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REVIEW

# Classification of analytics, sensorics, and bioanalytics with polyelectrolyte multilayer capsules

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## Abstract

Polyelectrolyte multilayer (PEM) capsules, constructed by LbL (layer-by-layer)-adsorbing polymers on sacrificial templates, have become important carriers due to multifunctionality of materials adsorbed on their surface or encapsulated into their interior. They have been also been used broadly used as analytical tools. Chronologically and traditionally, chemical analytics has been developed first, which has long been synonymous with all analytics. But it is not the only development. To the best of our knowledge, a summary of all advances including their classification is not available to date. Here, we classify analytics, sensorics, and biosensorics functionalities implemented with polyelectrolyte multilayer capsules and coated particles according to the respective stimuli and application areas. In this classification, three distinct categories are identified: (I) chemical analytics (pH; $K^+$, $Na^+$, and $Pb^{2+}$ ion; oxygen; and hydrogen peroxide sensors and chemical sensing with surface-enhanced Raman scattering (SERS)); (II) physical sensorics (temperature, mechanical properties and forces, and osmotic pressure); and (III) biosensorics and bioanalytics (fluorescence, glucose, urea, and protease biosensing and theranostics). In addition to this classification, we discuss also principles of detection using the above-mentioned stimuli. These application areas are expected to grow further, but the classification provided here should help (a) to realize the wealth of already available analytical and bioanalytical tools developed with capsules using inputs of chemical, physical, and biological stimuli and (b) to position future developments in their respective fields according to employed stimuli and application areas.

**Keywords** Polyelectrolyte multilayer capsules · Analytics · Sensors · Theranostics · Temperature · pH · Mechano-biology · Ions · pH · Glucose · Urea · Layer-by-Layer

## Introduction

Polyelectrolyte multilayer capsules have captivated the attention of scientists since their invention, which took place after extending the LbL (layer-by-layer) deposition technique to spherical [1, 2], as opposed to polyelectrolyte thin [3] and thicker, exponentially grown [4] films. The capsules are produced by sequentially depositing a desired number of polyelectrolyte multilayers (PEM) on a sacrificial template. After the desired number of layers has been absorbed, the template is typically dissolved, because ions can and some small molecules can diffuse through the polyelectrolyte multilayer shall, while larger molecules (peptides, proteins, and some smaller molecules) typically cannot diffuse through the polymeric shell. This process produces hollow polymeric shells, called capsules, fully suspended in such aqueous solution as water, physiological buffer, etc. Due to the described above dissolution process, templates, on which the polymeric shells are assembled, are then dissolved leaving a hollow shell. Due to potentially high consumable costs of templates, this process

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necessitates a proper choice of the template [5]. Novel methods of preparation and encapsulation of microcapsules are constantly under development [6], including, for example, the spraying methods [7] and interweaving assembly [8].

A number of templates are available: melamine formaldehyde (MF), polystyrene (PS), silica ($SiO_2$), etc., but calcium carbonate ($CaCO_3$) has been identified as a practical and an inexpensive materials for templates [9], particularly because they are used as sacrificial cores. Calcium carbonate exists in three polymorphs: aragonite, calcite, and vaterite. And, although the latter type is least stable, it is also the most interesting type, because of extensive porosity, which is used for loading the molecules. It should be noted that the potential instability of vaterite is overcome in the area of polyelectrolyte multilayer capsules by the polyelectrolyte coatings of calcium carbonate. Calcium carbonate is produced by mixing the salts containing calcium and carbonate ions [10] and can be easily dissolved by using ethylenediaminetetraacetic acid (EDTA). Peculiarly, the area of template development has turned almost to a stand-alone area, stimulated by the needs and promoted by application of particles as carriers in place of capsules, as it was, for example, shown for enzyme-catalyzed reactions [11]. Therefore, small calcium carbonate templates [12], anisotropic templates with controllable loading [13], Janus capsules [14] including those with a controlled patchiness [15], and shape-adaptable microcapsules [16] have been developed. To further develop advanced templates, the control of porosity of calcium carbonate particles has been shown [17].

In the first steps, encapsulation methods have been developed using different stimuli [18, 19] including pH-, temperature-, and direct loading into template-based methods. Subsequently, release methods [20] were proposed including light-nanoparticle interaction, mechanical, enzymatic degradation, chemical, etc. The versatility of polyelectrolyte multilayer capsules has been immediately felt upon introducing various materials from organic molecules to inorganic nanoparticles and carbon nanotubes into their shell. That stimulated the development of numerous applications in diverse areas [20–22], among which are analytics and sensorics. Analyzing past research and published papers, it appears that sensorics and analytics with PEM microcapsules is forming to be a stand-alone research and application area or sub-area. But it can be noticed that in the analytical domain, chemical analytics has almost always been synonymous with overall analytics, sensorics, and bioanalytics—to the best of our knowledge, no reports are available unifying, summarizing, and classifying analytical, sensoric and bioanalytical functionalities, approaches and applications in all these areas.

Here, an overview of principles and applications of polyelectrolyte multilayer capsules in the area of analytics, sensorics, and biosensorics is presented. Furthermore, a classification of these areas into chemical analytics (chemical stimuli), physical sensorics (physical stimuli), and biosensorics (biological stimuli) is made. This classification is not strictly rigorous. That is because some sensors operating by chemical or physical stimuli are also used in biology, and, vice versa, fluorophores and fluorescence imaging, which are at the heart of biological applications, are broadly used in chemical analytics and physical sensing. But such a classification is useful in regard with including all functionalities, many of which, particularly those based on physical stimuli, were out of the scope of analytics, bioanalytics, and sensorics. Historically, this can be assigned to the enormous interest in applications of chemical analytics (pH, ion, hydrogen peroxide, oxygen sensing) in biology. But it should be added that, although this area has seen a broad interest and has undergone an extensive development phase in chemical analytics, the sensorics using physical stimuli (temperature, mechanical strength, osmotic pressure) and bioanalytics (glucose-, urea-, protease-sensing and theranostics) should be added to construe the full scope of analytical functionalities.

## Analytical, sensoric, and biosensoric functionalities with PEM capsules

Figure 1 presents an overview of various analytics and sensorics areas developed using polyelectrolyte multilayer capsules. This classification is based in accordance with

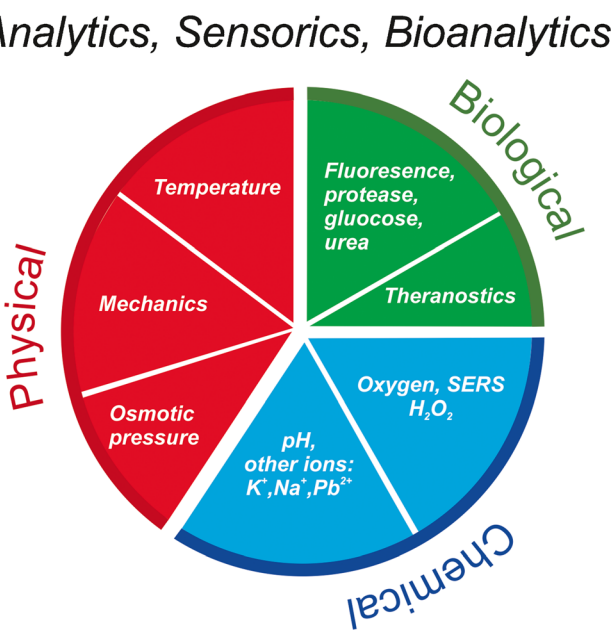


**Fig. 1** Overview of various analytics (pH-, ion-, oxygen-, hydrogen peroxide-, chemical surface-enhanced Raman scattering (SERS)-sensing), sensorics (temperature-, mechanical-, osmotic pressure-sensing), bioanalytics (fluorescence-, glucose-, urea-, and protease-biosensing and theranostics) functionalities implemented using polyelectrolyte multilayer capsules. The colors sub-division is made according to respective stimuli: blue (chemical/analytics), red (physical/sensorics), green (biological/bioanalytics)

various stimuli described earlier and hierarchically structured by Delcea et al. [18] according to their chemical, physical or biological nature. In subsequent discussion, we start with chemical analytics transferring to physical sensorics and bioanalytics.

