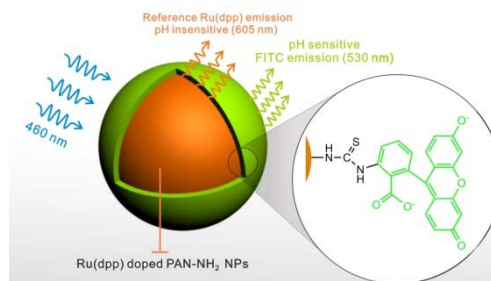
555. Photon Upconverting Nanoparticles for Luminescent Sensing of Temperature. A. Sedlmeier, D. E. Achatz, L. H. Fischer, H. H. Gorris, O. S. Wolfbeis; *Nanoscale* (2012), 4, 7090-7096. DOI: 10.1039/c2nr32314a. IF: 5.9.

Abstract: Nanoparticles displaying photon upconversion have advantages like the low background fluorescence of biological specimen due to near infrared (NIR) excitation and the presence of two or more emission bands. The ratio of these intensities of the main bands of upconverted emission of hexagonal NaYF₄ nanoparticles doped with Yb³⁺ as the sensitizer and with Er³⁺, Ho³⁺, or Tm³⁺ as the activators yields robust data for the determination of temperature in the “physiological” range (20 – 60°C). Resolutions of < ±1 °C can be achieved with particles consisting of a doped core and an inactive shell.



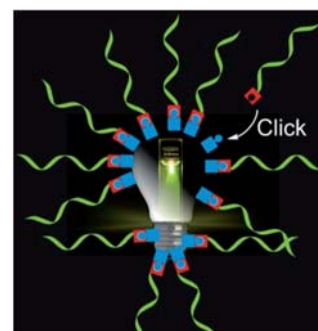
547. Fluorophore-Doped Polymer Nanomaterial for Referenced Imaging of pH and Temperature with Sub-Micrometer Resolution. X. Wang, R. J. Meier, O. S. Wolfbeis; *Adv. Funct. Mat.* (2012), 22, 4202-4207. DOI: 10.1002/adfm.201200813. IF: 8.5.

Abstract: We report on a new kind of pH and temperature (*T*)-sensitive material. It is composed of dye-doped polymer nanoparticles incorporated into a thin film of a polyurethane hydrogel. The new pH/*T*-sensitive nanoparticles were obtained by post-staining oxygen-impermeable amino-functionalized polyacrylonitrile nanoparticles with a long-lifetime reference dye. Staining is followed by covalently linking fluorescein isothiocyanate onto the surface of the nanoparticle. The sensor material has distinct features: (a) It enables imaging of pH via time domain dual-lifetime referencing (td-DLR); (b) effects of *T* on pH sensing may be compensated for; (c) *T* can simultaneously be visualized via rapid lifetime imaging; (d) It offers superior spatial resolution due to the use of nanosized sensor particles.



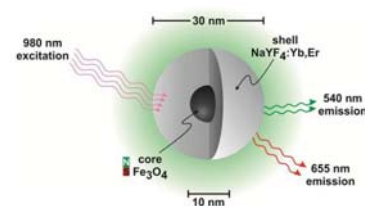
544. DNA "Nanolamps": Clicked DNA Conjugates with Photon Upconverting Nanoparticles as Highly Emissive Biomaterials. M. M. Rubner, D. E. Achatz, H. S. Mader, J. A. Stolwijk, J. Wegener, G. S. Harms, O. S. Wolfbeis, H.-A. Wagenknecht; *ChemPlusChem* (2012), 77, 129-134. DOI: 10.1002/cplu.201100055. IF: 3.3.

Abstract: Upconverting nanoparticles (UCNPs) display visible luminescence when excited in the near-infrared (NIR) region but have no biorecognition capabilities. However, functionalization of their surface with azido groups renders them conjugatable to ethynyl-modified oligonucleotides in a bioorthogonal fashion. Single-stranded DNA was covalently attached to the surface of UCNPs by click chemistry and purified by size exclusion chromatography (SEC) at elevated temperature. Covalent attachment was evidenced by diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy. DNA conjugation makes the particle soluble in water and enables it to recognize its counterstrand. Such UCNPs are capable of nonspecifically crossing cell membranes. Confocal microscopy reveals the high potential of the bright UCNPs for live cell imaging in the NIR, where the UCNP-DNA conjugates can be considered to act as a kind of nano-sized lamp. Furthermore, cross-linking of those DNA nanolamps yields highly emissive aggregates.



