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Microcapsules with three orthogonal reactive sites

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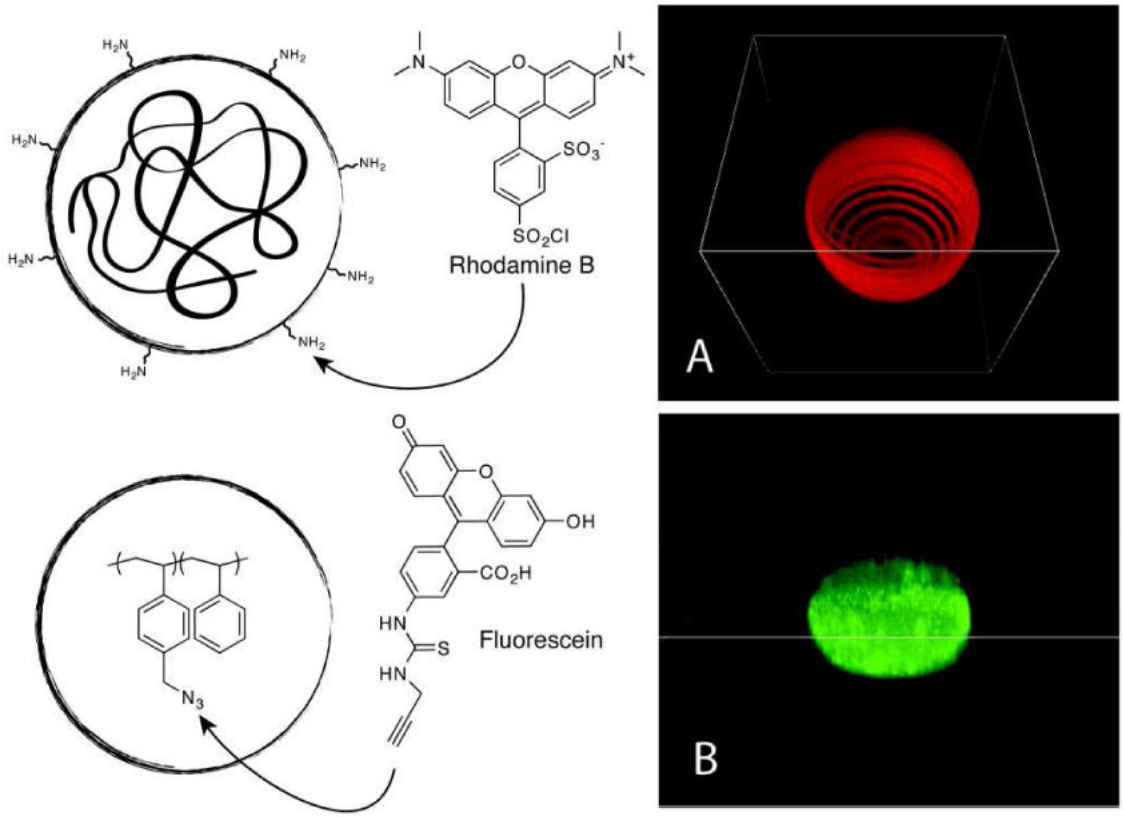
Abstract



Polymeric microcapsules containing reactive sites on the shell surface and two orthogonally reactive polymers encapsulated within the interior are selectively labeled. The capsules provide three spatially separate and differentially reactive sites. Confocal fluorescence microscopy is used to characterize the distribution of labels. Polymers encapsulated are distributed homogeneously within the core and do not interact with the shell even when oppositely charged.

Biological systems are replete with domains that contain disparate environments, such as the mitochondria, the endoplasmic reticulum, the golgi complex, and the nucleus.[1] These

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Supporting Information Available Experimental preparations, characterization, and depiction of shell formation and properties are available.

microenvironments enable the spatial distribution of ions, molecules, and macromolecules, ensuring that life-enabling gradients are maintained. The efforts put forth over the last 150 years to create and understand microenvironments has yielded a detailed understanding of micelles,[2] emulsions,[3] liposomes,[4] liquid crystals,[5] and bio-polymer assemblies,[6] as well as the creation of non-natural microenvironments derived from block-copolymers,[7] amphiphiles,[8] dendrimers,[9] star polymers,[10] and core-shell materials.[11] Each of these isolated cases often forms simple two component microenvironments; for example, lipid bilayers form both an interior and exterior aqueous phase with a small hydrophobic region in between. In fact, the differences in the microenvironments created by the lipid composition in lipid tubules are critical in protein organization at cellular interfaces. Recent studies have demonstrated that these phases can be selectively imaged by using staining methods that are specific to the micro-domains.[12] Microenvironments are also important in polymeric systems, where the micro-domain can be utilized for drug delivery, gene therapy, and catalysis.[13]

