Abstract: A process for the controlled release of at least one entity, said process including the steps of: (a) providing a core-shell material comprising: (i) a core comprising at least one releasable entity; (ii) a shell encapsulating the core; and (iii) at least one electromagnetic radiation absorbing material incorporated into the shell, and having tunable absorption properties; and (b) exposing the core-shell material to electromagnetic radiation of a predetermined wavelength and/or intensity wherein the shell is ruptured upon exposure to the electromagnetic radiation causing the release of the at least one entity.
ELECTROMAGNETIC RADIATION ADDRESSABLE MATERIALS

Field of the Invention

The present invention relates generally to a process for the controlled release of entities such as chemical compounds, analytical reaction compounds, pharmaceuticals, proteins, nucleic acids or functional biomaterials from electromagnetic radiation addressable core-shell materials. The present invention further relates to core-shell materials and a delivery system for the controlled release of at least one entity, wherein the entity is released from the core-shell material upon exposure to electromagnetic radiation. A further embodiment is the release of an entity from a single particle of the core-shell material by addressing the particle with a laser beam of electromagnetic radiation preferentially in the IR-spectrum.

Background

The present application utilises an electromagnetic radiation absorbing material, such as nanoparticles, in a shell material suitable for encapsulating an entity. Metallic nanoparticles, in particular, have attracted wide spread interest as components for integration into thin films because of their size dependent and unique optical and electronic properties. A common approach to prepare metallic nanoparticle based thin films has been via their direct adsorption onto substrates from organic or aqueous solutions. When deposited from aqueous media, typically sub-mono layer nanoparticle coverage is achieved.

Hollow polymer capsules have been developed by coating subsequent layers of positively and negatively charged polymeric (polyelectrolyte) coatings around a micro-particle core. The core is readily removed by dissolution leaving a hollow, largely spherical polyelectrolyte capsule held together by electrostatic forces.

A further development is to incorporate inorganic nanoparticles into the polyelectrolyte material. Typically gold or silver nanoparticles are used for such
purposes. The actual structure and composition of the capsule can be largely determined by the means for removing the core material. For example, if the core material is removed by heat alone and suitable nanoparticles are used, this leaves a largely ceramic material made up predominantly of the nanoparticle layer. If solvent is used to remove the core material, the material will largely be a composite of the polyelectrolyte layer and the nanoparticles.

Typically gold nanoparticles are utilised to add favourable optical activity to a material. By using such metallic nanoparticles with a controlled arrangement in a shell, additional freedom in tuning optical properties can be achieved. It is possible to vary the optical properties of complete hollow shells by varying the dimensions and packaging of the materials in the shell. That is, the optical properties of the shell may be tuned over a broad optical range. The same basic freedom in tuning the optical properties can be observed for the capsules made from such particulate materials.

Nano-shells made of a completely metal covered solid nanoparticles are discussed for diagnostic and therapeutic purposes by including a bio-conjugate in the paper by Loo et al. in Technol Cancer Res Treat. 2004 Feb; 3(1):33-40. The therapeutic purposes includes near infrared (NIR) absorbing nano-shells in thermal therapy of tumours. Similar use of nanoparticles in the thermal therapy for cancer is described in a paper by Hirsch published in Proc Natl Acad Sci USA 2003 November 11; 100(23):13549-54.

The discussion of documents, acts, materials, devices, articles and the like is included in this specification solely for the purpose of providing a context for the present invention. It is not suggested or represented that any or all of these matters formed part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

Summary
The present invention relates to a core-shell material that may be used to encapsulate entities such as chemical compounds, analytical reaction compounds, pharmaceuticals, proteins or functional biomaterials and release the encapsulated entities upon exposure of the core-shell material to electromagnetic radiation.

**Detailed Description**

The present invention provides a core-shell material for the controlled release of encapsulated entities such as chemical compounds like analytical reaction compounds, pharmaceuticals, proteins or functional biomaterials.

The core-shell material of the present invention comprises (i) a core comprising at least one releasable entity (ii) a shell encapsulating the core, and (iii) at least one electromagnetic radiation absorbing material incorporated into the shell and having tuneable properties. Upon exposure of the core-shell material to electromagnetic radiation of predetermined wavelength and/or intensity, the shell ruptures, causing the release of the entity. The ability of the core-shell material to respond in a controlled manner to an applied source of electromagnetic radiation enables a high degree of control and selectivity to be obtained for the release of the encapsulated entity.

The core-shell material of the present invention comprises a core having at least one releasable entity. The releasable entity is encapsulated in the core by the shell of the core-shell material. Where the core comprises more than one entity, they may be of the same type or they may be of different types of entities. Herein, we have used the term “entity” or “entities” to describe a broad range of entities that may be comprised in the core. The entity of the core may be of any suitable form such as solid, liquid, crystalline, particulate, amorphous or like and mixtures thereof, so long as it may be encapsulated. In a preferred embodiment, the core comprises at least one releasable entity selected from the group consisting of analytical reaction compounds, functional biomaterials, proteins, nucleic acids, carbohydrates, lipids, synthetic polymers, enzymes,
vitamins, drugs, reagents, dyes, fluorophores or a precursor of a drug, reagent, dye, fluorophore, nucleic acid, or a mixture of two or more thereof.

The core-shell material of the present invention comprises a shell that is responsive to applied electromagnetic radiation. The shell encapsulates the core of the core-shell material. The shell further incorporates at least one electromagnetic radiation absorbing material having tuneable absorption properties. In one preferred embodiment, the shell may be formed from a single layer or multiple layers of a polymer and/or lipid. Preferably, the shell comprises multiple polymeric layers. More preferably, the polymeric layers are cationic or anionic polyelectrolytes. The shell may comprise alternating layers of suitable polyelectrolytes, for example, a polyanion such as poly(sodium 4-styrenesulfonate (PSS) and a polycation such as poly(allylamine hydrochloride) (PAH). The shell may also comprise biodegradable polymers.

The shell incorporates an electromagnetic radiation absorbing material having tuneable absorption properties. The electromagnetic radiation absorbing material determines the energy source needed to release an encapsulated entity from the core of the core-shell material and may be tuned to absorb electromagnetic radiation of appropriate and predetermined intensities and/or wavelengths.

In a preferred embodiment, the electromagnetic radiation absorbing material is selected from metallic particles, semiconducting nanocrystals such as quantum dots (Qdots) and the like, chromophores, a metallic layer and metal ions, or a mixture thereof. In one preferred embodiment, the electromagnetic radiation absorbing material partially consists of particles. Preferably, the particles are nanoparticles. Even more preferably, the particles are metallic. The metallic particles preferably consist of gold, silver or coinage metal. Gold nanoparticles are particularly preferred. The particles may have a particle size in the range of from 0.001 to 100 microns, more preferably the particle size is in the range of from 0.1 to 10 microns, even more preferably the particle size is in the range of from 3 to 100 nm. The electromagnetic radiation absorbing material may be located on the outer surface of the shell or they may be located
within the structure of the shell. When the shell comprises multiple polymeric layers, the polymeric layers may interact with the electromagnetic radiation absorbing material, for example nanoparticles, to assist in the self-assembly of the material in the shell. When gold or silver particles are used to form the electromagnetic radiation absorbing material, it is preferred that they have a particle size in the range of from 3 to 100 nm.

When the electromagnetic radiation absorbing material comprises metallic particles, it is preferred that the metallic particles be ligand stabilised. The use of a ligand may assist with the incorporation of the material into the shell of the core-shell material. Preferably, the metallic particles are stabilised by the organic base 4-di-methylaminopyridine (DMAP). The person skilled in the art would appreciate that thiol compounds such as mercaptoundecanoic acid (MUA) and tri-n-octylphosphine (TOPO) and other ligands suitable for stabilising semiconductor nanocrystals may also be used. It is particularly preferred that the ligand stabilised particles are gold nanoparticles.

The electromagnetic radiation absorbing material may be tuned to absorb any desired intensity and/or wavelength of electromagnetic radiation, and it will appreciated by the person skilled in the art that the desired type and source of electromagnetic radiation will depend on a number of factors, including for example, the nature of the electromagnetic radiation absorbing material, the nature of the releasable entity and the desired application. Preferably, the material absorbs electromagnetic radiation in the optical region, for example, ultraviolet, visible, near infrared (NIR) or infrared. The material however, may also be tuned to absorb electromagnetic radiation outside the optical region for example, gamma, microwave and radio frequency radiation. In one embodiment, when the electromagnetic radiation absorbing material comprises metallic particles, the absorption properties of the material to be tuned by varying the thickness, length and/or width of the particles, so that the material is responsive to a range of predetermined intensities and/or wavelengths of electromagnetic radiation. In another embodiment, when the electromagnetic radiation absorbing material comprises a chromophore, the
chemical structure of the chromophore may be varied to enable tuning of the material.

The electromagnetic radiation may be provided by any suitable source. In a preferred embodiment, the electromagnetic radiation is provided by a beam of radiation that illuminates the core-shell material to cause release of the entity from the core of the core-shell material or particle. Preferably, the beam of electromagnetic radiation consists of a small diameter and is capable of selectively illuminating a single core-shell material. Preferably, the beam of electromagnetic radiation is provided by a laser, more preferably an infrared laser. The laser beam preferably has a diameter in the range of from 100 nm to 10 micrometer. The electromagnetic radiation may also be monochromatic.

Upon exposure of the core-shell material to predetermined intensities and/or wavelengths of electromagnetic radiation, the electromagnetic radiation absorbing material, e.g., gold nanoparticles, convert the radiation in heat, causing heating of the particles to several hundred degrees Celsius, and local heating of the surrounding shell material which leads to the perforation and rupture of the shell, which facilitates the release of one or more entities from the core encapsulated by the shell. An advantage of the current invention over the prior art is that only the shell is affected by the heat. The releasable entity in the core is not or is negligibly heated, therefore the releasable entity is not denatured in the case of proteins, or altered by the heat.

