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SUBJECT AREAS:

DRUG DELIVERY

SELF-ASSEMBLY

NANOPARTICLES

NANOPORES

Received
3 April 2013Accepted
23 May 2013Published
13 June 2013

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Yoctowells as a simple model system for the encapsulation and controlled release of bioactive molecules

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The development of nanosized drug delivery systems to transport drugs to target cells, are promising tools to improve the drug therapeutic index. Transport systems should have a simple design to control the release of loaded drug to the target areas, thereby increasing concentration and prolonging retention. Herein, we demonstrate the use of yoctoliter wells (1 yL = 10⁻²⁴ L) as simple model systems for the encapsulation and release of biologically active molecules, by manipulating pH. The drug molecule employed here is *doxorubicin*, which diffuses into the bottom of yoctowells from a bulk solution at pH 7. Capping of the yoctowells is achieved by addition of an anionic-porphyrin by electrostatic interaction. Furthermore, controlled release of the Doxorubicin and capping agent from the yoctowells is achieved by pH control. The effectiveness of the sustain release of the bioactive molecule from yoctowells, provides potential for development of a new generation of drug-delivery system for practical application.

Drug delivery systems which medically-transport active molecules, in a controlled manner, to diseased cells, have gained much attention in the past decade¹. Various drug delivery systems have been employed such as polymers², dendrimers³, micelles⁴, vesicles⁵, nanoparticles⁶ and vesicular supported particles⁷. However, although drug delivery systems have their own advantages, they also possess limitations, such as poor thermal and chemical elimination⁸, difficulties in controlling targeting and the well-organised release of the drug, as well as rapid elimination by the immune system⁹. Ideally, the transport system should be designed to control the release of loaded drug to the target areas¹⁰, thereby increasing its local concentration, bioavailability and prolonging its retention. In recent years, silica nanoparticles have generated a significant amount of interest because of their inherent properties as biocompatible alternatives¹¹. Several sophisticated drug delivery systems have been developed and employed using mesoporous silica nanoparticles¹². These controlled drug release systems are designed to react to cells with internal stimuli, such as pH¹³, or specific enzymes¹⁴, or to external stimuli such as light¹⁵, redox properties¹⁶ or temperature¹⁷. Dual-release mechanisms triggered by pH and reduction have also been proposed¹⁸.

Supramolecular self-assembly and self-organisation plays an important role in nanotechnology, biotechnology and in regenerative medicine¹⁹. The self-assembly is driven by non-covalent and reversible interactions, such as hydrophobic, electrostatic, van der Waals, metal-ligand interactions, hydrogen bonds and π - π stacking, with a wide range of possible applications²⁰⁻²⁴. 'Yoctowells' represent a self-assembled porous material in which a useful volume of the pore is in the yoctolitre scale (10⁻²⁴ L)^{25,26}. Yoctowells are generally prepared using a porphyrin template as a base, which allows for both electrochemical and photophysical handles with which to study inclusion and release phenomena. Yoctowells with various kinds of walls can be precisely constructed through a range of techniques depending upon the walls of the wells employed²⁷. The integrity of yoctowells can be confirmed by size-exclusion fluorescence quenching experiments such that, large molecules over 2.5 nm in diameter cannot enter into the gaps whereas molecules with size commensurate with that of the gap are able to enter and quench the fluorescence of the base porphyrin, whose role is both as a structural element and as a fluorescence reporter. The chemical versatility offered by the fabrication technique means that the walls of yoctowells can be designed with different phobicities which can provide simple models for biological systems²⁸. Yoctowells with hydrophobic, hydrophilic and peptidic side walls are easy to prepare and characterize²⁹. The non-swelling behaviour of the walls of the yoctowell allows for dissolution of molecules in a range of solvents. Modification of the walls has also enabled inclusion phenomena, typically with high association constants³⁰. The most characteristic property of yoctowells is their ability to induce the formation of well-filling "nanocrystals" in dilute (10⁻¹ M) aqueous



Construction of the yoctowell system. Yoctowells were constructed by a two-step self-assembly procedure on smooth, aminated silica particles. Firstly the flat-lying porphyrins were attached, followed by reaction of the diamido bolaamphiphiles **4** around the porphyrin islands **1**, leading to small wells of yoctoliter (10^{-24} L) size (see Supplementary Fig. S1). The colloidal carrier of choice consists of smooth amine-coated silica gel particles with a diameter of 100 nm with no pores, such as those introduced by van Blaaderen *et al.*³⁶. Their modestly curved, reactive, and photochemically inactive surface provides a perfect basis for the establishment of a closed monolayer²⁶. The absorption and fluorescence spectra of porphyrin **1** bound to the well surface, hardly changes after the two step self-assembly of bolaamphiphiles around the porphyrin, in water or organic solvents^{26,29}. Discriminative fluorescence spectroscopy showed that the yoctowell gaps formed are rigid and that the gaps are of the same size as the porphyrin (~ 2.2 nm), (see Supplementary Fig. S2). The aminated silica particles covered with porphyrin **1** and surrounded by bolaamphiphile **4** walls, were then functionalized at the bolaamphiphile double bond by the Michael addition of methylamine. A positive ring of methylammonium groups being formed directly below the triethylene glycol chain (TEG) headgroups³⁴.

