Supporting Information

Signal Self-Enhancement by Coordinated Assembly of Gold Nanoparticles Enables Accurate One-Step-Immunoassays

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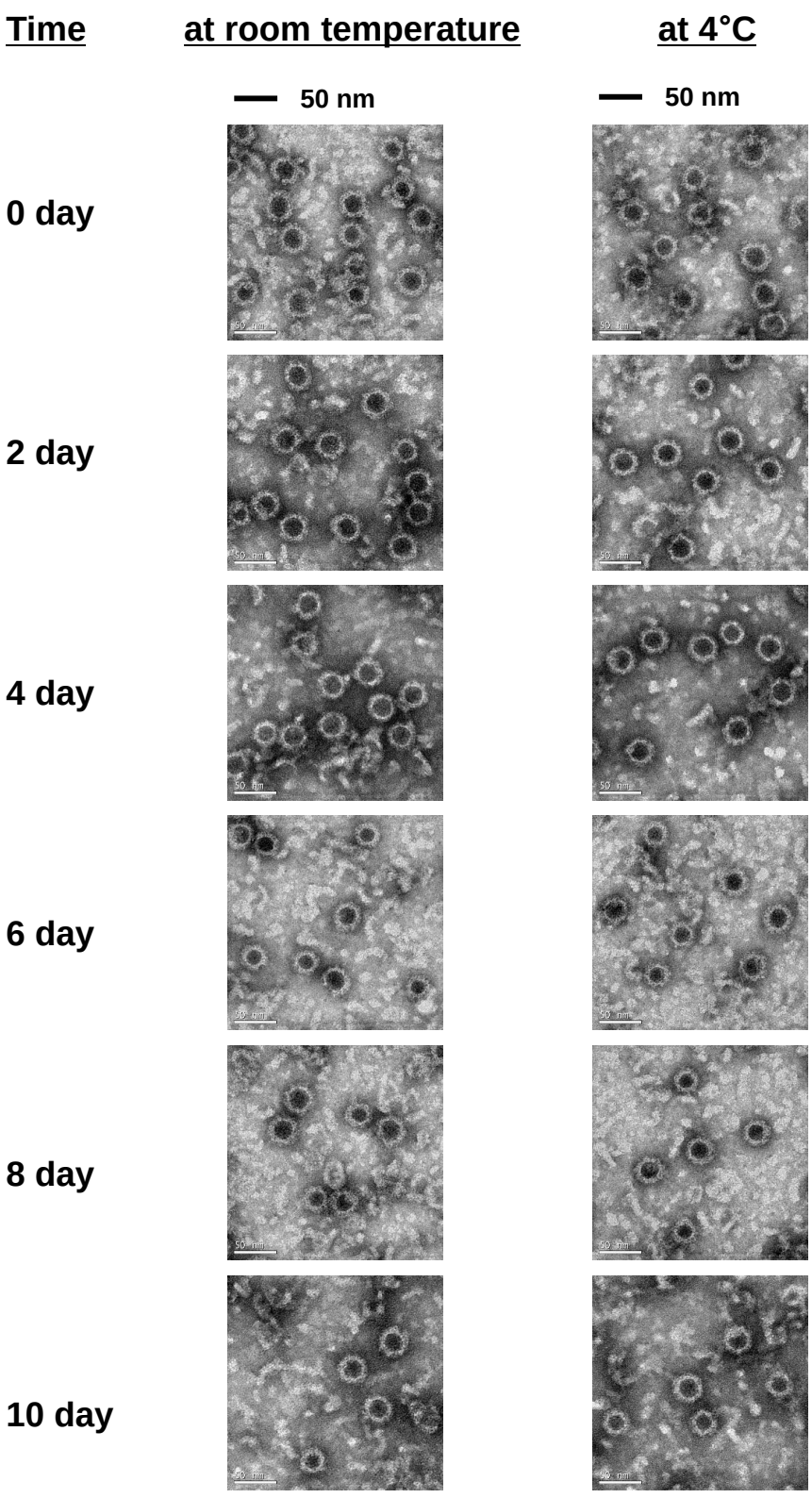
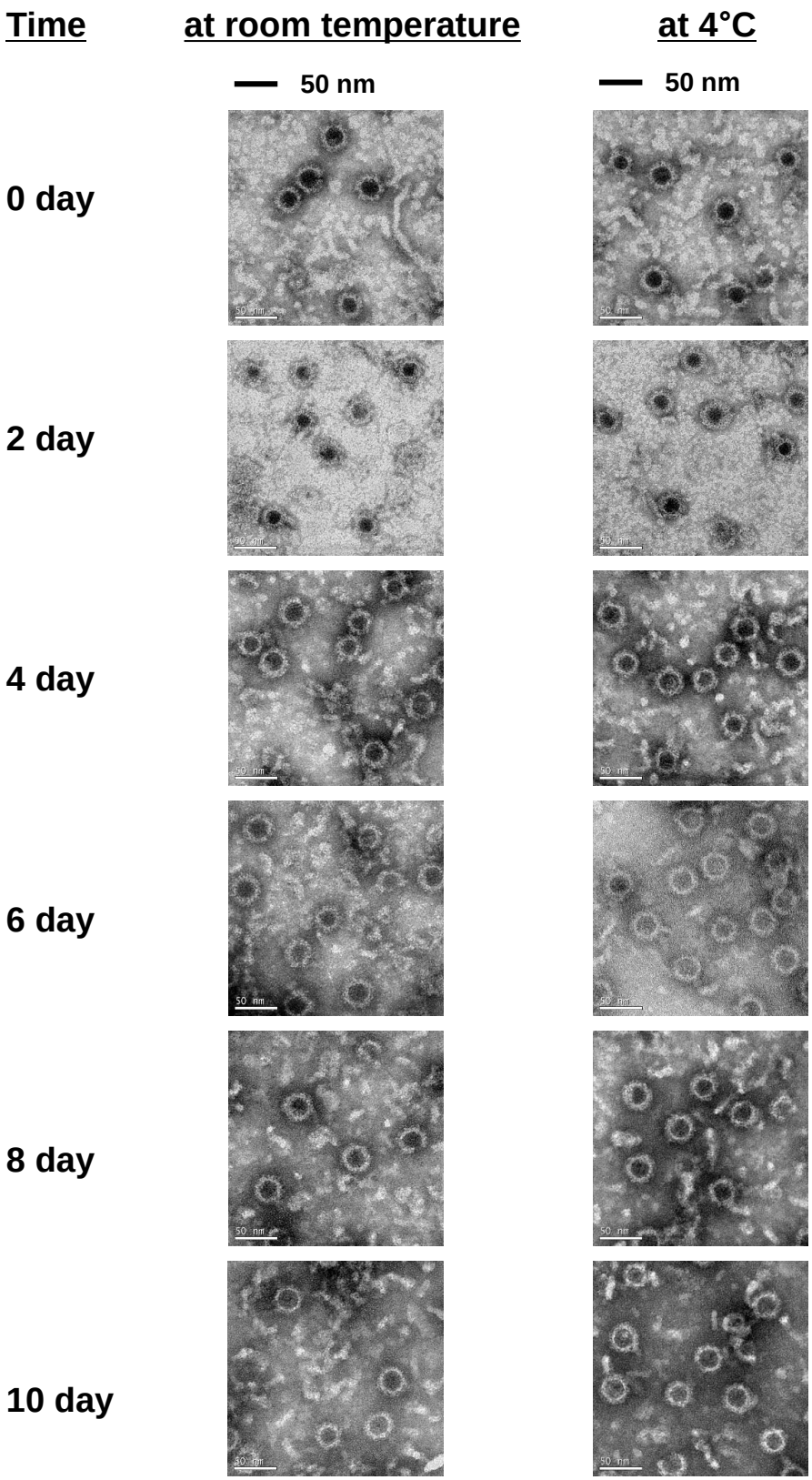
Figure S1

