Supporting Information

Fe-TAML Encapsulated inside Mesoporous Silica Nanoparticle as Peroxidase Mimic: Femtomolar Protein Detection

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(1) Materials and methods

Tetraethylorthosilicate (TEOS), Cetyltrimethylammonium bromide (CTAB), amino guanidine hydrochloride (AG.HCl), 3, 3′, 5, 5′-Tetramethylbenzidine (TMB), 3-Aminopropyl trimethoxy silane (APTES), DTT were obtained from Aldrich. EDTA, NaCl, H_2O_2 (30%), streptavidin, antigen hIgG, biotin were obtained from Merck. Human IgG from Jackson Smith. 96-well ELISA plates were purchased from R&D systems. Econo-Pac® Chromatography Columns, Bio-Gel P-6DG Gel, centrifugal filter tubes (MW = 10K) were purchased from BIO-RAD. CHO-S and CHO-IgG (Chinese Hamster Ovary) cells were obtained from Invitrogen for research use. In all the assays, de-ionized water was used. Alkyne tailed biuret modified Fe-TAML,[1] 3-azido-propyltriethoxysilane (AzPTES)[2] and tris (3-hydroxypropyl triazolyl methyl) amine (THPTA)[3], maleimideNHS,[4] and biotin NHS[5] were prepared as reported earlier.

(2) Synthesis and Characterizations

Synthesis

(a) Synthesis of AzPTES and Azide functionalized MSN (x-N_3-MSN)

Synthesis of 3-azidopropyltriethoxysilane, AzPTES: 3-Chloropropyltriethoxysilane (abbreviated as Cl-PTES; 2 g, 8.3 mmol) was added to a solution of sodium azide (1.08 g, 16.6 mmol) and tetrabutylammonium bromide (0.644 g, 2 mmol) in dry acetonitrile (50 mL), under nitrogen atmosphere. The reaction mixture was stirred under reflux for 18 h. After completion of the reaction, the solvent was removed under reduced pressure. The crude mixture was diluted in n-pentane and the suspension was filtered over Celite. Solvent was removed from the resulting filtrate and the crude oil obtained was distilled under reduced pressure of 0.025 mbar at 62 °C to give AzPTES (3-azidopropyltriethoxysilane) as a colorless liquid.[2] Yield: 1.52 g, 74%. 1H NMR

(500 MHz, CDCl3): δ 0.66 (t, 2H, J = 8.25 Hz), 1.21 (t, 3H, J = 6.88 Hz), 1.66–1.73 (m, 2H), 3.25 (t, 2H, J = 7.16 Hz), 3.80 (q, 2H, J = 6.88 Hz). 13C NMR (50 MHz, CDCl3) δ 7.59, 18.23, 22.64, 53.8, 58.41.

The azide functionalized MSN (x-N_3-MSN;x stands for % of azide loading) was synthesized by co-condensation of TEOS with AzPTES (3-azidopropyltriethoxysilane) by following procedure reported in literature[6, 7] with slight modifications. The density of azidopropyl groups on the surface of MSN was varied by varying the molar ratio of AzPTES with respect to TEOS during one pot co-condensation synthesis.

In a typical batch synthesis for 1% azide loading, CTAB (1 g, 2.744 mmol) was dissolved in 640 mL of water and 2M aqueous NaOH (3.5 mL, 7 mmol). The mixture was stirred thoroughly at 600 rpm for 30 min at 80 °C to dissolve the surfactant completely. To this clear solution, TEOS (4.702 g, 22.56 mmol) was injected rapidly followed by AzPTES (0.0563 g, 0.228 mmol). A white precipitate was observed within 1-2 min after the addition was completed. The resultant reaction mixture was allowed to stir at 600 rpm for 2 hr at 80 °C. The hot contents were then filtered and the white residue was washed with copious amounts of water and methanol and dried under vacuum at 100 °C over night (yield ~1.7 g). This azide grafted MSN will be simply denoted as 1-N_3-MSN. Similarly, 5-N_3-MSN and 10-N_3-MSN was synthesized using 0.2815 g (1.142 mmol) and 0.563 g (2.284 mmol) AzPTES respectively. All samples were characterized by SEM, TEM, XRD, FT-IR, TGA andnitrogen adsorption–desorption experiments. TGA was done to determine the azide incorporation in the x-N_3-MSN after removal of template. (Table SI 2)

(b) *Synthesis of 1-Fe-MSN, 5-Fe-MSN and 10-Fe-MSN*

Biuret modified Fe-TAML was grafted onto x-N_3-MSN using CuAAC. For CuAAC, 1-N_3-MSN was incubated with 3 equivalents of the alkyne tailed biuret modified Fe-TAML complex in 100 mM phosphate buffer containing THPTA (2.5 equivalent), AG.HCl (4 equivalent), $CuSO_4$ (0.5 equivalent) and sodium ascorbate (4 equivalent). In a typical click reaction, 1-N_3-MSN (10 mg, ~ 1.4μmol of azide) was incubated with alkyne tailed biuret modified Fe-TAML complex (2.7 mg, 4.2μmol) in 1mL of 100 mM phosphate buffer containing THPTA (1.5 mg, 3.5μmol), AG.HCl (0.62 mg, 5.6 μmol), $CuSO_4$ (0.18 mg, 0.7μmol). The reaction mixture was freeze-pump-thawed thrice, sodium ascorbate (1.1 mg, 5.6μmol) added and the mixture was stirred inside microwave at 55 °C for 15 min and then at room temperature for 24 hr. After completion of reaction, the reaction mixture was centrifuged and the residue was first washed with phosphate buffer twice and then sequentially washed with 10 mM N,N-diethyldithiocarbamate sodium solution in 100 mM phospate buffer and acetone respectively. The last two washings were repeated thrice. Finally, the yellowish white powder obtained was dried at 80°C in vacuum oven for 8 hr(Yield: ~9 mg). This biuret modified Fe-TAML functionalized MSN will be denoted as 1-Fe-MSN.

Similarly, CuAACclick reaction was carried out using 5-N_3-MSN and 10-N_3-MSN. 5-N_3-MSN (10 mg, ~ 5.1μmol of azide) was incubated with alkyne tailed biuret modified Fe-TAML complex (10 mg, 15.3μmol) in 1mL of 100 mM phosphate buffer containing THPTA (5.5 mg, 12.75μmol), AG.HCl (2.2 mg, 20.4μmol), $CuSO_4$ (0.64 mg, 2.55μmol). The reaction mixture was freeze-pump-thawed thrice and sodium ascorbate (4.0 mg, 20.4 μmol) was added and the mixture was stirred inside microwave at 55 °C for 15 min and then at room temperature for 24 hr.

In case of synthesis of 10-Fe-MSN, 10-N_3-MSN (10 mg, ~ 9.0μmol of azide) was incubated with alkyne tailed biuret modified Fe-TAML complex (17.7 mg, 27μmol) in 1mL, 100 mM phosphate buffer containing THPTA (9.8 mg, 22.5μmol), AG.HCl (3.96 mg, 36μmol), $CuSO_4$ (1.1 mg, 4.5μmol). The reaction mixture was freeze-pump-thawed thrice and sodium ascorbate (7.1 mg, 36μmol) was added and the mixture was stirred inside microwave at 55 °C for 15 min and then at room temperature for 24 hr.

The extent of click reaction was estimated by IR spectroscopy (Fig SI 7-9) and iron content in the 1-Fe-MSN, 5-Fe-MSN and 10-Fe-MSN was determined by ICP.

(c) Synthesis of Amine functionalized MSN (NH_2-N_3-MSN)

Using 3-amino-propyltriethoxysilane (APTES), by post synthetic grafting method amine groups were incorporated selectively on the outer surface of the 5-N_3-MSN and template was removed to yield amine functionalized MSN denoted as (NH_2-N_3-MSN).

