Supplementary Information (SI) for New Journal of Chemistry.
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Supplementary Information

A Universal and Programmable Platform for Fluorescent Detection and Profiling of Exosomes Based on Bispecific aptamers-programmed DNAzyme-switched CRISPR/Cas12a

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Table S1. Synthesized oligonucleotides sequences used in this work.

Oligonucleotides name	Sequence (5'to 3') description
$AP1_{CD63}$	CTCAACTTCAGCGATACTGTCGGTTTTTTTTTTTT CACCCCACCTCGCTCCCGTGACACTAATGCTAAG TTGGA
$AP2_{EPCAM}$	GACACTACAGAGGTTGCGTCTGTCCCACGTTGTC ATGGGGGGTTGGCCTGTTTTTTTTTTTTCCGACAG TCACCCATGTTAGTACCT
$AP2_{PD\text{-}L1}$	ACGGGCCACATCAACTCATTGATAGACAATGCGT CCACTGCCCGTTTTTTTTTTTTCCGACAGTCACC CATGTTAGTACCT
$AP2_{PTK7}$	ATCTAACTGCTGCGCCGCCGGGAAAATACTGTAC GGTTAGATTTTTTTTTTTTCCGACAGTCACCCATG TTAGTACCT
$AP2_{EGFR}$	TTGCTTTTGAGCATGCTGACGCATTCGGTTGACT TTTTTTTTTTTCCGACAGTCACCCATGTTAGTACC T
$AP2_{MUC1}$	GCAGTTGATCCTTTGGATACCCTGGTTTTTTTTTT TTCCGACAGTCACCCATGTTAGTACCT
HP	GGATATCACCAATAATTTCTACTAAGTGTAGATG AGG*GAGGTACTATrAGGAAGTTGAG*GGGGAGTTAA CGGACTGGGGTAATAAGGTGATATCC

BL	CACTTAGTAGAAATTATTGGTGATATCC
H	*CGACGATGGATGATATTCCACTATA*
crRNA	UAAUUUCUACUAAGUGUAGAU*UAUAGUGGAAUA UCAUCCAUCGUCG*
BL_{10}	GTAGAAATTATTGGTGATATCC
BL_{21}	ATCTACACTTAGTAGAAATTATTGGTGATATCC
H_{14}	*CGACGATGGATGAT*
H_{17}	*CGACGATGGATGATATT*
H_{20}	*CGACGATGGATGATATTCCA*
FQ	BHQ1-TTTATT-FAM
SD1	CTCAACTTCAGCGATACTGTCGGAAAAA
SD2	TTTTTCCGACAGTCACCCATGTTAGTACCT

The underlined sequence in AP1 and AP2 is aided by the proximity effect to assist DNAzyme in forming enzyme activity centers.The underlined sequence in HP is the universal activator, which is complementary to BL. The italicized sequence in HP is the part of HP that is complementary to the arm of DNAzyme. The underlined sequence in crRNA is complementary to the underlined sequence in BL. The italicized sequence of crRNA is the part that complements H to be used to activate Cas12a.