Targeting

The core-shell material of the present invention can be further modified to enable it to target specific sites of application and/or make it less immunogenic by the addition of a biocompatible layer on the outré surface of the shell. The biocompatible layer may comprise at least one biological component selected from the group consisting of lipid bi-layers, DNA, RNA, sugars, proteins and fragments thereof. The outer surface of the shell may further comprise a bio-recognition molecule. The bio-recognition molecule may be any one of a variety of standard recognition structures used in
biotechnology, such as antibodies, nucleic acids, lectins or receptors. Preferably, the bio-recognition molecule assists to deliver the capsule to specific sites of application and/or assists in resisting an immunogenic response being raised to the core-shell material.

In one preferred embodiment, a protein labelled lipid bi-layer may be provided on the outer surface of the core-shell material in order to direct the core-shell material to specific sites within the body. In a further embodiment, a phospholipid bi-layer may be applied to the core-shell material to assist in overcoming body rejection. The person skilled in the art would understand that standard recognising structures such as a combination or fragments of DNA, sugars, RNA and protein components, for example glycoproteins, neurotransmitters, receptors, lectins, ligands, antibodies, antigens and the like may be applied to the core-shell material, and the present invention need not be limited to the list of structures described herein. Application of these standard recognising structures to the capsule will enable the practitioner to target particular sites within the body.

Modification of the core-shell material to target specific sites, for example within the body or within a reaction chamber enables the core-shell materials to accumulate in a desired location within the body or reaction chamber for release of the encapsulated entity upon exposure of the core-shell material to electromagnetic radiation. It is desired that the electromagnetic radiation is applied either directly or endoscopically to effect release of the encapsulated entity.

The core-shell material of the present invention can also be modified with molecules or agents that influence the solubilization of the core-shell material. The molecules or agents may assist to render the core-shell material soluble or dispersible for example, in solution. This may be advantageous to enable the delivery of the core-shell material to the desired sites of application in the body or in a reaction chamber. Preferably, the molecule or agent is selected from the group consisting of ionic and non-ionic detergents, polyols such as polyethylene glycol, dextran and hydrogels. The core-shell material of the present invention
is preferably a colloidal system. In a preferred embodiment, the colloidal system is a suspension with a particle size of from 100 nm to 100 micrometers, more preferably the particle size is about 500 nm. The colloidal system is further preferably monodisperse.

The core-shell material may also be provided any in any form suitable for delivery to a site of application. For example, the core shell material may be provided in a pharmaceutical, therapeutic or diagnostic preparation. In this respect, the core shell material may be formulated with a suitable carrier. In one embodiment, the core-shell material is provided in a solid preparation. In another embodiment, the core-shell material is provided in a liquid preparation, such as a solution or a suspension. Preferably, the core-shell material is formed as an injectable suspension. In another embodiment, the core-shell material may be provided in the form of a film. The film may be provided in a patch for use at a site of application.

The present invention also provides a delivery system for the controlled release of at least one entity, the system comprising at least one core-shell material comprising:

(i) a core comprising at least one releasable entity,
(ii) a shell encapsulating the core,
(iii) at least one electromagnetic radiation absorbing material incorporated into the shell and having tuneable absorption properties;
and
(iv) an electromagnetic radiation source;

wherein upon exposure to electromagnetic radiation of a predetermined wavelength and/or intensity, the shell will be caused to rupture to enable release of the at least one entity.

Process of Material Formation

In one aspect, the present invention relates to a process for the preparation of a core-shell material for the controlled release of at least one entity, the process comprising the steps of:
(a) contacting a shell of the core-shell material with at least one electromagnetic radiation absorbing material to incorporate the electromagnetic radiation absorbing material into the shell; and

(b) introducing at least one entity,

wherein the entity is encapsulated in the shell.

The shell of the core-shell material of the present invention may be prepared using any suitable process known in the art. Examples of suitable general protocols are described in papers (1), (3), (14) and (15) as outlined in the references listed in this specification, which references are incorporated herein by reference. In one preferred embodiment, following the described general protocols of the prior art, ligand stabilised gold nanoparticles can be used to produce thin nanoparticle dense films on both planar (flat) and spherical (colloid) substrates via a single nanoparticle adsorption step from aqueous solutions. This may be achieved by exploiting nanoparticle self-assembly onto polyelectrolyte coated surfaces.

In a preferred embodiment, the shell is formed from a single layer or multiple layers of a polymer or lipids. When multiple layers are desired, the layers are preferably prepared using a layer-by-layer process involving the sequential deposition of suitable polyelectrolytes, for example, a polyanion such as poly(sodium 4-styrenesulfonate (PSS) and a polycation such as poly(allylamine hydrochloride) (PAH). Biodegradable polymers may also be used.

The shell of the core-shell material is contacted with an electromagnetic radiation absorbing material. In one embodiment, in which the electromagnetic radiation absorbing material comprises gold nanoparticles, the nanoparticles may be provided as a dispersion in an appropriate solvent. With reference to the preferred embodiment, the nanoparticle dispersion may be provided in an organic or an aqueous solution. It is preferred that an aqueous solution is used. In a preferred embodiment, an aqueous dispersion of nanoparticles may be obtained by first synthesising the nanoparticles in organic solvent, wherein the lack of aqueous ionic interactions during synthesis leads to higher
concentrations being produced. The organic base 4-di-methylaminopyridine (DMAP) may then be added to the nanoparticles to effect their phase transfer to water. The aqueous dispersion of nanoparticles are stabilised by DMAP and as a result are positively charged.

An attractive feature of using nanoparticles, in particular gold nanoparticles, stabilised with DMAP for the electromagnetic radiation absorbing material is that unlike other nanoparticles stabilised with covalently attached ligands, (eg. thiols), the DMAP ligand can be readily removed by simple water washing. This may be advantageous if the core-shell material is to be used in drug delivery applications where minimal levels of extrinsic materials are generally desirable.

In one preferred form, the shell is constructed on a core material. The core material may consist of the releasable entity or entities. Alternatively, the core material is removable template core and when removed, produces a hollow core. When the core material is removable template core, it is possible to remove the core material either before or after the shell is contacted with the electromagnetic radiation absorbing material. The removal template core material may be any suitable material that can be removed from the structure without disrupting the shell. Preferably, the removal template core material is comprised of melamine-formaldehyde (MF).

The entity may be introduced in a number of ways in order for the shell to encapsulate the entity. One means of introducing the entity to the core-shell material is to pre-form a shell incorporating an electromagnetic radiation absorbing material, wherein the shell surrounds a hollow core. The entity is then introduced into the hollow core by diffusion, resulting in the entity being encapsulated by the shell.

Another means of introducing the entity to the core-shell material is to pre-form the shell surrounding a hollow core. However, in this embodiment, the shell does not include the electromagnetic radiation absorbing material. In this embodiment, the entity may once again, be introduced into the hollow core by
diffusion. After encapsulation of the entity by the shell, the shell is then contacted with the electromagnetic radiation absorbing material to form the core-shell material.

Diffusion of the entity may be reduced due to presence of the electromagnetic radiation absorbing material if the material is incorporated into the shell before the entity is able to be encapsulated. Accordingly, it is preferred that the electromagnetic radiation absorbing material is incorporated into the shell after the entity encapsulated by the shell, rather than before.

A further means of introducing the entity to the core-shell material is by the use of pre-formed entities, for example crystals, which may be encapsulated by the shell at the same time that the core-shell material is being formed. For example, it is possible to construct the shell on the at least one entity to be encapsulated. The entity in this embodiment is used as the core material. After the shell has been constructed on the core comprising the entity, the shell is then contacted with an electromagnetic radiation absorbing material in order to form the core-shell material.

Specific examples of each of the general techniques for introducing the entity to the core-shell material are outlined in the Examples.

Preferably, when the entity is introduced into the capsule by diffusion, appropriate conditions are selected for the entity concerned. That is, the introduction of the entity takes place under pH conditions, temperature and salt levels (osmotic balance) that are appropriate for the particular entity and material used.

The protocols outlined in the prior art referred to represents preferred protocols of forming a core-shell material that is capable of being addressed by electromagnetic radiation, however the skilled practitioner working in this area would be appreciative of other protocols that have been developed for forming such core-shell materials.
The core-shell materials of the present invention may show resonant light absorption due to the surface plasmon resonance, the cavity plasmon resonance and mixed plasmon residences. It is possible to tune the absorption of the electromagnetic radiation absorption material incorporated in the shell of the core-shell material to the wavelength regime of between 520 nm and 10 microns, which has been found to be an appropriate range for the particularly preferred gold nanoparticles. Most preferably, absorption of the electromagnetic radiation absorbing material is tuned to the wavelength regime of between 700 nm and 1200 nm, as this is where the absorption and scattering of biological species is as low as possible.

Release of the entity

The present invention provides a process for the controlled release of at least one entity, wherein the process comprises the steps of:

(a) providing a core-shell material comprising:

(i) a core comprising at least one releasable entity;
(ii) a shell encapsulating the core; and
(iii) at least one electromagnetic radiation absorbing material incorporated in the shell having tuneable absorption properties;

and

(b) exposing the core-shell material to electromagnetic radiation of a predetermined wavelength and/or intensity, wherein the shell is ruptured upon exposure to electromagnetic radiation causing the release of the at least one entity.

The core-shell material of the present invention is electromagnetic radiation addressable, meaning that upon exposure to an electromagnetic radiation source for example, a short period of time, the core-shell material will cause the release an encapsulated entity. Preferably, the source of the electromagnetic radiation is a laser source, more preferably, an infrared laser, and it will be convenient to discuss the release mechanism of the invention in terms of the use of a laser or light source, but it should be appreciated that other electromagnetic radiation sources are contemplated within the invention.
The core-shell material comprises an electromagnetic radiation absorbing material, the absorption properties of which can be tuned to absorb the desired electromagnetic radiation wavelength and intensities. Most preferably, the electromagnetic radiation absorbing material is tuned to the absorption at a wavelength of a pulsed laser light source by exploiting resonant light absorption effects.

