

A novel SERS biosensor for ultrasensitive detection of HPV-E7 and OPN based on a cascade signal amplification strategy of catalytic hairpin assembly and hybridization chain reaction

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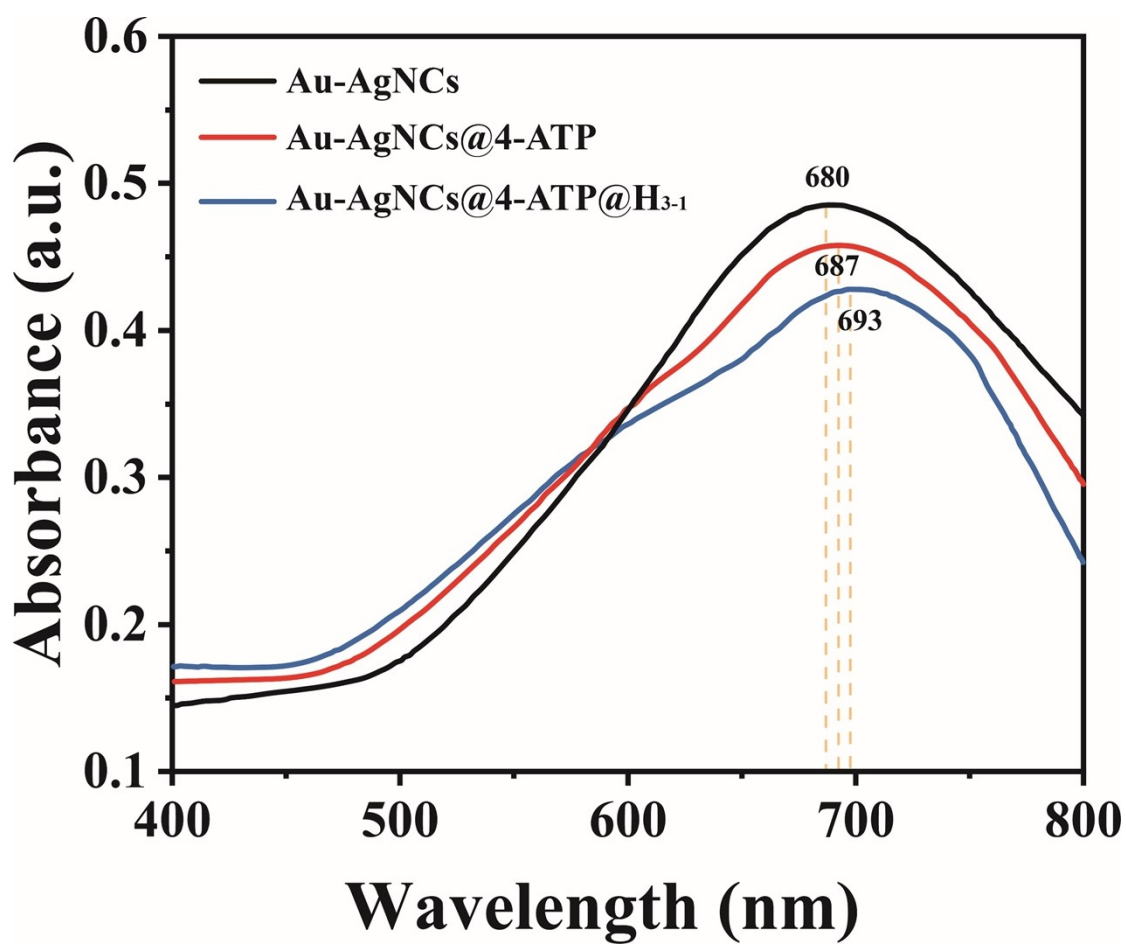


