Supporting Information

Application of Open-Tube Immobilized Enzyme Microreactor Constructed by Magnetic Macro-Porous Silica Beads in Proteomic Analysis

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Section 1 Experimental Procedures

1.1 Determination of protein concentration

Protein concentration was determined using the BCA assay. Briefly, different volumes (1, 2, 4, 8, 12, 16, and 20 μL) of standard protein solutions were added to a 96-well plate, and the total volume was adjusted to 20 μL with NH_4HCO_3 buffer. Then, 200 μL of BCA working reagent was added to each well, and the plate was incubated at 37°C for 30 min. The absorbance at 562 nm was measured using a microplate reader (SR-3518A), and a standard curve of absorbance versus protein concentration was established. The protein concentration of the samples was determined based on the measured absorbance, and each sample was measured in triplicate.

1.2 Database search

Raw Data of DIA were processed and analyzed by Spectronaut 19 (Biognosys AG, Switzerland) with default settings. The database was Homo_Sapiens (version 2024, 20608 entries) which downloaded from uniprot. Trypsin was the digestion enzyme and specific was the digest type. Carbamidomethyl on cysteine was specified as the fixed modification. Oxidation on methionine, Acetyl on protein N-term were specified as the variable modifications. Retention time prediction type was set to dynamic iRT. Data extraction was determined by Spectronaut based on the extensive mass calibration. Spectronaut will determine the ideal extraction window dynamically depending on iRT calibration and gradient stability. Qvalue (FDR) cutoff on precursor level was 1%, peptide level was 1% and protein level was 1%. Decoy generation was set to mutated which similar to scrambled but will only apply a random number of AA position swamps (min=2, max=length/2). Normalization strategy was set to local normalization. Peptides which passed the 1% Qvalue cutoff were used to calculate the major group quantities with MaxLFQ method.

1.3 Total immobilized enzyme amount inside the capillary

The enzyme immobilization amount inside the capillary was calculated according to the following formula:

$$m_e = \frac{1}{4}\Pi d_i^2 L \rho q_i$$

Where m_e (μg) is the enzyme immobilization amount inside the capillary; d_i (μm) is the capillary

inner diameter (75 μm in this work); L (cm) is the effective coating length of SiO_2@Fe_3O_4-nIMER; ρ (g/cm^3) is the density of SiO_2@Fe_3O_4, the bulk density (0.5126 g/cm^3) acquired from mercury intrusion porosimetry (MIP) was taken as the material density in the calculation; q_i (μg/mg) is the amount of immobilized enzyme per unit mass of carrier;