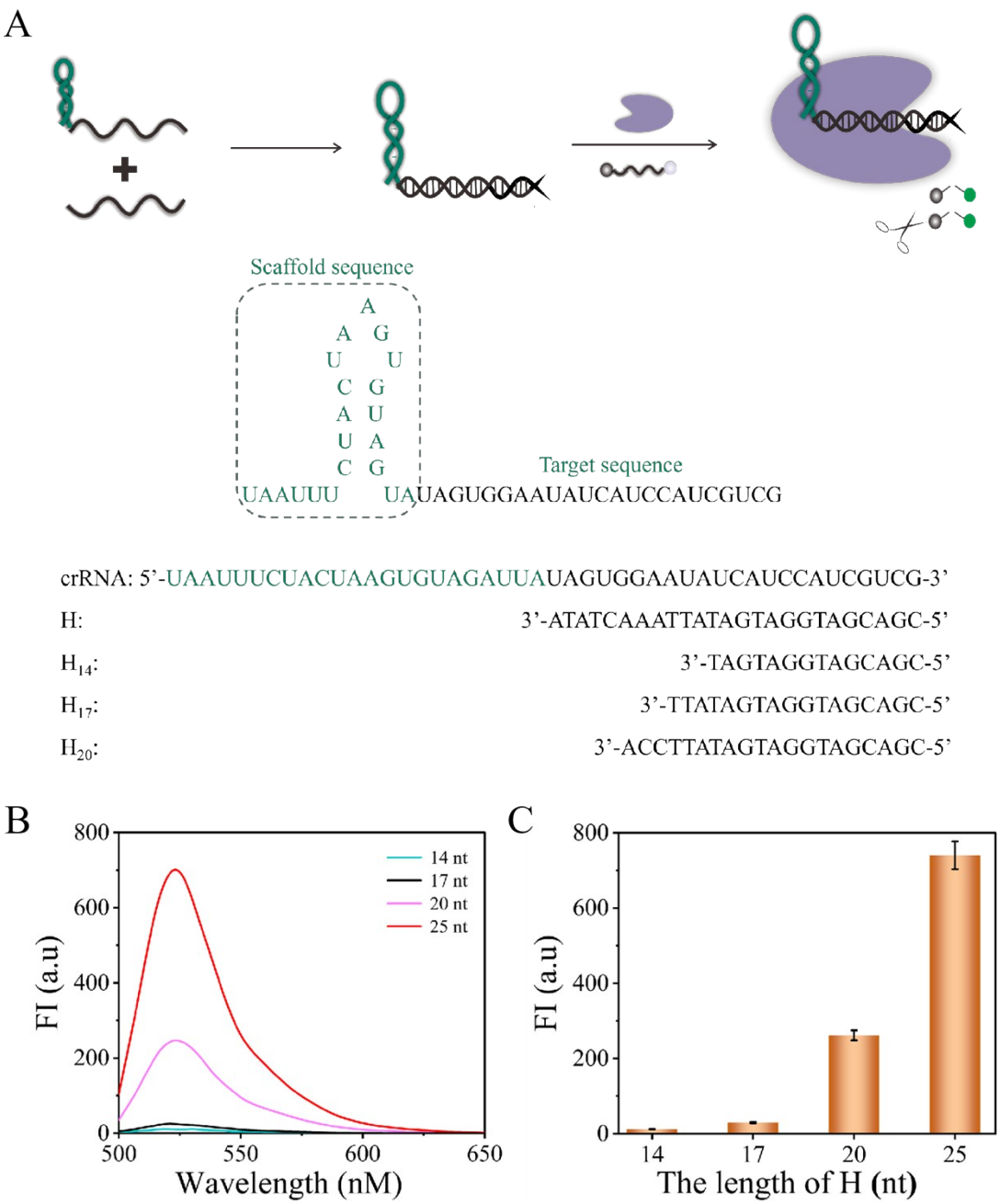

Figure S1. Schematic diagram (A) of activation of Cas12a with the assistance of helper (H) strand. Fluorescence spectra of H-crRNA to activate Cas12a (B) and column diagram (C) of the fluorescence intensity at 520 nm. Error bars are based on the standard deviations (SD) of three repetitive experiments.

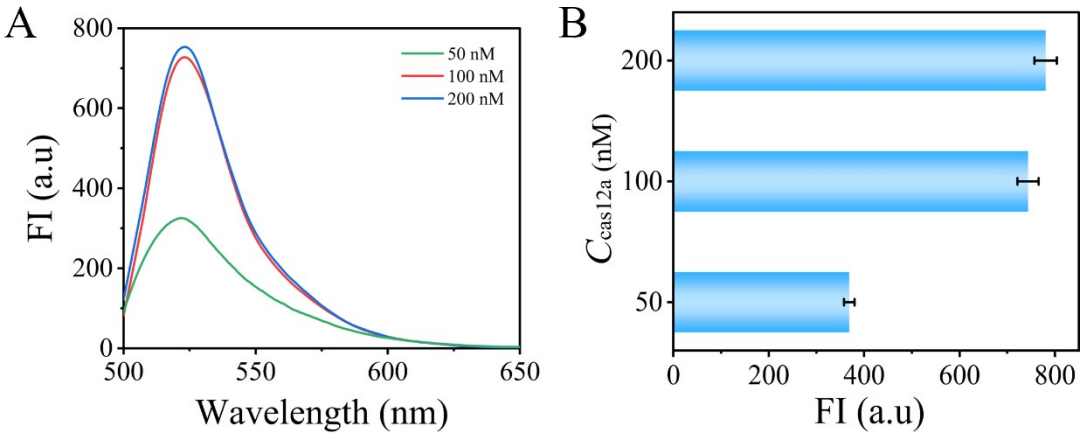

Figure S2. Concentration optimization of Cas12a of DDC system. Fluorescence spectra (A) and column diagram (C) of the fluorescence intensity at 520 nm. Error bars are based on the standard deviations (SD) of three repetitive experiments.

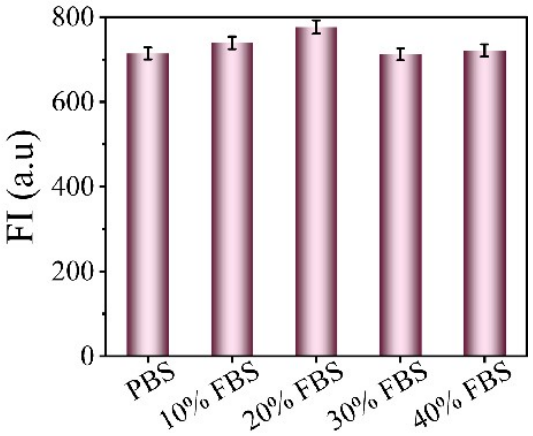

Figure S3. Fluorescence intensity at 520 nm obtained for the DDC system in the assay of stability. Error bars are based on the standard deviations (SD) of three repetitive experiments.

Table S2. An overview on recently reported methods for the determination of exosomes

Sensing mechanism	Biomarkers	LOD particles/μL	Linear range of detection particles/mL	Refs.
Fluorescence	EpCAM	13	5×10^{2}–5×10^{9}	1
Fluorescence	EpCAM	500	8.4×10^{3}–8.4×10^{8}	2
Fluorescence	PD-L1	6	7.78–7.78×10^{4}	3
Fluorescence	CD63, VEGF	1.02×10^{3}	1.75×10^{3}–3.5×10^{8}	4
Fluorescence	MUC1	1×10^{4}	2.4×10^{4}–1.18×10^{9}	5
Colorimetry	CD63, EpCAM	6.31×10^{4}	1.0×10^{5}–1.0×10^{9}	6
Colorimetry	CD63, EpCAM, and MUC1	CD63: 6.2×10^{7} EpCAM: 4.8×10^{7} MUC1: 5.4×10^{7}	CD63: 6.9×10^{7}-4.9×10^{8} EpCAM: 2.1×10^{8}–6.9×10^{8} MUC1: 0–6.2×10^{8}	7
Colorimetry	CD63	1.2×10^{6}	2.0×10^{6}–5.0×10^{8}	8
SERS	EpCAM	1.1×10^{5}	1.0×10^{6}–1.0×10^{10}	9
Fluorescence	CD63, PD-L1	3.5×10^{3}	6.3×10^{3}–6.3×10^{9}	This work

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