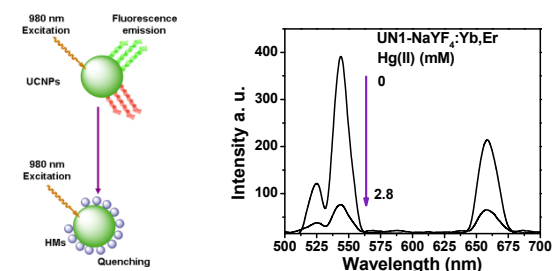
539. Magnetic Nanosensor Particles with Luminescence Upconversion Capability. S. Wilhelm, T. Hirsch, E. Scheucher, T. Mayr, O. S. Wolfbeis; *Angew. Chem. Intl. Ed.* (2011), 50(37), A59-A62. DOI: 10.1002/ange.201105813. IF: 12.7.

Abstract: Nanoparticles made of magnetite and with an average size of 10 nm have been used as seed crystals to further grow, on their surface, a layer of upconverting material consisting of Yb,Er-doped hexagonal NaYF₄. The resulting multifunctional upconverting and magnetic nanoparticles represent a novel material for potential use in magnetic resonance imaging, as “nanolamps”, and in bioimaging. Such particles may also form the basis for a new generation of optical immunosensors or gene sensors that can be separated from complex biomatter by magnetic force and where fluorescent signals can be generated using a 980-nm diode laser whose light easily penetrates most tissue.



537. Quenching of the Luminescence of Upconverting Luminescent Nanoparticles by Heavy Metal Ions. S. M. Saleh, R. Ali, O. S. Wolfbeis; *Chem. – Eur. J.* (2011), 17, 14611-14617. DOI: 10.1002/chem.201101860. IF: 5.5.

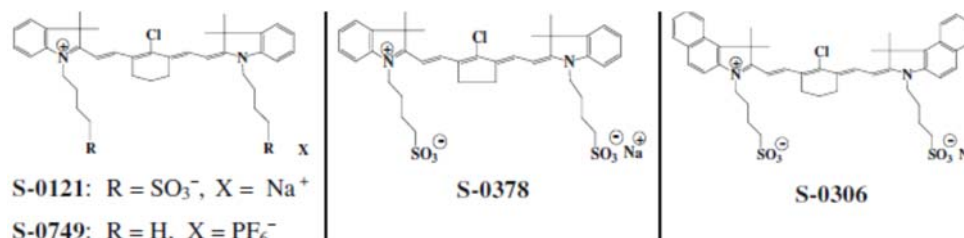
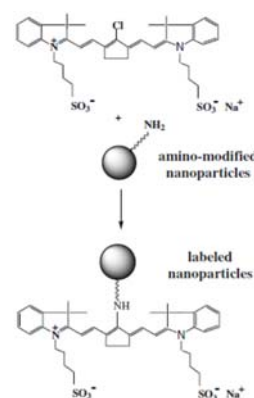
Abstract: The red and green luminescence of upconverting luminescent nanoparticles (photoexcited with a 980-nm diode laser) is dynamically and statically quenched by heavy metal ions (in particular by Hg(II) ions), and by bromide and iodide. Quantitative quenching studies are presented. The efficiency of quenching is different for the two main emission bands.



532. New Silica and Polystyrene Nanoparticles Labeled with Longwave Absorbing and Fluorescent Chameleon Dyes. S. M. Saleh, R. Ali, O. S. Wolfbeis; *Microchim Acta* (2011), 174, 429-434. DOI: 10.1007/s00604-011-0627-y. IF: 2.6.

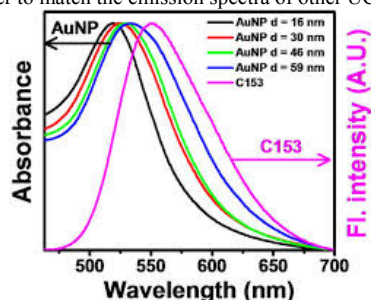
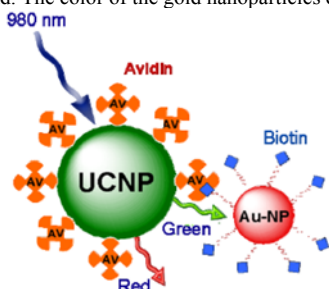
Abstract: We are presenting new fluorescent nanoparticles (NPs) made from silica or polystyrene. Such NPs are potentially useful for purposes of cellular imaging and sensing. The NPs were surface-modified with amino groups, and longwave absorbing and emitting dyes were then conjugated, via their reactive chloro atoms, to the NPs. The reactions proceeded at temperatures of around 65 °C and in predominantly aqueous solution, and are accompanied by a color change from typically green to blue. By analogy to other labels giving this effect, we refer to such dyes as chameleon labels. All NPs were characterized in terms of size, by absorption and emission spectroscopy, thermogravimetry and zeta potentials. The chameleon effect also was used to detect the presence of minute quantities of amino groups on the surface of NPs, both by absorptiometry and, with particular sensitivity, by fluorescence.