1 g of 5-N_3-MSN was suspended in 200 mL of dry toluene under continuous sonication for 10 minutes. To this APTES (0.358 g, 2mmol) was added, and the mixture was stirred for 18 hr at 80 °C under nitrogen atmosphere. After the completion of reaction, the contents were cooled, filtered and washed with toluene until it became free from APTES. The sample was then dried at 100 °C for 8hr in a vacuum oven. The template was extracted by stirring the as-synthesized sample (1 g) in 200 mL methanol and 2 ml concentrated hydrochloric acid at 60 °C for 6 hr. The resulting template removed solid product, was filtered and washed with methanol (100 mL) and 1% triethyl amine in methanol (50 mL). Then it was again washed with methanol (50 mL) and dried overnight under vacuum at 100 °C (yield ~0.64 g). This material will be referred as NH_2-N_3-MSN.TGA was done to determine the amine incorporation in the NH_2-N_3-MSN.

(d) Modification of NH_2-N_3-MSN with biuret modified Fe-TAML by Cu(I) catalyzed Azide-Alkyne Cycloaddition reaction (CuAAC) (NH_2-Fe-MSN)

For CuAAC, NH_2-N_3-MSN was incubated with 3 equivalents of the alkyne tailed biuret modified Fe-TAML complex in 100 mM phosphate buffer containing THPTA (2.5 equivalent), AG.HCl (4 equivalent), $CuSO_4$ (0.5 equivalent) and sodium ascorbate (4 equivalent). In a typical click reaction, NH_2-N_3-MSN (10 mg, ~ 5.1 μmol of azide) was incubated with alkyne tailed biuret modified Fe-TAML complex (10 mg, 15.3 μmol) in 1mL, 100 mM phosphate buffer containing THPTA (5.5 mg, 12.75μmol), AG.HCl (2.2 mg, 20.4 μmol), $CuSO_4$ (0.64 mg, 2.55 μmol). The reaction mixture was freeze-pump-thawed thrice and sodium ascorbate (4.0 mg, 20.4 μmol) was added and the mixture was stirred inside microwave at 55 °C for 15 min and then at room temperature for 24 h. After completion of reaction, the reaction mixture was centrifuged and the residue was first washed with phosphate buffer twice and then sequentially washed with 10 mM N,N-diethyldithiocarbamate sodium solution in 100 mM phosphate buffer and acetone respectively. The last two washings were repeated thrice. Finally, the yellowish white powder obtained was dried at 80 °C in vacuum oven for 8 hr (Yield: ~9 mg). This biuret modified Fe-TAML functionalized MSN will be simply denoted as NH_2-Fe-MSN. The extent of click reaction was estimated by IR spectroscopy (Fig SI 9) and ICP analysis.

(e) Synthesis of biotin immobilized MSN (biotin-Fe-MSN)

1mg of 5-Fe-MSN was added to a solution of NHS-Biotin (5 mg;14 μmol) in 2 mL of 100 mM, pH 7.4 PBS and stirred at room temperature for overnight as shown in Scheme 2(a). After completion of the reaction, the particles were washed extensively (5 times) with the buffer to

afford biotin-Fe-MSN. Ninhydrin test was performed to confirm that the free amine groups on the surface of NH_2-Fe-MSN were conjugated with biotin-NHS. NH_2-Fe-MSN gives a Ruhemann's purple with ninhydrin test whereas negligible color was observed for biotin-Fe-MSN. The samples were then dispersed in buffer and subsequently used for colorimetric assay.

Biotin modified MSN (no Fe-TAML present) was synthesized in a similar method for control experiments. NH_2-N_3-MSNwas used instead of NH_2-Fe-MSN for synthesis of Biotin-N_3-MSN for control experiments.

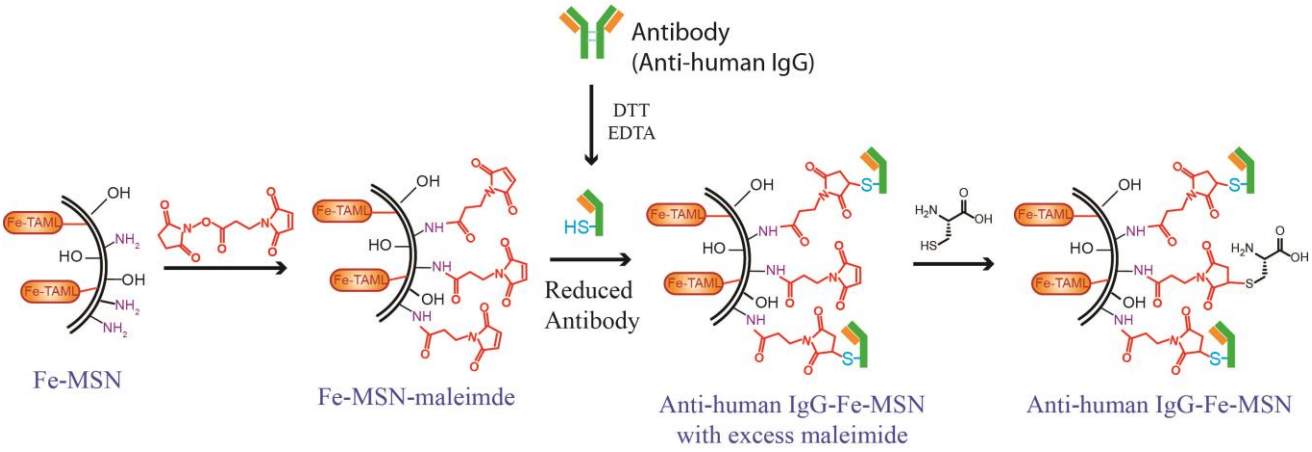
(f) Synthesis of maleimide functionalized MSN (maleimide-Fe-MSN)

To a dispersion of 5-Fe-MSN (1 mg) in PBS buffer was added maleimide NHS (5mg, 19µmol) in acetonitrile: water (1:1) and stirred at room temperature for 12 hr. After completion of the reaction, the particles were centrifuged and washed thoroughly several times with acetonitrile-buffer mixture. Ninhydrin test and TGA was performed to confirm that the free amine groups on the surface of NH_2-Fe-MSN were conjugated with maleimide-5-Fe-MSN. NH_2-Fe-MSN gives a Ruhemann's purple with ninhydrin test whereas negligible color was observed for maleimide-5-Fe-MSN. At the end of the centrifugation the particles were dispersed in 100 mM PBS buffer of pH 7.4 and used for the conjugation with reduced antibody.

(g) Conjugation of Antibodies to maleimide-Fe-MSN (anti-human IgG-5-Fe-MSN)

Anti-human IgG antibody was conjugated to maleimide-Fe-MSN using a methodology that is shown below (Scheme SI 1).The antibodies were first reduced with Dithiothreitol (DTT).Reduced antibody fragments were purified by gel filtration using a desalting resin.

Reduced antibody fragments with free sulfhydryl groups were conjugated to maleimide activated Fe-MSN through thiol-maleimide reaction. In a typical reaction, a 2.5 µmol antibody was mixed in 200 µL PBS-EDTA (100 mM, phosphate buffer of pH 7.2, 10 mM EDTA) with 6.5 µmol DTT and incubated at 37 °C for 2 hr. Then, 0.1 mg of NH_2-Fe-MSN was mixed with reduced IgG and the solution was incubated at room temperature for overnight. After 12 hrs of incubation, the solution was centrifuged twice (12000 rpm, 4 °C and 1 hr) and supernatant with unconjugated antibody fragments was discarded. Finally, cysteine was added to the conjugate (0.01 mg, 0.1µmol) to quench all the unreacted maleimides. The particle anti-human IgG-Fe-MSN was then dispersed in PBS buffer and used for antigen detection in immunoassay.



