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Hybrids of Polymeric Capsules, Lipids, and Nanoparticles: Thermodynamics and Temperature Rise at the Nanoscale and Emerging Applications

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Abstract. The importance of thermodynamics does not need to be emphasized. Indeed, elevated temperature processes govern not only industrial scale production, but also self-assembly, chemical reaction, interaction between molecules, *etc.* Not surprisingly, biological processes take typically place at defined temperature. Here, we look at possibilities to raise the localized temperature by a laser around noble metal nanoparticles incorporated into shells of layer-by-layer (LbL) polyelectrolyte microcapsules – freely suspended delivery vehicles in aqueous solution, developed in the Department of Interfaces, Max-Planck Institute of Colloids and Interfaces headed by Helmuth Möhwald. Understanding the mechanisms around localized temperature rise is essential, that is why we analyze thermodynamics at the nanoscale, the influence of incident intensity, nanoparticle size, their distribution and aggregation state. This leads us to scrutinize "global" (used for thermal encapsulation) versus "local" (used for release of encapsulated materials) temperature around nanoparticles. Similar analysis is extended to the lipid membrane system of vesicles and cells, on which nanoparticles are adsorbed. Insights are provided into the mechanisms of physico-chemical and biological effects, the nature of which has always been profoundly, interactively, and engagingly discussed in the Department. This analysis is combined with recent developments providing outlook and highlighting a broad range of emerging applications.

Introduction. The importance of Thermodynamics is difficult to overestimate[1]. With contributions and understanding of thermal heat exchange processes provided and inspired by Boltzman, Carnot, Boyle, Kelvin, Clausius, Thompson, Joule, Gibbs, and others essential developments have been made. One of the most tangible "products" of these developments is a refrigerator – nowadays used in many house-holds. In the early days, it was important to bring understanding of micro- processes to a macro- level, and gas was one of the most important subjects of research, not least because of the significance of the industrial revolution and the heat engine. The goals reflected in research subjects were big and grandiose then. Several hundred years fast forward and we are "back to future" looking at nano-scale processes, and significance of interconnected processes in biology brings water (and not gas) and molecules dissolved in water/solvents at the forefront of research subjects.

Here, we look into nanoscale thermal processes associated with temperature rise associated with nanoparticle immobilized in the shell of polyelectrolyte multilayer capsules which are freely suspended in an aqueous solution. First, we introduce polyelectrolyte multilayer capsules highlighting further the nature of absorption of nanoparticles and heat generation. We consider also spectral responsiveness, determined by the aggregation state of nanoparticles due to dipole-dipole interaction of neighboring nanoparticles. Subsequently, we analyze thermodynamics and heat propagation around nanoparticles. Thermodynamics processes of this system are discussed in light of nanoscale outlining phenomena and elaborating such fundamental processes associated with nanoscale heat conduction as encapsulation from and release into microcapsules. Nanoscale processes associated with temperature rise are analyzed in comparison to micro- and macroscale processes realizing that, on one hand, a temperature increase leads to encapsulation of molecules (thermal based encapsulation method, while on the other hand and in sharp contrast, while an increase temperature to similar values (albeit locally) induces release of the molecules from the same capsules? Then, we transfer to such captivating applications as localized permeability change of a hybrid system composed of polymers, lipids (an organic phase) and nanoparticles (an inorganic phase). We conclude by looking at a rich range of applications of microcapsules, many of which are associated with nanoscale heating, but also looking beyond.

A brief overview of polyelectrolyte multilayer capsules. Polyelectrolyte multilayer (PEM) capsules were invented just over twenty years ago in the Department of Möhwald, where scientific problems and questions, as for example self-assembly at the nanoscale,[2] have been taken under the "curiosity lens". This approach has been rewarding both in understanding fundamental science[3, 4] and in developing numerous and important applications. The invention itself was an insightful way of transferring the layer-by-layer (LbL)[5] coatings produced by depositing alternatively charged polyelectrolyte polymers – from flat substrates to spherical particles. The semi-permeable nature of LbL coatings enables dissolution of the particles, denoted as templates, leaving the polymeric coating intact.

An essential advantage of polyelectrolyte multilayer capsule is flexibility of their design and availability of various stimuli to control the interaction of polymers, encapsulation of molecules, release, mechanical properties.[6] At the beginning, right after the invention of PEM microcapsules, major efforts have been put to understand basic properties of the capsules. Such chemical stimuli as pH, ionic strength and physical stimuli such as temperature have been applied to control the permeability of the capsules, primarily for encapsulation. The flexibility of the polyelectrolyte shell upon modification by nanoparticles has led to a possibility of implementing remote release induced by laser-nanoparticle interaction. Other physical stimuli such as magnetic fields[7], ultrasound, mechanical action have been subsequently used to induce release of encapsulated molecules.

