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Synthesis of Customizable Macromolecular Conjugates as Building Blocks for Engineering Metal–Phenolic Network Capsules with Tailorable Properties

*Chan-Jin Kim,^{†,§} Francesca Ercole,^{‡,§} Yi Ju,[†] Shuaijun Pan,[†] Jingqu Chen,[†] Yijiao Qu,[†] John F.
Quinn,^{*,‡,#} and Frank Caruso^{*,†}*

[†]ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, and the
Department of Chemical Engineering, The University of Melbourne, Parkville, Victoria 3010,
Australia

[‡]ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, Drug Delivery,
Disposition and Dynamics Theme, Monash Institute of Pharmaceutical Sciences, Monash
University, Parkville, Victoria 3052, Australia

[#]Department of Chemical Engineering, Faculty of Engineering, Monash University, Clayton,
Victoria 3800, Australia

*Corresponding authors. E-mail: john.f.quinn@monash.edu (J.F.Q.); fcaruso@unimelb.edu.au
(F.C.)

ABSTRACT

Metal–phenolic networks (MPNs), formed through coordination bonding between phenolic molecules and metal ions, are a promising class of materials for engineering particle systems for diverse applications. However, the properties of such MPNs are inherently restricted due to the finite properties of naturally occurring phenolic molecules. Herein, we report a simple and robust approach to incorporate phenolic moieties into polymers, thereby providing customizable phenolic ligand building blocks that can be used to assemble capsules with a range of tailorable properties. The phenolic ligand building blocks were synthesized via carbonic anhydride coupling to terminal amines, a conjugation approach typically used for peptide coupling but applied herein for functionalizing polymers. The chemistry enabled optimized end group purity, thus affording a robust and efficient strategy to generate a library of macromolecular poly(ethylene glycol) (PEG) catechol building blocks with different architectures (i.e., 2-, 4-, and 8-arm) and molecular weights (from 2.5 to 20 kDa). The resulting phenolic building blocks were applied to fabricate capsules with shell thickness, permeability, and cell association properties that were controlled via variation of the macromolecular catechol architecture and molecular weight. Specifically, shell thickness was varied more than 19-fold (i.e., between 8.7 and 169.3 nm) by judicious selection of the polymer molecular weight, arm number, and template. Similarly, permeability of the resulting MPN capsules to 500 kDa dextran was tuned from >90% to <5% by varying the number of arms in the polymer structure while maintaining a constant PEG M_n -to-catechol group ratio. Further, cell association was reduced by a factor of 2.5 by employing 20 kDa 8-arm PEG instead of 2.5 kDa 2-arm PEG during film assembly. These results demonstrate that the applied macromolecular conjugation approach can be used to customize particle properties, potentially facilitating applications in therapeutic delivery, imaging, separations, and catalysis.

INTRODUCTION

In recent years, advancements in both macromolecular and small molecule synthesis have led to substantial progress in the engineering of particles with a range of tailorable properties. The breadth of particles that has been investigated and applied includes hollow capsules,^{1,2} functional inorganic particles,³ self-assembled polymer micelles,^{4,5} and biofunctional assemblies.^{6,7} Applications of such particles span a wide array of areas, including biomedical imaging,⁸ therapeutic delivery,^{9–16} catalysis,¹⁷ and environmental remediation.^{18,19} Advances in coating and thin film technologies have further enabled the optimization of particle surface and interfacial properties for specific applications.^{20,21} One such strategy for engineering particle coatings is via the assembly of supramolecular networks such as metal–phenolic networks (MPNs).^{1,2} This robust and modular coating method is based on the coordination chemistry between metal ions and naturally occurring phenolic molecules that contain catechol and galloyl groups (e.g., tannic acid (TA))¹ and enables the deposition of MPN films on a broad range of substrates, from macroscopic supports to synthetic particles and biological cells.^{1,2} The selection of specific phenolic molecules and metal ions for the network formation determines the mechanical properties, disassembly behavior, and functionality of the MPN films (or capsules when assembled on sacrificial particles).^{8,22} Broadening the range of phenolic compounds and metal ions that can be used in the assembly process would extend the range of properties exhibited by MPN films/capsules. However, this approach is hindered by several factors. Firstly, the choice of metal ions is finite and inherently dependent on available ions. Secondly, the number and distribution of functional groups in commercially available phenolic compounds, such as TA, dopamine, gallic acid, and pyrocatechol, is also somewhat restrictive. Thirdly, the more complex and potentially more useful phenolic compounds found in nature (e.g., gallocatechins) are often too challenging or costly to

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3 isolate and characterize in workable quantities. As such, there is a strong motivation to prepare
4 well-defined and functional phenolic building blocks that could provide functional and structural
5 diversity into the suite of MPN structures, and thus expand their range of accessible properties.
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7 One such solution to this is to prepare well-defined macromolecular phenolic building blocks. This
8 necessitates the development of high-yielding, robust modification strategies that can enable
9 quantitative incorporation of catechol groups into polymers.

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11 Poly(ethylene glycol) (PEG) is a well-known highly biocompatible and stealth polymer with
12 antifouling properties and high water solubility owing to steric repulsion and surface hydration.^{23–}
13
14 ²⁵ Inspired by the intrinsic properties of PEG and phenolic compounds (i.e., the ability to
15 coordinate with Fe³⁺ and to adhere to diverse surfaces), catechol-functionalized PEG building
16 blocks have been synthesized and applied to fabricate various biofunctional materials. For
17 example, we previously reported the preparation of low-fouling MPN capsules using dopamine-
18 conjugated 8-arm PEG.²⁶ Other reported examples include a pH-dependent, self-healing hydrogel
19 composed of catechol-functionalized 4-arm PEG and Fe³⁺ ions,²⁷ as well as a series of catechol-
20 functionalized PEG-based block copolymers, which provided significant antifouling capacity for
21 target surfaces.²⁸ These studies typically employ dopamine, dopamine analogues or dopamine
22 derivatives in the functionalization procedure.^{26–28} There are, of course, alternative phenolic
23 molecules that can be used to incorporate catechol functionality to macromolecules, although these
24 are often overlooked in favor of the well-characterized dopamine and its derivatives. One such
25 molecule is caffeic acid, which includes a carboxylic acid moiety that could potentially be linked
26 to an amine-functional polymer via amide formation, thus affording a polymeric caffeamide. Such
27 an approach could provide a convenient alternative for synthesizing well-defined PEG-based
28 phenolic macromolecules that could in turn be applied to MPN formation. Further, the
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3 development of robust, quantitative modification approaches will enable the systematic study of
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5 how the macromolecular architecture (number of arms) and size (molecular weights) of the
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7 macromolecular constituent (PEG) can be used to tune the properties of an assembled MPN. In the
8
9 absence of a quantitative and versatile conjugation technique, such studies are difficult to reliably
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11 conduct. Finally, the development of a modular modification strategy based on caffeic acid would
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13 enable conjugation of catechol groups not only to PEG, but more generally to other polymers
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15 incorporating pendent or terminal amine groups.
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19 Herein, we designed and synthesized six different catechol-functionalized PEG building blocks
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21 (PEG-caffeamides) with different architectures and molecular weights: α,ω -bisfunctional PEG,
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23 referred to as 2-arm PEG-CAF (2.5 and 5 kDa), 4-arm PEG-CAF (5 and 10 kDa), and 8-arm PEG-
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25 CAF (10 and 20 kDa) (Scheme 1b). Specifically, we developed a synthetic strategy for polymer-
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27 catechol conjugation using the acyl-protected and activated intermediate, caffeic acid carbonic
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29 anhydride, which was coupled with high specificity ($\sim 95\%$) and yield ($\sim 90\%$) to end-functional
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31 PEG-amines, thus affording a series of catechol-functionalized PEG building blocks. The applied
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33 synthetic approach is shown to be a versatile and robust strategy to synthesize the macromolecular
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35 catechols. The synthesized PEG-caffeamides were subsequently examined as phenolic building
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37 blocks for the fabrication of PEG capsules with a range of tailorable properties via MPN assembly.
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39 In previous studies, we demonstrated the tuning of MPN capsule permeability (high,²⁹ moderate,¹
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41 or low³⁰) by controlling the coordination kinetics. We also demonstrated the control of
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43 permeability of MPN capsules through the choice of phenolic compounds, metal ions, and their
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45 ratio, which influenced the intermolecular dynamics and network formation.³¹ In the present study,
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47 we focused on the effect of employing bespoke PEG-caffeamide building blocks to fabricate a
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49 range of MPN capsules with tailorable properties. Specifically, by varying the architecture and
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3 molecular weight of the PEG-caffeamide building blocks, the shell thickness, permeability, and
4 cell association behavior of the capsules could be tuned. Further engineering of the MPN capsule
5 properties was demonstrated by selecting different sacrificial templates, namely carboxylic acid-
6 functionalized polystyrene (PS-COOH, nonporous) and CaCO₃ (highly porous) particles. Using
7 the reported conjugation chemistry to yield high-purity macromolecular phenolic building blocks,
8 this study presents a straightforward and highly versatile approach for tailoring the properties of
9 MPN capsules and potentially opens up further opportunities for the synthesis of MPN composite
10 materials.
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22 EXPERIMENTAL SECTION

23
24 **Synthesis of Multi-Arm PEG-Caffeamides.** As a representative example, the synthesis of
25 PEG-caffeamide building block using 2.5 kDa 2-arm PEG is described. Typically, 2-arm PEG-
26 NH₂, [PEG₃₀-NH₂]₂, (2.6 kDa, 271 mg, 1.04×10^{-4} mol, Sigma-Aldrich) was weighed into a 4 mL
27 vial. Dry dichloromethane (DCM; 2.5 mL) was then added via a rubber septum, and the solution
28 was stirred under nitrogen for several minutes. In a separate vial, iBoc-protected caffeic acid-
29 carbonic anhydride (i-Boc caffeic acid, iBocCAF) was added (200 mg, 4.16×10^{-4} mol) followed
30 by dry DCM (2.0 mL). The solution was stirred under a gentle stream of nitrogen and cooled in an
31 ice bath. The solution of [PEG₃₀-NH₂]₂ was then taken up into a syringe and slowly added dropwise
32 to the solution of iBocCAF. After addition was complete, the solution was allowed to react
33 (stirring) over ice and nitrogen for 1 h and then allowed to equilibrate to ambient temperature for
34 15 min. The solution was then concentrated to half its original volume and then added dropwise to
35 excess cold diethyl ether to precipitate the product, [PEG₃₂-iBoc-caffeamide]₂ as a sticky clear
36 residue. Precipitation was carried out twice to remove excess iBocCAF (mass yield: ~90%, %
37 purity of end groups = 95%). Refer to Figure S12 for peak assignments.
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[PEG₃₂-iBoc-caffeamide]₂ from the previous step (250 mg, 7.1×10^{-5} mol) was dissolved in dry DCM (1.30 mL) in a vial. In a separate vial, a 32% v/v solution of isopropyl amine was prepared in dry DCM (500 μ L of isopropylamine in 1.06 mL DCM). The PEG-iBocCAF solution was then added dropwise to the stirring isopropylamine/DCM solution. The final concentration of isopropylamine in DCM was ~17% v/v or 2.1 M, which is approximately 20 equivalents of isopropylamine per isobutyl carbonate group. The solution was stirred for 16 h at ambient temperature. The darkened solution was then concentrated to half its original volume and added dropwise to excess cold diethyl ether to precipitate the product, [PEG₃₆-caffeamide]₂, as a sticky tan residue. The solid was then dissolved in 0.1 M HCl buffer and dialyzed against acidic water for 3 days. The product was then freeze dried to isolate the product as a powder (cream) (yield: ~90%). Refer to Figure S13 for peak assignments. The 2-arm PEG-CAF was used for capsule fabrication and further referred to as 2.5 kDa 2-arm PEG.

