Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Synthesis of Orthogonally Reactive Multilayered Microcapsule

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Experimental Section:

Materials: Branched poly-(ethyleneimine) (BPEI, MW~ 25,000 Da), dipentaerythritol penta-acrylate (5Acl, MW~ 524.51 g mol^{-1}), octadecylamine, octadecylacrylate, fluorescein isothiocyanate (FITC), 3-(dimethylamino)propylamine, [2-(acryloyloxy)ethyl]trimethyl-ammonium chloride, hydrogen fluoride (HF) and SiO_2 microparticles (diameter = 5 ± 0.35µm) were obtained from Sigma-Aldrich (Bangalore, India). Fluorescein cadaverine and tetramethylrhodamine cadaverine (TMRC) were purchased from Invitrogen (USA). Reagent grade tetrahydrofuran (THF) was purchased from RANKEM (Maharashtra). Absolute ethyl alcohol (CAS registry no. 64-17-5, Lot 1005150) was purchased from TEDIA Company (USA). Microscopic glass slides were obtained from JSGW (Jain Scientific Glass Works), India.

General Consideration:

ATR-IR spectra were recorded using PerkinElmer UATR Two at ambient conditions. Dynamic light scattering (DLS) study was performed by using a Zetasizer Nano ZS90 (model ZEN3690) instrument. Bright-field and fluorescence microscopic images were obtained using a ZEISS Axio Vert.A1 inverted microscope with 100X objective. Scanning electron microscope images were obtained using Carl Zeiss Gemini 300 FESEM (a thin layer of conductive gold coating was deposited on the non-conductive samples prior to imaging). Transmission electron microscope images were obtained using JEOL-2100F Field Emission Transmission Electron Microscope (FETEM). Atomic force microscope (AFM) images were acquired using OXFORD Cypher Atomic Force Microscope. Milli-Q grade water was used for all experiments.

Fabrication of Multilayered Microcapsules:

The solutions of 5Acl (132.5mg mL^{-1}) and BPEI (50 mg mL^{-1}) in ethanol were prepared in two separate glass vials. Then, 350 µl of BPEI solution was mixed with 5ml of 5Ac solution and kept for 40 minutes to initiate the formation of the chemically reactive polymeric nanocomplexes (referred as freshly prepared CRPNC). The silica (SiO_2) microparticles (diameter = 5 ± 0.35 µm, 1 µm and 500 nm) used as sacrificial templates, were washed with ethanol prior to starting the layer-by-layer (LbL) deposition process. Two distinct multilayered construction of CRPNC/BPEI and BPEI/5Acl were built by layer-by-layer deposition of BPEI with CRPNC and 5Acl, respectively through 1,4-conjugate addition reaction at ambient condition. The detailed LbL deposition process for constructing the

multilayers of CRPNC/BPEI is as follows; in the first step, the SiO_2 microparticles were suspended in 1ml of BPEI solution and kept under continuous agitation for 10 min. Then, the BPEI treated silica microparticles were centrifuged for 1 min at 1500 rpm, and the supernatant was removed carefully by pipet. In the second step, the BPEI treated microparticles were washed two times with ethanol bath and followed by centrifugation for removal of the supernatant. In the third step, the microparticles were suspended in 1 ml of freshly prepared CRPNC solution and kept in continuous agitation for 2 minutes. In the fourth step, the microparticles were washed as similar to the second step. This entire four-step process is considered as one cycle and the deposition of BPEI and CRPNC was denoted as 1 bilayer. The process was repeated for multiple cycles until the desired number of the bilayers were achieved. From the second bilayer onwards, BPEI treatment time is reduced to 2 min. After every two bilayer construction, freshly prepared CRPNC was used, and microparticles were placed in a fresh microcentrifuge tube to minimize particle aggregation. Similarly, multilayers of BPEI/5Acl were constructed on the silica microparticles, where the solution of CRPNC was replaced with the solution of 5Acl.

At the end, multilayers (both CRPNC/BPEI and BPEI/5Acl) coated silica microparticles were rinsed three times with THF prior to dispersion in 1ml of water. Then, respective multilayers coated silica microparticles were treated with hydrofluoric acid (HF, 2.0 M) to remove SiO_2 template cores following the standard reported protocol. *(**Caution!** Extreme care should be taken when handling HF. HF solutions and vapors are extremely poisonous and corrosive and may cause extreme burns that are not immediately painful. Handle with extreme caution in a chemical fume hood, and use appropriate protective equipment (gloves, face/eye protection, laboratory coat, etc.) and neutralize waste appropriately. Do not store in glass containers.).* The resulting hollow microcapsules were centrifuged at 4500 rpm for 5 min and rinsed five times with water, and characterized using bright-field and fluorescence microscopy.

