Supplementary Information for: Fluorescent silver nanocluster DNA probes for multiplexed detection using microfluidic capillary electrophoresis

Jackson Travis Del Bonis-O'Donnell, Deborah K Fygenson, and Sumita Pennathur

Table S1: Sequences for AgNC-DNA probes and targets listed from 5' to 3'. Underlined regions (green) indicate the target binding sequence. Upstream of the underlined sequence is the AgNC stabilizing region (red). Downstream of the underlined sequence is the blocking sequence (black) and poly-dT mobility modifying region (orange). Colors correspond to those from Fig. 1 of the manuscript.

HBV-probe	CCCTTAATCCCCTACCACATCATCCATATAACTGAAAGCCAAGGGGATT
HBV-target	TTGGCTTTCAGTTATATGGATGATGTGGTA
HAV-probe	CCCTTAATCCCCGAATTAATATTTACAAGCAAAACAAAGGAAGGGGATT
HAV-target	TTCCTTTGTTTTGCTTGTAAATATTAATTC
HCV-probe	CCCTTAATCCCCGCACCCTGTCAGGCAGTCACTCTCGAGCACGGGGATT
HCV-target	GTGCTCGAGAGTGACTGCCTGATAGGGTGC
HAV-probe-20T	CCCTTAATCCCCGAATTAATATTTACAAGCAAAACAAAGGAAGGGGATTT_{20}
HCV-probe-10T	CCCTTAATCCCCGCACCCTGTCAGGCAGTCACTCTCGAGCACGGGGATTT_{10}

Table S2: List of probe and target sequences from 5' to 3' for HCV that did not produce viable probes. Underlined regions (green) indicate the target binding sequence. Upstream of the underlined sequence is the AgNC stabilizing region (red). Downstream of the underlined sequence is the blocking sequence (black) and poly-dT mobility modifying region (orange). Colors correspond to those from Fig. 1 of the manuscript.

HCV-probe2	CCCTTAATCCCCTCACAGGGGAGTGATTCATGGTGGAGTGTCGGGGATT
HCV-target2	GACACTCCACCATGAATCACTCCCCTGTGA
HCV-probe3	CCCTTAATCCCCTTTCTGCCGTGAAACATGGCTAGACGCTTTGGGGATT
HCV-target3	AAAGCGTCTAGCCATGTTTCACGGCAGAAA
HCV-probe4	CCCTTAATCCCCTCATGGTGCACGGTCTACGAGACCTCCCGGGGGATT
HCV-target4	CCGGGAGGTCTCGTAGACCGTGCACCATGA