Scheme SI 1. Synthesis of anti-human IgG-Fe-MSN

Characterization techniques

Powder X-ray diffraction of all the samples was carried out in a PAN analytical X'pert Pro dual goniometer diffractometer. A proportional counter detector was used for low angle experiments and an X'celerator solid state detector was employed in the low angle experiments. The radiation used was CuKα (1.5418 Å) with a Ni filter and the data collection was carried out using a flat holder in Bragg–Brentano geometry (0.5 to 10°; 0.2° min^{-1}). Care was taken to avoid sample displacement effects.

SEM images were obtained on Leica Stereoscan 440 microscope. HR-TEM images were taken on a FEI Technai F30 operating at 300 kV with FEG. The samples were prepared by dispersing a large number of solid particles in isopropanol by sonication, and dropping the resulting suspension on a copper grid of 400 mesh and allowed to dry in air.

Nitrogen adsorption and desorption studies were carried out using Quantachrome instrument. Samples were preheated at 100 °C for 18 hrs in the vacuum line. Multi point BET surface area was obtained from adsorption isotherm from P/P_o 0.1-0.3. Pore size distributions were calculated from adsorption isotherm using the BJH method.

Semi-quantitative FT-IR spectra were recorded on Perkin Elmer FT-IR spectrum GX instrument by making KBr pellets. Pellets were prepared by mixing 3 mg of sample with 97 mg of KBr. Yields for CuAAC reactions were calculated from corrected area under the curve characteristic for the azide stretch at ~2100 cm^{-1}.

13C Cross Polarization Magic Angle Spinning (CPMAS) NMR experiments were carried out on a Bruker AVANCE 300 wide bore spectrometer equipped with a superconducting magnet with a field of 7.1 Tesla operating at 75.4 MHz. The samples were packed into a 4mm zirconia rotor and loaded into a 4mm BL MAS probe and spun about the magic angle (54.74) at 10 KHz

using a standard ramp-CP pulse sequence was used for the experiment. The RF-powers was 60 KHz 13C CPMAS experiments. The contact time was 3 ms for 13C CPMAS experiments. All the chemical shifts were referenced to TMS. Typically 10,000 to 25,000 scans with a recycle delay of 3s were collected depending on the sensitivity of the sample.

Confocal laser scanning microscopy (CLSM) images were taken with Carl Zeiss confocal system equipped with a 20x objective. Optical slices in the center of the particle were selected.

Thermogravimetric analysis (TGA) of the silica nanoparticles were carried out using a TA Instrument SDT Q600 analyzer between 100 and 750°C in air (flow 25 ml min^{-1}) at a heating rate of 10°C min^{-1}. All samples were stirred in water overnight, centrifuged and dried under vacuum at 80°C overnight prior to TGA runs. The amount of organic content on the silica surface was determined by TGA using thefollowing equation:

$$Graft\ density\ \left(\frac{mmol}{gm}\right) = \frac{\frac{W_{Grafted-MSN(150-750)}}{100-W_{Grafted-MSN(150-750)}} \times 100 - W_{MSN(150-750)}}{M \times 100}$$

Where $W_{Grafted\text{-}MSN(150\text{-}750)}$ is the weight loss between 150 ^{o}C and 750 ^{o}C corresponding to the decomposition of the organic substance from MSN corrected from the thermal degradation, while

$W_{MSN(150\text{-}750)}$represents the weight loss between 150 ^{o}C and 750 ^{o}C from functionalized moiety MSN. M is the molecular weight of the decomposed organic substance.

Determination of the concentration of 1-Fe-MSN and 5-Fe-MSN in a stock solution of 1 mg/mL.

At first the density of particles (N_3-MSN), that is the ratio of the mass of particle per its volume (excluding the void space between particles), was estimated using:

Density = mass of particles/volume of particles = mass of particles/(volume of walls + volume of internal pores)

Volume of internal pores: From gas adsorption 0.4 cm^3/g (used p/p_0 = 0.20 - 0.30 to exclude pore volume contribution from the inter particle porosity)

Volume of walls: mass divided by framework density (silica density of 2.20 g/cm^3 used since it is mostly SiO_2)

Therefore, Density of N_3-MSN = 1.176 gm/cm^3

This density estimated above and the amount of Fe-TAML loaded onto 1-Fe-MSN and 5-Fe-MSN (as determined by ICP) was used to calculate the concentration of 1-Fe-MSN and 5-Fe-MSN in solution. The concentration of 1mg/mL of 1-Fe-MSN was determined to be 3.96×10^{-8} M. Similarly, the concentration of 1mg/mL of 5-Fe-MSN was determined to be 2.5×10^{-8} M.

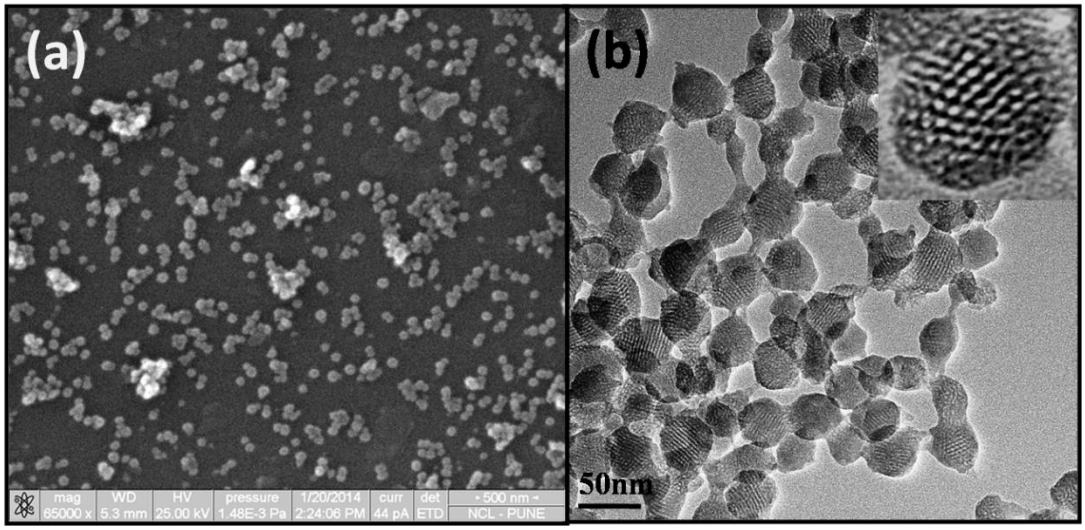


Figure SI 1. (a) SEM and (b) TEM images of 1-N_3-MSN showing formation of well-ordered two-dimensional hexagonal MSN with a spherical morphology having particle size of ~ 40 nm.

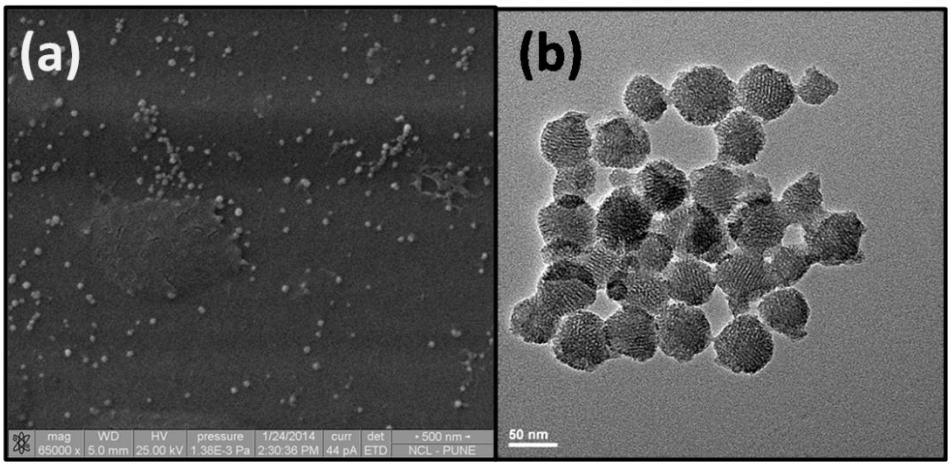


Figure SI 2. (a) SEM and (b) TEM images of 5-N_3-MSN showing formation of well-ordered two-dimensional hexagonal MSN with a spherical morphology having particle size of ~40 nm.