Post-Covalent Modifications of Multilayers of CRPNC/BPEI:

Multilayers of CRPNC/BPEI contains residual acrylate and primary amine groups, providing an opportunity for post-modification with other chemical functionalities. The residual acrylate groups were post-functionalized with amine-containing small molecules including 3-(dimethylamino)propylamine (0.5 mL in 10mL of THF), Octadecylamine (5 mg mL^{-1}, in THF) and also with fluorophore molecules containing primary amine functionality including fluorescein cadaverine (0.2 mg mL^{-1}, in ethanol), tetramethylrhodamine cadaverine (0.2 mg mL^{-1}, in ethanol). The multi-layered membrane of CRPNC/BPEI was thoroughly rinsed with THF/ethanol prior to treat with selected small molecules (nucleophiles) or fluorophores (e.g., TMRC, FITC etc.) for 1 hr under continuous agitation. Next, the residual amine groups were modified by reacting with various electrophiles including octadecylacrylate (0.5 mL in 10mL of THF), [2-(Acryloyloxy)ethyl]trimethyl-ammonium chloride (0.5 mL in 10mL ethanol) and fluorescein isothiocyanate (0.2 mg mL^{-1}, in ethanol).

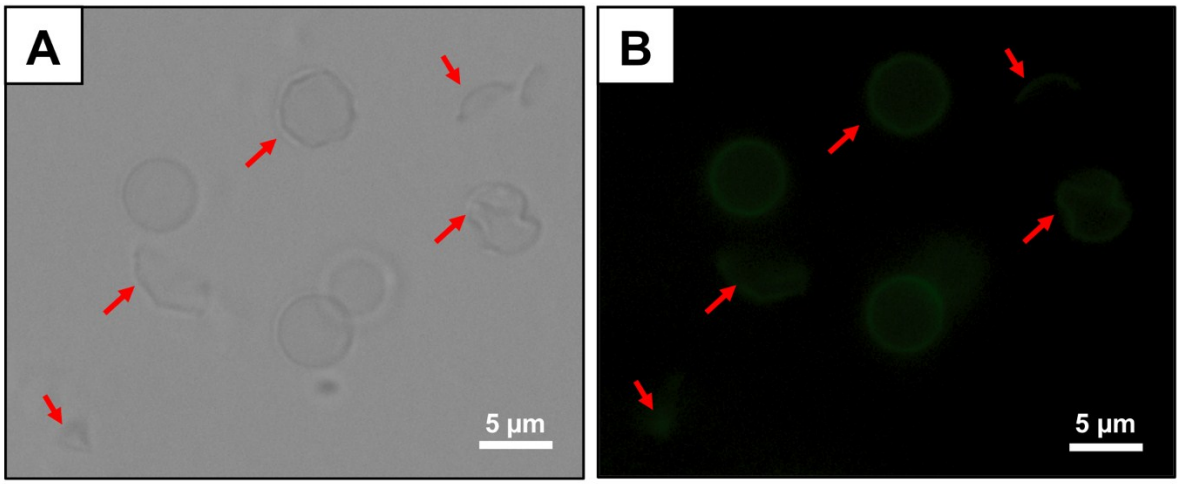

Figure S1. A-B) bright field (A), fluorescence (B) images illustrating the formation of mosly broken and distorted microcapsules of CRPNC by a half-diluted deposition solution (i.e., CRPNC) during Layer-by-Layer deposition process.

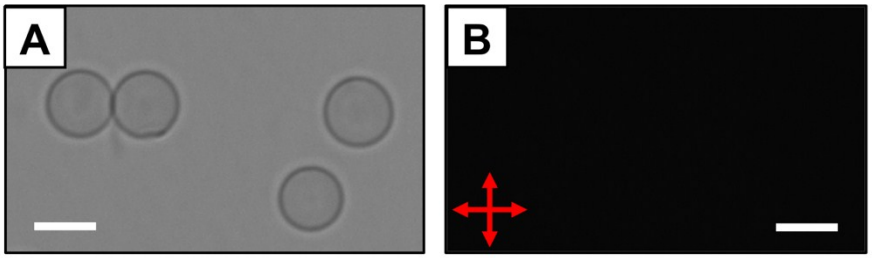

Figure S2. A-B) Bright field (A) and cross polarized light (B) microscopic images of $(CRPNC/BPEI)_5$ microcapsule. Scale bar is 5 µm.