Concept and design. The use of porphyrins as base for the yoctowell template, allows the introduction of both electrochemical and photophysical handles through which the study of inclusion and release phenomena can be pursued. DOX **2**, and Mn(III)TPPS **3** molecules were chosen to monitor the independent release processes due to their unique absorption and emission properties, and their presence and concentration can be judged by spectroscopy (UV-vis absorption and fluorescence). DOX is widely used in chemotherapy for the treatment of Kaposi's sarcoma³⁷, ovarian carcinoma³⁸ and breast cancer³⁹. However, it is highly toxic to the heart and kidneys, thereby limiting its application. Recently, a report of the release of DOX from mesoporous silica films has been described in cellular studies⁴⁰. Therefore, systems which are able to administer such drug molecules in a controlled and sustained fashion would be very beneficial.

Ordered molecules and structural studies. We explore the entrapment of DOX **2** *via* hydrophobic interactions, and Mn(III)TPPS **3** by electrostatic interactions, with the positive rim of the wells (Fig. 3a–c). DOX **2** (2.5×10^{-3} M) was added as an aqueous solution, and the decrease of fluorescence of well porphyrin **1** monitored over time at a 650 nm wavelength. The fluorescence quenching of **1** by **2** occurs almost immediately (< 1 s) if porphyrin **1** is bound to the aminated silicate without a surrounding wall of bolaamphiphiles **4** (Fig. 3d). However, when porphyrin **1** within the yoctowell *i.e.* surrounded by bolaamphiphile **4**, maximal quenching occurs after ~ 50 min. We took advantage of this slow immersion process to develop a procedure for the encapsulation of **2** within the yoctowells (Fig. 3e). To ensure complete and homogeneous binding of DOX **2**, fresh sample of the yoctowell material were suspended in Milli-Q water and the solution was treated with DOX **2** solution overnight. The excess DOX was removed by centrifugation, dispersion and repeated washing with Milli-Q water. Indeed, the inclusion of **2** into the pre-formed well was confirmed by the absence of an emission band at 650 nm due to **1**, and the appearance of a new band at 630 nm associated to DOX **2** (Fig. 3e). Fig. 3f shows UV-vis absorption spectra of **1** and **2**. A ratio of $\sim 2 : 0.1$ was obtained for the height of peaks, which is consistent with the $1 : 1 \pm 10\%$ stoichiometry expected based on molar extinction coefficients (**1**, $200,000 \text{ M}^{-1} \text{ cm}^{-1}$ and DOX **2**, $11,300 \text{ M}^{-1} \text{ cm}^{-1}$).

Following quantitative encapsulation of **2** within the pores, the potential for sequential addition of a third molecule into the yoctowell was explored. It was demonstrated that the walls of the wells bear enough positive charge at the rim to bind an anionic species, such as

Mn(III)TPPS **3**, *via* electrostatic interactions³⁰. Mn(III)TPPS **3** (2×10^{-3} M) was then added to the bulk water solution. Interestingly, the fluorescence spectrum of **1** remained quenched, implying that the encapsulated **2** remained intact within the pores. Upon addition of **3**, about 95% fluorescence quenching of the **2** was observed (Fig. 3e). After removal of excess **3** by centrifugation and washing with Milli-Q water, all three components **1**, **2** and **3** were detectable in the UV-vis spectra of the aqueous solution of the re-suspended particles. The fluorescence spectrum of **2** (Fig. 3) showed complete quenching of fluorescence by manganese porphyrin **3**. The UV-vis absorption spectrum of re-suspended particles was also measured in deionised water (Fig. 3f, red line), which clearly indicated an absorption maximum corresponding to **1** (420 nm) and a combined band of **2** and **3** at 450–470 nm. The ratio of the areas of the Soret band of well **1** at 420 nm, and the combined band of **2** and **3** at 440–470 nm in the UV-vis spectrum, was about the same as that in a 10^{-6} molar water solution of all three components. Importantly, capping of the pores in **1** by **3**, prevents the addition of **2**. Encapsulation not only depends on the sequential addition of the final adsorption process, but also on the limitation to only one molecule in each well at a time. These limitations allow the establishment of a $1 : 1 : 1 \pm 10\%$ ratio between the number of yoctowells and accessible molecules in aqueous solutions. We believe that a 20-fold excess of solute molecules are adsorbed on the outer surface of the particles or on the walls of the cuvettes, and is later removed quantitatively in the centrifugation and washing procedures. To evaluate the efficiency of encapsulation of DOX **2** within the yoctowells, loading experiments, investigating the attachment of DOX **2** to native silica particles, were performed. Typically, amino silica particles were titrated with aqueous DOX **2** solution (10^{-2} M) overnight. However, UV-Vis absorption spectra of the resultant redispersed silica particles (after centrifugation and washing) revealed no DOX **2** absorption peak, and thus the DOX had washed out.