Fig. S1 UV-vis spectra of the constructing process of Au-AgNCs@4-ATP@H₃₋₁

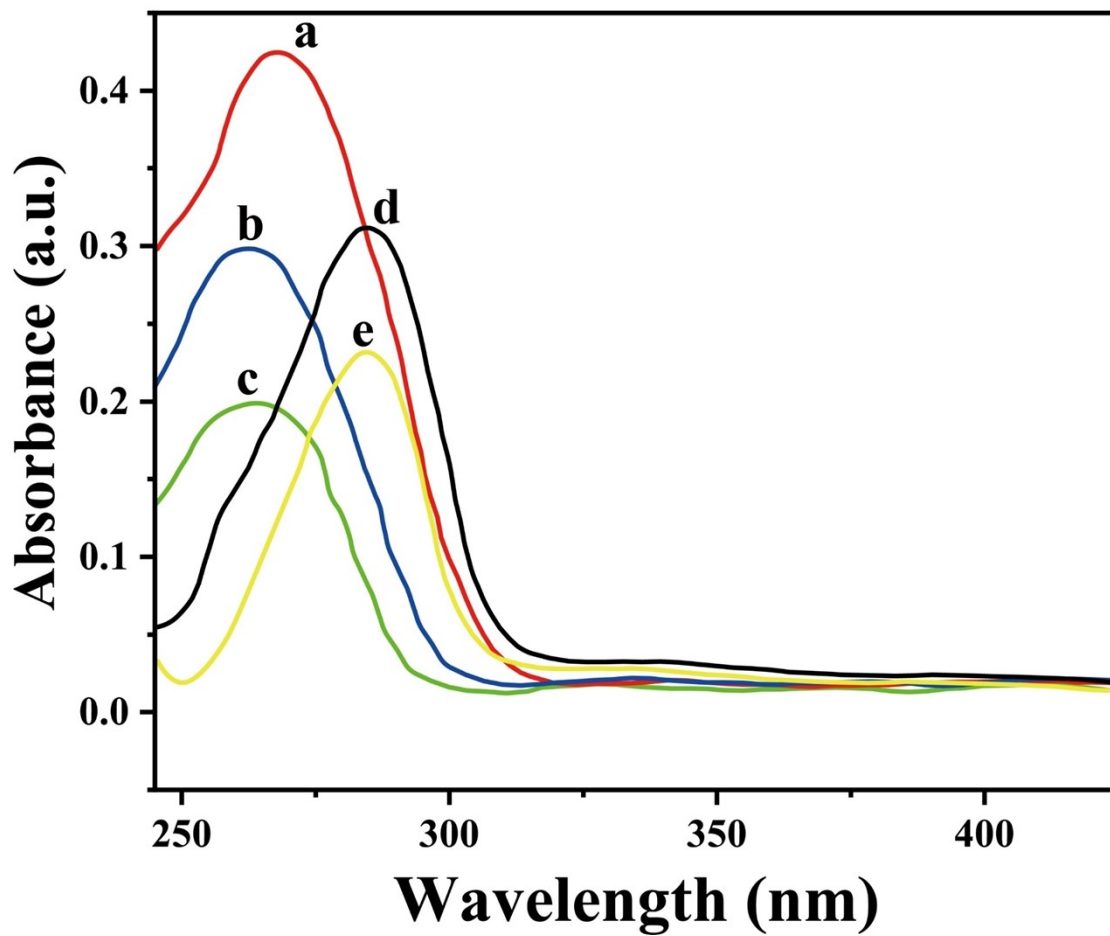


Fig. S2 Absorption spectra of (a) 1.0 mM Ab_{1-1} -DNA $_{1-1}$ -HPV-E7- Ab_{1-2} -DNA $_{1-2}$, (b) 1.0 mM DNA $_{1-1}$, (c) 1.0 mM DNA $_{1-2}$, (d) 0.2 mg mL $^{-1}$ Ab_{1-1} , (e) 0.2 mg mL $^{-1}$ Ab_{1-2} .