## Chemical analytics

Biochemical sensors implemented with PEM microcapsules have been discussed in a combination with their biological applications [23]. The mechanism of sensing is based on the entrapment and encapsulation of a reporter (often a fluorescent dye) in the interior of a microcapsule [24]. An analyte (ions or small molecules) penetrate inside a capsule, which holds and protects the reporter, after which a read-out by, for example, a confocal fluorescent microscope is carried out. Analytics is implemented through a reliable ratiometric detection method. For the ratiometric-based readout, either a co-encapsulation of a not sensitive reporter or a careful and thorough calibration is required. Using such methods, PEM microcapsules have been employed to measure ions, pH, and oxygen.

Sensing of different ions is considered as an important development in chemical analytics. In this case, a molecule relevant for ion monitoring is encapsulated inside microcapsules, while ions can freely penetrate inside capsules. Potassium ion sensing has been demonstrated by McShane and co-workers [25, 26], where a ratiometric method was used for polyelectrolyte multilayer capsules (PSS (polystyrene sulfonate)/PAH (polyallylamine hydrochloride)) with encapsulated potassium binding benzofuran isophthalate. A good leaching stability has been observed.

Figure 2 (I) shows UV-vis spectra of PBFI (benzofuran isophthalate tetraammonium salt)-loaded capsules upon changing the concentration of potassium ions from 0 to 150 mM in a solution surrounding the capsules - a blue shift and an increased normalized intensity of the peak between 335 and 360 nm can be clearly seen here. $Na^+$ was among other detected ions; in which case, a sodium sensitive dye, benzofuran isophthalate (SBFI), and a reference fluorophore, methoxycoumarin-3-carboxylic acid (MCA), were encapsulated in PEM capsules comprised of strong polylelectrolytes: PSS (polystyrene sulfonate)/PDADMAC (polydiallyldimethylammonium chloride) [27]. Furthermore, other ions were also detected: for $Pb^{2+}$ [28] detection capsules with CdSe quantum dots were used, and for $Cu^{2+}$, $Ag^+$ [29] detection-quantum dots of CdSe and rare-earth nanocrystals were applied.

Measuring the pH value of a solution, particularly relevant for intracellular monitoring, is another application of chemical analytics contributed to by Parak and co-workers [30], Fig. 2 (II). In this case, a pH-sensitive dye, SNARF, and the ratiometric method, Fig. 2 (II-a, b), have been used. The fluorescent reporter, SNARF, is a rather small molecule, so to hold it in microcapsules, it was connected to a larger dextran to make: SNARF-1-dextran. A peculiarity of SNARF-based detection is that this dye undergoes a spectral shift upon a change of pH. And although a read-out method requires a confocal laser scanning microscope equipped with spectral read-out capabilities, no reference fluorophore is required. The analytics of the ratiometric method is implemented by taking ratios of intensities at red (at ~ 650 nm) to green (at ~ 580 nm) spectral areas (with excitation at 540 nm or 488 nm). As it can be seen from Fig. 2 (II-c, d), microcapsules change their fluorescence upon changing pH values from green to red upon changing the pH value from 6 to 9, respectively. A clear distinction can be made between those capsules located inside and outside of cells, Fig. 2 (II, e, f). In another study, the pH sensitivity has been probed using microcapsules simultaneously containing three dyes, two pH-sensitive dyes: fluorescein, Oregon green and a pH-insensitive dye: Rhodamine B, which served as a solid control for radiometric pH sensing [31]. Furthermore, pH-sensitive microcapsules have been used for monitoring lysosomal pH changes [32] and for probing the interaction of HEK 293T cells with capsules serving as mobile pH sensors [33]. Sensing of pH has been done by incorporating quantum dots in microcapsules [34]. Sensors and biosensors have played a pivotal role in identifying variations in the surface charges caused by protonation and deprotonation of carboxylic groups upon pH changes, which affect cell growth [35]. Subsequent development in the area of pH sensing is multiplexing [36].

Another essential application of microcapsules in chemical analytics is oxygen sensing. On the one hand, oxygen (particularly singlet oxygen) can be generated [37] using gold nanoparticles. On the other hand, detection of oxygen is essential for many chemical and biochemical processes. The working principle of such sensors can be based on co-encapsulation of tris(2,20-bipyridyl) dichlororuthenium(II) hexahydrate (Ru(bpy)) serving the function of a sensor material co-encapsulated with a fluorescence reference (for example, fluorescein isothiocyanate (FITC)) [38]. Interestingly, due to broad absorption bands of both fluorophores (Ru((bpy)) and FITC, a single excitation (for example, with 460 nm) is possible to simultaneously monitor fluorescence at ~ 520–525 nm for FITC and ~ 620 nm for Ru(bpy). It should be noted that other porphyrins, namely Ru(dpp), have been also used for oxygen sensing.

In addition to oxygen, hydrogen peroxide has been also detected. In this case, capsules were filled with dihydrorhodamine 123 (DHR123)—a non-fluorescent molecule, which emits green fluorescence upon oxidation with hydrogen peroxide in the presence of peroxidase [39]. Peptide-labeled LbL coatings lost their red fluorescence after incubation in a trypsin solution, thus enabling protease sensors, which is relevant for active protease sensing in biological probes.