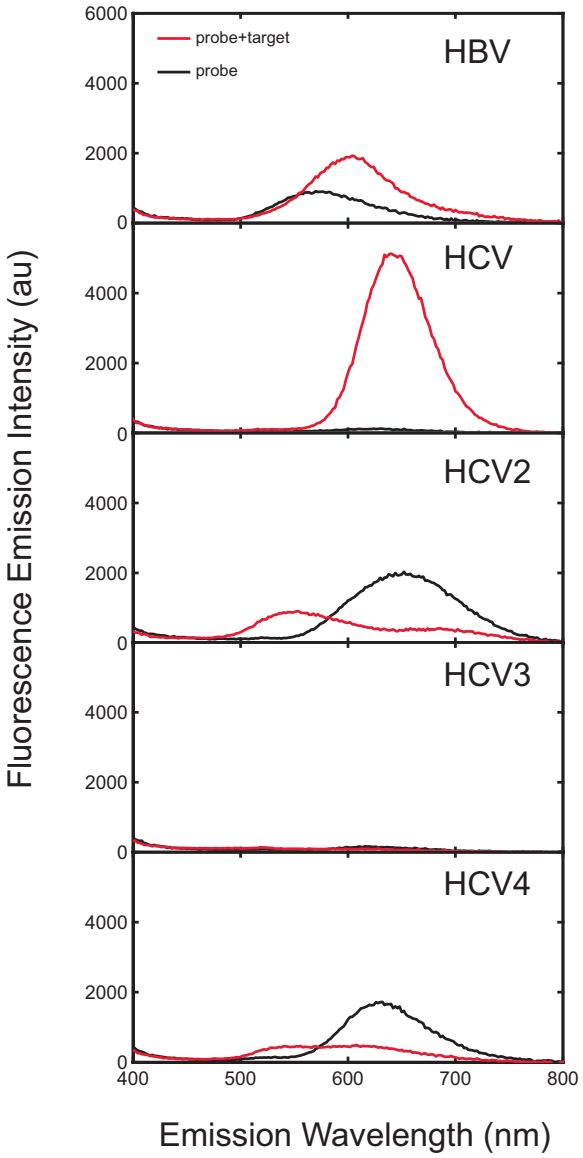

Figure S1: Emission spectra of the different HCV probe sequences under 260 nm excitation with and without corresponding target DNA. HCV2, HCV3 and HCV4 sequences did not produce viable probes. The HBV and HCV probes used in the manuscript are included in the first 2 panels for reference.

