Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
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Supplementary Information

Copolymerization of an indazole ligand into the self-polymerization of dopamine for enhanced binding with metal ions

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1. Synthesis

Materials. 3,4-dimethoxybenzaldehyde, bromine, hydrazine monohydrate, dopamine hydrochloride, tris(hydroxymethyl)aminomethane (TRIS, ≥99.8%), and Nile red were purchased from Sigma Aldrich. Ethyl acetate and 1,4-dioxan were purchased from Ajax Finechem Pty Ltd. Ethanol (95%) was purchased from VWR International. Chloroform was purchased from Chem-Supply Pty Ltd. Hydrochloric acid (32%) was purchased from RCI

Labscan Ltd. Deionized water was obtained from a Millipore water deionizing unit. All chemicals and solvents were used as received unless specified otherwise.

Synthesis of 2-bromo-4,5-dimethoxybenzaldehyde. The procedure was adapted from the one reported by Oliver *et al.* with some modifications.[1] 3,4-dimethoxybenzaldehyde (1 g, 6.02 mmol) was dissolved in dry chloroform (8 mL) while chilling over an ice-water bath. Bromine (3.2 mL, 6.25 mmol) was added dropwise to the chilled solution. The resulting mixture was refluxed at 60 °C overnight. The reaction was quenched by chilling upon completion, and the solvent was removed. The solid residue was redissolved in chloroform to remove residual bromine, followed by the removal of the solvent. The solid was recrystallized from ethanol to obtain a yellowish white product with a yield of 56% after drying. δ_H (300 MHz; $CDCl_3$; Me_4Si) 3.90 (3 H, s, OMe), 3.94 (3 H, s, OMe), 7.03 (1 H, s, Ar H), 7.39 (1 H, s, Ar H), 10.16 (1 H, s, COH). δ_C (75 MHz; $CDCl_3$; Me_4Si) 6.26, 56.61, 110.5, 115.6, 120.5, 126.6, 149.0, 154.6, 190.9.

Synthesis of 5,6-dimethoxy-*1H*-indazole. The procedure was adapted from the one reported by Lukin *et al.*[2] 2-bromo-4,5-dimethoxybenzaldehyde (1 g, 4.08 mmol) was added to 1,4-dioxane (4 mL) while chilling over an ice-water bath. Hydrazine (98%, 4 mL, 79.9 mmol) was added dropwise to the chilled mixture over 5 minutes. The resulting mixture was refluxed at 80 °C overnight. The mixture was chilled upon completion, and the volume was halved by removing the solvent under reduced pressure. The concentrate was added carefully to deionized water (4-8 mL) which resulted in precipitation of a white solid as the product with a yield of 99% after drying. δ_H (300 MHz; $CDCl_3$; Me_4Si) 3.85 (3 H, s, OMe), 3.87 (3 H, s, OMe), 6.93 (1 H, s, Ar H), 7.35 (1 H, s, Ar H), 8.00 (1 H, s, CH). δ_C (75 MHz; $CDCl_3$; Me_4Si) 56.07, 56.26, 108.6, 113.8, 115.1, 126.5, 142.0, 148.8, 150.2.