Synthesis of PEG-Caffeamide-Fe^{III} MPN Capsules from PS-COOH Templates. A particle dispersion (100 μ L) of PS-COOH (1.86 ± 0.03 μ m, 50 mg mL⁻¹) was transferred into a 1.7 mL microcentrifuge tube and washed twice with water. To wash the PS-COOH particles, water (500 μ L) was added. The suspension was mixed by vortexing and sonication for 1–2 min, after which the particles were pelleted by centrifugation (2000g, 2 min). The supernatant (500 μ L) was then discarded, and the process was repeated for a second wash. The PEG-caffeamide stock solution and FeCl₃·6H₂O solution were added to obtain final concentrations of 0.5 mM and 1–4 mM (1 mM for 2-arm PEG-CAF, 2 mM for 4-arm PEG-CAF, and 4 mM for 8-arm PEG-CAF), respectively, and mixed by vortexing for 2 min. The final concentration of the particles was 10 mg mL⁻¹. 3-(*N*-Morpholino)propanesulfonic acid buffer (25 mM, pH 7.4, 0.7 mL) was added to raise the pH to >7, leading to the formation of bis- and tris-coordination complexes between catechol

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3 PEG and Fe^{III}. After increasing the pH, excess and unreacted materials were removed by pelleting
4 the particles (2000g, 2 min) and the supernatant was discarded. The MPN-coated particles were
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6 the particles (2000g, 2 min) and the supernatant was discarded. The MPN-coated particles were
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8 washed three times with water (500 μ L) by repeated centrifugation (2000g, 2 min). The particles
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10 were then resuspended in water (50 μ L), and tetrahydrofuran (THF; 1 mL) was added to remove
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12 the PS-COOH templates. The MPN capsules were pelleted through centrifugation (2000g, 2 min)
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14 and washed with THF (500 μ L) five times. At the final THF washing step, the capsules were
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16 pelleted through centrifugation (2000g, 3 min) and the supernatant was discarded. The resulting
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18 PEG-caffeamide-Fe^{III} MPN capsules were washed with water once and resuspended in water (250
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20 μ L).
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24 **Permeability Studies.** To assess the permeability of the PEG-caffeamide-Fe^{III} MPN capsules,
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26 the dispersion of MPN capsules was mixed with an equal volume of fluorescein isothiocyanate-
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28 tagged dextran (FITC-dextran) solution (1 mg mL⁻¹) through pipetting onto a glass slide. After 5–
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30 10 min, confocal laser scanning microscopy (CLSM) images of the capsules were taken. Dark
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32 capsule interiors were considered to indicate the formation of an impermeable film, whereas
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34 capsules with interiors of similar fluorescence intensity to the outer environment were considered
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36 permeable. Around 50–100 capsules were examined. The permeability of the capsules was
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38 reported as a percentage of capsules permeable to FITC-dextran.
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42 **Cell Association Experiments.** For flow cytometry analysis, RAW 264.7 cells were seeded in
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44 a 24-well plate at a 5×10^4 cells per well and then cultured in Dulbecco's Modified Eagle Medium
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46 (DMEM) with 10% fetal bovine serum (FBS) at 37 °C with 5% CO₂ for 20 h to allow cellular
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48 adhesion on the plates. The prepared fluorescent PEG-caffeamide-Fe^{III} MPN capsules were added
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50 to the cells at a capsule-to-cell ratio of 50:1 and then incubated for 4 h to examine cell association.
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53 After incubation, the supernatant was discarded, and the cells were washed with Dulbecco's
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3 phosphate-buffered saline (DPBS; 500 μ L) three times. Trypsin solution (1 \times , 200 μ L) was added
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5 to facilitate cell detachment, and the solution was maintained in the incubator (37 $^{\circ}$ C with 5%
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7 CO₂) for 5 min. For neutralization, DMEM solution (300 μ L) was added and the detached cells
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9 were washed with DPBS (1 mL) three times through centrifugation (350g, 5 min, 4 $^{\circ}$ C). The cells
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11 were dispersed in DPBS (400 μ L) and cell association was analyzed by flow cytometry. Relative
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13 fluorescence intensity (RFI) refers to the fluorescence intensity of treated cells relative to the
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15 fluorescence intensity of untreated cells.
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19 For the CLSM analysis, RAW 264.7 cells were seeded into 8-well Lab-Tek chambered
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21 coverglass slides (Thermo Fisher Scientific, Waltham, MA, USA) at a cell density of 4×10^4 cells
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23 per well and then cultured in DMEM with 10% FBS at 37 $^{\circ}$ C with 5% CO₂ for 20 h to allow
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25 cellular adhesion on the plates. The prepared fluorescent PEG-caffeamide-Fe^{III} MPN capsules
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27 were added to the cells at a capsule-to-cell ratio of 50:1 and then incubated for 4 h to evaluate cell
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29 association. After the incubation time had elapsed, the supernatant was discarded and the cells
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31 were washed with DPBS (400 μ L) twice. Cells were fixed by adding 4% paraformaldehyde (200
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33 μ L) for 30 min at 37 $^{\circ}$ C, then washed with DPBS (400 μ L) twice. The cell membranes were stained
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35 with wheat germ agglutinin-Alexa Fluor 594 conjugate (WGA594) for 5 min on ice, and the cell
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37 nucleus was dyed with Hoechst 33342 for 10 min at room temperature. CLSM imaging was
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39 performed to monitor the cell association of the capsules on a microscope equipped with a Plan
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41 Apo λ 60 \times 1.4 NA oil immersion objective, 405, 488, and 561 nm lasers, and 450/50, 525/50, and
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43 595/50 bandpass emission filters. The images were processed by Fiji software.
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49 **Cytotoxicity Studies of PEG-Caffeamide-Fe^{III} MPN Capsules.** A 2,3-bis[2-methoxy-4-nitro-
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51 5-sulfophenyl]-2*H*-tetrazolium-5-carboxyanilide inner salt (XTT) assay was performed to
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53 examine the cytotoxicity of the capsules. RAW 264.7 cells were seeded on a 96-well plate at a
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3 density of 8000 cells per well and incubated overnight. The culture media was then removed and
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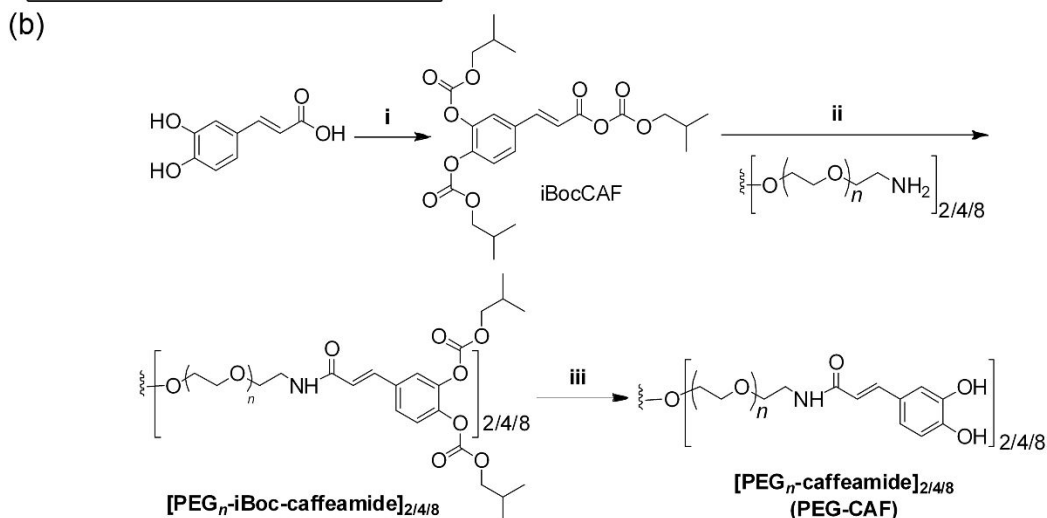
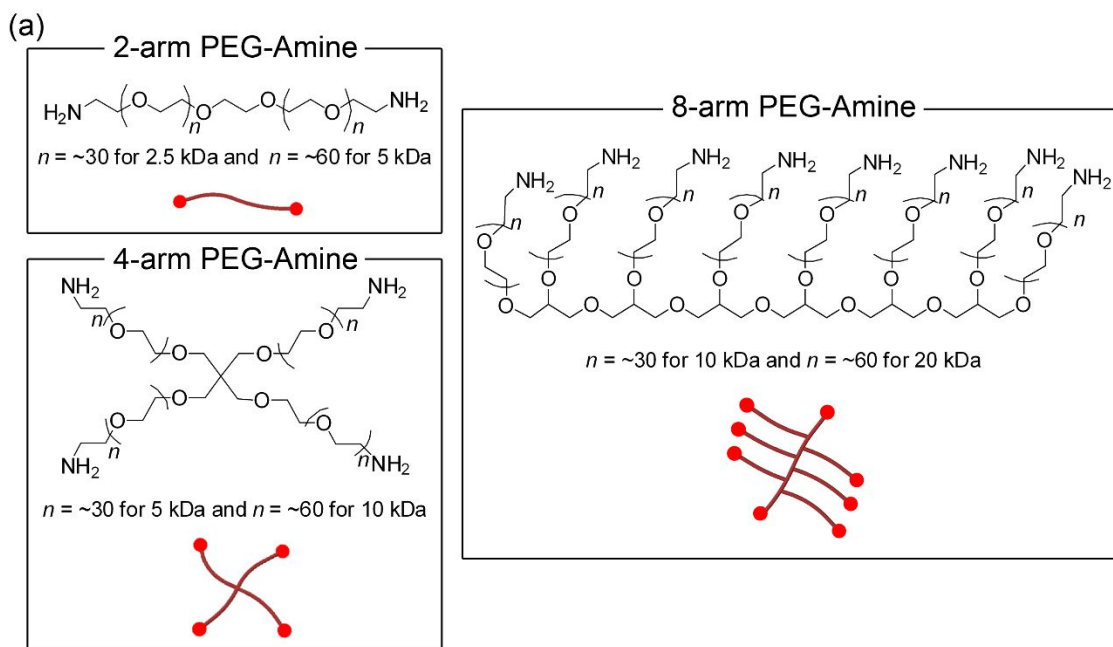
density of 8000 cells per well and incubated overnight. The culture media was then removed and replaced with either fresh media (for the cell control) or fresh media containing fluorescent PEG-caffeamide-Fe^{III} MPN capsules at a particle-to-cell ratio of 100:1 for 24 or 48 h. After the treatment, the media was replaced with fresh media containing activated XTT (9 mL of 0.2 mg mL⁻¹ XTT in DMEM with 10% FBS was activated by adding 22.5 μL of 0.6 mg mL⁻¹ phenazine methosulfate in DPBS), and cells were further incubated for 3 h. Finally, cells were screened on an Infinite M200 microplate reader (Tecan, Switzerland). Absorbance readings were measured at 475 nm with 30 s shaking, and a reference wavelength of 675 nm was used. Cell viability was expressed as a percentage by normalizing the absorbance to that obtained for untreated cells. All experiments were performed in triplicate, and data are presented as the mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Design and Synthesis of PEG-Caffeamides. Direct amidation or esterification of phenolic carboxylic acid small molecules can be conducted without the implementation of protective strategies for phenolic hydroxyl groups.³²⁻³⁴ However, with such an approach, the yields can be diminished by side reactions, such as the self-condensation of phenol hydroxyl and carboxylic acid groups.³⁵⁻³⁸ Reasonable conversions (>60%) to the desired esters or amides are more likely guaranteed using successive steps, namely (1) protection (of the phenolic hydroxyl group); (2) activation of the carboxylic acid group (for example using coupling agents such as 1-hydroxybenzotriazole, *N,N'*-dicyclohexylcarbodiimide, or acid chlorides); (3) condensation with alcohol or amine; and finally (4) deprotection.^{39,40} A range of protective groups and conjugation chemistries have been applied to yield libraries of phenolic acid esters and amide derivatives such

as caffeates and caffeamides. These have been shown to possess important properties such as anti-inflammatory, antioxidant, antitumor, and antibacterial activity.⁴¹

