Electronic Supplementary Information: A universal design for a DNA probe providing ratiometric fluorescence detection by generation of silver nanoclusters

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Table 1. Target DNA sequences

HCV1	GACACTCCACCATGAATCACTCCCCTGTGA
HCV2	AAAGCGTCTAGCCATGTTTCACGGCAGAAA
HCV3	GTGCTCGAGAGTGACTGCCTGATAGGGTGC
HCV4	CCGGGAGGTCTCGTAGACCGTGCACCATGA
HAV	TTCCTTTGTTTTGCTTGTAAATATTAATTC
HBV	TTGGCTTTCAGTTATATGGATGATGTGGTA
miR182	TTTGGCAATGGTAGAACTCACACT
HrasMUT	TTGCCCACACCGACGGCG
HrasWT	TTGCCCACACCGCCGGCG

Table 2. AgNC22-MB probe sequences

HCV1	TTCCCACCCACCCCGGCCCGTTTTTTTCACAGGGGAGTGATTCATGGTGGAAACGGGCCGG
HCV2	TTCCCACCCACCCCGGCCCGTTTTTTTTTCTGCCGTGAAACATGGCTAGACAACGGGCCGG
HCV3	TTCCCACCCACCCCGGCCCGTTTTTTGCACCCTGTCAGGCAGTCACTCTCGAACGGGCCGG
HCV4	TTCCCACCCACCCCGGCCCGTTTTTTTCATGGTGCACGGTCTACGAGACCTAACGGGCCGG
mr182	TTCCCACCCACCCCGGCCCGTTTTTTAGTGTGAGTTCTACCATTGCCAAAAACGGGCCGG
HrasMUT	TTCCCACCCACCCCGGCCCGTTTTTTCGCCGTCGGTGTGGGCAAAACGGGCCGG
HAV	TTCCCACCCACCCCGGCCCGTTTTTTGAATTAATATTTACAAGCAAAACAAAACGGGCCGG
HBV[1]	TTCCCACCCACCCCGGCCCGTTTTTTTACCACATCATCCATATAACTGAAAGCCAAAACGGGCCGG

Table 3. AgNC12-MB probe sequences

HCV1	CCCTTAATCCCCTCACAGGGGAGTGATTCATGGTGGAGTGTCGGGGATT
HCV2	CCCTTAATCCCCTTTCTGCCGTGAAACATGGCTAGACGCTTTGGGGATT
HCV3	CCCTTAATCCCCGCACCCTATCAGGCAGTCACTCTCGAGCACGGGGATT

[1] The binding domain of HBV contains 30 bases for consistency with Xiao *et al.*[1] The remainder of the NC22 probes contain only 25 bases in the binding domain to reduce cost and improve synthesis yield. While the difference in binding domain length may alter binding affinity, we expect the effect is negligible in this case and could be optimized for any particular target on a case-by-case basis.

HCV4	CCCTTAATCCCCTCATGGTGCACGGTCTACGAGACCTCCCGGGGGGATT
HAV	CCCTTAATCCCCGAATTAATATTTACAAGCAAAACAAAGGAAGGGGATT
HBV	CCCTTAATCCCCTACCACATCATCCATATAACTGAAAGCCAAGGGGATT

Table 4. AgNC12-MB containing a 4T spacer between AgNC12 domain and binding domain

HCV1	CCCTTAATCCCCTTTTTCACAGGGGAGTGATTCATGGTGGAGTGTCGGGGATT
HCV2	CCCTTAATCCCCTTTTTTTCTGCCGTGAAACATGGCTAGACGCTTTGGGGATT
HCV3	CCCTTAATCCCCTTTTGCACCCTATCAGGCAGTCACTCTCGAGCACGGGGATT
HCV4	CCCTTAATCCCCTTTTTCATGGTGCACGGTCTACGAGACCTCCCGGGGGGATT
HAV	CCCTTAATCCCCTTTTGAATTAATATTTACAAGCAAAACAAAGGAAGGGGATT
HBV	CCCTTAATCCCCTTTTTACCACATCATCCATATAACTGAAAGCCAAGGGGATT