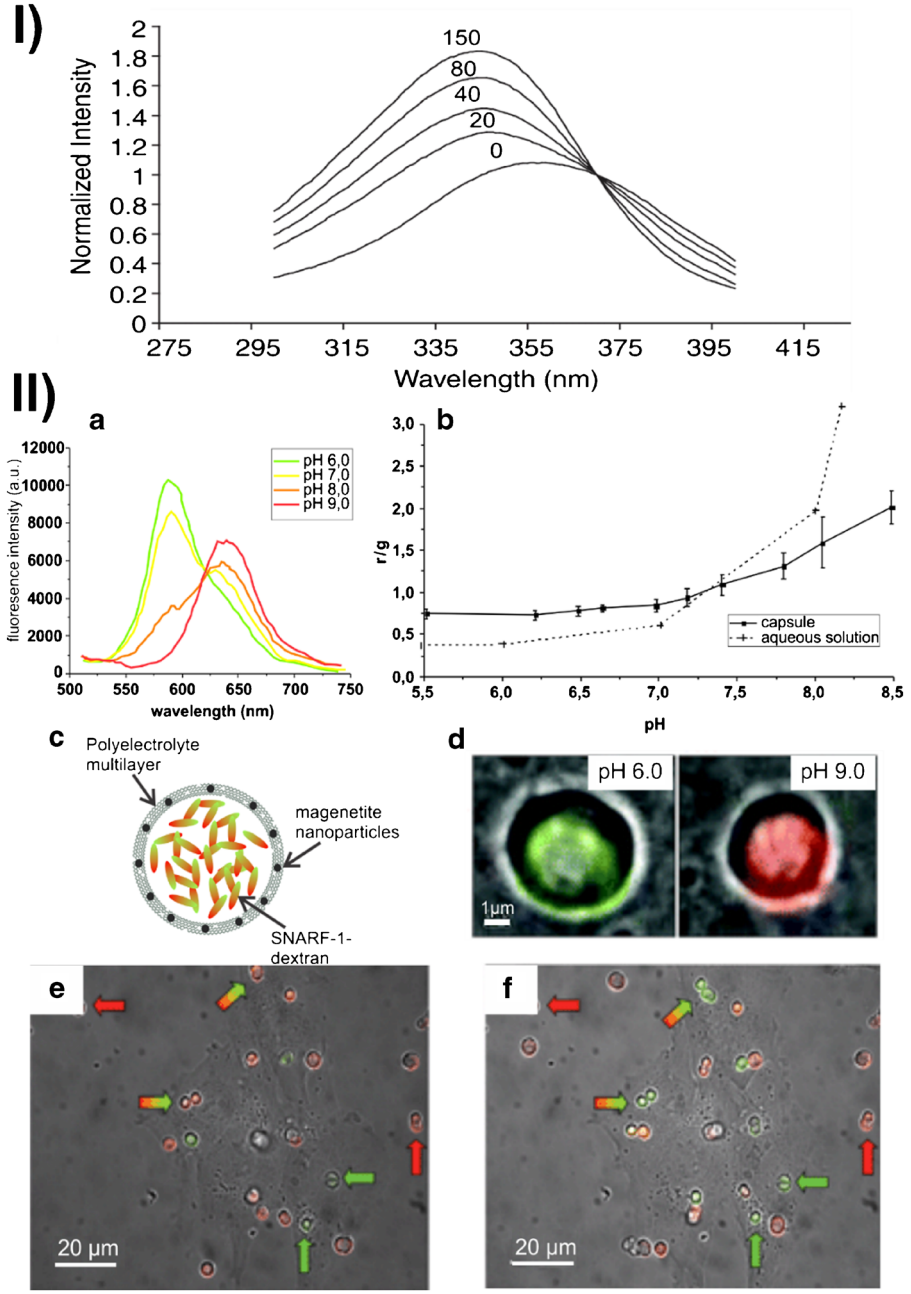


**Fig. 2** I) Excitation spectra of PBFI-loaded nanocapsules (normalized to 370 nm) with increasing concentration of potassium concentration. Concentrations are given in mM units above each spectrum. Reprinted with permission of IEEE from [26]. II) PEM capsules as mobile pH sensors: (**a**) Fluorescence spectra of SNARF-1-dextran (MW = 70 000; 10 mg/ml) in aqueous solution measured at indicated pH values (excitation = 488 nm). Upon alkalinization, SNARF-1 exhibits a shift from green to red fluorescence. (**b**) Ratio of red to green fluorescence (r/g) of SNARF-1-dextran in aqueous solution (dashed curve) and in capsules (straight curve). The latter was derived from spectral analysis of single capsules at different pH values. The slope of the r/g versus pH curve is lower for SNARF-dextran embedded inside the capsules indicating a lower sensitivity of the encapsulated dye. (**c**) Scheme of the capsule geometry. The walls of the capsules are composed of onion-like arranged layers of oppositely charged polymers with embedded magnetic nanoparticles. The capsule interior is filled with SNARF-1-dextran. (**d**) Overlay of phase contrast and fluorescence microscopy images taken at 580 nm and 650 nm (green and red channel) of single capsules in acidic and alkaline pH. The lower panels ((**e**) and (**f**)) depict - SNARF-loaded capsules changing from red to green fluorescence upon internalization by MDA-MB435S breast cancer cells. (**e**) SNARF-fluorescence after adding the capsules to the cell culture and 30 min equilibration. Most of the capsules are outside of the cells and exhibit red fluorescence due to the alkaline pH of the medium. (**f**) The same cells after another 30 min of incubation. Capsules remaining in the cell medium retain their red fluorescence (red arrows). Capsules that were already incorporated in the acidic endosome in the first image retain their green fluorescence (green arrows). Some capsules were incorporated in endosomal/lysosomal compartments inside cells within the period of 30 min, which is indicated by their change in fluorescence from red to green (red to green arrows). Both images comprise an overlay of fluorescence (using a red and a green filter sets) with phase contrast microscopy images. Reprinted modified with permission of the Royal Society of Chemistry from [30]

Analytical functionality of fluorescence sensing has been also demonstrated to determine hydrogen peroxide concentrations inside living cells [40] by BSA (bovine serum albumin)-AuNCs (gold nanoclusters), sensitive to $H_2O_2$, co-encapsulated with insensitive to $H_2O$ and serving as control fluorospheres.

Chemical fingerprint SERS sensing has been applied in different fields. A typical approach in this area involves encapsulation or adsorption of noble metal (gold, silver, etc.) nanoparticles in the interior or on the surface of capsules or particles. An analyte would encounter metal nanoparticles, which enhance label-free scattering signals. A readout is carried out by, for example, a Raman microscope. It should be noted that either detection of analytes through their chemical fingerprinting or a detection of capsules is possible. In one instance, RNA detection using PEM microcapsules with encapsulated gold nanoparticles [41] has been made and is presented in Fig. 3 (I). SERS sensing can be also done either by functionalizing non-porous particles with such highly amplifying materials as carbon nanotubes and gold nanoparticles [43] or by using porous calcium carbonate particles where SERS amplifying nanoparticles are located in the pores [42], Fig. 3 (II). In this area, it is essential to design the assembly of amplifying nanoparticles and to control surface chemistry [44]. Another interesting functionality is the capability of displacement and mobility induced by a magnetic field to position the sensors at a desired location [45]. In this application, the detection of a model molecule, Rhodamine 6G, was demonstrated to be in the range of $10^{-5}$ to $10^{-3}$ M with enhancement factors of up to $10^5$. In addition to magnetic field, another possibility of achieving mobility of SERS sensors can be implemented through optical tweezers [46]. Furthermore, pH and urea sensing have been also realized using SERS [47]: SERS pH tracking has been reported by using SERS-sensitive pH reporter molecule (4-mercaptopyridine functionalized with silver nanoparticles protected by bovine serum albumin (BSA)) to monitor urea concentrations in the range of 0, 0.1 mM, 1 mM, and 10 mM. Peculiarly, BSA has been used to prevent aggregation of microcapsules, which ultimately improves the sensing range and sensitivity. Gold nanoparticle sensing has been carried out for Rhodamine 6G molecule in the presence of BSA protein [48]. Another in vivo application of SERS has been realized inside a worm, *Caenorhabditis elegans*, where laser was used for releasing encapsulated materials [49]. Reaction compartments for bioassays [50]. And special hedgehog particles with LbL coatings have been also developed for SERS sensing [51]. In addition, using Raman label-free sensing, optically controlled redox processes can be followed [52]. It can be noted that PEM microcapsules represent the so-called free-space carriers, which have some advantages but also some disadvantages compared to waveguide-based SERS sensors or planar affinity biosensor [53]. In addition, label-free Raman monitoring allows a label-free detection of various chemical including carbon-like structures upon laser destruction of microcapsules [54].