(a)


Time
at room temperature
at 4°C
50 nm
50 nm
0 day
2 day
4 day
6 day
8 day
10 day

(b)


Time
at room temperature
at 4°C
50 nm
50 nm
0 day
2 day
4 day
6 day
8 day
10 day

(c)

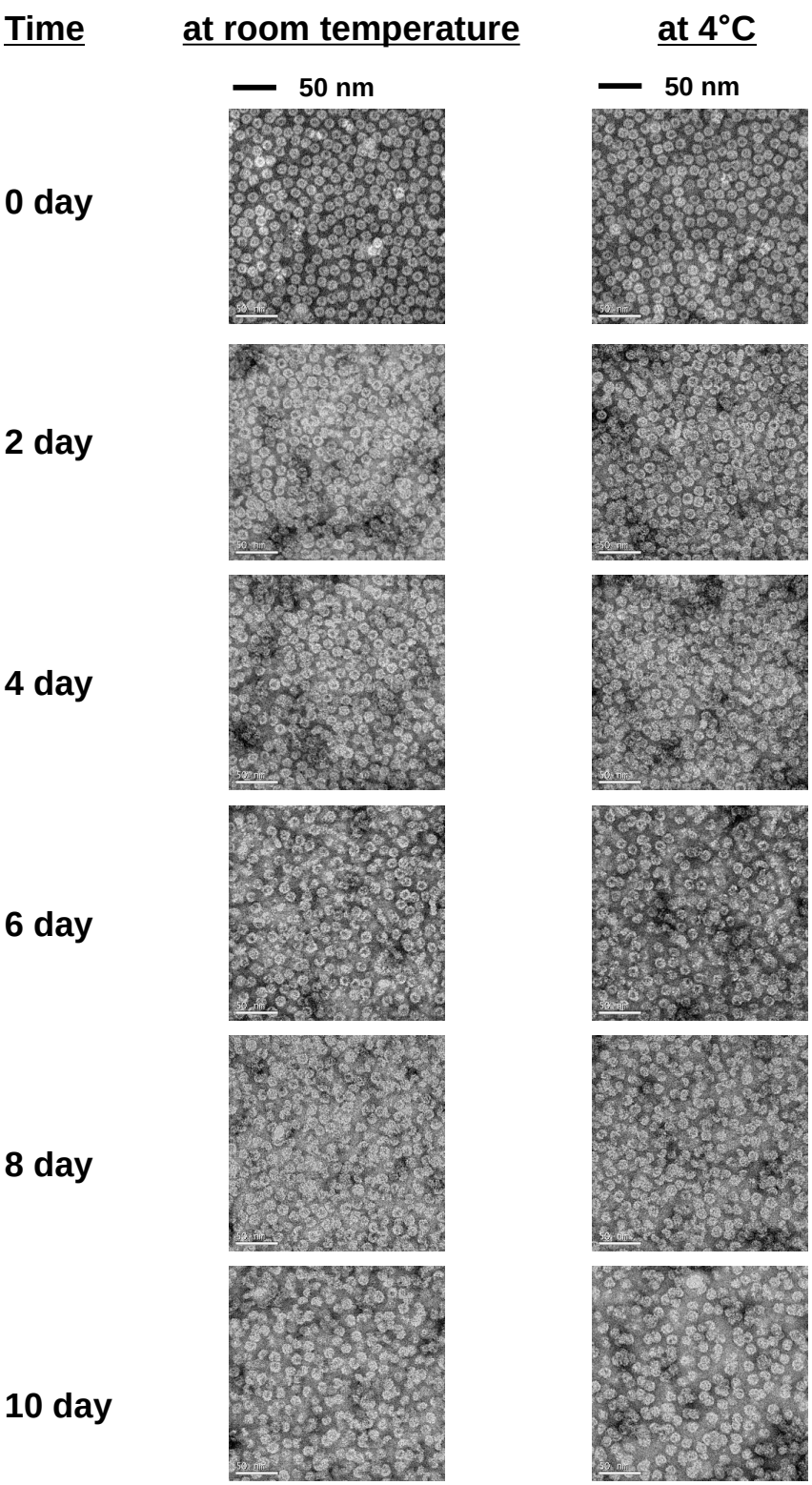
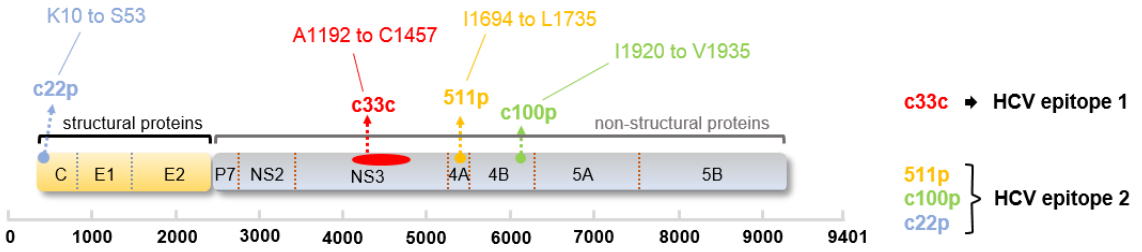


Figure S1. TEM images of 3D bioprobes (a) H_6-SPA_B-capsid, (b) capsid-HCV1, and (c) hFTH-HCV2, stored for 10 days at room temperature and 4 ºC.