Scheme 1. (a) Structures and Molecular Weights of End-Functional PEG-Amine Precursors. (b) Synthesis of 2-, 4-, and 8-Arm PEG-CAF Building Blocks Using the Caffeic Acid Carbonic Anhydride Method: (i) isoButyl Chloroformate, *N*-Methyl Morpholine, and Triethylamine in THF at $-15\text{ }^{\circ}\text{C}$; (ii) $0\text{ }^{\circ}\text{C}$, DCM, 1 h; and (iii) Isopropyl Amine in Excess, DCM, Room Temperature.



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3 Using mixed carboxylic-carbonic anhydrides, which are activated forms of carboxylic acids, has
4 proven successful for the formation of amide bonds, particularly for peptide linkages,^{42–44} and this
5 approach has also been applied to the synthesis of low molecular weight caffeates and
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Using mixed carboxylic-carbonic anhydrides, which are activated forms of carboxylic acids, has proven successful for the formation of amide bonds, particularly for peptide linkages,^{42–44} and this approach has also been applied to the synthesis of low molecular weight caffeates and caffeamides.⁴⁵ Using an alkyl chloroformate, simultaneous protection (of hydroxyls) and activation (of the carboxylic acid group) can be conducted to yield the protected and activated caffeic acid-carbonic anhydride. Subsequent reaction of the carboxylic-carbonic anhydride with an alcohol or amine then affords the corresponding ester or amide, with deprotection facilitated using an excess of amine. In this work, caffeic acid-carbonic anhydride was used to synthesize a series of PEG-caffeamide building blocks with different molecular weights and architectures by conjugating to a range of end-functional PEG-amine molecules as starting materials (Scheme 1). The synthesis of iBocCAF (Scheme 1b) was performed in one step using isobutyl chloroformate, *N*-methyl morpholine, and triethylamine. Both hydroxyls in caffeic acid are protected as isobutyl carbonate groups, while the carboxylic acid is activated as the carbonic anhydride. Amide bond coupling between iBocCAF and the PEG-amine was then performed in DCM for 1 h by mixing the two solutions while cooling to afford a series of protected caffeamide-functionalized PEG building blocks, [PEG_{*n*}-iBoc-caffeamide]_{*m*}, where *m* is 2, 4, or 8. In this step, amide bond formation occurs via reaction of the amine with the caffeic acid carbonyl, with a loss of CO₂ and isobutanol (Scheme S1). The protected PEG-caffeamide building blocks ([PEG_{*n*}-iBoc-caffeamide]_{2/4/8}) were isolated by precipitation and analyzed before deprotection (see Figures S1–S26 for synthetic details and analysis of the end-functional PEG-amine, and protected and isolated PEG-caffeamide building blocks). In the case of small molecules, a one-pot conjugation followed by deprotection is typically conducted.^{42,45–47} However, in the present work, we implemented a two-step strategy to monitor the formation of the carbamate side product. This is formed upon

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3 reaction of the amine with carbonate groups during the coupling step, in this case yielding [iBoc-
4 PEG_n carbamate] arms (as shown in Scheme S2). This side reaction is known to occur during
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6 peptide synthesis and is thought to be influenced by the nature of the amine (i.e., primary or
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8 secondary), temperature, and solvent.⁴⁸ However, in the case of peptide coupling, racemization is
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10 an additional factor that needs to be considered. In our conjugation, the PEG carbamate product
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12 was not functionalized with catechols and could not be removed from the final product. Therefore,
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14 it was important to monitor the side product and identify conditions for minimizing its formation.
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17 A low molecular weight PEG-carbamate, i.e., [iBoc-PEG₇ carbamate]₂ was synthesized (Figure
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19 S9) to allow the identification and quantitation of the side product by ¹H NMR spectroscopy. Using
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21 an excess of iBocCAF for coupling (1.5–2 equivalents vs. amine) was key to yielding minimal
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23 amounts of carbamate side product (~5% vs. the desired [PEG_n-iBoc-caffeamide]_{2/4/8} product). In
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25 the next step, a low molecular weight amine (primary or secondary) was required to deprotect the
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27 carbonate groups—herein, we used isopropyl amine. A small molecule carbamate was thus formed
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29 upon deprotection via reaction of the small molecule amine with isobutyl carbonate groups. The
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31 carbamate was extracted from the polymer mixture by precipitation, and the polymer subsequently
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33 purified by dialysis. For each polymer, a large excess of amine was added to completely deprotect
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35 the isobutyl carbonate groups, yielding [PEG_n-caffeamide]_{2/4/8} products in high purity and
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37 specificity. In the case of [PEG₆₀-caffeamide]₄, a small amount of unprotected arm remained in the
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39 mixture but this was deemed insignificant by ¹H NMR analysis (<5%).
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47 It is worth noting that a series of end-functional PEG-amine building blocks used in this study
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49 were synthesized in-house using commercially sourced hydroxyl end-functional PEG (see Figures
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51 S3–S8). Specifically, six end-functionalized PEG amine molecules with different structures and
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53 molecular weights were prepared: α,ω -bisfunctional 2-arm PEG-NH₂ (2.5 and 5 kDa), 4-arm PEG-
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3 NH₂ (5 and 10 kDa), and 8-arm PEG-NH₂ (10 and 20 kDa) (structures displayed in Scheme 1a).
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5 As part of this procedure, the Staudinger reaction was used to produce iminophosphorane end
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7 groups from corresponding azides, which were then converted into the amines in situ.⁴⁹ This is by
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9 no means the only methodology for introducing amine functionality to a polymer and ought not be
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11 construed as an exclusive and essential step in the caffeamide functionalization: an alternative
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13 amination approach could equally be used. Further, while conversion of the iminophosphorane
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15 end groups into amines is commonly achieved via hydrolysis in water, methanolysis can be used
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17 as an alternative to hydrolysis to produce the amine. Methanolysis was applied in the current study,
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19 owing to the ease of reagent removal. We note that the application of methanolysis results in a
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21 small amount of methylated amine end groups (secondary and tertiary) in addition to the primary
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23 amine. This was particularly evident for the 2-arm PEG-NH₂ (2.5 and 5 kDa, Figures S3 and S4).
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25 Therefore, the converted [PEG_n-iBoc-caffeamide]_{2/4/8} products also showed some small methyl
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27 peaks in their ¹H NMR spectra (3.0–3.3 ppm in Figures S10, S11, S15, and S16). Commercial
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29 PEG amines were therefore substituted as starting materials to generate the 2-arm PEG-CAF
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31 products ([PEG_n-caffeamide]₂) and accordingly their ¹H NMR spectra did not feature such peaks.
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33 Nevertheless, this factor had no apparent effect on subsequent capsule properties, and further
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35 optimization of amine synthesis was therefore not pursued. It is also important to note that amine
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37 functionality might be incorporated into the polymer via chemistries other than the Staudinger
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39 reaction, or, as noted above, commercially available polymeric amines might be used as the
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41 substrate for functionalization. Moreover, although we have exemplified the approach here using
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43 a PEG-amine library, it is important to note that functionalization via iBocCAF is equally
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45 applicable to other amine functional polymers.
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The resulting $[\text{PEG}_n\text{-caffeamide}]_{2/4/8}$ polymers with different architectures (2-, 4-, and 8-arms) and molecular weights were used as MPN building blocks for the capsule studies and referred to as: 2.5 kDa 2-arm PEG-CAF, 5 kDa 2-arm PEG-CAF, 5 kDa 4-arm PEG-CAF, 10 kDa 4-arm PEG-CAF, and 10 kDa 8-arm PEG-CAF and 20 kDa 8-arm PEG-CAF, as outlined in Table S1.

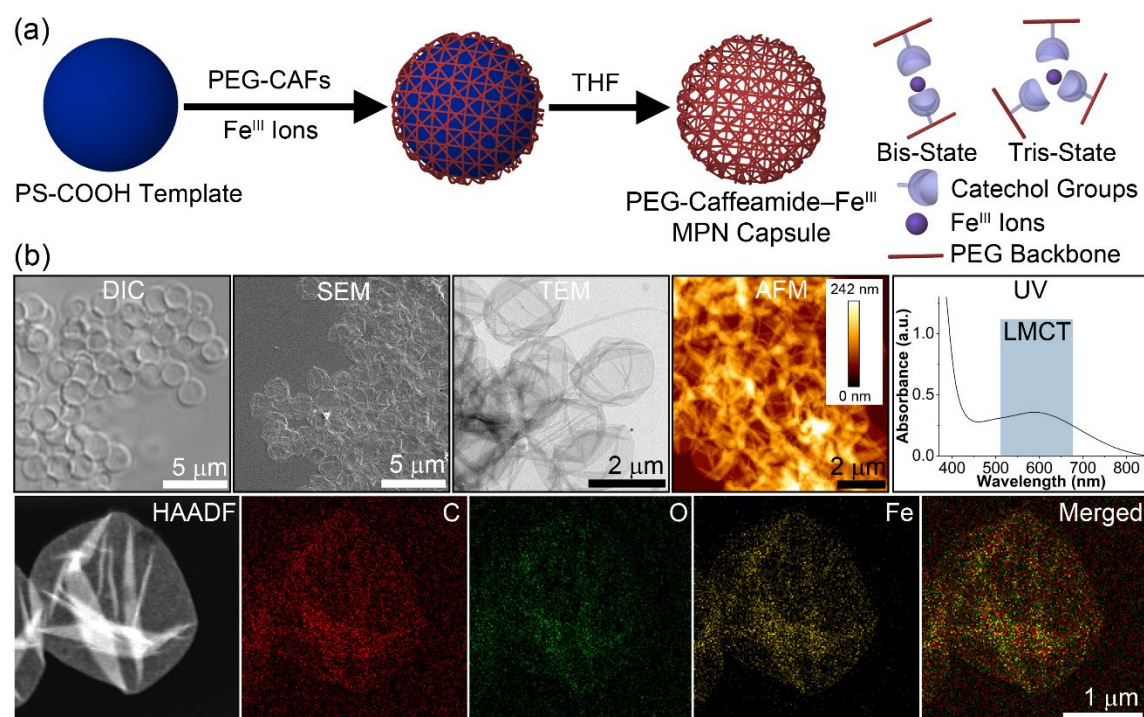


Figure 1. (a) Schematic of the preparation of PEG-caffeamide- Fe^{III} MPN capsules using PS-COOH templates. (b) Characterization of PEG-caffeamide- Fe^{III} MPN capsules (using 10 kDa 4-arm PEG-CAF) derived from PS-COOH sacrificial particles using DIC, SEM, TEM, AFM, UV-vis spectroscopy, HAADF, and EDX elemental mapping. The UV-vis absorption spectrum of the PEG-caffeamide- Fe^{III} system shows an LMCT band, which indicates the formation of bis- and tris-state MPN complexes.