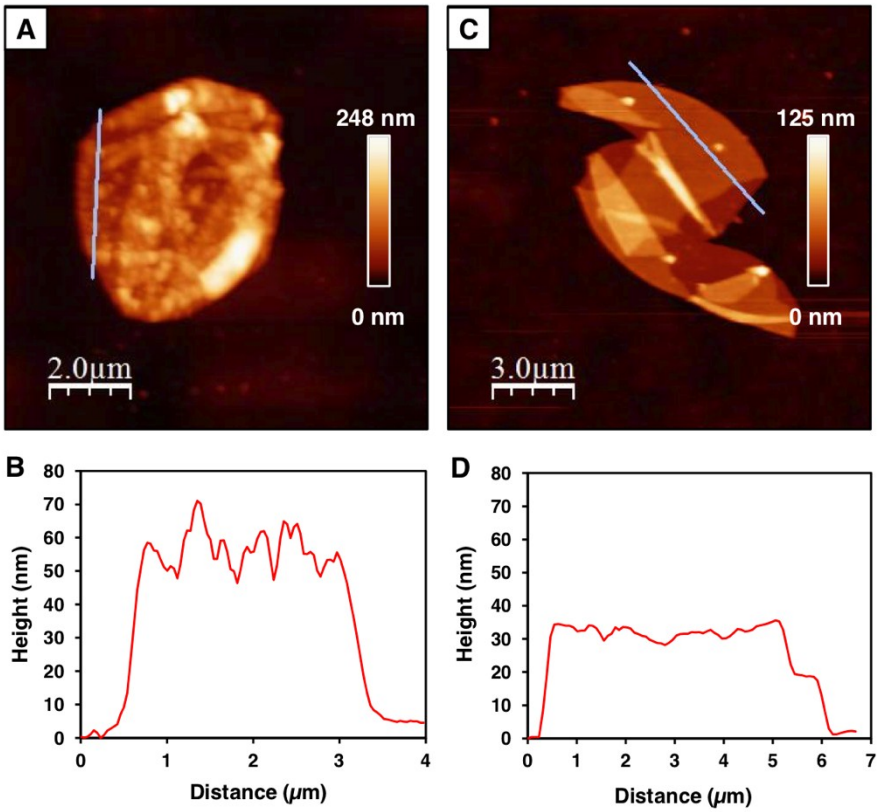

Figure S3. A-D) AFM images (A,C) and line profiles (B, D) illustrating the topographical changes in multilayers membranes of ((CRPNC/BPEI)$_5$, A-B) and (BPEI/5AcI)$_5$, C-D)

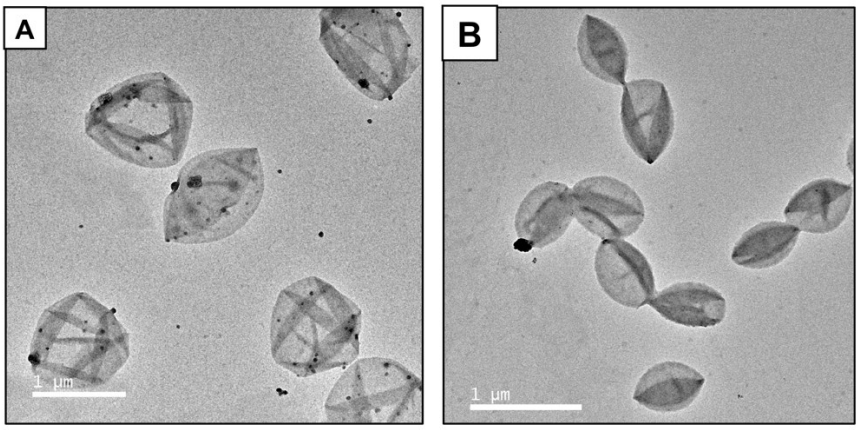

Figure S4. A-B) FETEM images of multi-layered microcapsules (CRPNC/BPEI)$_5$, which was prepared using 1 μm and 500 nm silica particles, respectively. Scale bar: 1 μm.