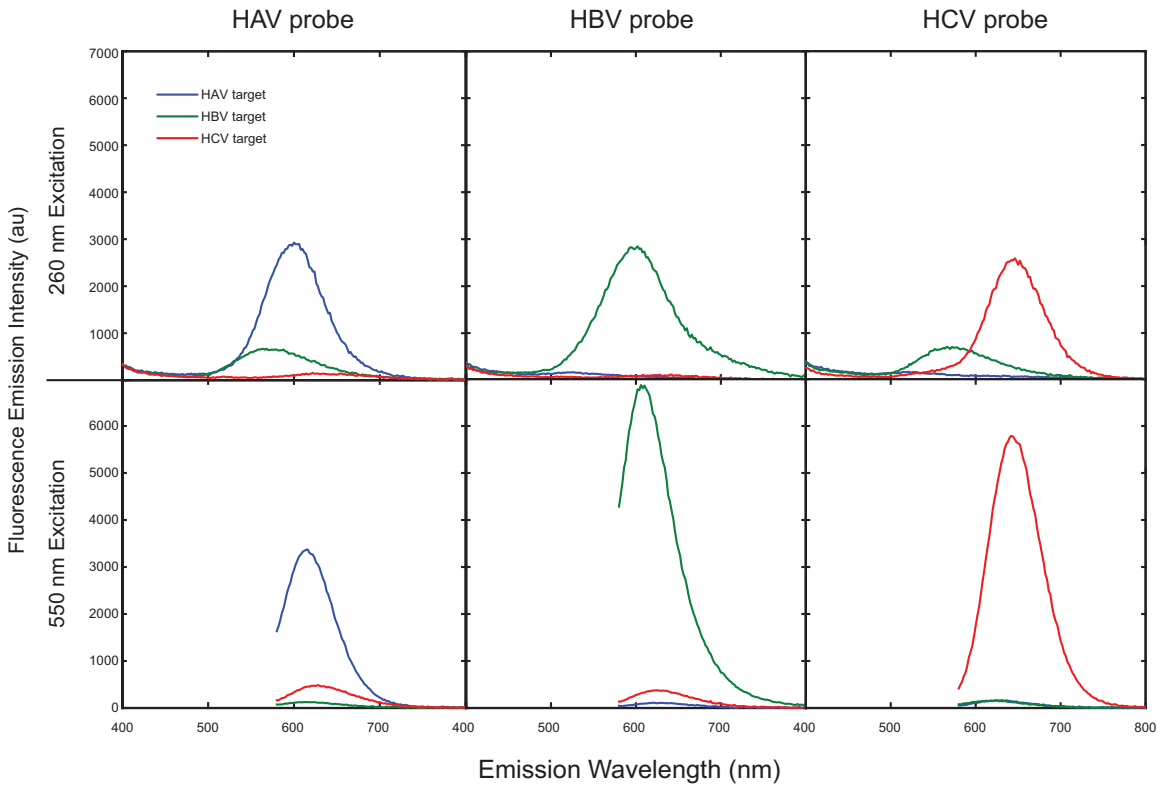

Figure S2: Emission spectra of each probe in the presence of all three different DNA targets. Top row shows emission at 260 nm excitation. Bottom row shows emission at 550 nm excitation. Each probe shows bright fluorescence in the presence of its complementary target and weak to no fluorescence when in the presence of mismatched target. Excitation at 550 nm further minimizes any fluorescent emission from probes with mismatched targets.

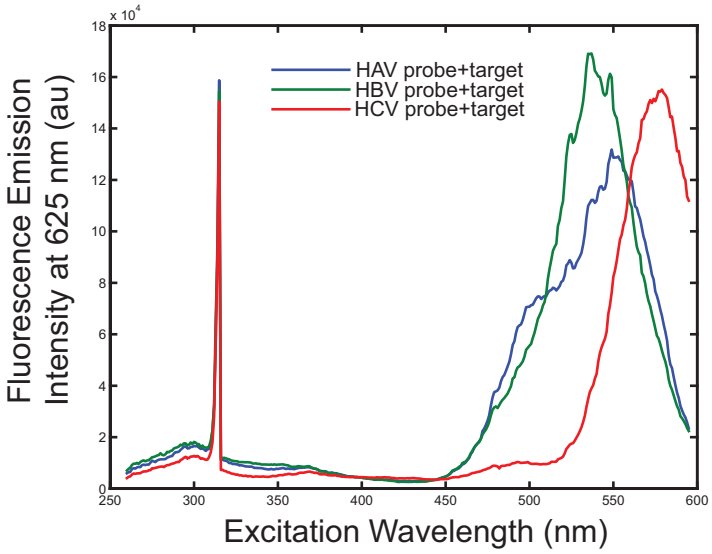

Figure S3: Fluorescence excitation scan of AgNC-DNA probes for HAV, HBV and HCV in the presence of their respective target DNA. The detector is set to an emission wavelength of 625 nm. The large spike near 312 nm is an artifact related to a harmonic of the detector wavelength, 625 nm. These spectra show that excitation at 550 nm produces approximately equal emission for each probe.

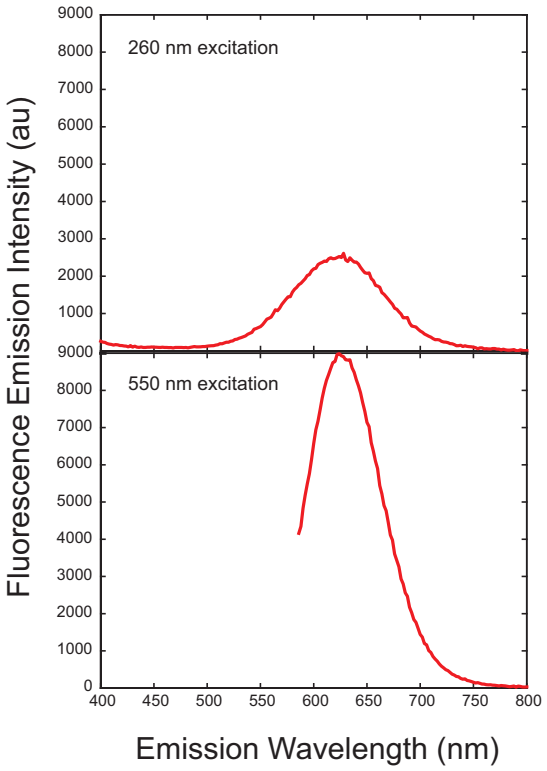

Figure S4: Fluorescence emission intensity of a mixture of AgNC-DNA probes for HAV, HBV and HCV with a mixture of all three targets. The top plot shows fluorescence emission of the mixture under 260 nm excitation. The bottom plot shows fluorescence emission under 550 nm excitation. The emission spectra appears to be the sum of each probe's emission spectra with a peak at 625 nm, which falls between the peak emissions of the individual probes.