With encapsulation and release functionalities, numerous applications using polyelectrolyte multilayer capsules have been developed, including intracellular delivery and release[8], enhancement of mechanical properties of microcapsules,[9] using microcapsules as sensors for intracellular pressure upon uptake,[10] protection of enzymes[11] and monitoring their activity,[12] a combination with polymeric coatings, investigation of the strength of the interaction of the polyelectrolyte multilayer shells with cells[13]. In parallel, development of multicompartment and anisotropic microcapsules has followed[14].
Templates or particles, on which microcapsules are made, i.e. on which the layers are adsorbed, can make preparation expensive. Indeed, a significant bottle-neck for design of polyelectrolyte multilayer capsules are the templates or particles, on which polyelectrolytes are assembled. Here, a potential toxicity to cells and the production price are some of the most important characteristics. It is not surprising, therefore, that among various available templates[15] essential efforts have been put into developing biocompatible and bio-friendly cores, among which calcium carbonate is an excellent example[16].

Here we look into three main parts:

(a) analysis of the localized temperature rise and thermodynamics at the nanoscale;
(b) insights into the state of behavior of polymers and lipids upon localized heating;
(c) outlook and future directions associated with nanoscale thermodynamics and beyond.

Localized temperature rise around metal nanoparticles. Peculiarly, the bulk properties of metals are different from those in the form of nanostructures. This is because the mean free path of electrons determines absorption of nanoparticles and this mean free path is located in the nanometer size range[17]. For the conditions of experiments, optical properties of nanoparticles have been calculated[18] and were reported to be important for biomedical appliations[19]. Among various nanoplasmonic systems,[20] gold and silver nanoparticles are used most frequently, and the latter are used in our analysis due to their inertness.

In this report, we first overview applicability of laser-nanoparticle interaction to microcapsules and specifically for a controlled release from microcapsules. But we start with analysis of localized temperature rise, which was investigated theoretically and measured experimentally[21]. At the level of a single nanoparticle, an exact analytical solution for temperature rise under equilibrium conditions exists[21]:

$$T(r) = \frac{A\, r_0^3}{3\, K\, r} \qquad (1)$$

where A [W m^{-3}] is the heating per unit volume, K [W m^{-1} K^{-1}] is the thermal conductivity, r_0 [m] is the radius of the nanoparticle heated by a laser, and r [m] is the radial distance away from the nanoparticle, $r > r_0$. It can be seen from equation (1) that the temperature rise is proportional to the volume of a nanoparticle and the heating rate and is reciprocal to thermal conductivity. The heating rate is proportional to the applied laser power, or the power density of laser intensity and absorption coefficient, which depends on two factors: the size of nanoparticles (the surface plasmon of nanoparticles shifts to the red part of spectrum with increasing sizes of nanoparticles), and proximity of nanoparticles to each other, i.e. the formation of aggregates. These aggregates determine absorption of light, which was the subject of earlier research on microcapsules[22] and micelles[23]. Specifically for the considered system, optical properties of colloidal particles[24] have been calculated, where aggregates have been identified as important constituents for near-infrared (near-IR) absorption.[25] Related to heating under biological conditions also other parameters need to be taken into account, such as the presence of salts and proteins[26].

The aggregate formation can be controlled under specialized nanoparticle adsorption conditions upon adsorption, by adding polymers serving the function of “wedges” between the nanoparticles. Such a concept can be used, for example, for DNA sensing[27]. Alternatively, it can be performed by direct adsorption either at high[28] or low[29] concentrations of nanoparticles relative to the surface of microcapsules. On the surface, the average distance between the nanoparticles $< d >$ is reciprocally proportional to the concentration of nanoparticles, which can be also expressed through their surface filling factor F_S[21]:

$$< d >= \frac{2\, r_0}{\sqrt{F_S}} \quad (2)$$

The surface filling factor F_S is the normalized concentration of nanoparticles. It would be interesting to look into thermodynamics at the macro- and nano- scales.

Thermodynamics at different scales. Nanotechnology opened extensive opportunities and a diversified range of new applications, many of which are associated with thermodynamics. Taking into consideration the sizes of the system nano-thermodynamics is central to the system. Some consideration here were set by Hill, which extended Gibbs energy to include the chemical potential of the nano-system. Decreasing the size of the system leads to a necessity to encounter the effects of both the nanoparticle system and the environment. In regard with nanoparticles, quantum size effects will need to be taken into consideration for a system smaller than a few nanometers. For the environment surrounding the heating centers, nanoparticles, open or closed system needs to be considered. The free energy of a system at the nanoscale is written as follows nanothermodynamics[30]:

$$\mathrm{d}E = T\,\mathrm{d}S - p\,\mathrm{d}V + \sum_i \mu_i\,\mathrm{d}N_i + \mathcal{E}\,d\mathcal{N} \quad (3)$$

$\mu_i = (\delta E/\delta N_i)_{S,V,Nj}$ is the chemical potential of component *i*, $\mathcal{E} = \delta E_t/\delta\mathcal{N}_{St,Vt,Nt}$ is the subdivision potential, and the entropy S, the volume V, and the pressure p – are environmental parameters, as introduced by Hill[30].

