

# Controlled Release in Hydrogels Using DNA Nanotechnology

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**Table S1.** List of DNA sequences used in this study. Sequences are from 5' to 3'.

| Base Design           |                                                                              |
|-----------------------|------------------------------------------------------------------------------|
| Name                  | Sequence                                                                     |
| 3WJ-1                 | CTGACCGAGCGTGGCTACAGCTTC TTT GGTTCACCGCTTGCCTTCTGCTCT<br>CGCTGTGGCACCTGCACG  |
| 3WJ-2                 | CCTGCTTGCTGATCCACATCTGCT TTT GAAGCTGTAGCCACGCTCGGTCAG<br>GCATCGGGTATCCAGTGG  |
| 3WJ-3                 | AGAGCAGAAGGCAAGCGGTGAACC TTT AGCAGATGTGGATCAGCAAGCAGG<br>TACGGTCGGCGCTGTATG  |
| 4WJ-3                 | AGAGCAGAAGGCAAGCGGTGAACC TTT CGTGA CTGCACCTGAATTGGCACT<br>TGCGCCATCCCTTCAGAG |
| 4WJ-4                 | AGTGCCAATTCAGGTGCAGTCACG TTT AGCAGATGTGGATCAGCAAGCAGG<br>TACGGTCGGCGCTGTATG  |
| DNA Bait              | /Azide/ TTT CGTGCAGGTGCCACAGCG                                               |
| Trigger 1             | CATAACACA TCT CACAATCCA TCT CACCACCCA                                        |
| Payload 1 Top         | CACAATCCA TCT CACCACCCA /Iowa Black FQ/                                      |
| Payload 1 Bottom      | /HEX/ TGGGTGGTG AGA TGGATTGTG AGA TGTG AGA CATA CAGCGCCGACCGTA               |
| Trigger 2             | CACAATCCA TCT CACCACCCA TCT CAAA AACTCA                                      |
| Payload 2 Top         | CACCACCCA TCT CAAA AACTCA /Iowa Black FQ/                                    |
| Payload 2 Bottom      | /FAM/ TGAGTTT TG AGA TGGGTGGTG AGA TGG A AGA CCACTGGATACCCGATGC              |
| Trigger 3             | CACCACCCA TCT CAAA AACTCA TCT CATCCAACA                                      |
| Payload 3 Top         | CAAA AACTCA TCT CATCCAACA /Iowa Black SQ/                                    |
| Payload 3 Bottom      | /Texas-Red/ TGT TGGATG AGA TGAGTTT TG AGA TGGG AGA CTCTGAAGGGATGGCGCA        |
| Bait Probe            | /HEX/ TTT CGCTGTGGCACCTGCACG                                                 |
| Mismatched Triggers   |                                                                              |
| Name                  | Sequence                                                                     |
| 1bp-center<br>(TrigA) | CATAACACA TCT CACA T TCCA TCT CACCACCCA                                      |
| 2bp-center<br>(TrigB) | CATAACACA TCT CACA TG CCA TCT CACCACCCA                                      |
| 3bp-center<br>(TrigC) | CATAACACA TCT CAC TTG CCA TCT CACCACCCA                                      |

|                       |                                         |
|-----------------------|-----------------------------------------|
| 5bp-center<br>(TrigD) | CATAACACA TCT CA ATTGG CA TCT CACCACCCA |
| 1bp-3' end<br>(TrigE) | CATAACACA TCT CACAATCCA TCT CACC G CCA  |
| 2bp-3' end<br>(TrigF) | CATAACACA TCT CACAATCCA TCT CACC GG CCA |
| 3bp-3' end<br>(TrigG) | CATAACACA TCT CACAATCCA TCT CAC AGG CCA |
| 5bp-3' end<br>(TrigH) | CATAACACA TCT CACAATCCA TCT CA AAGGT CA |

**Table S2.** Individual measurements of gelatin modification quantification via fluorescamine.

| Theoretical [DBCO] ( $\mu\text{M}$ ) | Measured [DBCO] ( $\mu\text{M}$ ) | Efficiency |
|--------------------------------------|-----------------------------------|------------|
| 135                                  | 142.43                            | 106%       |
| 135                                  | 78.87                             | 58%        |
| 135                                  | 74.83                             | 55%        |
| 135                                  | 113.09                            | 84%        |
| 135                                  | 122.14                            | 90%        |
| 135                                  | 166.90                            | 124%       |