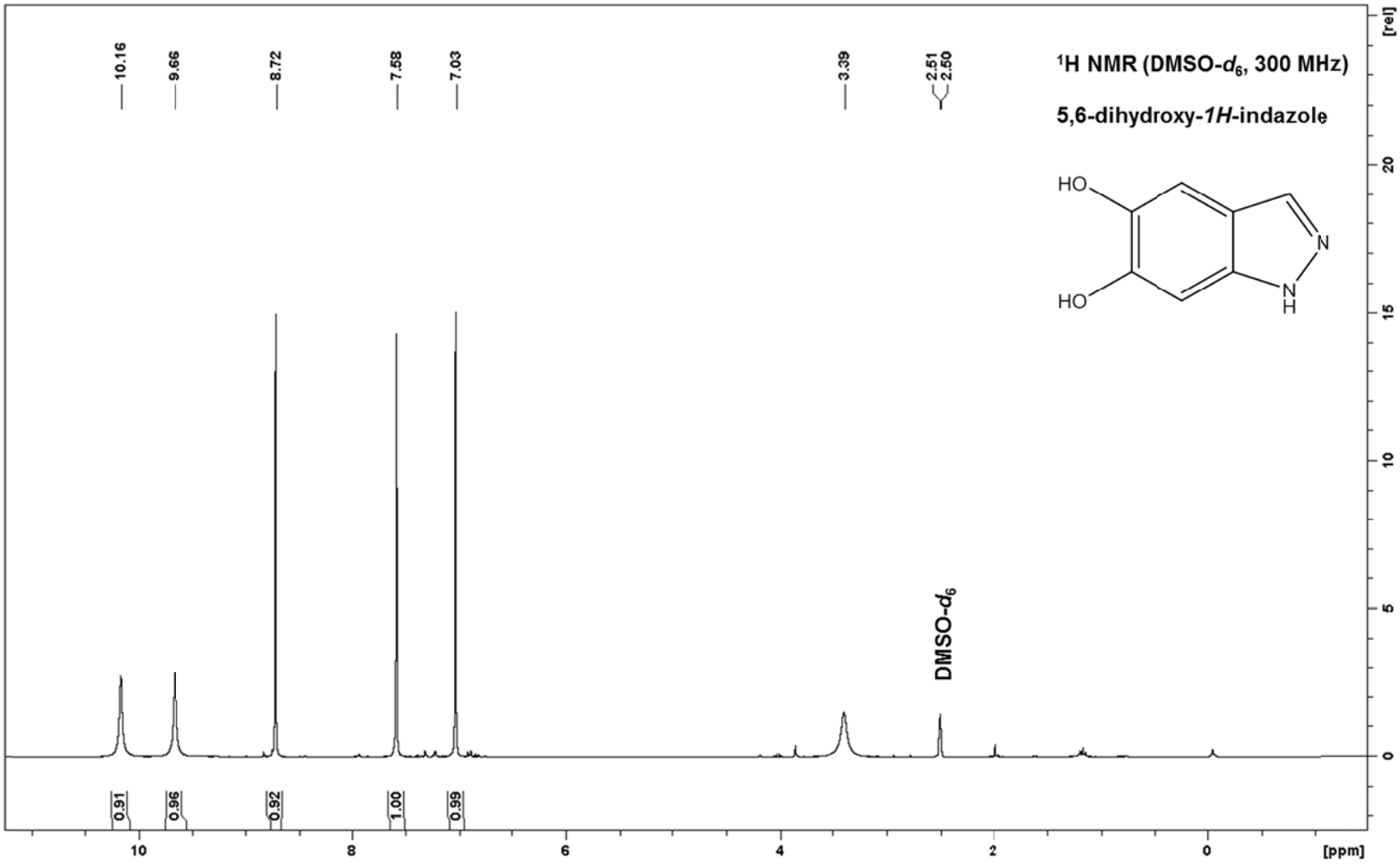

Fig. S1 ^{1}H NMR of 5,6-dihydroxy-*1H*-indazole in DMSO-d_6.

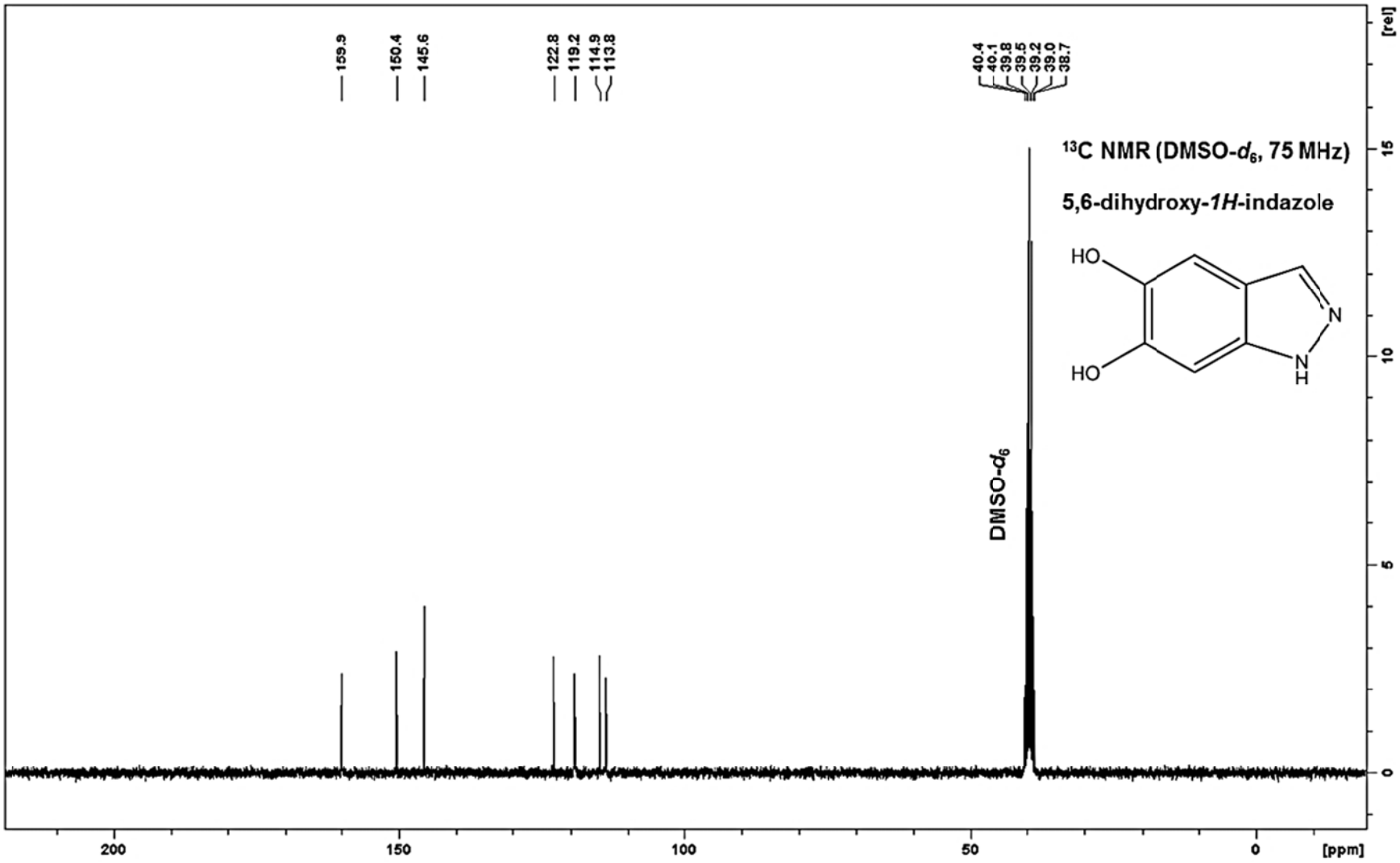

Fig. S2 ^{13}C NMR of 5,6-dihydroxy-*1H*-indazole in DMSO-d_6.

Preparation of homopolymers and copolymers. The procedure was similar to the preparation of the polymer-coated particles while excluding the addition of SiO_2 template. The quantities of monomers and solvents required are summarized in the table below.