The difference of nano-thermodynamics from thermodynamics has been considered in the follow-up of Hill’s approach distinguishing the whole system, Figure 1 (a) driven by thermodynamics laws, from that where a sub-ensemble of mobile nanoparticles needs to be taken in to consideration[31], Figure 1 (b) driven by nano-thermodynamics. But in the case of localized heating around nanoparticles bound on a membrane of capsules of vesicles the nanoparticles are immobilized, as depicted in Figure 1 (c), and, therefore, in this case thermodynamics should be applicable for these particles.

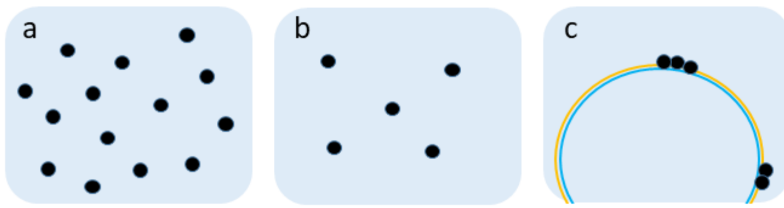

Figure 1. Release from a polyelectrolyte multilayer capsule (black frames at the top) upon controllable on-and-off application of a laser.

With a constant pressure and minimal volume change, the first term of equation 3 becomes particularly important. And it can be expressed in terms of a change of the entropy of the system, ΔS_{syst} of polyelectrolytes with nanoparticles; and the surrounding, $\Delta S_{surround}$, (environment in an aqueous solution). Typically, ΔS_{syst} << $\Delta S_{surround}$. But essential interest at this scale range is for a situation when:

$$\Delta S_{syst} > 0 \; and \; \Delta S_{surrond} \approx 0 \qquad (4)$$

which satisfies also: $\Delta S_{univ} = \Delta S_{syst} + \Delta S_{surrond} \geq 0$. At these conditions, one would look for localized system changes and what temperature can do to the local state of the system under more or less constant surrounding conditions. Polyelectrolyte multilayer capsules, assembled using a variety of effects (electrostatic interaction, hydrophobic interaction, glass transition, etc), represent an interesting system for studying thermal effects. This is because polymers in this case are freely suspended in an aqueous solution.

We consider now two very different reactions of polyelectrolyte multilayer capsules to supplied heat, Figure 2: (a) on one hand, elevated temperatures have been identified as one of the mechanisms of a controllable shrinking of capsules; while (b) on the other hand, laser heating of nanoparticles has been used for release of encapsulated materials. As a consequence, whereas in the first case (a) the polyelectrolyte shell gets stabilized, in the second case (b) it either disintegrates[21] or is getting perturbed[32] locally.

Analyzing these situations, we note that in the first case, the so-called "global" temperature rise, i.e. when the solution, in which capsules are immersed, is heated, affects the whole capsule. Upon heating the volume of an aqueous solution containing the capsule needs to be heated above the glass transition temperature of the polyelectrolyte polymer complex, T_g (Differential Scanning Calorimetry (DSC) has revealed[33] that for a pair of polystyrene sulfonate/polydimethyldiallylammonium chloride (PSS/PDADMAC) polymers T_g is around 41 ˚C). And in this case, it affects the whole capsule. Analyzing this situation in Figure 2 (a), one can see that in the case of micro- and macro-heating of the whole volume containing the microcapsule. F_{RES} is the resulting force of hydrophobic $F_{H\text{-}ph}$ (due to hydrophobic moieties on one polymer, for example, PSS), osmotic F_{Osm}, and elastic F_{Elast}, which also include the interaction of polymers. This leads to a shrinking in the case of a microcapsule with an even number of layers layers[33] led by hydrophobic force aiming to take the minimal energy configuration and repel from water, leading to a smaller sphere. This has been used for encapsulation (thicker grey arrows in Figure 2 (a) correspond to the case of shrinking; it should be noted that a charge dis-balance in the case of an odd number of layers leads to an opposite effect, expansion[33]). But most importantly, there is a perfect symmetry here in regard with the net force ΔF_{RES} along the circumference of the capsule: $\frac{\Delta F_{RES}}{\Delta \xi} = 0$ (where ξ is the circumference of the capsule, provided that the polymers are evenly distributed, which is the case here).

