Supplementary Information

Table S1. A list of all oligonucleotide sequences used for the experiments. Colored domains indicate complementarity.

DNA Sequence Name	Sequence	Nucleotide Length	GC content	Molecular Weight(g/mol)
H1 Unmodified (Gel)	GGAGTGAT-TCATGGT-GGAGTGTC-GAGAGAAC-GTGATGAA-GACACTCC-ACCATGA	54	50 %	16823.9
H 1 5' Thiol(AuNP)	5ThioMC6-D/iSp18/GGAGTGAT-TCATGGT-GGAGTGTC-GAGAGAAC-GTGATGAA-GACACTCC-ACCATGA	54	50 %	17496.5
FAM-H1-quencher	GGAGTGAT/i6-FAMK/TCATGGT-GGAGTGTC-GAGAGAAC-GTGATGAA-GACACTCC-ACCATGA/3IABkFQ/	54	50%	18120.0
H2 Unmodified(Gel)	TCATGGT-GGAGTGTC-TTCATCAC-GTTCTCTC-ATCACTCC-CTTGTCTC-GAGAGAAC-GTGATGAA-ACCATGA	70	47 %	21480.9
H2 5' Thiol(AuNP)	5ThioMC6-D/iSp18/TCATGGT-GGAGTGTC-TTCATCAC-GTTCTCTC-ATCACTCC-CTTGTCTC-GAGAGAAC-GTGATGAA-ACCATGA	70	47 %	22153.6
H3 Unmodified(Gel)	GTTCTCTC-GAGACAAG-GGAGTGAT-GACACTCC-ACCATGA-ATCACTCC-CTTGTCTC	55	51 %	16805.9
H3 5' Thiol(AuNP)	5ThioMC6-D/iSp18/GTTCTCTC-GAGACAAG-GGAGTGAT-GACACTCC-ACCATGA-ATCACTCC-CTTGTCTC	55	51 %	17478.6
H3-quencher	GTTCTCTC-GAGACAAG-GGAGTGAT-GACACTCC-ACCATGA/BHQ1/ATCACTCC-CTTGTCTC	55	51 %	17766.8
HCV RNA	GACACUCC-ACCAUGA-AUCACUCC	23	52 %	6841.4

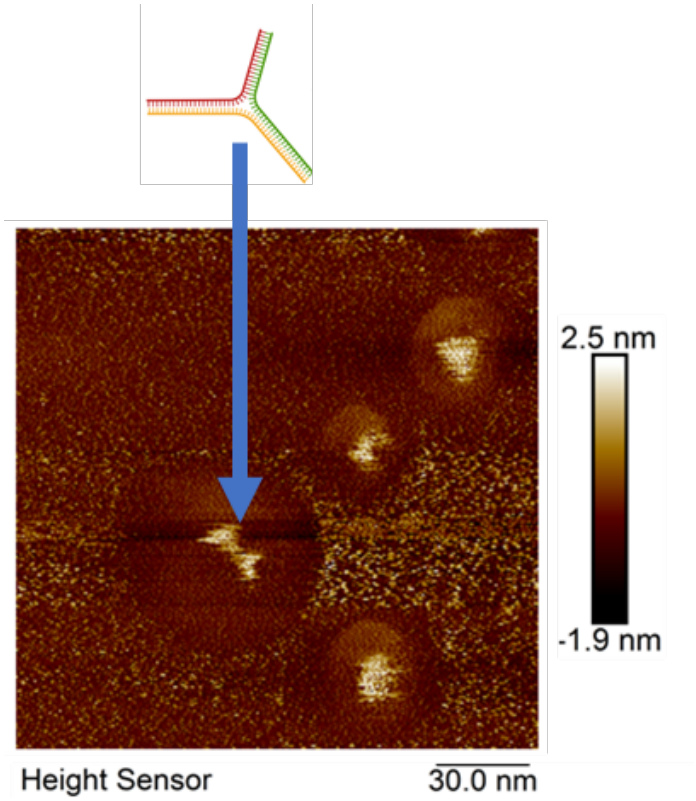

Figure S1. AFM image of 3-way junction CHA product formation. The sample was prepared by mixing the CHA reaction in 1 × TAE/Mg buffer (40 mM Tris, 20 mM acetic acid, 2 mM EDTA, 12.5 mM MgCl2, pH = 7.5) before depositing on mica treated with 10 mM $NiCl_2$. The scale Bar is 30 nm. The image area is 200 x 200 nm^2. Z range is 4.4 nm