Encapsulation of bioactive molecules in the yoctowell on gold surfaces. The encapsulation of DOX **2**, and the capping of the wells by Mn(III)TPPS **3**, was confirmed by an independent electrochemical method (Fig. 3g). Following an earlier protocol, yoctowells prepared on gold electrodes were constructed by two step-self-assemblies (see Supplementary Fig. S3), followed by functionalization of the double bond of the bolaamphiphiles³⁵. Upon confirmation of a positive rim around the wells of the yoctowell, electrodes were then immersed in DOX **2** solution (10^{-3} M) overnight, followed by washing with MilliQ water. Cyclic-voltammetry confirmed the encapsulation of **2** within the wells. Finally, the electrode was immersed into an aqueous solution of tetrasulphanato-porphyrin **3** (10^{-3} M) for 2 h. Complete closing of the gaps with **3** was confirmed by cyclic voltammetry using ferricyanide as redox-active compound.

Release of bioactive molecules by pH stimuli. Yoctowell loaded with DOX **2** and capped with Mn(III)TPPS **3** was prepared, and the pH stimulated control release process subsequently investigated (Fig. 4a–c). It is worth mentioning here that it is most important to wash the nanoparticles with Milli-Q water (pH 7) after each step, as excess molecules (**2** and **3**) can adsorb onto the outer surface, and may be released before the operating pH is attained. Yoctowells bearing **2** and **3** molecules on silica particles were placed in a cuvette and water was added. The release of **2** and **3** from yoctowells was monitored *via* the recovery of fluorescence of **2** and **1** at 650 nm ($\lambda_{\text{ex}} = 430$ nm) respectively, which was measured as a function of time. A flat baseline showed that **2** and **3** were held within the yoctowells at neutral pH without premature release. The pH of the solution was then lowered to 4.5 by addition of 0.1 M HCl, and recovery of the fluorescence emission of **2** (which was quenched by **3**) was observed within less than an hour (Fig. 4d). Mn(III)TPPS **3** was released simply by lowering the pH, the protonated (SO_3^- of **3**