**Table S3.** Individual measurements of DNA overhang quantification via overhang probe.

| Theoretical [DNA Overhang]<br>( $\mu\text{M}$ ) | Measured [DNA Overhang]<br>( $\mu\text{M}$ ) | Efficiency |
|-------------------------------------------------|----------------------------------------------|------------|
| 45                                              | 20.71                                        | 46%        |
| 45                                              | 31.06                                        | 69%        |
| 45                                              | 42.78                                        | 95%        |
| 45                                              | 19.14                                        | 43%        |
| 45                                              | 21.49                                        | 48%        |

**Table S4.** 50% Release Time ( $T_{50}$ ) for 4WJ.

| 4WJ Type | Measured $T_{50}$ (mins) | Fitted $T_{50}$ (mins) |
|----------|--------------------------|------------------------|
| HEX      | 9.5                      | 9.71                   |
| FAM      | 11.7                     | 12.01                  |
| TR       | 10.6                     | 9.69                   |

**Table S5.** Dunn's Kruskal-Wallis Multiple Comparison *post-hoc* analysis table for mismatch trigger release, which corresponds to Figure 3d,f in the main text.

| No. | Comparison                        | Z Score    | p Value     |
|-----|-----------------------------------|------------|-------------|
| 1   | 4WJ_HEX_MT1_13' - 4WJ_HEX_MT1_1C  | 1.2396648  | 0.2150994   |
| 2   | 4WJ_HEX_MT1_13' - 4WJ_HEX_MT1_23' | 1.1986678  | 0.2306572   |
| 3   | 4WJ_HEX_MT1_1C - 4WJ_HEX_MT1_23'  | -0.102012  | 0.9187471   |
| 4   | 4WJ_HEX_MT1_13' - 4WJ_HEX_MT1_2C  | 2.5746262  | 0.01003485  |
| 5   | 4WJ_HEX_MT1_1C - 4WJ_HEX_MT1_2C   | 1.2082823  | 0.2269387   |
| 6   | 4WJ_HEX_MT1_23' - 4WJ_HEX_MT1_2C  | 1.3862501  | 0.1656706   |
| 7   | 4WJ_HEX_MT1_13' - 4WJ_HEX_MT1_33' | 3.421316   | 0.000623189 |
| 8   | 4WJ_HEX_MT1_1C - 4WJ_HEX_MT1_33'  | 2.058649   | 0.03952788  |
| 9   | 4WJ_HEX_MT1_23' - 4WJ_HEX_MT1_33' | 2.2775367  | 0.0227542   |
| 10  | 4WJ_HEX_MT1_2C - 4WJ_HEX_MT1_33'  | 0.9402715  | 0.3470783   |
| 11  | 4WJ_HEX_MT1_13' - 4WJ_HEX_MT1_3C  | 4.4411638  | 8.94737E-06 |
| 12  | 4WJ_HEX_MT1_1C - 4WJ_HEX_MT1_3C   | 3.0207058  | 0.002521862 |
| 13  | 4WJ_HEX_MT1_23' - 4WJ_HEX_MT1_3C  | 3.3008608  | 0.000963887 |
| 14  | 4WJ_HEX_MT1_2C - 4WJ_HEX_MT1_3C   | 1.9576411  | 0.05027214  |
| 15  | 4WJ_HEX_MT1_33' - 4WJ_HEX_MT1_3C  | 0.9743393  | 0.3298881   |
| 16  | 4WJ_HEX_MT1_13' - 4WJ_HEX_MT1_53  | 5.4375361  | 5.40224E-08 |
| 17  | 4WJ_HEX_MT1_1C - 4WJ_HEX_MT1_53   | 3.9881917  | 6.65789E-05 |
| 18  | 4WJ_HEX_MT1_23' - 4WJ_HEX_MT1_53  | 4.3228951  | 1.53995E-05 |
| 19  | 4WJ_HEX_MT1_2C - 4WJ_HEX_MT1_53   | 3.0026453  | 0.002676442 |
| 20  | 4WJ_HEX_MT1_33' - 4WJ_HEX_MT1_53  | 1.9963735  | 0.04589328  |
| 21  | 4WJ_HEX_MT1_3C - 4WJ_HEX_MT1_53   | 1.0450042  | 0.296021    |
| 22  | 4WJ_HEX_MT1_13' - 4WJ_HEX_MT1_5C  | 6.2147066  | 5.14206E-10 |
| 23  | 4WJ_HEX_MT1_1C - 4WJ_HEX_MT1_5C   | 4.7428306  | 2.10752E-06 |
| 24  | 4WJ_HEX_MT1_23' - 4WJ_HEX_MT1_5C  | 5.1200818  | 3.05403E-07 |
| 25  | 4WJ_HEX_MT1_2C - 4WJ_HEX_MT1_5C   | 3.8177485  | 0.000134675 |
| 26  | 4WJ_HEX_MT1_33' - 4WJ_HEX_MT1_5C  | 2.7935602  | 0.005213132 |
| 27  | 4WJ_HEX_MT1_3C - 4WJ_HEX_MT1_5C   | 1.8601074  | 0.06287033  |
| 28  | 4WJ_HEX_MT1_53 - 4WJ_HEX_MT1_5C   | 0.8151032  | 0.4150132   |
| 29  | 4WJ_HEX_MT1_13' - 4WJ_HEX_T1      | -0.6403353 | 0.5219546   |
| 30  | 4WJ_HEX_MT1_1C - 4WJ_HEX_T1       | -1.913472  | 0.05568765  |
| 31  | 4WJ_HEX_MT1_23' - 4WJ_HEX_T1      | -1.9115137 | 0.0559386   |
| 32  | 4WJ_HEX_MT1_2C - 4WJ_HEX_T1       | -3.3718801 | 0.00074657  |
| 33  | 4WJ_HEX_MT1_33' - 4WJ_HEX_T1      | -4.2380353 | 2.25484E-05 |
| 34  | 4WJ_HEX_MT1_3C - 4WJ_HEX_T1       | -5.3295212 | 9.8472E-08  |
| 35  | 4WJ_HEX_MT1_53 - 4WJ_HEX_T1       | -6.3745254 | 1.83531E-10 |
| 36  | 4WJ_HEX_MT1_5C - 4WJ_HEX_T1       | -7.1896286 | 6.49678E-13 |