Figure S2



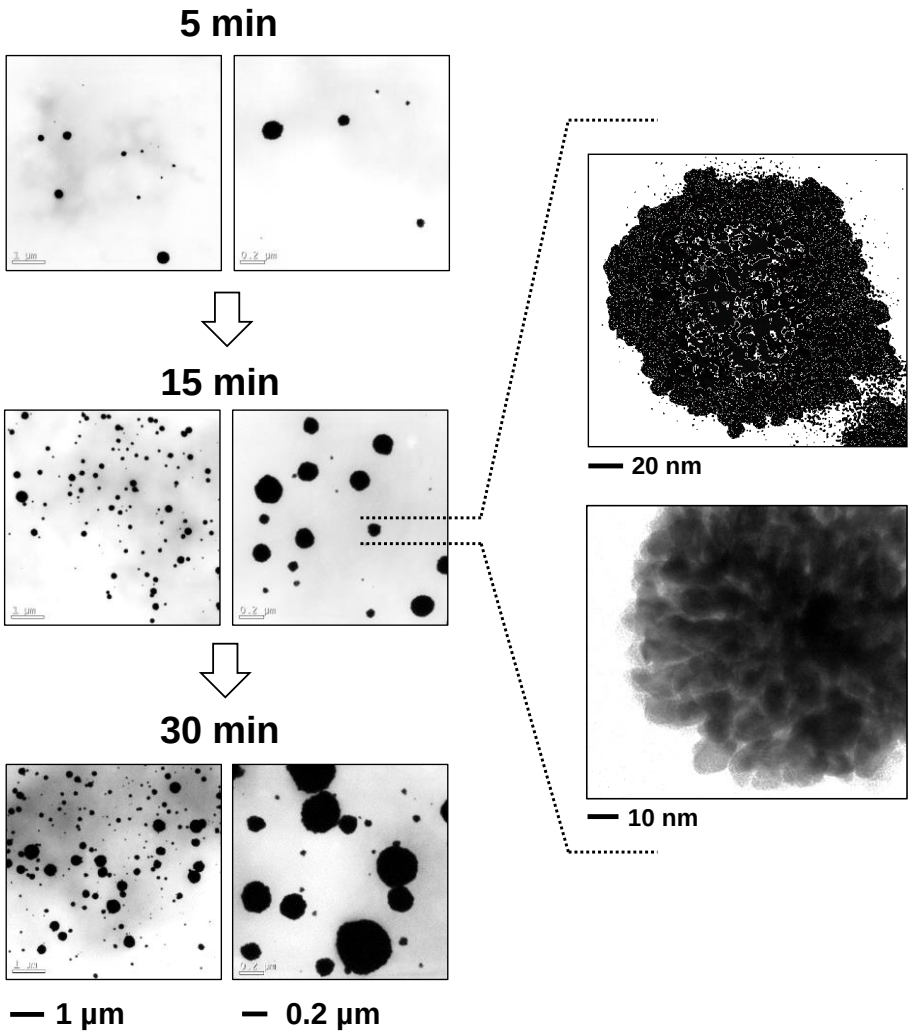
HCV genome sequence

MSTNPKPQK **K10** **NKRNTNRRPQDVKFPGGGQIVGGVYLLPRRGPRLGVRATRKT** **S53** ERSQPRG RRQPIPKARR PEGRTWAQPG YPWPLYGNEG CGWAGWLLSP 100 RGSRPSWGPT DPRRRSRNLG KVIDTLTCGF ADLMGYIPLV GAPLGGAARA LAHGVRVLED GVNYATGNLP GCSFSIFLLA LLSCLTVPAS AYQVRNSTGL 200 YHVTNDCPNS SIVYEAADAI LHTPGCVPCV REGNASRCWV AMTPTVATRD GKLPATQLRR HIDLLVGSAT LCSALYVGDL CGSVFLVGQL FTFSPRRHWT 300 ---------- 1001 PVSARRGREI LLGPADGMVS KGWRLLAPIT AYAQQTRGLL GCIITSLTGR DKNQVEGEVQ IVSTAAQTFL ATCINGVCWT VYHGAGTRTI ASPKGPVIQM 1100 YTNVDQDLVG WPAPQGSRSL TPCTCGSSDL YLVTRHADVI PVRRRGDSRG SLLSPRPISY LKGSSGGPLL CPAGHAVGIF RAAVCTRGVA K **A1192** **VDFIPVEN LETTMRSPVF TDNSSPPVVP QSFQVAHLHA PTGSGKSTKV PAAYAAQGYK VLVLNPSVAA TLGFGAYMSK AHGIDPNIRT GVRTITTGSP ITYSTYGKFL ADGGCSGGAY DIIICDECHS TDATSILGIG TVLDQAETAG ARLVVLATAT PPGSVTVPHP NIEEVALSTT GEIPFYGKAI PLEVIKGGRH LIFCHSKKKC DELAAKLVAL GINAVAYYRG LDVSVIPTSG DVVVVATDAL MTGYTGDFDS VIDCNT C1457** VTQ TVDFSLDPTF TIETITLPQD AVSRTQRRGR TGRGKPGIYR 1500 FVAPGERPSG MFDSSVLCEC YDAGCAWYEL TPAETTVRLR AYMNTPGLPV 1550 CQDHLEFWEG VFTGLTHIDA HFLSQTKQSG ENLPYLVAYQ ATVCARAQAP 1600 PPSWDQMWKC LIRLKPTLHG PTPLLYRLGA VQNEITLTHP VTKYIMTCMS 1650 ADLEVVTSTW VLVGGVLAAL AAYCLSTGCV VIVGRVVLSG KPA **I1694** **IPDREVLYREFDEMEE CSQHLPYIEQGMMLAEQFKQKALG** **L1735** LQTAS RQAEVIAPAV 1750 QTNWQKLETF WAKHMWNFIS GIQYLAGLST LPGNPAIASL MAFTAAVTSP 1800 LTTSQTLLFN ILGGWVAAQL AAPGAATAFV GAGLAGAAIG SVGLGKVLID ILAGYGAGVA GALVAFKIMS GEVPSTEDLV NLLPAILSPG ALVVGVVCAA 1900 ILRRHVGPGE GAVQWMNRL **I1920** **AFASRGNHVSPTHYV1935** PESDA AARVTAILSS LTVTQLLRRL HQWISSECTT PCSGSWLRDI WDWICEVLSD FKTWLKAKLM 2000 PQLPGIPFVS CQRGYKGVWR VDGIMHTRCH CGAEITGHVK NGTMRIVGPR TCRNMWSGTF PINAYTTGPC TPLPAPNYTF ALWRVSAEEY VEIRQVGDFH 2100 ---------- 2801 VYYLTRDPTT PLARAAWETA RHTPVNSWLG NIIMFAPTLW ARMILMTHFF SVLIARDQLE QALDCEIYGA CYSIEPLDLP PIIQRLHGLS AFSLHSYSPG 2900 EINRVAACLR KLGVPPLRAW RHRARSVRAR LLARGGRAAI CGKYLFNWAV RTKLKLTPIA AAGQLDLSGW FTAGYSGGDI YHSVSHARPR WIWFCLLLLA 3000 AGVGIYLLPN R 3011