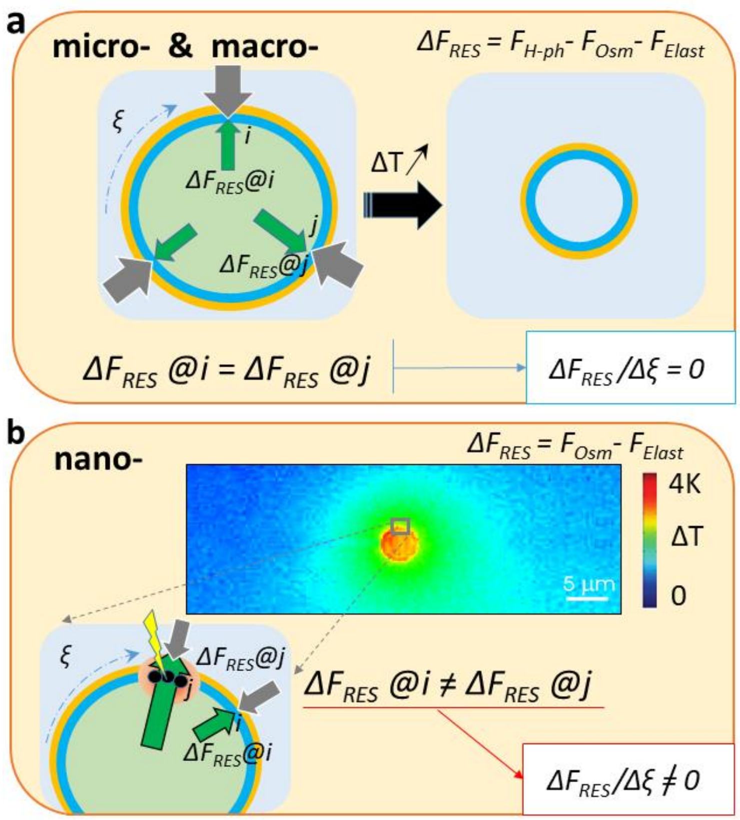

Figure 2. "Global" effect on the whole microcapsule system heating ΔT (top panel, a), versus the effect of "localized" heating (bottom panel, b) comparing the energy change along the circumference ξ of a polymeric microcapsule. ΔF_{RES} is the resulting force, comprised of $F_{H\text{-}ph}$ (hybrophobic), F_{Osm}(osmotic), F_{Elast} (elastic). (a) Schematic illustrating the effect of shrinking of a microcapsule upon "global" heating. The net force acting on points *i* and *j* along the circumference of the capsule are zero. (b) Schematic illustrating the effect of "local" temperature rise induced by an action of a laser on unevenly distributed nanoparticle (aggregates). The net force acting on a point *i* and *j* along the perimeter of the capsule are not zero. A color image (digitalized from previous work[21]) in the bottom panel stems from the fluorescent and temperature sensitive dye (BCECF, Molecular Probes) effectively providing the reading on temperature rise around this microcapsule (in this case ~ 4 ˚C temperature rise was measured[21]

upon illuminating the capsule with 25 mW of laser operating at 980 nm), this image is reproduced modified from previously work[21] with permission of the American Chemical Society.

In the case of nano- or "local" heating upon laser excitation, Figure 2 (b), the symmetry breaks $\frac{\Delta F_{RES}}{\Delta \xi} \neq 0$, because nanoparticles are unevenly distributed in the shell. F_{RES} in this case is noted as the sum of F_{Osm} and F_{Elast} encountering for a general case when no polymer with hydrophobic groups is present (even if a polymer with hydrophobic groups would be present the symmetry would still break, because of unevenness of the heat supply in this case). Under such circumstances release will take place, i.e. an opposite effect to what occurred in the case (a). Thus, these seemingly contradictory phenomena are both accounted for with the above analysis.

Release by gently "moving" polymers in a temperature gradient

Release from a microcapsule upon laser illumination is shown in Figure 3 (a-g), which was demonstrated in a solution[34, 35] (Figure 3 (a)) or under a microscope[21, 36] (Figure 3b). In addition to the above mentioned conditions, release has been realized from a shell-in-shell assembly, Figure 3 (c)[37], on-and-off controllable, portion-wise release, Figure 3 (d)[32], fusion of microcapsules by laser light, Figure 3 (e)[38], release by pushing a capsule from a multicompartment shell-in-shell structure, Figure 3 (f)[37], direction specific release, Figure 3 (g)[39]. It should be noted that if the concentration of metal nanoparticles is high (almost complete coverage), then explosive release takes place,[36] which can be used for killing cells, similarly to that shown in Figure 3 (h).