It is generally considered that it is the thickness:diameter ratios and the surface coverage of the electromagnetic radiation absorbing material that determines the wavelengths of radiation which are absorbed. In particular, in the preferred embodiment when the electromagnetic radiation absorbing material comprises metallic particles, it is considered that it is the thickness and diameter of the particles, and the packing of the particles in the electromagnetic radiation absorbing material, which influences the absorption wavelengths and/or intensities.

The electromagnetic radiation source is applied for a period of time and at an intensity adjusted to the electromagnetic radiation absorbing material, and the specific application for which core-shell material is to be used. This determines the energy dissipation out of the electromagnetic radiation absorbing material and the resulting physical effects that contribute to the entity release mechanism.

In order to release the encapsulated entity from the core-shell material, it is therefore possible to choose from a number of physical effects, such as the generation of free electrons, thermal and mechanical stresses, cavitation, melting or thermal damage as contributors to the release by adapting the pulse duration of the electromagnetic radiation to the absorption properties of the electromagnetic radiation absorbing material.

The pulse duration range used for adapting the effects is preferably from about femto seconds to several seconds. The preferred range for a preferred embodiment of medicinal drug delivery is short enough to prevent substantial
energy dissipation into the interior or the surrounding of the core-shell material. This prevents undesirable heating in the vicinity of the encapsulated entity. The pulse duration range is for example, in the range of from femto seconds to nano seconds.

Application of the electromagnetic radiation source will disrupt the electromagnetic radiation absorbing material within the core-shell material of the present invention. Without being bound by theory, it is thought that the application of an electromagnetic radiation source, such as a light source or a laser source, will porate the electromagnetic radiation absorbing material. The application of the electromagnetic radiation source may be a direct source or the radiation can be applied endoscopically.

The release of the encapsulated entity from the core-shell material is instant release upon exposure of the core-shell material to electromagnetic radiation. Therefore, there is controlled release of the entity, such as a drug, so that the entity may be released at the appropriate time to suit various applications, for example, after a drug has been delivered to a site of application, or when it is desired to mix analytical compounds together for reaction.

Use of an appropriate pulse length of electromagnetic radiation can also prevent unnecessary heating. For example, use of a radiation pulse length from a laser source of about a millisecond may cause heating of the material. A laser pulse of about femto seconds to nano seconds with the appropriately tuned light absorbing material allows for release of the entity without undue heating.

For some applications, it may be desirable to provide two or more core-shell materials in accordance with the present invention. When a plurality of core-shell materials are provided, each of the core-shell materials may be exposed to electromagnetic radiation individually or together, to release the encapsulated entities.
In one embodiment, when an application requires the presence of two or more core-shell materials, it may be desirable for some of the core-shell materials to have different encapsulated entities. Preferably, at least one of the two-core shell materials in a composition comprising a plurality of core-shell materials have different encapsulated entities. When the shells of the core-shell materials are ruptured on exposure to electromagnetic radiation of predetermined wavelength and/or intensity, the different encapsulated entities are released at the same time. In one preferred embodiment, two or more particles of core-shell material comprising at least two different encapsulated entities are brought into close proximity to each other and are exposed to electromagnetic radiation to cause release and mixture of the entities. More preferably, the two or more particles of core-shell material are brought into direct contact with each other.

In another embodiment, at least two core-shell materials in a composition comprising a plurality of core-shell materials comprise electromagnetic radiation absorbing materials incorporated in the shell that are tuned so that the materials are capable of absorbing different wavelengths and/or intensities of electromagnetic radiation. The at least two core-shell materials may encapsulate the same type of entity or different types of entities. Exposure of the at least two core-shell materials to different wavelengths and/or intensities of electromagnetic radiation, will cause the release to the encapsulated entities at different times. This may be advantageous where sequential release of the entities is desired.

The present invention enables a high degree of control and selectivity to be achieved in the release of entities for desired application. The tuneable absorption properties of the electromagnetic radiation absorbing material within the core-shell material and its electromagnetic radiation addressable nature coupled with the ability to use electromagnetic radiation beams of narrow diameters enables the user to for example, select a single core-shell material form a plurality of core-shell materials and expose it to electromagnetic to cause the release of an encapsulated entity at a desired time.
Applications

The core-shell material has particular application for the encapsulation of pharmaceuticals or chemical entities for utilisation in the body. This is particularly so, given that it is envisaged that it is possible to target particular sites within the body and control the timing and quantity of release of an entity such as a pharmaceutical substance. The specificity of the site to which an entity such as a pharmaceutical may be directed within the body and the control of the release of the entity is considered to be a particular advantage of the core-shell material of the present invention.

The tunability of the electromagnetic radiation absorbing material combined with suitable pulsed electromagnetic radiation sources enables the release of an encapsulated entity, such as a drug, under desirable energy conditions without significant heating, thus avoiding potential damage to the organ or the drug. This has particular advantages in the treatment of conditions on organs, which may be damaged by the application of heat.

The core-shell material may also have application in biomedical research. That is, it is possible to chemically alter single cells or modify cells, or inject a gene into a cell by targeting cells with the core-shell material and releasing a functional biomaterial at the appropriate time. By attaching core-shell materials to a cell, it may be possible to open the cell to enable a gene to be placed within the cell.

The core-shell material may also be used in other non biological applications. For example, they may find utilisation in chemical applications where a chemical reaction can be controlled by delaying the chemical reaction until such time that a chemical is released by exposure of the core-shell material to electromagnetic radiation such as light activation. That is, mixing of the chemical entities can be delayed. This can be achieved in a number of ways, for example a number of different chemicals may be encapsulated in different core-shell materials, which are tuned to different wavelengths and/or intensities of electromagnetic radiation. Therefore they can be multi-
sequentially released. Another way is to provide different entities in a single core-shell material. These uses may also have application for medicinal purposes, where more than one drug may be applied sequentially by controlling the release of two or more drugs.

These concepts also have application in microchips, where reaction occurs in micro fluid channels where the chemicals can not be mixed. The core-shell material can be in specific channels and the release of the chemical reaction can be timed.

The range of applications of such electromagnetic radiation addressable materials is broad in that they can be utilised in a number of environments where it is desirable to control the release of the encapsulated content.

**Brief Description of the Figures**

Figure 1 shows the basic concept and extinction spectra of core-shell materials in accordance with the present invention.

Figure 2 shows a plot in which the proportion of core-shell materials damaged versus radiant exposure from either an 8 ns Nd:YAG laser pulse at 532 nm or a 10 ns Nd:YAG laser pulse at 1064 nm.

Figure 3 shows scanning electron microscope (SEM) images and transmission electron microscope (TEM) images of the hollow core-shell material at various stages of the experiments.

Figure 3 shows (A) SEM image of the hollow core-shell material before irradiation, (B) SEM image of the hollow core-shell material after moderate radiant exposures (30mJ/cm²) and (C) SEM image of the hollow core-shell material after radiant exposures of 50mJ/cm².
Figure 4A shows light microscope images of a representative lysozyme-loaded core-shell material before and after irradiation by a Nd:YAG laser pulse (10 ns, 1064 nm) of radiant exposure 50mJ/cm².

Figure 4B shows a plot of lysozyme substrate extinction (at 450 nm) versus time for: encapsulated crystalline samples (control- no irradiation or mechanical release; circles), laser induced release (rectangles), and mechanically-induced release (squares).

Figure 5 shows the chemical structure of FITC-dextran

Figures 6 and 7 show the core-shell material loaded with FITC-dextran at pH 3 and 5, respectively, at 100x and 20x magnifications.

Figure 8 shows the chemical structure of R6G

Figure 9 shows the chemical structure of DMAP

Figure 10 shows the fluorescence emission spectra of samples 3.1 (8) and 5.1 (9) listed in Table 1.

Figure 11 shows the fluorescence emission spectra of samples 5.1 (10) and 6.1 (11) listed in Table 1.

Figure 12 shows the fluorescence emission spectra of samples 5.2 (12) and 5.3 (13) listed in Table 1.

Figure 13(a) shows the fluorescence emission spectra of samples 2.3 (14) and 4.3 (15) listed in Table 1.

Figure 13(b) shows the fluorescence emission spectrum of sample 4.3 (16) and fluorescence excitation spectrum of sample 4.3 (17) listed in Table 1.
Figure 14(a) shows the fluorescence emission spectra of samples 2.1 (18), 2.2 (19), and 2.3 (20) listed in Table 1.

Figure 14(b) shows the fluorescence emission spectra of samples 4.1 (21), 4.2 (22), and 4.3 (23) listed in Table 1.

Figure 15 shows the fluorescence emission spectra of samples 6.1 (24) and 8.1 (25) listed in Table 1.

Figure 16 shows TEM images of DMAP-gold nanoparticle coated, dried (deflated) hollow core-shell material.

Figure 17 shows extinction spectrum of DMAP-gold encapsulated lysozyme crystals. The insert shows a fluorescence microscope image of the core-shell material before DMAP-gold coating after using FITC-labeled PAH for LbL coating of the crystals.

Figure 18 shows absorption spectrum of water and the most important chromophores of tissue, with the tuning possibilities of spherical core shell structures like hollow core-shell material indicated.

Figure 20 shows TEM images of encapsulated and DMAP gold nanoparticle coated MF particles after irradiation using 532 nm light; 10 ns pulses.

Figure 21 shows the extinction spectra of irradiated and non irradiated core-shell material after irradiation using 532 nm and at 1064 nm radiation.

Figure 22 shows light microscopy images of core-shell material functionalized with the help of a lipid layer coated onto the surface and a subsequent binding of recognizing structures to this lipid layer.
Detailed Description of Preferred Embodiments

With reference to Figure 1, the basic concept and extinction spectra of an optically addressable core-shell material is shown. The core-shell material (A) containing a releasable entity consists of a shell in which electromagnetic radiation absorbing material like gold nanoparticles is incorporated. The entity is released by irradiating the entity loaded core-shell material with a short (fs to ns) laser pulse. (B) Schematic of a cross-section of the shell (not to scale). The shell comprises multiple polyelectrolyte layers (1), gold nanoparticles (2), an outer lipid bi-layer (3), and recognizing surface molecules (4). (C) Extinction spectra of 1.38 µm core-shell material (normalized at 450 nm) incorporating 1 layer of gold nanoparticles (5) or three layers of gold nanoparticles (6) in comparison to single 6 nm DMAP stabilized gold nanoparticles (7). The extinction spectra show that it is possible to tune the plasmon resonances of the gold loaded shell to the near infrared region where most drugs and tissues do not show strong electromagnetic radiation absorption.