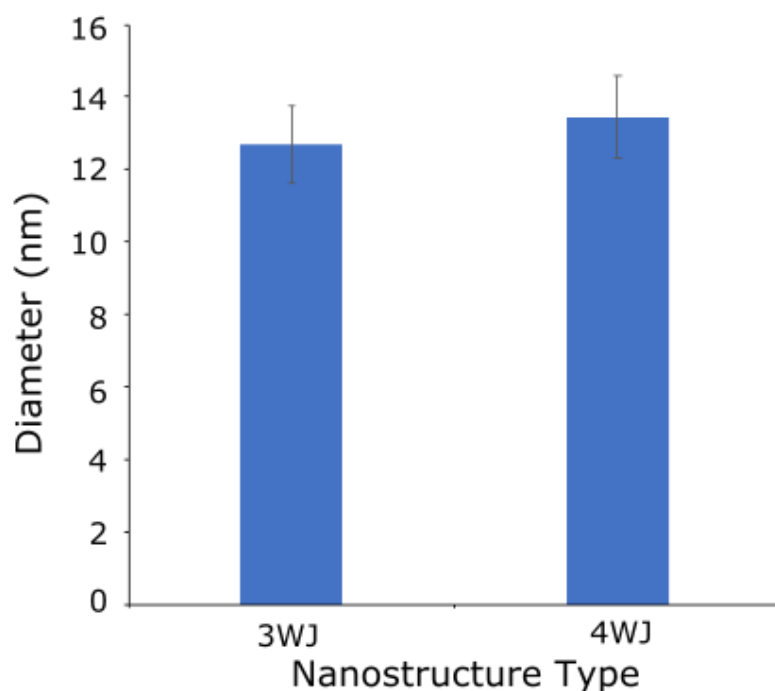


**Figure S1.** DLS Size Measurements for Bare 3WJ and 4WJ. (Welch two sample t-test,  $n = 9$ ,  $p > 0.05$ ).

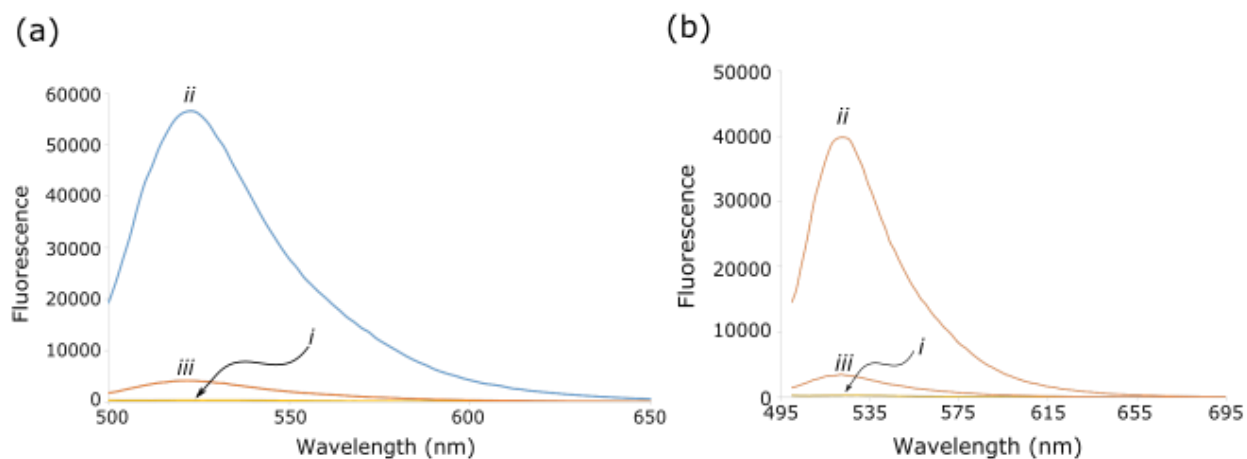


**Figure S2.** Fluorescent quantification of 4WJ (a) Fluorescent quantification of 4WJ-FAM. The excitation wavelength of FAM was 455 nm and emission spectrum was recorded from 500 nm to 800 nm. (b) Fluorescent quantification of 4WJ-TR. The excitation wavelength of Texas-Red was 