Shown below (↓) are chemical structures of representative reactive labels.



531. Detection of Biotin-Avidin Affinity Binding by Exploiting a Self-Referenced System Composed of Upconverting Nanoparticles and Gold Nanoparticles. S. M. Saleh, R. Ali, T. Hirsch, O. S. Wolfbeis; *J. Nanoparticle Res.* (2011), 13, 4603-4611. DOI: 10.1007/s11051-011-0424-x. IF 3.3.

Abstract: We describe an affinity system based on the interaction of two types of nanoparticles. The first consists of upconverting luminescent NaYF₄:Yb,Er nanoparticles (UCLNPs) with a size of 40 – 100 nm, absorbing light in the infrared and showing luminescence at 521, 543 and at 657 nm. The second consists of (red) gold nanoparticles (Au-NPs) with a size of about 50 nm and capable of absorbing the green luminescence of the UCLNPs. The UCLNPs were coated with silica and then labeled with avidin, while the AuNPs were coated with biotin. The two kinds of NPs represent a self referenced bioaffinity system that is applicable to biosensing in biological samples. In the presence of avidin-modified UCLNPs, the biotinylated Au-NPs can be detected in the range from 12 to 250 µg·mL⁻¹ by ratioing the intensity of the red (analyte-independent) emission to that of the green (analyte-dependent) emission band. The color of the gold nanoparticles can be adjusted via their charge in order to match the emission spectra of other UCLNPs.



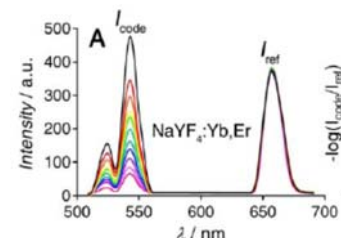
530. Review. Fluorescent Sensing, Biosensing, and Screening Using Upconverting Nanoparticles. D. E. Achatz, A. Reham, O. S. Wolfbeis; *Topics Curr. Chem.* (2011), 300, 29-50. DOI: 10.1007/128_2010_98. IF: 4.3.

Abstract: Upconverting nanoparticles (UCNPs) display the unique property of converting near-infrared light (with wavelengths of typically 800 to 1000 nm) into visible luminescence. The main classes of materials are discussed. We also review the state of the art of using UCNPs (a) to label biomolecules such as antibodies and (synthetic) oligomers for use in affinity assay and flow assays; (b) to act as nanolamps whose emission intensity is modulated by chemical indicators, thus leading to a novel kind of chemical sensors; and (c), in FRET-based chemical sensors and biosensors.



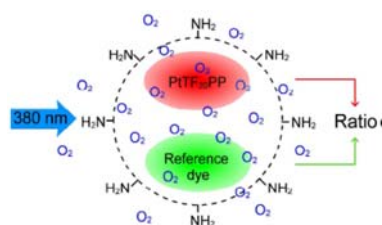
528. Tuning the Dual Emission of Photon-Upconverting Nanoparticles for Ratiometric Multiplexed Encoding. H.-H. Gorris, R. Ali, S. M. Saleh, O. S. Wolfbeis; *Adv. Mater.* (2011) 23, 1652-1655. DOI: 10.1002/adma.201004697. IF: 10.9.

Abstract: Commercially available longwave dyes were immobilized in different concentrations on the surface of upconverting luminescent nanoparticles in order to selectively tune one of the dual emission bands (I_{code}). The second emission band (I_{ref}), by contrast, serves as an internal reference to obtain ratiometric codes. Combining UCNPs with distinct emission bands expands the coding capacity exponentially.

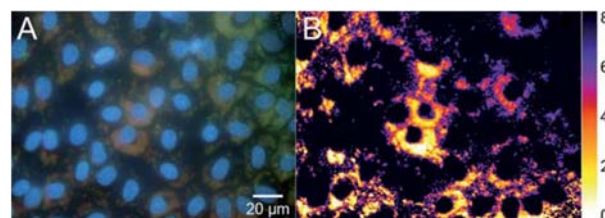


527. Self-Referenced RGB Colour Imaging of Intracellular Oxygen. X. D. Wang, H. H. Gorris, J. A. Stolwijk, R. J. Meier, D. B. M. Groegel, J. Wegener, O. S. Wolfbeis; *Chem. Sci. (Cambridge)* (2011) 2, 901-906. DOI: 10.1039/c0sc00610f. IF: 7.5.