Creating and imaging microenvironments where the shell, interior, and exterior have different properties is difficult to achieve. Many new microcapsule and liposome materials have been demonstrated that use orthogonal organic chemistry approaches.[14] We have used emulsion templated polymerizations for synthesis of nano and microcapsules have been used to produce polymeric capsules[1542-48] that exhibit multiple domains, which can then be functionalized using well-known and reliable reactions, such as the Huisgen cycloaddition.[16] Such microcapsules can be reacted with dye moieties to allow the microenvironments to be visualized by optical microscopy.[17] Herein, we demonstrate the synthesis and three-dimensional confocal imaging of the microcapsules by 'optically barcoding' the microdomains using orthogonal functionalization of shell walls and sites within the capsules (Figure 1).

The desired triply functional microcapsules were produced via a copolymerization of polymethylene polyphenyl isocyanate (PMPPI) and tetraethylenepentamine (TEPA), forming a shell comprised of a highly cross-linked polyurea membrane (Scheme 1). The reaction of both the amine and isocyanate are incomplete, leaving the shell adorned with reactive amines and isocyanates. Treating fully formed capsules with *N*,*N*-dimethylaminopyridine (DMAP) in water efficiently converts the unreacted isocyanates to amines as determined by loss of the isocyanate stretch by IR and by measuring the increase of reactive amines using fluorine labeling (IR spectra shown in supplemental data). The amine loading for DMAP treated capsules was determined by capping them with trifluoroacetic anhydride and measuring fluorine content by elemental analysis (0.04 mmol/g amine).

To assess the ease of functionalization, we proceeded to dye label each reactive group in the microcapsule. By using three organic dye molecules the polymeric microcapsule and the encapsulated polymers (polyazidomethylstyrene (pAMS) and polychloromethyl styrene (pCMS)) can be differentiated by labeling the exposed polymer surface amine moieties with a red emitting dye (rhodamine B, λ_{ex} = 561nm and the emission collected 605/75 nm), the encapsulated internal polymer pAMS azide moiety by a green emitting dye (fluorescein-alkyne, λ_{ex} = 488 nm and the emission collected 515/30 nm), and the exposed chloromethyl group on pCMS by a blue emitting dye (cascade blue, λ_{ex} = 404 nm and the emission collected 450/35 nm). The shell labeling by rhodamine B is accomplished by a sulfamide-forming reaction. The encapsulated pAMS is labeled with an alkyne-fluorescein conjugate[16d] using the Huisgen 'click' reaction. The encapsulated PCMS is coded by an S_N2 displacement.

Barcoding of the microcapsule and its constituents can be carried out step-wise or simultaneously in a single reaction. The labeling of the microdomains of the microcapsule were imaged by confocal laser scanning microscopy (CLSM). The approach to simultaneous barcoding of the microcapsule is shown in Scheme 2. The triple labeling was performed in a single step by combining all of the dyes in equal proportion and rocking them overnight in a

suspension of THF-swollen microcapsules at room temperature, and excess dye was removed by washing with DMF. In labeling control experiments using fluorine labeled conjugates, we found that the labeling is nearly quantitative for these three types of conjugation chemistry. The domains of the functionalized microcapsules were examined by confocal microscopy by selectively exciting each dye moiety. The overlaid image in Figure 2 reveals a blue-green interior and a pink shell.

By separating the image into the individual dye contributions and projecting the intensity across the capsules, the localization of the polymer components can be analyzed (Figure 3). Inspection of Figure 3 reveals that cascade blue hydrazide and fluorescein-alkyne selectively label the interior volume of the microcapsules through specific polymers labeling of pCMS and pAMS, respectively. The fluorescein dye (Figure 3A, 3B) is evenly distributed within the capsule interior, while the cascade blue dye shows slight increased intensity at the shell wall boundary. In contrast, the exterior shell is labeled with rhodamine B (C).

The non-uniform distribution of rhodamine B around the shell surface most likely arises from the rough, crenated morphology of the polymer shell. The increase in cascade blue emission intensity on the shell wall indicates either localization of the polymer near the walls of the polymer shell via an electrostatic interaction of the more negatively charged pCMS-cascade blue polymer with the positive charge of the capsule shell arising from rhodamine B labeling. The same labeling results were obtained for singly labeled and dual labeled capsules.