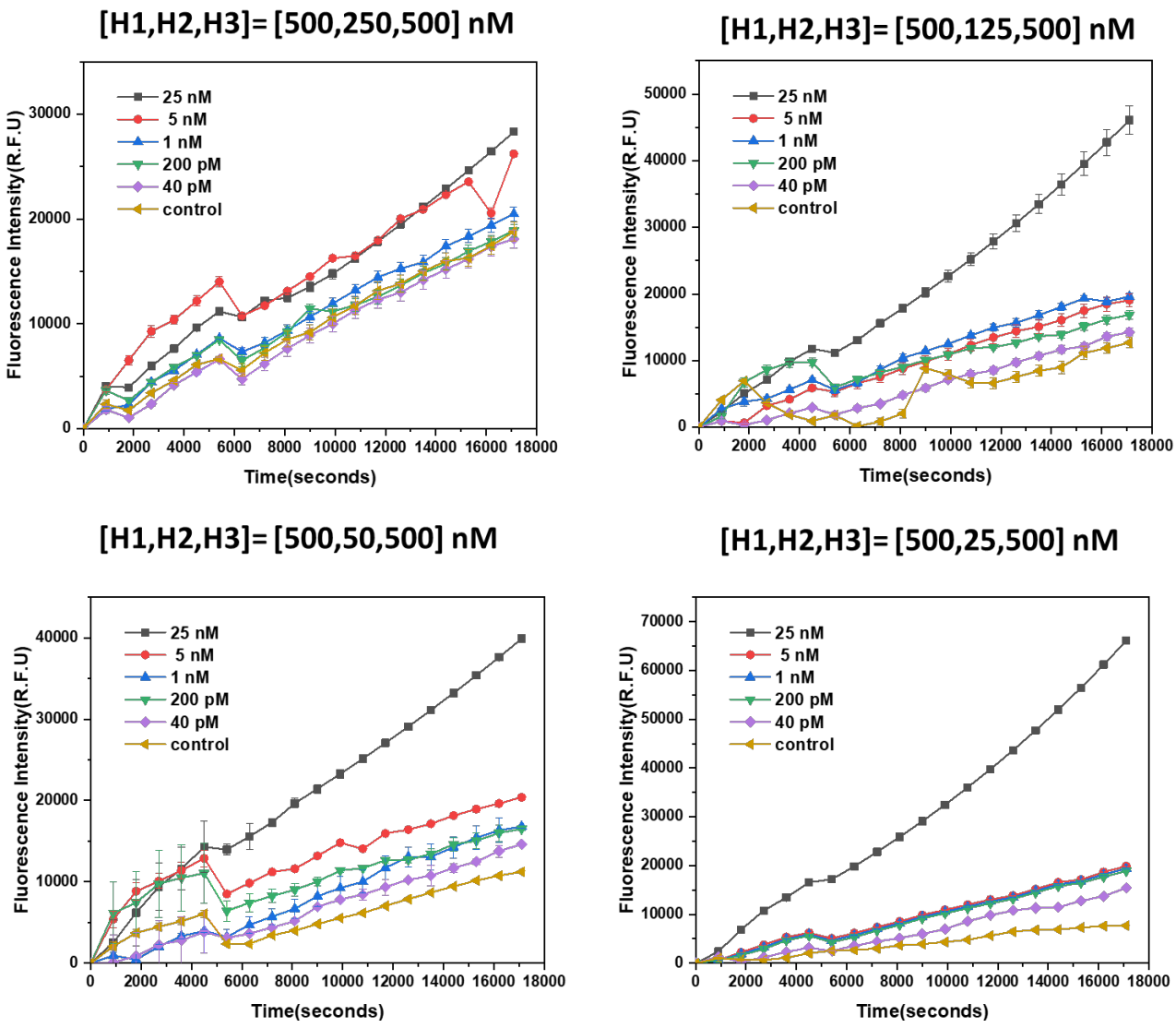

Figure S2. Fluorescence spectroscopy for optimizing the concentration of H2. For all experiments, H1 and H3 concentrations were kept at 500 nM. H2 concentrations were varied as follows: 25, 50, 125, and 250 nM. Clear discrimination between the control and the lowest assayed concentration target at 40 pM can be seen for H2 concentrations of 25 and 50 nM.