PDA and the 1:1- and 1:3-copolymers of DHI and dopamine were collected by Millipore filtration. The polymer of DHI (PDHI) however, required to be precipitated out by acidification with hydrochloric acid (HCl, ca. 20 mL, 10%) prior to filtration. The collected materials were rinsed by several portions of deionized water and dried at 40 °C in the vacuum oven overnight.

Table S1 Quantities of monomer and solvent used in polymerization and copolymerization.

Sample	**PDHI**	**PDA**	**1:1-copolymer**	**1:3-copolymer**
m(DHI), mg	300.3	0.00	150.1	75.1
n(DHI), mmol	2.00	0.00	1.00	0.50
m(DA*), mg	0.00	379.3	189.6	284.5
n(DA*), mmol	0.00	2.00	1.00	1.50
Molar Ratio	N/A	N/A	1:1	1:3
V(Water), mL	120	200	120	120
V(Ethanol), mL	80	0	80	80
Volume Ratio	3:2	N/A	3:2	3:2

*Dopamine (as a hydrochloride)

2. Characterization

^{1}H, ^{13}C and 2D nuclear magnetic resonance (NMR) spectroscopy. Chloroform-d_1 ($CDCl_3$, D-99.8%) and dimethyl sulfoxide-d_6 (DMSO-d_6, D-99.9%) purchased from Cambridge Isotope Laboratories, Inc. have been used to dissolve the samples depending on the solubility. Analyses of samples were performed on a Bruker Avance III 300 MHz spectrometer equipped with a SampleXpress automatic sample changer and BBFO z-gradient probe. Typical acquisition parameters are as followed: 8 scans for ^{1}H spectra (at 300 MHz), and 256 scans for ^{13}C spectra (at 75 MHz). 2D NMR spectra were acquired by performing heteronuclear single-quantum correlation (HSQC) and heteronuclear multiple-bond correlation (HMBC) spectroscopy. Typically 2 scans were performed for HSQC, while 4

scans were performed for HMBC. The ^{1}H spectrum (acquired at 300 MHz) was plotted on the x-axis, while the ^{13}C spectrum (acquired at 75 MHz) was plotted on the y-axis to deduce the structure of 5,6-dihydroxy-*1H*-indazole.

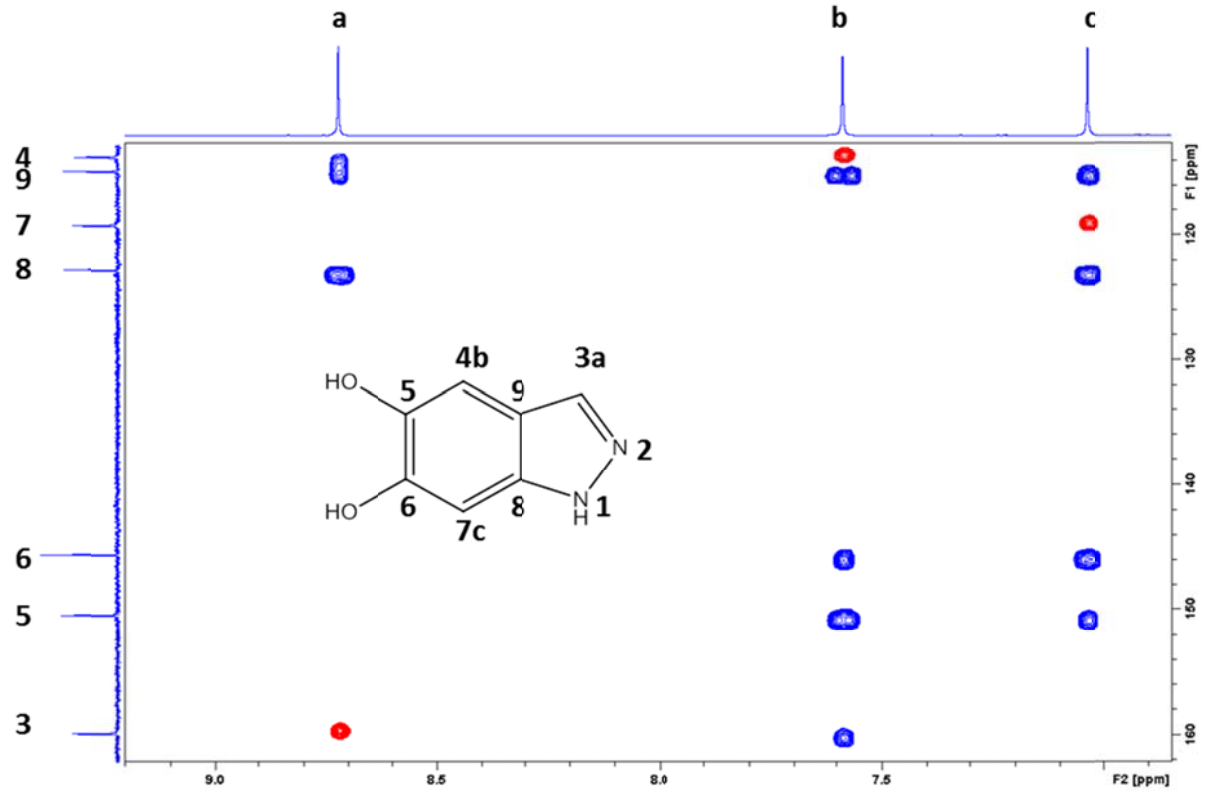

Fig. S3 2D NMR of 5,6-dihydroxy-*1H*-indazole in DMSO-d_6 (HSQC = red signals; HMBC = blue signals).