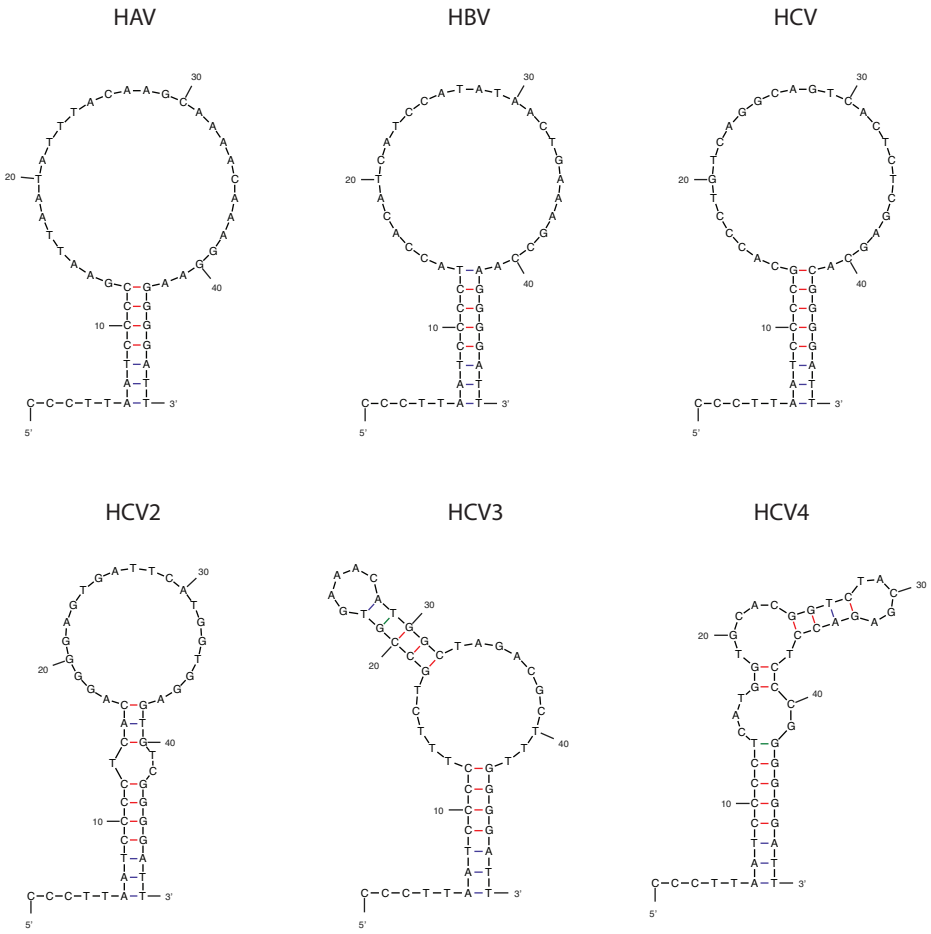

Figure S5: Predicted lowest free energy secondary structures of the native probes generated using the DINAMelt Web Server tool "Two-state Folding"(`http://mfold.rna.albany.edu/?q=DINAMelt/software`)[2, 1]. The conditions set for the calculations were: $[Na^+]$=20 mM, Temperature=25° C and [DNA]=15 μM.