555 nm and emission spectrum was recorded from 590 nm to 800 nm. (i) bare 4WJ (ii) 4WJ-1F (iii) 4WJ-1FQ.

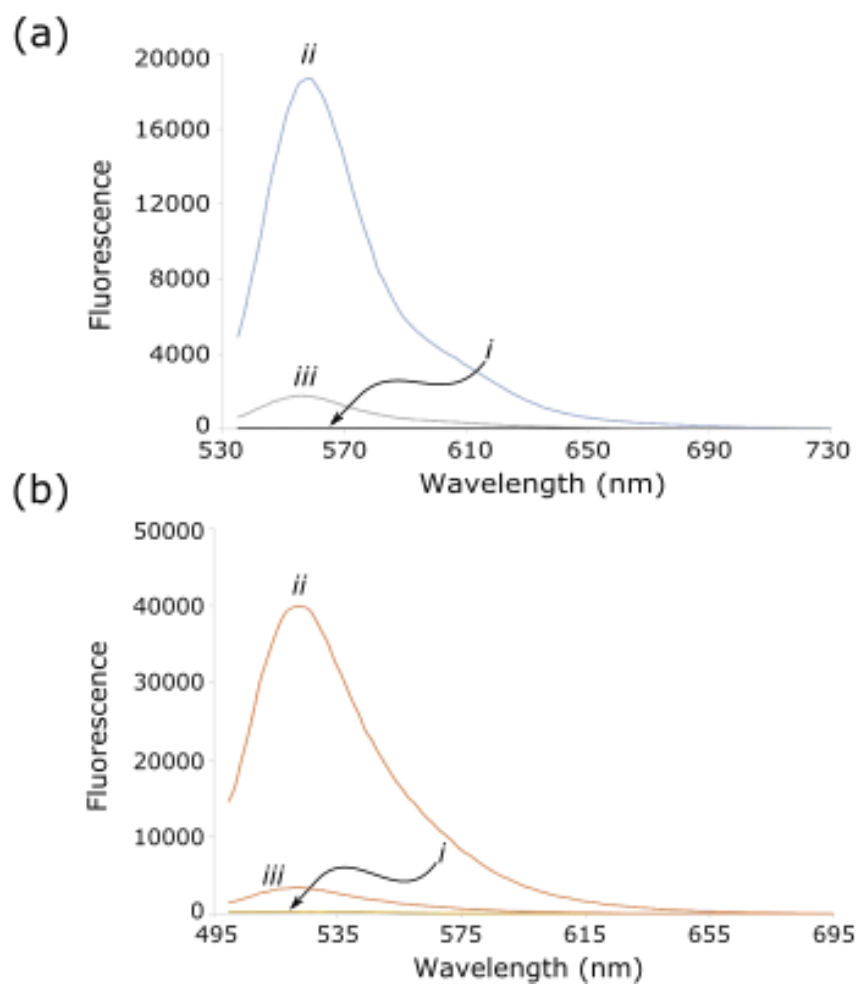


**Figure S3.** Fluorescent quantification of 3WJ **(a)** 3WJ-HEX. The excitation wavelength of HEX was 495 nm and emission spectrum was recorded from 535 nm to 800 nm **(b)** 3WJ-FAM. The excitation wavelength of FAM was 455 nm and emission spectrum was recorded from 500 nm to 800 nm. (i) bare 3WJ (ii) 3WJ-1F (iii) 3WJ-1FQ.