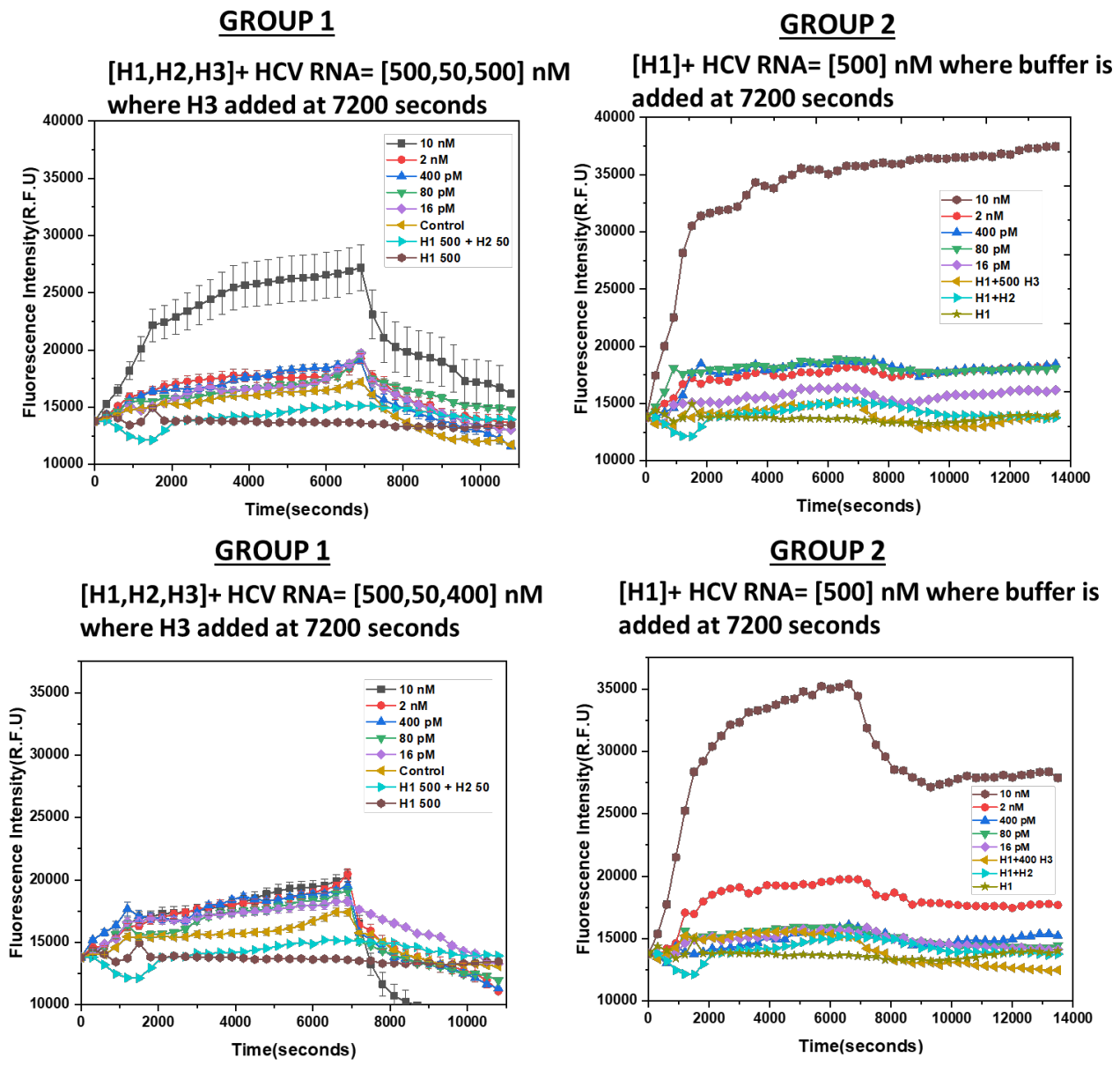

Figure S3. Fluorescence spectroscopy for determining the appropriate concentration of H3. **Group 1**- 500 nm H1 +50 nM H2 + HCV RNA concentrations + 500 nM H3-quencher (**top**), 500 nm H1 +50 nM H2 + HCV RNA concentrations + 400 nM H3-quencher (**bottom**). **Group 2**- 500 nM H1+ HCV RNA concentrations+ water +buffer (500 nM and 400 nM H3) (represented by the gold spectrum at **top** and **bottom** respectively). In the case of group 1, H3, buffer and water are added at 7200 seconds. The larger precipitous drop in fluorescence in group 1 indicates target recycling.