Section 2 Supplementary Figures and Tables

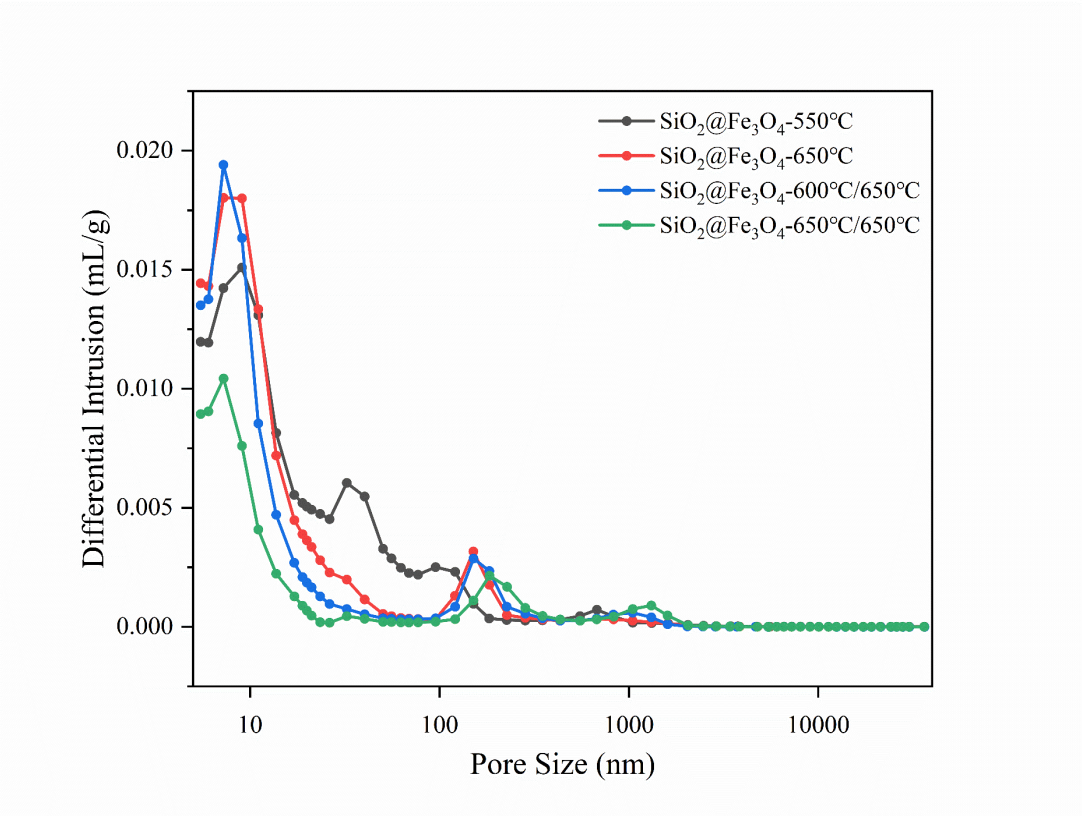

Figure S-1. The pore size distribution of SiO_2@Fe_3O_4-550℃, SiO_2@Fe_3O_4-650℃, SiO_2@Fe_3O_4-600℃/650℃ and SiO_2@Fe_3O_4-650℃/650℃ measured by MIP.

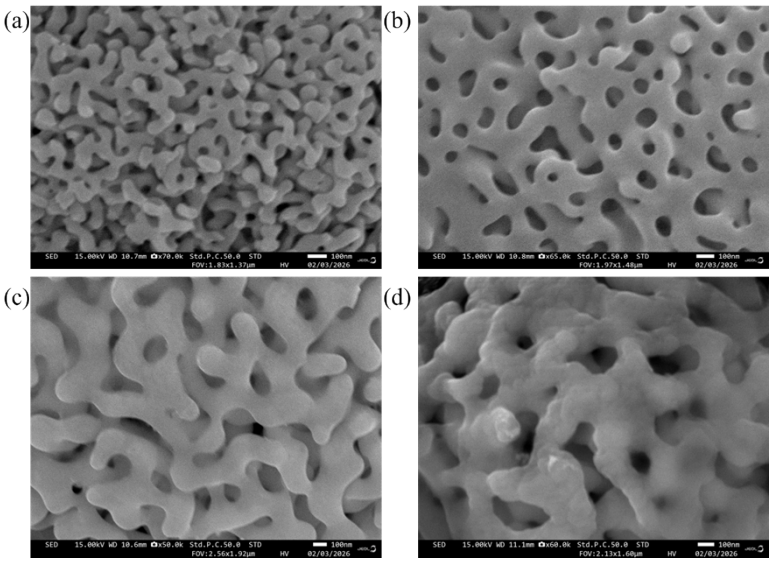

Figure S-2. SEM images of macro-porous silica materials synthesized at 550℃ (a), 650℃ (b), 600℃/650℃ (c) and 650℃/650℃ (d) calcination temperature.