A very special case is depicted in the middle of Figure 3 as carried out by gently "moving" polymers by a laser. The term "moving" is important here, because it both allows to study fundamental processes associated with equilibrium of a polymeric system in solution and carry out biologically relevant processes (for example, intracellular release) non-destructively. This is done based on the principle of non-destructive modulation of polymers locally. From the fundamental science point of view, it is a very elegant way to "move" only those polymers located next to or around a heating metal nanoparticle. A practical application of this phenomenon would be to enable release of a portion of encapsulated materials by controlling the traffic of molecules through the polymeric membrane. The results of such a modulation can be seen in Figure 3 (d), where a slice of the membrane is shown in red color with an aggregate of nanoparticles incorporated in the center of the membrane.[32] Application of light to the membrane produces heat locally around the nanoparticle aggregate, which moves very slightly only those polymers located in the vicinity of these nanoparticles. The rest of polymers, molecules and aqueous solution remains unaffected by this localized heating. The insets to this schematics (black panels above) show two capsules with one capsule at the bottom serving as a control, which is not exposed to laser excitation. The content of the left capsule (inset to Figure 3 (d)) is released upon laser illumination, but when the laser light is turned off the capsule seals itself, thus releasing only some of its contents. Upon another exposure to laser light, the whole content of the capsule is released, see the inset to the right panel in Figure 3 (d).

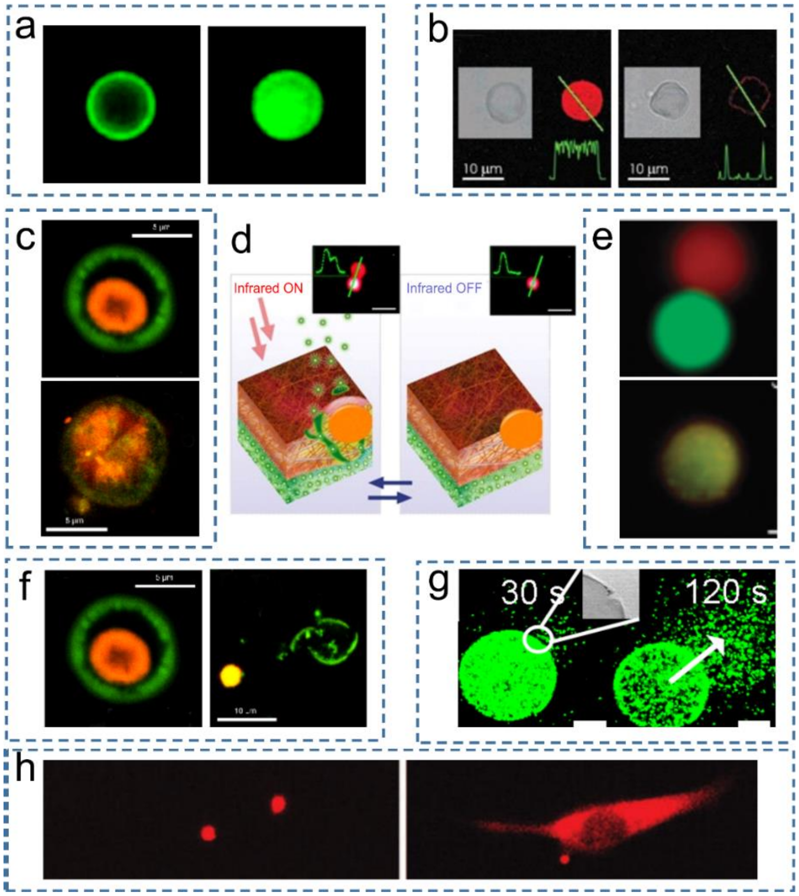

Figure 3. Release from a microcapsule, in which the shell were functionalized with metal nanoparticles, are upon laser illumination: (a) a capsule before the exposure to laser irradiation (left) and another capsule after exposure to an intense laser pulse, reproduced[24] with permission of the American Chemical Society; (b) a microcapsule possessing dextran labelled rhodamine molecules (left) and the same capsule after the laser illumination (insets show transmission microscopy images), reproduced[21] with permission of the American Chemical Society; (c) a shell-in-shell multicompartment microcapsule (top), where the interior of two subcompartments were mixed after the exposure to a laser, reproduced[37] modified with permission of Wiley-VCH; (d) on-and-off release from a microcapsule realized by switching the laser (on-and-off, respectively) and thus moving polymers around nanoparticles, reproduced[32] with permission of the American Chemical Society; (e) fusion of microcapsules showing two capsules before the fusion (top) and the resultant fused microcapsule after laser exposure, reproduced[38] with permission of the American Chemical Society; (f) a shell-in-shell microcapsule, in which the inner capsule was knocked out of the outer shell upon exposure to a laser, reproduced modified[37] with permission of Wiley-VCH; (g) a giant microgel-based capsule, from which a direction-specific release was carried out, reproduced modified[39] with permission of the Royal Society of Chemistry; (h) two microcapsules inside a cell before the release (left) and after releasing the contents of one capsule inside a cell (right), reproduced modified[40] with permission of the American Chemical Society.