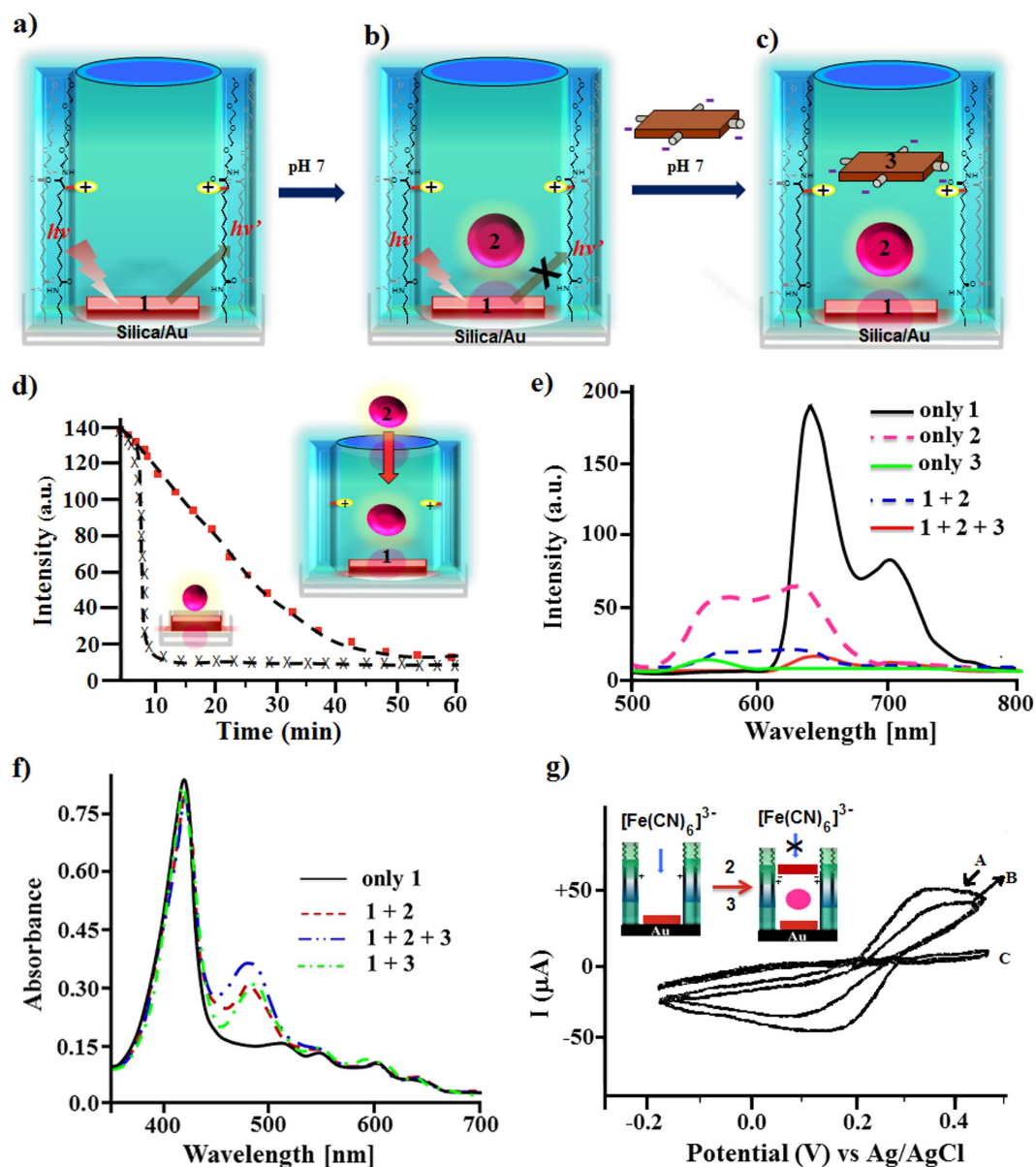


Figure 3 | Schematic diagrams for the encapsulation of bioactive molecules. **a**, Vacant yoctowells on the silica/Au surface. **b**, Encapsulation of DOX 2. **c**, Capping of the yoctowells by Mn(III)TPPS 3 held in place by electrostatic interactions. **d**, Fluorescence quenching of bound porphyrin 1 upon addition of DOX 2, with or without bolaamphiphiles surrounding the porphyrin 1 ($\lambda_{ex} = 430$ nm). Fluorescence quenching with water-soluble DOX 2 occurs immediately (black line) if the only porphyrin 1 attached to the silicate particles (see model), whereas maximal quenching is only reached after ~ 50 min when porphyrin 1 is within the yoctowells. **e**, Fluorescence spectra of: well porphyrin 1 (1, black solid line) alone; well porphyrin 1 + DOX 2 (blue dotted line), well porphyrin 1 + DOX 2 + Mn(III)TPPS 3 (red solid line) and spectra's of pure 10^{-6} M solution of DOX 2 (pink line) and Mn(III)TPPS 3 (green line) in water respectively. **f**, The UV-Vis absorption spectra of well porphyrin 1 (1, black solid line); well porphyrin 1 + DOX 2 (dark red), well porphyrin 1 + DOX 2 + Mn(III)TPPS 3 (blue) and well porphyrin 1 + Mn(III)TPPS 3 (green) in water, respectively. **g**, Cyclic-voltammograms of an aqueous solution of ferricyanide ions (10^{-3} M, 1 M KCl), measured at 100 mV s^{-1} in water at pH 7: forms yoctowell (curve A), which shows open clefts; the blocking effect after treatment with 10^{-3} M DOX 2 (curve B), where the yoctowells contain sufficient positive charge, as a result of the generated ammonium groups, to hold a Mn(III)TPPS 3 molecule electrostatically above the surface, thus inhibiting the inclusion of ferricyanide as indicated by a complete loss of the CV current (curve C).

into SO_3H) form of dye 3 being expelled from the wells. Thereafter, increasing the pH by addition of NaOH (0.1 M), enabled the release of 2 from the yoctowell cavities. Upon release of 2, a rapid increase of fluorescence intensity around 650 nm vs time was detected. This was attributed to the recovery of fluorescence of the base porphyrin 1 (Fig. 4d). Fluorescence intensity measurements were used to calculate the % release of molecules from the yoctowells. To confirm pH dependent release, yoctowell bearing only 2 was employed in the release process - a flat baseline at pH 7 indicated

that 2 were held within the yoctowells, and thereafter no release was observed upon reducing the pH to 4.5. However, upon increasing the pH of the solution to 7.8, release of DOX 2 was observed as a function of time, and approximately 15% of 2 were released from 3 mg of yoctowells after ~ 6 h (Fig. 4d, dash line).

In order to evaluate the pH dependency for release of the encapsulated molecules, we have performed two independent protocols. The first to determine the exact time of release of 3, which was only held within the wells by electrostatic interactions, and the second, to