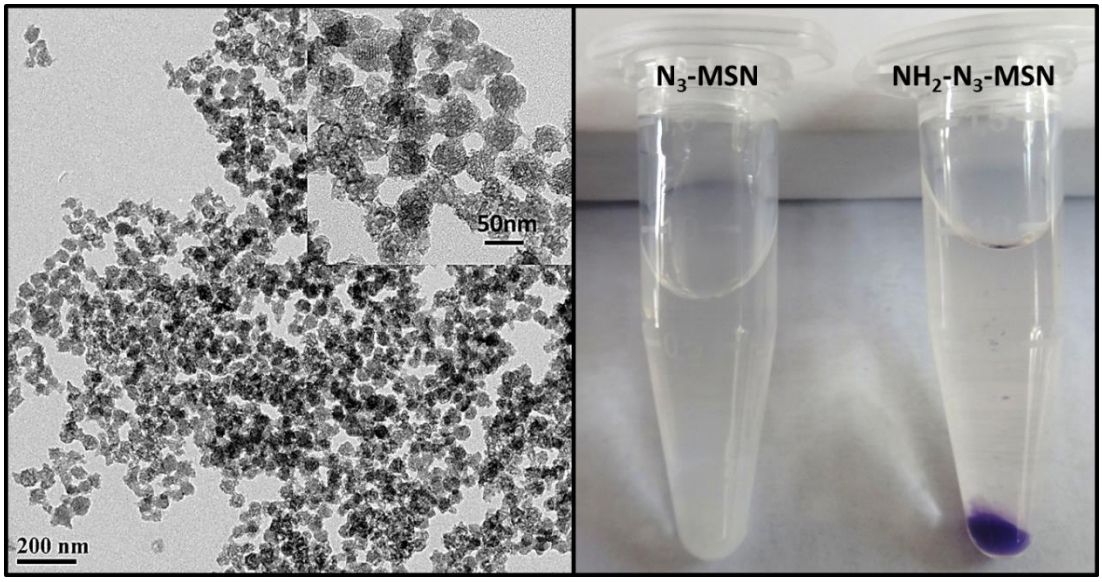


Figure SI 3. (a)TEM image of NH_2-N_3-MSN shows the absence of clustering in mesoporous silica nanoparticles after surface modification. (b) Ninhydrin test before and after amine grafting on MSN. Formation of dark violet color confirms the presence of amine groups on the surface of MSN.

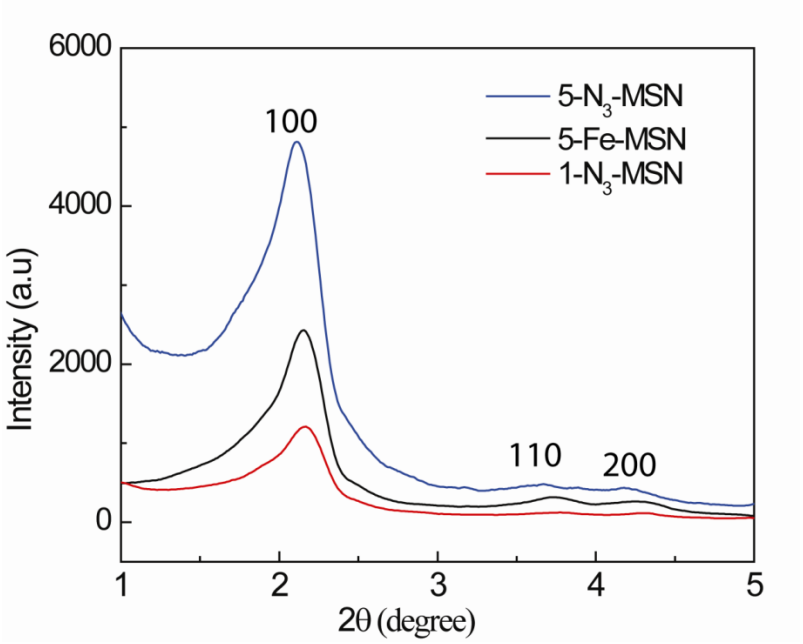


Figure SI 4. Powder XRD patterns of 1-N_3-MSN, 5-N_3-MSN and 5-Fe-MSN. All materials showed the characteristic high intensity (100) diffraction peak at $2\theta \sim 2.3^{o}$. The other significant peaks corresponding to (110) and (200) diffractions were also observed indicating that well-ordered one dimensional hexagonal mesoporous channels of MSN were formed and remained intact after functionalization.

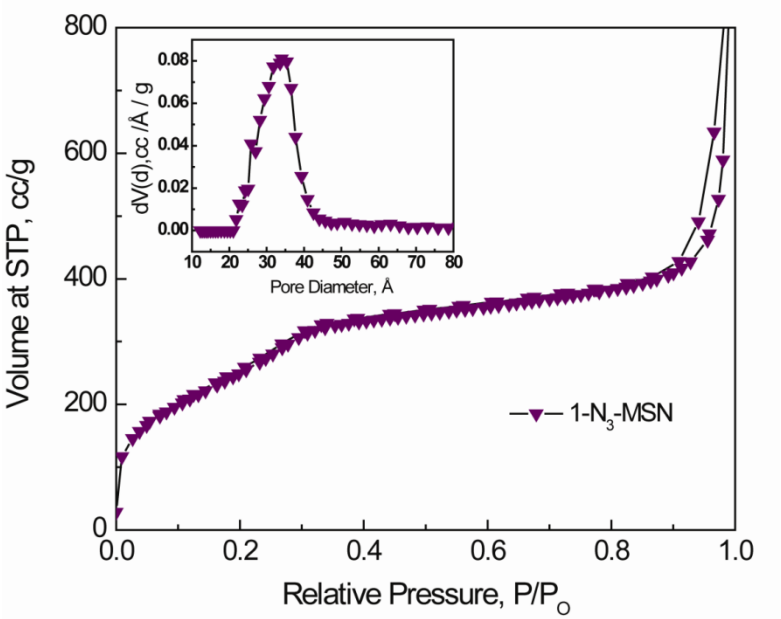


Figure SI 5. Nitrogen adsorption-desorption isotherms for1-N_3-MSN (*inset* shows pore size distribution)

