Magnetically Remote-Controlled Optical Sensor Spheres for Monitoring Oxygen or pH

Günter Mistlberger,* Klaus Koren, Sergey M. Borisov, and Ingo Klimant

Institute of Analytical Chemistry and Food Chemistry, Graz University of Technology, Graz, Austria

Magnetic sensor macrospheres (MagSeMacs), i.e., stainless steel spheres coated with optical chemical sensors, are presented as an alternative to existing optical sensor patches and fiber-optical dip-probes. Such spheres can either be reversibly attached to the tip of an optical fiber (dip-probe) or trapped inside a vessel for read-out through the side wall. Moving the magnetic separator at the exterior enables measurements at varying positions with a single sensor. Moreover, the sensor's replacement is rapid and contactless. We measured dissolved oxygen or pH in stirred liquids, rotating flasks, and 24-well plates with a SensorDish-reader device for parallel cell culture monitoring. In these applications, MagSeMacs proved to be advantageous over conventional sensor patches and magnetic optical sensor particles because of their magnetism, spherical shape, reflectance, and size. These properties resulted in strong but reversible fixation, magnetic remote-controllability, short response times, high signal intensities, and simplified handling.

Bioprocess developments rely on the strict control of process parameters, such as pO_2, pH, temperature, and metabolite concentrations.[1–5] For process monitoring, optical sensors represent a reliable, robust, and cheap alternative to conventional mostly electrochemical or physical sensors. Among optical sensors, sensor patches fixed in transparent reaction vessels or coated onto optical fiber probes (micrometer or millimeter scale) have been used most frequently.[6–13] Although sensor patches are widespread they have some limitations. (1) Price: photobleaching and biofouling of the sensors require periodical exchanges of vessels as well as fiber-optic probes. (2) Time: the sensor spots need to be fixed long time before the measurement. (3) Mobility: once a sensor patch is fixed, its position cannot be changed. Consequently, measurement information can only be acquired at a single position. The identification of concentration gradients would require many sensor spots or the application of a dip-probe.

Measuring analyte concentrations at different positions with mobile sensors was recently solved by applying magnetic optical sensor particles (MOSePs).[14,15] With MOSePs, sensor spots can be formed in situ in front of an optical fiber by using special magnetic separators.[16] The brightness and inducible magnetic force of such "sensor swarms" is higher compared to single particles.[14] Despite the suitability of MOSePs for complex applications, where fixed sensor patches fail, further improvements are essential for bioprocess monitoring. Stability against shear forces and time required for sensor spot formation play a crucial role for the sensor performance and are difficult to control with micro- and nanoparticles. Nelson and co-workers recently reported on wireless optical sensors as MEMS prototypes for intraocular oxygen measurements.[17] Focusing the sphere in a certain distance from the magnets, however, resulted in a relatively complex setup.

This technical note describes the spray coating of stainless steel spheres with polymeric matrixes and sensor dyes for the production of highly ferromagnetic, user-friendly, remote-controlled optical sensor macrospheres (MagSeMacs) in transparent vessels (Figure 1). These spheres can also be used as disposable sensors for dip-probes. The response times and calibration curves of two types of MagSeMacs (pH or oxygen sensitive) were measured. Their suitability as an alternative but also as an extension to currently available sensor patches is discussed. Finally, magnetic separators are described which enabled the application of MagSeMacs as dip-probes and remote-controlled sensors.

EXPERIMENTAL SECTION

Chemicals and Materials. Polysulfone (MW 35 000; Sigma), polystyrene (PS, MW 250 000; Acros Organics), poly(vinylidene chloride-*co*-acrylonitrile) (PViCl−PAN; 20% (w/w) polyacryloni-

* To whom correspondence should be addressed. E-mail: mistlberger@tugraz.at.