^{13}C solid-state (CP-MAS) spectroscopy. The ^{13}C SSNMR spectra were acquired by a Bruker Avance III 300 Solid State spectrometer. The sample was densely packed into a 4 mm o.d. rotor and spun at 12 kHz inside a 4 mm 1H/X broadband CPMAS (cross polarization-magic angle spinning) probe. Acquisition of the ^{13}C spectra were performed with 3 µs recycle delay and 13 ms acquisition time. ^{1}H decoupling was performed with SPINAL64. 1024 scans were acquired for all samples. Reference was set to the carbonyl peak at 176 ppm on the ^{13}C spectrum of glycine.

Gel permeation chromatography (GPC). Mass distribution of the polymer sample was determined by a GPC unit purchased from Shimadzu Scientific Instruments, which included a SIL-10AD VP auto-sampler, a LC-20AT pump, a CTO-10A VP column oven, and a RID-10A refractive index (RI) detector. The unit was operated using *N,N*-dimethylacetamide (DMAc) as the eluent [containing 0.3 g L^{-1} of lithium bromide and 0.5 g L^{-1} of 2,6-bis(1,1-dimethyl-ethyl)-4-methylphenol]. In a typical analysis, the sample was prepare with DMAc at a concentration of 2–3 mg mL^{-1}, which was eluted through the guard column (Phenomenex, 5 μm bead size) and the chromatography columns (Phenomenex, Phenogel – 10^5, 10^4 and 10^3 Å pore size) over 60 minutes at a regulated temperature of 50°C. Calibration of the unit was performed on a regular basis with Polystyrene High EasiVials purchased from Agilent Technologies.

Dynamic light scattering (DLS). Malvern Zetasizer Nano ZS equipped with a 4mW He-Ne laser (λ = 632.8 nm) was used to determine the particle size distribution of the dispersed sample in deionized water (RI = 1.33, absorption = 0.01). Three measurements were performed for each sample, while each measurement consisted of 12 – 14 scans. Poly(dopamine) was chosen as the reference material [RI = 1.59,[3] absorption = 0.01, (same values for the silica particle templates)] since the RI of poly(5,6-dihydroxy-*1H*-indazole) and the copolymer were unknown. The instrument was calibrated with titanium oxide standard (RI = 2.40, absorption = 0.01).

Thermogravimetric analysis (TGA). The thermal degradation of the sample was monitored on TGA Q5000 (V3.15 Build 263) purchased from TA instruments. Sample was preloaded onto a high-temperature platinum pan, which was then sent into the furnace supplied with streams of air (at 25.0 mL min^{-1}) and nitrogen (at 15.0 mL min^{-1}). In a typical procedure, the temperature was ramped from 25 °C to 100 °C at 20 °C min^{-1}, and set to stay isothermal for

10 minutes for the removal of residual moisture. Thermal degradation of the sample began when the temperature was ramped from 100 °C to 900 °C at 10 °C min^{-1}.

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. Absorption spectra were acquired by a Bruker IFS 66/S single-beam spectrometer. The instrument has been equipped with a mid-infrared lamp and diffuse reflectance sampling accessories. 32 scans were performed for each sample with a resolution of 4 cm^{-1}.

Ultraviolet-visible (UV-Vis) spectroscopy. Absorption spectra were acquired by a Varian Cary 300 UV-Visible Spectrophotometer with a resolution of ≤ 0.24 nm. All samples were dispersed in deionized water and analyzed in a quartz cuvette unless specified.

Transmission electron microscopy (TEM). Images were acquired with a JEOL 1400 transmission electron microscope. The sample was first dispersed in deionized water with the aid of sonication, which was then applied on the copper grids coated with formvar. Water was allowed to evaporate off completely prior to perform imaging.

Scanning electron microscopy (SEM). Images of the chromium-coated sample were acquired with a Nova NanoSEM 230 filed-emission scanning electron microscope at accelerating voltage of 3KV with spot size of 2.5. Details of the photographic setting were shown on individual images.

Energy dispersive X-ray spectroscopy (EDS). The elemental analysis of the prepared copolymer-coated particles and copolymer capsules was performed with a FEI Tecnai G2 20 transmission electron microscope equipped with a Bruker XFlash® detector 5030. Sample preparation was similar to that for TEM analysis; however, the formvar-coated copper grids used were also coated with a layer of carbon. TEM images have also been taken with the instrument to illustrate the area that has been analyzed by EDS.

Laser scanning confocal microscopy (LSCM). The copolymer capsules (3.3 mg) were incubated in an ethanol solution of Nile red (2 mg mL^{-1}) for 18 hours on an orbital shaker at 27 °C in the dark. The dye-loaded capsules were collected by filtration, rinsed with a small portion of ethanol, followed by deionized water. The dye-loaded capsules were then stored in the dark.

The Nile red-loaded and dye-free (control) capsules were dispersed in deionized water and then added into a 35-mm glass bottom Fluorodish and incubated for one day at room temperature in the dark. The dishes were rinsed once with deionized water and the nanoparticles adhered on the surface were observed under a Zeiss LSM780 confocal microscope. The LSM780 system was equipped with a DPSS 561-10 laser connected to a Zeiss Axio Observer.Z1 inverted microscope (oil immersion ×100 /1.4 NA objective). The ZEN2011 imaging software (Zeiss) was used for image acquisition and processing.