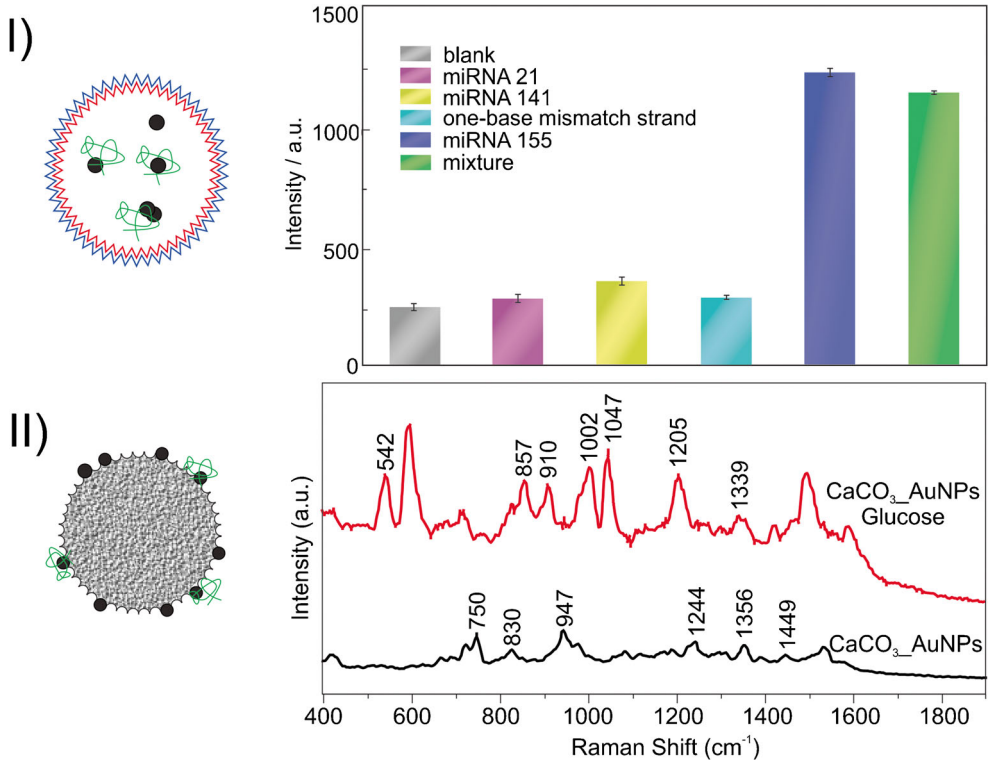


**Fig. 3** I) The concept of SERS sensing in a microcapsule (left); and (right): SERS biosensor with different solutions (the concentration of miRNA 155 is 1 pM, each interference is 100 pM, the mixture is 1 pM of miRNA 21 and 100 pM of three interferences). Reproduced with permission of the American Chemical Society from [41]. II) The concept of SERS sensing of molecules on the surface of particles (left); and (right): the SERS amplification of D-glucose molecules. Reproduced with permission of Wiley-VCH from [42]

## Physical sensorics

Physical stimuli enabling sensorics include temperature measurements, probing mechanical strength of capsules as well as the forces exerted by cells, and osmotic pressure. Unlike the uniformity of approaches in the area of chemical analytics, the approaches and stimuli in this area vary substantially.

Temperature measurement is of importance to various processes, because temperature affects not only polymers and biomolecules but also the stability of nanoparticles, carriers, and capsules [55]. Temperature release has been shown for microcapsules functionalized with metal (inorganic [56]) nanoparticles, which compose and can be referred to as hybrid systems [57]. Temperature has been shown to transform the shape of poly-(acrylic acid)/poly-(allylamine hydrochloride) LbL-based carriers in the form of microtubes [58]. Temperature has also been shown to act as the mechanism for a controlled release from polyelectrolyte multilayer capsules both on the individual capsule level [59] and in a solution [60, 61]. In this area, it is important to understand the mechanisms and influence of the localized [62] versus global temperature rise. Indeed, global temperature rise is used to shrink microcapsules leading to encapsulation, while local temperature rise is used to release encapsulated materials from polyelectrolyte multilayer capsules [63]. These two opposite effects are achieved using the same phenomenon—a temperature rise, as this has been recently explained [63]. The molecular mechanisms of the interaction of molecules lead to polymeric capsule shrinking [64]. On the other hand, upon a localized heating, the overall temperature in a solution and the temperature around the polymeric shell remain unchanged, while the localized temperature, raised by heating of nanoparticles, induces the permeability changes of a polymeric shell near nanoparticles. The temperature rise around nanoparticles has been measured employing a fluorescent microscope and a temperature sensitive dye, 2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF) [65], as it is shown in Fig. 4. Initially, the capsule appears in a fluorescence microscope (Fig. 4 (a) in a black-and-white mode). Upon illuminating this capsule with a laser beam, the fluorescence around the capsule appears to be darker, Fig. 4(b), which corresponds to a temperature rise. Quantification of temperature rise is presented in Fig. 4(c). It should be noticed that this temperature rise depends on the applied laser power, the density of nanoparticles, which can be controlled either by a direct synthesis or adsorption of pre-synthesized nanoparticles [66] and the aggregation state of nanoparticles. At a relatively low density of nanoparticles with near-infrared absorption coefficient, the global temperature rise was reported to reach several degrees (4 K degree rise in [65]). At high aggregation and the filling factor (the density) of nanoparticles, the localized temperature on nanoparticles will be higher [67], as it can be also assessed from simulation [67]. It should be noted that, on one hand,

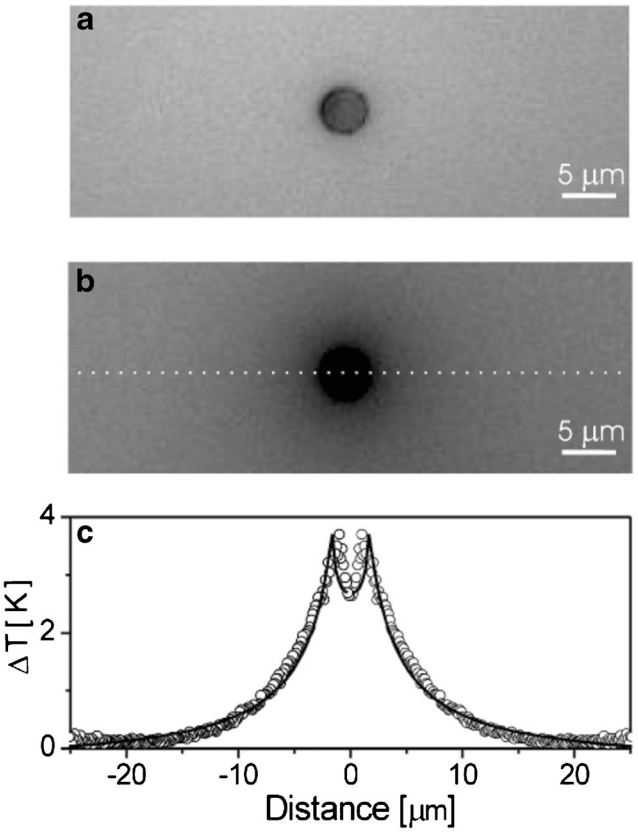


**Fig. 4** (a) Typical image of a hollow capsule containing gold sulfide core/gold shell nanoparticles in its walls is shown immersed in a fluorescent dye solution without laser light. (b) When the laser is switched on, the temperature around the capsule increases and fluorescence of the dye solution around the capsule becomes darker. (c) Experimentally measured data for temperature change ΔT (hollow dots), extracted along the dotted line in (b), are shown together with simulated (solid line) temperature distribution induced by 980 nm laser diode operated at 25 mW. Zero coordinate is located at the center of the capsule. Reproduced with permission of the American Chemical Society from [65]

measurement of temperature rise is essential for assuring the non-destructive character of release upon intracellular delivery, while on the other hand, it is essential to assure that sufficient temperature rise is achieved for photothermal therapy or for killing cells [68]. It should be noticed that, in addition to thermally responsive microcapsules due to metal nanoparticles, organic molecules can be also used to disrupt LbL multilayers upon laser illumination [59, 69, 70]. In addition to temperature measurements and motivated by poor performance of microcapsules upon uptake by cells, mechanical properties of capsules have been extensively investigated.