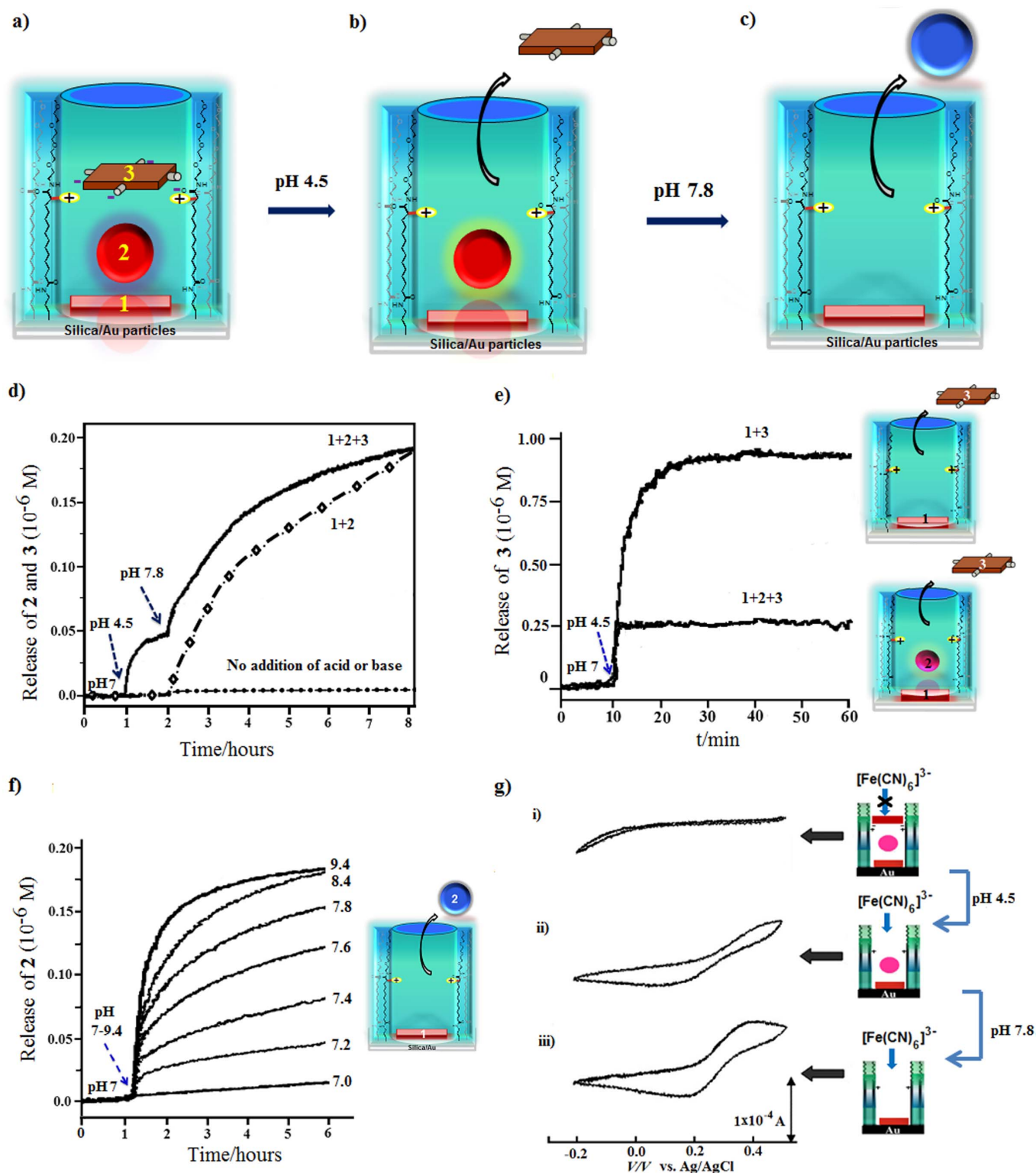


Figure 4 | Evaluation of release of bioactive molecules. **a**, Schematic illustration of the dual-release process by pH stimuli, **b**, release of electrostatically bound Mn(III)TPPS 3 at lower pH (4.5), and **c**, by DOX 2 at higher pH (>7.8). **d**, Release profiles of 2 and 3, while monitoring increase of emission at 650 nm ($\lambda_{\text{ex}} = 430$ nm) as a function of time; **e**, only release of 3 at pH 4.5 and no release of DOX 2 at this pH. **f**, pH triggered release of DOX 2 at increased pH 7.0–9.4 confirms that 2 can be only released at higher pH, and **g**, Cyclic-voltammograms (CVs) of ferricyanide (10^{-3} M) in the presence of aqueous KCl solution observed with gold electrodes covered with (i) blocked yectowells with 2 and 3, (ii) release of 3 at pH 4.5, shows current regeneration and (iii) after release of Doxorubicin 2 shows 50% regeneration of current, which is comparable to the empty wells shows in Fig. 3g.

test whether Mn(III)TPPS 3 interferes in the second release process, by independent release of Mn(III)TPPS 3 (Fig. 4e). At pH 7, a flat baseline indicated that the base porphyrin 1 was quenched when 3 was bound to the positive rim of the wells, mainly due to electron

transfer from porphyrin 1 to 3^{3+} . The fluorescence of porphyrin 1 remained quenched at pH 7 indicating that no premature release of 3 occurred. The pH of the solution was adjusted to 4.5 by adding HCl (0.1 M) and a rapid increase in the emission intensity at 650 nm



($\lambda_{\text{ex}} = 430 \text{ nm}$) was observed i.e. the recovering fluorescence of **1** indicated that Mn(III)TPPS **3** was released immediately from the yoctowells upon lowering the pH (> 20 minutes). However, yoctowell loaded with **2** and **3** at pH 4.5 only released **3** i.e. DOX **2** remained intact within the cavity at pH 4.5. pH dependency for the release of **2** was evaluated by varying pH from 7 to 9.4 (Fig. 4f). The flat baseline indicated that **2** remains held within the wells at pH 7 even after 6 h in aqueous solution. As the pH of the solution was increased (pH 7.2–9.4) gradually, we observed an overall increase in fluorescence intensity ($\lambda_{\text{em}} = 650$) as a function of time with increasing pH. At pH 8.4 and 9.4, a rapid increase in the fluorescence of **1** was observed; however, pH in the range of 7.4–7.8 gave a slow and sustained release of **2**. The release of DOX **2** from the yoctowells was further investigated at physiological salt concentration, using Phosphate Buffered Saline (PBS, pH 7.2), between pH 7.2–9.5 (see Supplementary Fig. S4). The results obtained were similar to the release of DOX **2** from the wells in Milli-Q water.

Independent electrochemical methods were also used to study the pH dependent release process (Fig. 4g). Cyclic voltammetric curves of ferricyanide (10^{-3} M) were recorded in the presence of aqueous KCl solution. Yoctowell bearing **2** and **3** showed a complete loss of CV current, thereby confirming that the encapsulated guest remained intact. Upon immersing the electrode in a solution of pH 4.5 for 60 minutes, a cyclic voltammogram with a current regeneration of about 20% was recorded, thus confirming the re-opening of the wells. When the electrode was immersed in an aqueous medium of pH 7.8 for 3 days and the CV measured, about 50% of the current was observed which was similar to the empty yoctowells (Fig. 3g). Therefore, cyclic voltammetry also supports the pH dependence of the release process of **2** and **3** by pH control.