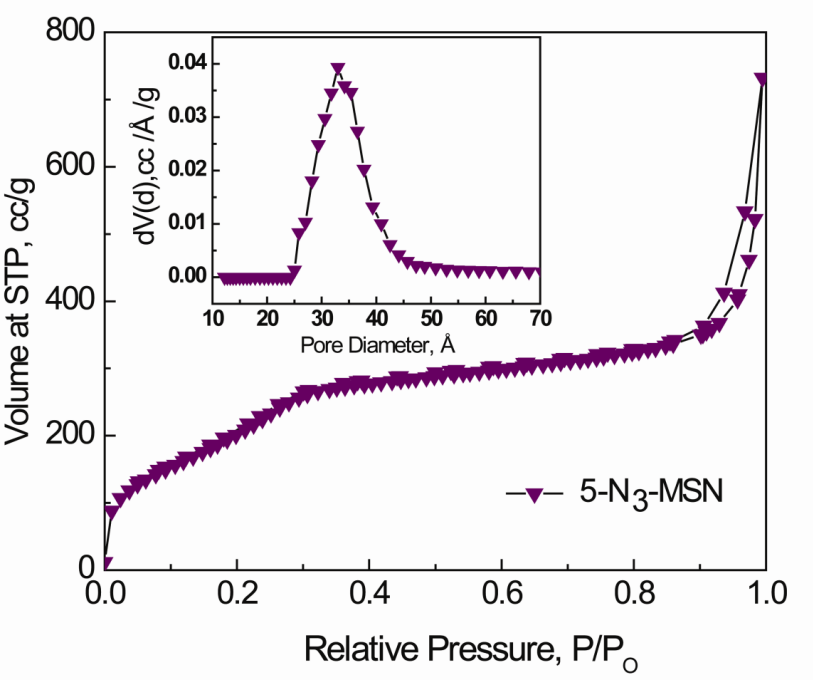


Figure SI 6. Nitrogen adsorption-desorption isotherms for 5-N_3-MSN (*inset* shows pore size distribution)

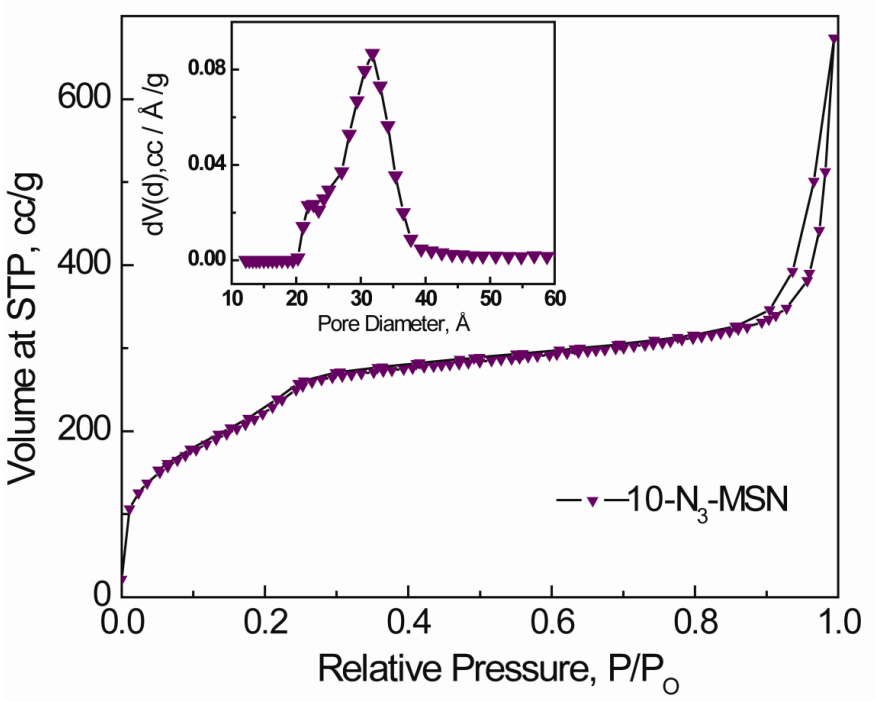


Figure SI 7. Nitrogen adsorption-desorption isotherms for 10-N_3-MSN (*inset* shows pore size distribution)

Table SI 1. Physical Characterization of x-N_3-MSN

Sample Name	**Azide content mmol/g**	**Pore diameter (nm)**	**M_{BET} (m^2/g)**	**Pore Volume (cm^3/g)**
1-N_3-MSN	0.14	3.54	947	0.925
5-N_3-MSN	0.51	3.30	851	0.779
10-N_3-MSN	0.90	3.18	844	0.731

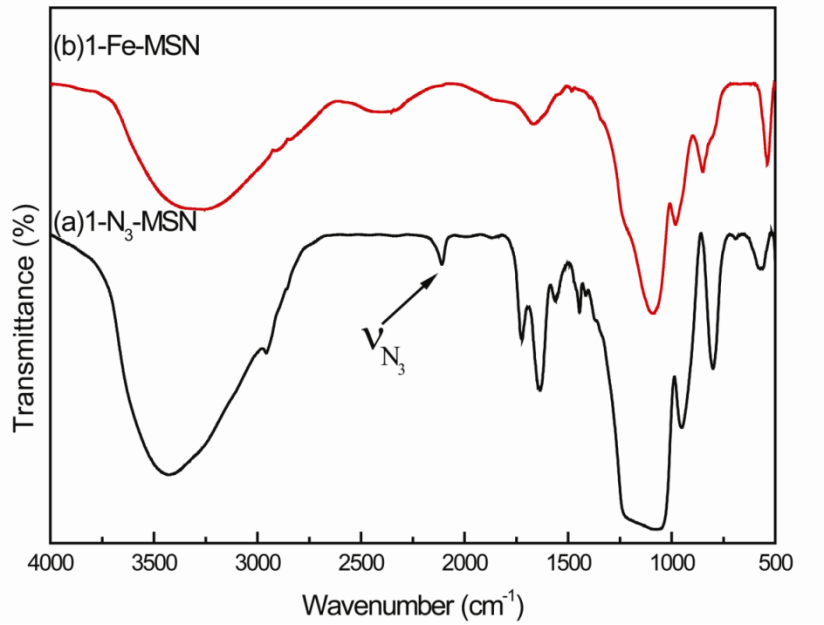


Figure SI 8. FT-IR spectra of (a) 1-N_3-MSN and (b) 1-Fe-MSN: IR spectroscopy shows about 98% decrease in the integrated intensity of ν_{N3}at 2100 cm^{-1}.

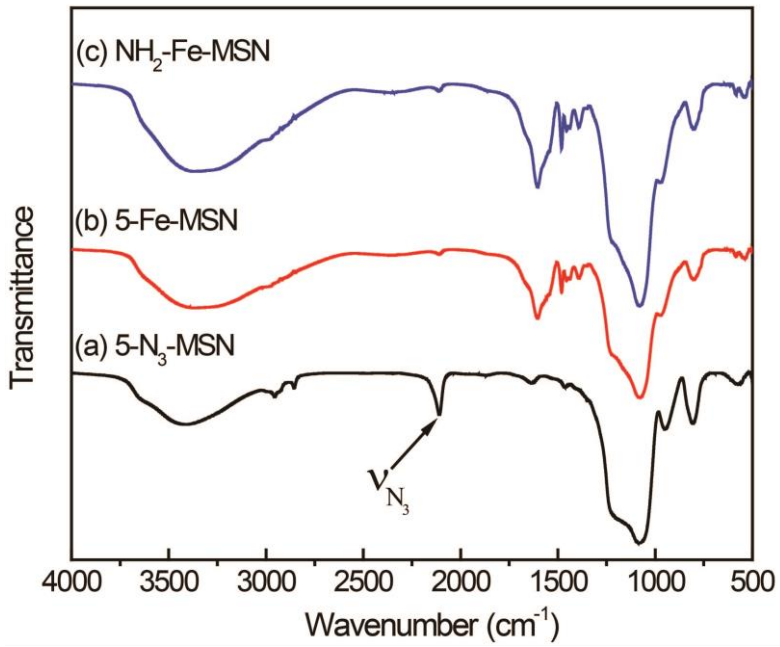


Figure SI 9. FT-IR spectra of (a) 5-N_3-MSN and (b) 5-Fe-MSN (c) NH_2-Fe-MSN: IR spectroscopy shows about 95% decrease in the integrated intensity of ν_{N3}at 2100 cm^{-1}.