Fabrication of PEG-Caffeamide- Fe^{III} MPN Capsules. MPN film formation via the coordination of PEG-caffeamide building blocks and Fe^{III} ions was achieved on sacrificial template particles, namely, PS-COOH ($1.86 \pm 0.03 \mu\text{m}$) particles (Figure 1a and S27a). PEG-

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3 caffeamide solutions and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ were successively added to the suspension of sacrificial
4 particles, followed by the addition of buffer solution (pH 7.4) to increase the pH of the suspension.
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6 The change in pH resulted in the cross-linking of the PEG-caffeamide- Fe^{III} MPNs based on the
7 formation of bis- and tris- coordination states. MPN capsules were obtained following dissolution
8 of the PS-COOH template particles in THF. The coordination between the Fe^{III} ions and PEG-
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caffeamide building blocks in the MPN capsules was characterized by UV-vis spectroscopy analysis. This coordination exhibited the pH-dependent transitions expected between mono-, bis-, and tris-complex states (Figure S28). A characteristic ligand-to-metal charge transfer (LMCT) band was detected around 565 nm, indicating bis- and tris-type coordination between Fe^{III} and PEG-caffeamide, where the bis-state was dominant, in the capsules (Figures 1b and S28).^{1,50} The preparation of MPN capsules composed of natural phenolic compounds (e.g. TA, gallic acid, pyrogallol, and pyrocatechol) and Fe^{III} ions was previously reported using PS particles as sacrificial templates.^{1,22} In the present work, the PEG-caffeamide- Fe^{III} MPN films (using PEG-caffeamide building blocks) were successfully deposited on carboxylic acid-functionalized PS particles instead of bare PS particles via hydrogen bonding between the PEG backbone and carboxylic acid groups, which appears to be a key factor for MPN film deposition (Figure S29).

As a representative sample, the characterization of PEG-caffeamide- Fe^{III} MPN capsules prepared from PS-COOH templates using 10 kDa 4-arm PEG-CAF and Fe^{III} is shown in Figure 1b. The capsules were well dispersed in aqueous solution, as observed from the differential interference contrast (DIC) microscopy image in Figure 1b. Scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), and high-angle annular dark-field (HAADF) microscopy revealed collapsed capsule structures with folds and creases, which are characteristic of collapsed capsules following drying in air. Energy-dispersive

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3 X-ray spectroscopy (EDX) elemental mapping revealed that C, O, and Fe were all uniformly
4 distributed throughout the PEG-caffeamide-Fe^{III} MPN capsules. Fourier transform infrared
5 spectroscopy additionally confirmed the presence of PEG in the resulting capsules (Figure S30).
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10 A different MPN capsule system (Figure S31) was additionally fabricated using 10 kDa 4-arm
11 PEG-CAF and porous CaCO₃ particles (3.19 ± 0.34 μm) as sacrificial templates (Figure S27b).
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13 The templates were selectively dissolved using ethylenediaminetetraacetic acid (EDTA). The
14 AFM data showed that the overall shell thickness of the capsules prepared using CaCO₃ templates
15 were thicker (163 nm vs 16 nm) than the PS-COOH-templated capsules (Figure 1b, Figure S31,
16 and Table S2). Coordination between catechol-PEG and Fe^{III} has been reported to occur within the
17 highly porous CaCO₃ particles,¹² generating thicker shells. Overall, these results demonstrate that
18 well-defined capsules can be prepared via MPN formation between the synthesized PEG-
19 caffeamides and Fe^{III} ions.
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32 **Scheme 2. PEG-Caffeamide-Fe^{III} MPN Capsules Prepared from Coordination-Driven**
33 **Cross-Linking of Fe^{III} Ions with Different 2-, 4-, or 8-arm PEG-CAF Building Blocks Using**
34 **PS-COOH Templates.**
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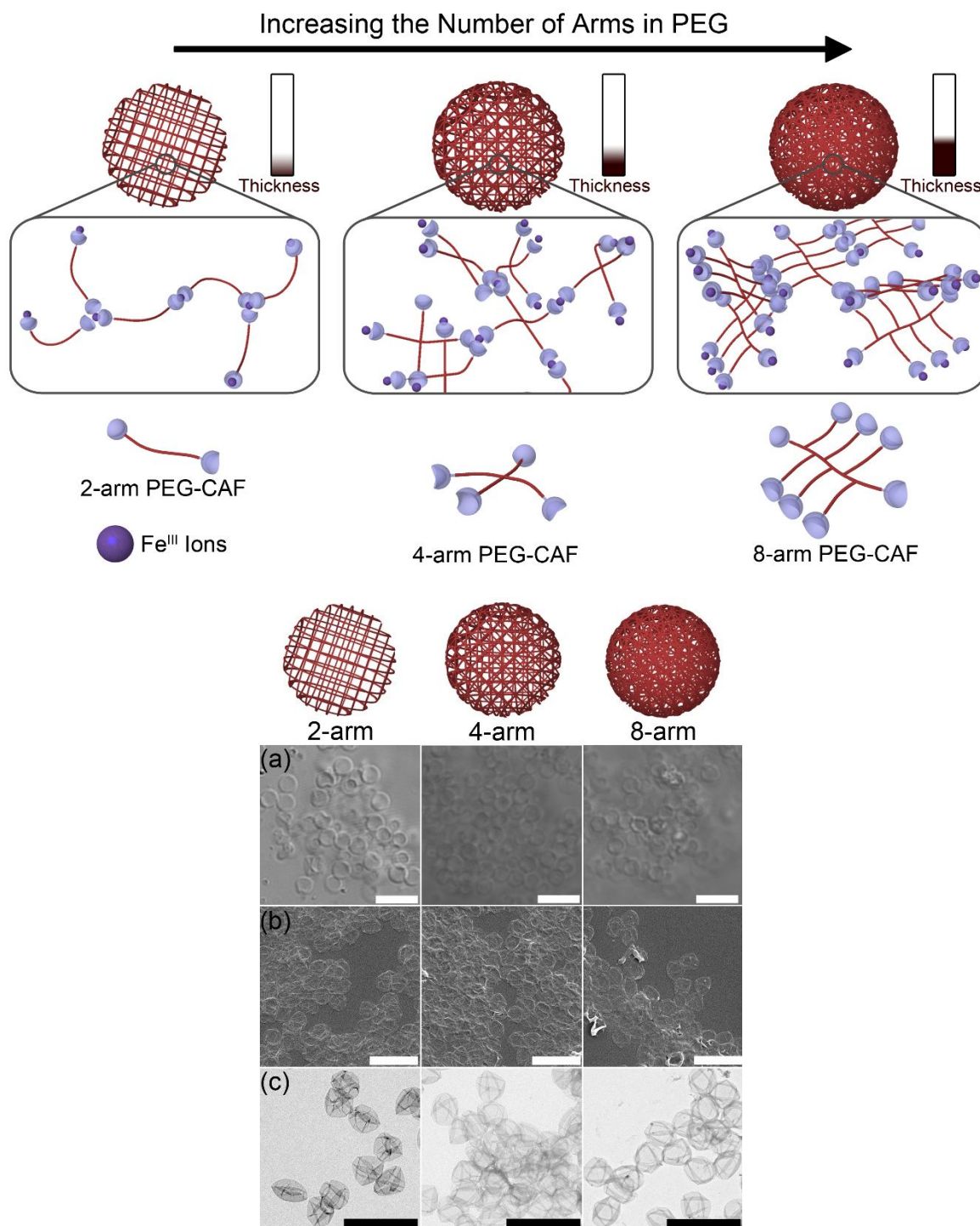


Figure 2. Microscopy images of PEG-caffeamide-Fe^{III} MPN capsules prepared from 2.5 kDa 2-arm, 5 kDa 4-arm, and 10 kDa 8-arm PEG-CAFs and PS-COOH templates: (a) DIC, (b) SEM, and (c) TEM images. All scale bars are 5 μm .

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3 **Engineering the Shell Thickness of MPN Capsules.** The conventional MPN film formation,
4 which occurs via a discrete assembly process, generally creates hollow capsules with a film
5 thickness of approximately 10 nm regardless of the metal ion, phenolic building block, or phenolic
6 compound-to-metal molar ratio selected.^{1,8,22} Additionally, it is difficult to control the film
7 thickness owing to rapid film growth (<1 min) using these parameters.^{1,30} Using alternative
8 approaches, i.e., by repeating the MPN coating cycle¹² or via continuous assembly,⁵¹ MPN
9 capsules with different shell thicknesses can be engineered. However, these methods require
10 repetitive and multiple steps or longer times for film formation (~4 h). In contrast, in the present
11 work, we postulated that a simple and more rapid methodology (<2 min), involving only one
12 discrete deposition step, could be used to tune the shell thickness of the capsules by varying both
13 the architecture (2-, 4-, and 8-arm) and the molecular weight (M_n 2.5–20 kDa) of the PEG-
14 caffeamide building blocks prepared herein (Scheme 2). To investigate this approach, six types of
15 PEG-caffeamide–Fe^{III} MPN capsules were first fabricated from PS-COOH sacrificial templates
16 and using the six PEG-caffeamide building blocks (2-arm PEG-CAF (2.5 and 5 kDa), 4-arm PEG-
17 CAF (5 and 10 kDa), and 8-arm PEG-CAF (10 and 20 kDa)). The capsules were characterized via
18 DIC, SEM, TEM, and dynamic light scattering (DLS) (Figures 2, S32, and S33a). As observed
19 from the DIC images and DLS analysis, the PEG-caffeamide–Fe^{III} MPN capsules were stable and
20 monodisperse. Moreover, SEM and TEM images revealed the typical surface morphology of
21 collapsed capsules.
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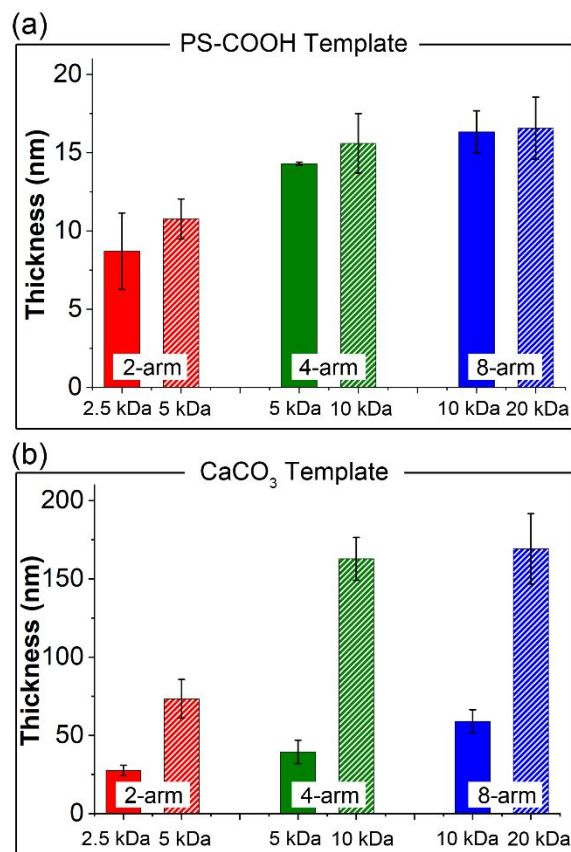


Figure 3. Comparison of the shell thickness of PEG-caffeamide-Fe^{III} MPN capsules prepared from (a) PS-COOH templates and (b) CaCO₃ templates and various PEG-caffeamide building blocks: 2.5 kDa 2-arm and 5 kDa 2-arm; 5 kDa 4-arm and 10 kDa 4-arm; and 10 kDa 8-arm and 20 kDa 8-arm. The PEG-to-catechol ratios were 1250:1 (solid bars) and 2500:1 (diagonal stripes). The thickness was determined by height-distance AFM graphs and the data are shown as the mean \pm SD of three independent AFM measurements. Scale bars are 5 μ m.