Perspectives for fundamental research and selected applications

Investigating localized nanoscale heating is of interest to both fundamental research and practical applications. Fundamental research in this direction is not necessarily limited to the area of physical chemistry. Some examples of fundamental research in biology include the investigation of surface presentation of small peptides upon intracellular release of peptides.

Directional control of temperature and release

It is worth mentioning that directional control of release is somewhat more straight forward, but still challenging. This control was shown to take place also by controlling the distribution of nanoparticles and/or the location of laser illumination on the polymeric shell. Osmotic pressure is the driving force enabling the release.

Heat flow is governed by the laws of thermodynamics, which state that heat cannot flow from a cooler object to a hotter object without any work. Controlling the direction of heat flow – one of the most lucrative goals – is challenging, because of scarcity of available tools or parameters enabling that. At the nano-scale, a metal nanoparticles illuminated by a laser act as the source of the localized heating, which flows from them to the surrounding environment. One parameter available for controlling the heat flow is thermal conductivity. By situating a nanoparticle at the border between a medium with a higher thermal conductivity, *e.g.* an aqueous medium with polymers, and that with a lower thermal conductivity, *e.g.* air, one can assure the directional heat flow mostly to the polymer medium, see Figure 4. Practical application of directional heat control is linked with specific and direction-controlled release.[39]

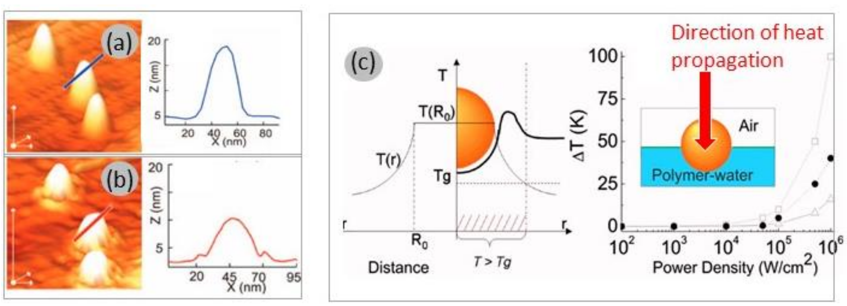

Figure 4. Embedding nanoparticles into polymeric layers: (a) nanoparticles before laser exposure; and (b) the same nanoparticles after laser exposure. (c) temperature profile around a nanoparticle upon laser illumination (left panel) together with schematics of direction heat propagation (the red arrow). Reproduced modified from previously published work[41] with permission of American Physical Society.

We note that a directional heat flow can be used for studying fundamental processes of heat exchange at the nanoscale. But protecting nanoparticles can be used for creating, for example Janus nanoparticles, which are envisioned to drive a phoretic self-propulsion, i.e. machine-free movement of particles at the nanoscale.

Localized heating of lipid membranes and cells by nanoparticles

Controlling the behavior of lipids is essential for understanding the function and protection of cells. In this area, a very precise ion current monitoring upon modulation and, if necessary, disruption of the membrane was shown. Controlled the permeability through a lipid membrane monitored by ion current has been also demonstrated,[42] Figure 5.

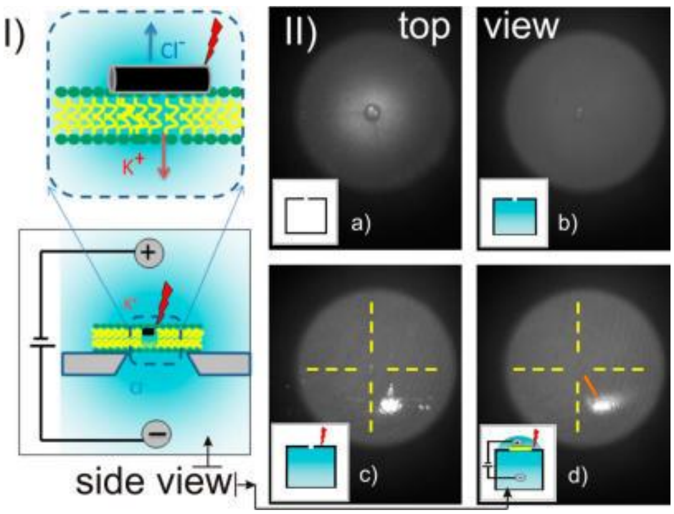

Figure 5. Schematic setup (I) and reflection microscopy images (II) of experiments for the effect of the localized temperature rise of nanoparticles onto a lipid membrane, on which they were adsorbed. Reproduced from previously published work[42] with permission of the American Chemical Society.

Research on understanding of the influence of nanoparticles on a lipid membrane[43] is important not only for fundamental science, but it has essential practical implications for inducing the permeation through lipid membranes[44] relevant for cell biology. This phenomenon has been recently utilized for delivery of molecules inside living cells,[45] which was also realized with advanced patterning[46], see Figure 6.