Mechanical properties of capsules have been measured using atomic force microscopy (AFM) employing the so-called colloidal probe AFM technique [71, 72], where a large bead presses on a polymeric capsule to measure Young's modulus, stiffness, and forces upon deformation of capsules. Mechanical properties play an important role in assuring intracellular delivery of biomolecules, overall their functionality

as well as sensorics [73]. Interesting results in investigating mechanics of microcapsules were obtained by coupling a fluorescent microscope (from the bottom) with an AFM (from the top) [74]; in such an approach, one can assess not only the mechanical properties of capsules by following mechanics with AFM but also and simultaneously the release of encapsulated materials by following the fluorescent signal. It was found in that study [31] that polymeric capsules comprised of 8 layers of alternatively deposited poly(diallyl dimethylammonium chloride) and poly(styrene sulfonate) can withstand ~ 18% of relative deformation before they start to release encapsulated molecules and the same capsule would undergo plastic deformation (irreversible shape change) upon deformation of over 21%, Fig. 5 (I). Mechanical properties of similar capsules can be controlled by fabricating capsules with different wall thicknesses, Fig. 5 (II), which can be achieved through a thermal (global temperature rise) shrinking. Subsequently, capsules were used as sensors for measuring the pressure exerted by cells upon uptake, Fig. 5 (III), where it was found that cells exert approximately 0.2 μN of force, Fig. 5 (III), upon uptake of capsules [75]. From that study, it was incurred which forces cells exert upon uptake and which mechanical stiffness microcapsules should have to assure a successful intracellular delivery. In other applications, mechano-responsive microcapsules have been also shown to undergo a self-propelled motion [76]. Successful circulation of capsules can be achieved, for example, by designing their mechanical properties to be similar to those of red blood cells (RBC). A discussion about soft (RBC-like) cells or carriers versus hard capsules—both capable of circulating and delivering encapsulated molecules—has been performed comparing red blood cells and polyelectrolyte multilayer capsules [77]. Interestingly, the elasticity of carriers has been reported to affect the uptake of carriers by cells [78]. Peculiarly, mechanical properties of polymeric capsules and planar LbL layers can be enhanced with nanoparticles [79]. A similar enhancement of mechanical properties of soft films has been observed [15], where the embedding depth of particles, from which microcapsules are made, serve as a sensor. The layer-by-layer approach has been suggested and discussed for sensor applications [80], among which are electrochemical sensors very relevant for coatings [81] and osmotic pressure sensors.

Osmotic pressure, which can be also used for sensing applications, has a close relationship with mechanical sensors. In this area, the similarity of approaches has been identified between capsules and polymeric films in regard to sensing osmotic pressure [82] and also binary mixed polymer brushes [83]. Mechanisms of osmotic buckling have been recently developed and discussed [84]. Osmotic pressure sensing has been shown for giant capsules with encapsulated fluorescent probes (green fluorescent marker beads of 500 nm co-encapsulated with dextran-hydroxy-ethyl methacrylate (dex-HEMA)) [85], Fig. 6 (I). In this approach, capsules containing various concentrations of encapsulated materials (L-dex-HEMA (low concentration) and H-dex-HEMA (high concentration)) and producing different osmotic pressure have been subjected to an external stimulus (laser light acting on nanoparticles incorporated in the shell of giant capsules) to release those encapsulated materials. An important feature of such an experiment is a possibility to trace the released encapsulated materials, for example, by a fluorescence microscope. A higher concentration of encapsulated marker beads (corresponding to higher osmotic pressure inside capsules) should result in longer distances traveled by released fluorescence markers. This is exactly what is observed in Fig. 6 (I and II), where the higher the osmotic pressure (P) inside microcapsules resulted in larger distances (L) of released fluorescence marker beads. Induced through a mechanical disruption of polyelectrolyte multilayers, the described above method of release resembles a direction-specific release from capsules carried out remotely by a laser beam [86]. Other experiments with osmotic pressure were carried out for hydrogen-bonded PVPON/PMAA (poly(N-vinylpyrrolidone/poly(methacrylic acid)) capsules, as they have recovered from an osmotic shock generated by a solution of 18 wt.% of PSS (polystyrene sulfonate) polymers [87]. It should be noted that osmotic pressure has played an essential role in the fusion of capsules generated by a high concentration salt solution [88] as an alternative to pH-based [89] or laser-based [90] methods for capsule fusion. In many of the above areas, fluorescence sensing is used as a detection mechanism which, due to its broad applicability in biology, relates to biochemical sensors and bioanalytics.

## Biosensorics and bioanalytics

Early work on fluorescent sensing using polyelectrolyte multilayer capsules has employed sodium dye and a reference fluorophore [27], and luminescent components entrapped inside capsules [91]. Biosensing and biological stimuli discussed and proposed here possess elements of both chemical and physical sensing.

For example, fluorescence sensing is essential to identifying various sub-compartments of cells [92]. Also, fluorescence sensing has been used for identifying bacterial endotoxin upon exposure of liquid crystal–based droplets encapsulated in a LbL shell [93], which is also capable of detecting bacteria and viruses [94].

For biosensor applications, it is essential to develop biomimetic capsules as it was recently discussed by Li and co-workers [95]. Further biosensor applications have been realized for probing capsule internalization by cells [96]. Other realized sensing functionalities include a Janus micromotor–based luminescence sensor for active TNT (trinitrotoluene) detection [97], carbohydrate sensing where PMAA-co-AAPBA (Poly(methacrylic acid)-co-3-(Acrylamido)phenylboronic Acid) block co-polymer