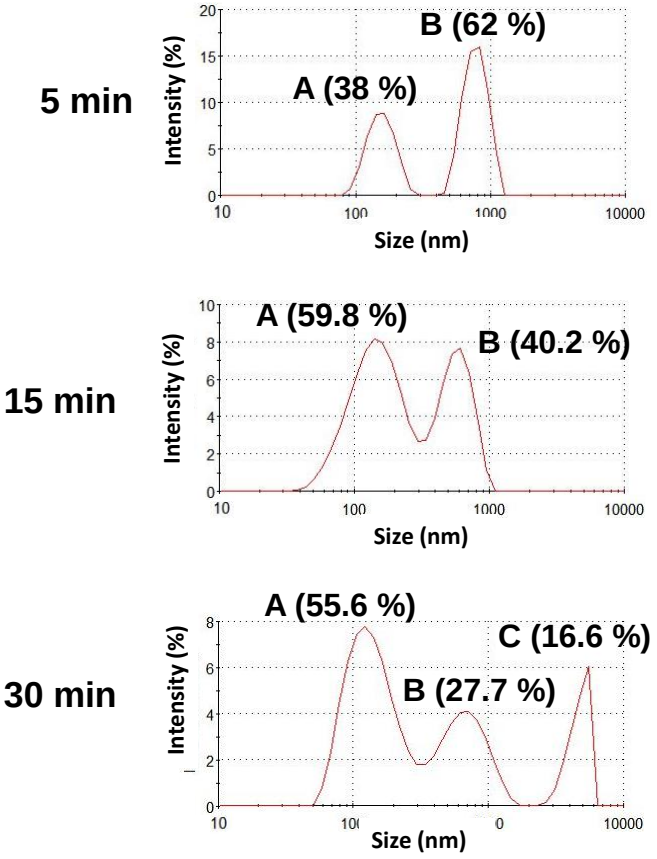
Figure S2. Sequences of antigenic epitopes (c33c, c22p, c100p, and 511p) of HCV that are used to detect anti-HCV antibody markers in patient sera in this study.

Figure S3

(a)



(b)



(c)

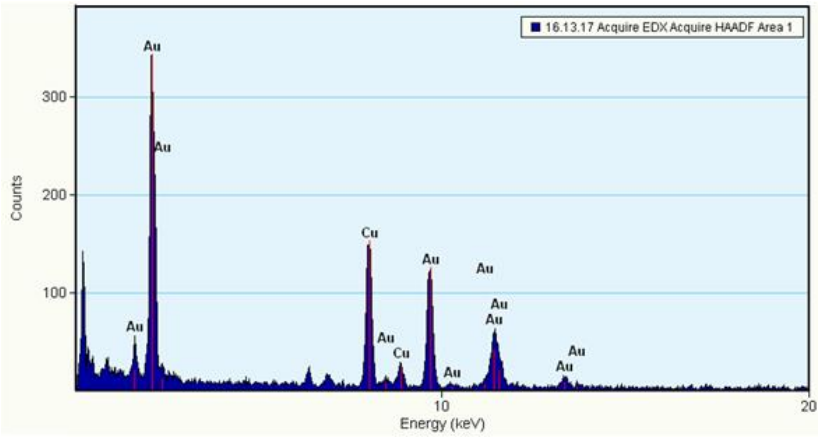
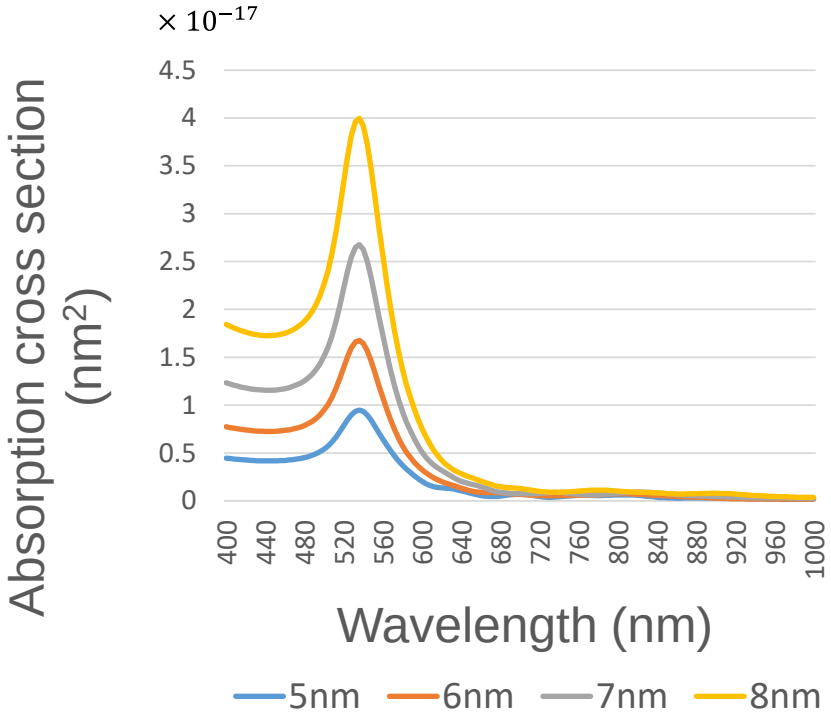


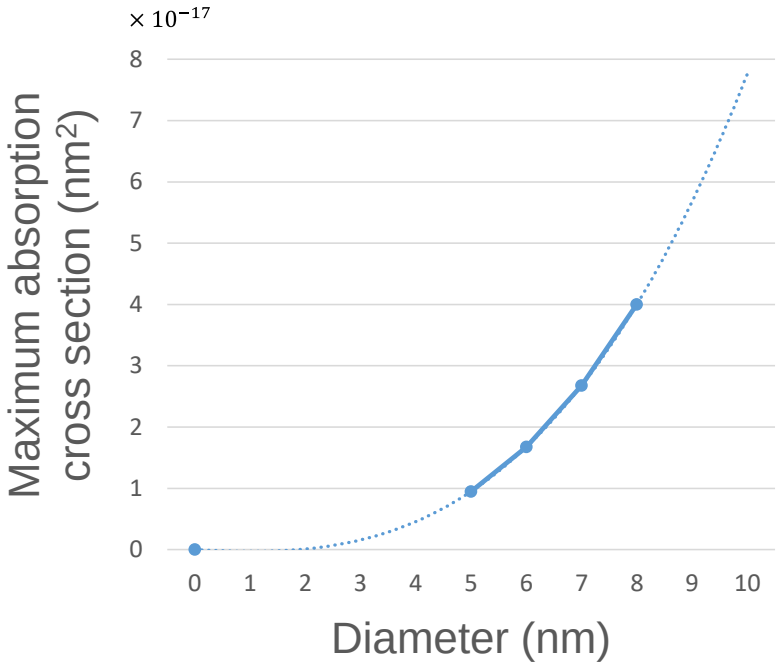
Figure S3. Results of **(a)** TEM, **(b)** DLS, and **(c)** EDX spectroscopy analyses of the assay solution of AMI patient serum.