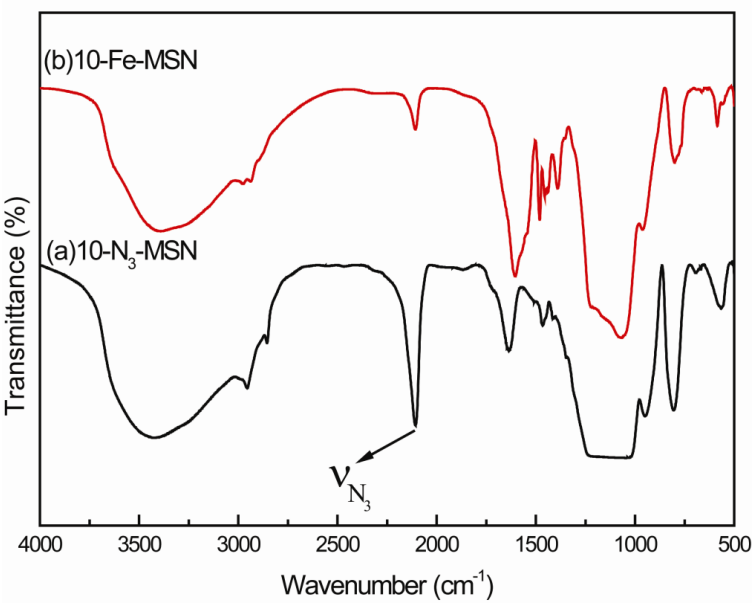


Figure SI 10. FT-IR spectra of (a) 10-N_3-MSN and (b) 10-Fe-MSN: IR spectroscopy shows about 80% decrease in the integrated intensity of ν_{N3}at 2100 cm^{-1}.

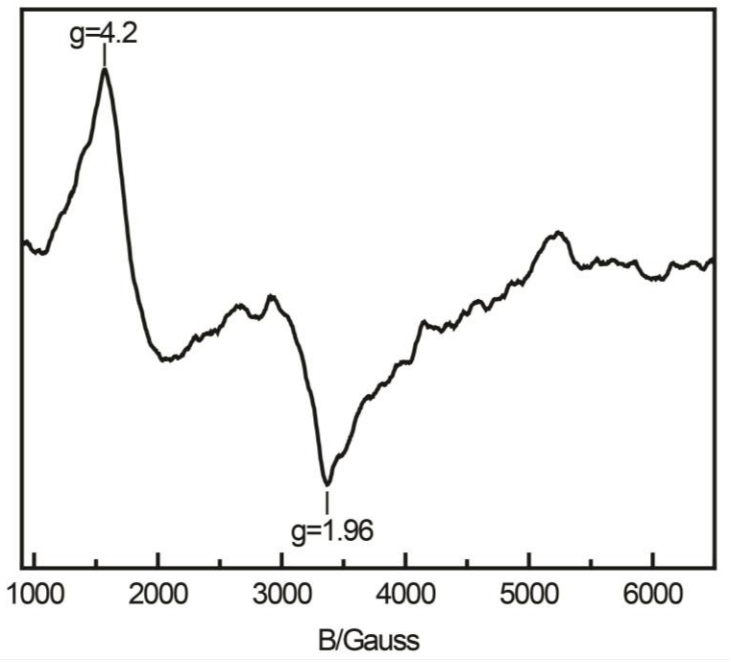


Figure SI 11. X-band EPR spectrum of 1-Fe-MSN Solid at 94 K

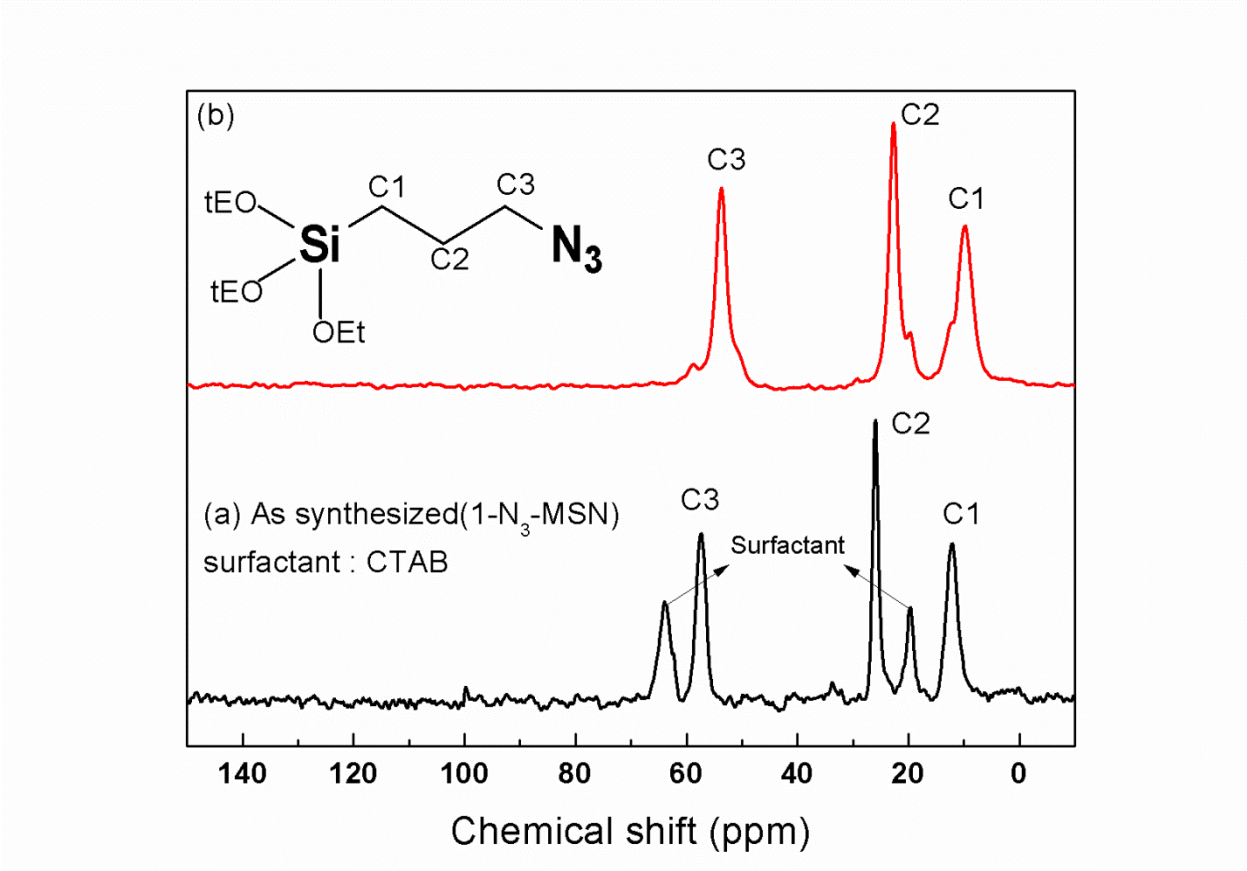


Figure SI 12. ^{13}C CPMAS-NMR for 1-N_3-MSN.NMR spectrum of 1-N_3-MSN (a, black) showed three distinct peaks corresponding to the C1, C2 and C3 carbon atoms which is similar to three peaks present in azido-propyl silane (b, red) C1 (8.68 ppm), C2 (22 ppm), C3 (53 ppm).