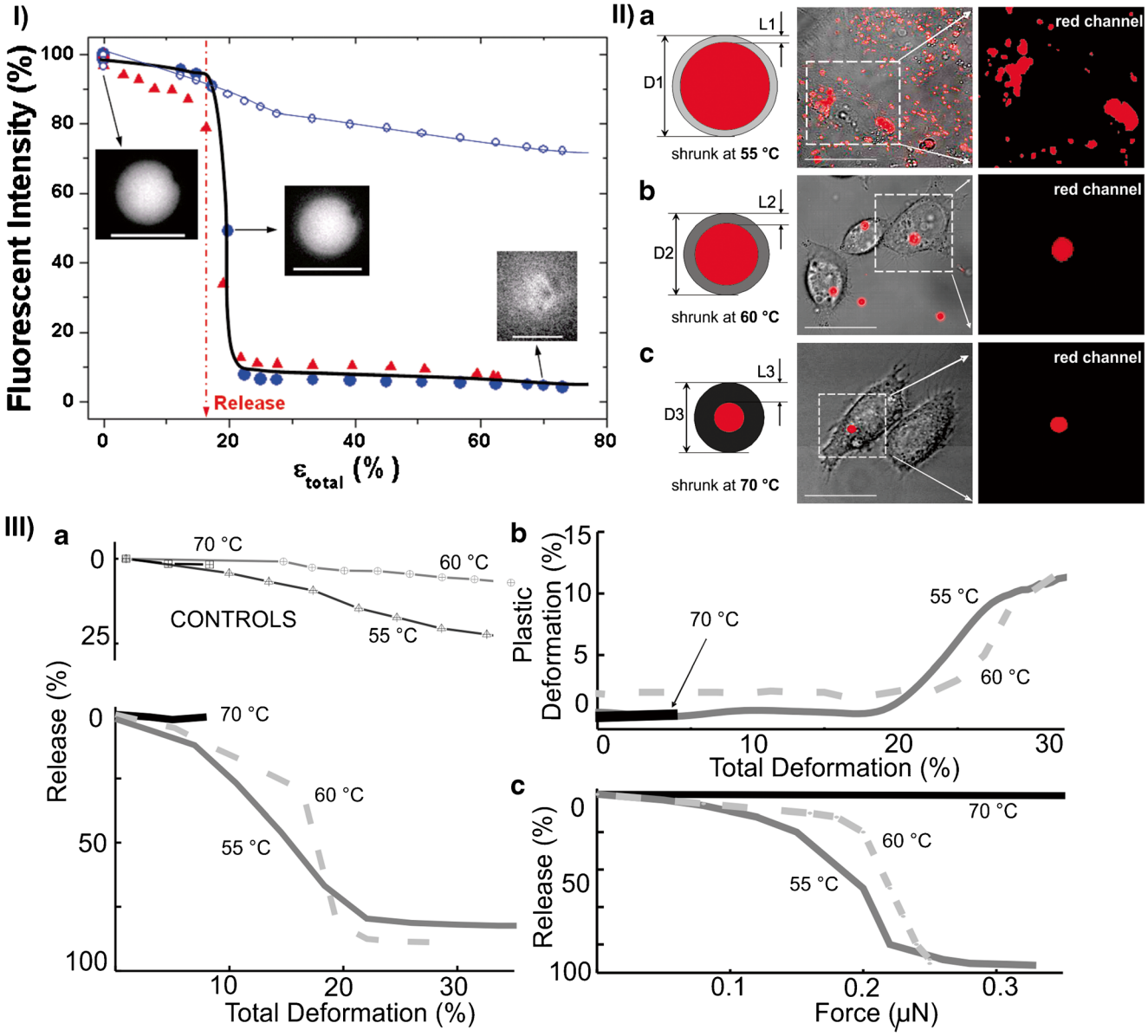


**Fig. 5** I) Investigation of mechanical properties of polyelectrolyte multilayer capsules using a colloidal probe AFM coupled with a fluorescence microscope: average fluorescence intensity from a typical microcapsule subjected to mechanical deformation (filled circles) and a control microcapsule not subjected to mechanical deformation (open circles) calculated from images (some are shown) taken after each push–pull cycle as a function of total capsule deformation. The scale bar in the images corresponds to 5 micrometers. As an example, reproducibility is evidenced by similar results on another capsule (triangles). The full lines are just guides for the eye. The dashed-dotted line indicates the threshold total deformation (~18%) beyond which release is triggered. Reproduced with permission of the Royal Society of Chemistry from [74]. II) Mechano-biology with polyelectrolyte multilayer capsules: microscopy images (differential interference contrast and red fluorescence channels are superimposed, in the middle) of live Vero (African green monkey kidney) cells after intracellular incorporation of filled polymeric microcapsules shrunk at a) 55 °C, b) 60 °C, and c) 70 °C. Left-hand side images show schematics of microcapsules, while the right-hand side images show enlarged sections (the red fluorescence channel only) of the corresponding images presented in the middle column. The scale bars correspond to 15 μm. Reproduced modified with permission of Wiley-VCH from [75]. III) a) Release (measured by the average fluorescence intensity) for capsules shrunk at 55 °C (gray line), 60 °C (dashed light gray line), and 70 °C (black line) from images taken after each AFM push–pull cycle as a function of capsule's applied deformation by the colloidal probe of AFM. A control on top (in the panel marked CONTROLS) depicts release-versus-total-deformation curves for microcapsules not pressed on but simultaneously present in the corresponding images; b) plastic deformation as a function of total deformations applied during each push–pull cycle for 55 °C (gray line), 60 °C (dashed light gray line), and 70 °C (black line) shrunk capsules; c) release–force curves for 55 °C (gray line), 60 °C (dashed light gray line), and 70 °C (black line) shrunk capsules. Reproduced with permission of Wiley-VCH from [75]

has shown to interact and distinguish such monosaccharides as fructose, glucose, galactose, ribose, and disaccharides such as lactose, maltose, sucrose, and trehalose [98]. Microarray chambers [99] and patterning [100] are viewed as another significant

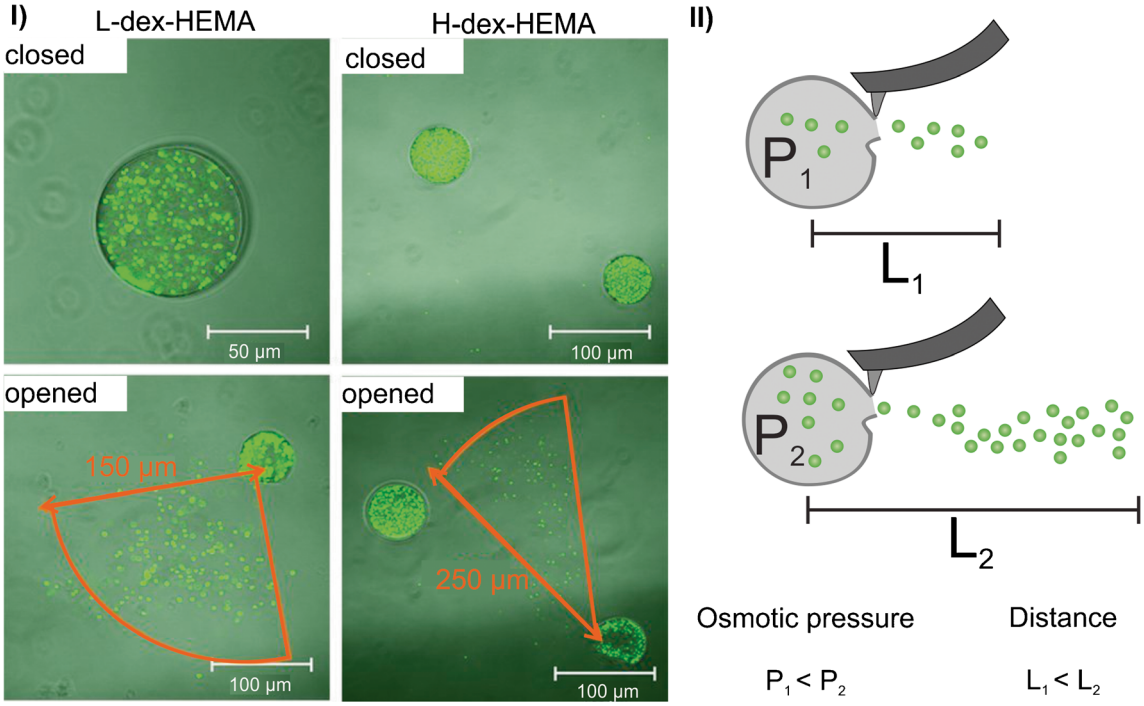


**Fig. 6** I) Giant hydrogel-based microcapsules with the shell comprised of polyelectrolyte multilayers are used for assessing the osmotic pressure in their interior upon laser induced opening of the shell: left-hand side images correspond to a low concentration of encapsulated dextran (L-dex-HEMA), while images on the right-hand side correspond to a higher concentration of encapsulated dextran (H-dex-HEMA). Opening the shell of pressurized microcapsules (lower panel) results in rapid release and large spread distances of the encapsulated marker beads. The area enclosed by the arc marker contains ≈ 90% of the released marker beads. As indicated, the distance (L) is larger for H-dex-HEMA in comparison to that for L-dex-HEMA capsules. Please note a different magnification in the upper left figure: the H-dex-HEMA and L-dex-HEMA capsules are actually of similar size. Reproduced with permission of Wiley-VCH from [85]. II) Schematics of experiments depicted in (I), where the osmotic pressure (P) is related to the distance of (L) traveled by the released encapsulated dextran