Abstract: The nanosensors contain two luminophores matching the red and the green channels of red-green-blue (RGB) color cameras. The red emission of the

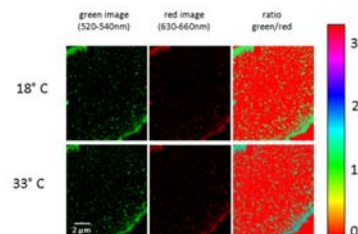


oxygen probe is quenched by oxygen, while the (constant) blue-green emission of the second fluorophore serves as a reference. The dyes are incorporated into polystyrene particles for ic-sensing of oxygen. The image on the right shows (A) a conventional photographic picture, and (B), the ratioed picture in pseudo-colors that reflects the concentration of oxygen (in ppm) according to the calibration bar (right).



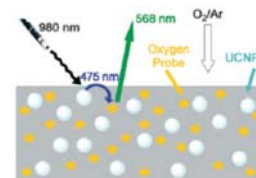
526. Highlight Article. Upconversion Nanoparticles for Nanoscale Thermometry. L. H. Fischer, G. S. Harms, O. S. Wolfbeis; *Angew. Chem. Intl. Ed.* (2011) 50, 4546-4548. DOI: 10.1002/anie.201006835. IF: 12.7.

Abstract: Lanthanide ion-doped nanoparticles display a strongly temperature-dependent luminescence that can be used to sense temperature in sub-μm dimensions, for example in cells or nanofluidics. Unlike molecular probes, the use of such nanoparticles with their anti-Stokes emission is not interfered by background luminescence, and the excitation wavelengths (> 700 nm) are hardly absorbed by cells.



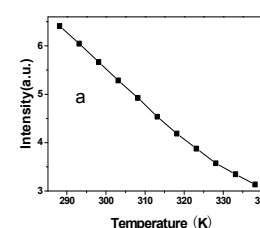
518. Luminescent Sensing of Oxygen Using a Quenchable Probe Along with Upconverting Nanoparticles. D. E. Achatz, R. J. Meier, L. H. Fischer, O. S. Wolfbeis; *Angew. Chem. Intl. Ed.* (2011), 50, 260-263. DOI: 10.1002/anie.201004902. IF 12.7.

Abstract: We are presenting the first sensor for oxygen that can be excited with NIR light. It makes use of upconverting nanoparticles of the type NaYF₄:Yb,Tm that are photo-excited with a 980-nm laser and whose visible luminescence is used to photoexcite a quenchable iridium probe for oxygen, thereby overcoming the lack of NIR-excitable probes for oxygen. The components and materials used are readily available, and merits of working at such long excitation wavelength include the complete absence of luminescence background that can be strong in many samples to be analyzed. A new type of ratiometric readout also is demonstrated.



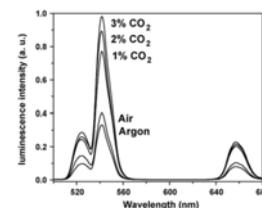
511. Temperature-Sensitive Luminescent Nanoparticles and Films Based on a Terbium(III) Complex Probe. L. Sun, J. Yu, H. Peng, J. Z. Zhang, L. Shi, O. S. Wolfbeis; *J. Phys. Chem. C* (2010), 114, 12642-12648. DOI: 10.1021/jp1028323. IF: 4.2.

Abstract: The terbium-tris[(2-hydroxy-benzoyl)-2-aminoethyl]amine complex (Tb-THBA) with its high color purity, long luminescence lifetime and high quantum yield has been found to be a viable indicator for optical sensing of temperature (T). Both its luminescence intensity and lifetime strongly depend on T in the range from 15 to 65 °C. When photoexcited at 341 nm, it displays typical Tb³⁺ ion emission bands with the strongest peak at 546 nm and a typical decay time of 1.15 ms at 15 °C. The probe is shown to be an excellent for sensing T , as demonstrated in two kinds of optical sensor membranes.



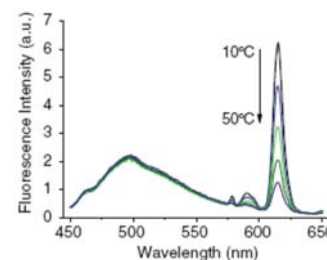
510. Upconverting Nanoparticle Based Optical Sensor for Carbon Dioxide. R. Ali, S. M. Saleh, R. J. Meier, H. A. Azab, I. Elgawad, O. S. Wolfbeis; *Sensors Actuat. B (Chemical)* (2010), 150, 126–131. DOI: 10.1016/j.snb.2010.07.031. IF 3.1.