Figure S4

(a)


× 10⁻¹⁷
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Absorption cross section (nm²)
400
440
480
520
560
600
640
680
720
760
800
840
880
920
960
1000
Wavelength (nm)
5nm
6nm
7nm
8nm

(b)


× 10⁻¹⁷
8
7
6
5
4
3
2
1
0
Maximum absorption cross section (nm²)
0
1
2
3
4
5
6
7
8
9
10
Diameter (nm)

(c)

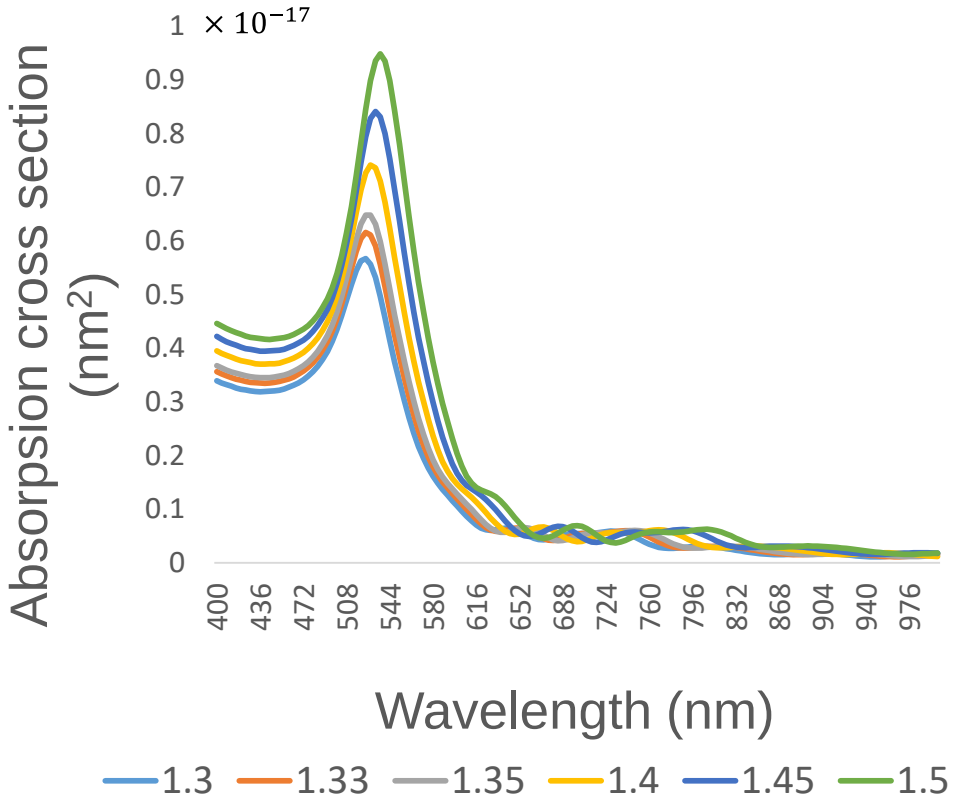


Figure S4. Calculated absorption cross section of a single gold nanoparticle. **(a)** Absorption cross section spectra for different nanoparticle diameter. The peak absorption wavelength is nearly constant. **(b)** The maximum absorption cross section at the peak wavelength as a function of particle diameter. Cubic dependence (fitting curve) is apparent. **(c)** The absorption cross section spectra for various refractive indices of the host material. As the index of the host material is increased the peak increases and redshifts slightly.