The results suggested a good capping efficiency of Mn(III)TPPS **3**, *via* electrostatic interaction of the DOX **2** molecule, versus the undesired leaching problem at neutral pH. Addition of HCl to the aqueous suspension of Mn(III)TPPS-capped yoctowells triggered a rapid release of the Mn(III)TPPS **3** (< 20 minutes) and the release reached 100%. Interestingly, only $\sim 15\%$ of the encapsulated DOX was released after 6 h, and thus the total release of DOX **2** within 3 days. This large difference between the portion of **2** and **3** released from the yoctowells implies that Mn(III)TPPS **3** was only electrostatically attached within the wells, whereas DOX molecules were more strongly physisorbed to the walls of the yoctowell *via* hydrophobic interactions. These results were in agreement with our earlier

hypothesis that the blockage of small molecules is caused by the “immobile hydration water” of solutes, which binds to the hydrophobic walls³². Upon increasing to pH > 7 , DOX **2** disperses in the yoctowell water and is released from the cavity. There appears to be a critical point in the rate of release, as at pH > 7.8 the DOX **2** is released quickly, whilst at pH's which are very slightly basic but less than pH 7.8, the release of DOX **2** takes place in a slower fashion. This critical pH point may coincide with a critical point in the deprotonation-protonation equilibrium, where at pH < 7.8 the hydroxyl groups are probably in the deprotonated oxide form.

The absorption and fluorescence spectra of the solution, which were examined before and after the release of **2**, were totally different and comparable to those of pure DOX **2** in aqueous solution (Fig. 5). The particles (5 mg) were dispersed in water (5 mL) and the pH of solution lowered to 4.5 by addition of 0.1 M HCl (25 μL). After 1 h, the particles were isolated by centrifugation and washing with Milli-Q water. Complete release of **3** was achieved by dispersion of the particles in water at pH 7, as confirmed by the fluorescence recovery of **2** in (Fig. 5a). On increasing the pH of the solution by addition of 0.1 M NaOH solution (25 μL), the release of **2** was observed. After recovering the particles by centrifugation, the supernatant showed only emission of **2** and redissolving the particles in water only gave the fluorescence emission of **1**. Furthermore, absorption spectroscopy was used to estimate the amount of **2** after its release from the wells (Fig. 5b). The absorption band for the encapsulated DOX **2** within the yoctowell appears at $\sim 460 \text{ nm}$ at neutral pH, whereas released DOX **2** at pH 7.8 gave absorption bands at 490 and 570 nm, and was comparable to that of pure DOX **2** in solution. However, the UV-vis absorption bands of **2** at pH > 8 appeared at 520 and 620 nm. UV-vis absorption spectroscopy therefore confirmed that DOX **2** (10^{-6} M) was completely released from yoctowells after 3 days.

For the preparation aminated silica colloidal particles in 1–10 g quantities we followed Blaaderen³⁶ protocol with slight modified procedure for achieving 100 nm size with smooth surface. The smoothness, size, and chemical self-assembly procedures were optimized in order to establish a yoctowell with modest curvature and containing functional gaps. Furthermore, the smoothness and size of particles have been determined by transmission electron microscopy (TEM) and observed ill-defined networks of 100-nm spheres with a smooth surface (Fig. 6a). TEM always showed perfectly smooth particles even after the self-assembly of porphyrin **1** and the bolaamphiphile **4**