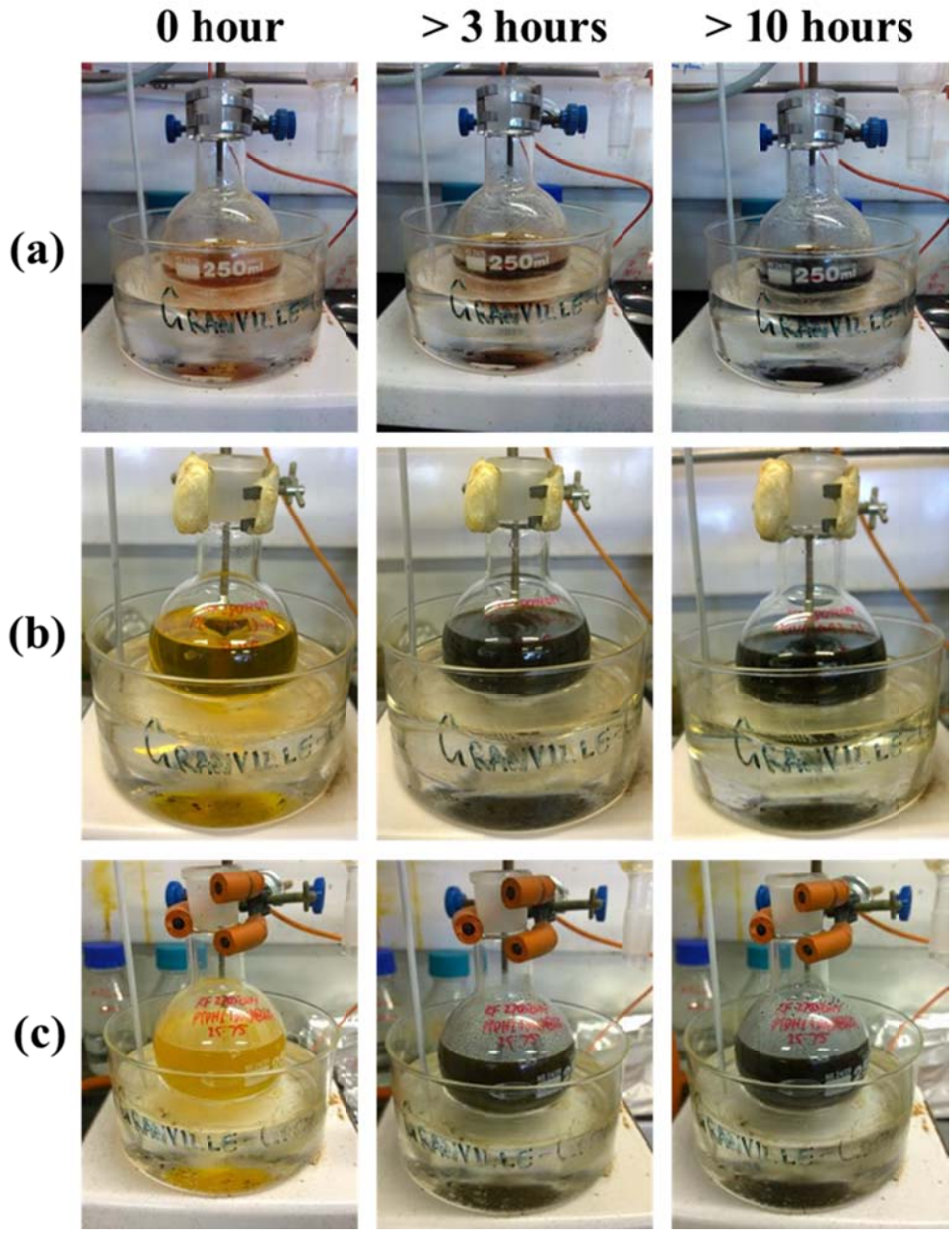

Fig. S4 Polymerization of 5,6-dihydroxy-*1H*-indazole (a), and copolymerization of 5,6-dihydroxy-*1H*-indazole and dopamine in 1:1 (b) and 1:3 (c) molar ratio.

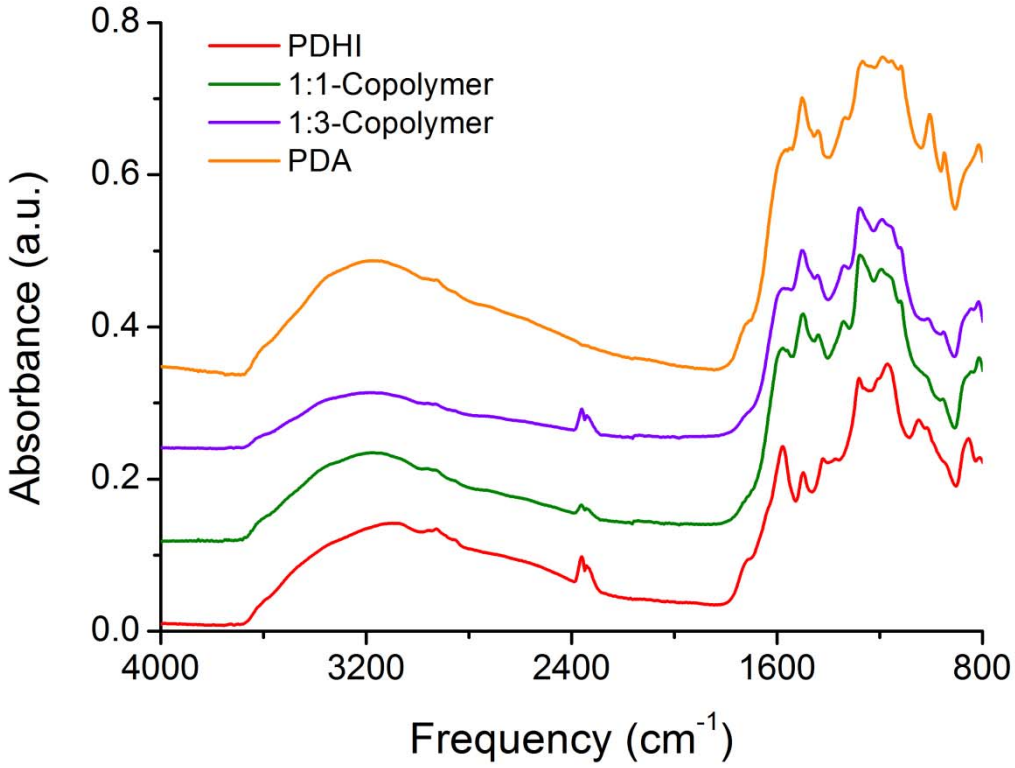

Fig. S5 Comparison of the ATR-FTIR spectra of the synthesized materials.