development in the area of microcapsules for a higher throughput readout. In addition, assembling microcapsules using microfluidics [101, 102], filtration [103], and capillary assembly of PEM multilayers [104, 105] has been proposed and it is seen to enhance the capability of capsule fabrication. Catalytic reactions were also monitored using highly sensitive membrane-based sensors [106]. Other sensor functionalities were achieved by phenylboronic acid functionalization of capsules [107]. Functionalization of microcapsules by quantum dots has been shown to facilitate the detection of microcapsules with targeting to cells [108]. Another example of an effective calcium carbonate–templated capsule is based on alginate coating with silver nanoparticles, where a very effective SERS capsule is presented and applied for intracellular sensing [109]. Optical sensor functions have been performed with polymer brushes [110] functionalized similarly to those used for microcapsules. It should be also noted that biosensorics involves not only soluble molecules but also gases. In this regard, silica-templated hemoproteins assembled through electrostatic interaction were shown capable of sensing sulfide gas [111]. Molecular detection has been developed using sensors based on surface-enhanced Raman scattering (SERS) amplification effect [112].

In this area, a number of different glucose sensors [113] based on glucose oxidase and an oxygen-quenched fluorophore has been developed [114]. In addition, glucose-sensitive capsules based on hemoglobin and glucose oxidase [115] have also been proposed and successfully tested. Glucose (and lactate)-sensitive microcapsules based on fluorescence signals have been also developed, where Ru(dpp) (tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) dichloride was proposed as a mechanism for fluorescence-based sensing methods [116]. Glucose sensing is also relevant for in vivo monitoring of the level of glucose [117].

Furthermore, protease sensors have been further demonstrated [118]. In this application area, a rather different approach has been taken: LbL coatings of PSS/PAH polymer pair has been applied to microcapsules containing a peptide trypsin substrate–encoded green fluorescent polystyrene memobeads (with a size of ~ 40 μm). Red fluorescently labeled trypsin substrate (peptide) was also incorporated into the LbL multilayers. Upon subsequent incubation in a trypsin solution, the memobead was observed to lose the red fluorescence. Furthermore, magnetic nanoparticles have been also incorporated into the shell of capsules to enhance handling capabilities.

Urea-sensing highlights various ways that detection of the same substance can be made. On the one hand, chemical sensing of urea has been already described by monitoring through

SERS [47]. But biochemical sensing employing enzyme is also possible: in this case, co-encapsulation of an enzyme, urease, together with a pH-sensitive dye SNARF-1 coupled to dextran is carried out [119]. In this case, urea was monitored in the concentration range from $10^{-6}$ to $10^{-1}$ M by following the fluorescence in the 600–680-nm spectral range upon a confocal laser scanning microscope with the excitation wavelength of 543 nm (Fig. 7 (I)). The principle of operation is based on a pH shift (to more alkaline values) upon degradation of urea ($CO(NH_2)_2$), and this pH shift is monitored by the co-encapsulated SNARF-1 dye; the coupling to dextran is used in such cases to assure that this molecule stays inside capsules (Fig. 7 (II)). It should be noticed that, although the enzyme is co-encapsulated with SNARF-1-dextran inside capsules, the sensor dye (SNARF-1) is shown in Fig. 7 (II) as if it is located in the shell of capsules. This representation depicted in Fig. 7 (II) corresponds to fluorescence microscopy images shown in Fig. 7 (I), where fluorescence is seen mostly around the polyelectrolyte multilayer shell. This can be assigned, at least in part, to the presence of uncompensated charges in the polyelectrolyte multilayer shell, and that would be consistent with a slight shift of pKa of SNARF-1-dextran (from 7.58 for these molecules dissolved in water) to 7.15–7.25 (for these molecules encapsulated into different polyelectrolyte multilayer capsules, as attributed to the influence of PAH) [118].

Biosensing is also essential for theranostics—an emerging area in medicine and biology. In the area of theranostics, the diagnostics or sensorics is coupled to therapy by integrating these functionalities in an approach, devices or capsules. Some of the best suited carriers for theragnostic applications are those based on multicompartment capsules [120]. Figure 8 (the left panel) presents several configurations of the so-called multicompartment polyelectrolyte multilayer capsules. Concentric, pericentric, innercentric, and acentric polyelectrolyte multilayer capsules have been already demonstrated. Concentric capsules are constructed by double-synthesis of, for example, calcium carbonate particles followed by a separation step of concentric capsules. Pericentric capsules are built by adsorbing smaller particles or capsules onto a larger capsule. Innercentric capsules can be realized by co-encapsulating smaller capsules or particles into an inner part of the capsules. In this area, several methods for developing

