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# Polymeric template assisted synthesis of monodisperse-porous manganese oxide microspheres: A new nanozyme with oxidase-like activity allowing biomolecule determination via bimodal sensing

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## Supporting Information

### 1. Materials

The chemicals used for synthesis of monodisperse-porous poly(methacrylic acid-co-ethylene dimethacrylate), poly(MAA-co-EDMA) microspheres were obtained from Aldrich Chemical Co., Milwaukee, WI, U.S.A., as given previously.[1] Potassium permanganate ($KMnO_4$), concentrated nitric acid (containing 65 % w/w $HNO_3$), potassium hydrogen phosphate and potassium dihydrogen phosphate , ascorbic acid (AA) , o-phenylenediamine (OPDA) and concentrated HCl (37 % w/w) were also obtained from Aldrich and used without further purification. All runs were performed with distilled-deionized (DDI) water with 18 MΩ resistance obtained from Direct-Q3 UV System (Millipore S.A.S, Molsheim, France).

### 2. Synthesis of poly(MAA-co-EDMA) microspheres

Poly(glycidyl methacrylate) seed latex 1.8 μm in size was obtained by dispersion polymerization of glycidyl methacrylate (3 mL) within absolute ethanol (30 mL) including azobisizobutyronitrile (AIBN, 0.28 g) as the initiator and poly(vinyl pyrrolidone (PVP-K-30, Mw: 40.000 Da) as the stabilizer.[2] For the synthesis of

monodisperse-porous poly(MAA-co-EDMA) microspheres 5.3 μm in size by multistep seeded polymerization, poly(glycidyl methacrylate), poly(GMA) seed latex (0.3 g) was swollen by ethylbenzene (EB, 4 mL) in an aqueous emulsion (40 mL) containing SDS (5 mg/mL) by stirring at room temperature for overnight. The swollen seed latex was then reswollen by a monomer phase containing methacrylic acid (MAA, 2 mL), ethylene dimethacrylate (EDMA, 4 mL) and benzoyl peroxide (BPO, 0.30 g) in an aqueous emulsion medium (40 mL) containing SDS (5 mg/mL) as the stabilizer by stirring at room temperature for 4 h. Monodisperse-porous polymer microspheres including the porogen mixture were obtained by the polymerization performed at 80$^{o}$C for 16 h. The polymer microspheres were extracted by THF to obtain monodisperse-porous poly(MAA-co-EDMA) microspheres 5.3 μm in size, by the removal of porogen. After washing with ethanol and water, poly(MAA-co-EDMA) microspheres were dispersed within DDI water and the solid concentration was determined gravimetrically.[3] The monomer conversion in the multistage microsuspension polymerization was determined by calculating the percent ratio of mass of poly(MAA-co-EDMA) microspheres to the total mass of MAA and EDMA loaded into the polymerization medium. The synthesis runs were also performed by changing the amount of seed latex between 0.15-0.60 g for obtaining poly(MAA-co-EDMA) microspheres with different mean size values.

### 3. Characterization of manganese oxide microspheres

The mean size and size distribution of $MnO_x$ microspheres were determined by Scanning Electron Microscope (FEI, Quanta 200, FEG, , a part of Thermo Fisher Scientific, Waltham, MA, U.S.A.). X-ray powder diffraction (XRD) analyses of microspheres were carried out on X-ray diffractometer with CuKa1 radiation (λ) 1.54060 nm) operating at 30 mA and 40 kV (Scintag, a part of Thermo Fisher Scientific, Waltham, MA, U.S.A.). The porous properties (i.e. pore size distribution, median pore size, pore volume and specific surface area, SSA) were determined by a surface area and pore size analyzer (Nova 2200e, Quantachrome, a part of Thermo Fisher Scientific, Waltham, MA, U.S.A.), by using nitrogen adsorption/desorption method.

