Supporting Information
TO

Multiplexed Aptasensor for Detection of Acute Myocardial Infraction (AMI) Biomarkers

Duygu Beduk †[a], Tutku Beduk†[b,c], Abdellatif Ait Lahcen[d], Veerappan Mani[c], Emine Guler Celik[e], Gamze Iskenderoglu[f], Ferhat Demirci[g], Soysal Turhan[h], Oner Ozdogan[h], Su Ozgur[i], Tuncay Goksel[i,j], Kutsal Turhan[k], Khaled Nabil Salama*[c], Suna Timur*[a,l]

[a]Central Research Test and Analysis Laboratory Application and Research Center, Ege University, 35100 Bornova, Izmir, Turkey
[b]Silicon Austria Labs (SAL) GmbH, Europastraße 12, 9500, Villach, Austria
[c]Sensors Lab, Advanced Membranes and Porous Materials Center, Computer, Electrical, and Mathematical Science and Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900 Saudi Arabia
[d]Weill Cornell Medicine,Cornell University, New York, NY 10021, USA
[e]Department of Bioengineering, Faculty of Engineering, Ege University, 35100, Bornova, Izmir, Turkey
[f]Department of Cardiovascular Surgery, Health Sciences University Izmir Tepecik Education And Research Hospital, 35180, Konak, Izmir, Turkey
[g]Department of Medical Biochemistry, Health Sciences University Izmir Tepecik Education And Research Hospital, 35180, Konak, Izmir, Turkey
[h]Department of Cardiology, Health Sciences University Izmir Tepecik Education And Research Hospital, 35180, Konak, Izmir, Turkey
[i]EGESAM-Ege University Translational Pulmonary Research Center, 35100, Bornova, Izmir, Turkey
[j]Department of Pulmonary Medicine, Faculty of Medicine, Ege University, 35100, Bornova, Izmir, Turkey
[k]Department of Thoracic Surgery, Ege University School of Medicine, 35100, Bornova, İzmir, Turkey
[l]Department of Biochemistry, Faculty of Science, Ege University, 35100 Bornova, Izmir, Turkey, suna.timur@ege.edu.tr

† Duygu Beduk and Tutku Beduk contributed equally to this manuscript.

1. Laser-Scribed Graphene Electrode Fabrication

The fabrication procedure was followed as previously explained.[1] The predesigned LSG three-electrode system was prepared on a commercially available substrate PI sheet (Kapton Width: 12”, Utech Products, USA). A CO_2 laser (Universal Laser Systems® PLS6.75) with a wavelength of 10.6 μm and a spot size of ~150 μm was used to irradiate a PI sheet to produce graphene electrodes as the photothermal process leads to molecular rearrangements. A polymer precursor was cleaned before the scribing process for the fabrication of three three-electrode sensing systems (2.8 cm × 1.2 cm). To achieve the best graphene quality formation, the laser scribing speed and power were optimized to fabricate the LSG electrodes while using an inert gas flow to minimize the heteroatom bonding effect. Laser power, speed, and z distance were recorded as 3.2 W, 2.8 cm/s, and 2.5 mm, respectively, at 1000 pulses per inch to produce the best-quality multilayer graphene with high conductivity.

2. Electrochemical Measurements

The electrochemical responses of AuLSG aptamer electrodes were investigated using the cyclic voltammetry (CV) method. The CV method employed a scan rate of 50 mV/s, sweeping the potential from -0.6 V to +0.4 V. Measurements were carried out at room temperature in a solution of 0.1 M KCl containing 2.5 mM $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ as a redox probe, with LSG serving as the reference and counter electrodes. The purpose was to examine the electrochemical changes in response to the interaction of the aptamer-modified electrodes with cTnT cTnI, and CRP proteins. The variations in current intensities were indicative of the different amounts of target analytes captured by the DNA aptamer immobilized on the electrode surface. Notably, all electrochemical measurements were conducted in triplicate to ensure the reliability and reproducibility of the results.