Abstract: We demonstrate a novel optical sensor for carbon dioxide in concentrations between 0 and 3%. The sensing scheme is based on the optical interrogation of a 12- μm polystyrene (PS) film containing upconverting nanoparticles (UCNPs; 40–100 nm in size) of the type $\text{NaYF}_4:\text{Yb,Er}$, and the longwave absorption pH probe bromothymol blue (BTB) in its anionic (blue) form. PS is chosen as a matrix because it displays permeation selectivity for CO_2 and rejects protons. The color of BTB in the PS matrix depends on the partial pressure of CO_2 gas. The UCNPs are photoexcited with a 980-nm laser diode to give a green (542 nm) and a red (657 nm) emission whose intensity is screened off (depending on whether BTB is present in its blue or yellow form) due to an inner filter effect.



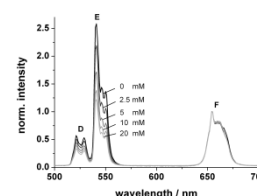
509. Ratiometric Fluorescent Nanoparticles for Sensing Temperature. H. Peng, S. Huang, O. S. Wolfbeis; *J. Nanoparticle Res.* (2010), 12, 2729–2733. DOI: 10.1007/s11051-010-0046-8. IF: 2.5.

Abstract: Nanoparticles made from a poly(methyl methacrylate)-co-1,2-bis(trimethoxysilyl)decane composite and containing a red-luminescent europium(III) complex were prepared by the encapsulation-precipitation method. By introducing a green-emitting naphthalimide reference dye, the NPs display both a green and a red fluorescence under single-wavelength excitation. The ratio of fluorescence intensities is highly temperature dependent in the 25–45 $^{\circ}\text{C}$ range, with a sensitivity of $\sim 4.0\%$ per $^{\circ}\text{C}$. Given their small size (20–30 nm) and biocompatibility (due to the presence of an outer layer of silica), such NPs are useful *T* sensors for cellular sensing and imaging.



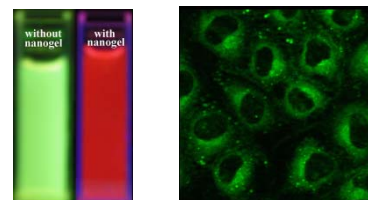
504. Optical Ammonia Sensor Based on Upconverting Luminescent Nanoparticles. H. S. Mader, O. S. Wolfbeis; *Anal. Chem.* (2010), 82, 5002–5004. DOI: 10.1021/ac1007283. IF: 5.2.

Abstract: The sensor exploits the phenomenon of upconversion luminescence and is based on (a) the use of upconverting nanoparticles (UCNPs) of the $\text{NaYF}_4:\text{Yb,Er}$ type that can be excited with 980 nm laser light to give a green and red luminescence and (b) the pH probe phenol red immobilized in a polystyrene matrix. Exposure of the sensor film to ammonia causes a strong increase in the 560 nm absorption of the pH probe which, in turn, causes the green emission of the UCNPs to be screened off. The red emission of the UCNPs, in contrast, remains unaffected by ammonia and can serve as a reference signal. Due to the use of 980 nm as the excitation light source, the optical signal obtained is completely free of background visible luminescence of the sample and of scattered light. This is advantageous in the case of sensing ammonia in complex matrices.



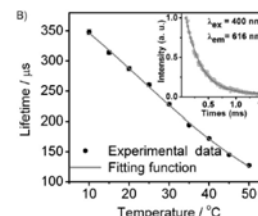
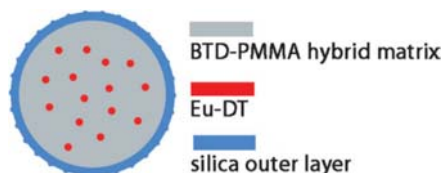
500. A Nanogel for Ratiometric Fluorescent Sensing of Intracellular pH Values. H. Peng, J. A. Stolwijk, L. Sun, J. Wegener, O. S. Wolfbeis; *Angew. Chem. Intl. Ed.* (2010), 49, 4246–4249. DOI: 10.1002/anie.200906926. IF: 11.8.

Abstract: We are reporting on the first ratiometric fluorescent nanogel (NG) for sensing pH. It can be easily prepared and made pH-responsive via addition of a pH probe and a (ratiometric) FRET system. It has been used to ratiometric imaging of pH inside cells.