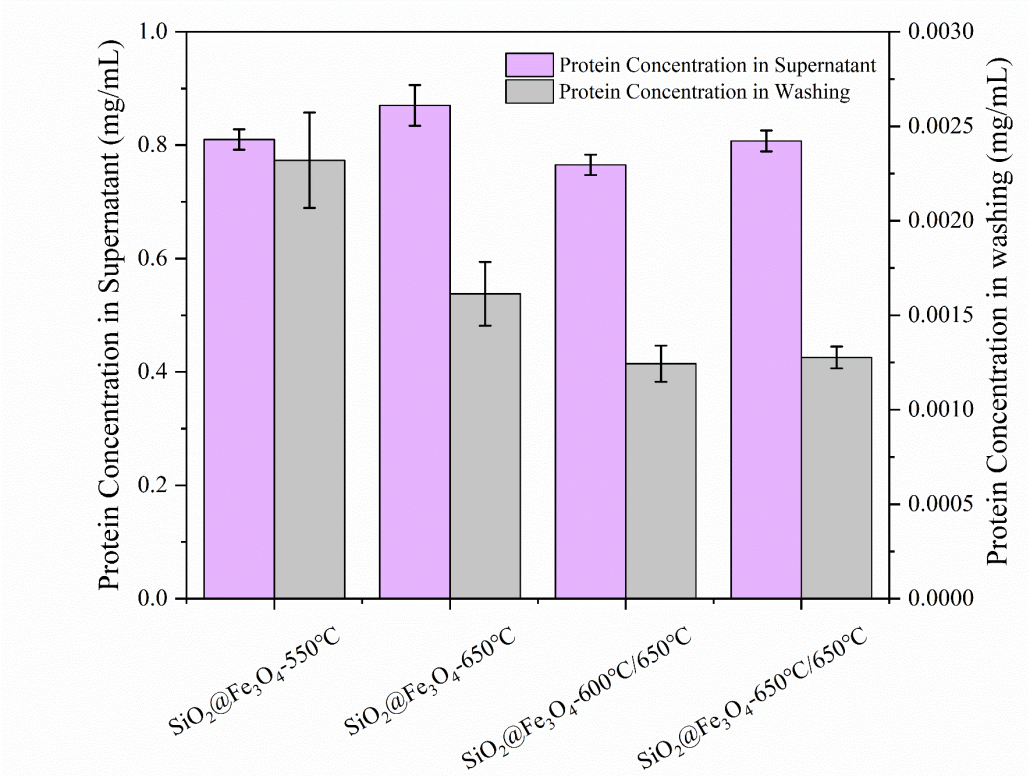

Figure S-3. protein concentration in supernatant and washing solution of SiO_2@Fe_3O_4-550°C, SiO_2@Fe_3O_4-650°C, SiO_2@Fe_3O_4-600°C/650°C and SiO_2@Fe_3O_4-650°C/650°C carriers.

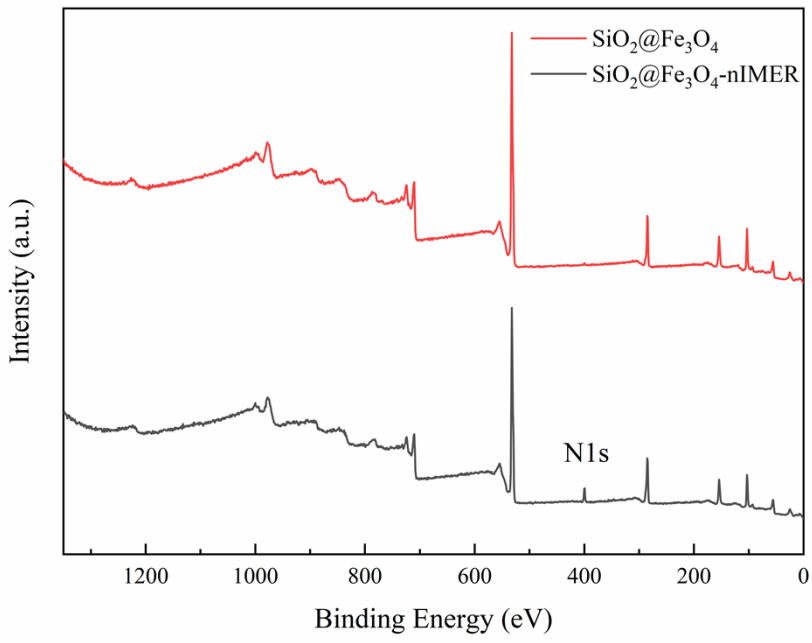

Figure S-4. XPS survey spectrum of SiO_2@Fe_3O_4 and SiO_2@Fe_3O_4-nIMER.

