Supporting Information

for

Novel test device and colorimetric quantitative method for detection of human chorionic gonadotropin (hCG) based on Au@Zn-Salen MOF for POCT applications

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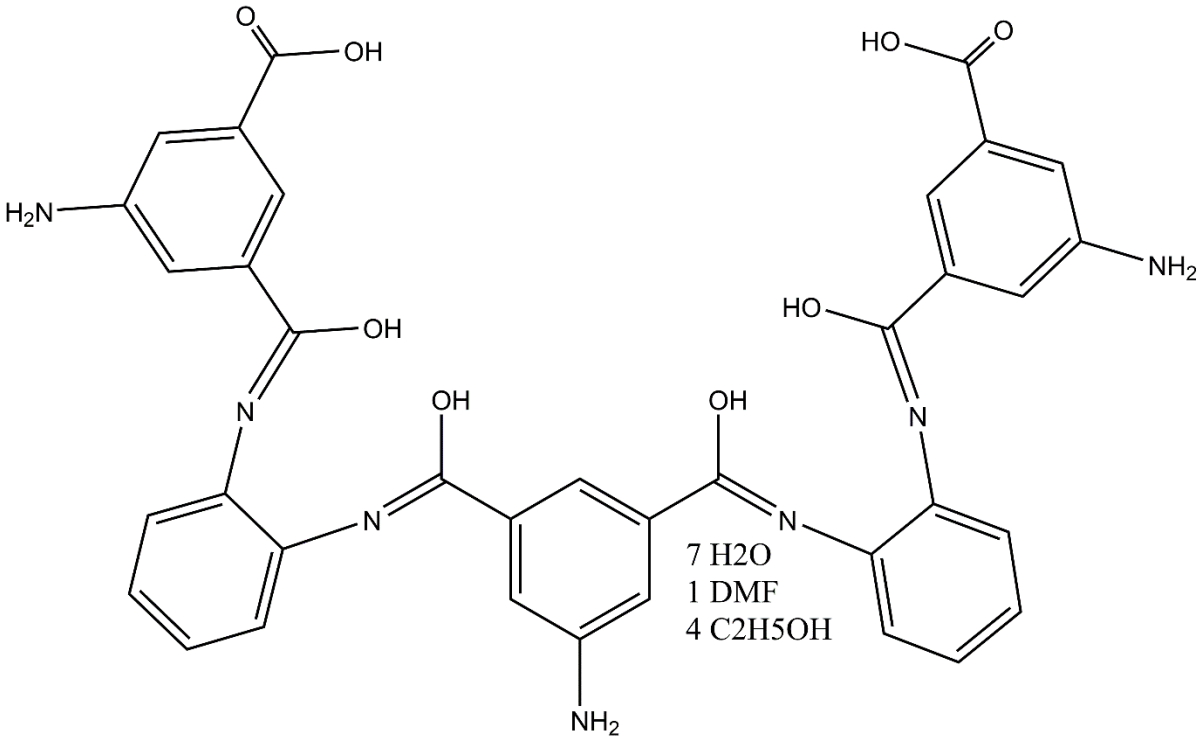

Fig. S1: Organic nano-linker chemical structure.

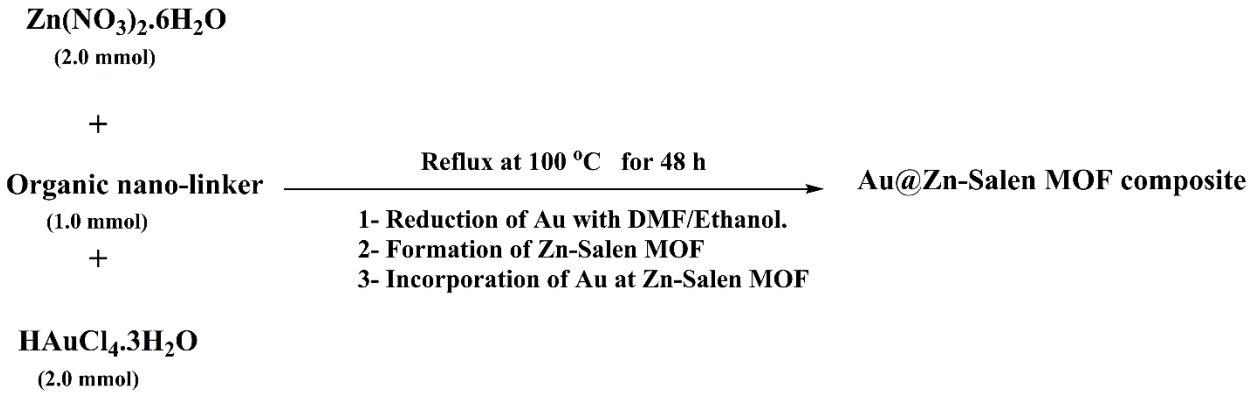

Fig. S2: The proposed reaction mechanism Scheme of the Au-Zn-Sln-MOF composite synthesis.