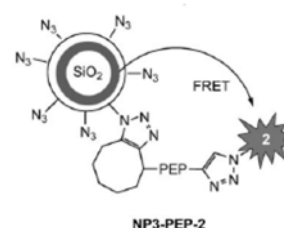
496. Luminescent Europium(III) Nanoparticles for Sensing and Imaging of Temperature in the Physiological Range. H. Peng, M. I. J. Stich, J. Yu, L. Sun, L. H. Fischer, O. S. Wolfbeis; *Adv. Mat.* (2010), 22, 716–719. DOI: 10.1002/adma.200901614. IF: 8.4.

Abstract: Lanthanide-based nano-particles with a diameter of 20–30 nm are introduced for use in luminescent sensing and imaging of physiological temperatures. They are characterized by (i) visible-light photo-excitation, (ii) line-like emission (which facilitates multicolor (dual) sensing), (iii) inert-ness to external perturbors as a result of encapsulation of the europium probe into a biocompatible protective nanoshell, (iv) high photostability, (v) a dynamic range that covers *T*'s encountered in medicine, (cellular) biology, and biotechnology, and (vi) good resolution (typically $\pm 0.3\text{ }^{\circ}\text{C}$).



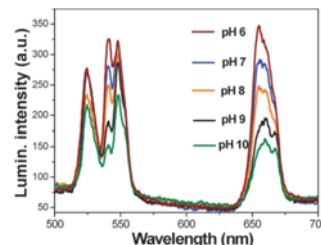
491. Probing the Activity of Matrix Metalloproteinase II Using a Sequentially Click-Labeled Silica Nanoparticle FRET Probe. D. E. Achatz, G. Mezö, P. Kele, O. S. Wolfbeis; *ChemBioChem* (2009), 10, 2316–1320. DOI: 10.1002/cbic.200900261. IF: 3.3.

Abstract: Fluorescent core-shell silica nanoparticles (SiNPs) bearing a fluorescently labeled substrate for the tumor marker protease MMP-2 were prepared. FRET from the SiNP to the label is observed but suppressed once the substrate is hydrolyzed by the enzyme. The reaction rate is a direct parameter for determining the activity of MMP-2.



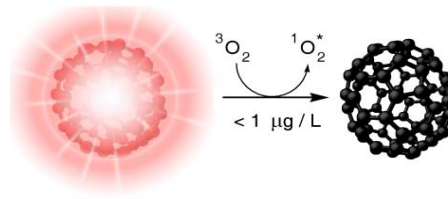
486. pH Sensor Based on Upconverting Luminescent Lanthanide Nanorods. L. Sun, H. Peng, M. I. J. Stich, D. E. Achatz, O. S. Wolfbeis; *Chem. Comm.* (2009), 33, 5000-5002. DOI: 10.1039/b907822c. IF: 5.5.

Abstract: The pH sensor exploits the phenomenon of upconversion luminescence and is based on a hydrogel matrix containing (a) nanorods of the NaYF₄:Er,Yb type that can be excited with 980-nm laser light to give a green and red (dual) emission, and (b) a longwave absorbing pH probe that causes a pH-dependent inner filter effect.



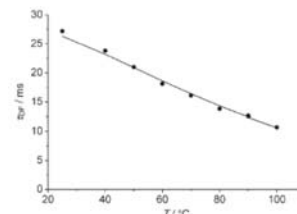
447. Optical Sensing and Imaging of Trace Oxygen with Record Response. S. Nagl, C. Baleizão, S. M. Borisov, M. Schaeferling, M. N. Berberan-Santos, O. S. Wolfbeis; *Angew. Chem. Intl. Ed.* (2007), 46, 2317-2319. DOI: 10.1002/anie.200603754. IF: 10.3.

Abstract: Ultratrace quantities of oxygen can be determined over a temperature range of more than 100 °C by exploiting the extremely efficient quenching of the delayed fluorescence of fullerene C₇₀ incorporated into organosilica or ethyl cellulose.



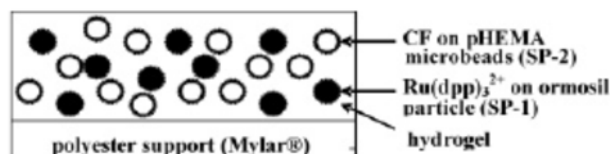
444. An Optical Thermometer Based on the Delayed Fluorescence of C₇₀. C. Baleizao, S. Nagl, S. M. Borisov, M. Schaeferling, O. S. Wolfbeis, M. N. Berberan-Santos; *Chem. Eur. J.* (2007), 13, 3643-3651. DOI: 10.1002/chem.200601580. IF: 5.0.