More insight into the three-dimensional distribution of the dyes in the capsule can be obtained by simultaneously exciting all three fluorescent dyes and z-stack imaging using 1 μm cross-sections (Figure 4A-D). Half of the three-dimensional image reconstruction is shown for clarity and visualization of the center of the microcapsule and its components. Imaging cascade blue and fluorescein reveals that the pCMS and pAMS polymers are uniformly distributed throughout the interior of the vesicle (Figure 4A and 4B). The slight increase in concentration of fluorescein along the shell wall may arise from the emission bleed through of cascade blue into the green channel, which was minimized in Figure 3 by single laser excitation and single channel emission acquisition. Even with single wavelength excitation and channel detection some color from the interior dyes appears to encrotch on the shell dye. We suggest that this over lay is due to the interior polymers binding to the shell wall. In control experiments where we encapsulated polymer bound dyes, we always observed a slight enhancement of fluorescence at the shell compared to the interior.

Due to the propensity of the molecular dyes chosen to photobleach, no attempt to utilize single laser excitation methods was carried out for z-stack imaging. The z-stack image for the red dye rhodamine B appears as a red hoop except for the bottom of the microcapsule, where details of the microcapsule deformation can clearly be seen due to the microcapsule — glass coverslip physical interaction. Stacking each of the 1 μm image cross sections together into a single reconstructed image provides convincing evidence that only the shell is functionalized with the rhodamine B dye (Figure 4C). An overlay of the functionalized cascade blue center and rhodamine B shell is shown to demonstrate clear and distinct interior polymer and shell labeling (Figure 4D). From the triply labeled microcapsule data sets, we conclude that selective functionalization of one functional group in the presence of another is possible and that confocal microscopy is an effective method to distinguish the labels and their spatial arrangement.

Generally, existing microcapsule systems lack orthogonal reactive sites. By installing three differentially reactive groups on the wall and within the capsule interior, we have realized a material that is readily functionalized at three locations post microcapsule formation. The three functional groups can be reacted separately or simultaneously, resulting in labels that reside sufficiently far away from other labels as to prevent energy transfer. We also demonstrated

that the soluble polymers residing within the interior remain in the interior and that despite being oppositely charged relative to the shell, the dyes have only modest affinity for binding to the microcapsule wall. In conclusion, using the basic organic chemistry strategy of orthogonal reactivity, we have created microcapsules with three reactive handles that are distributed in two distinct environments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