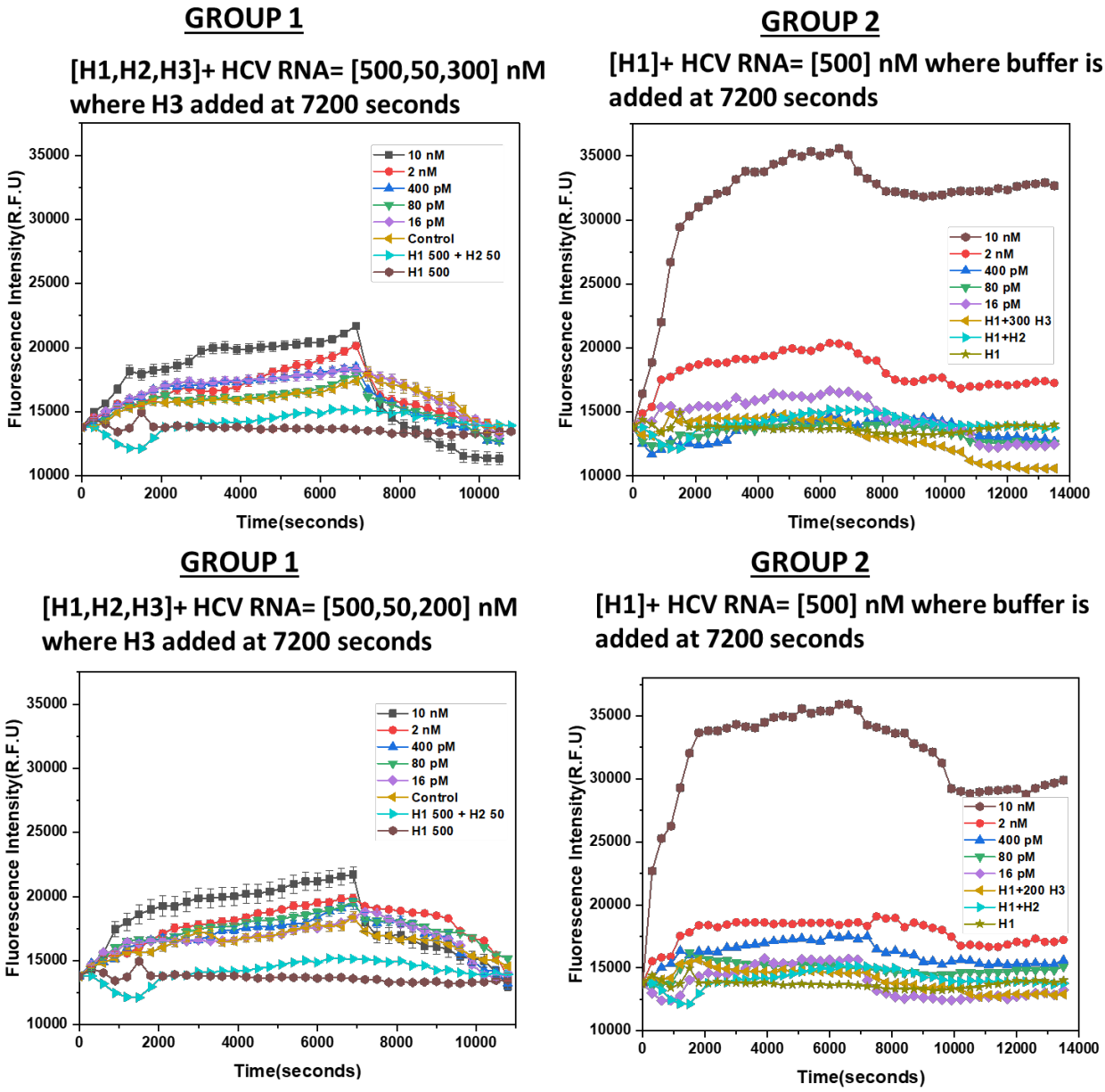

Figure S4. Fluorescence spectroscopy for determining the appropriate concentration of H3. **Group 1**- 500 nm H1 +50 nM H2 + HCV RNA concentrations + 300 nM H3-quencher (**top**), 500 nm H1 +50 nM H2 + HCV RNA concentrations + 200 nM H3-quencher (**bottom**). **Group 2**- 500 nM H1+ HCV RNA concentrations + water + buffer (300 nM, 200 nM H3) (represented by the gold spectrum at **top** and **bottom** respectively). In the case of group 1, H3, buffer and water are added at 7200 seconds. The larger precipitous drop in fluorescence in group 1 indicates target recycling.