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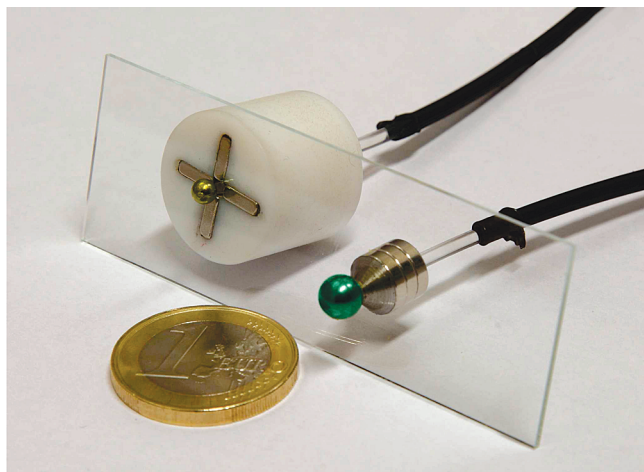
Figure 1. Magnetic sensor macrospheres captured in front of an optical fiber with a radial (left) or axial (right) separator.

trile; MW 150 000; Polysciences), polyurethane hydrogel D4 (Cardiotech), glucose oxidase from *Aspergillus niger* (GOx, Fluka), trichloromethane, ethanol, glucose monohydrate, and phosphate and citrate buffers (all purchased from Carl Roth GmbH, Germany) were used as received without further purification. Iridium(III)((benzothiazol-2-yl)-7-(diethylamino)-coumarin))(acetylacetonate) ($Ir(C_S)_2(acac)$),[18] 1-hydroxypyrene-3,6,8-tris-bis(2-ethylhexyl)sulfonamide ($HPTS(DHA)_3$)[19] and platinum(II)-tetraphenyltetrabenzoporphyrin (PtTPTBP)[20] were synthesized in our lab as described in the literature. Spectra and structures of the dyes can be seen in Figure S1 in the Supporting Information. Magnetic steel spheres (stainless steel or class 3 DIN5401) with diameters of 2, 3.2, 4, and 5 mm, respectively, were purchased from Kugel Pompel (www.kugelpompel.at). NdFeB block and ring magnets were purchased from ChenYang Technologies (www.cy-magnetics.com).

Magnetic Separator Design. The magnetic separators were designed as described elsewhere.[16] Dip-probes for magnetically fixed MagSeMacs had additional barriers around the sphere in order to avoid the sphere's accidental wiping off from the fiber tip (Figure 2).

Sensor Preparation. Steel spheres were coated by spraying a solution of dye and polymer in organic solvent ("cocktail") with an airbrush on rapidly shaking spheres. A total of 100 stainless steel spheres ($d = 3.2$ mm) were heated in a crystallizing dish to 70 °C with a heat gun. The crystallizing dish was fixed to a vibrating device (Vibramax 100, Heidolph) with double-faced adhesive tape and shaken at 1000 min^{-1} (shaking orbit 3 mm) in order to avoid the sticking of the spheres to the dish and to each other, respectively.

For oxygen sensitive MagSeMacs, a cocktail of 14.6 mg of polystyrene or polysulfone, 0.22 mg of an indicator dye (PtTPTBP or $Ir(C_S)_2(acac)$) and 0.732 g (0.5 mL) of $CHCl_3$ was sprayed onto the preheated spheres from a distance of 30 mm with a cocktail flow-rate of 1.6 mL min^{-1} and a shear gas pressure of

3 bar. The airbrush was moved in circles above the crystallizing dish to additionally agitate the spheres and to avoid their sticking to the dish.

A dual lifetime referencing (DLR) system[21,22] was utilized for the production of pH-sensitive MagSeMacs. We incorporated $HPTS(DHA)_3$ as pH-sensitive and $Ir(C_S)_2(acac)$ as a reference dye in the D4 hydrogel (Figure 3 and Figure S2 in the Supporting Information). In order to avoid cross-sensitivity of $Ir(C_S)_2(acac)$ to oxygen, this reference dye was first incorporated in PViCl−PAN nanoparticles, which is a gas-impermeable material.[23] For the spraying procedure, the cocktail consisted of 116 mg of D4, 1.4 mg of $HPTS(DHA)_3$, 14.6 mg of PViCl−PAN nanoparticles containing 0.15 mg of $Ir(C_S)_2(acac)$, 5 g of ethanol, and 0.5 g of deionized water. A volume of 1 mL of this cocktail was used for spray-coating.

The oxygen sensing performance of MagSeMacs, a sensor patch, dispersed nanoparticle sensors, and magnetic optical sensor particles (MOSePs) was compared. Except for MagSeMacs, these sensors were previously employed for monitoring the analyte concentration in multiwell plates with a SensorDish-reader device. We used a 4 μm thick PS foil containing 2% $Ir(C_S)_2(acac)$ as a sensor patch. Nonmagnetic nanoparticles (PSPVP-NP)[24] and MOSePs[25] were prepared in our lab as described elsewhere.