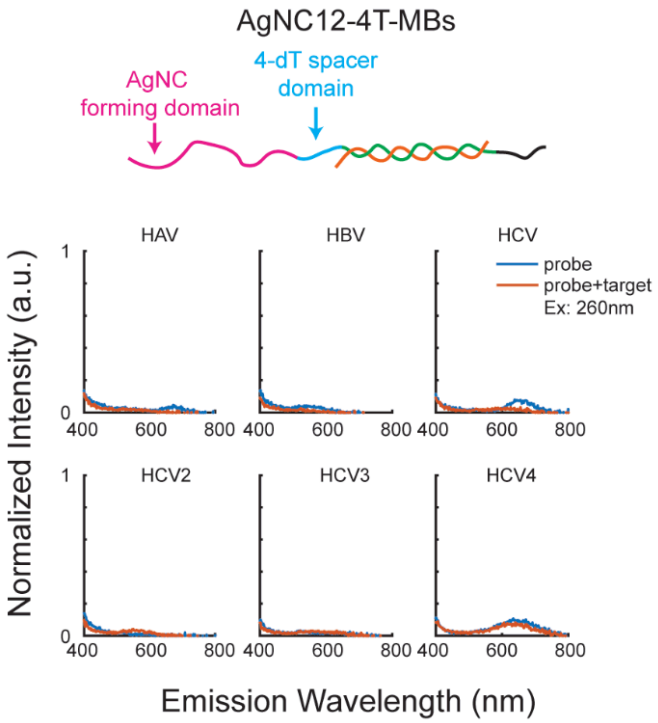

Figure S1 Fluorescence emission of AgNC12-T-MB probes that include a 4-dT spacer between the AgNC forming domain and the target binding domain. Samples were excited using 260 nm light. Fluorescence emission is normalized to the peak AgNC12 emission intensity in Figure 2 of the manuscript.

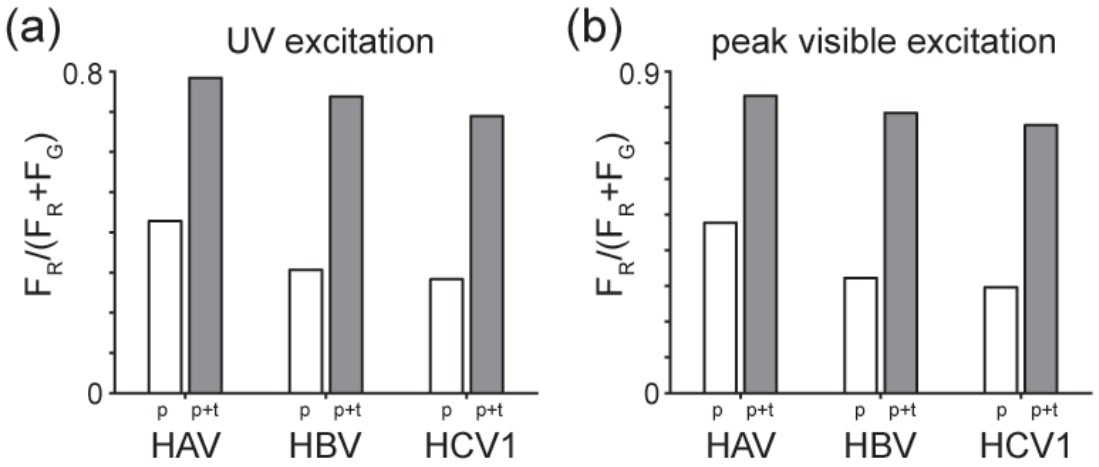

Figure S2 Ratiometric fluorescence emission of AgNC22-MB probes measured using 260 nm excitation (a) and peak visible excitation of red (ex. 572 nm, em. 640 nm) and green (ex. 475 nm, em. 550 nm) peaks (b). The two excitation modes produce comparable ratiometric fluorescence values and can be used interchangeably.