The following examples are presented in a way that the underlying scientific train of thought can be easily understood in each example.

Example 1
Response of addressable core-shell material to laser irradiation

The first stage involved in the construction of the delivery system for the controlled release of at least entity is determining the optimal laser parameters for addressing the core-shell material. In this work, melamine formaldehyde (MF) particles of diameter 1.38±0.01 µm were used as templating cores to produce monodisperse core-shell material. This relatively small particle size was selected because the ability of crossing barriers within the body increases with decreasing drug carrier size. A seven-layer polyelectrolyte shell was constructed around each of the templating cores by the sequential deposition of the strong polyanion poly(sodium 4-styrenesulfonate) (PSS, average MW 70 kDa) and the relatively weak polycation poly(allylamine hydrochloride) (PAH, average MW 70 kDa) onto the positively charged MF particle surface, as described previously (see reference 1 and supporting material). This
polyelectrolyte pair was chosen as it is one of the most widely used for producing multi-layer films on planar and colloidal surfaces (reference 2). The core-shell material may however be fabricated from biodegradable polymers. Following the layer-by-layer self-assembly, 6 nm gold nano particles (NPs) stabilized by the ligand 4-(dimethylamino)pyridine (DMAP) (reference 3) were infiltrated into the polyelectrolyte shell, and then two additional layers (PSS, PAH) were adsorbed. DMAP was employed as the stabilising ligand because it readily dissociates from the gold nanoparticles after they penetrate the polyelectrolyte shell and after water washing, resulting in a relatively high gold loading and a final DMAP amount in the core-shell material below the detection limit. Hollow core-shell materials were prepared by treating the core-shell material with 0.1M hydrochloric acid to decompose the templating MF cores. These hollow core-shell materials were used to study the properties of the polyelectrolyte-gold shell with regard to laser induced damage in further detail. The extinction spectrum recorded for the non-irradiated core-shell material is shown in Figure 1.

The maximum of the broad extinction increasing from 450 nm up to 800 nm and then decreasing down to 1200 nm was red-shifted relative to the single gold-NP extinction. Consequently, several different laser sources may be used to address the core-shell material, such as Ti-Sapphire lasers (800 nm) or Nd-YAG lasers (532 nm or 1064 nm). Secondly, no absorption peak of individual gold nanoparticles was observed, which suggests that the gold is closely packed within the shell. With reference to Figure 3 (see insert A), the transmission electron microscopy (TEM) images of the non-irradiated hollow core-shell material showing only the gold nanoparticle structures within the shell because of their high electron contrast compared to the polymer structures confirmed the apparent high density of the gold packing. Thirdly, the extinction intensity in the NIR increased with increasing gold density (shell thickness), as expected. The extinction spectrum recorded for the non-irradiated hollow core-shell material (see supporting material) was very similar to that recorded for the non-irradiated core-shell material, the main difference being that the red-shift was less pronounced.
Having established which laser sources are the most appropriate, the next step was to irradiate the core-shell material to investigate the various laser parameters involved. In a first series of laser experiments, core-shell materials were immobilised onto glass slides and subjected to increasing radiant exposure from either an 8 ns Nd-YAG laser pulse at 532 nm or a 10 ns Nd-YAG laser pulse at 1064 nm. In order to estimate the fraction of core-shell material damaged by a given radiant exposure, a light microscope was used to count the number of intact core-shell material on the glass slide before and after irradiation. With reference to Figure 2, the results obtained show that to visually damage 50% of the core-shell material, the radiant exposure required with the 1064 nm laser pulse is roughly 20 mJ/cm² greater than that required with the 532 nm laser pulse. The light microscope images presented in Figure 2 illustrate typical morphological changes observed for radiant exposures above 10 mJ/cm² namely bleaching of the core-shell material (30mJ/cm² image), clustering of the core-shell material on the glass slide (50 mJ/cm² image), and desorption of the core-shell material from the glass slide (100 mJ/cm² image).

Figure 2 shows in plot the proportion of core-shell material damaged versus radiant exposure from either an 8 ns Nd:YAG laser pulse at 532 nm or a 10 ns Nd:YAG laser pulse at 1064 nm. To damage a specified percentage of the core-shell material, the radiant exposure required with the 1064 nm laser pulse is greater than that required with the 532 nm laser pulse. The light microscope image insets illustrate the typical morphological changes observed: A) intact core-shell material, B) bleaching of the core-shell material (30mJ/cm², C) clustering of the core-shell material on the glass slide (50mJ/cm²), and D) desorption of the core-shell material from the glass slide (100 mJ/cm²).

The results of these laser experiments suggest that the core-shell material can be addressed by a single laser pulse of reasonable radiant exposure, which would be suitable for medical applications (reference 4). For example in laser skin treatment and tattoo removal radiant exposures up to 650mJ/cm² are applied using short pulses (references 5, 6). Although these pulses may induce particle mediated damage to the skin the benefits would
potentially outweigh the side effects. Therefore in case of cancer treatment the benefits using the core-shell material are even more obvious.

In a second series of laser experiments designed for irradiating larger amounts of core-shell material, an aqueous dispersion of core-shell material in a 1 cm cuvette equipped with a magnetic stirrer was irradiated (Nd-YAG laser pulse (10 ns, 1064 nm, variable radiant exposure) for 3 mins at a frequency of 10 Hz; An area of 5mm in diameter was irradiated to ensure that each particle was exposed to at least one laser pulse). TEM images of the irradiated core-shell material revealed that while the MF cores suffered no obvious damage like cracking or shrinkage even with radiant exposures up to ten times that at which morphological changes in the immobilized core-shell material were first observed under the light microscope (see supporting material), holes had developed within the polyelectrolyte-gold shells. These TEM images also support the observation made in the first series of experiments, notably laser irradiation does not cause core-shell material in solution to cluster. In order to further investigate how laser irradiation affects the polyelectrolyte-gold shell, a third series of laser experiments was conducted on an aqueous dispersion of hollow core-shell material.

With reference to Figure 3 scanning electron microscope (SEM) images and transmission electron microscope (TEM) images of the hollow core-shell material at various stages of the experiments are shown. Before irradiation, the hollow core-shell materials were spherical in solution but resembled deflated balloons after they were dried down for the electron microscope images (see insert A of Figure 3). The distribution of gold nanoparticles, which are visible as a bright electron dense structure within the invisible polymer shell was homogeneous (insert i). After moderate radiant exposures (20 mJ/cm²), the outlines of individual hollow core-shell material could still be discerned (see insert B of Figure 3). The contrast and distribution of the gold nanoparticles changed notably which may be interpreted as formation of aggregates of varying sizes within the shells (insert ii). After radiant exposures of 50mJ/cm² and higher, the outlines of individual hollow core-shell materials were no longer readily discernable (see insert C of Figure 3), and the shells were completely
porated (insert iii). The results of these laser experiments show that pores may be induced within the shell.

Figure 3 shows (A) SEM image of the hollow core-shell material before irradiation: the hollow core-shell material resemble deflated balloons after being dried for electron microscopy. The inset shows a TEM image of a typical hollow core-shell material before irradiation: Due to the high contrast of the gold nanoparticles only the bright gold nanoparticles can be seen in the insets and the shell is nearly invisible. The distribution of gold nanoparticles within the shell is relatively homogeneous. (B) SEM image of the hollow core-shell material after moderate radiant exposures (30mJ/cm²): the outlines of individual hollow core-shell material can still be discerned. The inset is a corresponding TEM image: the gold nanoparticles have formed aggregates of varying sizes within the shell. (C) SEM image of the hollow core-shell material after radiant exposures of 50mJ/cm² and higher: the outlines of individual hollow core-shell material are no longer readily discernable. The inset is a corresponding TEM image: the shell is highly porated.

As the MF cores suffered no obvious damage upon irradiation (see supporting material) and MF is not a particularly stable material, the drugs are not likely to be affected by that treatment.

Example 2
Release of enzymes from the core-shell material

The construction of the core-shell material involved studying the release of a model drug from therein to determine whether the laser irradiation affects the activity of the drug. The protein lysozyme was selected for this purpose as it has been well researched with regard to thermal and chemical denaturation (references 7, 8). In order to achieve the highest possible loading of the model drug, lysozyme crystals were prepared and encapsulated via the approach described for the MF templating cores. The crystallisation conditions employed produced mainly cubic and tetrahedral crystals, with a broad size distribution ranging from 500 nm up to 10 μm (maximum of size distribution at 2 μm). Larger crystals were removed in a mild centrifugation step (100 g, 1 min)
following crystallization. Importantly, the polyelectrolytes were solubilized in the same buffer solution used for lysozyme crystallization to ensure that the lysozyme crystals did not dissolve during the encapsulation process (see supporting material). As expected, the extinction spectrum recorded for the lysozyme crystals encapsulated by polyelectrolyte-gold shells was relatively broad due to the different crystal sizes and shapes within the sample (see supporting material).

In order to ascertain whether laser-induced release affects the activity of lysozyme, the encapsulated crystals were irradiated using a Nd-YAG laser pulse (10 ns, 1064 nm) of high radiant exposure (50 mJ/cm²) to ensure that all the core-shell material were addressed. Afterwards the sample was mixed with the strongly scattering bacterium micrococcus lysodeikicus (i.e. a substrate for the enzymatic action of lysozyme (reference 9)).

With reference to Figure 4A, light microscope images of a representative lysozyme-loaded core-shell material before and after irradiation by a Nd:YAG laser pulse (10 ns, 1064 nm) of radiant exposure 50mJ/cm² is shown. With reference to Figure 4B, a plot of lysozyme substrate extinction (@ 450 nm) versus time for: encapsulated crystalline samples (control- no irradiation or mechanical release; circles), laser induced release (rectangles), and mechanically induced release (squares) is shown. These data suggest that the lysozyme-loaded core-shell material were effectively leakage-proof in the absence of initiated damage, and that the loss in lysozyme activity caused by the laser pulse was comparable to that caused by crushing the core-shell material open.