Measurement Setup. MagSeMacs were placed in a 10 mL glass vial (calibration) or a 200 mL beaker (response time) and trapped with the above-mentioned magnetic separators. The luminescence phase shift was read out with a 2 mm optical fiber and a phase fluorimeter (pH-Mini, PreSens GmbH, Germany). Alternatively, for PtTPTBP a 625 nm LED (Roithner Laser Technik, www.roithner-laser.com) was modulated with a two-phase lock-in amplifier (SR830, Stanford Research Inc., www.thinksrs.com). A bifurcated fiber bundle was used to guide the excitation light (filtered through a Calflex K filter, Linos) to the MagSeMac and the luminescence back to the detector after being filtered through an RG9 (Schott) glass filter. Luminescence was detected with a PMT (H5701-02, Hamamatsu, www.sales.hamamatsu.com). The modulation frequencies were adjusted to 5 kHz for PtTPTBP and 20 kHz for $Ir(C_S)_2(acac)$. For the measurements in a 24-well plate, a SensorDish-reader device (PreSens GmbH, Germany) was modified with magnets as described elsewhere[25] and the modulation frequency was adjusted to 20 kHz, which is the ideal frequency for $Ir(C_S)_2(acac)$. Gas mixtures for pO_2-calibration were obtained using a gas mixing device (MKS Instruments, www.mksinst.com). Nitrogen, synthetic air, and oxygen were purchased from Air Liquide. Calibration of pH-MagSeMacs was carried out in 20 mM phosphate or phosphate−citrate buffers (ionic strength adjusted to 100 mM with NaCl) with pH values from 3.69 to 11.9. The response time was measured upon rapid mixing of two solutions with analyte concentrations in the dynamic range of the sensors. Here, dip-probe type separators were employed.

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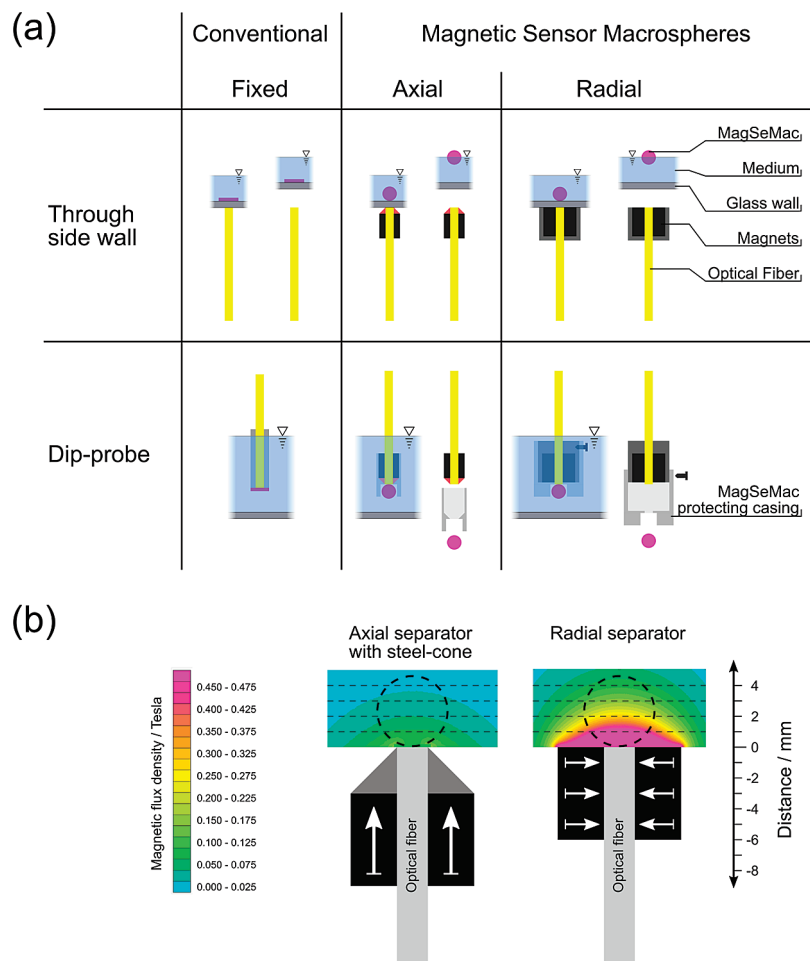(25) Mistlberger, G.; Borisov, S. M.; Klimant, I. *Sens. Actuators, B* **2009**, *139*, 174−180.