## 4. Characterization of $MnO_x$ microspheres

**Table S1**. EDX results obtained by SEM examination of $MnO_x$ microspheres synthesized with different calcination temperatures.

| Calcination temperature (°C) | C | O (% w/w) | Mn |
|---|---|---|---|
| 380 | 7.31 | 37.63 | 55.05 |
| 410 | 5.01 | 34.69 | 60.29 |
| 430 | 5.84 | 38.71 | 55.45 |
| 470 | 5.90 | 46.16 | 47.94 |
| 500 | 6.27 | 44.17 | 49.56 |
| 560 | 4.48 | 37.41 | 58.11 |

**Table S2**. Gravimetric data obtained after adsorption of $MnO_x$ onto poly(MAA-co-EDMA) microspheres and after calcination of poly(MAA-co-EDMA)/MnOx composite microspheres at different temperatures.

| **Adsorption of $MnO_x$ onto poly(MAA-co-EDMA) microspheres** | | |
|---|---|---|
| Poly(MAA-co-EDMA) ($M_S$, Seed loaded) (g) | Poly(MAA-co-EDMA)/$MnO_x$ composite ($M_D$, Dried material from adsorption stage) (g) | $MnO_x$ loaded in adsorption ($M_L$= $M_S$-$M_D$) (g) |
| 0.10 | 0.167 | 0.067 |

**Gravimetric data obtained after calcination of poly(MAA-co-EDMA)/MnOx composite microspheres**

| Calcination Temperature (°C) | 380 | 410 | 450 | 500 | 560 |
|---|---|---|---|---|---|
| $M_T$ (g) | 0.084 | 0.087 | 0.078 | 0.082 | 0.081 |
| $M_C$ (g) | 0.017 | 0.019 | 0.011 | 0.014 | 0.014 |
| $M_M/M_C$ (%) | 79.8/20.2 | 78.2/21.8 | 85.9/14.1 | 83.0/17.0 | 82.7/17.3 |

*$M_T$ : Total weight of $MnO_x$ microspheres after calcination including both MnOx and carbonized material.*
*$M_C$ (g): Weight of carbonized material after calcination, $M_C$= $M_T$-$M_L$*
*$M_M/M_C$ (%): Weight percent ratio of $MnO_x$ to carbon based organic material in the final composition of $MnO_x$ microspheres.*

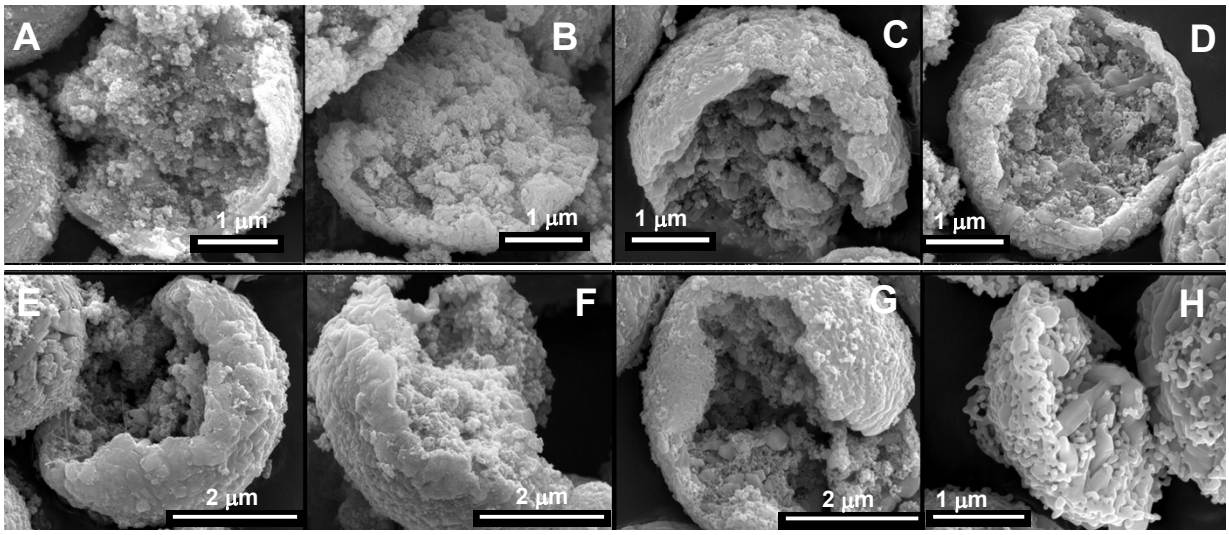


**Figure S1**. SEM photographs of broken $MnO_x$ microspheres showing their internal macroporous structure. Calcination temperature (°C) and Magnification: (A) 380, X60.000, (B) 410, X60.000, (C) 430, X60.000, (D) 450, X60.000, (E) 470, X55.000, (F) 500, X50.000, (G) 530, X55.000, (H) 560, X70.000.