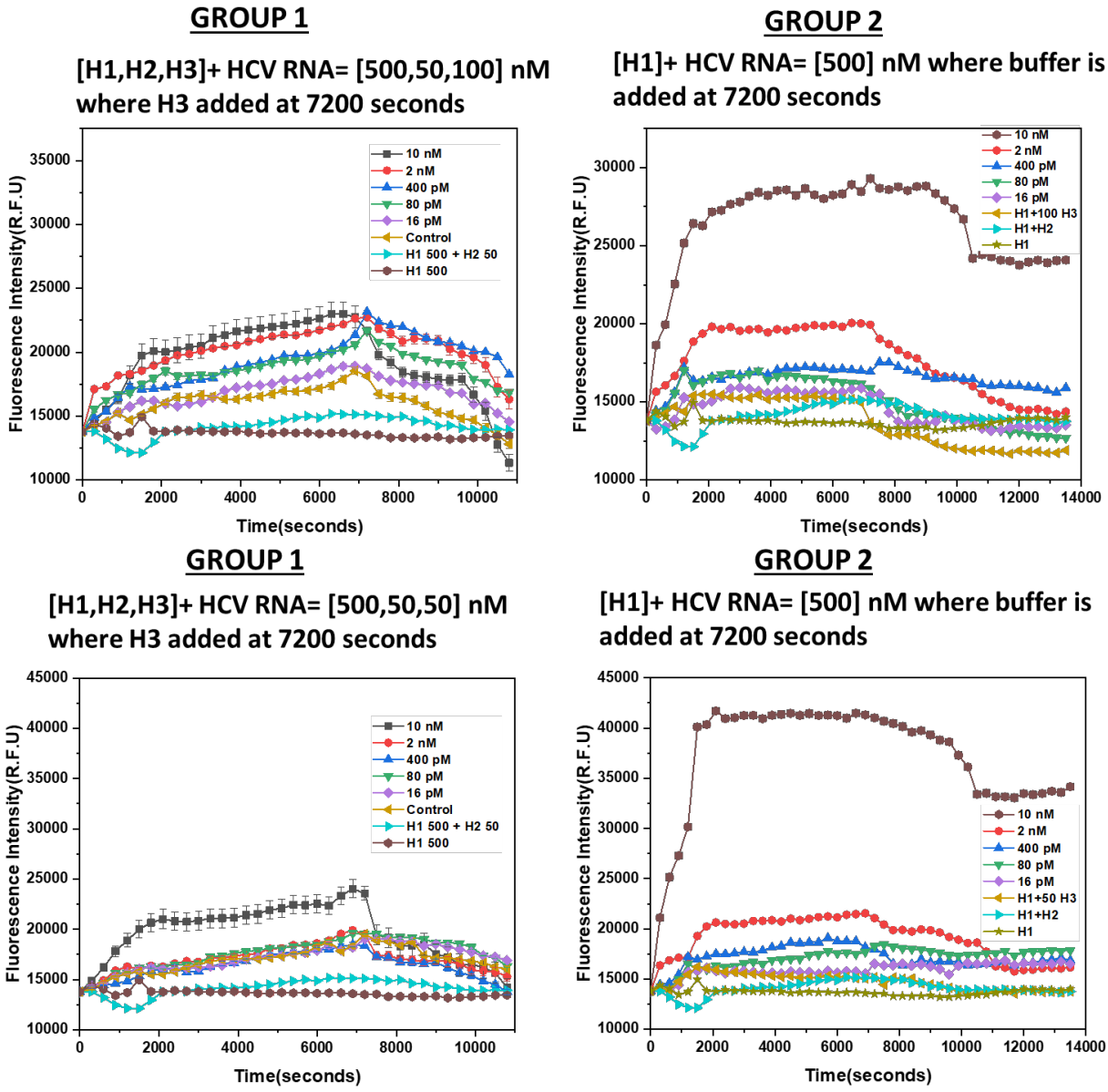

Figure S5. Fluorescence spectroscopy for determining the appropriate concentration of H3. **Group 1**- 500 nm H1 +50 nM H2 + HCV RNA concentrations + 100 nM H3-quencher (**top**), 500 nm H1 +50 nM H2 + HCV RNA concentrations + 50 nM H3-quencher (**bottom**). **Group 2**- 500 nM H1+ HCV RNA concentrations+ water +buffer (100 nM, 50 nM H3) (represented by the gold spectrum at **top** and **bottom** respectively). In the case of group 1, H3, buffer and water are added at 7200 seconds. The larger precipitous drop in fluorescence in group 1 indicates target recycling.