Subsequently, we explored assembly of the same six polymers using porous CaCO₃ particles as the template. In this case, PEG-caffeamide-Fe^{III} MPN film deposition occurs within the porous structures of the particles¹² (Scheme S3). The obtained capsules were characterized by DIC, SEM, TEM, and DLS (Figures S33b and S34). The CaCO₃-templated MPN capsules exhibited fewer folds and creases than the PS-COOH-templated MPN capsules, suggesting thicker capsule walls

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(see below for a quantitative discussion). Overall, these results indicate that variation of the template has a significant effect on shell properties.

To provide a quantitative comparison of the shell thickness of the capsules, the PEG-caffeamide-Fe^{III} MPN capsules were examined by AFM (Figures 3, S35, and S36). The thickness of the PS-COOH-templated MPN capsules showed a dependency on the type of the PEG-caffeamide building block used (which is detailed in subsequent discussion). A thin film layer ranging from 8.7 to 16.6 nm was observed (Figures 3 and S35 and Table S2). This thickness range is comparable with the shell thickness typically observed for conventionally prepared TA-Fe^{III} MPN capsules (~10 nm).¹ In contrast, the CaCO₃-templated PEG-caffeamide-Fe^{III} MPN capsules displayed thicker shells with a broad range of attainable thickness (27.6–169.3 nm) (Figures 3 and S36 and Table S2), further confirming that the sacrificial template greatly influences the thickness of the capsules. The shell thickness was also affected by the concentration of PEG-caffeamide in the assembly solution (Figure S37a). Specifically, PEG-caffeamide-Fe^{III} MPN capsules prepared using 0.25 mM 10 kDa 4-arm PEG-caffeamide have thinner shells than capsules prepared using 0.5 and 1 mM 10 kDa 4-arm PEG-caffeamide. Further, incubating the template particles for different times in the assembly solution (i.e., 2, 20, and 60 min) led to similar shell thicknesses (Figure S37b).

Regardless of the particle template employed, the shell thickness was largely dependent on the structure and M_n of the PEG-caffeamide building blocks. One primary factor affecting shell thickness is the polymer structure itself. Across the range of PS-COOH-templated capsules prepared, the increase in shell thickness correlated with an increase in the number of arms in the PEG-caffeamide building blocks (Figure 3a). During the deposition process, network formation is facilitated by the coordination of the Fe^{III} ions with the catechol end groups, which leads to

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3 bridging between individual polymer chains. A higher number of PEG arms thus corresponds to a
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5 higher number of catechol end groups and therefore the concurrent formation of more
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7 interconnections. Overall, this results in enhanced polymer deposition and therefore the formation
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9 of thicker films. Maintaining the same number of PEG arms (and therefore number of catechol
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11 end groups) while increasing the M_n of PEG also resulted in thicker capsule films (Figure 3a). This
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13 trend can be attributed to hydrogen bonding interactions between the polymer chains in the
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15 network, as well as with water and the template itself. The hydrogen bonding interactions are more
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17 significant in films with a higher proportion of PEG. It is notable that for the PS-COOH-templated
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19 capsules, the increase in shell thickness while increasing PEG M_n at a given PEG arm number is
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21 not significant. However, in the case of the CaCO₃-templated capsules, the effect of the PEG M_n
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23 (at a given PEG arm number) on the shell thickness was more pronounced. This was particularly
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25 evident for the 4-arm PEG capsules, where the capsules prepared from 10 kDa PEG-CAF were
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27 approximately 4× thicker than the capsules prepared from 5 kDa PEG-CAF (Figure 3b). To better
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29 understand the thickness trend obtained, the PEG M_n -to-catechol (end group) ratio was examined,
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31 as this determines both the concentration of anchor points in the final network and the length of
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33 the PEG chain that separates each anchor point. Within the PEG-cafeamide building block series,
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35 two groups were distinguished based on the PEG M_n -to-catechol ratio (1250:1 and 2500:1 groups,
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37 refer to Table S1). The higher ratio group (2500:1) had a higher M_n PEG chain separating each
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39 catechol-Fe^{III} anchor point, and this group consistently displayed higher thickness values across
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41 the PEG-arm series (Figure 3), likely due to hydrogen bonding interactions that significantly
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43 affected film growth.
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51 For the CaCO₃-templated particles, a different trend was observed from that observed for the
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53 PS-COOH-templated capsules. The thickness of the capsules formed using the 5 kDa 2-arm PEG-
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3 CAF was larger than that for the capsules formed from the corresponding 5 kDa 4-arm PEG-CAF.
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5 The same effect was evident for the 10 kDa 4-arm PEG-CAF capsules, which were thicker than
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7 the 10 kDa 8-arm PEG-CAF capsules. In these cases, at a given PEG M_n , a significantly thicker
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9 capsule wall was formed using fewer arms (i.e., less catechol end groups). The greater number of
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11 anchor points available for complexation is likely to result in larger aggregates being formed more
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13 rapidly, thus restricting diffusion of the polymer into the pores of the CaCO_3 .
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17 Overall, we demonstrate a rapid and straightforward methodology for tuning film thickness of
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19 PEG-caffeamide- Fe^{III} MPN capsules via AFM analysis. By changing the properties of PEG (i.e.
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21 architectures and molecular weights), combined with the type of sacrificial particles (porous or
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23 nonporous), large adjustments in capsule shell thickness can be engineered, ranging from 8.7 to
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25 169.3 nm. In the case of PS-COOH templated capsules, which have thinner walls overall compared
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27 with the CaCO_3 -templated capsules, minor increases in capsule thickness were achieved by
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29 increasing the proportion of PEG in the PEG-caffeamide building block. This was facilitated by
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31 either increasing the number of arms or the M_n of PEG itself. For the CaCO_3 -templated capsules,
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33 thicker walls were formed due to infiltration of the network into the porous structure of the
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35 template. However, by simply increasing the number of catechol end groups in the building blocks,
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37 which form the anchor points in the network, a reduction in film thickness was achieved due to
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39 likely reduced diffusion of the Fe^{III} -complexed polymer chains into the pores of the CaCO_3
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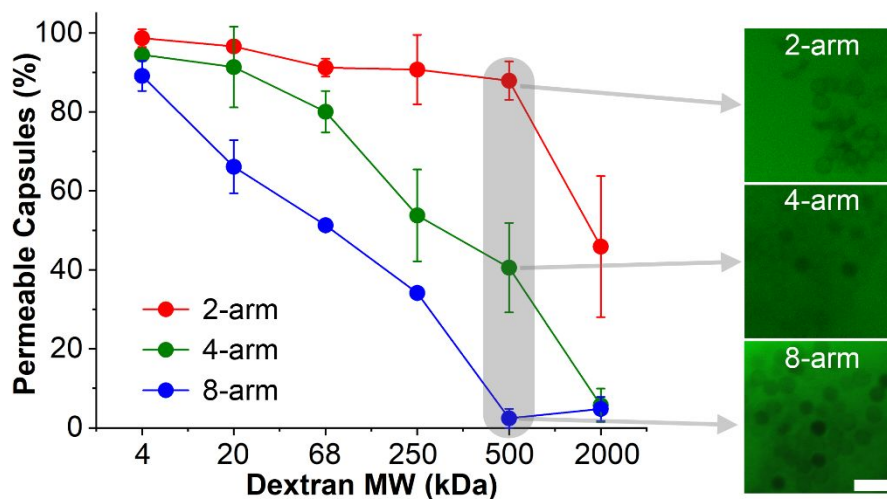


Figure 4. Comparison of the permeability of PEG-caffeamide-Fe^{III} MPN capsules prepared using PS-COOH templates and 2.5 kDa 2-arm, 5 kDa 4-arm, and 10 kDa 8-arm PEG-CAF against FITC-dextran with molecular weight (MW) ranging from 4 to 2000 kDa. The PEG M_n -to-catechol ratio was 1250:1. Scale bars are 5 μ m. Error bars represent the SD of three independent experiments.

Modulating Film Permeability. Capsule properties such as stiffness, permeability, and cell association behavior are influenced by the capsule shell thickness.^{12,51,52} As the thickness of the capsule wall was influenced by the structure and composition of the PEG-caffeamide building block, we investigated whether the permeability of the PEG-caffeamide-Fe^{III} MPN capsules could be controlled in an analogous manner. The permeability of the PEG-caffeamide-Fe^{III} MPN capsules was probed by incubating the capsules with FITC-dextran of different molecular weights, ranging from 4 to 2000 kDa (Figures 4, S38–S41). As observed, the PS-COOH-templated MPN capsules, prepared from different PEG-caffeamide building blocks, displayed variable permeability against FITC-dextran molecules (Figures 4, S38, and S39). The permeability of the PEG-caffeamide-Fe^{III} MPN capsules prepared from 2-arm, 4-arm, or 8-arm PEG-CAFs with a PEG M_n -to-catechol ratio of 1250:1 was examined. Increasing the number of arms (and therefore