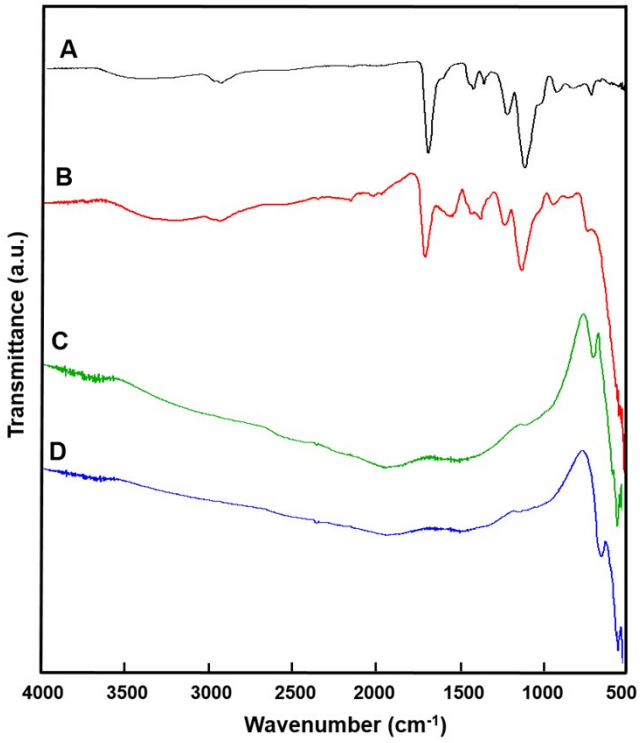


**Figure S2.** FTIR spectra of (A) poly(MAA-co-EDMA) microspheres, (B) poly(MAA-co-EDMA)/$MnO_x$ composite microspheres, (C) $MnO_x$ microspheres calcined at 410°C, (D) $MnO_x$ microspheres calcined at 500°C.