Extinction spectra were recorded at a series of time intervals after the laser-induced release to monitor the amount of bacteria remaining in the sample, as this provides a measure of the amount of bacteria digested by the lysozyme and hence the activity of the released lysozyme. The two control experiments were a non-irradiated mixture of intact encapsulated crystals plus bacteria (i.e. no release), and a non-irradiated mixture of mechanically crushed encapsulated crystals plus bacteria (i.e. mechanically-induced release). In the
latter control experiment, encapsulated crystals were crushed via a polymer ball inserted into the Eppendorf sample tube, and then vortexed for 15 mins before introducing the bacteria.

Figure 4B shows the decay in the extinction at 450 nm with time for each of the three experiments: no release induced, laser-induced release and mechanically induced release. Note that these plots are directly comparable as the same amounts of encapsulated crystals and bacteria were employed in each experiment. When no release was induced, the extinction was essentially constant over the time range investigated. This implies that the lysozyme-loaded core-shell material were effectively leakage-proof despite the high osmotic pressure (reference 10) that developed within due to the change in buffer conditions from 1.4 M NaCl, pH 4.7 (during crystallisation and encapsulation) to 153 mM PBS, pH 7 (during the assay). When release was induced via laser irradiation, the extinction decreased abruptly and then leveled out to the mechanical release induced case. This indicates that a significant proportion of the bacteria was digested following the laser-induced release, and hence the lysozyme activity remained stable after the laser irradiation. When release was induced mechanically, the decay in the extinction at 450 nm with time was almost identical to that for the laser-induced release case. This indicates that any loss in lysozyme activity caused by the laser pulse is similar to that caused by mechanically crushing the core-shell material open, the latter being a technique widely used for preparing seed crystals for protein crystallization (reference 11). The above data clearly demonstrates that encapsulated biologically active compounds can be released via the laser-induced core-shell material release mechanism.

Example 3

Targeting of core-shell material using antibodies

To demonstrate that the core-shell material can be coated with lipid layers and bio-recognition molecules attached to the surface we performed a lipid coating (reference 12) of the core-shell material, which was functionalized covalently with antibodies afterwards. Using DPH-staining in fluorescence microscopy it was shown that lipids cover all core-shell material (see supporting
material). A primary antibody (mouse IgG) was coupled to the core-shell material using the standard EDC coupling, as reported by Hermanson (reference 13). After blocking and washing the homogenous coverage of the core-shell material with the secondary fluorescence tagged antibody can be seen (see supporting material). As the lipid and protein coupling to the surface is not limited to antibodies, and works straightforward for any amine and carboxylic acid containing compounds, it can be concluded that the core-shell material are suitable for drug delivery.

Example 4
Release of FITC-Dextran

4.2 Drug Loading and Release

4.2.1 Preparation of hollow core-shell material

The second stage of this work involved investigating the loading and release behaviour of multilayer PSS/PAH core-shell material. It was necessary therefore to prepare some hollow core-shell material.

The hollow core-shell material were fabricated from particles prepared (namely, 3.14 μm MF particles with 8 PSS/PAH layers) by using HCl to decompose the templating cores, and then adsorbing an additional layer of PSS to yield core-shell material with a negative surface charge. These relatively ‘large’ core-shell material were used in the subsequent loading experiments because, although they are less suitable for drug carrier application, they are much more readily observed under the light microscope.

4.2.2 Loading with FITC-dextran

The next step was to load the multilayer PSS/PAH core-shell material with FITC-dextran. The chemical structure of FITC-dextran is shown in Figure 5.

FITC-dextran comprises a polymer of anhydroglucose with FITC randomly conjugated to the free hydroxyl groups at a frequency of 0.003 to 0.02 moles of FITC per mole of glucose (Figure 5). Dextrans of $M_w$ 2 000 to 10 000
exhibit the properties of an expandable coil. Hence, dextran (average \( M_w 10500 \)) was selected for this work as it provides a convenient model of high molecular weight therapeutic agents with a helical structure, namely DNA. The dextran was obviously labeled with FITC to permit investigation of its loading and release.

Sukhorukov and coworkers (2001) have shown that the permeability of the multilayer PSS/PAH shell to FITC-dextran can be tuned via pH: below pH 6, FITC-dextran permeates the core-shell material wall, while above pH 8, FITC-dextran is excluded from the core-shell material interior; at a pH value between 6 and 8, 'open' and 'closed' core-shell material exist simultaneously. Therefore, the loading experiments involved incubating the hollow core-shell material in a solution of FITC-dextran at low pH (3 or 5), and then increasing the pH to 9 to remove the excess FITC-dextran from the bulk solution.

With reference to figures 6 and 7, the core-shell material loaded with FITC-dextran at pH 3 and 5, respectively, at 100x and 20x magnifications is shown. It would appear, within the resolution of the light microscopy technique, that roughly the same degree of loading is achieved at the two different pH values. In addition, a large proportion of the core-shell material are deformed whether the loading is carried out at pH 3 or pH 5; no such core-shell material deformation was observed by Nayak et al. (2003) and Sukhorukov et al. (2001) in their equivalent studies.

In order to determine the point at which the core-shell material become damaged, a sample from the core-shell material stock solution was treated with the red dye R6G (Figure 8) to permit visualisation of the hollow core-shell material, and the loading experiments were repeated to obtain samples at the low pH stage and at the high pH stage (before removal of the excess FITC-dextran). Examination of these samples under the light microscope revealed that while the hollow core-shell material in the stock solution are mostly intact, a large proportion of the core-shell material are deformed at the low pH stage and at the high pH stage. This implies that the core-shell material damage occurs primarily during the loading process (specifically the low pH stage) rather than
in the subsequent centrifugation/washing cycles. Perhaps changes in polyelectrolyte charge upon lowering the pH, combined with the force of the bulky FITC-dextran molecules diffusing through the shell, are sufficient to deform the polyelectrolyte network irreversibly in the case of the shape altered core-shell material.

Therefore, multilayer PSS/PAH core-shell material can be loaded with FITC-dextran to roughly the same extent at pH 3 and pH 5. However, a large proportion of the core-shell materials become shape altered in the loading process at each pH.

4.2.3 Laser-induced release

The final step in this second stage of the work was to investigate the laser-induced release of FITC-dextran from the core-shell material loaded at pH 3 and pH 5.

Prior to the laser experiments, the loaded core-shell material were incubated (either for a couple of hours or overnight) in DMAP-Au, i.e. a solution of gold nanoparticles stabilised by the ligand 4-(dimethylamino)pyridine (Figure 9). This novel colloidal system (Gittins et al., 2001 and 2002) was employed to coat the multilayer PSS/PAH shells with gold, and hence render the core-shell material optically addressable.

The laser experiments involved irradiating the samples for approximately five minutes with a series of nanosecond laser pulses. The associated ‘controls’ comprised non-irradiated loaded core-shell material, with and without the gold coating.

To investigate the loading and release behaviour of the core-shell material, fluorescence emission spectra were then recorded for each (i) control/irradiated sample, (ii) supernatant produced by centrifuging (i) at low speed (800 g for 5 minutes), and (iii) supernatant produced by centrifuging (ii) at high speed (10 000 g for 10 minutes). Table 1 details the various samples for which spectra were recorded.
Table 1. Samples for which fluorescence spectra were recorded

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH during loading</th>
<th>Au coating?</th>
<th>Au incubation time</th>
<th>Irradiated?</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>3</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>capsule dispersion</td>
</tr>
<tr>
<td>1.2</td>
<td>3</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>1st supernatant</td>
</tr>
<tr>
<td>1.3</td>
<td>3</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>2nd supernatant</td>
</tr>
<tr>
<td>2.1</td>
<td>5</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>capsule dispersion</td>
</tr>
<tr>
<td>2.2</td>
<td>5</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>1st supernatant</td>
</tr>
<tr>
<td>2.3</td>
<td>5</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>2nd supernatant</td>
</tr>
<tr>
<td>3.1</td>
<td>3</td>
<td>✓</td>
<td>short</td>
<td>×</td>
<td>capsule dispersion</td>
</tr>
<tr>
<td>3.2</td>
<td>3</td>
<td>✓</td>
<td>short</td>
<td>×</td>
<td>1st supernatant</td>
</tr>
<tr>
<td>3.3</td>
<td>3</td>
<td>✓</td>
<td>short</td>
<td>×</td>
<td>2nd supernatant</td>
</tr>
<tr>
<td>4.1</td>
<td>5</td>
<td>✓</td>
<td>short</td>
<td>×</td>
<td>capsule dispersion</td>
</tr>
<tr>
<td>4.2</td>
<td>5</td>
<td>✓</td>
<td>short</td>
<td>×</td>
<td>1st supernatant</td>
</tr>
<tr>
<td>4.3</td>
<td>5</td>
<td>✓</td>
<td>short</td>
<td>×</td>
<td>2nd supernatant</td>
</tr>
<tr>
<td>5.1</td>
<td>3</td>
<td>✓</td>
<td>short</td>
<td>✓</td>
<td>irradiated fluid</td>
</tr>
<tr>
<td>5.2</td>
<td>3</td>
<td>✓</td>
<td>short</td>
<td>✓</td>
<td>1st supernatant</td>
</tr>
<tr>
<td>5.3</td>
<td>3</td>
<td>✓</td>
<td>short</td>
<td>✓</td>
<td>2nd supernatant</td>
</tr>
<tr>
<td>6.1</td>
<td>5</td>
<td>✓</td>
<td>short</td>
<td>✓</td>
<td>irradiated fluid</td>
</tr>
<tr>
<td>6.2</td>
<td>5</td>
<td>✓</td>
<td>short</td>
<td>✓</td>
<td>1st supernatant</td>
</tr>
<tr>
<td>6.3</td>
<td>5</td>
<td>✓</td>
<td>short</td>
<td>✓</td>
<td>2nd supernatant</td>
</tr>
<tr>
<td>7.1</td>
<td>3</td>
<td>✓</td>
<td>long</td>
<td>✓</td>
<td>irradiated fluid</td>
</tr>
<tr>
<td>7.2</td>
<td>3</td>
<td>✓</td>
<td>long</td>
<td>✓</td>
<td>1st supernatant</td>
</tr>
<tr>
<td>7.3</td>
<td>3</td>
<td>✓</td>
<td>long</td>
<td>✓</td>
<td>2nd supernatant</td>
</tr>
<tr>
<td>8.1</td>
<td>5</td>
<td>✓</td>
<td>long</td>
<td>✓</td>
<td>irradiated fluid</td>
</tr>
<tr>
<td>8.2</td>
<td>5</td>
<td>✓</td>
<td>long</td>
<td>✓</td>
<td>1st supernatant</td>
</tr>
<tr>
<td>8.3</td>
<td>5</td>
<td>✓</td>
<td>long</td>
<td>✓</td>
<td>2nd supernatant</td>
</tr>
</tbody>
</table>

Examination of the fluorescence emission spectra obtained revealed the following:

- The fluorescence from an irradiated sample is considerably stronger than that from the corresponding non-irradiated sample (see Figure 10). This confirms that core-shell material were indeed loaded with FITC-dextran.

