Supporting Information

Reconfigurable DNA Origami Nanocapsule for pH-Controlled Encapsulation and Display of Cargo

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Supplementary methods

Agarose gel electrophoresis (AGE). 2% agarose gels were prepared in two different buffer systems: 1× TAE with 11 mM $MgCl_2$ (pH 8.2) or 45 mM MES, 25 mM Tris with 11 mM $MgCl_2$ (pH 6.4). Gels were run for 50 minutes with 90 V on an ice bath. Normal gels were dyed with ethidium bromide and samples loaded with New England Biolabs 6× loading dye, and imaged with BioRad ChemiDoc XRS+. Fluorescent gels were prepared by loading samples with self-made loading dye (1× containing 2.5 w-% Ficoll 400, 3.3 mM Tris-HCl and 0.015 w-% bromophenol blue) and imaged with BioRad ChemiDoc MP imaging system using channels Alexa Fluor 488 (excitation filter 470/30 and emission filter 532/28) and Alexa Fluor 546 (excitation filter 530/28 and emission filter 602/50).

$MgCl_2$ depletion FRET experiments. To study the stability of the closed pHL nanocapsules in low Mg^{2+} (0.6 mM) concentration and subsequent introduction into physiological (150 mM) NaCl concentration, pHL nanocapsules in 1× FOB (pH 6.4) were exchanged to low-Mg^{2+} buffer by spin-filtration. Samples were first diluted with 11 mM Tris buffer at pH 6.4, so that final concentrations of buffer components were 11.2 mM Tris, 0.8 mM acetic acid, 0.04 mM EDTA, 0.6 mM $MgCl_2$, and 0.2 mM NaCl. The diluted sample was then concentrated back to original volume with 100 kDa MWCO Amicon Ultra 100 kDa cutoff centrifugal filters (6000 rcf). For 150 mM NaCl sample, NaCl concentration was adjusted after spin-filtration by adding 5 M NaCl. Emission spectra were collected similarly to previous FRET experiments described in the text

FRET measurements in the presence of blood plasma. In order to test whether closed pHL nanocapsules remain in a closed state after introduction to plasma, emission spectra were measured from pHL nanocapsules in 1× FOB (pH 6.4) after addition of either 1% or 10% plasma from human (Sigma). Emission spectrum of 1% plasma sample was measured 110 min after addition of plasma. 10% plasma sample was first incubated for 72 min in the presence of 1% plasma, after which plasma volume was increased to 10% and spectrum was collected after 40 minutes of incubation. Changes in the FRET signal were first followed with kinetic measurements to ensure that fluorescence signals had stabilized. Emission spectra were then collected similarly to previous FRET experiments described in the text.

Supplementary figures

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- S2. Agarose gel elctrophoresis (AGE)
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- S3. Sample aggregation at low pH and at high Mg^{2+} concentrations
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Supplementary tables S1–S5. List of staple strands.