Table S2. Percentage of soluble materials in the copolymers.

Sample	**1:1-Copolymer**	**1:3-Copolymer**
Mass of New Filter (mg)	2735.1	2736.0
Mass of Used Filter (dried, mg)	2740.6	2744.2
Mass Difference (mg)	5.5	8.2
Sample Mass (mg)	11.3	11.1
% Soluble Material	51%	26%

Table S3 GPC results of 1:3-copolymer, 1:1-copolymer and PDHI.

Sample	**Molecular Weight (M_n, g mol^{-1})**	**Molecular Weight (M_w, g mol^{-1})**	**Dispersity (Đ)**
1:3-Copolymer	18,404	33,476	1.81
1:1-Copolymer	22,142	43,091	1.95
PDHI	22,910	34,003	1.48

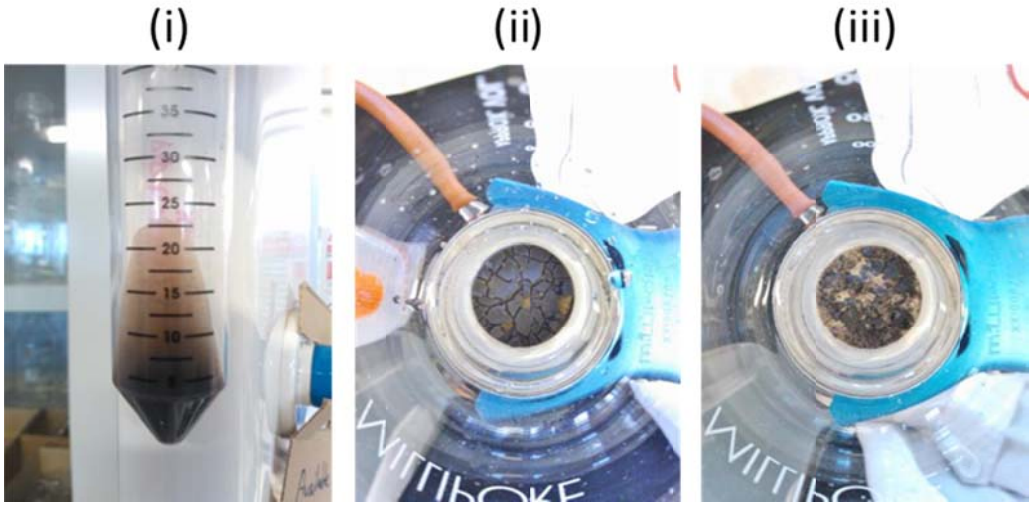

Fig. S6 Evidence of instability of 1:1-copolymer coating prepared on silica particle templates: polymer-coated particles after being purified *via* centrifuging-decanting-redispersing cycles which already showed a lighter color (i), polymer-coated particles collected by Millipore filtration (ii), and 1:1-copolymer delaminated from the coated particles after being rinsed with ethyl acetate while the particle templates were retained (iii).

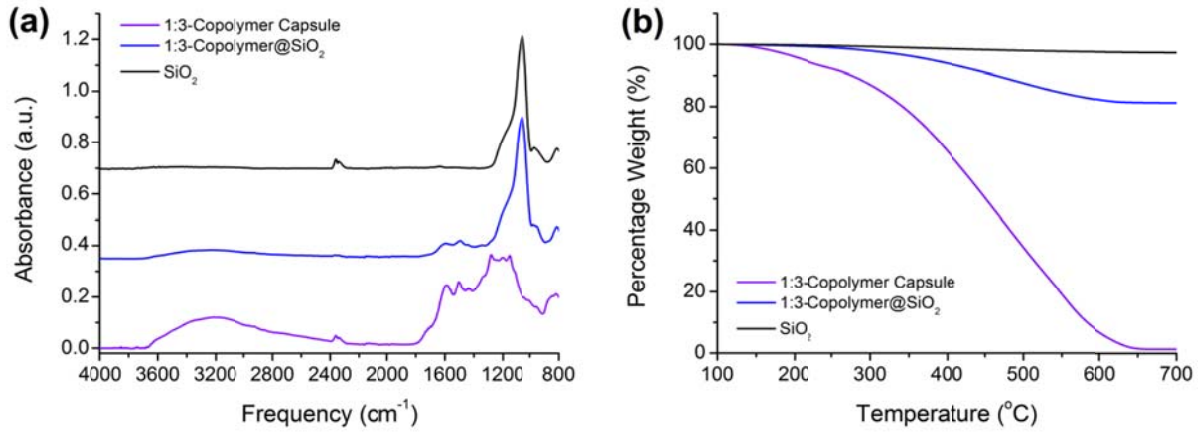

Fig. S7 Proof of the complete removal of silica particle templates through hydrofluoric acid etching by comparing against the copolymer-coated silica (*i.e.* 1:3-copolymer@SiO_2) and bare silica with: ATR-FTIR, in which the overwhelming absorption peak of silica at 1200 – 1000 cm^{-1} is no longer observable while the signals of the copolymer is revealed (a); TGA, in which the complete thermal degradation copolymer capsules indicates the absence of silica (b).