Figure 2. (a) Overview of possible sensor configurations with MagSeMacs (right) compared to fixed sensor patches (left). MagSeMacs can replace both fixed sensor spots on glass walls and coated fiber optical dip-probes. (b) The employed magnetic separators ensure a reliable localization of the magnetic sphere in the field of view of the optical fiber. The radial magnetization results in a higher magnetic field density and, consequently, in a stronger attraction of the sphere by the separator.
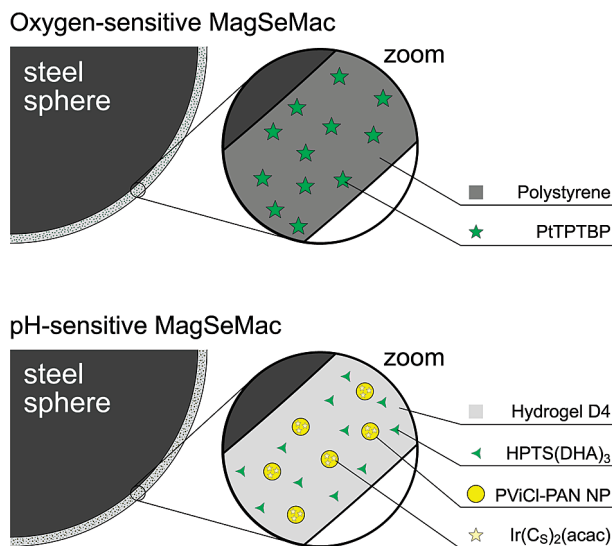

Figure 3. Schematic representation of the chemically sensitive coatings of MagSeMacs.

RESULTS AND DISCUSSION

Magnetic stainless steel spheres were coated with an optical sensor by spray-painting. This high-throughput method resulted in fast responding, bright, and mechanically stable coatings.

Stainless steel was necessary to avoid signal changes due to corrosion. Oxygen and pH-sensitive MagSeMacs, respectively, were prepared and their response times as well as their sensor characteristics were investigated. The Stern−Volmer plot of PtTPTBP-stained MagSeMacs (Figure 4a) shows a good correlation with the simplified two-site model of Carraway and Demas,[26] where one fraction of the dye molecules is assumed to be unquenchable, i.e., its $K_{SV2} = 0$ (see eqs 1 and 2).[27]

$$\frac{\tau}{\tau_0} = \frac{P}{1 + K_{SV1}pO_2} + \frac{1 - P}{1 + K_{SV2}pO_2} \quad (1)$$

$$K_{SV2} = 0 \Rightarrow \frac{\tau}{\tau_0} = \frac{P}{1 + K_{SV1}pO_2} + 1 - P \quad (2)$$

The quenchable fraction of the dye (93%) has a K_{SV1} of 0.014 hPa^{-1}.

The response time of oxygen-sensitive MagSeMacs ($t_{90} = 1.8$ s, Figure 4b) is sufficiently fast for real-time monitoring of most biological processes. As a consequence of the small contact area

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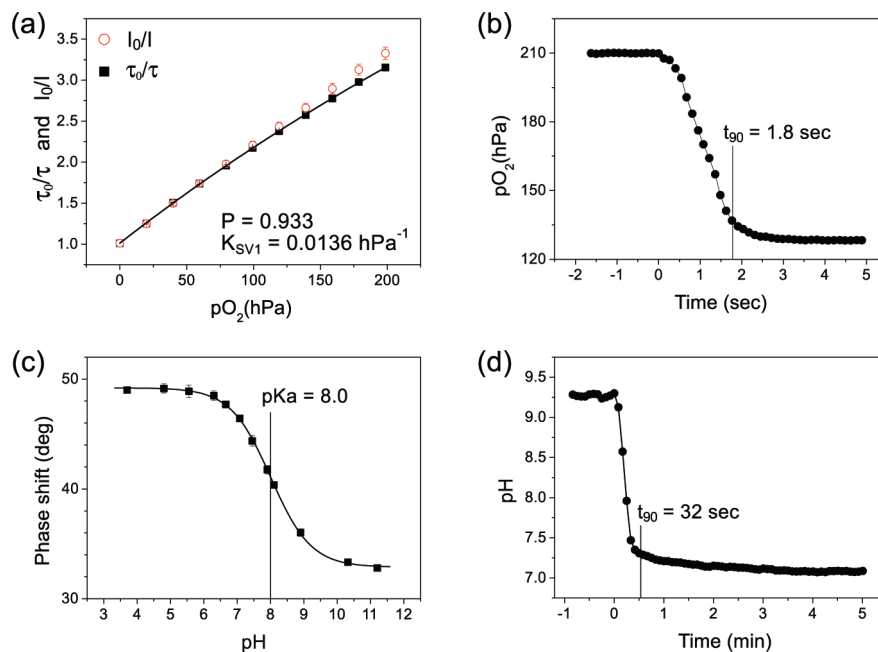
Figure 4. Calibration and response curve of an oxygen sensitive, PtTPTBP-stained MagSeMac (a,b) and pH-sensitive MagSeMacs (c,d).