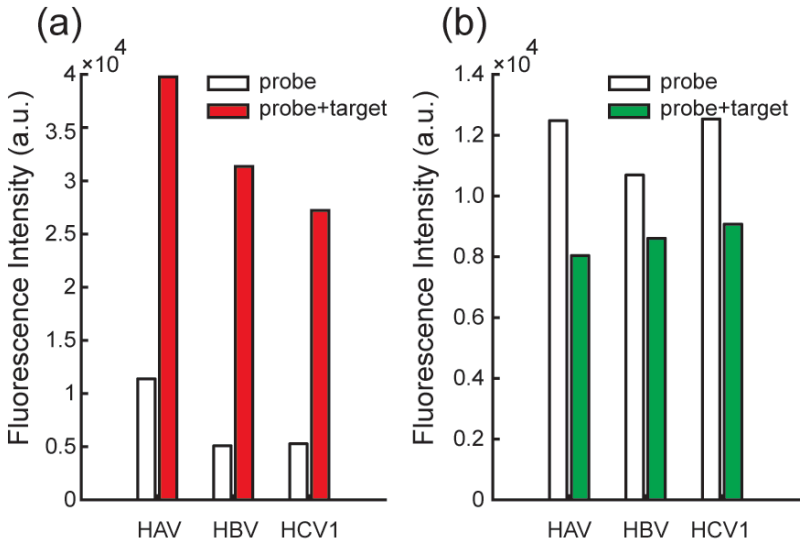

Figure S3 Peak fluorescence emission intensity of AgNC22-MBs when excited at peak cluster excitation (a) ex. 572 nm, em. 640 nm, (b) ex. 475 nm, em. 550 nm. In the presence of target, red fluorescence emission intensity of the probes increases, while green intensity decreases.

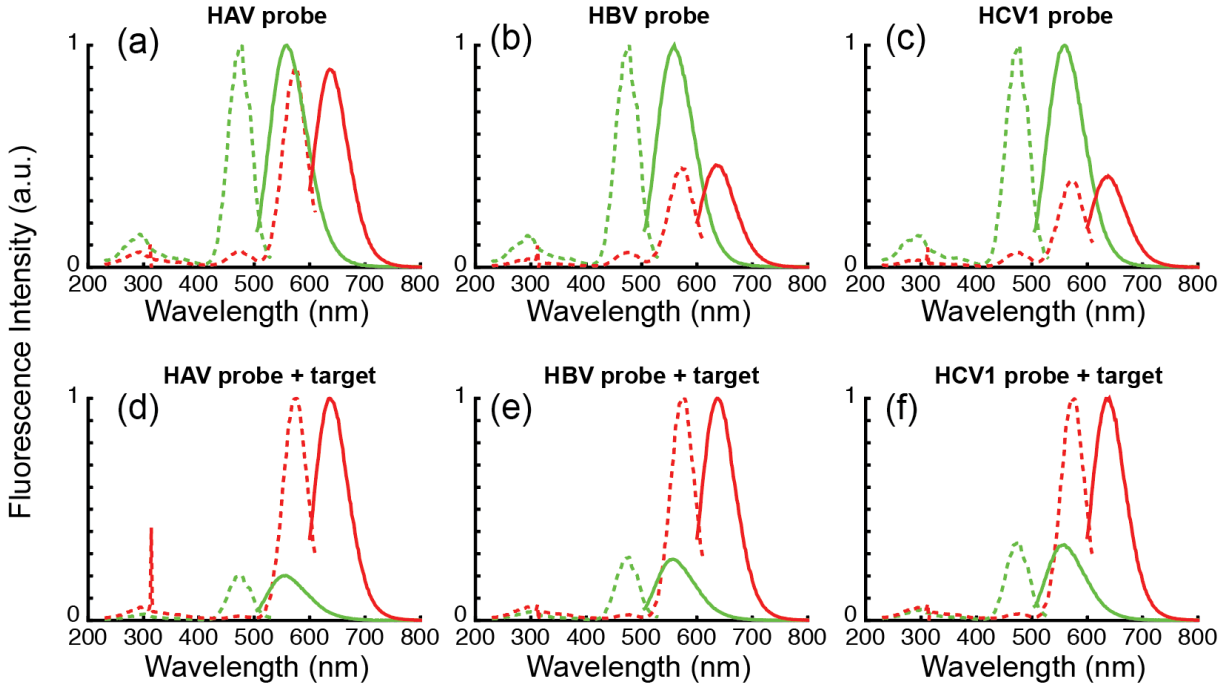

Figure S4 Excitation and emission spectra of AgNCNC22-MB probes for HAV, HBV and HCV1 before (a)-(c) and after (d)-(f) addition of target DNA. Peak excitation and emission wavelengths are independent of the choice of binding domain sequence.