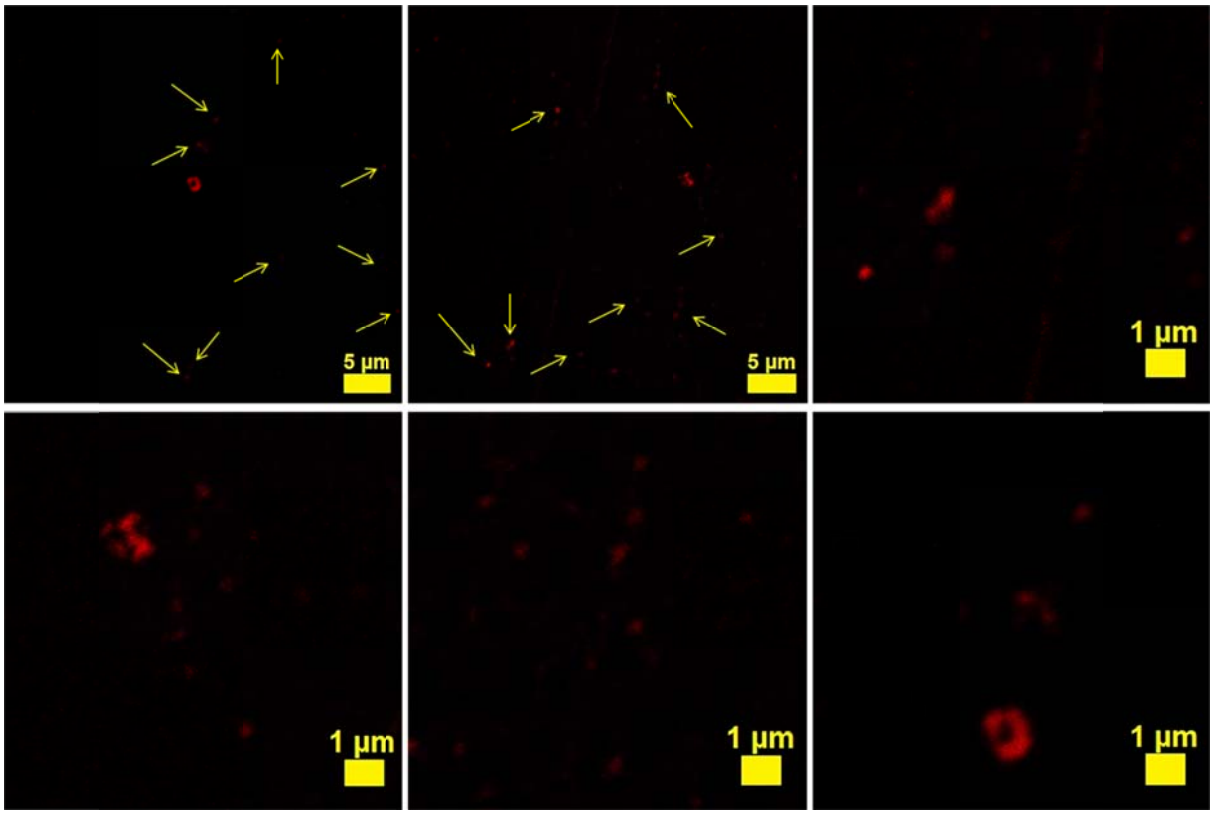

Fig. S8 Laser scanning confocal microphotographs of the copolymer capsules loaded with Nile red (scale bar = 5 µm and 1 µm respectively). Arrows indicate some of the dye-loaded capsules.

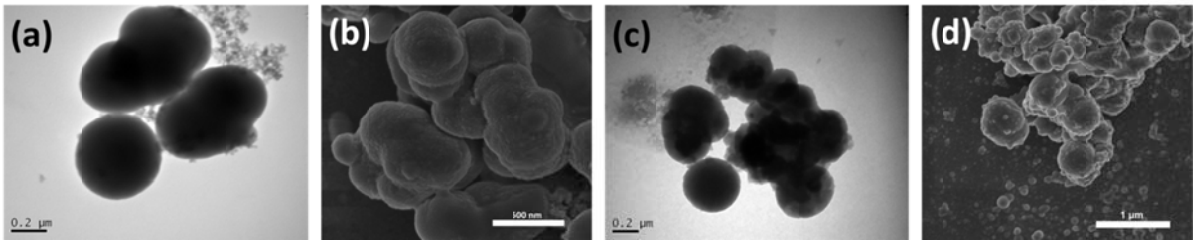

Fig. S9 TEM (scale bar = 0.2 µm) and SEM (scale bar = 500 nm and 1µm) images of 1:3-copolymer-coated silica particles (a, b) and 1:3-copolymer capsules (c, d), which illustrate the wide particle distribution and occasional particle aggregation of the samples.

Table S4 Summary of the zeta potentials exhibited by the coated particles, polymeric capsules and bare silica particles.