Table 1. Intra- and Intersphere Variations of the Analyte Signal of Oxygen (PtTPTBP-Stained) or pH-Sensitive MagSeMacs

	$(\tau_0/\tau_{air}) \pm s$	s_{rel} (%)	$\tau_0 \pm s$ (10^{-6} s)	s_{rel} (%)	pH $\pm s$	s_{rel} (%)
intrasphere	3.28 ± 0.03	1.0	47.3 ± 0.2	0.3	7.16 ± 0.04	0.6
intersphere	3.28 ± 0.07	2.1	47.5 ± 0.4	0.7	7.0 ± 0.1	1.8

between the sphere and wall, the analyte exchange at the sensing spot is rapid and the diffusion distance is reduced to the thickness of the sensor coating. Finally, the signal is enhanced by doubling the optical path length and yield of luminescence light by the reflective surface of the stainless steel spheres.

To further characterize the applicability of such sensors, we tested the variability of the analyte signal by repeated release and collection cycles of a single sensor-sphere ("intrasphere"). In addition, the signal of different spheres was compared ("intersphere"). The summarized data can be seen in Table 1. The intrasphere variation of (τ_0/τ_{air}) of an oxygen sensitive Mag-SeMac given as the relative standard deviation of 15 measurements was 1%, which equals 3 hPa error in oxygen determination. The intersphere variation at air saturation was 2.1% (6.3 hPa). Variations of τ_0 were below 1% in both cases. These results suggested that a one-point calibration at air saturation is sufficient for each sphere and that no recalibration is required whenever the same sphere is released and trapped again. MagSeMacs sensitive to pH also showed little variation in both intra- and intersphere measurements (0.04 and 0.1 pH units, respectively).

Because of the intrinsic or self-referenced detection principle, the analyte signal was not affected by slightly inhomogeneous coatings. The variable thickness among different spheres (8 ± 2 μm) resulted in a varying signal intensity ($I = 40 \pm 20$ mV), whereas the values for (τ_0/τ_{air}) changed only marginally (Table 1).

Bioprocess-monitoring applications usually require sterile sensor spheres. Polystyrene is unsuitable due to its low glass

transition temperature of approximately 105 °C. Therefore, we produced MagSeMacs coated with polysulfone and $Ir(C_S)_2(acac)$ incorporated as an oxygen sensor. The ratio (τ_0/τ) of such sensors did not change significantly upon autoclaving, i.e., less than the intrasphere variability. The decreased sensitivity ($(\tau_0/\tau_{air}) = 1.37$ compared to 1.62 for $Ir(C_S)_2(acac)$ in PS) might be overcome by using PtTPTBP as an oxygen indicator. Another potential problem of using MagSeMacs for bioprocess-monitoring are strong magnetic field gradients in close proximity to the sphere. Such fields might capture the sensor irreversibly. Moreover, moving MagSeMacs might damage fragile adherent cells.