- There is little difference between the spectra of the pH 3 samples and the corresponding spectra of the pH 5 samples (see Figure 11). This implies that the degree of loading achieved at the two different pH values is very similar, as suggested earlier (section 4.2.2).

- The fluorescence intensity from the first supernatant is roughly equal to that from the second supernatant (see Figure 12). This indicates that any suspended material in the original sample is removed almost entirely during the first centrifugation.
• For samples without the gold coating, the wavelength of maximum fluorescence emission (max) was ~ 520 nm, as expected for FITC-dextran; whereas for samples with the gold coating, max was ~ 510 nm (Figure 13). This small blue-shift suggests that the vibrational structure of FITC-dextran increases slightly in the presence of gold.

• For non-irradiated loaded core-shell material without the gold coating, the fluorescence from the original sample is markedly stronger than that from the two supernatants (see Figure 14a); whereas for non-irradiated loaded core-shell material with the gold coating, the fluorescence intensity from the original sample is very similar to that from the two supernatants (see Figure 14b). This implies that the gold coating effectively 'quenches' the fluorescence from the FITC-dextran weakly bound to the multilayer PSS/PAH shells, and 'masks' the fluorescence from the FITC-dextran within the core-shell material.

• Following irradiation, the fluorescence from a long-gold-incubation sample is significantly weaker than that from the corresponding short-gold-incubation sample (see Figure 15). This suggests that the longer the gold incubation, the denser the gold coating, the higher the gold content of the sample after irradiation, the greater the quenching effect, and hence the weaker the fluorescence. The concept that the density of the gold coating increases with incubation time is evidenced by the appearance of the centrifugation pellets during sample preparation: the short-gold-incubation pellet exhibited the characteristic ruby red colour of colloidal gold, which has been attributed to the surface plasmon absorption of the nanoparticles (Mie, 1908); whereas the long-gold-incubation pellet appeared black due to the relatively dense packing of nanoparticles within the gold coating.

On the basis of the fluorescence emission spectra obtained, the percent loading was estimated as 280% and 260% for the core-shell material loaded at pH 3 and pH 5, respectively. Allowing for the numerous sources of experimental error involved, plus the various simplifying assumptions underlying the calculations, these figures are quite reasonable since they are out by less than a factor of ten. In addition, the fact that the two figures are very similar
further substantiates the conclusion that roughly the same degree of loading is achieved at the two different pH values.

Clearly, the use of fluorescence spectroscopy here has provided valuable information on not only the loading and release behaviour of the core-shell material, but also on the physicochemical effects of the gold coating.

Supporting Material

Multi-layer Shell Formation

The core-shell material were produced based on the self-assembly of polyelectrolytes and nanoparticles following the general protocols reported by Caruso et al. and Gittins et al. (references 1, 3, 14, 15). Briefly, an aqueous PSS solution (concentration 1mg/ml, NaCl concentration 0.5 M) was added to an aqueous suspension of MF particles (final PSS concentration 0.5mg/ml) and PSS was allowed to adsorb on the colloids for 20 min with continual stirring. The dispersion was then centrifuged for 3 min at 1000 g. The supernatant solution was replaced with water, and the particles were redispersed by shaking. The cleaning process (centrifugation / supernatant exchange/redispersion) was repeated a further two times to remove non-adsorbed PSS. PAH (concentration 1mg/ml, NaCl concentration 0.5 M) was subsequently adsorbed onto the PSS-coated MF particles using the same procedure. The whole process was repeated a further three times to produce an 8-layer PSS/PAH film on the MF particles.

Layer-by-Layer (LbL) Encapsulation of Protein Crystals

Recrystallisation Procedure

Lysozyme was crystallized in a batch crystallization process following a protocol from Hampton Research Inc., which was derived from the basic protocols of Osaterman (references 11, 16). Lysozyme was purchased from Fluka. Briefly, equal amounts of lysozyme solution (50mg/ml in 0.1M sodium acetate, pH 4.8) were mixed with the following reagent (8% w/v sodium chloride in 0.1M sodium acetate, pH 4.8) and incubated at 20°C. The crystal growth was monitored microscopically (40x Objective). Once the crystals reached a medium size of 2 µm the crystal suspension was washed with the
storage buffer (4-5% w/v sodium chloride in 0.1M sodium acetate, pH 4.8). The crystals stopped growing in this buffer and were stable for performing the Layer-by-Layer (LbL) assembly on the crystal surface.

The polymer layers were then assembled onto the enzyme crystals by the sequential deposition of poly(sodium 4-styrenesulfonate) (PSS), 70 kDa MW and poly(allylamine hydrochloride) (PAH), 70 kDa MW. The first layer was deposited by adding a 0.25 mL aliquot of a 5mg/ml aqueous PSS solution (containing 4% w/v NaCl in sodium acetate 0.1 M, pH 4.8) to 0.2 ml of the crystal suspension, occasionally shaking the suspension, and allowing 25 minutes for adsorption. The excess polyelectrolyte was removed by three repeated centrifugation (500 g, 1 min, 20°C) buffer wash/redispersion cycles. The next layer, PAH, was deposited from a 5mg/ml solution in the same buffer as used for PSS, using the same procedure and conditions. Subsequent alternating PSS and PAH layers were deposited in an identical fashion until the desired number of polymer multilayers was achieved. A homogenous coating was proven by using 0.1% FITC-PAH mixed into the PAH solution. With reference to Figure 2, fluorescence microscopy images shown in the insert of the Figure that illustrates that the protein crystals are homogenously covered with these polyelectrolytes.

**Gold Nanoparticle Coating**

Core-shell material were coated with gold nanoparticles following the general protocol of Gittins et al. (references 3, 15). A suspension of core-shell material in water was added to DMAP-stabilized 6 nm gold particles (NPs) and the NPs were allowed to adsorb on the core-shell material overnight under continual stirring. The dispersion was then centrifuged 5 times for 5 min at 500 g, the supernatant solution was replaced with appropriate buffer, and the gold nanoparticle coated core-shell material were redispersed by shaking. Figure 16 shows TEM images of DMAP-gold nanoparticle coated, dried (deflated) hollow core-shell material. The coating is extremely homogenous.

To minimise adhesion of the gold coated core-shell material to each other and to plastic ware, three additional layers of PSS/PAH/PSS were
subsequently adsorbed as described before. Extinction spectra of the hollow core-shell material are shown in Figure 1 of the letter. The extinction spectrum of the gold-NP coated enzyme crystals is shown in Figure 17.

It should be noted that the red shift of the core-shell material extinction compared to the single DMAP-gold particles falls into the wavelength regime between 750 nm and 1000 nm, which is useful in biological or medical applications, as in this wavelength regime tissue penetration of light is the highest possible. This is shown in Figure 18 together with the principle tuning possibilities of hollow core-shell material and the most versatile laser wavelengths. Tissue data are derived from Venugopalan et al. (reference 17).

The red-shift depends mainly on the gold surface coverage of the core-shell material and the ratio of gold layer thickness to core-shell material diameter. A theoretical description of the redshift based on a full gold coverage can be found in the recent work of Prodan et al. (reference 18, 19). According to this work, a red-shift up to several micrometers can be expected for the geometries used for this report. The reason for a less pronounced and more broad shift is to be seen in the particular coverage of the core-shell material, which is not complete.

**Preparation of hollow core-shell material**

The hollow PSS/PAH core-shell material were prepared by exposing an aqueous suspension of DMAP-gold NP coated 1.38μm MF-core-shell particles to HCl (final HCl concentration 0.1 M) for 20 min. The dispersion was then centrifuged for 5 min at 5000 g, the supernatant solution was replaced with water, and the core-shell material were redispersed by shaking. To produce hollow core-shell material with a negative surface charge, a layer of PSS was subsequently adsorbed onto the 7-layer PSS/PAH hollow core-shell material, as mentioned before, by adding aqueous PSS solution (final PSS concentration 0.5mg/ml), allowing 20 min for PSS adsorption, and removing excess PSS (and residual MF oligomers) by four repeated centrifugation (5000 g, 5 min)/redispersion (in water) cycles. To observe the hollow core-shell material
under the light microscope, Rhodamine 6G solution was added to an aqueous suspension of core-shell material (final R6G concentration 1 μM).

**Lysozyme assay**

The rate of lysis of Micrococcus lysodeikticus was determined as outlined by Shugar (reference 9). Lysozyme activity can be measured as a decrease in turbidity at 450 nm at pH 7.0 and 25°C.