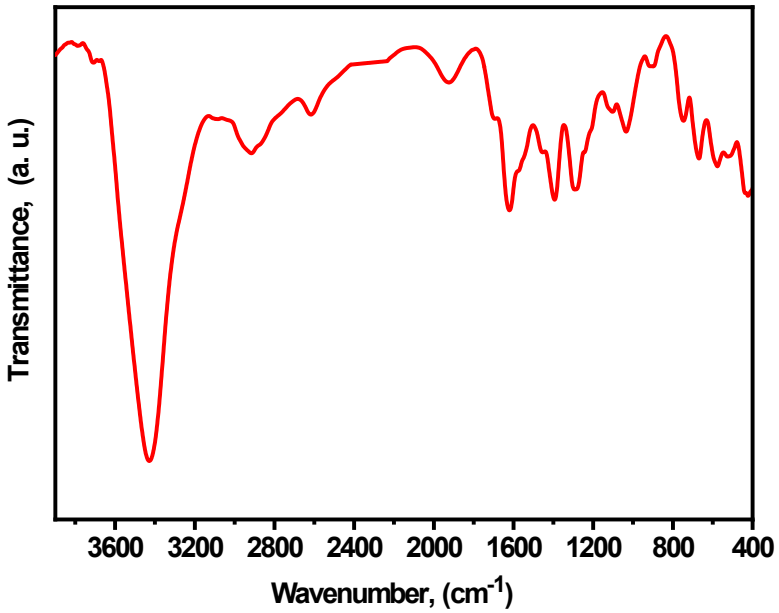

Fig. S3: The FT-IR spectrum of the organic linker.

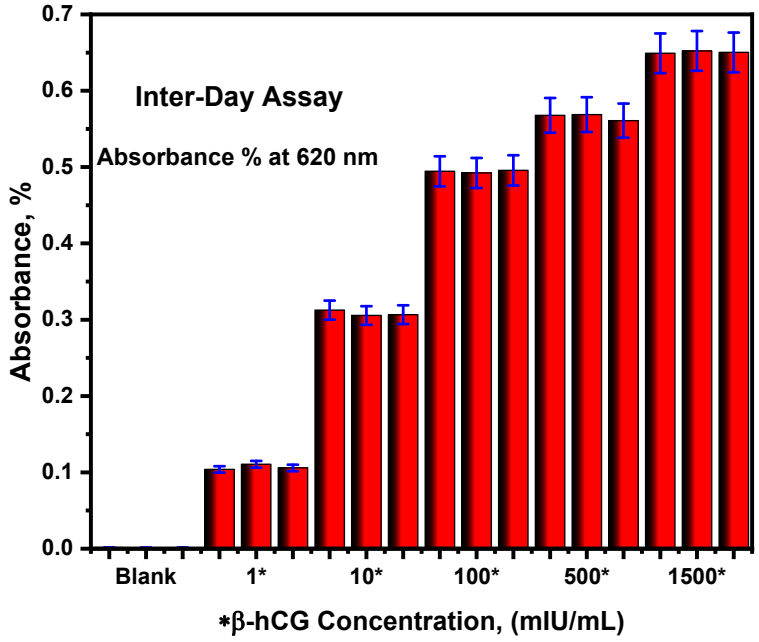

Fig. S4. A histogram of evaluation of inter-day accuracy, and precision for the colorimetric biosensor.

