

SERS based Y-shaped aptasensor for early diagnosis of acute kidney injury

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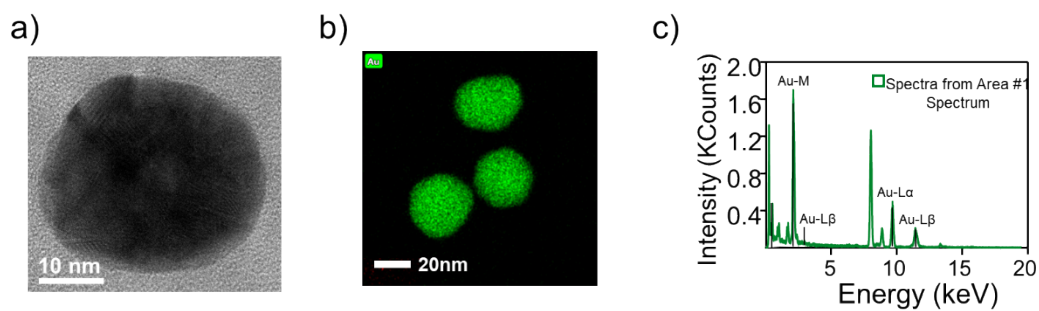


Figure S1 a) HR-TEM image of AuNPs. b) TEM-EDS mapping of AuNPs. c) EDS spectrum of AuNPs.

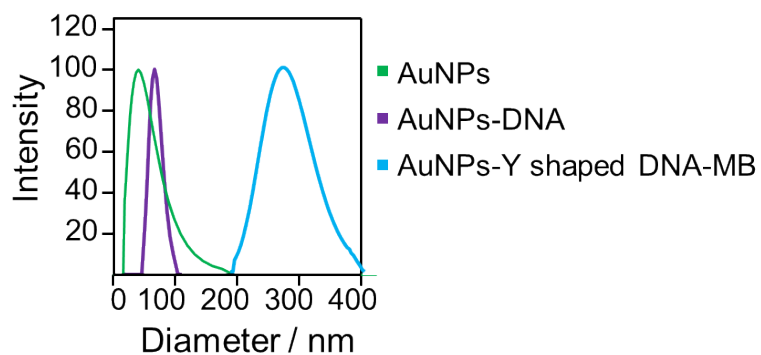


Figure S2 DLS data of AuNPs (green line), AuNPs-DNA (purple line) and AuNPs-Y shaped DNA-MB (blue line).

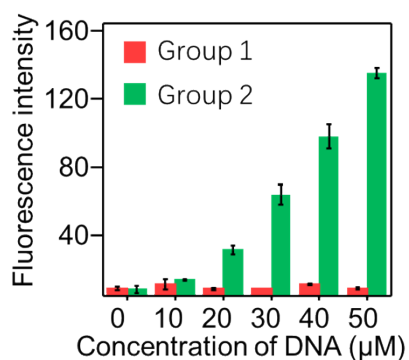


Figure S3 Fluorescent intensity of safe green nucleic acid dye in two groups of biotinylated DNA conjugated streptavidin-coated magnetic beads with various concentrations of biotinylated probe DNA₂ from 0 μM to 50 μM. $\lambda_{\text{ex}} = 254 \text{ nm}$, $\lambda_{\text{em}} = 520 \text{ nm}$.