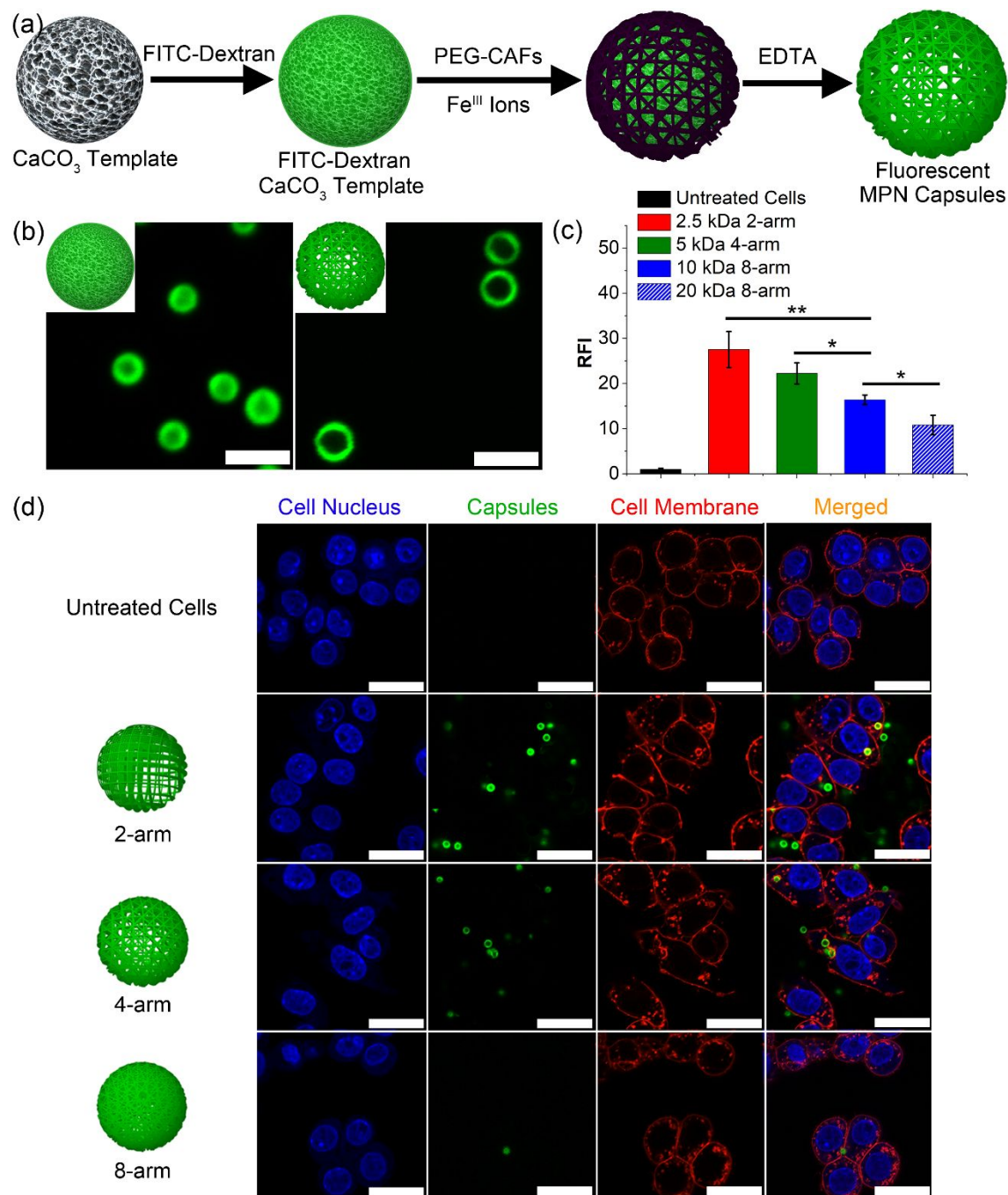
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3 number of catechols) resulted in a reduction in permeability. Specifically, 87.9% of 2-arm PEG-
4 caffeamide-Fe^{III} MPN capsules (regarded as highly permeable), 40.6% of 4-arm PEG-caffeamide-
5 Fe^{III} MPN capsules (regarded as moderately permeable), and 2.4% of 8-arm PEG-caffeamide-Fe^{III}
6 MPN capsules (regarded as nearly impermeable) were permeable to varying extents to 500 kDa
7 FITC-dextran (Figures 4 and S39a). When a higher PEG-to-catechol ratio of 2500:1 was
8 employed, the capsules followed a similar permeability trend, with similar levels achieved (Figure
9 S38). Specifically, 89.1% of 2-arm PEG-caffeamide-Fe^{III} MPN capsules (highly permeable),
10 45.8% of 4-arm PEG-caffeamide-Fe^{III} MPN capsules (moderately permeable), and 19.6% of 8-
11 arm PEG-caffeamide-Fe^{III} MPN capsules (nearly impermeable) were permeable to varying
12 degrees to 500 kDa FITC-dextran (Figures S38 and S39b). Higher permeability is associated with
13 a less dense network structure, i.e., with a higher proportion of PEG and fewer Fe^{III}-catechol cross-
14 links.³¹ This was achieved using a PEG-caffeamide building block with fewer catechol-
15 functionalized PEG arms (2 arm vs. 4 arm vs. 8 arm). The results correlate with the shell thickness
16 trends, where a significant reduction in capsule thickness is obtained by reducing the number of
17 arms, resulting in a network structure with fewer crosslinks (anchor points).

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38 In contrast, the CaCO₃-templated PEG-caffeamide-Fe^{III} MPN capsules showed significantly
39 reduced permeability, with less than 25% of the 2-arm PEG-caffeamide-Fe^{III} MPN capsules and
40 close to 0% of the 4- and 8-arm PEG-caffeamide-Fe^{III} MPN capsules being permeable to a range
41 of FITC-dextran (4–2000 kDa) (Figures S40 and S41). These results are likely due to the thicker
42 shell layers formed on these capsules (ranging from 27.6 to 169.3 nm) (Figure 3b and Table S2).
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49 Interestingly, the PEG-caffeamide-Fe^{III} MPN capsules prepared from 10 kDa 4-arm PEG-CAF
50 and 20 kDa 8-arm PEG-CAF, which had the thickest shells (162.9 and 169.3 nm, respectively),
51 were impermeable even to 4 kDa FITC dextran (Figure S40b), thus showing the robust and
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3 impermeable feature of the shell. The low permeability achieved herein is lower than that reported
4 for MPN capsules prepared from discrete assembly^{1,31} or oxidation-mediated coordination
5 assembly,³⁰ thus showing promise for loading small molecule drugs. These results demonstrate
6 that with proper selection of the PEG building block and sacrificial template, the permeability of
7 PEG-caffeamide-Fe^{III} MPN capsules can be tuned from 0 to 100% over a wide molecular weight
8 range (e.g., 4–2000 kDa dextran). The present methodology, which allows fine and broad tuning
9 of capsule permeability, could potentially be employed for engineering capsules for various
10 applications, including therapeutic delivery, sensing, separations, and catalysis.^{12–16,30}

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12 **Controlling Cell Association of PEG-Caffeamide-Fe^{III} MPN Capsules.** PEG has attracted
13 much interest in biomedical applications owing to the stealth effect derived from its excellent
14 antifouling behavior and high biocompatibility.^{23–25,28} For instance, to prolong the blood
15 circulation time of drug delivery systems, their surfaces have commonly been modified with PEG
16 chains, which results in reduced nonspecific interactions.⁵³ The PEG-caffeamide-Fe^{III} MPN
17 capsules are expected to have tunable stealth properties arising from the different architectures and
18 molecular weights of the PEG building blocks used in the network system. To compare the stealth
19 properties of the different PEG-caffeamide-Fe^{III} MPN capsules, fluorescent MPN capsules were
20 first prepared (Figure 5a). Briefly, fluorescent sacrificial template particles were prepared via
21 physical interactions between CaCO₃ particles and 2000 kDa FITC-dextran. Capsules exhibiting
22 green fluorescence were then obtained through successive MPN film deposition on the as-prepared
23 FITC-dextran labeled CaCO₃ templates and template removal with EDTA. The FITC-dextran-
24 labeled CaCO₃ particles and fluorescent PEG-caffeamide-Fe^{III} MPN capsules were characterized
25 by CLSM (Figure 5b). The observed fluorescent ring structures of the capsules likely derive from
26 hydrogen bonding interactions between dextran and the PEG film. To investigate the stealth
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properties of the fluorescent PEG-caffeamide- Fe^{III} MPN capsules, their cell association profiles were analyzed by flow cytometry and CLSM to determine the green fluorescence intensity originating from the cell-associated fluorescent MPN capsules (Figure S42).



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3 **Figure 5.** (a) Schematic illustration of the preparation of fluorescent MPN capsules using FITC-
4 dextran-labeled CaCO_3 particles. (b) Fluorescence microscopy images of FITC-dextran (2000
5 kDa)-labeled CaCO_3 particles (left) and fluorescent PEG-caffeamide- Fe^{III} MPN capsules (right).
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10 (c) Flow cytometry analysis of RAW 264.7 cells treated with fluorescent PEG-caffeamide- Fe^{III}
11 MPN capsules prepared from 2.5 kDa 2-arm, 5 kDa 4-arm, 10 kDa 8-arm, and 20 kDa 8-arm PEG-
12 CAF building blocks, following incubation for 4 h at 37 °C. The PEG M_n -to-catechol ratios were
13 1250:1 (solid bars) and 2500:1 (diagonal stripes). RFI to untreated cells is shown. Error bars
14 represent the SD of three independent experiments. Statistical significance was determined by one-
15 way ANOVA analysis: ** $p < 0.01$ and * $p < 0.05$. (d) CLSM images of Raw 264.7 cells incubated
16 with fluorescent PEG-caffeamide- Fe^{III} MPN capsules prepared from 2.5 kDa 2-arm, 5 kDa 4-arm,
17 and 10 kDa 8-arm PEG-CAF for 4 h at 37 °C. Cell membranes and nuclei were stained with
18 WGA594 (red) and Hoechst 33342 (blue), respectively. Green fluorescence represents fluorescent
19 MPN capsules. Scale bars are 5 μm (b) and 20 μm (d).
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34 Capsules prepared from different PEG-caffeamide building blocks (2-, 4-, and 8-arm PEG-CAF
35 with a PEG M_n -to-catechol ratio of 1250:1) were incubated with RAW 264.7 macrophage cells for
36 4 h at 37 °C at a capsule-to-cell ratio of 50:1. As observed in Figure 5c, the fluorescence intensity
37 (relative to untreated cells) of RAW 264.7 cells treated with PEG-caffeamide- Fe^{III} MPN capsules
38 decreased from 27.5 to 22.2 and 16.4 RFI with increasing number of arms from 2 to 8 in the PEG
39 building blocks. Moreover, the RFI decreased further to 10.8 RFI by increasing the molecular
40 weight of 8-arm PEG-CAF from 10 to 20 kDa. For visualization of the cell–capsule interactions,
41 the cell nucleus and membrane were stained by Hoechst 33342 (blue) and WGA594 (red), which
42 do not overlap with the fluorescence from the MPN capsules (green). Consistent with the flow
43 cytometry results, 2-arm PEG-caffeamide- Fe^{III} MPN capsules associated more significantly with
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3 cells in comparison with 8-arm PEG-CAF capsules, which associated less with cells (Figure 5d).
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5 Increasing the proportion of PEG in the building blocks (to 2500:1, Figure S43) showed a similar
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7 trend in the RFI of the cells, that is, PEG-caffeamide-Fe^{III} capsules prepared from PEG building
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9 blocks with more arms were more stealthy. The cytotoxicity of the resulting capsules was
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11 evaluated via XTT assay. No detectable toxicity was observed across the range of particle
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13 concentrations tested (Figure S44). These results demonstrate that the biological properties of the
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15 MPN capsules can also be controlled via judicious selection of the PEG-CAF building block, with
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17 an increase in the proportion of PEG-CAF in the building block resulting in reduced nonspecific
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19 cell association. The proposed methodology could therefore be used to control the fouling
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21 properties of capsules with potential in the development of advanced delivery vehicles for drugs
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23 or therapeutic nucleic acids.^{10,54}
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29 CONCLUSIONS