3. Characterizations

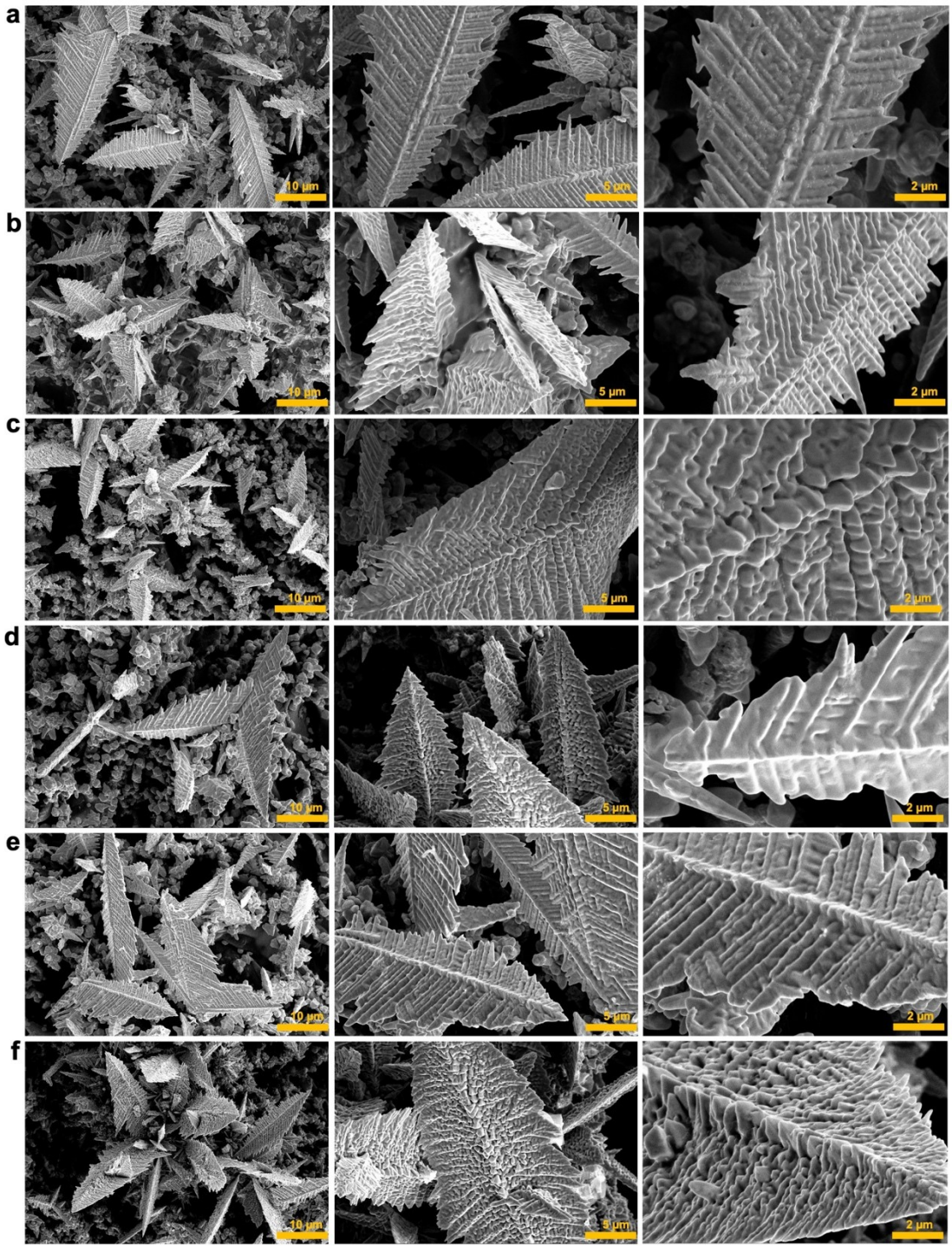

Figure S1. Scanning Electron Microscopy (SEM) images of (a) AuLSG/cTnI Aptamer/MCH Sensor, (b) AuLSG/cTnT Aptamer/MCH Sensor, (c) AuLSG/CRP Aptamer/MCH Sensor, (d) AuLSG/cTnI Aptamer/MCH/BSA Sensor, (e) AuLSG/cTnT Aptamer/MCH/BSA Sensor, (f) AuLSG/CRP Aptamer/MCH/BSA Sensor.