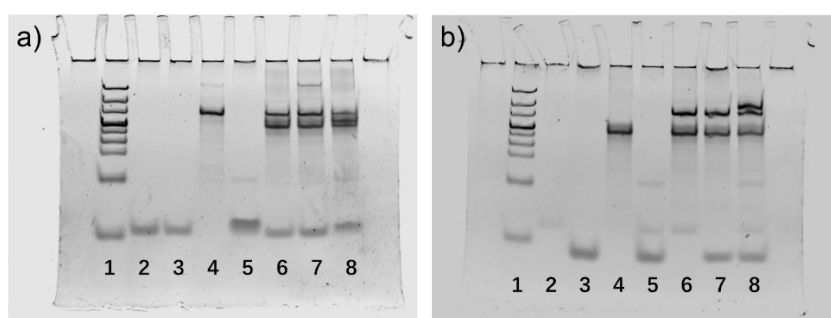


Figure S4 Electrophoresis characteristics of the Y-shaped aptamer. a) Formation of Y-shaped aptasensor of NGAL (Lane 1 - Lane 8). Lane 1: marker, Lane 2: 1.0 μM Probe₁, Lane3: 1.0 μM Probe₂, Lane 4: 0.3 μM NGAL aptamer, Lane 5: 1.0 μM Probe₁+1.0 μM Probe₂, Lane 6: 1.0 μM Probe₁ + 0.3 μM NGAL aptamer, Lane 7: 1.0 μM Probe₂ + 0.3 μM NGAL aptamer, Lane 8: 1.0 μM Probe₁ + 1.0 μM Probe₂ + 0.3 μM NGAL aptamer. b) Formation of Y-shaped aptasensor of Cys C (Lane 1 - Lane 8). Lane 1: marker, Lane 2: 1.0 μM Probe₁, Lane3: 1.0 μM Probe₂, Lane 4: 0.3 μM Cys C aptamer, Lane 5: 1.0 μM Probe₁+1.0 μM Probe₂, Lane 6: 1.0 μM Probe₁ + 0.3 μM Cys C aptamer, Lane 7: 1.0 μM Probe₂ + 0.3 μM Cys C aptamer, Lane 8: 1.0 μM Probe₁ + 1.0 μM Probe₂ + 0.3 μM Cys C aptamer.

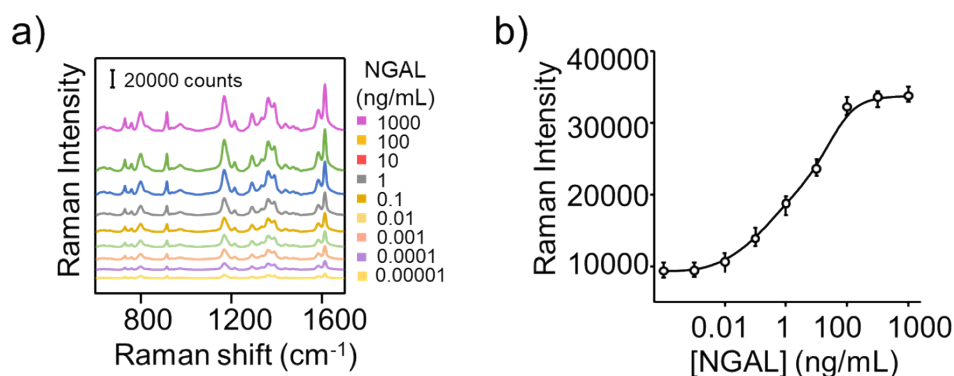


Figure S5 Standard working curve for NGAL. a) SERS spectra of AuNPs-Y shaped DNA-MB (NGAL) after incubation at various concentrations, respectively. b) SERS signals at 1618 cm^{-1} in the spectra of a.

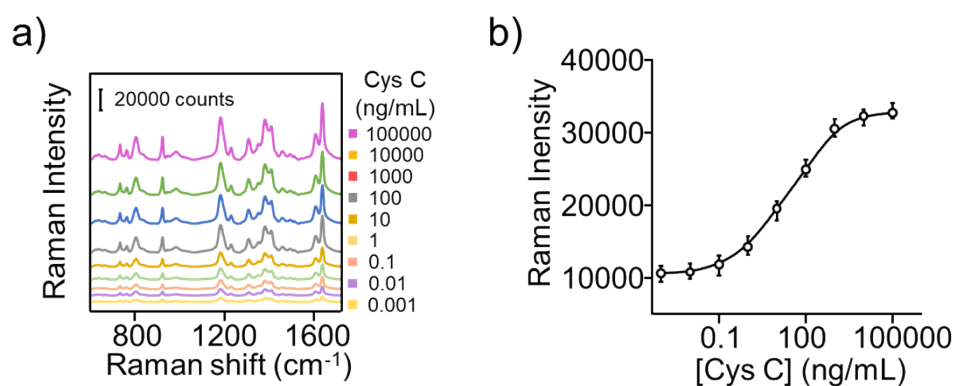


Figure S6 Standard working curve for Cys C. a) SERS spectra of AuNPs-Y shaped DNA-MB (Cys C) after incubation at various concentrations, respectively. b) SERS signals at 1618 cm^{-1} in the spectra of a.