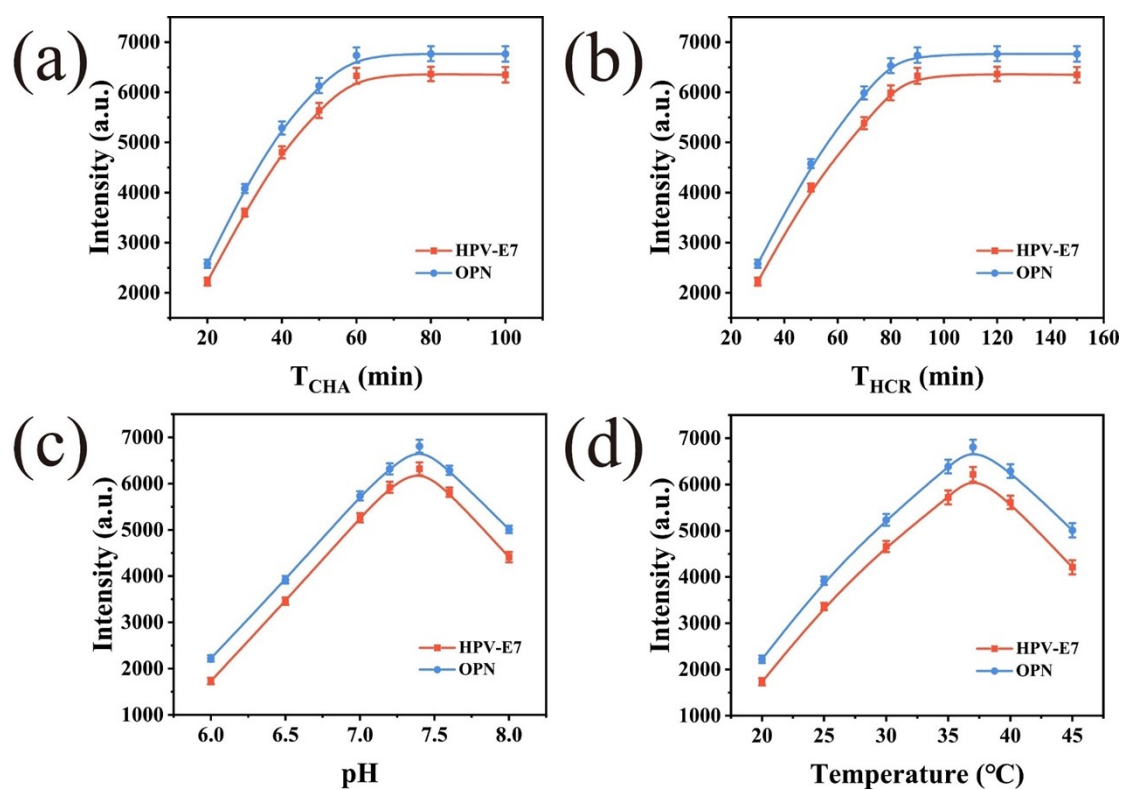


Fig. S3 Optimization parameters. (a) CHA time. (b) HCR time. (c) pH. (d) reaction temperature.

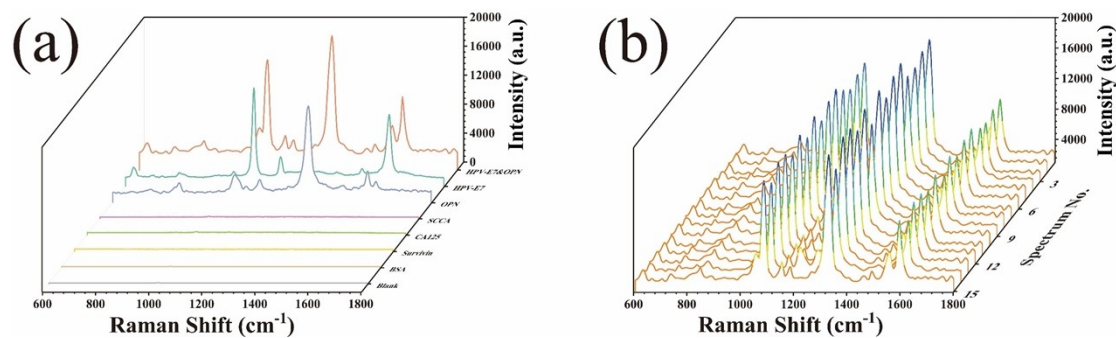


Fig. S4 Specificity and reproducibility study of the proposed SERS biosensor. (a) The averaged spectra of the SERS signals with a range of protein interferences. (b) SERS spectra from parallel measurements on fifteen independent substrates.