Abstract: The thermo-meter is based on the thermally activated delayed fluorescence of fullerene C₇₀. It consists of C₇₀ molecularly dispersed in a various polymer films. In the absence of oxygen and for temp. above 20 °C, the red fluorescence of C₇₀ in the films is so intense that it is easily perceived by the bare eye. The fluorescence intensity of C₇₀ increases with temp. by a factor of up to 90, depending on the polymer. This results in a working range from – 80 °C to at least 140 °C. Perylene was incorporated as an internal reference in order to enable ratiometric measurements. The sensitivity of the lifetime of the delayed fluorescence to temp. is also high, and results in an even wider working range. The graph shows the temp. dependence of the experimental (circles) and calculated (solid line) lifetimes the delayed fluorescence of C₇₀ at 700 nm.



441. Indicator-Loaded Permeation-Selective Microbeads for Use in Fiber Optic Simultaneous Sensing of pH and Dissolved Oxygen. G. S. Vasylevska, S. M. Borisov, C. Krause, O. S. Wolfbeis; *Chem. Mat.* (2006), 18, 4609-4616. DOI: 10.1021/cm060967n. IF: 5.1.

Abstract: New materials are described that lead to sensors capable of simultaneously sensing of pH and oxygen via a single fiber optic sensor. They make use of pH probe based on carboxyfluorescein, and of a ruthenium(II) complex acting as a probe for dissolved oxygen. The selectivity of the probes was considerably improved by incorporating them into two kinds of microparticles, each of specific permeation selectivity. The pH probe was immobilized on particles made from proton permeable amino-modified poly(hydroxy-ethyl methacrylate), while the oxygen probe was physically immobilized in beads made from an organically modified sol-gel. Both kinds of beads were dispersed into a hydrogel matrix and placed at the distal end of an optical fiber waveguide for optical interrogation. A phase-modulated blue-green LED serves as the light source for exciting luminescence whose average decay times or phase shifts serve as the analytical information.



374. Book Chapter. Advanced Luminescent Labels, Probes and Beads, and Their Application to Luminescence Bioassay and Imaging. O. S. Wolfbeis, M. Boehmer, A. Duerkop, J. Enderlein, M. Gruber, I. Klimant, Ch. Krause, J. Kuerner, G. Liebsch, Zh. Lin, B. Oswald, M. Wu; *Springer Series in Fluorescence Spectroscopy*, vol. 2 (R. Kraayenhof, A. J. W. G. Visser, H. C. Gerritsen, eds.); Springer Verlag, Berlin-Heidelberg, 2002; pp. 3-42. DOI: 10.1007/978-3-642-56067-5_1.

Abstract: An overview is given on our recent activities in the following areas: (1) general logics for designing fluorescent probes and labels; (2) new diode laser-excitable probes for non-covalent protein detection; (3) diode laser-compatible amine-reactive covalent labels; (4) diode laser-assisted fluorescent single molecule detection of dyes and labeled proteins; (5) new labels for flow cytometric determination of HSA; (6) new DNA labels; (7) fluorescence resonance energy transfer gene assays; (8) reactive ruthenium ligand complexes as markers for bioassays; (9) diode laser-excitable fluorescent polymer beads; (10) polyaniline-coated nanobeads as fluorescent pH probes; (11) phosphorescent poly(acrylonitrile) nanospheres as markers for optical assays; (12) competitive binding of streptavidin to biotinylated nanobeads as studied by resonance energy transfer; (13) nanobeads as reference dyes in luminescent lifetime imaging using DLR; (14) phosphorescent nanospheres for use in advanced time-resolved multiplexed bioassays; (15) beads dyed with a europium-based label and excitable with the 405-nm diode laser; and (16) a europium(III)-based probe for use in oxidase-associated reactions.