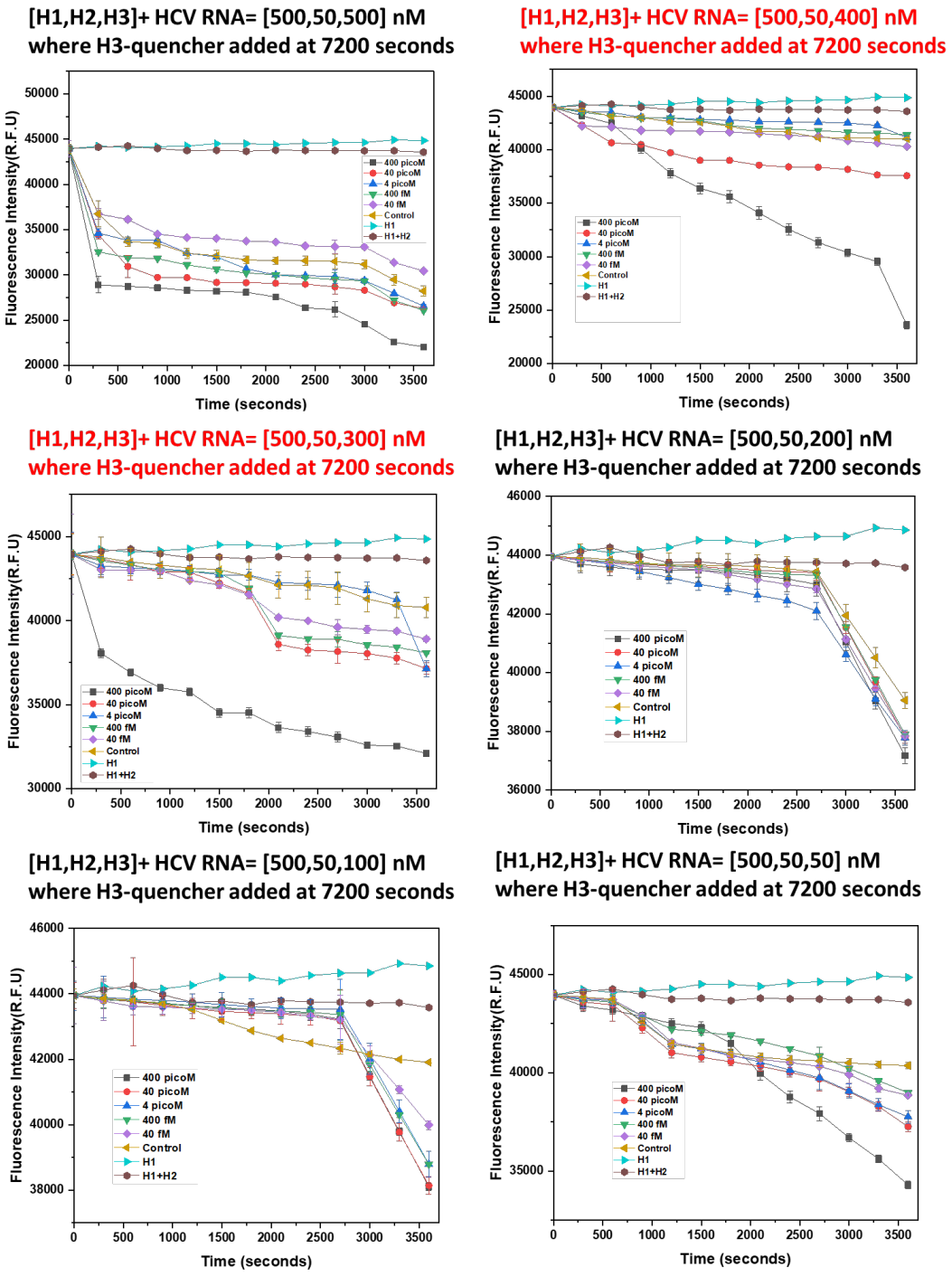

Figure S6. Fluorescence spectroscopy quantification towards LOD determination for HCV RNA concentrations (400 pM - 40 fM, 10-fold dilutions). H1 and H2 are present in concentrations of 500 nM and 50 nM respectively. H3 concentrations of 300 and 400 nM (highlighted in red) appear to provide the best discrimination.