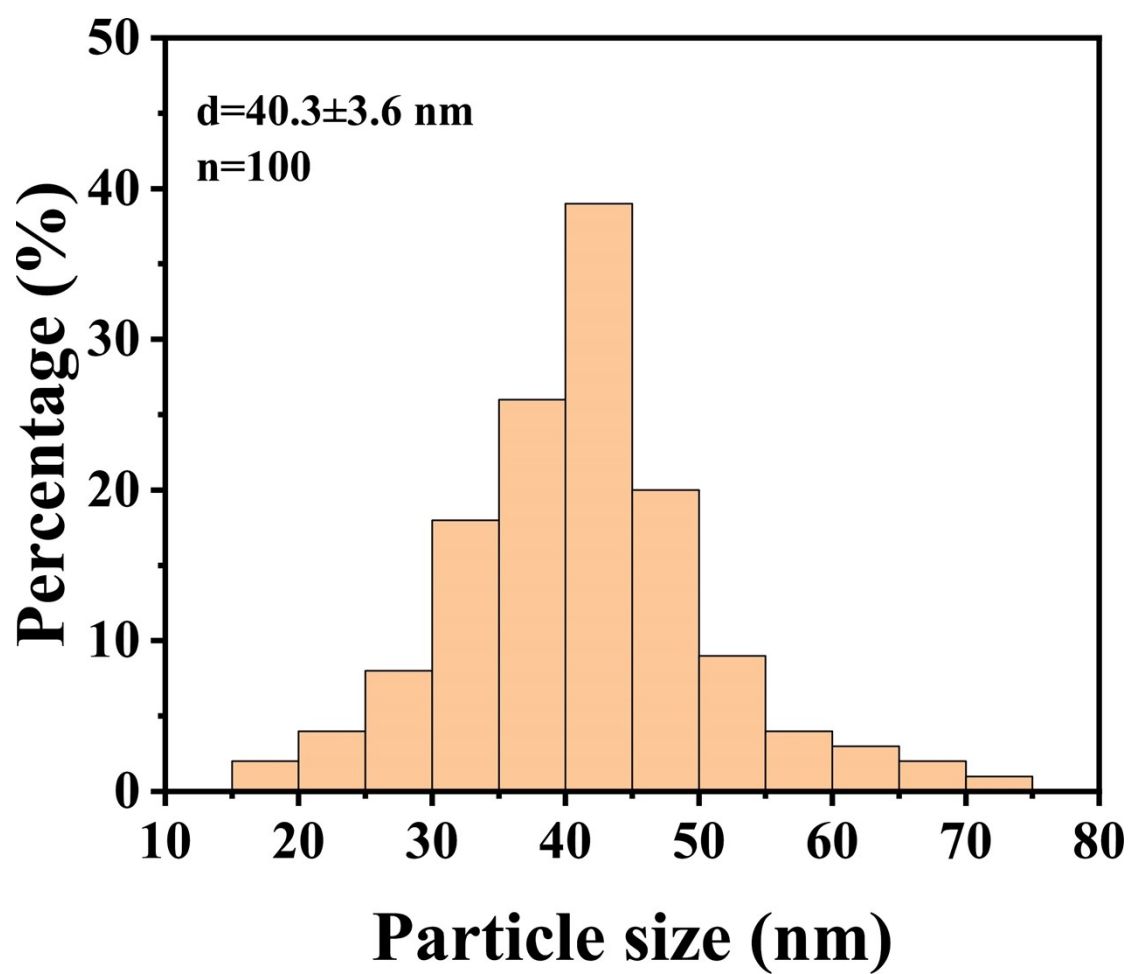


Fig. S5 Size distribution histogram of Au-AgNCs from SEM.