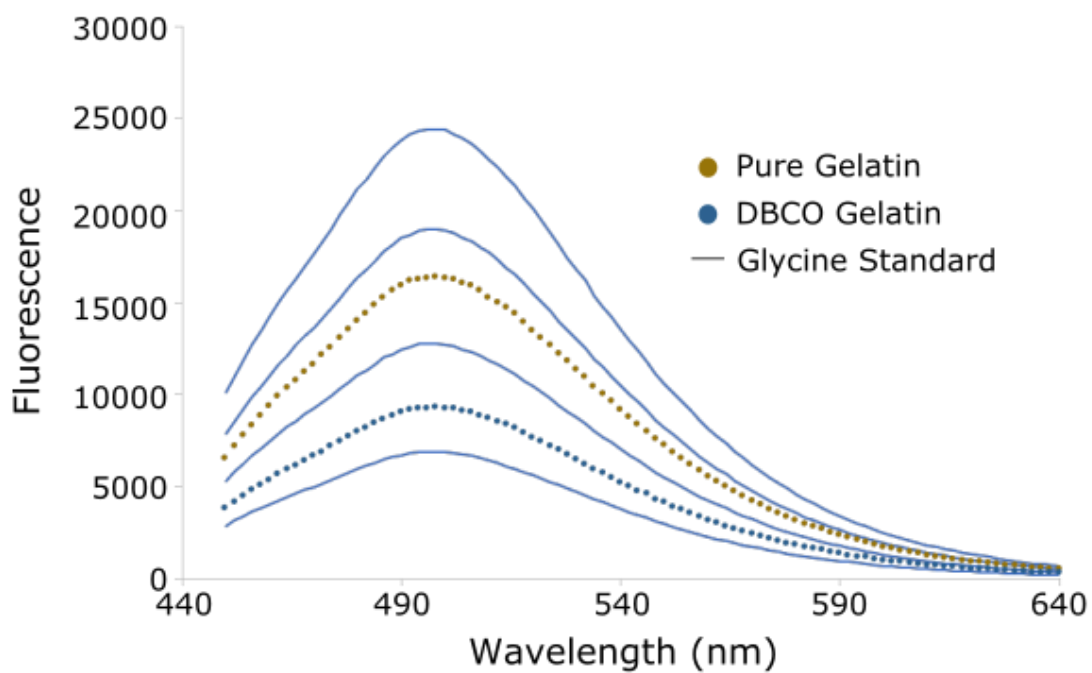


**Figure S4.** Representative Fluorescence Curves of Fluorescamine for Both Glycine Standard and Pure and DBCO Gelatin Samples. Standard range from 50–200 nM of Glycine. Fluorescamine was excited at a wavelength of 380 nm and the emission spectrum was recorded from 450 nm to 750 nm with step size of 2 nm.

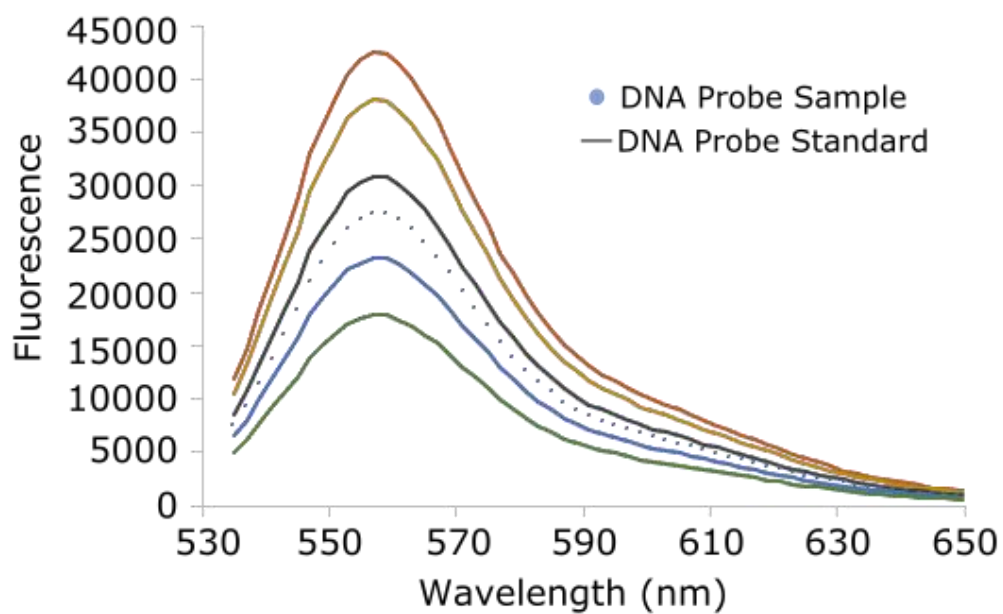


**Figure S5.** Representative Fluorescence Curves of DNA Bait Quantification for Both DNA Probe Standard and Sample. Standard range from 300 to 700 nM of DNA Probe. The excitation wavelength of HEX was 495 nm and emission spectrum was recorded from 535 nm to 800 nm.