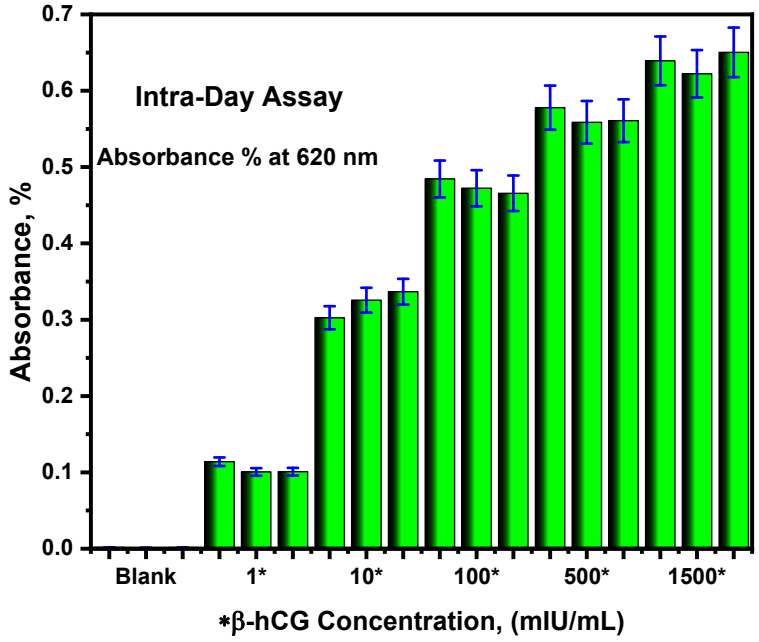

Fig. S5. A histogram of evaluation of intra-day accuracy, and precision for the colorimetric biosensor.

Table S1: EDX analysis of the Au-Zn-Sln-MOF composite.

Element	Weight %	Atomic %	Net Int.	Error %
C	27.23	66.73	9.83	14.84
N	1.89	3.98	0.29	96.75
O	9.39	17.27	4.42	21.49
Zn	9.41	4.24	5.21	24.97
Au	52.08	7.78	7.54	23.85

Table S2: Evaluation of swabs test device in compared with two product pregnancy hCG cassettes rapid test kits in the market in different real samples.

Sample type	No. of samples	NOVA-test-Kit			Medicaldisposables .US-Kit			Swaps test device		
		+ve	-ve	false	+ve	-ve	false	+ve	-ve	false
Serum	25 +ve	19	0	6	22	0	3	25	0	0
	25 -ve	0	23	2	0	21	4	0	24	1
Plasma	25 +ve	Not applicable			Not applicable			24	0	1
	25 -ve							0	24	1
Urine	25 +ve	21	0	4	18	0	7	25	0	0
	25 -ve	0	22	3	0	19	6	0	23	2

** **+ve**, Positive pregnancy samples; **-ve,** Negative pregnancy samples; **false,** false positive or negative result.*

Table S3. Comparison between the current colorimetric immunoassay biosensor and some published methods for the determination of hCG hormone.

Method	Linear detection range	LOD	Reference
A plasmonic thermal sensing based portable device (lateral flow assay)	----	2.8 mIU/mL	[5]
Electrochemiluminescence immunoassay using silver carbon quantum dots	0.001 - 500 mIU/mL	0.33 μIU/mL	[8]
Colorimetric immunoassay using peroxidase-mimicking MnO2 nanorods	0.5 - 400 mIU/mL	0.36 mIU/mL	[9]
Quantitative automated method based Modular Analytics E170 module (Roche)	6.0 - 800 mU/L	0.7 U/L	[12]
Microfluidic Lateral Flow Assay	---	1.26 ng/mL	[19]
A smartphone-based lateral flow strip	6–300 ng/mL	3.0 ng/mL	[20]
Voltametric immunosensor using a glassy carbon electrode modified with silver nanoparticles	0.0212 - 530 mIU/mL	0.066 mIU/mL	[27]
Three-dimensional CoNi-MOF nanosheet array-based immunosensor	0.005 - 250 mIU/mL	0.0185 mIU/mL	[26]
Colorimetric immunoassay method based on Au-Zn-Sln-MOF composite	0.01 – 3000 mIU/mL	0.055 mIU/mL	The present work

Table S4: Sensitivity and regression parameters for colorimetric immunoassay biosensor

Parameter	Method
Absorbance, nm	620
Limit of detection (LOD), mIU/mL	0.055
Limit of quantification (LOQ), mIU/mL	0.167
Regression equation	(Y=a+bX)*
Intercept (a)	0.150
Slope (b)	0.159
Standard deviation	0.0027
Correlation coefficient (r^2)	0.998

**Y, is the absorbance intensities; X, is the concentration of- hCG in mIU/mL; a, is intercept; b, is slope.*

Table S5: Evaluation of intra-day, inter-day accuracy, and precision study.

hCG mIU/mL	Intra-day assays*				Inter-day assays*			
	X	SD	CV	RE%	X	SD	CV	RE%
1.0	1.036	0.068	0.005	0.965	1.005	0.006	0.009	0.995
10.0	9.972	0.293	0.086	1.003	10.55	0.208	0.043	0.948
100.0	99.67	0.175	0.031	1.003	103.3	0.681	0.463	0.968
500.0	497.6	1.285	1.653	1.005	496.7	2.606	6.79	1.007
1500.0	1501	2.478	6.142	0.999	1469	3.183	10.13	1.021

* Each reading was repeated three times; X, mean values; SD, standard deviation; CV, the coefficient of variation; %RE, percent of relative error.