MagSeMacs with a pH sensing capability were accomplished with a DLR system.[22] We incorporated $HPTS(DHA)_3$ as the pH-sensitive component and $Ir(C_S)_2(acac)$ as a reference dye in a polyurethane hydrogel matrix. To avoid oxygen cross-sensitivity of the reference dye, we incorporated the dye in virtually gas-impermeable PViCl−PAN nanoparticles.[23] The emission spectra of the reference dye and the pH-indicator in its basic form match perfectly (Supporting Information, Figure S2), which is a prerequisite for DLR.[22] The calibration curve (Figure 4c) shows an apparent pK_a value of 8.0, a typical value for sensors applied in marine biology but also suitable under physiological conditions (pH 7.4). The pK_a value is in good agreement with the results reported previously.[19] Such a high apparent pK_a value for the sulfonamide derivative results from the localization of the indicator in apolar regions of the hydrogel. The formation of charged species upon dissociation of the hydroxyl group is not favored in such an environment. As a consequence, the
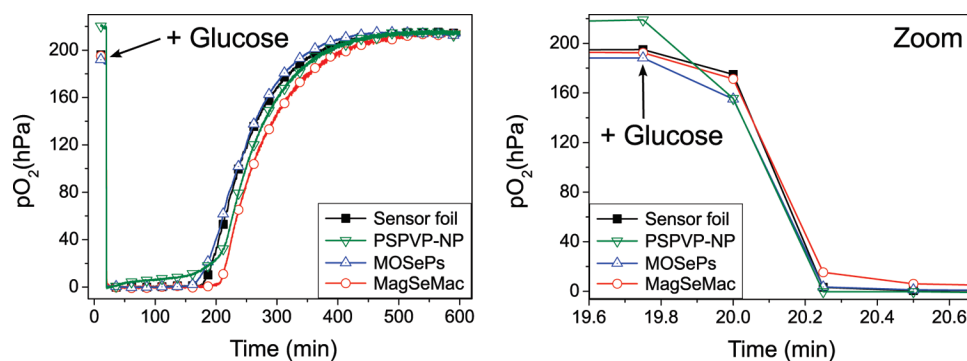
Figure 5. Dissolved oxygen concentration monitored by a sensor foil, a dispersed optical nanosensor (PSPVP-NP), magnetic, optical sensor particles, (MOSePs) and MagSeMacs. The addition of 5 mol of glucose to the solution containing 25 U mL^{-1} GOx was followed by a steep drop in oxygenation. After all the glucose was consumed, the medium was slowly reoxygenated. The right plot represents a zoom into the time frame in which the glucose was added to the solution. All tested sensors equilibrated within two data points.

pK_a value increases significantly compared to the aqueous solution (pK_a = 5.6 for 8-hydroxypyrene 1,3,6-trisdimethylsulfonamide)[28] and more polar materials such as poly(vinylpyrrolidone) (pK_a = 6.9).[19] The response time (t_{90} = 32 s, Figure 4d) was significantly higher than the one for oxygen-MagSeMacs but still in an acceptable range for most biological systems.

Finally, we evaluated MagSeMacs for oxygen monitoring in a commercial SensorDish-Reader suitable for simultaneous monitoring in standard 24-well microplates (see Figure S6e in the Supporting Information). To compare the performance of Mag-SeMacs with other sensor systems, we measured the oxygen level in a solution of glucose oxidase upon addition of glucose with four different systems: a fixed sensor patch, a dispersion of dye doped PSPVP nanoparticles,[24] spray-dried MOSePs,[25] and the here presented MagSeMacs. For all sensors, the Ir-coumarin dye was chosen as an oxygen indicator due to the spectral compatibility with the SensorDish-reader. All sensors reflected the steep drop in oxygen concentration upon mixing and the slow reoxygenation (Figure 5).

CONCLUSIONS

Magnetic sensor macrospheres (MagSeMacs) were prepared for monitoring analyte concentrations in both the solution and gas phases. Sphere sizes in the milimeter range ensure strong and rapid magnetic retention of MagSeMacs. This enables analyte monitoring in stirred liquids, in rotating and shaking vessels, and along gradients in plug-flow reactors. The size of the spheres also allows one to retract the sensor from the medium without a contamination of the sample. Sensor chemistry and readout equipment of sensor patches can be used for MagSeMacs without major modifications.

In summary, we successfully extended the concept of magnetically guided, optical sensors[14,15] to millimeter sized, highly ferromagnetic sensor spheres. Such MagSeMacs are suitable as versatile remote-controlled sensors and as exchangeable sensor caps for dip-probes.

SUPPORTING INFORMATION AVAILABLE

Structures and spectra of the used dyes, surface images of MagSeMacs and a discussion on potential applications of Mag-SeMacs (including schematic drawings and movies). This material is available free of charge via the Internet at http://pubs.acs.org.

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