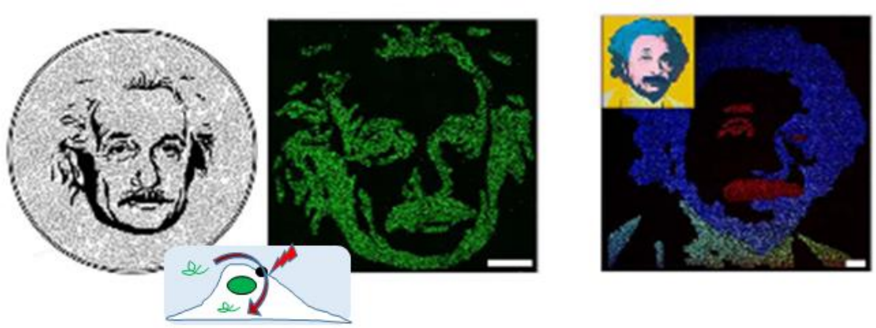

Figure 6. A “live” painting made by the delivery of biomolecules into living cells by laser-nanoparticle interaction upon selectively choosing the cells for delivering biomolecules. Reprinted modified from previous work[46] developed by Braeckmans *et al* with permission of Elsevier.

Remote release inside cells – study of viral peptides surface presentation in immunology

Remote release inside living cells[47] demonstrated on a single cell level as well as on a single capsule level[48] has already enabled investigation of surface presentation of small peptides[49], release of enzymes and corresponding substrates,[50] as well as release of agents for immunostaining[51]. An established concept in immunology is that viral peptides are transported through ER (erythrocytic reticulum) onto the surface of cells, said to be surface presented. The only “clean” way of verifying this concept is to release such peptides from within cells, and this is precisely what was accomplished with polyelectrolyte multilayer capsules equipped with nanoplasmonic nanoparticles.

Remote release in-vivo

In the next step, release from a multicellular organism would be of interest. Implementing remote release in such organisms would enable studying various signaling pathways and influence development of these organisms. Recently, this was done inside a special type of worms – *C elegans*.[52] Uptake of capsules took place spontaneously, but capsules were situated for some time in a semi-moist environment. A special type of capsules based on alginate and capable of withstanding semi-dry conditions have been used there.[53] Laser remote release was also used to study Wnt/beta-catenin signaling pathways, relevant for translational medicine, in multicellular organisms[54]. This signaling was switched in vivo upon an external laser action. Effectively, the presented approach allows for a remote and non-destructive method of initiating release inside cells and microorganisms.

Conclusions

Polyelectrolyte multilayer capsules represent an interesting system well suited to study the behavior of polymers in a solution, because the polymers are freely suspended in a solution, which is relevant for fundamental understanding of physico-chemical processes. At the same time, they represent an important system relevant for a numerous applications, which is relevant for applied bio-sciences and chemical production processes. One direction of research extensively studied on microcapsules is a nanoscale temperature rise, also termed nanoplasmonics. It provides understanding of the state of polymers around their glass transition temperature and explained the motion of polymers as the mechanism of portion-wise release from capsules. Localized temperature rise has been investigated around nanoparticles on microcapsules, and the temperature rise around microcapsules was measured and linked with calculated thermal rise. The aggregated state of nanoparticles determines the spectral range of absorption, which allows to tune the wavelength of laser excitation. Nanothermodynamics, an area dedicated to studying thermodynamics at the nanoscale, is discussed in the context of localized temperature rise around nanoparticles and linked with the situation of nanoparticles fixed in the shells of microcapsules. On the other hand, polyelectrolyte multilayer capsules represent an excellent system for practical applications enabling intracellular release, investigation of killing cells by applied laser light, the surface presentation of small peptides, in vivo release in insects and worms, etc. “We should do more of it” is an expression, often referred to microcapsules - indeed, there is which is to say that for interesting applications should always find room for further studies.

Outlook and emerging applications

- A large part of polymer assemblies covered in this perspective is based on the LbL approach, which has been an important driving force for development of various applicaiotns.[55] The following emerging applications can be considered as stand-alone ones or incorporate a localized temperature rise mechanism.

- *Assembly of capsules and encapsulation*. Further research is taking place on developing novel ways of the polymer shell assembly of capsules.[56] There, metallo-supramolecular complexes can play an important role[57], and these developments are particularly relevant for enzyme catalyzed reactions.[58] It is worth noting that in the area of enzyme catalyzed reactions traditional layer-by-layer coatings have shown to be very promising.[11, 59, 60] In addition to above mentioned new methods of LbL assembly, new assembly mechanisms[61, 62], such as exploring hydrogen bonding[63] would be beneficial. Investigation of fundamental physico-chemical properties[64, 65, 66] of LbL remains relevant both for fundamental and applied science. Capsules are perfect structures for gaining fundamental understanding of the interaction of polymers, for example, a fusion of capsules, which has been shown by temperature and salts[67] as well as by a localized heating using a laser[38].