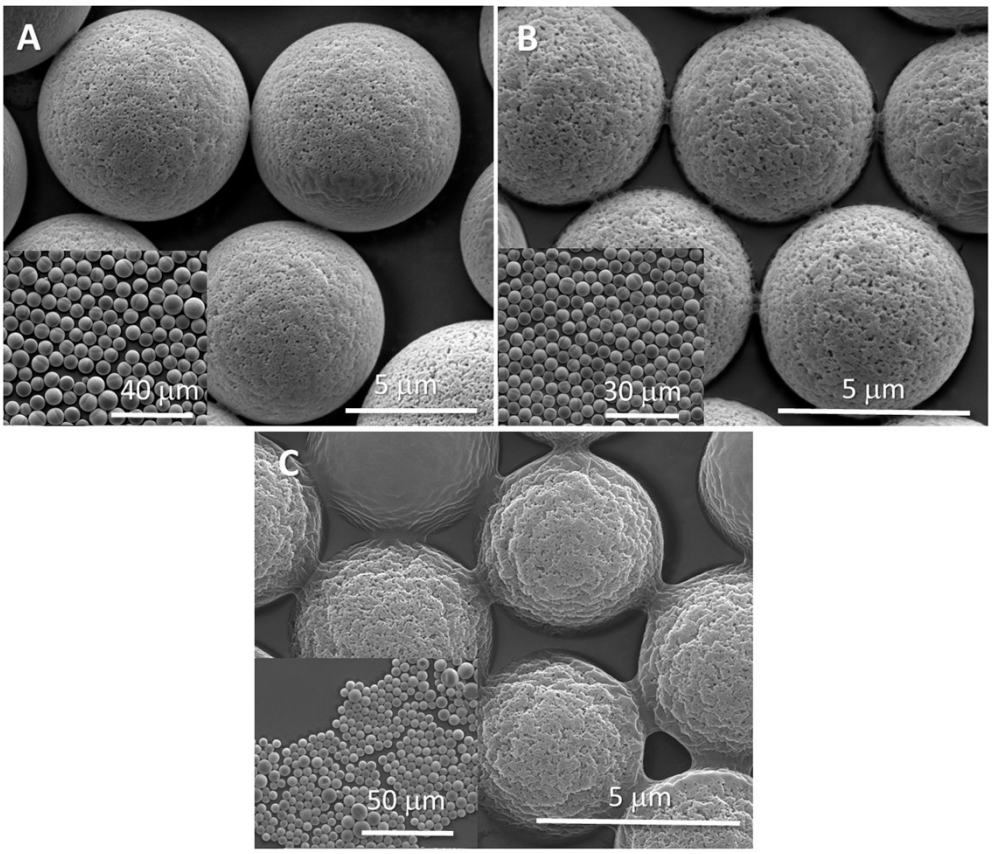


**Figure S3.** SEM photographs of poly(MAA-co-EDMA) microspheres obtained with different monomer/seed latex ratios. Monomer/seed latex ratio (mL/g): (A) 10, (B) 20, (C) 40. Amount of poly(GMA) latex: Variable between 0.6-0.15 g, Monomer phase: 6.0 mL (EGDMA: 4 mL, MAA: 2 mL), EB: 4 mL, Polymerization: 80$^{o}$C, 16 h, 120 cpm. Magnification: (A) X14.000 (X2.500), (B) X20.000 (X3.000), (C) X20.500 (X2.000). The magnifications for inset photos are given in the paranthesis.

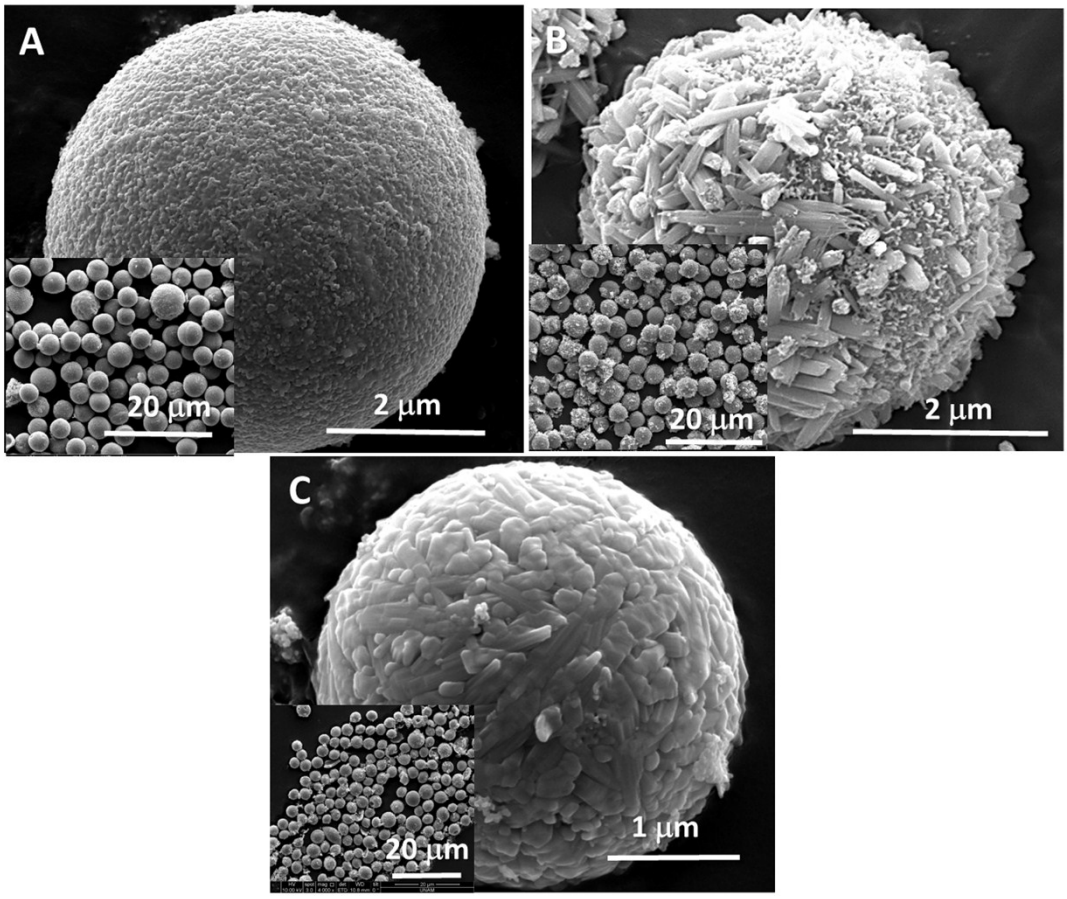


**Figure S4.** SEM photographs of $MnO_x$ microspheres obtained by using poly(MAA-co-EDMA) microspheres with different mean size values. Mean size of poly(MAA-co-EDMA) microspheres used as starting material (μm): (A) 4.5, (B) 5.3, (C) 7.1. Magnification: (A) X39.000 (X5.000), (B) X46.549 (X3.426), (C) X64.000 (X4.000). The magnifications for inset photos are given in the paranthesis.