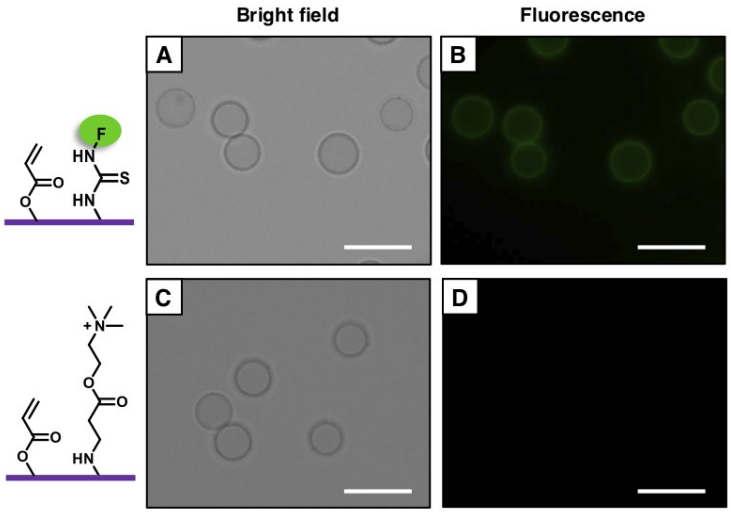

Figure. S5. A-D) Bright field (A, C) and fluorescence (B, D) images of freshly prepared (A-B) and 2-(acryloyloxy)ethyltrimethyl-ammonium chloride (AETMAC) modified (C-D) microcapsules (CRPNC/BPEI, 5 bilayers), after treatment with FITC. Scale bar: 10 μm.

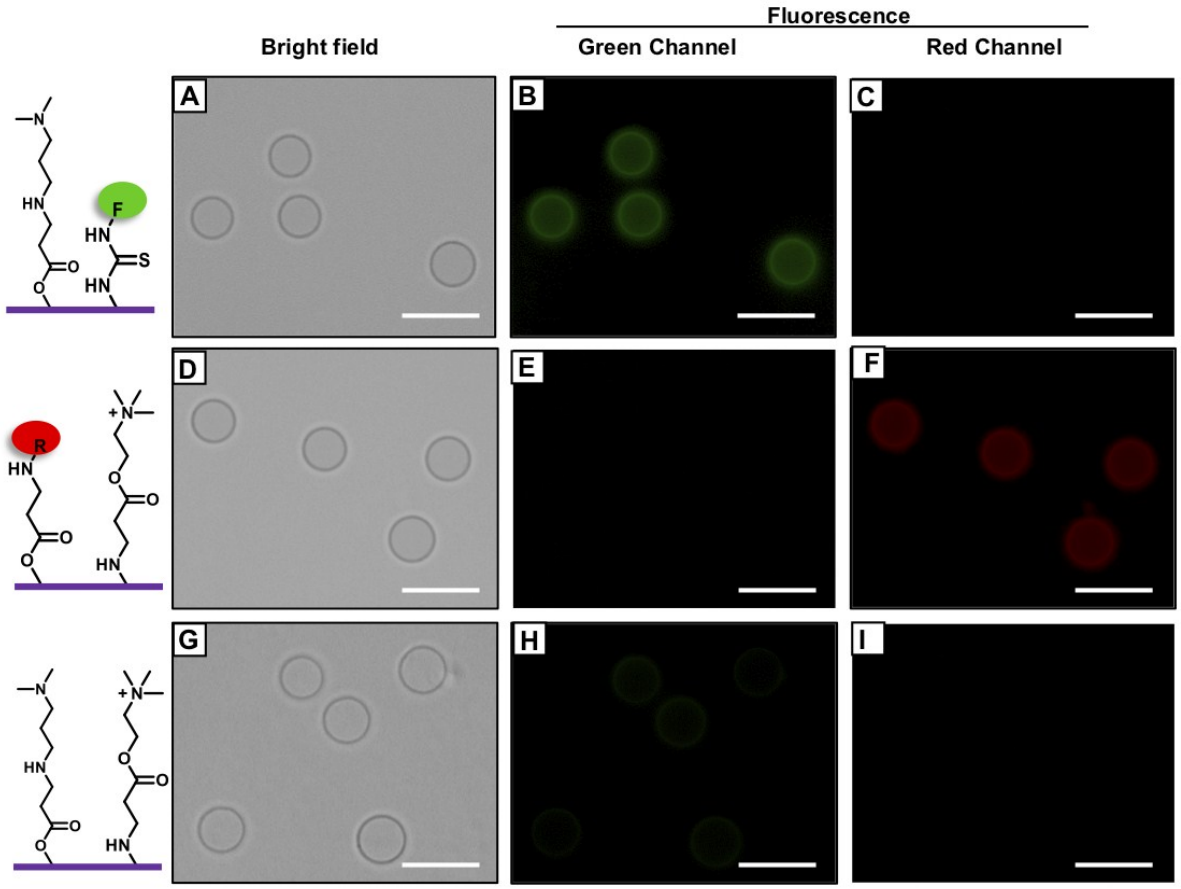

Figure. S6. A-I) Bright field (A, D, G) and fluorescence (B-C, E-F, H-I) images of DMAPA-modified (A-C), AETMAC-modified (D-F) and both DMAPA/ AETMAC-modified (G-I) microcapsules (CRPNC/BPEI, 5 bilayers), after sequential treatments of FITC and TMRC. Scale bar: 10 μm.