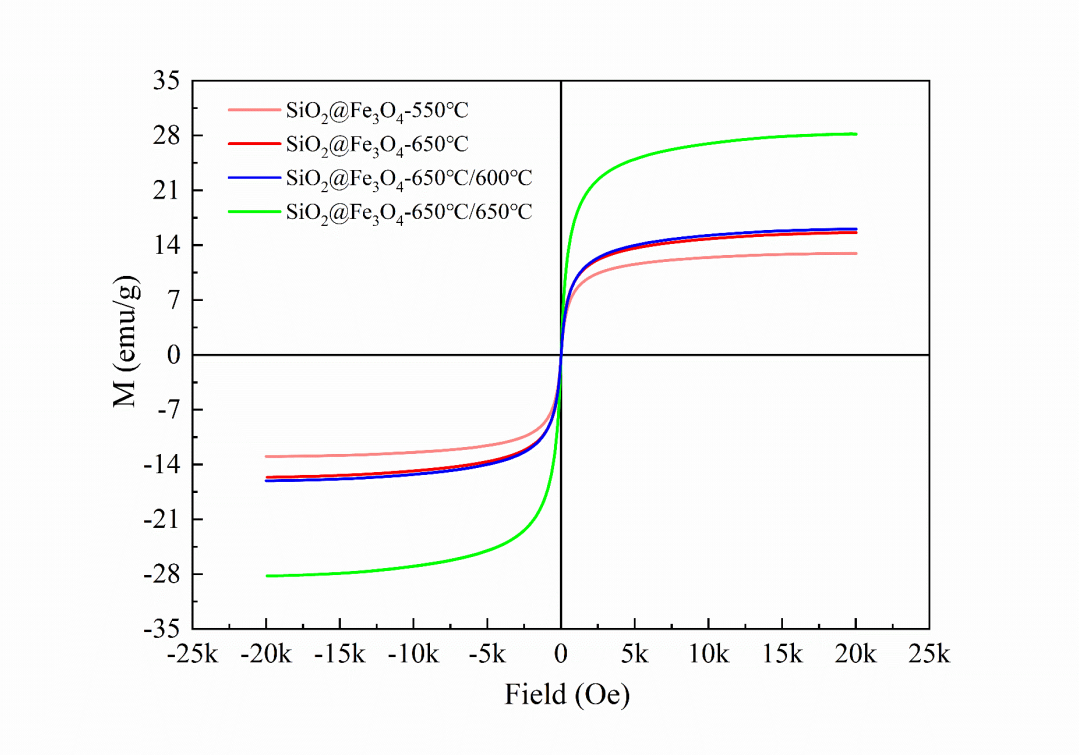

Figure S-5. The hysteresis loop of SiO_2@Fe_3O_4-550℃, SiO_2@Fe_3O_4-650℃, SiO_2@Fe_3O_4-600℃/650℃ and SiO_2@Fe_3O_4-650℃/650℃ carriers.

Table S-1. Influence of Protein Concentration on Enzymatic digestion efficiency.

Total protein concentration (mg/mL)	1	0.1	0.01	0.001	0.0001
BSA					
Sequence Coverage	90%	76%	80%	78%	74%
Peptides matched	139	64	82	80	73
Cytochrome C					
Sequence Coverage	97%	71%	83%	88%	94%
Peptides matched	32	20	18	18	21

Digestion conditions: effective coating length: 5.4 cm; Flow rate: 10 μL/min; 37℃.