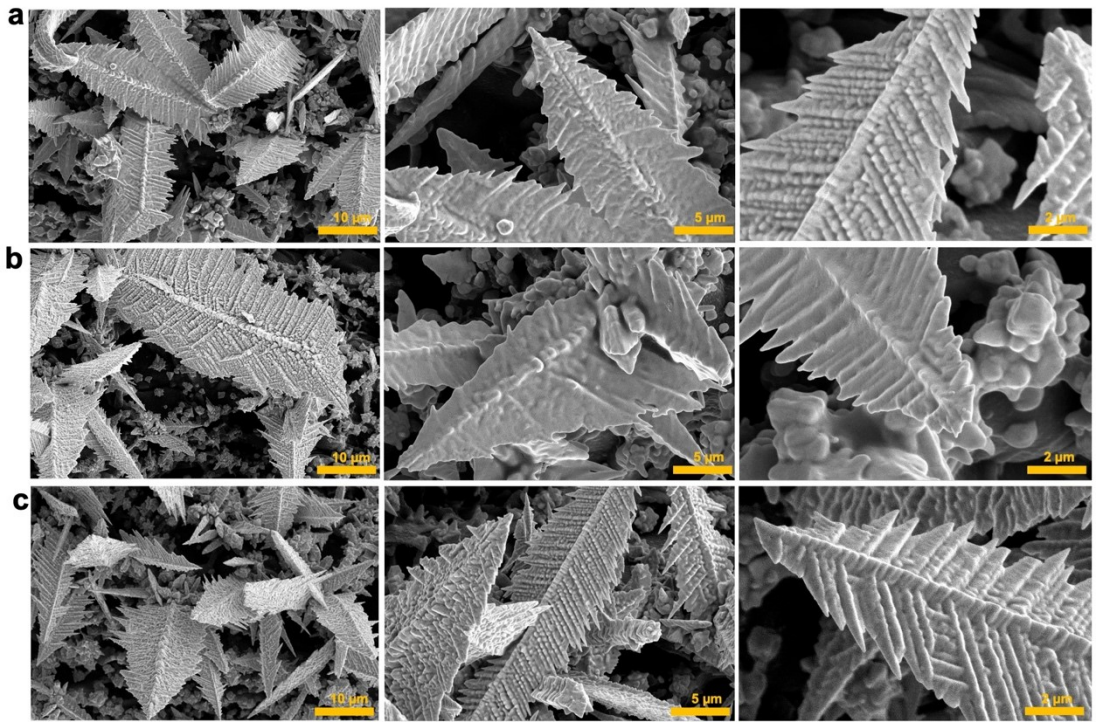

Figure S2. Scanning Electron Microscopy (SEM) images of (a) AuLSG/cTnI Aptamer/MCH/BSA Sensor/Serum Sample (b) AuLSG/cTnT Aptamer/MCH/BSA Sensor/Serum Sample (c) AuLSG/CRP Aptamer/MCH/BSA Sensor/Serum Sample in different magnifications.