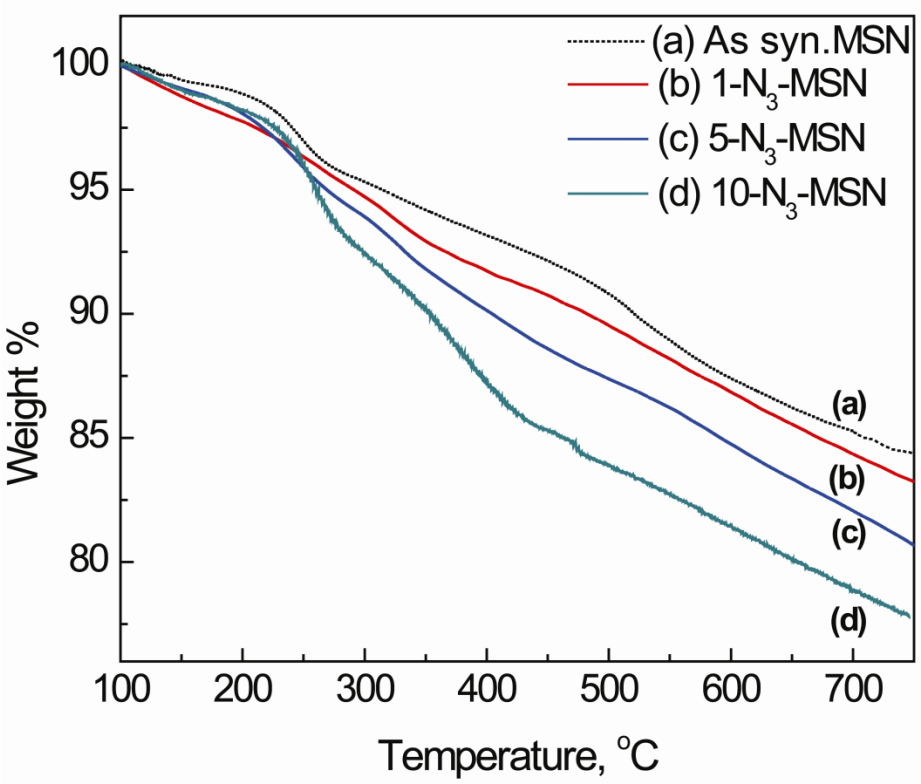


Figure SI 13. TGA for MSN before and after azide grafting. (a) As synthesized MSN, (b) 1-N_3-MSN (c) 5-N_3-MSNand (d) 10-N_3-MSN stand for 0, 1, 5 and 10% N_3 grafting MSN respectively.

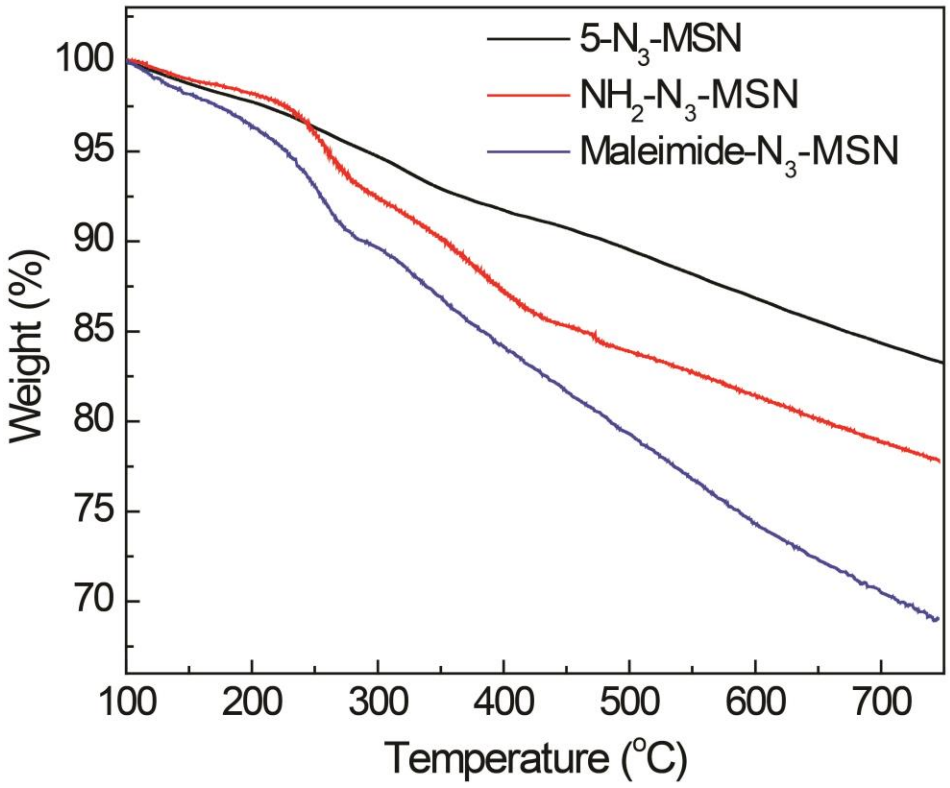


Figure SI 14. TGA for NH_2-N_3-MSN and maleimide-N_3-MSN.

Table SI 2. Grafting density of functional moiety for 5-N_3-MSN

Sample	Grafting Density (mmol/g)
5-N_3-MSN	0.51
NH_2-N_3-MSN	1.04
Maleimide-N_3-MSN	0.45

(3) Kinetics of TMB Oxidation

Experimental methodology

The determination of K_m and K_{cat} values of 1-Fe-MSN and 5-Fe-MSN for the oxidation of TMB in presence of H_2O_2 was carried out at the physiological pH 7.4. At this pH, upon oxidation TMB forms three products i) one electron oxidized product (TMB^+; λ_{max} = 900 nm), ii) a charge transfer complex consisting of one unreacted TMB and one two electron oxidized species (TMB^{2+}:TMB; λ_{max} = 650, 370 nm) and iii) a two electron oxidized product (TMB^{2+}; λ_{max} = 450 nm). All these three species remain in equilibrium with each other.[8] However, when the pH of the solution is lowered below 2, the equilibrium is shifted towards the complete formation of TMB^{2+} (characteristic peak at 450 nm; ε = 59,000 $M^{-1}cm^{-1}$) together with the disappearance of [TMB^{2+}:TMB] and TMB^+. So estimation of rate constants using normal scanning kinetics at any one of the above mentioned λ_{max} (which would represent only one oxidation products of TMB) would not be representative of the "true" rate constants of Fe-MSN. Therefore all kinetic runs the reactions were initiated at pH 7.4 and were quenched to pH < 2 by addition of HCl at different time intervals. The absorbance of the product formed after quenching was measured at 450 nm (See Figure SI 14). The initial rate was measured according to absorbance change at 450 nm for different time intervals. For detailed kinetic studies, keeping the catalyst (1-Fe-MSN and 5-Fe-MSN) concentration fixed, [TMB] or [H_2O_2] was varied separately as substrate keeping the other constant. The plots of initial rate *vs.* substrate concentrations were fitted according to Michaelis–Menten equation: $v = V_{max} \times [S]/(K_m + [S])$ where "v" stands for initial rate or initial velocity, V_{max} is the maximal velocity, [S] is the concentration of the substrate and K_m is the Michaelis-Menten constant. K_m and V_{max} values were determined as per fitting (Fig. SI 17, Table 2) within 10% error limit.