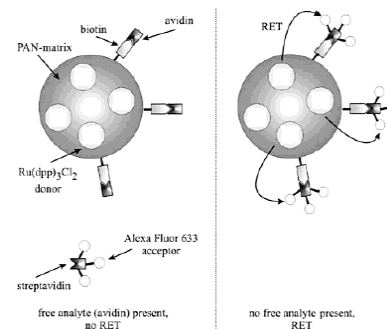


367. Homogeneous Luminescence Decay Time-Based Bioassay Using Energy

Transfer from Nanospheres, J. M. Kuerner, O. S. Wolfbeis, I. Klimant; *Anal.*

***Chem.* 74 (2002) 2151-2156. DOI: 10.1021/ac0111098. IF: 5.7.**

Abstract: A novel scheme is presented for homogeneous assays based on resonance energy transfer (RET) from phosphorescent biotinylated nanospheres to fluorescently-labeled streptavidin (SA). The nanospheres, with a diameter of well below 50 nm, are made from carboxylated polyacrylonitrile (PAN) and dyed with the ruthenium complex Ru(dpp). RET occurs from Ru(dpp) to the label if labeled SA binds to the surface of the nanospheres. Luminescence quenching by oxygen or other species can be neglected due to the shielding effect of the polymer matrix. A competitive binding assay was established, where avidin and labeled SA compete for the biotin binding sites on the nanosphere. The process of binding to the surface can be detected by measurement of the luminescence intensity or the apparent decay time which is in the order of 2.5 to 4.5 μ s.



361. Inert Phosphorescent Nanospheres as Markers in Optical Assays, J. M. Kuerner, I. Klimant, Ch. Krause, H. Preu, W.

Kunz, O. S. Wolfbeis; *Bioconjug. Chem.* 12 (2001) 883-889. DOI: 10.1021/bc000130x. IF: 3.8.

Abstract: An encapsulation technique is presented to produce highly phosphorescent, inert nanospheres which are suitable luminescent markers. It is based on the co-precipitation of phosphorescent ruthenium(II)-tris(polypyridyl) complexes and polyacrylonitrile derivatives from a solution in N,N-dimethylformamide. The typical particle diameter is less than 50 nm. The nanospheres were characterized with respect to their spectral properties, quantum yields of the luminophores, luminescence decay time, stability in aqueous buffered suspensions, and in terms of size, shape and surface charge of the particles, as well as storage stability, quenching by oxygen, and dye leaching.



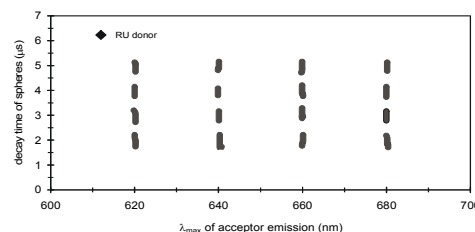
357. A New Type of Phosphorescent Nanospheres for Use in Advanced

Time-Resolved Multiplexed Bioassays, J. M. Kuerner, I. Klimant, Ch.

Krause, E. Pringsheim, O. S. Wolfbeis; *Anal. Biochem.* 297 (2001) 32-41.

DOI: 10.1006/abio.2001.5295. IF: 3.0.

Abstract: Optically encoded phosphorescent nanospheres are presented. They are distinguishable by their individual decay time and spectral distribution of their emission spectra. They are composed of a phosphorescent ruthenium metal ligand complex (MLC) dissolved, along with certain strongly fluorescent cyanine dyes, in modified polyacrylonitrile-based nanospheres. Since the MLC (the donor) and the cyanine (the acceptor) are in close spatial proximity, efficient resonance energy transfer (RET) does occur. Thus, the nanospheres emit dual luminescence, one from the acceptor dye, the other from the donor MLC. Variation of the concentrations of the acceptor dye results in a varying efficiency of RET, thus making the spheres distinguishable. Hence, a set of multiplexable sphere labels is obtained by using one MLC (acting as the phosphorescent donor and present in constant concentration) and one acceptor dye (which varies both in terms of spectral properties and concentration).

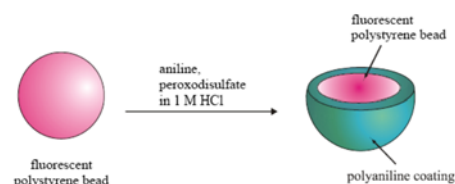


353. Fluorescent Beads Coated with Polyaniline: A Novel Nano-Material for Optical Sensing of pH. E. Pringsheim, D.

Zimin, O. S. Wolfbeis; *Adv. Mat.* 13 (2001) 819-822. DOI: 10.1002/1521-4095(200106)13:11<819::AID-

ADMA819>3.0.CO;2-D. IF: 7.9.

Abstract: Fluorescent nanobeads are presented that can be used for optical sensing of pH. The sensing scheme is based on the finding that aniline, if oxidized in presence of fluorescent polystyrene beads, is deposited on the particles as a thin film of polyaniline (PANI). The resulting coated beads, typically 360 nm in diameter, were characterized by fluorescence spectroscopy, atomic force microscopy and flow cytometry. It is found that the fluorescence intensity of the PANI-coated beads undergoes pH-dependent changes even though the fluorophore is inert to pH. This is due to an inner filter effect caused by the pH-sensitive PANI coating which modulates fluorescence intensity. The beads thus can act as fluorescent bead probes for physiological pHs.



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