Sample	**Zeta Potential (mV)**
1:3-Copolymer-Coated Particles	-35.8 ± 0.2
1:3-Copolymer Capsules	-42.9 ± 0.8

PDA-Coated Particles	-32.4 ± 0.9
PDA Capsules	-8.9 ± 1.3
Silica Particles	-36.3 ± 1.2

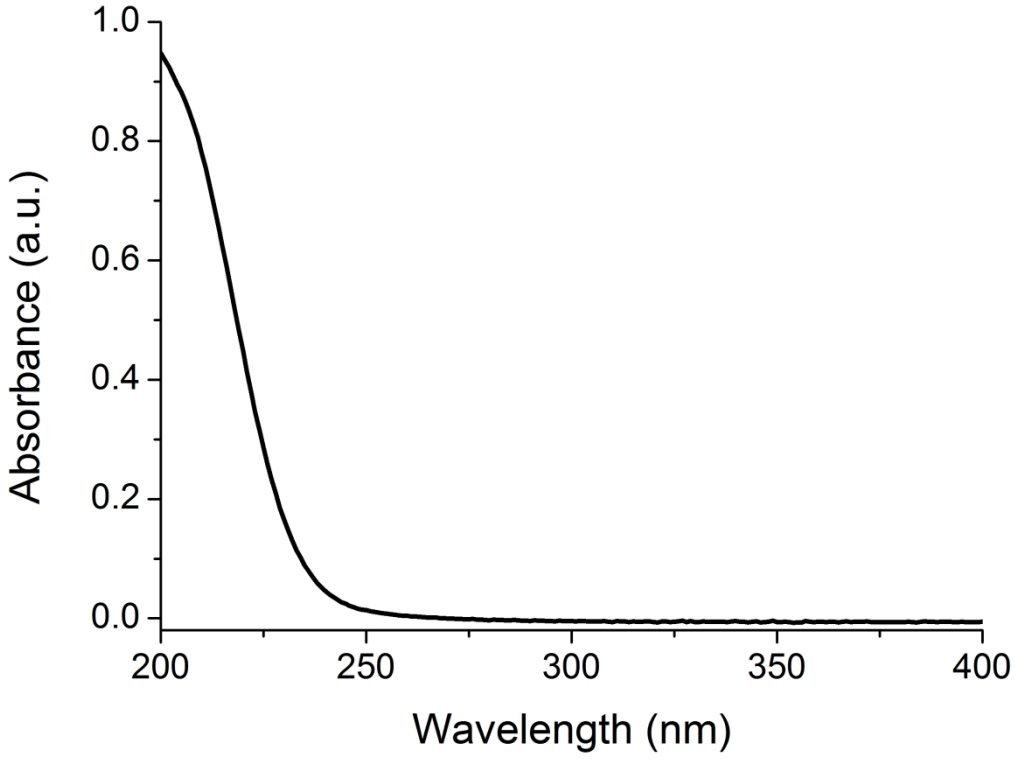

Fig. S10 UV-Vis spectrum of copper(II) sulfate.

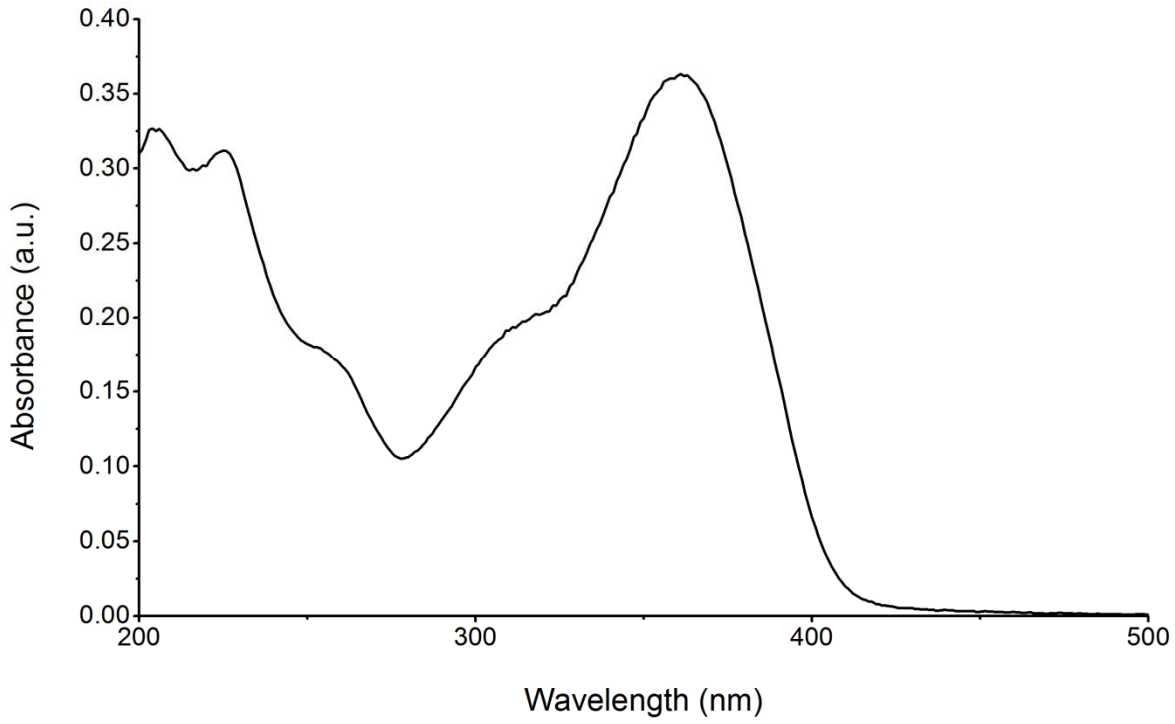

Fig. S11 UV-Vis spectrum of 5,6-dihydroxy-*1H*-indazole measured in water/ethanol mixture.

3. Reference

1. R. Olivera, R. SanMartin, E. Domínguez, X. Solans, M. K. Urtiaga and M. I. Arriortua, *The Journal of Organic Chemistry*, 2000, **65**, 6398-6411.
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3. Y. Zhang, B. Thingholm, K. N. Goldie, R. Ogaki and B. Städler, *Langmuir*, 2012, **28**, 17585-17592.