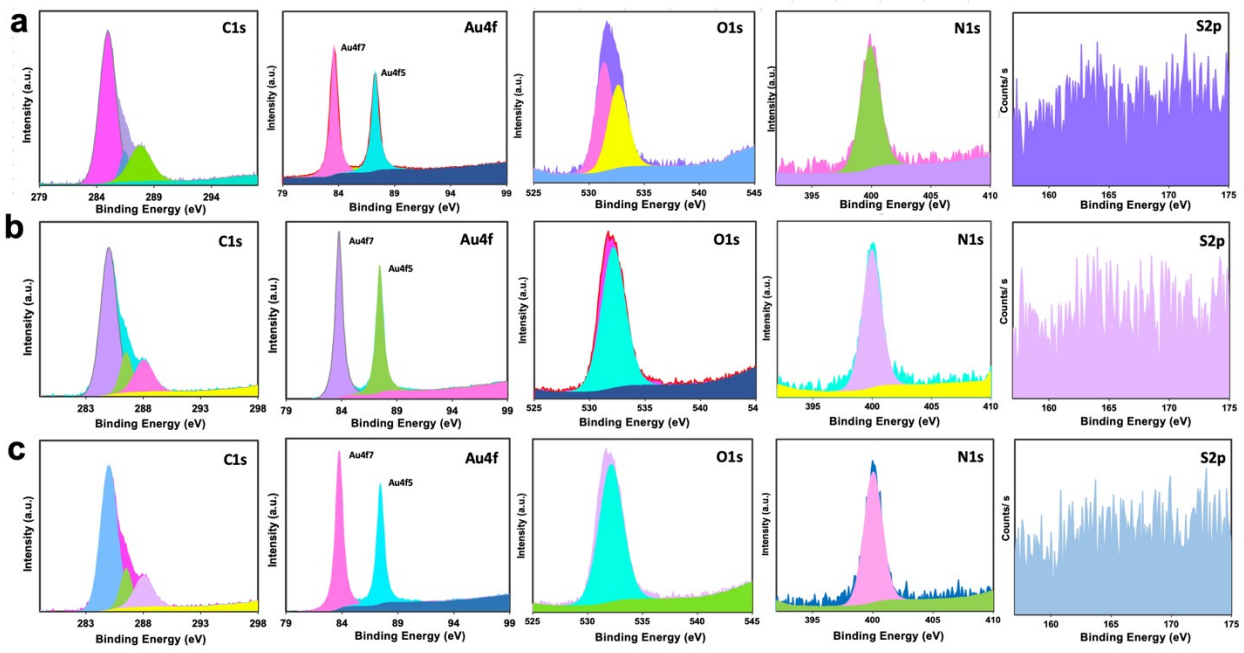

Figure S3. XPS High-resolution spectra of (a) AuLSG/cTnI aptamer/BSA/cTnI protein, (b) AuLSG/cTnT aptamer/BSA/cTnT protein, (c) AuLSG/CRP aptamer/ BSA/CRP protein

Table S1. Elemental composition of the sensor surface at each fabrication step.

	Atomic %				
Elements	**LSG**	**AuLSG**	**AuLSG/cTnI Aptamer/MCH**	**AuLSG/cTnT Aptamer/MCH**	**AuLSG/CRP Aptamer/MCH**
Au4f	-	22.05	5.4	3.41	2.62
C1s	77.71	64.32	52.18	50.43	49.39
N1s	3.51	-	8.17	9.84	8.74
O1s	17.53	11	21.15	20.76	19.38
S2p	-	2.64	-	-	-

Table S2. Elemental composition of the sensor surface at each fabrication step.

	Atomic %					
Elements	**AuLSG/cTnI Aptamer/MCH/BSA**	**AuLSG/cTnT Aptamer/MCH/BSA**	**AuLSG/CRP Aptamer/MCH/BSA**	**AuLSG/cTnI Aptamer/MCH/ BSA/cTnI Protein**	**AuLSG/cTnT Aptamer/MCH/ BSA/cTnT Protein**	**AuLSG/CRP Aptamer/MCH/ BSA/CRP Protein**
Au4f	9.06	8.1	9.61	3.44	1.98	3.48
C1s	50.47	54.41	53.62	64.61	66.84	65.74
N1s	6.64	7.75	9.15	10.21	9.84	10.05
O1s	18.85	17.02	18.25	16.37	17.46	17.46