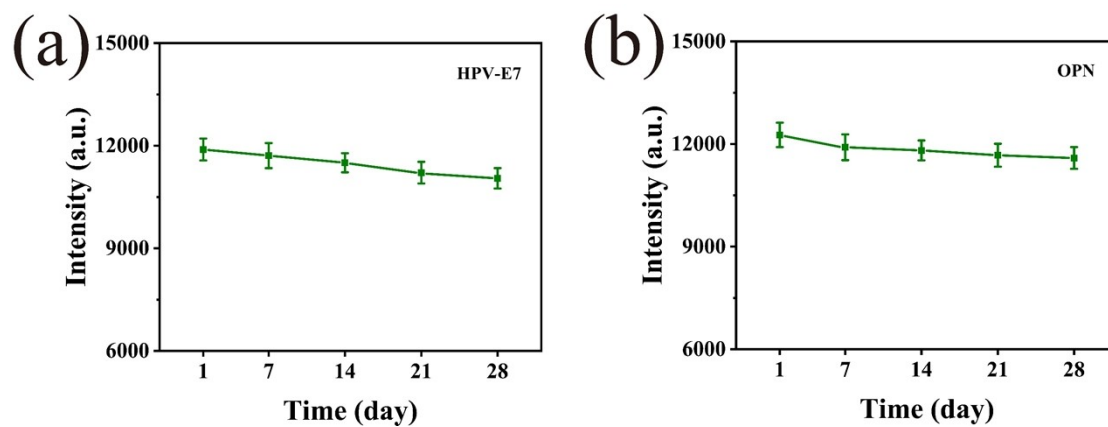


Fig. S6 Stability of the SERS biosensor. (a) SERS intensity measured at 1078 cm^{-1} for HPV-E7. (b) SERS intensity measured at 1336 cm^{-1} for OPN.

Table S1. The sequences used in this work.

DNA	Sequences (from 5' to 3')
DNA ₁₋₁	HS-TTAATCCCATATTTCTGACCATTTTTTTTTTTTTTTAGCTTCCACGTTAGACCACTT
DNA ₁₋₂	ATCTCCACAACCTGAAAACAGGTTTTTTTTTTTTTTAGATGGTCAGACATATCGGAT-SH
DNA ₂₋₁	HS-TACGTCCAGAACTTTACCAATTTTTTTTTTTTTTTGTGAGGCACGTTAGACCACTT
DNA ₂₋₂	ATCTCCACAACCTGAACCTCACTTTTTTTTTTTTTTTGGCTCTAGCGTATGCTATTG-SH
H ₁₋₁	ACAACTGAACACGTTAGACCACTTCCATCCTCGCAAATCTCCACAACCTAAGTGGTCTAACGTGTTCAAGTTGTGGAGAT
H ₂₋₁	ACCTCCACAACCTGAACACGTTAGACCACTTAGTTGTGGAGATTTGCGAGGATGGAAGTGGTCTAACAGAAGAAGGTGTTTAAGT
H ₁₋₂	ACAACTGAACACGTTAGACCACTTCCATCCTCGCAAATCTCCACAACCTAAGTGGTCTAACGTGTTCAAGTTGTGGAGAT
H ₂₋₂	TGCTCTCCACTTCCATCCTCGCAAATCTCCACAACCTGAACACGTTAGACCACTTAGTTGTGGAGATTTGCGAGGATGGAAGTGGTCTAAC
H ₃₋₁	HS-AGGGCGGGTGGGTGTTTAAGTTGGAGAATTGTACTTAAACACCTTCTTCTTGGGGTTCTCGGCAGTATCATTTGACACATGATACG
H ₄₋₁	TGGGTCAATTCTCCAACCTTAAACTAGAGAAGGTGTTTAAGTTGGGTAGGGCGGGTGTCAAATGATACTGCCGAGAACTAGGAGCTA-SH
H ₃₋₂	HS-CATCTCTTCAGCGATCTTTTTCGAGGATGGAAGTGGAGAGCAATCTCCTGCTCTCCACTTCCATCCAAGCACCCATGTTAGTTGGT
H ₄₋₂	CATCTCTTCAGCGATCTTTGCTCTCCACTTCCATCCTCGCAATGGATGGAAGTGGAGAGCAGGAGATAAGCACCCATGTTAGTTGGT-SH
refDNA	TTGGTAAAGTTCTGGACGTATCTCCCATTGTATTATCTTGTATTATCTCTTATCTCCAGTCAATAGCATACGCTAGAGCC

Table S2. Comparison of our strategy with other methods in protein biomarker detection.

Method	target	Linear range	LOD	Ref.
ECL	HPV-E7	2-60 ng mL ⁻¹	0.1 ng mL ⁻¹	1
Fluorescence	HPV-E6	5-300 pg mL ⁻¹	50.6 pg mL ⁻¹	1
LDI MS	HPV-L1	2-80 ng mL ⁻¹	58.8 pg mL ⁻¹	2
Electrochemical	OPN	0.05-10.0 ng mL ⁻¹	0.98 fg mL ⁻¹	3
ELISA	OPN	0.15-60 ng mL ⁻¹	0.015 ng mL ⁻¹	4
LFB	OPN	10-500 ng mL ⁻¹	0.1 ng mL ⁻¹	5
SERS	PSA	0.01-100 ng mL ⁻¹	0.01 ng mL ⁻¹	6
SERS	PSA	0.005-50 ng mL ⁻¹	0.012 ng mL ⁻¹	7
SERS	HPV-E7	1 pg mL ⁻¹ -1 µg mL ⁻¹	0.76 pg mL ⁻¹	This work
	OPN		0.62 pg mL ⁻¹	

ECL: electrochemiluminescence, LDI MS: laser desorption ionization mass spectrometry, LFB: lateral flow biosensor

Table S3. Information of clinical serum samples.