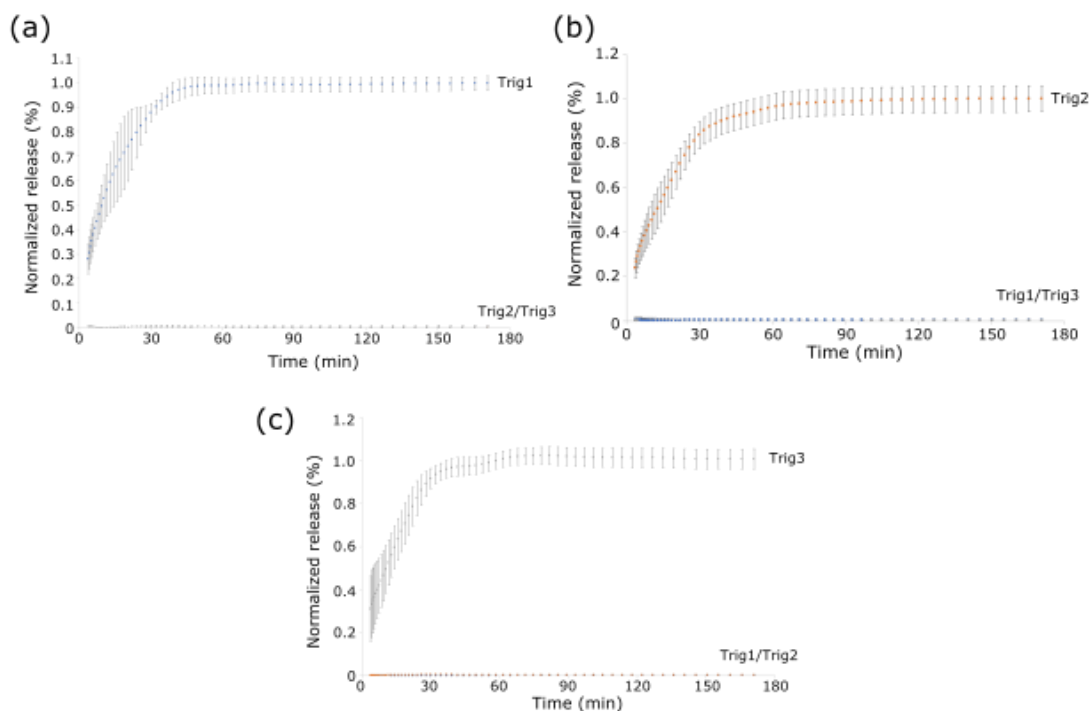


**Figure S6.** 4WJ Release Calibration within Solution. (a) 4WJ-HEX. The excitation for HEX is 540 nm and the emission was recorded at 570 nm. (b) 4WJ-FAM. The excitation for FAM is 465 nm and the emission was recorded at 510 nm. (c) 4WJ-TR. The excitation for TR is 585 nm and the emission was recorded at 618 nm.