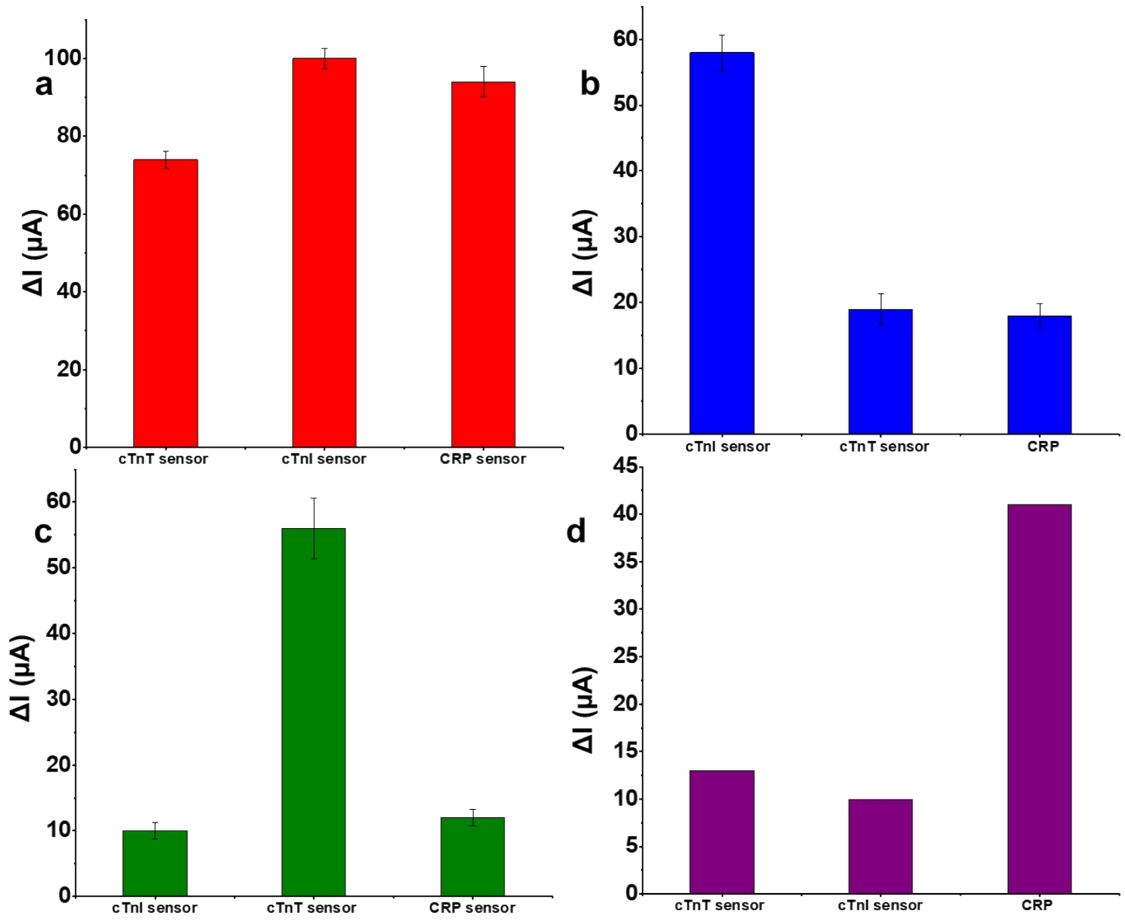

Figure S4. a. Oxidation current change of aptasensors when a patient serum is introduced. The oxidation current change of cTnT, cTnI, CRP aptasensors when b, 05 ng/ml of cTnI protein, c. 50 ng/ml of cTnT protein, d. 50 ng/ml of CRP introduced as analyte.

Table S3. Summary of reported electrochemical platforms for cardiac biomarkers.