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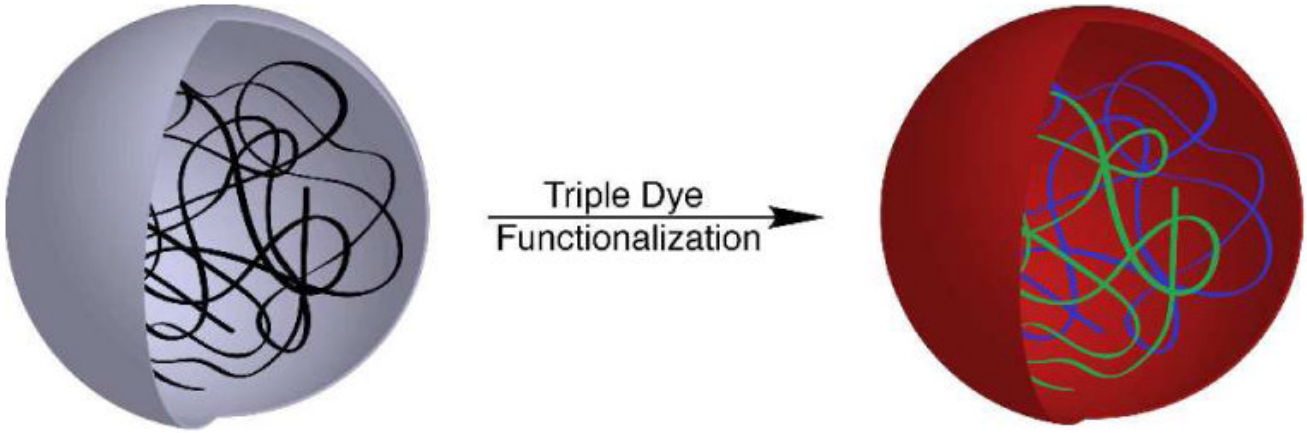
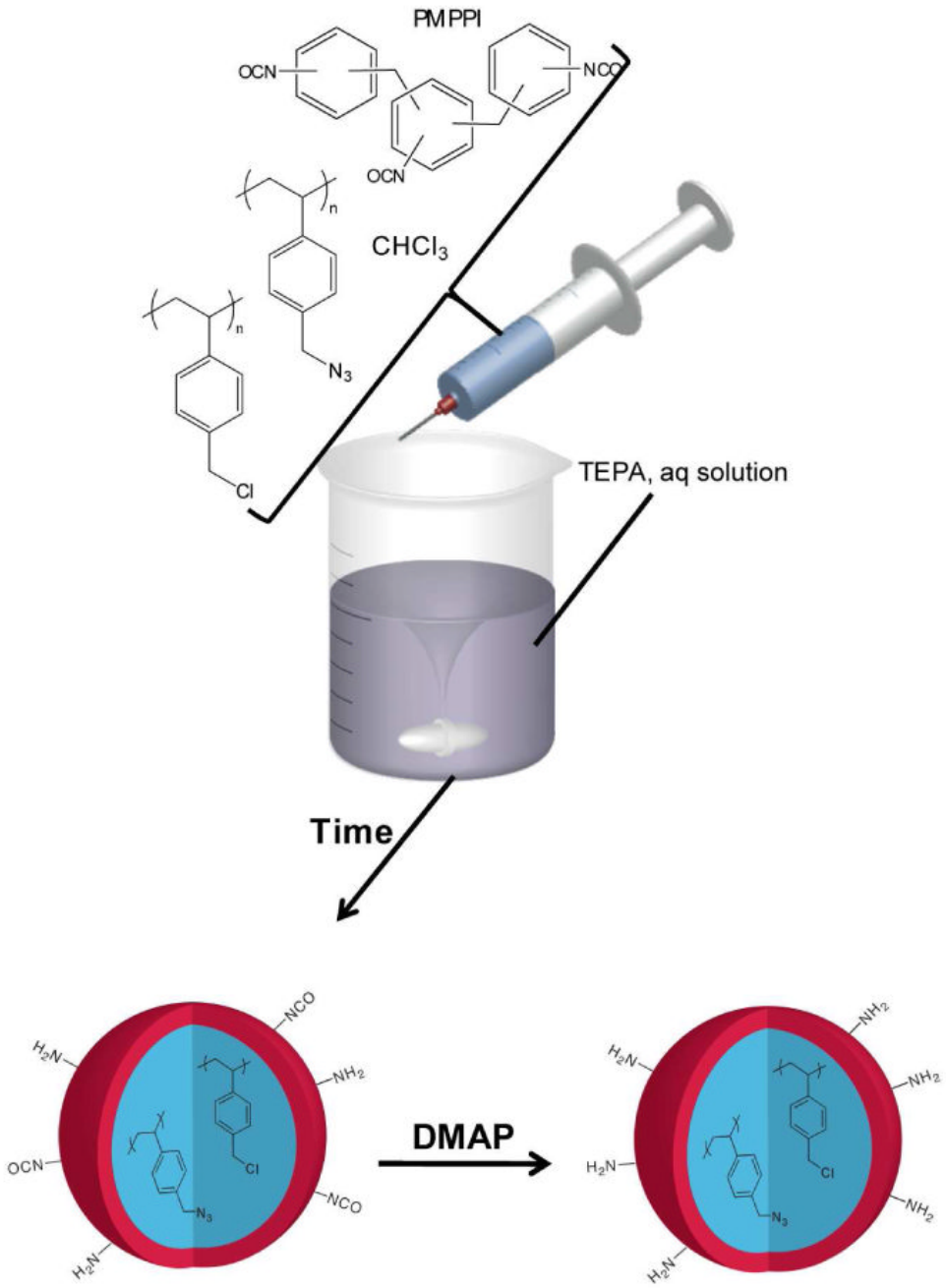


Figure 1.
Microcapsules with three orthogonal reactive groups, one group located in the shell and two groups entrapped within the core.



Scheme 1.

Triply labeled microcapsules via interfacial polymerization. Polymers, pCMS and pAMS, are combined with PMPPI in chloroform and added to a stirred poly(vinyl alcohol) aqueous solution. TEPA is added to further cross-link the shell. Capsules are swelled in THF/water and stirred with DMAP overnight to convert residual isocyanates to amines.