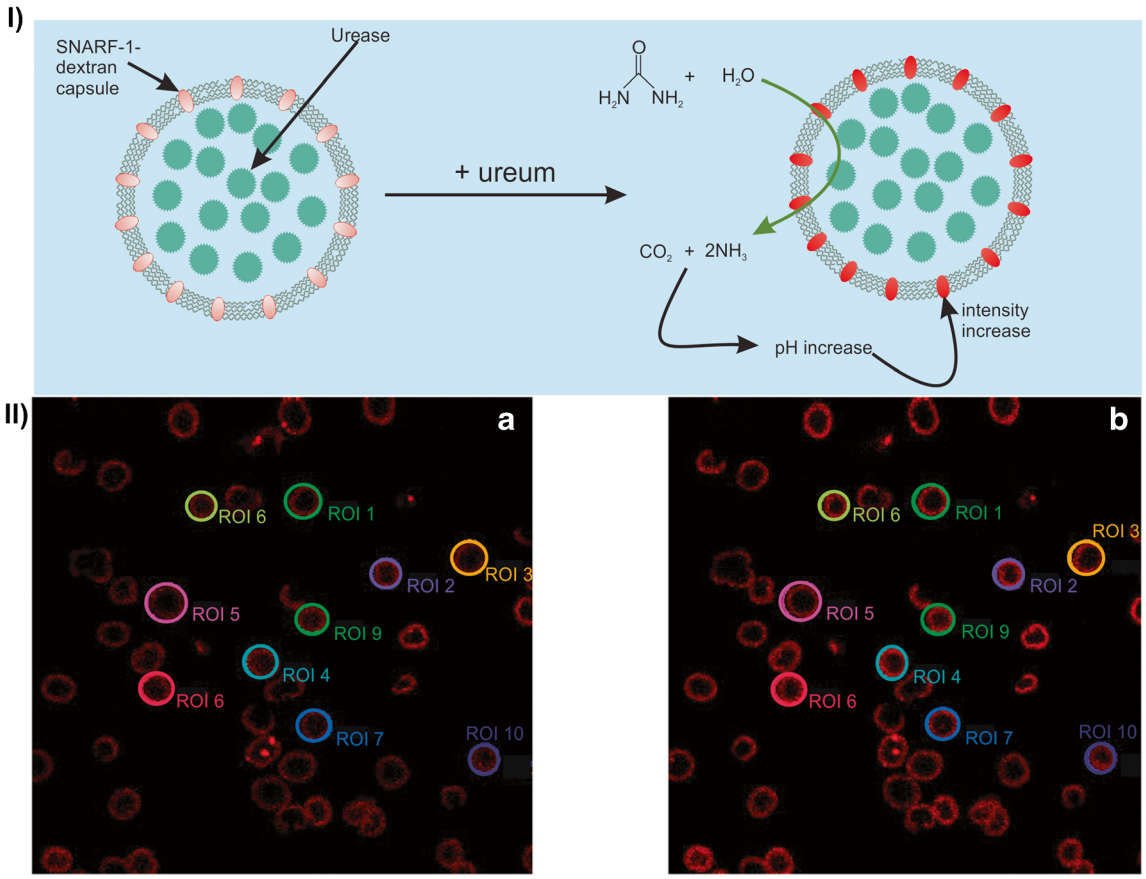


**Fig. 7** I) Confocal fluorescence microscopy images of (PSS/PAH)4PSS capsules loaded with SNARF-1 dextran (MW = 70 kDa) and urease enzyme in water (image A) and 0.1 M urea concentrations (image B). The red fluorescence emission was accumulated at 600–680 nm after excitation by the FITC-TRITC-TRANS laser at 543 nm. Reproduced with permission of the Royal Society of Chemistry from [119]. II) Schematics of urea sensing with polyelectrolyte multilayer capsules, where the substrate is shown to penetrate inside microcapsules encapsulating the enzyme

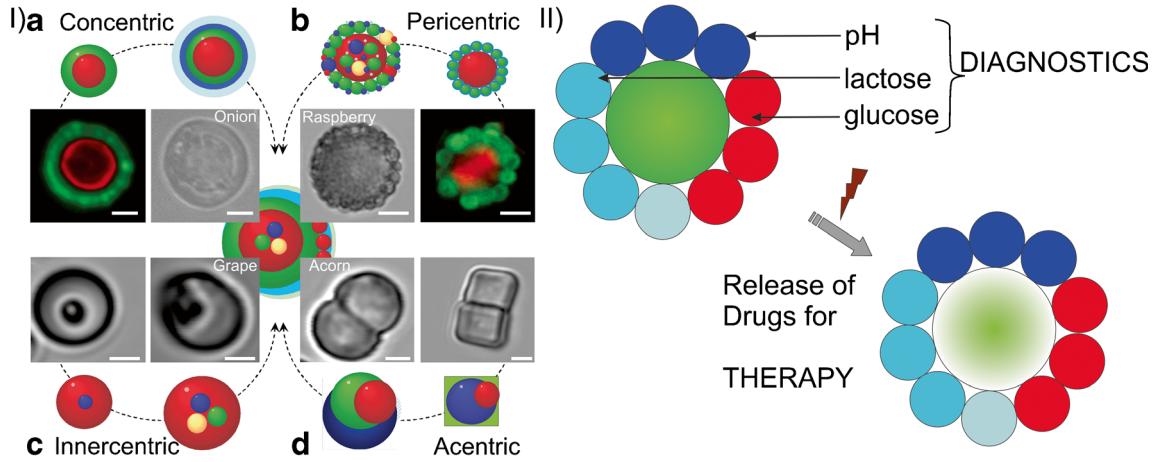


**Fig. 8** I) Multicompartment polyelectrolyte multilayer capsules are depicted in various configurations: concentric, pericentric, innercentric, and acentric. Reproduced with permission of Wiley from [120]. II) An example of the application of microcapsules in theragnostic areas is shown for multicompartment PEM capsules, where some sub-compartments are used for diagnostics, while others are responsible for the release of drugs. Reproduced with permission of Theranostics from [121]

asymmetric capsules, where the original synthesis of asymmetric templates would directly lead to such asymmetric capsules. Capsules of different shapes can be either made by designing different templates, by deforming existing templates [122] or by assembling on anisotropic calcium carbonate particles [123]. Multicompartment particles and capsules have been also used for coupled enzyme-catalyzed reactions [124]. Multicompartment capsules and particles are ideal candidates for an emerging area of theranostics [121], where some compartments are responsible for detection and sensing, while other sub-compartments are responsible for therapy, Fig. 8 (the right panel). The latter can be achieved by a controlled release of encapsulated materials after the feedback obtained from the sensing sub-compartments. Another biosensor related capability can be linked to LbL coated particles, demonstrated on an example of latex particles, and containing gold nanoparticles. Such a platform has been shown to perform as an immunoassay [125].

It can be noted that the polyelectrolyte multilayer assembly method, used for producing capsules, has been also applied on flat surfaces, where interesting sensors and analytical tools have been proposed. In this regard, various oxygen and other ion detection [38] as well as humidity sensors [126] have been developed based on LbL thin films, while electrochemical sensors have been implemented by incorporating gold nanoparticles [127].

## Conclusion and outlook

We have presented an overview of analytical, sensor and biosensor functionalities developed using polyelectrolyte multilayer capsules and classified them according to respective stimuli and application areas. Microcapsules provide means of both protecting encapsulated sensor molecules and selective permeability of analytes. In the above-mentioned classification, we distinguish chemical, physical, and biological analytical and sensoric functionalities. In chemical analytics, pH sensing as well as ion sensing such as potassium and sodium was developed. In addition, oxygen- and hydrogen peroxide-sensing and surface-enhanced Raman scattering (SERS) chemical analytics was implemented. Further chemical analytics can be applied to detect various ions, including $Zn^{2+}$ [128], $Ca^{2+}$ [129], etc. In physical sensorics, microcapsules have been used for intracellular delivery of encapsulated biomolecules, where the localized temperature rise was used as a trigger mechanism. Therefore, it is of paramount importance for intracellular delivery to measure temperature rise on and around of microcapsules. Although the localized temperature rise could reach several tens of degrees, the overall, global, temperature rise was measured to be only a few degrees. Further, for intracellular delivery, mechanical stiffness and overall mechanical stability determine successful delivery. Employing AFM (atomic force microscopy), as a control tool, the capsules were found to be elastically deformed with deformations ranging up to 18%, after which a release of encapsulated materials takes place. It was found from AFM measurements that deformations above 21% lead to plastic (destructive) deformation. Peculiarly, microcapsules themselves were used as bio-mechanical sensors, revealing that cells exert ~ 0.2 μN forces upon uptake. Sensing the osmotic pressure linked with the strength of release is another functionality. Biosensing with polyelectrolyte multilayer capsules was shown to detect fluorescence, glucose, urea, enzymes, and protease, which is particularly interesting for sensing inside cells. Theranostics has also been identified as an emerging area of bioanalytics. Developed for flat substrates and curved micro- and nano-particles, polyelectrolyte

multilayer assembly deposited through the layer-by-layer technology and their analytical and sensorics applications have a bright future, which is largely driven by an ever-increasing range of potential and real applications. We would like to pay a tribute to one person, Helmuth Möhwald, who has seen this potential and has profoundly influenced this area [130].

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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