Cardiac Biomarker	Detection Platform	Electrode Type	Media	LOD	Ref.
Troponin I	Electrochemiluminescence Immunosensor	Bipolar Electrode	Human Serum Samples	0.4416 pg/mL	2
Troponin I	Sandwich-Type Aptasensor	Au/Zr–C/SPCE	Standard Solutions	1.24×10^{-3} pg/mL	3
Troponin I	Aptasensor	LSG/$ZnFe_2O_4$	Spiked human serum samples	1.0 pg/mL	4
Troponin I	Labeled Free Immunosensor	CMK-3/AuNPs-Modified Gold Electrode	Human Serum Samples	2.4 fg/mL	5
Troponin I, Myoglobin	Sandwich-Type Aptasensor	CAu/CC/SPCE	Standard Solutions	cTnI: 3.8 fg/mL Myo: 35 fg/mL	6
Troponin I	Sandwich-Type Aptasensor	HMCS/PDA/AuNPs	Human Serum Samples	2.3 fg/mL	7
Troponin I	DNAzyme-Driven Aptasensor	Au/$NiCO_2S_4$/GCE	Human Serum Samples	0.26 pg/mL	8
Troponin I	Sandwich-Type Immunosensor	PtPdCu/BNC/GCE	Serum Samples	4.27 fg/mL	9
Creatine Kinase	Immunosensor	GP/AuNP Electrode	Blood Samples	0.045 pg/mL	10
Troponin T	Immunosensor	O-Aminobenzoic Acid Film/GCE	Human Serum Samples	0.016 ng/mL	11
Troponin T, Troponin I and Myoglobin	Electrogenerated Chemiluminescence Biosensor	Gold Electrode	Human Serum Samples	Myo: 0.30 ng/mL, cTnI: 0.79 pg/mL cTnT: 31 pg/mL	12
BNP-32, Troponin I	Aptasensor	Gold-Based Screen-Printed Electrode	Spiked Human Serum Samples	BNP-32: 1 pg/mL cTnI: 1 pg/mL	13
Troponin I	Immunosensor	Gold Electrode	Human Serum Samples	1.7 pg/mL	14
Creatine Kinase-MB	Label-Free Immmunosensor	AuPdCu NWNs/GCE	Human Serum Samples	0.88 pg/mL	15

TNF-A and NT-proBNP	Surface Plasmon Resonance (SPR)	Single Planar Gold Chip	Human Serum Samples	NT-proBNP: 0.06 pM, TNF-A: 0.03 nM	[16]
Troponin I	Immmunosensor	GQD/ITO Electrode	Standard Solutions	29 pM	[17]
Troponin T	Immmunosensor	QTF Electrode	Human Serum Samples	0.24 fg/mL	[18]
Troponin I	CRISPR/Cas12a-Based Biosensor	Gold Electrode	Standard Solutions	10 pg/mL	[19]
Troponin T	Label-Free Immunosensor	ITO/PET Electrode	Standard Solutions	0.571 fg/mL	[20]
C-Reactive Protein	Label-Free Biosensor	AuNPs/BP/PDA Gold Electrode	Human Blood Samples	0.7 ng/mL	[21]
C-Reactive Protein	Sandwich-Like Immunosensor	Au-N-CNSs/GCE	Human Serum Samples	7.70 pg/mL	[22]
C-Reactive Protein	Immmunosensor	AuNPs/IL-MoS_2/GCE	Human Serum Samples	3.3 pg/mL	[23]
C-Reactive Protein	Immmunosensor	AuNPs/SPCE	Human Serum Samples	0.058 µg/mL	[24]
Troponin T, Troponin I, C-Reactive Protein	Aptasensor	AuLSG Electrode	Human Serum Samples	cTnT: 1.65 ng/mL cTnI: 2.58 ng/mL CRP: 1.84 ng/mL	This work

Abbreviations:

ECL: Electrochemiluminescence, SPCE: Screen Printed Carbon Electrode, Au/Zr-C: Zirconium-Carbon loaded Gold, CMK-3/AuNPs: Nitrogen-Doped Ordered Mesoporous Carbon AuNPs Nanocomposites, GP/AuNP: Gold-Graphite Paper, AuPdCu Nwns: Gold palladium copper Alloyed Nanowire Networks, CAu: Coralloid Au, CC: Zirconium-Based Carbon Architecture, HMCS/PDA/AuNPs Polydopamine Modified Hollow Mesoporous Carbon Spheres, GQDs: Graphene Quantum Dots, ITO: Indium Tin Oxide, GCE: Glassy Carbon Electrode, PtPdCu/BNC: Trimetallic PtPdCu Mulberry-Like Nanospheres, ITO/PET: Indium Tin Oxide Coated Polyethylene Terephthalate, Au-N-CNSs: Mono-Dispersed Nitrogen-Rich Porous Carbon Nanospheres Decorated with Au Nanoparticles, QTF: Quartz Tuning Forks, TNF-A: Tumor necrosis factor (TNF)-alpha, NT-pro-BNP: B-type natriuretic peptide.

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