**Laser-induced release**

The morphological change of core-shell material upon irradiation with one pulse was studied using light microscopy by immobilizing core-shell material on a glass slide coated with polyelectrolyte layers (their outer layer was oppositely charged to the outer layer of the core-shell material). The backside of the 1mm thick glass slide was coated with a black varnish (Edding), which became damaged due to the irradiation and thus was a marker for finding the irradiated spots in subsequent investigation steps. These core-shell material coated slides were irradiated in a water filled glass cuvette. The laser (Continuum Inc., USA) was operated at high pump power (400 mJ per pulse IR output) for achieving a homogenous top head, multimode laser profile and was attenuated to the needed lower energies using an adjustable polarizing beam splitter for keeping the beam profile constant in all experiments. The setup is drawn schematically in Figure 19A.

Figure 19 shows the setup for irradiating the core-shell material with nanosecond laser pulses. A) For irradiating the core-shell material with 1 pulse, the core-shell materials were immobilized on a glass slide, which was coated with a varnish on the backside for detecting the irradiated areas. The slide was placed in a water filled cuvette. B) For irradiating larger sample volumes, core-shell materials were filled into a cuvette with a magnetic stirrer. Due to that stirrer they statistically passed the irradiated volume. Therefore they were irradiated with multiple pulses. For irradiating the core-shell material, a Q-switched Nd:Yag laser was used either at 532 nm or at 1064 nm. The beam diameter was 5mm and the top head beam profile was held constant by attenuating the beam using a polarizing beam splitter.
Irradiation of larger sample volumes was achieved by using stirred samples in a 1 cm (3 ml) cuvette for 5 min with a 10Hz series of 10 ns laser pulses applying a beam 5mm in diameter (see Figure 20). Due to the mixing, most of the core-shell material pass the beam once in that irradiation time (It was assumed complete mixing was achieved in the cuvette after 1 min, as determined by observing the time taken for a dye droplet to become homogeneously mixed through the solution). The associated controls comprised non-irradiated loaded core-shell material, with and without the gold coating.

Irradiation of gold nanoparticle coated MF-core core-shell material results in rupture and desorption of the shell without damaging the core as shown in TEM. Even using radiant exposures about 10 times (200 mJ/cm²) above the onset of morphology changes of the core-shell material (30 mJ/cm²) as observed by light microscopy, no significant damage to the cores was observed (see Figure 20).

With reference to Figure 20, TEM images of encapsulated and DMAP gold nanoparticle coated MF particles after irradiation using 532 nm light; 10 ns pulses is shown. Even for very high radiant exposures (10 times above the onset for an optically detectable shell modification) no obvious damage visible in TEM can be induced on the cores.

Irradiation of larger sample volumes enabled us to study the extinction properties after irradiation. With reference to Figure 21, the extinction spectra of irradiated and non irradiated core-shell material is shown. The irradiated core-shell material were either irradiated using 532 nm green light or 1064 nm NIR light. The extinction spectra after irradiation with the same radiant exposure of 50 mJ/cm² show the significant difference, that after green irradiation the spectrum changes to the spectrum characteristic for single gold nanoparticles. In contrast to that after NIR irradiation the spectrum shows characteristics of gold nanoparticle clusters with NIR extinction.

Using green light (532 nm) the extinction spectrum is changed towards an spectrum typical for larger single gold nanoparticles (compare Figure 1 or
literature (references 20,21)). Using NIR irradiation (1064 nm) results in a small increase in extinction around 520-600nm which is typical for single gold nanoparticles and nanoparticle clusters (compare (reference 22)) but in contrast to the irradiation using green light the NIR extinction does not vanish as effectively after irradiation with the same radiant exposure (50 mJ/cm²). No qualitative differences were observed in TEM images of these samples. In all images we observed the features shown in Fig.3, gold nanoparticle clustering, a changed size distribution of the gold nanoparticles, some gold nanoparticles free in solution and ruptured core-shell material. Therefore the change indicates that there are less single gold nanoparticles in the solution after NIR irradiation and that the gold remains associated with the remaining of the destroyed core-shell material. This may be due to a different damage mechanism and will be studied in the future work.

Functionalization with antibodies

Following general approaches on lipid coating (reference 12) of the core-shell material, the core-shell material were functionalized with a mixture of lipids forming a layer around the core-shell material, which was functionalized covalently afterwards. Using DPH-staining in fluorescence microscopy it was shown, that lipids cover all core-shell material (see Figure 22A and AA). The amine groups of the lipid coating were functionalized using FITC following a general FITC coupling protocol (reference 13). To make sure that only amine groups of the lipids and not of the PAH were functionalized, the core-shell material used in these experiments were built with PDMAC instead of PAH as a positive polyelectrolyte. FITC staining shows that the core-shell material are homogenously covered with functional groups for covalent protein coupling. In the last step a primary antibody (mouse IgG) was coupled to the core-shell material using the standard EDC coupling as reported by Hermanson (reference 13). Afterwards the core-shell material were blocked for 30 min using 10% w/v BSA and incubated for 30 min with a secondary FITC labeled antibody (rabbit anti mouse IgG) for 30 min in PBS. After washing the sample three times the homogenous coverage of the core-shell material with the secondary antibody can be seen (see Figure 22C). A schematic drawing of this functionalization and staining is shown in Figure 22D. As the lipid and protein coupling to the
surface is not limited to antibodies, and works straight forward for any amine and carboxylic acid containing compounds, it can be concluded that the core-shell material are suitable for drug delivery as soon as good recognizing structures are available for the desired target.

Figure 22 shows light microscopy images of core-shell material functionalized with the help of a lipid layer coated onto the surface and a subsequent binding of recognizing structures to this lipid layer. A, AA) A is a fluorescence microscopy image of DPH stained lipid coated core-shell material. By comparing this image to the bright light image it becomes clear, that all particles are covered with lipids. B shows an image of FITC coupled lipid coated core-shell material. The image shows that the core-shell material is homogenously covered with functional amine groups after the lipid coating. C shows an image of lipid coated core-shell material which were functionalized with a primary mouse IgG and incubated with a secondary FITC coupled rabbit anti mouse IgG. In this case the core-shell material are covered homogenously as well. The scheme of this functionalization is shown in Figure 22D.

In the experiments, it turned out that the core-shell material are strong and even pH changes between 4.8 and 11 will not open the core-shell material. This proved to be somewhat unexpected, as it can be expected that the enzyme crystals dissolve under these changing conditions and a huge osmotic pressure is acting on the shell. This was true also for changes in ionic strength between 150mM NaCl and 1.5M NaCl over the whole pH range. Therefore, it can be expected that such core-shell material are strong enough to withstand all physiological conditions without leaking.

It was successfully demonstrated that it is possible to build nanostructured addressable core-shell material for encapsulating chemical entities. It was shown that the core-shell material are extremely stable and that the release mechanism does not induce significant damage to the drugs. The release mechanism is fast enabling an opening of the core-shell material within 10 nanoseconds. The needed radiant exposure of 20mJ/cm at 1064nm is low in comparison to other medical laser applications used on skin.
Finally, it is to be understood that various other modifications and/or alterations may be made without departing from the spirit of the present invention as outlined herein.

5  **Instruments**

**Light microscopy**

The particles and core-shell material fabricated were studied by means of an Olympus BHS microscope equipped with a mercury lamp and Model BH2-RFL attachments for fluorescence microscopy, plus a Leica DC300 F digital camera for the acquisition of digital images. Samples were prepared by applying a small aliquot of the particle suspension to glass slides.

**Scanning Electron Microscopy (SEM)**

The surface morphology of core-shell material was examined using a Philips XL30 FEG field emission scanning electron microscope operated at an acceleration voltage of 2 kV. Samples were prepared by applying a small aliquot of the core-shell material suspension to glass wafers.

20  **Determination of core-shell material concentration**

The concentration of gold coated core-shell material in non-irradiated samples was determined using a Neubauer type hemocytometer. The graduated counting chamber was charged with 10 μl of the core-shell material suspension and then viewed under a light microscope (40x objective) to determine the core-shell material concentration.

**Materials**

The following materials purchased from Sigma-Aldrich if not otherwise stated were employed in the course of this work:

- aqueous poly(sodium styrenesulfonate) (PSS) solution average PSS Mw 70 000, PSS;
- aqueous suspension of melamine formaldehyde (MF) particles 1 wt %, core diameter 1.39 ± 0.06 μm, core HCl-decomposable purchased from microparticles GmbH Germany;
• water (resistivity 18.2 M.cm);
• aqueous poly(allylamine hydrochloride) (PAH) solution average PAH Mw 70 000, PAH;
• 1,2-didodecanoyl-sn-glycero-3-phosphoethanolamine (DLPE); ethanol;
• 1,6-diphenyl-1,3,5-hexatriene (DPH) solution concentration 0.1 mM; phosphate buffered saline (PBS) concentration 0.1 M, pH 7;
• fluorescein isothiocyanate (FITC) solution 1 mg of FITC per mL of 0.1 M PBS, pH 7;
• hydrochloric acid (HCl) concentration 1 M;
• primary antibody solution 1 mg of purified mouse immunoglobulin G (IgG) per mL of 10 mM PBS, sodium azide concentration 15 mM, pH 7;
• secondary antibody solution 15 mg of FITC labeled rabbit anti-mouse IgG per mL of 10 mM PBS, sodium azide concentration 15 mM, pH 7;
• bovine serum albumin (BSA) solution 1 mg of BSA per mL of 0.1 M PBS, pH 7;
• rhodamine 6G (R6G) chloride solution concentration 1 mM;
• solution of 4-(dimethylamino)pyridine stabilised gold nanoparticles (DMAPAu) average particle diameter 6 nm produced following the protocol reported by Gittins (3, 15);
• Lysozyme (Fluka Cas no : 62971)

General notes
(i) All experiments were performed at room temperature (20°C) unless stated otherwise.
(ii) All samples were stored in the fridge (4°C) to prevent gradual thermal degradation.
(iii) Samples involving lipid, antibody and/or FITC were prepared and stored in opaque centrifuge tubes to prevent photo-bleaching.
References


THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A process for the controlled release of at least one entity, said process including the steps of:
   (a) providing a core-shell material comprising:
       (i) a core comprising at least one releasable entity;
       (ii) a shell encapsulating the core; and
       (iii) at least one electromagnetic radiation absorbing material incorporated into the shell, and having tuneable absorption properties; and
   (b) exposing the core-shell material to electromagnetic radiation of a predetermined wavelength and/or intensity wherein the shell is ruptured upon exposure to the electromagnetic radiation causing the release of the at least one entity.