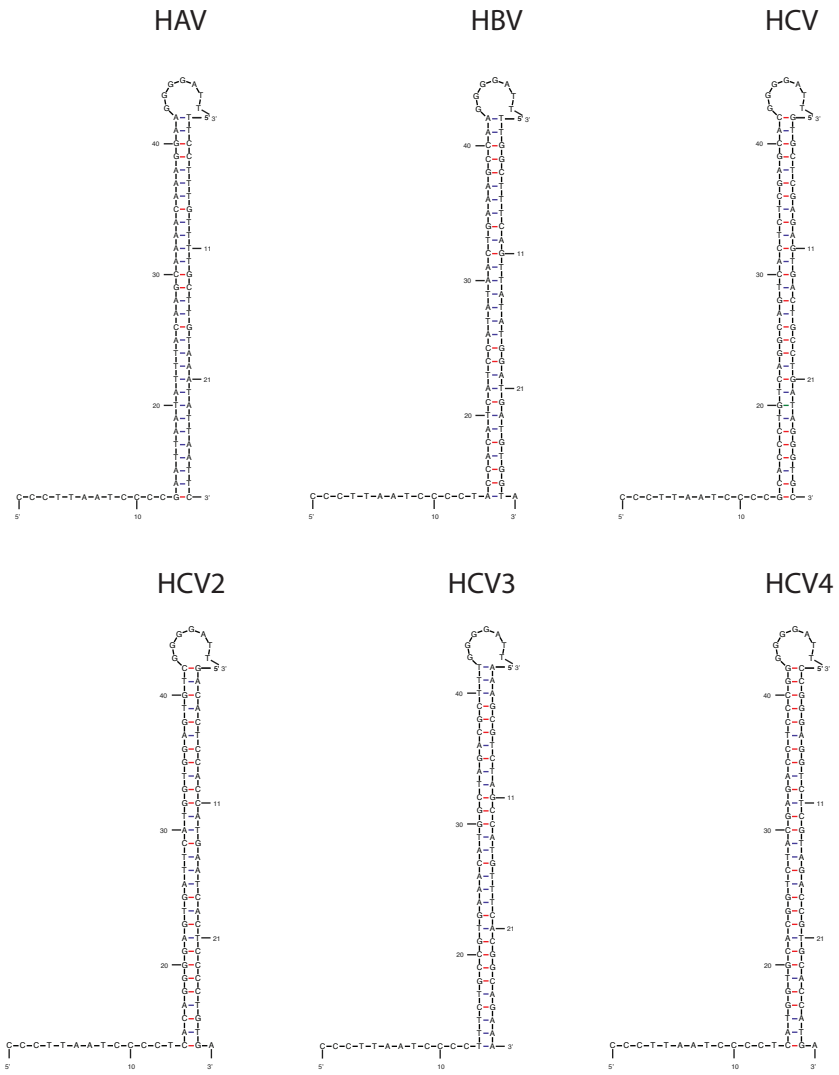

Figure S6: Predicted lowest free energy secondary structures of the probe-target complexes generated using the DINAMelt Web Server tool "Two State melting (hybridization)"(`http://mfold.rna.albany.edu/?q=DINAMelt/software`)[2, 1]. The conditions set for the calculations were: [Na^+]=20 mM, Temperature=25° C and [DNA]=15 μM.

Table S3: Thermodynamic parameters for probes in their native state and bound to target calculated using the DINAMelt Web Server tools "Two-state Folding" and "Two State melting (hybridization)"(`http://mfold.rna.albany.edu/?q=DINAMelt/software`)[2, 1]. The conditions set for the calculations were: $[Na^+]$=20 mM, Temperature=25 C and [DNA]=15 μM. Free energy and enthalpy are in kcal/mol; entropy is in e.u. (cal/mol/K).