DLS characterization of core satellite nanoparticles

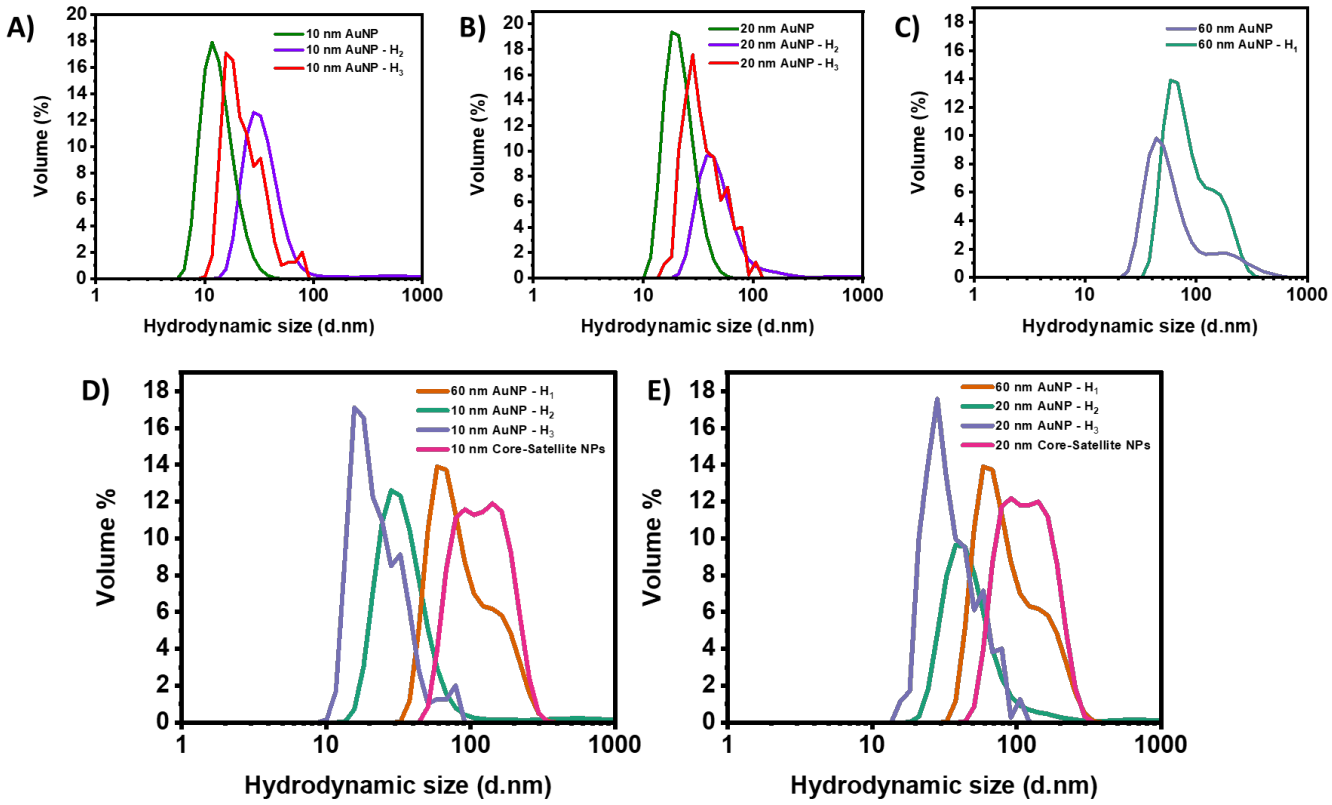

Figure S7. DLS characterization of core (60 nm), satellite (10 and 20 nm) and core-satellite assemblies. A) 10 nm satellite particles before (green) and after the addition of H2 (purple) and H3 (red). B) 20 nm satellite particles before (green) and after the addition of H2 (purple) and H3 (red). C) 60 nm core particles before (purple) and after the addition of H1 (green). D) Comparison of individual core, satellite particles before reaction- 60 nm-H1 (orange), 10 nm-H2 (green), 10 nm-H3 (purple) with the 60-10 nm core satellite assembly (pink). E) Comparison of individual core, satellite particles