Figure S5

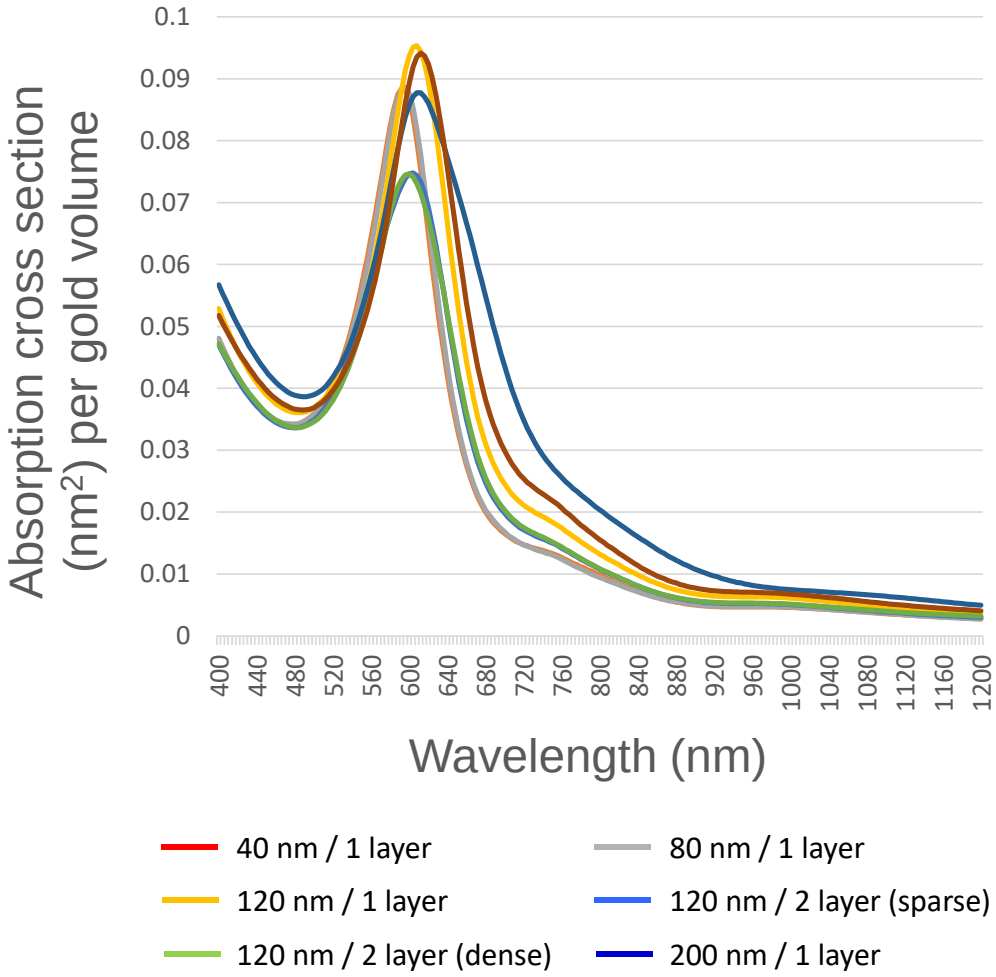
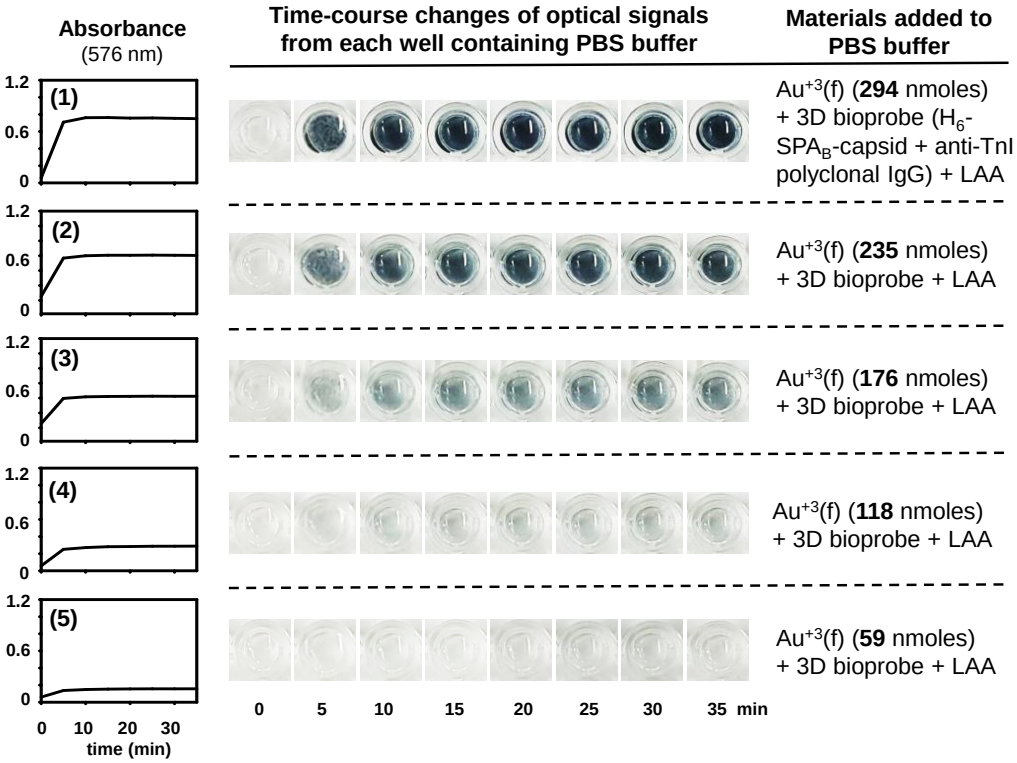


Figure S5. Absorption cross section normalized by the total volume of gold for various configurations. The absorption spectrum shows weak dependency of configuration.

Figure S6

(a)


Absorbance
(576 nm)
Time-course changes of optical signals from each well containing PBS buffer
Materials added to PBS buffer
(1)
(2)
(3)
(4)
(5)
1.2
0.6
0
0 10 20 30
time (min)
Au+3(f) (294 nmoles) + 3D bioprobe (H6-SPAB-capsid + anti-TnI polyclonal IgG) + LAA
Au+3(f) (235 nmoles) + 3D bioprobe + LAA
Au+3(f) (176 nmoles) + 3D bioprobe + LAA
Au+3(f) (118 nmoles) + 3D bioprobe + LAA
Au+3(f) (59 nmoles) + 3D bioprobe + LAA
0 5 10 15 20 25 30 35 min

(b)

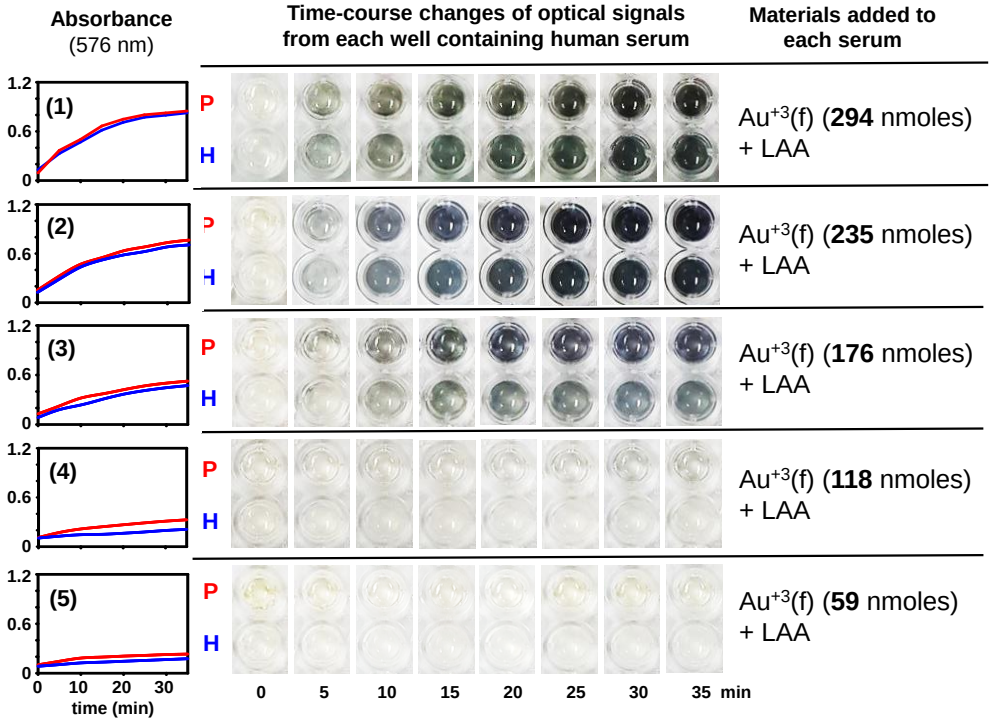

Absorbance
(576 nm)
Time-course changes of optical signals from each well containing human serum
Materials added to each serum
(1)
(2)
(3)
(4)
(5)
1.2
0.6
0
P
H
0 10 20 30
time (min)
Au+3(f) (294 nmoles) + LAA
Au+3(f) (235 nmoles) + LAA
Au+3(f) (176 nmoles) + LAA
Au+3(f) (118 nmoles) + LAA
Au+3(f) (59 nmoles) + LAA
0 5 10 15 20 25 30 35 min