**Table S3.** The size and porous properties of poly(MAA-co-EDMA) microspheres synthesized with different monomer/seed latex ratios.

| Monomer/seed latex ratio (mL/g) | $C_M$ (% w/w) | Mean size (μm) | CV (%) | Vp (cc/g) | Median pore size (nm) | SSA ($m^2/g$) |
|---|---|---|---|---|---|---|
| 10 | 91.2 | 4.5 | 5.2 | 0.20 | 20 | 77.0 |
| 20 | 88.3 | 5.3 | 4.0 | 0.23 | 20 | 83.0 |
| 40 | 82.8 | 7.1 | 5.5 | 0.24 | 20 | 82.8 |

*CV: Coefficient of variation for size distribution, Vp: Pore volume, SSA: Specific surface area. $C_M$: Percent monomer conversion obtained in the multistage microsuspension polymerization based on the feed mass of MAA and EDMA.*

**Table S4.** The size and porous properties of $MnO_x$ microspheres obtained using poly(MAA-co-EDMA) microspheres synthesized with different mean size values.

| Mean size of poly(MAA-co-EDMA) microspheres | Mean size (μm) | CV (%) | Vp (cc/g) | Median pore size (nm) | SSA ($m^2/g$) |
|---|---|---|---|---|---|
| 4.5 | 3.2 | 7.1 | 0.09 | 40 | 16.4 |
| 5.3 | 3.8 | 6.8 | 0.09 | 53 | 26.7 |
| 7.1 | 5.1 | 6.9 | 0.10 | 41 | 16.7 |

*CV: Coefficient of variation for size distribution, Vp: Pore volume, SSA: Specific surface area.*

## 5. Effect of pH on oxidase-like activity of $MnO_x$ microspheres

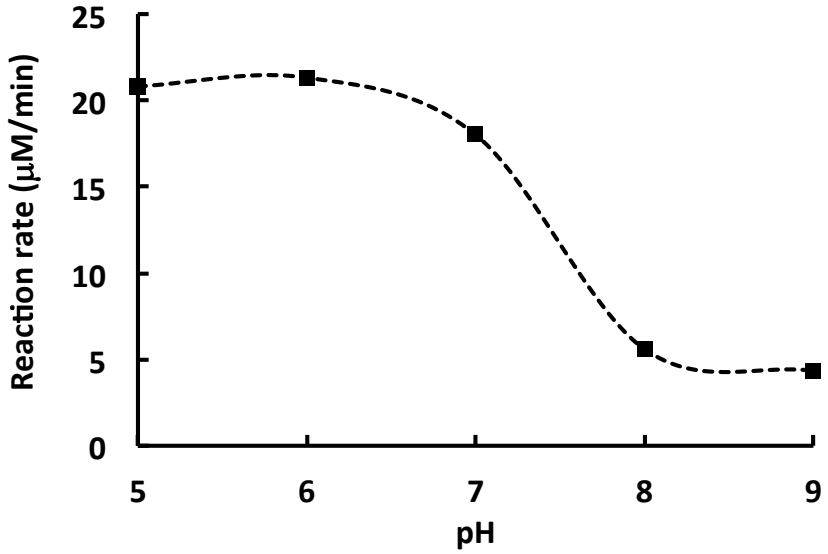


**Figure S5.** The effect of pH on the oxidase-like activity of $MnO_x$ microspheres. $MnO_x$ microspheres produced with the calcination temperature of 500°C. pH 5,6: 50 mM citrate buffer, pH 7.0: 50 mM phosphate buffer, pH 8,9: 50 mM HEPES buffer, OPDA concentration: 100 μM, $MnO_x$ concentration: 1.25 mg/mL, Volume: 4 mL, Room temperature, 100 rpm, 20 min.

### References for Supporting Information


1 K. Salimi, D.-D. Usta, I. Koçer, E. Çelik and A. Tuncel, *RSC Adv.,* 2017*,* **7**, 8718-8726.

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3 T. Camli, M. Tuncel, S. Senel and A. Tuncel, *J. Appl. Polym. Sci.,* 2002, **84**, 414-429.