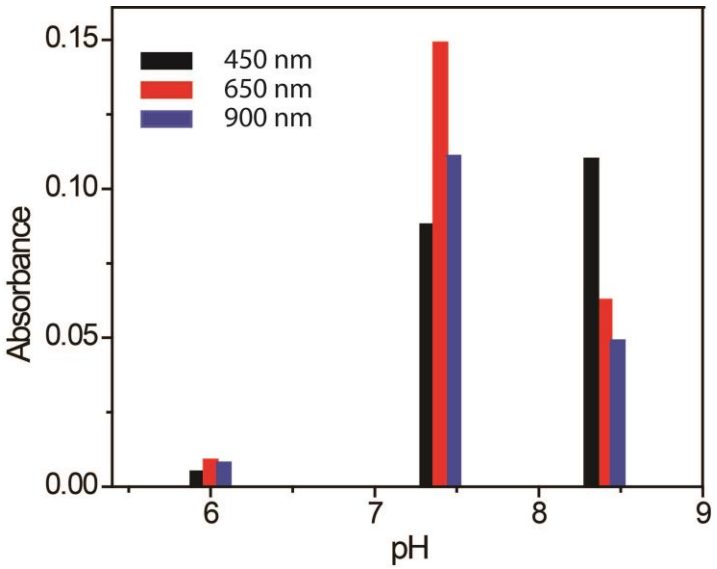


Figure SI 15. Absorbance of the three different peaks (450, 650 and 900 nm) generated for TMB oxidation by 5-Fe-MSN and H_2O_2 at pH 6, 7.4 and 8.4. All the absorbance was measured after 60 seconds of the reaction. Reaction condition: [TMB] = 3×10^{-4} M, [H_2O_2] = 1.2×10^{-4} M, [5-Fe-MSN] = 12.5×10^{-14}, PBS buffer of 10mM (pH 6, 7.4 and 8.4).

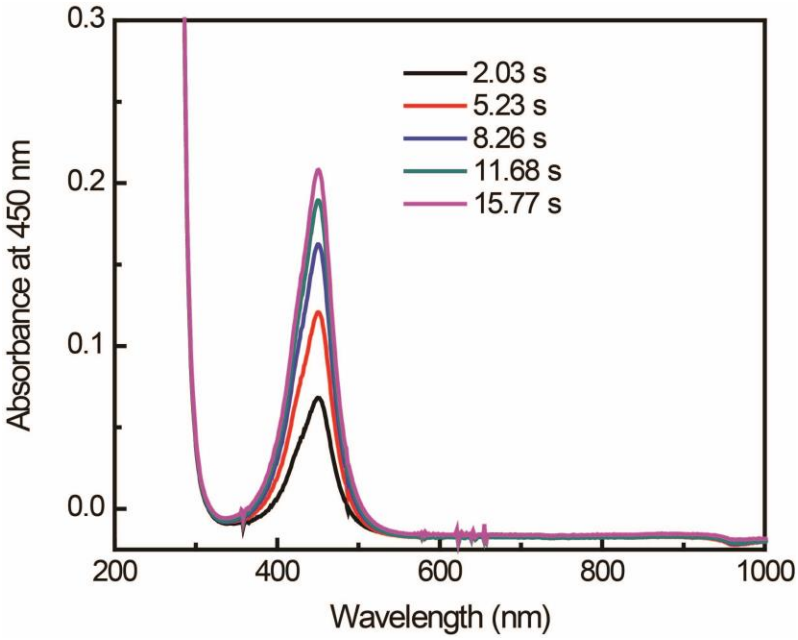


Figure SI 16. UV-vis spectra of quenched reaction mixture for TMB oxidation using 5-Fe-MSN and H_2O_2. The reaction was quenched at different time intervals' using HCl and the UV-vis spectra was measured. The peak at 450 nm is only observed. Reaction condition: [TMB] = 2×10^{-4} M, [H_2O_2] = 1.5×10^{-4} M, [5-Fe-MSN] = 12.5×10^{-14}, PBS buffer (pH 7.4, 10 mM).

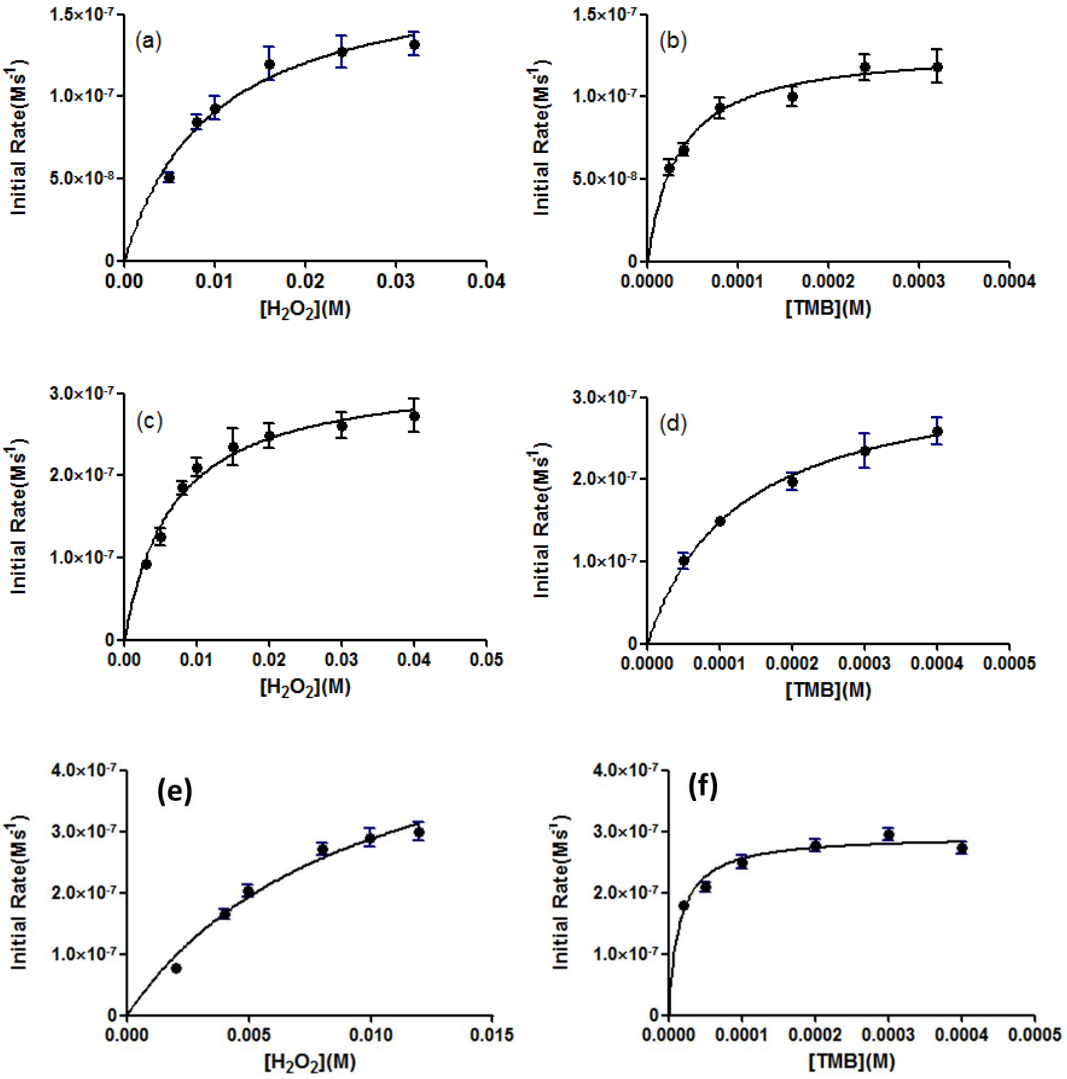


Figure SI 17. Michaelis-Menten fit for H_2O_2 and TMB variation; (a) and (b) for 1-Fe-MSN, (c) and (d) for 5-Fe-MSN , (e) and (f) for 10-Fe-MSN .

The K_{cat} values for 10-Fe-MSN with H_2O_2 and TMB as the substrate was found to be 1×10^{7} and 5.4×10^{6} respectively. K_{cat} value has been calculated using formulae $K_{cat} = V_{max}/[E]$. Where [E] $=5.4\times 10^{-14}$ and V_{max} for H_2O_2 and TMB was found to be 5.598×10^{-7} and 2.933×10^{-7}. Error bars shown represent the standard error derived from three repeated measurements.

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