HBV	ΔG = -4.2	ΔH = -62.5	ΔS = -195.4	T_m= 46.7° C
HAV	ΔG = -3.4	ΔH = -57.1	ΔS = -180.0	T_m= 44.0° C
HCV	ΔG = -5.6	ΔH = -65.4	ΔS = -200.6	T_m= 52.9° C
HCV2	ΔG = -4.7	ΔH = -79.7	ΔS = -251.7	T_m= 43.5° C
HCV3	ΔG = -3.9	ΔH = -93.0	ΔS = -298.9	T_m= 37.9° C
HCV4	ΔG = -4.0	ΔH = -108.7	ΔS = -351.1	T_m= 36.5° C
HBV+target	ΔG = -30.1	ΔH = -228.0	ΔS = -663.8	T_m= 57.9° C
HAV+target	ΔG = -26.3	ΔH = -231.7	ΔS = -689.0	T_m= 51.5° C
HCV+target	ΔG = -34.4	ΔH = -234.3	ΔS = -670.6	T_m= 63.8° C
HCV2+target	ΔG = -34.3	ΔH = -234.1	ΔS = -670.1	T_m= 63.7° C
HCV3+target	ΔG = -34.9	ΔH = -240.4	ΔS = -689.3	T_m= 63.5° C
HCV4+target	ΔG = -38.5	ΔH = -246.0	ΔS = -696.1	T_m= 68.1° C

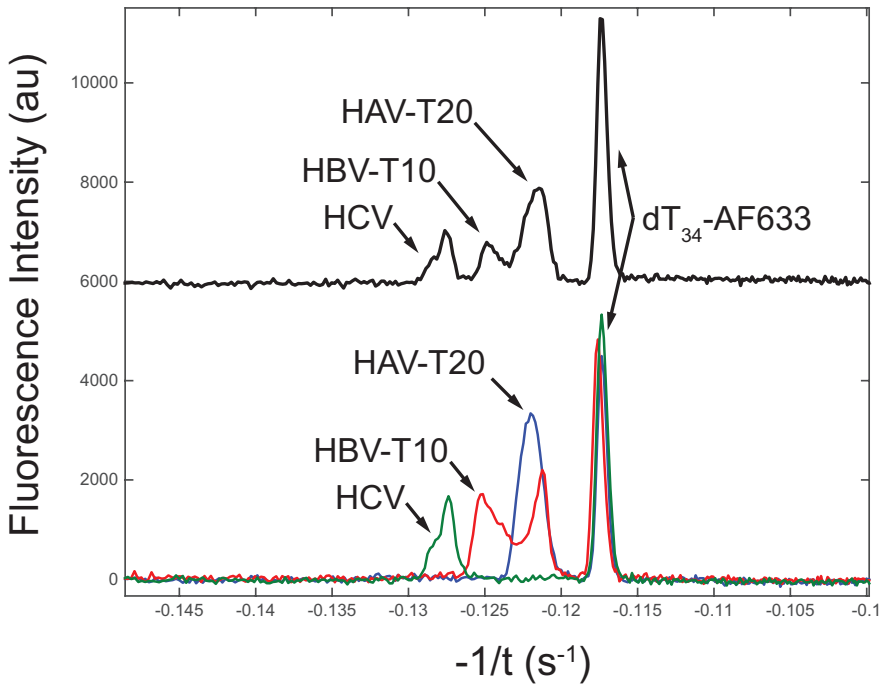

Figure S7: mCE separation of a sample mixture containing probe+target for HAV-T20, HBV-T10, HCV and a poly-dT_{34} reference peak with an Alexafluor633 tag. The HBV-T10 probe+target complex produces two elution peaks indicating that there are two stable conformations that yield similar AgNCs. However, improved separation resolution is necessary to separate the secondary HBV-T10 peak and the HAV-T20 and eliminate a false-positive for HAV target in the presence of HBV target.

References

[1] Nicholas R Markham and Michael Zuker. DINAMelt web server for nucleic acid melting prediction. *Nucleic Acids Res.*, 33(suppl 2):W577–W581, 2005.

[2] Nicholas R Markham and Michael Zuker. UNAFold: software for nucleic acid folding and hybridization. *Methods Mol. Biol.*, 453:3–31, January 2008.