- *Release from capsules*. Developing new release[68, 69] mechanisms[70], where organic molecules[71, 72, 73] would complement inorganic nanoparticles[74, 75]. pH dependency stands[76, 77] apart in the row of other available release modalities, but it is not applicable for the subset of biological applications, in which pH must be kept constant. Therefore, biodegradability is viewed as an essential functionality.[78] Further development in the area of novel composition of both release stimulating nanoabsorbers[79] and capsules includes new types of containers inherently incorporating metal nanoparticles in their shells.[53] Another trend in this area is to deliver molecules directly inside a particle or a templates, and not necessarily at a pre-defined location. There, controlling pores[80] as well as developing smaller carriers[81] is essential. Temperature[82] of the aqueous solution has been identified as an important mechanism to govern polymer re-arrangements, and thus encapsulation.[33] Shrinking[83] of microcapsules based on calcium carbonate particles, which are difficult to control,[84] will be useful for release.

- *Nanoplasmonics* is an area, devoted to study surface plasmon resonances upon interaction of electromagnetic waves with predominantly of noble metal nanoparticles, which enables a localized temperature rise and is closely linked with thermodynamics at the nanoscale. In addition to above mentioned perspective for research, determine of phase transitions of materials has been identified an interesting applicaiton.[85] Furthermore, another development there is chiral plasmonic structures[86, 87] as well as cell-selective delivery has been already mentioned, but one of the following developments is selective patterning.[46] *Other selected applications*. Sensors[88] and micropackaaging[89] for arrays[90] is another prominent application area of microcapsules. Propulsion has been also realized using nanoplasmonics; here, autonomous movement can be executed by Janus particles and capsules[91] as well as bio-mimetic propulsion particularly effective on hydrophobic surfaces.[92] Corrosion protection is yet another application of nanoplasmonics, in which the localized temperature rise has been used for a triggered release.[93] Another developing trend is to combine new assembly blocks, for example halloysites tubes, with polymers and their assembly[94].

- *Coatings*. Novel ways of coatings have been proposed.[95] One example is the incorporation of nanoparticles[96, 97] and microcapsules[98] into polymeric coatings, where nanoparticles can serve both as hardness controllers[99] and as centers for a localized temperature rise including the release of encapsulated materials. Thermal annealing of LbL coatings has been shown to facilitate cell growth,[100] while LbL has been shown to control bacterial growth by bacteriostatic effect.[101] A combination of hydrogels[102], and specifically hyaluronic acid hydrogels,[103] with LbL is another development, where properties of both types of materials are utilized to advantage. In addition, functionalization of hydrogels[104] with calcium carbonate particles,[105] serving the templates in capsules, opens new routes for initiation of biomineralization processes. Modification of such hydrogels by nanoparticles allows to control hardness for cell adhesion and fabrication of custom-tuned Janus particles[99].
- *Biological applications: cells and in-vivo. Mechanical properties* have always been of significance[106, 107] and they are relevant for cell uptake[108]. Enhancement of mechanical properties is essential for reliable delivery, where new materials such as silk-on-silk[109] or inorganic carbon nanotubes[110] appear to be promising candidates. The latter carry a dual function: on the one hand, they enhance mechanical properties, while on the other hand they serve as centers with localized temperature rise upon laser illumination. In addition to enhancement of mechanical properties, a combination of carbon nanotubes with polymers has been shown as a flame retardant.[111] *Cell* studies[112] and targeting[113] have always been some of the most relevant applications in the area of microcapsules, where shape,[114] including Janus character of particles,[115] has been identified as an important feature for efficient cell uptake.[116] Non-destructive intracellular release[47] has been used to study the surface presentation of small peptides[49] – a phenomenon particularly relevant for immunology. Further studies are certainly to take place utilizing these methods. *In-vivo* studies represent an important milestone after cell and intracellular studies. Performed on multicellular organisms and small animals,[117] recent progress in this area is particularly encouraging.
- *Hybrid materials.* Localized temperature rise discussed here has been induced through hybrid materials combining organic (polymer) matrices and inorganic (nanoparticles) components. These *inorganics-in-organics* materials have been recently systemically classified[118] in regard with other *organics-in-inorganics*, and this classification should be helpful for further design and optimization of these materials.
- *Nanomedicine and theranostics. Theranostics*[119] is an areas of research, where diagnostics and therapy are combined into the same entity – and is closely linked with in-vivo studies.

Targeted drug delivery is seen as an important application.[120] Here, multicompartment microcapsules[14, 121, 122] and capsules with different shapes,[123] including Janus microcapsules[124] possess attractive multi-functionality in regard with delivery, sensing, targeting. *Nanomedicine*. Ultimately, all these trends would emerge for novel applications into a burgeoning area of nanomedicine.[125]

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