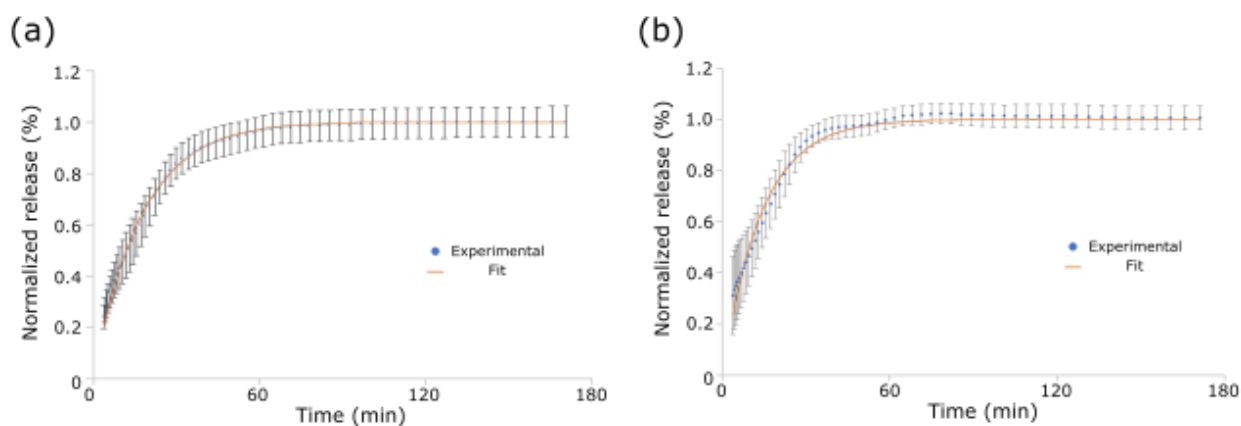


**Figure S7.** 4WJ release in solution (a) 4WJ-FAM. The excitation for FAM is 465 nm and the emission was recorded at 510 nm. (b) 4WJ-TR. The excitation for TR is 585 nm and the emission was recorded at 618 nm.

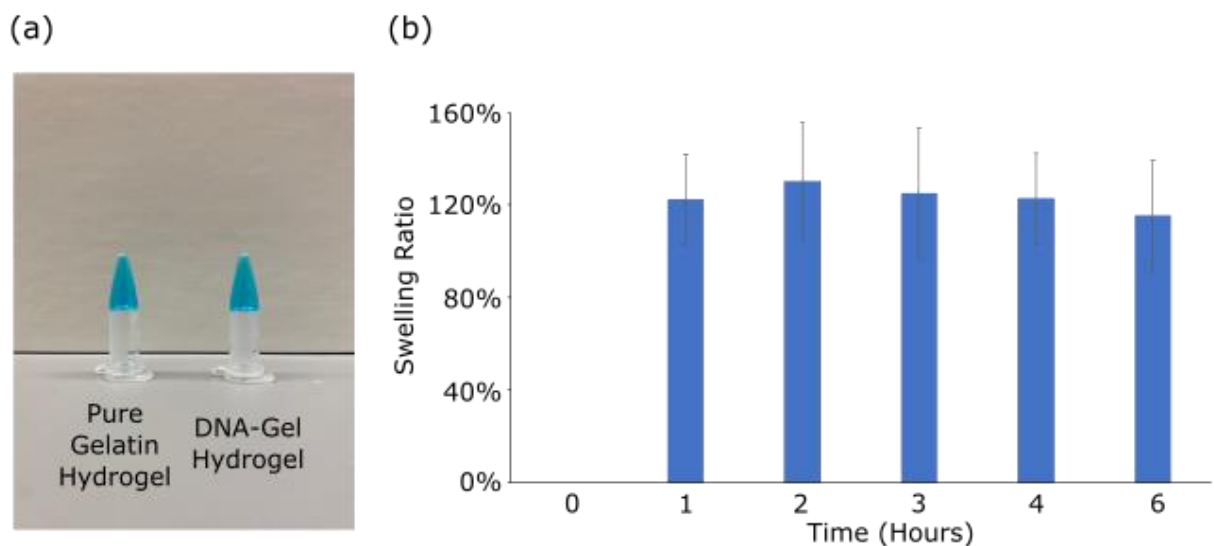


**Figure S8.** Hydrogel characterization (a) Image of both pure gelatin hydrogel and DNA-Gel hydrogel in a tube. Blue ink was added to better visualize the hydrogel within the tube. (b) Swelling ratio of the pure gelatin hydrogel in 1X PBS (pH 7.4) at room temperature over 6 h (n = 3).