Figure S6. Time-course changes in photographic image and absorbance (at 576 nm) for 35 min in **(a)** each PBS buffer solution where a different amount of $HAuCl_4$ (free Au^{+3}, 59 to 294 nmoles) and 3D bioprobe were present with LAA and in **(b)** each human (patient and healthy) serum solution where a different amount of $HAuCl_4$ (free Au^{+3}, 59 to 294 nmoles) was present with LAA without 3D bioprobes. This demonstrates that when the amount of free Au^{+3} of Figure 4-1 (294 nmoles) was reduced to its 40% (118 nmoles), the blue-colored AuNP clusters never formed in the solutions of both patient and healthy sera, indicating that a significant fraction of the free Au^{+3} should be adsorbed to 3D IgG-probes as in the pre-assay solution of the new one-step-immunoassay to avoid the false positive signals in healthy control assays that is caused by the rapid reduction of a large amount of free Au^{+3}. [P and H represent patient and healthy control serum, respectively, and red and blue curves represent the absorbance signals of the assay solutions of patient and healthy serum, respectively.]

Figure S7

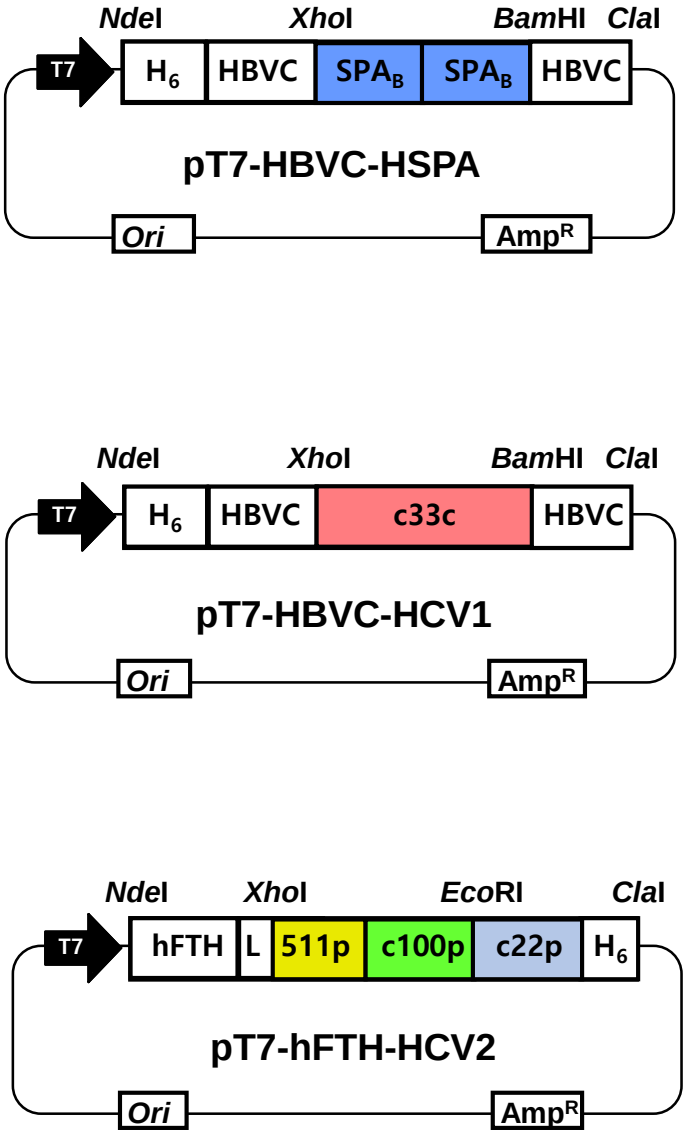


Figure S7. Schematic illustration of genetic modification of HBV core protein and human ferritin heavy chain and synthesis of surface-engineered HBV capsid (H_6-SPA_B-capsid and capsid-HCV1) and human ferritin nanoparticle (hFTH-HCV2). (HCV1 and HCV2 represent HCV antigenic peptides, c33c and 511p-c100p-c22p, respectively.)