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32 In this work, we have demonstrated a versatile and robust approach for conjugating caffeic acid
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34 to amine functional polymers, thereby yielding an array of macromolecular phenolic building
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36 blocks for MPN assembly. Specifically, by reacting caffeic acid with isobutyl chloroformate, we
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38 were able to activate the carboxylic acid to amine addition (as the carbonic anhydride) while
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40 simultaneously protecting the hydroxyls as isobutyl carbonate groups. The resulting iBoc-
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42 protected caffeic acid-carbonic anhydride could be conjugated quantitatively to PEG-amines and
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44 deprotected under mild conditions to reveal a series of PEG-caffeamides. Specifically, PEG-
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46 caffeamide building blocks with different architectures and molecular weights (2-arm PEG (2.5
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48 and 5 kDa), 4-arm PEG (5 and 10 kDa), and 8-arm PEG (10 and 20 kDa)) were synthesized. Well-
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50 defined MPN capsules were subsequently fabricated using the various PEG-caffeamide building
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52 blocks on different sacrificial particles via the coordination of catechol-end groups with Fe^{III} ions.
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3 The resulting particles displayed tunable capsule properties (shell thickness, permeability, and cell
4 association), dictated by the nature of the PEG-caffeamide building block. Independent of the
5 template chosen (PS-COOH or CaCO₃), the increase in shell thickness of the capsules correlated
6 with an increase in the number of arms. Increases in PEG M_n also resulted in thicker capsule films,
7 due to hydrogen bonding, although this increase was more significant in the CaCO₃-templated
8 capsules. Furthermore, the use of nonporous PS-COOH template particles afforded thinner films,
9 whereas the use of porous CaCO₃ particles led to thicker films. The permeability profiles of the
10 capsules were controlled by varying the structure of the PEG-caffeamide building block, with a
11 systematic increase obtained by using fewer catechol-functionalized arms, as consistent with shell
12 thickness trends. In the case of the porous CaCO₃-templated capsules, where capsule walls were
13 significantly thicker, the capsules were significantly less permeable. The degree of cell association
14 of the CaCO₃-templated capsules decreased by increasing the number of arms in the PEG-
15 caffeamide building blocks and decreased further by increasing the proportion of PEG in the
16 formed shell structure. These results demonstrate that, by combining a high yielding and versatile
17 polymer conjugation approach with the judicious choice of capsule template, it is possible to tune
18 MPN capsule properties over a wide range. Such micrometer-sized capsules could be of potential
19 interest for controlled vaccine and pulmonary delivery applications.^{12–16} Moreover, given that the
20 reported conjugation strategy is applicable to almost any amine-functional substrate, the reported
21 approach should provide a platform to further the biological application of MPN capsules as
22 delivery vehicles^{10,12–16,30,54,55} and for surface coating technologies.⁵⁶

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52 ASSOCIATED CONTENT
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3 **Supporting Information.** Materials and instrumentation; synthesis of PEG-caffeamide building
4 blocks, CaCO₃ particles, FITC-dextran-labeled CaCO₃ particles, and fluorescent MPN capsules;
5 thickness comparison of MPN capsules; and NMR; DIC; SEM; TEM; permeability; cell
6
7 association; cytotoxicity data; and MIRIBEL checklist for reporting research in bio–nano science.
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9 This material is available free of charge via the Internet at <http://pubs.acs.org>.
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15 AUTHOR INFORMATION

16 17 18 **Corresponding Author**

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21 *E-mail: john.f.quinn@monash.edu (J.F.Q.) and fcarus@unimelb.edu.au (F.C.)
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24 **Author Contributions**

25
26 §C.-J. K. and F.E. contributed equally to this work. The manuscript was written through
27 contributions of all authors. All authors have given approval to the final version of the
28
29 manuscript.
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34 **Notes**

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37 The authors declare no competing financial interest.
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8 REFERENCES

9
10 (1) Ejima, H.; Richardson, J. J.; Liang, K.; Best, J. P.; van Koeverden, M. P.; Such, G. K.; Cui,
11 J.; Caruso, F. One-Step Assembly of Coordination Complexes for Versatile Film and Particle
12 Engineering. *Science* **2013**, *341*, 154–157.
13
14
15

16
17 (2) Guo, J.; Tardy, B. L.; Christofferson, A. J.; Dai, Y.; Richardson, J. J.; Zhu, W.; Hu, M.; Ju,
18 Y.; Cui, J.; Dagastine, R. R. et al. Modular Assembly of Superstructures from Polyphenol-
19 Functionalized Building Blocks. *Nat. Nanotechnol.* **2016**, *11*, 1105–1111.
20
21
22
23

24
25 (3) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. A DNA-Based Method for
26 Rationally Assembling Nanoparticles into Macroscopic Materials. *Nature* **1996**, *382*, 607–609.
27
28
29

30
31 (4) Lu, Y.; Lin, J.; Wang, L.; Zhang, L.; Cai, C. Self-Assembly of Copolymer Micelles:
32 Higher-Level Assembly for Constructing Hierarchical Structure. *Chem. Rev.* **2020**, *120*, 4111–
33 4140.
34
35
36
37

38
39 (5) Chan, L. W.; Anahtar, M. N.; Ong, T. H.; Hern, K. E.; Kunz, R. R.; Bhatia, S. N.
40 Engineering Synthetic Breath Biomarkers for Respiratory Disease. *Nat. Nanotechnol.* **2020**, *15*,
41 792–800.
42
43
44
45

46
47 (6) Kim, C.-J.; Park, J.-e.; Hu, X.; Albert, S. K.; Park, S.-J. Peptide-Driven Shape Control of
48 Low-Dimensional DNA Nanostructures. *ACS Nano* **2020**, *14*, 2276–2284.
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 (7) Samanta, D.; Ebrahimi, S. B.; Kusmierz, C. D.; Cheng, H. F.; Mirkin, C. A. Protein
4 Spherical Nucleic Acids for Live-Cell Chemical Analysis. *J. Am. Chem. Soc.* **2020**, *142*, 13350–
5 13355.
6
7

8
9
10 (8) Guo, J.; Ping, Y.; Ejima, H.; Alt, K.; Meissner, M.; Richardson, J. J.; Yan, Y.; Peter, K.;
11 Elverfeldt, D. v.; Hagemeyer, C. E. et al. Engineering Multifunctional Capsules through the
12 Assembly of Metal-Phenolic Networks. *Angew. Chem., Int. Ed.* **2014**, *53*, 5546–5551.
13
14

15
16 (9) Wang, X.; Yan, J.-J.; Wang, L.; Pan, D.; Yang, R.; Xu, Y.; Sheng, J.; Huang, Q.; Zhao, H.;
17 Yang, M. Rational Design of Polyphenol-Poloxamer Nanovesicles for Targeting Inflammatory
18 Bowel Disease Therapy. *Chem. Mater.* **2018**, *30*, 4073–4080.
19
20
21

22
23 (10) Blum, A. P.; Kammeyer, J. K.; Rush, A. M.; Callmann, C. E.; Hahn, M. E.; Gianneschi, N.
24 C. Stimuli-Responsive Nanomaterials for Biomedical Applications. *J. Am. Chem. Soc.* **2015**, *137*,
25 2140–2154.
26
27

28
29 (11) Cheng, L.; Wang, C.; Feng, L.; Yang, K.; Liu, Z. Functional Nanomaterials for
30 Phototherapies of Cancer. *Chem. Rev.* **2014**, *114*, 10869–10939.
31
32
33

34 (12) Ju, Y.; Cortez-Jugo, C.; Chen, J.; Wang, T. Y.; Mitchell, A. J.; Tsantikos, E.;
35 Bertleff-Zieschang, N.; Lin, Y. W.; Song, J.; Cheng, Y.; et al. Engineering of Nebulized Metal–
36 Phenolic Capsules for Controlled Pulmonary Deposition. *Adv. Sci.* **2020**, *7*, 1902650.
37
38

39 (13) Gause, K. T.; Yan, Y.; O'Brien-Simpson, N. M.; Cui, J.; Lenzo, J. C.; Reynolds, E. C.;
40 Caruso, F. Codelivery of NOD2 and TLR9 Ligands via Nanoengineered Protein Antigen Particles
41 for Improving and Tuning Immune Responses. *Adv. Funct. Mater.* **2016**, *26*, 7526–7536
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 (14) Cui, J.; De Rose, R.; Best, J. P.; Johnston, A. P. R.; Alcantara, S.; Liang, K.; Such, G. K.;
4 Kent, S. J.; Caruso, F. Mechanically Tunable, Self-Adjuvanting Nanoengineered Polypeptide
5 Particles. *Adv. Mater.* **2013**, *25*, 3468–3472
6
7

8
9
10 (15) Sexton, A.; Whitney, P. G.; Chong, S.-F.; Zelikin, A. N.; Johnston, A. P. R.; De Rose, R.;
11 Brooks, A. G.; Caruso, F.; Kent, S. J. A Protective Vaccine Delivery System for In Vivo T Cell
12 Stimulation Using Nanoengineered Polymer Hydrogel Capsules. *ACS Nano* **2009**, *3*, 3391–3400.
13
14

15
16 (16) Chong, S.-F.; Sexton, A.; De Rose, R.; Kent, S. J.; Zelikin, A. N.; Caruso, F. A Paradigm
17 for Peptide Vaccine Delivery Using Viral Epitopes Encapsulated in Degradable Polymer Hydrogel
18 Capsules. *Biomaterials* **2009**, *30*, 5178–5186.
19
20
21

22
23 (17) Lian, X.; Fang, Y.; Joseph, E.; Wang, Q.; Li, J.; Banerjee, S.; Lollar, C.; Wang, X.; Zhou,
24 H.-C. Enzyme–MOF (Metal–Organic Framework) Composites. *Chem. Soc. Rev.* **2017**, *46*, 3386–
25 3401.
26
27
28

29
30 (18) Zhu, Q.; Pan, Q. Mussel-Inspired Direct Immobilization of Nanoparticles and Application
31 for Oil–Water Separation. *ACS Nano* **2014**, *8*, 1402–1409.
32
33

34
35 (19) Deng, Y.; Qi, D.; Deng, C.; Zhang, X.; Zhao, D. Superparamagnetic High-Magnetization
36 Microspheres with an Fe₃O₄@SiO₂ Core and Perpendicularly Aligned Mesoporous SiO₂ Shell for
37 Removal of Microcystins. *J. Am. Chem. Soc.* **2008**, *130*, 28–29.
38
39

40
41 (20) Richardson, J. J.; Björnmalm, M.; Caruso, F. Technology-Driven Layer-by-Layer
42 Assembly of Nanofilms. *Science* **2015**, *348*, aaa2491.
43
44

45
46 (21) Caruso, F.; Caruso, R. A.; Möhwald, H. Nanoengineering of Inorganic and Hybrid Hollow
47 Spheres by Colloidal Templating. *Science* **1998**, *282*, 1111–1114.
48
49
50
51
52