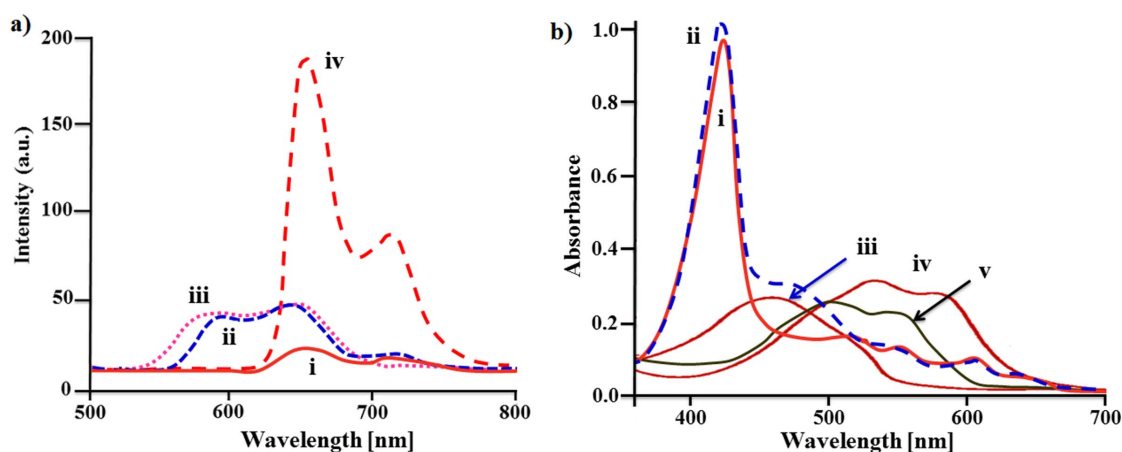


Figure 5 | **a**, Fluorescence spectra of **1**, **2** after completion of the release process: **i**) **1** + **2** + **3** within the yoctowells, **ii**) after release of **3** from the yoctowells at pH 4.5, **iii**) **2** was released at pH 7.8 after 3 days and was isolated from supernatant by removing particles by centrifugation, **iv**) after release of **2** and **3**, redispersed particles shows only emission of bound base porphyrin **1**. **b**, UV-vis absorption spectra before and after release: **i**) isolated particles after release of **2** and **3**, gives only bands of porphyrin **1** (10^{-6} M) in water at pH 7, **ii**) complex **1** + **2** (10^{-6} M) in water at pH 7, **iii** & **iv**) **2** (10^{-6} M) in water at pH 7 and pH 9, respectively, and **v**) released **2** 10^{-6} M from the yoctowells (after 3 days) at pH 7.8, which was obtained from the supernatant by removing particles by centrifugation and spectrum is similar to that of pure **2** in water i.e. spectrum **iv**.

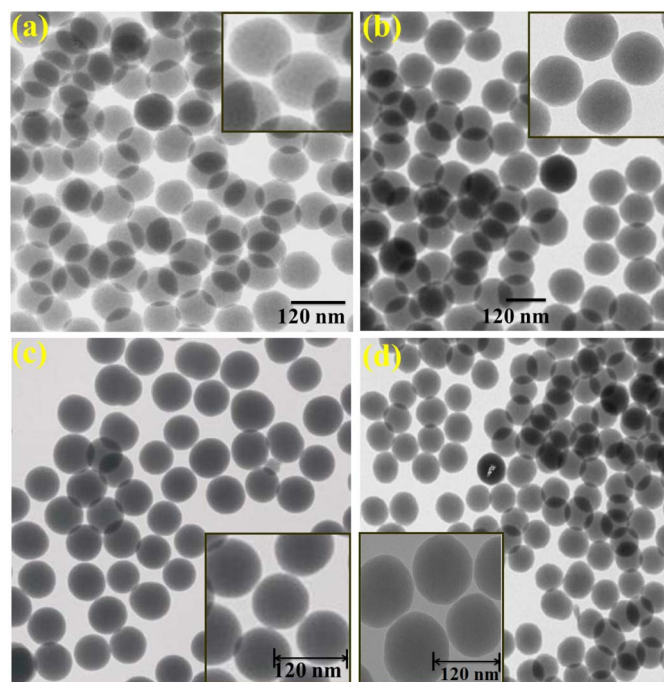


Figure 6 | Transmission electron micrographs (TEM). **a**, Synthetic aminated silica particles. **b**, after the self-assembly of porphyrin 1 and the bolaamphiphile 4 monolayers. **c**, Upon encapsulation of DOX 2 and capping of the yoctowells by Mn(III)TPPS 3. **d**, After release of DOX 2 and Mn(III)TPPS 3 from the yoctowells.

monolayers. The particles examined by transmission electron microscopy (TEM) and observed ill-defined networks of 100-nm spheres with a smooth surface after two step self-assembly (Fig. 6b). TEMs of silica colloidal particles after loading and release of DOX 2 and Mn(III)TPPS 3 shows ill-defined 100 nm spheres, see figures 6c and 6d, respectively. Thus, silica based yoctowell system for drug delivery *in-vivo* will be biocompatible.

Discussion

Our results demonstrate a new approach to the use of yoctoliter wells ($1 \text{ yL} = 10^{-24} \text{ L}$) as simple model systems for the encapsulation and release of biologically active molecules, by employing a yoctowell system in conjunction with naturally occurring stimuli *in vivo*, i.e. pH. The drug molecule employed here is doxorubicin, which diffuses into the bottom of yoctowells from a bulk solution at pH 7. Capping of the yoctowells is achieved by addition of an anionic-porphyrin by electrostatic interaction at neutral pH (7.0). Importantly, the non-swelling hydrophobic behaviour of the walls of the yoctowell allowed for the stable encapsulation of doxorubicin. Modification of the walls with ammonium rings enabled the binding of a third species which acts as a cover for the wells by electrostatic interaction, as shown in Figure 3.

Furthermore, controlled release of the capping agent i.e. anionic-porphyrin at pH 4.5 by addition of 0.1 M HCl solution, followed by Doxorubicin at pH 7.8 by addition of 0.1 M NaOH solution from the yoctowells were achieved respectively, as shown in Figure 4. The effectiveness of the sustain release of the bioactive molecule from yoctowells, provides potential for development of a new generation of drug-delivery system for practical application, as $\sim 15\%$ of Doxorubicin were released from 3 mg of yoctowells after ~ 6 h.

This drug delivery yoctowell system has the potential to change radically the practice of therapy for a variety of diseases and disorders by manipulating guest molecules. These yoctowells will enhance the properties of already existing drugs in terms of solubility, bioavailability and prolonged circulation times. Furthermore they can be

tailor-made in such a manner that they selectively release bioactive molecules at the desired site of action by modification of the transferring system using magnetic nanoparticles in future. The yoctowells system therefore presents an excellent starting point for a new approach for encapsulation of drugs and sustain release with pH control. The success of such a simple yoctowell systems based on this strategy promises well for future, more sophisticated designs that employ for a similar approach.