Table S6: Determination of hCG in different real samples using colorimetric immunoassay biosensor

Sample	Initial hCG concentration in sample mIU/mL	hCG Spiked mIU/mL	Found (mIU/mL)			X	RSD	RC%
Serum samples	3.16	0.5	3.639	3.701	3.696	3.678	0.034	100.5
		10	12.92	13.4	13.27	13.2	0.245	100.3
		100	102.9	102	101	102	0.94	98.84
		1000	1002	1001	998.4	1000	1.839	99.73
Plasma samples	2.74	0.5	3.171	3.169	3.182	3.174	0.007	97.96
		10	12.38	12.33	13.18	12.63	0.474	99.15
		100	99.16	100.3	98.29	99.25	1.004	96.6
		1000	998	998.5	994.7	997.1	2.07	99.44
Urine samples	4.14	0.5	4.501	4.479	4.465	4.482	0.018	96.59
		10	13.62	13.45	13.69	13.59	0.123	96.1
		100	98.75	99.4	106.4	101.5	4.249	97.48
		1000	979.6	972.5	966.6	972.9	6.536	96.89

* X, mean values; RSD, relative standard deviation; RC %, Recovery percent.

Appendix A: Materials & Instrumentation

Materials

1, 2-phenylenediamine $C_6H_8N_2$, $Zn(NO_3)_2.6H_2O$, and $HAuCl_4.3H_2O$ were purchased from Sigma-Aldrich. 5-aminoisophthalic acid $C_8H_7NO_4$, was purchased from Acros-organics. All proteins and hormones standards like bovine serum albumin (BSA), human HCG antigen, anti-human HCG monoclonal antibody (Anti-HCG), Alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), prostate-specific-antigen (PSA), and α-fetoprotein (AFP), were purchased from Monobind, Inc. Company, USA. All solvents and chemicals used in this study were of analytical reagent grade and were purchased from Sigma Aldrich and used as received. High purity deionized water (resistivity: 18.2 MΩ cm, TOC: 3 ppb) obtained by using a Milli-Q Plus system (Millipore Corp., Bedford, MA, USA) was used throughout.

Characterization

The characterization and applications were performed using different analytical techniques: The FE-SEM images and EDX spectroscopy spectra were recorded with a combination of field emission scanning electron microscopy (FE-SEM), and element mapping by spatially resolved energy-dispersive X-ray spectroscopy (EDX) (JEOL JSM-6510LV, Japan). The structures of the phases formed were examined by using a high-resolution transmission electron microscope (HR-TEM) with an acceleration voltage up to 200 kV (JEM-2100-JEOL, Japan). The Fourier-transform infrared (FT-IR) spectra were recorded with a JASCO FT/IR-460 spectrophotometer with the use of KBr tablets in the range from 400 to 4000 cm^{-1} at room temperature (JASCO, USA). The UV-vis spectra were obtained using V-770 UV-Visible/NIR spectrophotometer over a range from 200 to 2200 nm (JASCO, USA), and the band gap was calculated with Optbandgap-204B soft wear. The X-ray diffraction (XRD) analysis of Au-Zn-Sln-MOF composite was performed with a D8-AVANCE X-ray diffractometer (Bruker, Germany) with Cu-Kα radiation (λ =0.154056 nm) for identification of the crystalline phase, relative crystallinity, and crystal size of as-prepared Au-Zn-Sln-MOF composite. The XRD analysis was performed in the 2θ range from 3.0° to 80.0° with a 0.020° step at a scan speed of 0.4 s. ^{1}H-NMR spectrum is done in DMSO-d_6 using Gemini-300 MHz NMR spectrometer (ECA 500 II, JEOL,

Japan). Differential Scanning Calorimetry/Thermogravimetric Analysis (TGA/dTG) of the sample was carried out with a Universal V4.5-TA Instruments (USA), under atmospheric nitrogen gas with a rate of 10 °C min^{-1}. The output data obtained from different analytical techniques for characterization step like the spectra of FT-IR, UV, NMR, and TGA/dTG as well the XRD pattern, in addition to the histograms and Figures for statistical evaluation and validations of the proposed biosensor were analyzed with Origin-8.