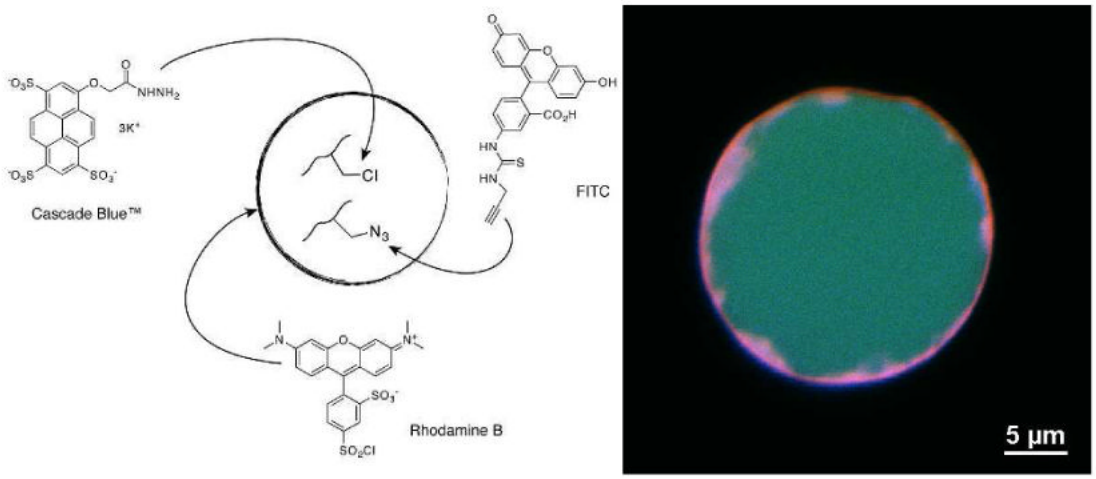


Figure 2.
Confocal overlay of triply functionalized microcapsules containing Cascade Blue hydrazide, FITC-alkyne, and rhodamine B sulphonyl chloride molecular dyes. The image is representive of the entire capsule population in each preparation.

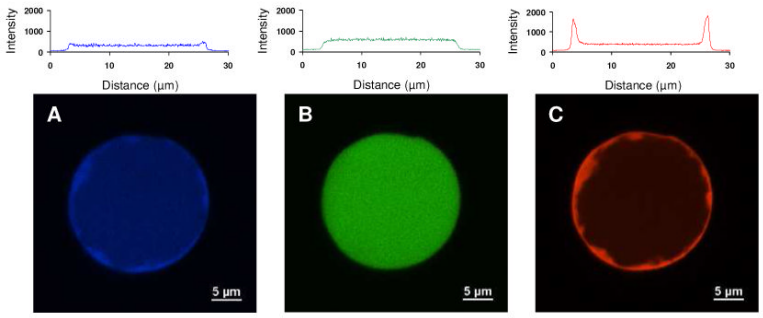


Figure 3.

Microcapsules were imaged using confocal laser scanning microscopy (CLSM) to evaluate the extent of Cascade Blue hydrazine (A), Fluorescein isothiocyanate (FITC) -alkyne (B), and Lissamine Rhodamine B (C) dye functionalization. The fluorophores were selectively excited using a 404, 488, and 561 nm laser and imaged in the blue (450/35), green (515/30), and red (605/75) channels for image A-C, respectively. The corresponding channel intensity across each image is shown above the individual micrographs to demonstrate dye selective functionalization on the inside of the microcapsule, as well as the exterior shell wall.

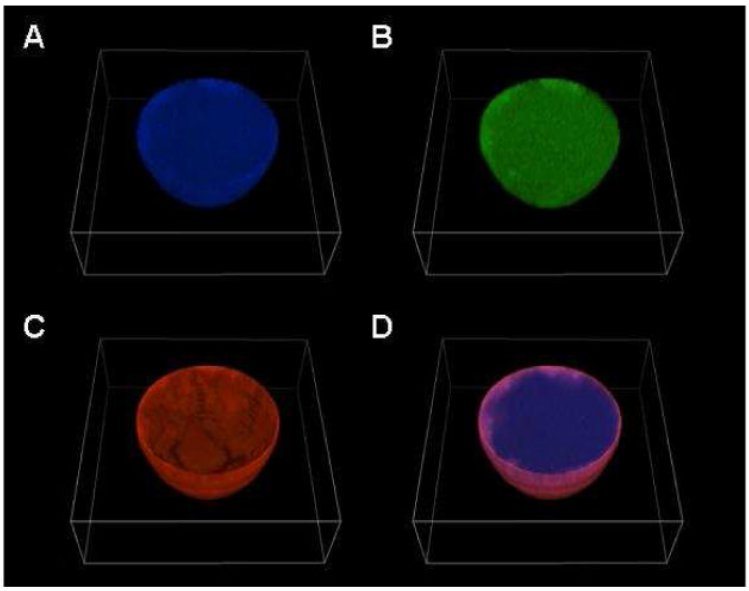


Figure 4.
Three-dimensional volume reconstruction of a triply functionalized polyurea microcapsule collected from a series of confocal micrographs at a 1 μm step-size (A-D). Images A-C depict the blue, green, and red individual components. Image D is an overlay of both the blue and red components showing the site specific labeling of the fluorescent dyes to their respective microcapsule functionalities.