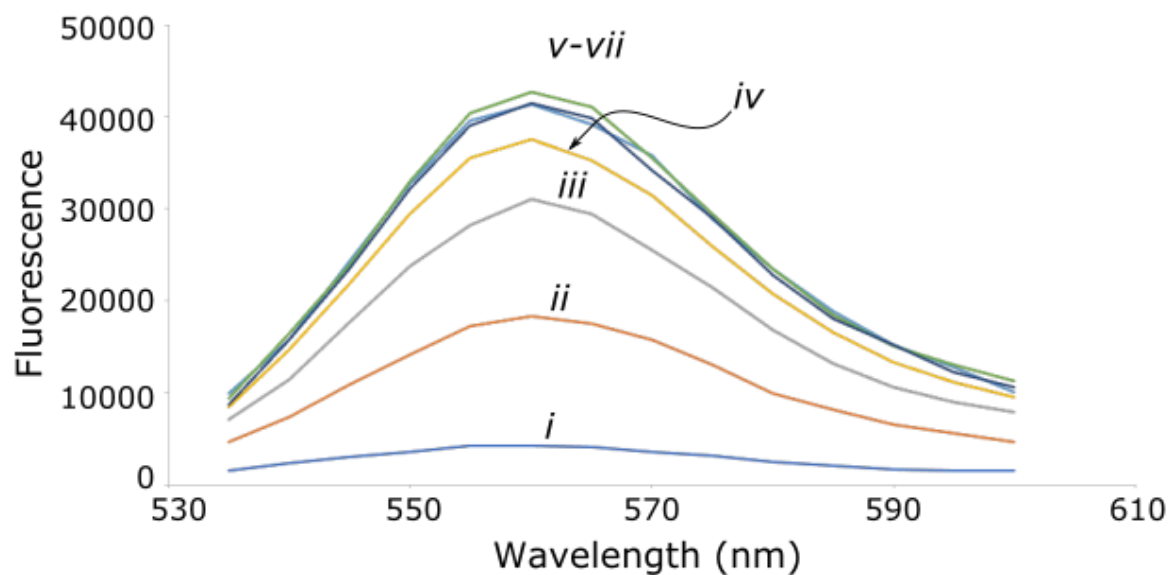


**Figure S9.** Representative fluorescence curves for the 4WJ-HEX release in DNA-Gel. (i) 0 h (ii) 1 h (iii) 3 h (iv) 5 h (v) 7 h (vi) 12 h (vii) 17 h.

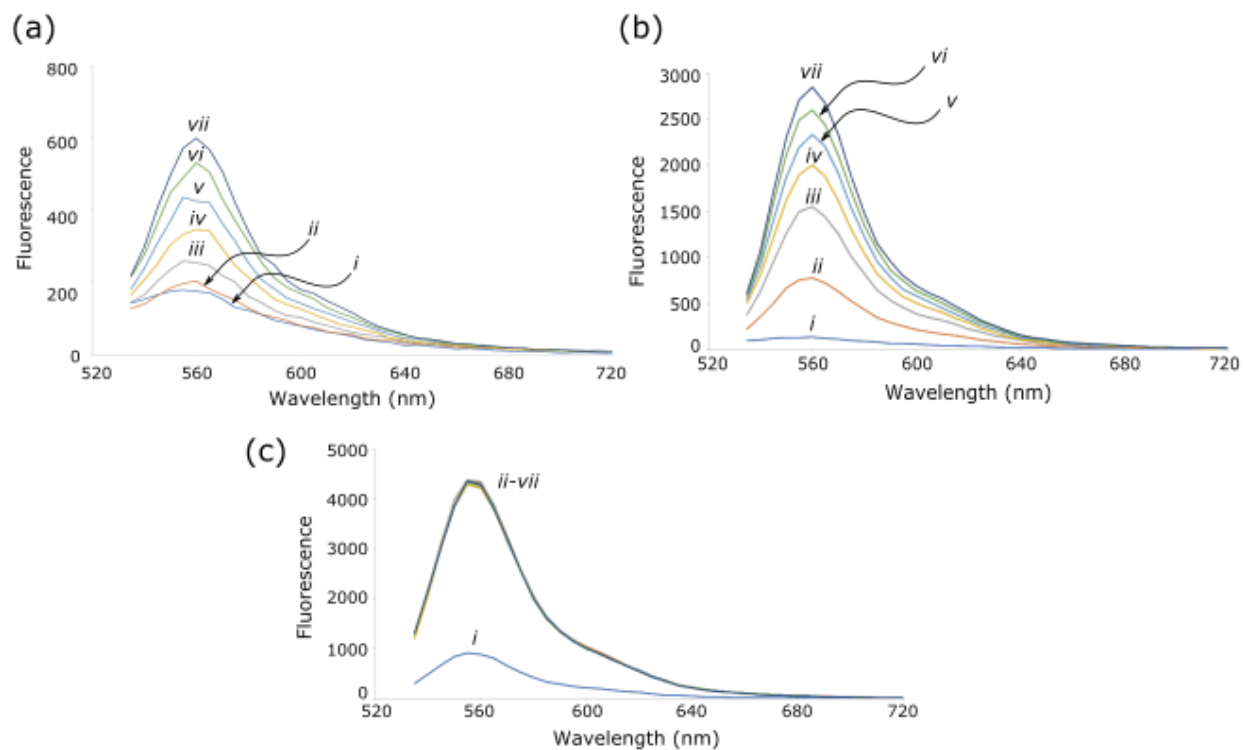


**Figure S10.** Representative fluorescence curves for the stability study for 4WJ-HEX **(a)** under serum condition in DNA-Gel hydrogel, **(b)** under DNase condition in DNA-Gel hydrogel, and **(c)** under DNase condition in solution. (i) 0 h (ii) 4 h (iii) 8 h (iv) 12 h (v) 16 hours (vi) 20 h (vii) 24 h.