Figure S8

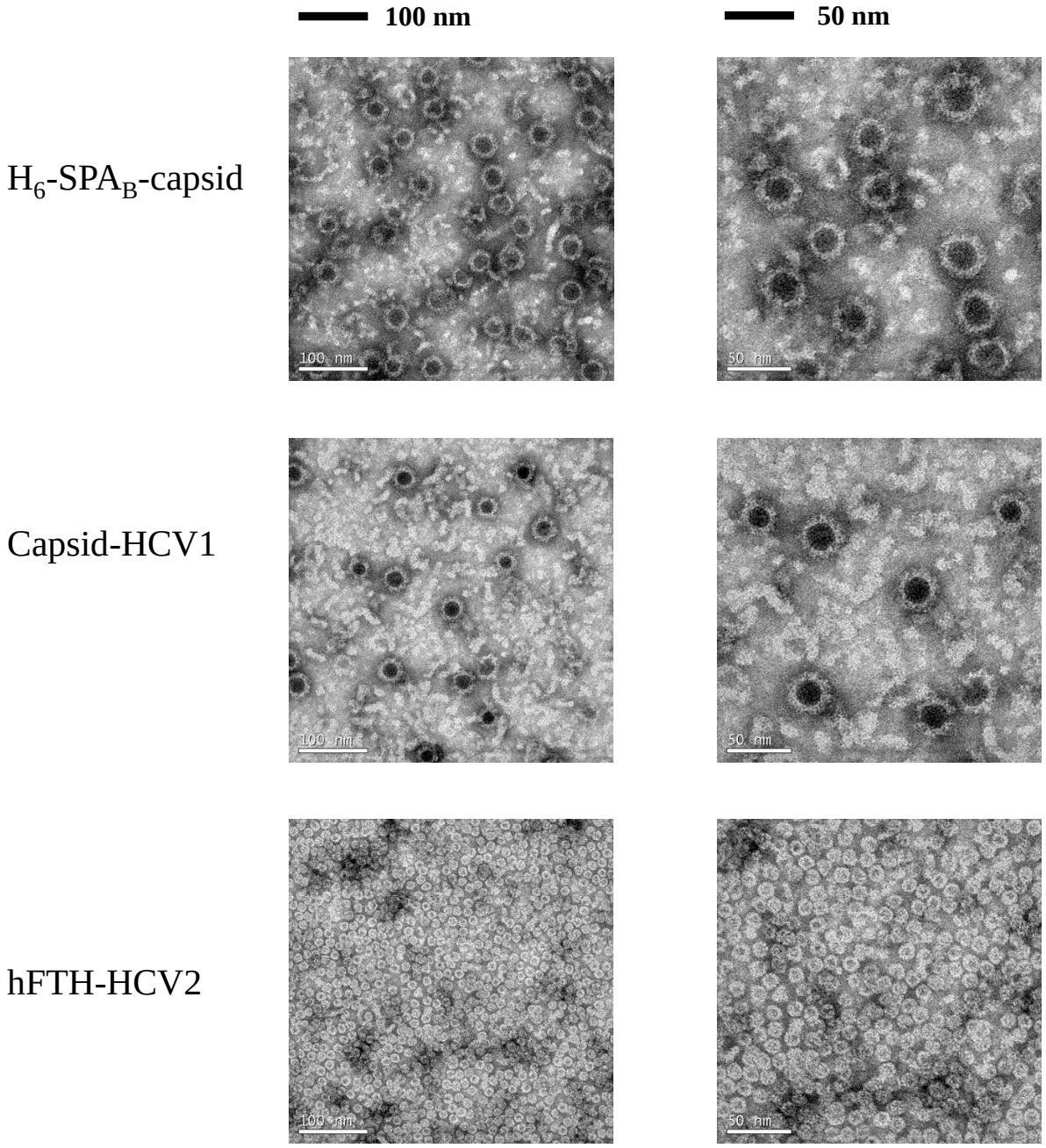


Figure S8. TEM images of H_6-SPA_B-capsid, capsid-HCV1 and hFTH-HCV2 used for one-step-immunoassay of hepatitis C.

Figure S9

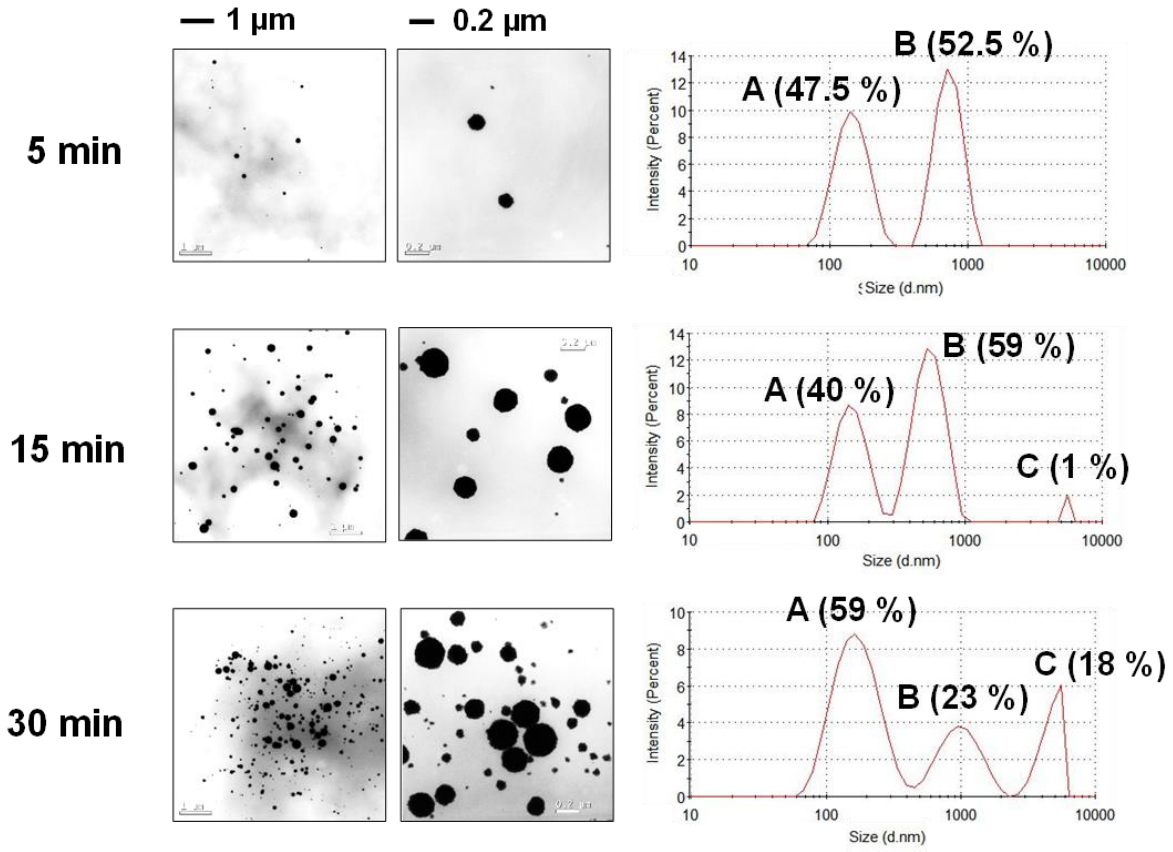


Figure S9. Time-course TEM images (left) and results of DLS analysis (right) of the assay solutions of hepatitis C patient serum.

Tables

Table S1

Information about 20 AMI patient sera (P1 to P20, Figure 2a) from College of Medicine at Seoul National University, Seoul, Republic of Korea

Patient No.	Age	Gender	Indication
1	65	M	NSTEMI
2	54	M	STEMI
3	45	M	NSTEMI
4	63	M	h/o AMI
5	58	M	h/o AMI
6	72	M	NSTEMI
7	75	M	NSTEMI
8	68	F	NSTEMI
9	45	M	NSTEMI
10	81	M	STEMI
11	78	M	STEMI
12	54	M	NSTEMI
13	54	M	NSTEMI
14	52	M	STEMI
15	63	M	NSTEMI
16	73	M	STEMI
17	67	M	NSTEMI
18	67	M	recent MI
19	65	M	STEMI
20	63	M	STEMI

STEMI, NSTEMI, recent MI, and h/o AMI represent serum sample collected within 1.5 h, 24~72 h, 7~30 day, and more than 30 day, respectively, after AMI is diagnosed.

Table S2

Information about 6 AMI patient sera (P21 to P26, Figure 2e) from College of Medicine at Seoul National University, Seoul, Republic of Korea.

Patient No.	Age	Gender	Indication
21	52	M	STEMI
22	75	M	NSTEMI
23	67	M	NSTEMI
24	63	M	STEMI
25	61	M	STEMI
26	75	M	STEMI

STEMI and NSTEMI represent serum sample collected within 1.5 h and 24~72 h, respectively, after AMI is diagnosed.