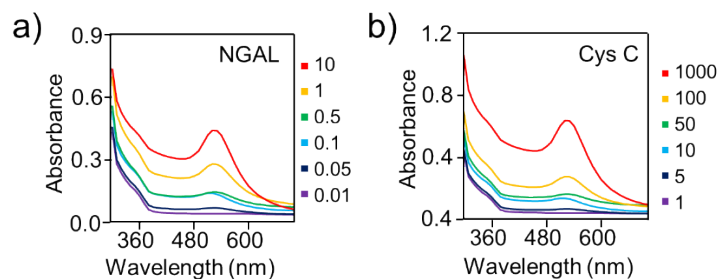


Figure S7 a, b) UV-vis spectra of the probe supernatant after adding each target protein with different concentration and incubating, respectively.

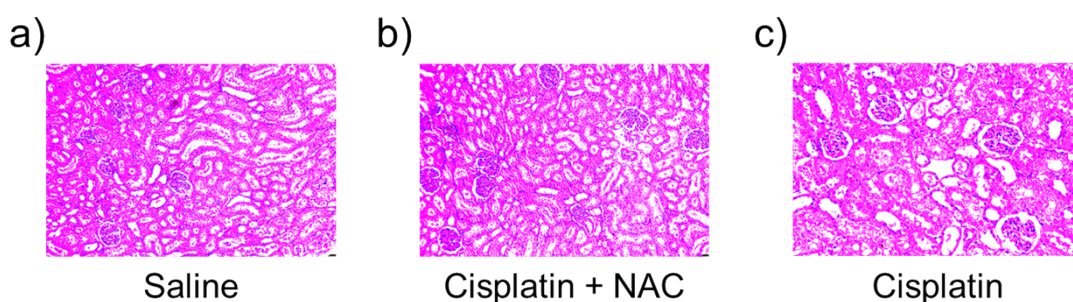


Figure S8 Photographs of H&E staining of paraffin-embedded sections of rat kidneys after drug treatment. a) Saline injection at 24 h. b) Cisplatin + NAC injection at 24 h. c) Cisplatin injection at 24 h. Scale bar, 50 μ m.

Table S1 Sequence list

NGAL aptamer	(5'-)AGCAGCACAGAGGTCAGATGGCGCTGGATAGCAAGATCACGTTATCATC GTAAACCCTATGCGTGCTACCGTGAA (-3')	
NGAL	Probe ₁	(5'-) CTGTGACTGCTGCT (-3') [-(CH ₂) ₆ -SH-3']
	Probe ₂	(5'-) ACCTCGTGTCACAG (-3') [-biotin]
Cys-C aptamer	(5'-)CCTAACCGATATCACACTCACGAACTGTCGGAACCTCGGGCCAAATGGAC GAGCGACCATTGGTTGTTTCGTCATTGGAGTATC(-3')	
Cys-C	Probe ₁	(5'-) CTGTGACGGTTAGG (-3') [-(CH ₂) ₆ -SH-3']
	Probe ₂	(5'-) TGATATCGTCACAG (-3') [-biotin]

Table S2 Comparison of the proposed method and other reported methods.

	Assay	Biomarker	Range (ng/mL)	LOD (ng/mL)	Assay Time	Dual assay	Ref.
1	SERS-based Y-shaped aptasensor	NGAL	0.01-10	0.052	5 mins	Yes	This method
		Cys C	1-1000	0.34			
2	ELISA	NGAL	0.25-2	0.032	4 h	No	[1]
		Cys C	0.5-31.3	0.5			[2]
3	RIA	NGAL	4-25	4	3.5 h	No	[3]
		Cys C	0.125-62.5	0.125	ND		[4]
4	UPT-LFA	NGAL	7.68-1000	7.68	30 mins	No	[5]
5	LC-MS/MS	Cys C	250-15000	30	7-8 mins	No	[6]
6	fluorescence-based immunoassay	NGAL	60-1300	60	20 mins	No	[7]
7	bFQICA	Cys C	0.0-100	0.69	15 mins	No	[8]

ELISA: enzyme-linked immunosorbent assay; RIA: radioimmunoassay; UPT-LFA: UCP technology-based lateral flow assay; LC-MS/MS: Liquid chromatography-tandem mass spectrometry; bFQICA: background fluorescence quenching immune chromatographic assay.

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