2. A process according to claim 1, wherein two or more core-shell materials are provided, wherein the entity in at least two of the core shell materials is different, and wherein the shells are ruptured upon exposure to electromagnetic radiation of predetermined wavelength and/or intensity to release the entities at the same time.

3. A process according to claim 1, wherein the releasable entity is selected from proteins, nucleic acids, carbohydrates, lipids, synthetic polymers, enzymes, drugs, reagents, dyes, vitamins, antioxidants and fluorophores; or a precursor of a drug, reagent, dye, fluorophore or nucleic acid; or a mixture of two or more thereof.

4. A process according to claim 1, wherein the shell is formed from a single or multiple layer of a polymer and/or lipids.

5. A process according to claim 1 wherein the polymer is a cationic or anionic polyelectrolyte.
6. A process according to claim 5, wherein the shell carries on its outer surface bio-recognition molecules selected from antibodies, nucleic acids, lectins, receptors and ligands or a mixture thereof.

7. A process according to claim 5 or 6, wherein the shell carries on its outer surface solubilisation molecules selected from ionic and non-ionic detergents, polyethylene glycol, hydrogel, and dextranes to render the core shell material dispersible.

8. A process according to claim 1, wherein the electromagnetic radioactive absorbing material incorporated into the shell is selected from metallic particles, quantum dots, chromophores, a metallic layer and metal ions, or a mixture thereof.

9. A process according to claim 8, wherein the thickness, length and/or width of the metallic particles or quantum dots is varied to enable tuning of absorption properties.

10. A process according to claim 8, wherein the metallic particles are gold or silver particles of 3 nm to 100 nm.

11. A process according to claim 8, wherein the metallic particles are ligand stabilised by a ligand selected from 4-di-methylaminopyridine (DMAP), mercaptoundecanoic acid (MUA) and tri-n-octylphosphine (TOPO).

12. A process according to claim 1, wherein the core shell material is a colloidal system.

13. A process according to claim 12, wherein the colloidal system is a suspension with a particle size of from 100 nm to 100 micrometer.

14. A process according to claim 13, wherein the particle size is 500 nm.
15. A process according to claim 12, wherein the colloidal system is monodisperse.

16. A process according to claim 1 wherein the core-shell material is in the form of an injectable suspension.

17. A process according to claim 1, wherein the core is crystalline, liquid, or an amorphous material, or a mixture thereof.

18. A process according to claim 1, wherein the electromagnetic radiation is infrared or near infrared radiation.

19. A process according to claim 1, wherein the electromagnetic radiation is monochromatic.

20. A process according to claim 1, wherein the electromagnetic radiation is a beam of small diameter capable of addressing a single particle of the core-shell material by illumination causing release of the entity from the single particle.

21. A process according to claim 20, wherein the diameter of the beam is a laser beam of from 100 nm to 10 micrometer.

22. A process according to claim 20, wherein two or more particles of the core-shell material containing at least two different entities in two particles are brought into close proximity to each other and exposed to electromagnetic radiation causing release and mixture of the entities.

23. A process according to claim 22, wherein the two or more particles are brought into direct contact with each other.

24. A process according to claim 1, wherein two or more core-shell materials are provided and may contain the same or different entity, and wherein the
at least two core-shell materials are capable of absorbing different intensities and/or wavelengths of electromagnetic radiation.

25. A core-shell material for the controlled release of at least one entity, said material comprising:
   (i) a core comprising at least one releasable entity,
   (ii) a shell encapsulating the core, and
   (iii) at least one electromagnetic radiation absorbing material incorporated into the shell capable and having tunable absorption properties;
   wherein upon exposure to electromagnetic radiation of a predetermined wavelength and/or intensity, the shell will be caused to rupture causing release of the at least one entity.

26. A delivery system for the controlled release of at least one entity, the system comprising at least one core-shell material, comprising:
   (i) a core of at least one releasable entity,
   (ii) a shell encapsulating the core,
   (iii) at least one electromagnetic radiation absorbing material incorporated into the shell and having tunable absorption properties; and
   (iv) an electromagnetic radiation source;
   wherein upon exposure to electromagnetic radiation of a predetermined wavelength and/or intensity the shell will be caused to rupture causing release of the at least one entity.

27. A core-shell material according to claim 25 or a delivery system according to claim 26, wherein the releasable entity is selected from proteins, nucleic acids, carbohydrates, lipids, synthetic polymers, enzymes, drugs, reagents, dyes, vitamins, antioxidants and fluorophores; or precursors of a drug, reagent, dye, fluorophore, nucleic acid; or a mixture of two or more thereof.
28. A core-shell material according to claim 25 or a delivery system according to claim 26, wherein the shell is formed from a single or multiple layers of a polymer and/or lipids.

29. A core-shell material according to claim 25 or a delivery system according to claim 26, wherein the polymer is a cationic or anionic polyelectrolyte.

30. A core-shell material according to claim 25 or a delivery system according to claim 26, wherein the shell also carries on its outer surface bio-recognition molecules selected from antibodies, nucleic acids, lectins, receptors, ligands or a mixture thereof.

31. A core-shell material according to claim 25 or a delivery system according to claim 26, wherein the shell material also carries solublisation molecules selected from ionic and non-ionic detergents, polyethylene glycol, hydrogels or dextrans to render the core-shell material dispersible.

32. A core-shell material according to claim 25 or a delivery system according to claim 26, wherein the core-shell material is a colloidal system.

33. A core-shell material according to claim 25 or a delivery system according to claim 26, wherein the colloidal system is a suspension having a particle size of from 100 nm to 100 micrometer.

34. A core-shell material according to claim 25 or a delivery system according to claim 26, wherein the particle size is about 500 nm.

35. A core-shell material according to claim 25 or a delivery system according to claim 26, wherein the colloidal system is monodisperse.

36. A core-shell material according to claim 25 or a delivery system according to claim 26, wherein the core-shell material is formed as an injectable suspension.
37. A core-shell material according to claim 25 or a delivery system according to claim 26, wherein the core is crystalline, liquid or an amorphous material or a mixture thereof.

38. A core-shell material according to claim 25 or a delivery system according to claim 26, wherein the electromagnetic radiation absorbing material incorporated into the shell is selected from metallic particles, quantum dots, chromophores, a metallic layer and metal ions, or a mixture thereof.

39. A core-shell material according to claim 25 or a delivery system according to claim 26, wherein the electromagnetic radiation absorbing material is gold or silver particles of 3nm to 100nm.

40. A process according to claim 1 or material according to claim 25 or a delivery system according to claim 26 for use in medicinal, pharmaceutical, cosmetic or diagnostic applications.
* the site of attachment of FITC is assumed to be randomly associated with any free hydroxyl group

FIG 5
FIG 12

FIG 13a
FIG 13b

FIG 14a
FIG 21

after 1064 nm irradiation

after 532 nm irradiation

hollow capsules before irradiation

wavelength [nm]

320 420 520 620 720

Extinction [a.u.]

0.3 0.25 0.2 0.15 0.1 0.05 0
## INTERNATIONAL SEARCH REPORT

### A. CLASSIFICATION OF SUBJECT MATTER

**Int. Cl.** A61K 9/00, 41/00, 49/00

According to International Patent Classification (IPC) or to both national classification and IPC

### B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

- DWPI: Nano, Particle, Nanoshell, Radiation, Infrared, Metal, Gold, Silver, Cadmium, Quantum dots, Qdots, Chromophore, A61K-009/IC, A61K-041/IC, A61K-049/IC
- CA: Nanoshell, Infrared, Radiation.
- MEDLINE: Same as CA.

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>

* Further documents are listed in the continuation of Box C

* See patent family annex

**Date of actual completion of the international search**

6 April 2005

**Date of mailing of the international search report**

13 APR 2005

**Name and mailing address of the ISA/AU**

AUSTRALIAN PATENT OFFICE  
PO BOX 200, WODEN ACT 2606, AUSTRALIA  
E-mail address: pct@ipaustralia.gov.au  
Facsimile No. (02) 6285 3929

**Authorized officer**

SHUBHRA CHANDRA  
Telephone No: (02) 6283 2264

Form PCT/ISA/210 (second sheet) (January 2004)
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Loo, Christopher et al, NANOSHELL-ENABLED PHOTONICS-BASED IMAGING AND THERAPY OF CANCER, Technology in Cancer Research and Treatment (2004 Feb) 3 (1) 33-40, Whole Document.</td>
<td>1-40</td>
</tr>
</tbody>
</table>
This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>US 6428811</td>
<td>AU 11285/02 AU 29947/99 AU 96374/01</td>
</tr>
<tr>
<td>CA 2329436</td>
<td>CA 2377722 CA 2379357</td>
</tr>
<tr>
<td>US 6344272</td>
<td>US 6645517 US 6685986</td>
</tr>
<tr>
<td>US 6699724</td>
<td>US 6852252 US 2001002275</td>
</tr>
<tr>
<td>US 2002045675</td>
<td>US 2002061363 US 2002132045</td>
</tr>
<tr>
<td>US 2002169235</td>
<td>US 2002187347 US 2004214001</td>
</tr>
<tr>
<td>WO 0105586</td>
<td>WO 0106257 WO 0228551</td>
</tr>
<tr>
<td>WO 0228552</td>
<td>WO 9946351</td>
</tr>
<tr>
<td>US 6479146</td>
<td>DE 19812083 DE 19907552 EP 0972563</td>
</tr>
<tr>
<td>US 6699501</td>
<td>US 2003219384 WO 9947253</td>
</tr>
<tr>
<td>WO 9947252</td>
<td>WO 9947253</td>
</tr>
<tr>
<td>WO 0158458</td>
<td>AU 36798/01 CA 2399293 EP 1263447</td>
</tr>
<tr>
<td>US 6530944</td>
<td>US 2002103517</td>
</tr>
</tbody>
</table>

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.