before reaction- 60 nm-H1 (orange), 20 nm-H2 (green), 20 nm-H3 (purple) with the 60-20 nm core satellite assembly (pink).

Qualitative colorimetric data upon completion of 3wJ CHA of core-satellite nanostructures

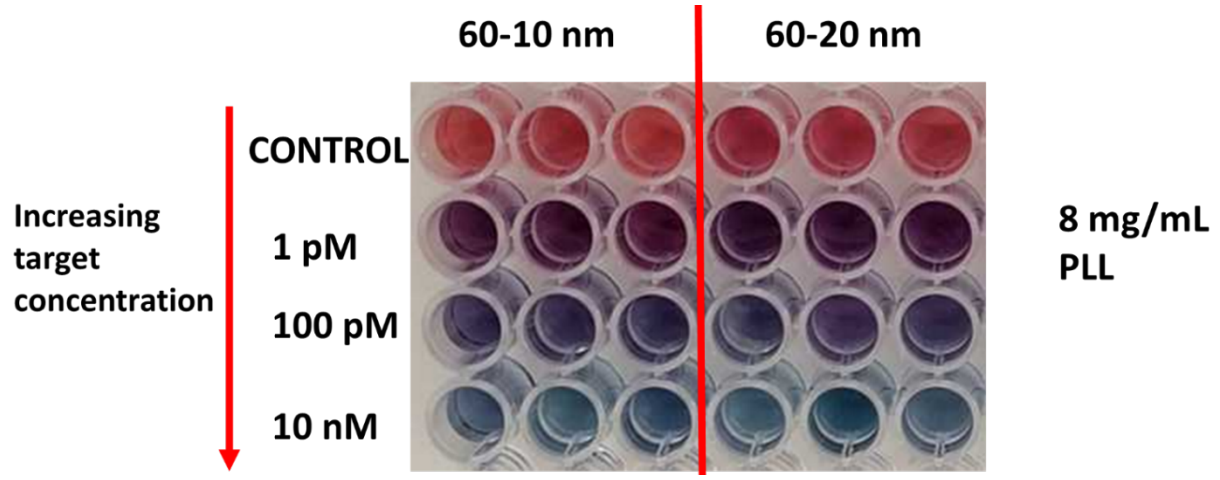

Figure S8. Visual representation of 3-way junction CHA for the 60-10 & 60-20 nm core-satellite assemblies in a clear 96 well plate. An increase in blue color corresponds to an increase in core-satellite assemblies and a decrease in nanoparticle distance which happens with increasing HCV RNA target concentrations10. H1, H2, and H3 were present in 500, 50, and 400 nM respectively with target concentrations (0, 1 pM, 100 pM, and 10 nM).