Patient ID	FIGO Stage	Age(y)	Pathology	Patient ID	FIGO Stage	Age(y)	Pathology
1	IVA	49	Malignant	31	health	51	Control
2	IIIC	37	Malignant	32	health	46	Control
3	IIIB	56	Malignant	33	health	42	Control
4	IIIB	43	Malignant	34	health	39	Control
5	IIIA	61	Malignant	35	health	56	Control
6	IIIA	48	Malignant	36	health	47	Control
7	IIB	36	Malignant	37	health	53	Control
8	IIB	52	Malignant	38	health	60	Control
9	IIB	45	Malignant	39	health	48	Control
10	IIB	42	Malignant	40	health	55	Control
11	IIB	50	Malignant	41	health	39	Control
12	IIB	53	Malignant	42	health	50	Control
13	IIA	41	Malignant	43	health	46	Control
14	IIA	37	Malignant	44	health	44	Control
15	IIA	40	Malignant	45	health	56	Control
16	IIA	38	Malignant	46	health	38	Control
17	IIA	47	Malignant	47	health	41	Control
18	IIA	52	Malignant	48	health	53	Control
19	IIA	39	Malignant	49	health	66	Control
20	IIA	61	Malignant	50	health	57	Control
21	IB	35	Malignant	51	health	61	Control
22	IB	36	Malignant	52	health	44	Control
23	IB	44	Malignant	53	health	50	Control
24	IB	56	Malignant	54	health	39	Control
25	IA	42	Malignant	55	health	34	Control
26	IA	36	Malignant	56	health	47	Control
27	IA	48	Malignant	57	health	38	Control
28	IA	52	Malignant	58	health	40	Control
29	IA	41	Malignant	59	health	36	Control
30	IA	46	Malignant	60	health	43	Control

Table S4. The levels of HPV-E7 and OPN detected by our method and ELISA in cervical cancer patients.

Patient ID	Our method (ng mL ⁻¹)		ELISA (ng mL ⁻¹)	
	HPV-E7	OPN	HPV-E7	OPN
1	1.27	1.51	1.22	1.57
2	1.23	1.48	1.34	1.43
3	1.25	1.39	1.28	1.33
4	1.19	1.38	1.17	1.42
5	1.21	1.37	1.22	1.37
6	1.17	1.36	1.21	1.39
7	1.18	1.32	1.16	1.32
8	1.17	1.31	1.17	1.29
9	1.15	1.30	1.16	1.38
10	1.08	1.29	1.12	1.24
11	0.97	1.29	0.93	1.29
12	0.96	1.28	0.95	1.27
13	0.92	1.26	0.96	1.23
14	1.02	1.10	1.08	1.21
15	1.03	1.07	0.99	1.10
16	1.15	1.17	1.13	1.19
17	1.08	1.18	1.07	1.17
18	1.06	1.20	1.03	1.19
19	1.16	1.21	1.14	1.17
20	1.05	1.23	1.13	1.22
21	0.87	1.27	0.88	1.21
22	0.91	1.27	0.86	1.24
23	0.91	1.18	0.90	1.19
24	0.77	0.94	0.79	0.95
25	0.68	1.02	0.67	1.02
26	0.66	1.00	0.65	0.98
27	0.81	1.21	0.78	1.16
28	0.65	1.30	0.63	1.30
29	0.87	1.31	0.84	1.30
30	0.89	1.13	0.93	1.15

Table S5. Diagnostic value of detection of HPV-E7 and OPN levels in cervical cancer.

Markers	Sensitivity (%)	Specificity (%)	Youden Index	AUC
HPV-E7	83.72	87.68	0.714	0.853
OPN	82.51	81.42	0.639	0.822
HPV-E7+OPN	91.23	95.45	0.867	0.963

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