Methods

Spectroscopic measurements. UV/vis absorption spectra of membrane-coated silica colloids were acquired using a Varian Cary 1 Bio spectrophotometer at 25°C . Fluorescence measurements and quenching experiments were performed on a FluoroMax-4 equipped with an injector port and stirrer 25°C . Cyclic voltammetric experiments were performed in a one-compartment three-electrode cell using PG310 equipped with a potentiostat (HEKA, Dr Schulz GmbH, Germany). The working electrode was a circular bar gold electrode or monolayer-coated gold electrode with a surface area of 0.5 cm^2 . A Pt wire counter electrode and aqueous Scanning Calomel Electrode reference electrode were used. An aqueous solution containing 0.1 M KCl and 1 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$ were used as electrolyte.

Fluorescence Quenching Decay Measurements Experiments. Colloidal silica decorated with yoctowells (3 mg) was dispersed in 3 mL of water and placed in a quartz cuvette. A 30 μL aliquot of an aqueous solution of Doxorubicin 2 (10^{-3} M) was added. The fluorescence spectrum of the well base porphyrin 1 was continuously monitored. The same quenching procedure was applied for attachments of anionic Mn(III)TPPS 3 (ref. 26).

Encapsulation of Doxorubicin 2 and Mn(III)TPPS 3. Yoctowells with functionalised walls, with a positive rim, on silica particles (20 mg) were dispersed in 14 mL of Milli-Q water and placed in a clean glass tube and soaked with Doxorubicin 2 (3 mL of 10^{-3} M), at pH 7, overnight at room temperature, to allow the Doxorubicin 2 to diffuse into the wells of the silica nanoparticles. Excess doxorubicin was then removed by centrifugation, dispersion and ultrasonication. This was followed by addition of Mn(III)TPPS 3 (3 mL, 10^{-3} M of pH 7.0). After 30 min, and washing with Milli-Q water (pH 7.0) three times to remove excess adsorbed molecules 3, silica particles with a trimeric system were obtained. The obtained particles in heterotrimer form were confirmed by UV-vis and fluorescence spectroscopy thereby indicating the presence of a strong inclusion complex. Most importantly, the yoctowell obtained in trimeric form can be stored in a dried form for more than three months without release of any of the molecules from the cavities. In each step, UV-visible and fluorescence spectroscopy were used to confirm the encapsulation and stability of guest molecules.

Release of the molecules from yoctowells on silica particles. Release of the encapsulated molecules from yoctowells was examined by fluorescence spectroscopy. A silica nanoparticles (3 mg) containing yoctowells along with Doxorubicin 2 and Mn(III)TPPS 3 were placed in a fluorescence cuvette ($t = 0 \text{ sec}$). Milli-Q water (2 mL) was then added and a 1 mm magnetic stirrer bar was placed into the bottom of the cuvette. Upon stirring of the mixture for 2 minutes, the fluorescence emission ($\lambda_{\text{em}} = 650 \text{ nm}$) intensity was measured using an excitation wavelength, ($\lambda_{\text{ex}} = 430 \text{ nm}$), as a function of time during addition: after a flat baseline at pH 7 had been recorded for 60 minutes, to ensure no leaching had occurred, we then added 0.1 M HCl (25 μL) to pH 4.5, and allowed the system to stabilise until there was no further increase in fluorescence intensity. This was followed by the addition of 0.1 M of NaOH (25 μL) to pH 7.8, after which a rapid increase in the fluorescence intensity of the porphyrin 1 was collected at 1 s interval.

Transmission Electron Microscopy (TEM). TEM measurements were performed on an electron microscopy Igor 1200EX, operating 80 kv of an accelerating voltage. Freshly prepared samples were prepared by dropping 5 μL aliquots of silica colloidal particle solution (H_2O) onto a TEM grid (400-mesh copper grid coated with carbon). After a few minutes, remaining solution was blotted off with a filter paper and images were collected.

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Acknowledgements

This research was supported financially by the Australian Research Council, under a Future Fellowship Scheme (FT110100152) to RMIT S.V.B. S.V.B. also acknowledges the School of Applied Sciences (RMIT University) for facilities and support. We also acknowledge A. Gupta and K. Latham, (RMIT) for proof reading of the manuscript and M. Al Kobaisi for help in the drawing.

Author contributions

RMIT S.V.B. designed the experiment and performed synthesis of bolaamphiphiles, particle preparation, encapsulation and release of bioactive molecules using fluorescence, UV-Vis absorption spectroscopic measurements. IICT S.V.B. performed synthesis of porphyrins and electrochemical experiment. RMIT S.V.B. analysed the data. RMIT S.V.B. has written the manuscript and both co-authors reviewed the manuscript.

Additional information

Supplementary information accompanies this paper at <http://www.nature.com/scientificreports>

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Bhosale, S.V. & Bhosale, S.V. Yocrowells as a simple model system for the encapsulation and controlled release of bioactive molecules. *Sci. Rep.* **3**, 1982; DOI:10.1038/srep01982 (2013).



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