1
2
3 (22) Rahim, M. A.; Kempe, K.; Mullner, M.; Ejima, H.; Ju, Y.; van Koeeverden, M. P.; Suma,
4 T.; Braunger, J. A.; Leeming, M. G.; Abrahams, B. F. et al. Surface-Confined Amorphous Films
5 from Metal-Coordinated Simple Phenolic Ligands. *Chem. Mater.* **2015**, *27*, 5825–5832.
6
7

8
9
10 (23) Ulbrich, K.; Hola, K.; Subr, V.; Bakandritsos, A.; Tucek, J.; Zboril, R. Targeted Drug
11 Delivery with Polymers and Magnetic Nanoparticles: Covalent and Noncovalent Approaches,
12 Release Control, and Clinical Studies. *Chem. Rev.* **2016**, *116*, 5338–5431.
13
14
15

16
17 (24) Delplace, V.; Nicolas, J. Degradable Vinyl Polymers for Biomedical Applications. *Nat.*
18 *Chem.* **2015**, *7*, 771–784.
19
20
21

22
23 (25) Jia, F.; Lu, X.; Wang, D.; Cao, X.; Tan, X.; Lu, H.; Zhang, K. Depth-Profiling the Nuclease
24 Stability and the Gene Silencing Efficacy of Brush-Architected Poly(ethylene glycol)–DNA
25 Conjugates. *J. Am. Chem. Soc.* **2017**, *139*, 10605–10608.
26
27
28

29
30 (26) Ju, Y.; Cui, J.; Mullner, M.; Suma, T.; Hu, M.; Caruso, F. Engineering Low-Fouling and
31 pH-Degradable Capsules through the Assembly of Metal-Phenolic Networks. *Biomacromolecules*
32 **2015**, *16*, 807–814.
33
34
35

36
37 (27) Holten-Andersen, N.; Harrington, M. J.; Birkedal, H.; Lee, B. P.; Messersmith, P. B.; Lee,
38 K. Y. C.; Waite, J. H. pH-Induced Metal-Ligand Cross-Links Inspired by Mussel Yield Self-
39 Healing Polymer Networks with near-Covalent Elastic Moduli. *Proc. Natl. Acad. Sci. U. S. A.*
40 **2011**, *108*, 2651–2655.
41
42
43

44
45 (28) Shin, E.; Lim, C.; Kang, U. J.; Kim, M.; Park, J.; Kim, D.; Choi, W.; Hong, J.; Baig, C.;
46 Lee, D. W. Mussel-Inspired Copolyether Loop with Superior Antifouling Behavior.
47 *Macromolecules* **2020**, *53*, 3551–3562.
48
49
50
51
52
53
54
55

1
2
3 (29) Yun, G.; Besford, Q. A.; Johnston, S. T.; Richardson, J. J.; Pan, S.; Biviano, M.; Caruso,
4 F. Self-Assembly of Nano- to Macroscopic Metal–Phenolic Materials. *Chem. Mater.* **2018**, *30*,
5 5750–5758.
6
7

8
9
10 (30) Zhong, Q.-Z.; Li, S.; Chen, J.; Xie, K.; Pan, S.; Richardson, J. J.; Caruso, F. Oxidation-
11 Mediated Kinetic Strategies for Engineering Metal–Phenolic Networks. *Angew. Chem., Int. Ed.*
12 **2019**, *58*, 12563–12568.
13
14
15

16
17 (31) Chen, J.; Pan, S.; Zhou, J.; Zhong, Q.-Z.; Qu, Y.; Richardson, J. J.; Caruso, F.
18 Programmable Permeability of Metal–Phenolic Network Microcapsules. *Chem. Mater.* **2020**, *32*,
19 6975–6982.
20
21
22
23

24
25 (32) Shi, Z.-H.; Li, N.-G.; Shi, Q.-P.; Tang, H.; Tang, Y.-P.; Li, W.; Yin, L.; Yang, J.-P.; Duan,
26 J.-A. Synthesis and Structure–Activity Relationship Analysis of Caffeic Acid Amides as Selective
27 Matrix Metalloproteinase Inhibitors. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1206–1211.
28
29
30
31

32
33 (33) Misra, K.; Maity, H. S.; Nag, A.; Sonawane, A. Radical Scavenging and Antibacterial
34 Activity of Caffemides against Gram Positive, Gram Negative and Clinical Drug Resistance
35 Bacteria. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 5943–5946.
36
37
38
39

40
41 (34) Fu, J.; Cheng, K.; Zhang, Z.-m.; Fang, R.-q.; Zhu, H.-l. Synthesis, Structure and Structure–
42 Activity Relationship Analysis of Caffeic Acid Amides as Potential Antimicrobials. *Eur. J. Med.*
43 *Chem.* **2010**, *45*, 2638–2643.
44
45
46
47

48
49 (35) Uwai, K.; Osanai, Y.; Imaizumi, T.; Kanno, S.-i.; Takeshita, M.; Ishikawa, M. Inhibitory
50 Effect of the Alkyl Side Chain of Caffeic Acid Analogues on Lipopolysaccharide-Induced Nitric
51 Oxide Production in Raw264. 7 Macrophages. *Bioorg. Med. Chem.* **2008**, *16*, 7795–7803.
52
53
54
55

1
2
3 (36) Fancelli, D.; Abate, A.; Amici, R.; Bernardi, P.; Ballarini, M.; Cappa, A.; Carezzi, G.;
4 Colombo, A.; Contursi, C.; Di Lisa, F. Cinnamic Anilides as New Mitochondrial Permeability
5 Transition Pore Inhibitors Endowed with Ischemia-Reperfusion Injury Protective Effect in Vivo.
6
7
8
9
10 *J. Med. Chem.* **2014**, *57*, 5333–5347.

11
12
13 (37) Chen, Z.; Digiacomio, M.; Tu, Y.; Gu, Q.; Wang, S.; Yang, X.; Chu, J.; Chen, Q.; Han, Y.;
14 Chen, J. Discovery of Novel Rivastigmine-Hydroxycinnamic Acid Hybrids as Multi-Targeted
15 Agents for Alzheimer's Disease. *Eur. J. Med. Chem.* **2017**, *125*, 784–792.

16
17
18 (38) Liu, Z.; Fu, J.; Shan, L.; Sun, Q.; Zhang, W. Synthesis, Preliminary Bioevaluation and
19 Computational Analysis of Caffeic Acid Analogues. *Int. J. Mol. Sci.* **2014**, *15*, 8808–8820.

20
21 (39) Nomura, E.; Kashiwada, A.; Hosoda, A.; Nakamura, K.; Morishita, H.; Tsuno, T.;
22 Taniguchi, H. Synthesis of Amide Compounds of Ferulic Acid, and Their Stimulatory Effects on
23 Insulin Secretion in Vitro. *Bioorg. Med. Chem.* **2003**, *11*, 3807–3813.

24
25
26 (40) Hunneche, C. S.; Lund, M. N.; Skibsted, L. H.; Nielsen, J. Antioxidant Activity of a
27 Combinatorial Library of Emulsifier–Antioxidant Bioconjugates. *J. Agric. Food Chem.* **2008**, *56*,
28 9258–9268.

29
30
31 (41) de Armas-Ricard, M.; Ruiz-Reyes, E.; Ramírez-Rodríguez, O. Caffeates and Caffeamides:
32 Synthetic Methodologies and Their Antioxidant Properties. *Int. J. Med. Chem.* **2019**, *2019*,
33 2592609.

34
35
36 (42) Tarbell, D. S. Carboxylic Carbonic Anhydrides and Related Compounds. *Acc. Chem. Res.*
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
1969, *2*, 296–300.

1
2
3 (43) Tarbell, D. S.; Leister, N. A. The Stability of Mixed Carboxylic-Carbonic Anhydrides. *J.*
4
5 *Org. Chem.* **1958**, *23*, 1149–1152.
6

7
8 (44) Vaughan Jr, J. R.; Eichler, J. A. The Preparation of Optically-Active Peptides Using Mixed
9
10 Carbonic—Carboxylic Acid Anhydrides. *J. Am. Chem. Soc.* **1953**, *75*, 5556-5560.
11
12

13
14 (45) Nakamura, K.; Nakajima, T.; Aoyama, T.; Okitsu, S.; Koyama, M. One-Pot Esterification
15
16 and Amidation of Phenolic Acids. *Tetrahedron* **2014**, *70*, 8097–8107.
17
18

19 (46) DellaGreca, M.; Longobardo, L. Protection and Activation of Hydroxycinnamic Acids in
20
21 Water. *ChemistrySelect* **2020**, *5*, 4588–4591.
22
23

24 (47) Li, L.; Li, Y.; Luo, X.; Deng, J.; Yang, W. Helical Poly(*N*-Propargylamide)s with
25
26 Functional Catechol Groups: Synthesis and Adsorption of Metal Ions in Aqueous Solution. *React.*
27
28 *Funct. Polym.* **2010**, *70*, 938–943.
29
30

31 (48) Chen, F. M. F.; Lee, Y.; Steinauer, R.; Benoiton, N. L. Mixed Anhydrides in Peptide
32
33 Synthesis. A Study of Urethane Formation with a Contribution on Minimization of Racemization.
34
35 *Can. J. Chem.* **1987**, *65*, 613–618.
36
37

38 (49) Pal, B.; Jaisankar, P.; Giri, V. S. Versatile Reagent for Reduction of Azides to Amines.
39
40
41 *Synth. Commun.* **2004**, *34*, 1317–1323.
42
43

44 (50) Sever, M. J.; Wilker, J. J. Visible Absorption Spectra of Metal–Catecholate and Metal–
45
46 Tironate Complexes. *Dalton Trans.* **2004**, 1061–1072.
47
48

49 (51) Rahim, M. A.; Björnmalm, M.; Bertleff-Zieschang, N.; Besford, Q.; Mettu, S.; Suma, T.;
50
51 Faria, M.; Caruso, F. Rust-Mediated Continuous Assembly of Metal–Phenolic Networks. *Adv.*
52
53 *Mater.* **2017**, *29*, 1606717.
54
55
56
57
58
59
60

1
2
3 (52) Sun, H.; Wong, E. H.; Yan, Y.; Cui, J.; Dai, Q.; Guo, J.; Qiao, G. G.; Caruso, F. The Role
4 of Capsule Stiffness on Cellular Processing. *Chem. Sci.* **2015**, *6*, 3505–3514.
5
6
7

8 (53) Amoozgar, Z.; Yeo, Y. Recent Advances in Stealth Coating of Nanoparticle Drug Delivery
9 Systems. *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol.* **2012**, *4*, 219–233.
10
11
12

13 (54) Fan, W.; Yung, B.; Huang, P.; Chen, X. Nanotechnology for Multimodal Synergistic
14 Cancer Therapy. *Chem. Rev.* **2017**, *117*, 13566–13638.
15
16
17

18 (55) Guo, J.; Suma, T.; Richardson, J. J.; Ejima, H. Modular Assembly of Biomaterials Using
19 Polyphenols as Building Blocks. *ACS Biomater. Sci. Eng.* **2019**, *5*, 5578–5596.
20
21
22
23

24 (56) Lee, H. A.; Ma, Y.; Zhou, F.; Hong, S.; Lee, H. Material-Independent Surface Chemistry
25 Beyond Polydopamine Coating. *Acc. Chem. Res.* **2019**, *52*, 704–713.
26
27
28
29
30
31
32
33
34
35
36
37
38
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