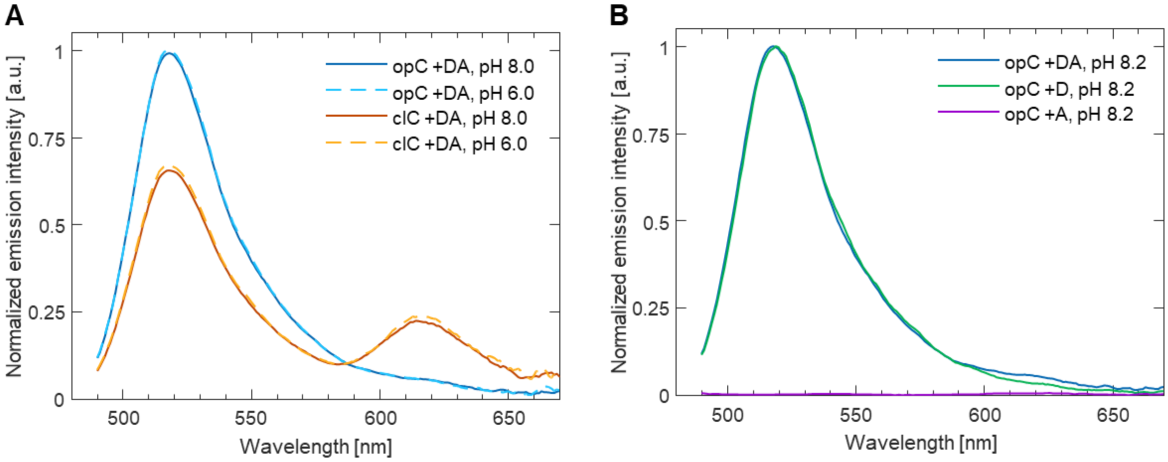

Figure S1. Additional FRET controls. (A) Emission spectra of permanently open (opC) and closed (clC) control samples labeled with both A488 (D) and A594 (A), and measured at pH 6.0 and 8.0 after 460 nm excitation. Emission profiles and FRET efficiencies of the samples are not affected by pH change, indicating that the conformation of opC and clC is not affected by pH and furthermore, that pH change alone does not affect the emission properties of the FRET pair. (B) Comparison of opC sample emission after 460 nm excitation when labeled either with both FRET dyes (opC +DA), or partially labeled with only the donor (opC +D) or acceptor (opC +A). Direct acceptor excitation at the applied donor excitation wavelength is minimal, as seen in the lack of fluorescence in the opC +A sample. Comparison of opC +D to the opC +DA sample shows that even in the open state, presence of the donor leads to a low acceptor emission indicating FRET. All spectra have been normalized to acceptor emission intensity in the sample after excitation at 560 nm. Acceptor intensity after direct excitation at a wavelength where the donor does not absorb can be used as an internal reference for fluorophore concentration.

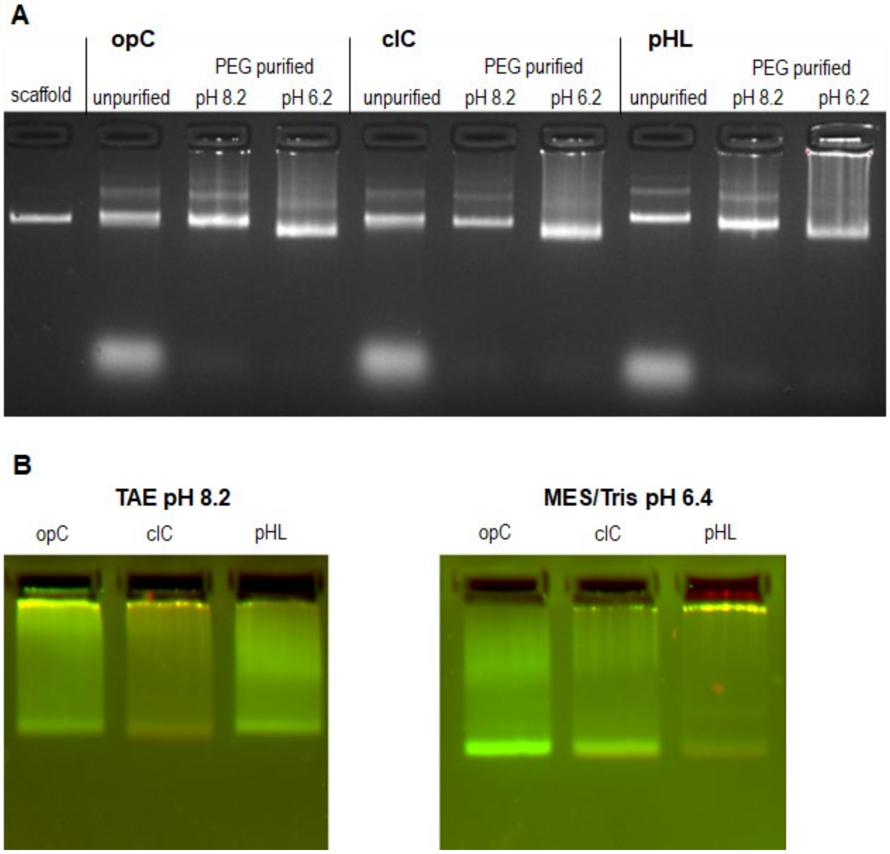

Figure S2. Characterization of the folding quality and electrophoretic mobility of opC, clC, and pHL nanocapsules with agarose gel electrophoresis (AGE). (A) Comparison of the electrophoretic mobility of different sample types before PEG purification of excess staples, and after PEG purification of excess staples and resuspension into 1× FOB (1× TAE, 15 mM $MgCl_2$, 5 mM NaCl) at either pH 8.2 or pH 6.2. Electrophoretic mobility of the nanocapsules is not observably affected by the open/closed state. (B) FRET-labeled capsule samples in 2% agarose gels at either pH 8.2 or pH 6.4. Green channel: donor excitation and detection; red channel: acceptor excitation and detection.

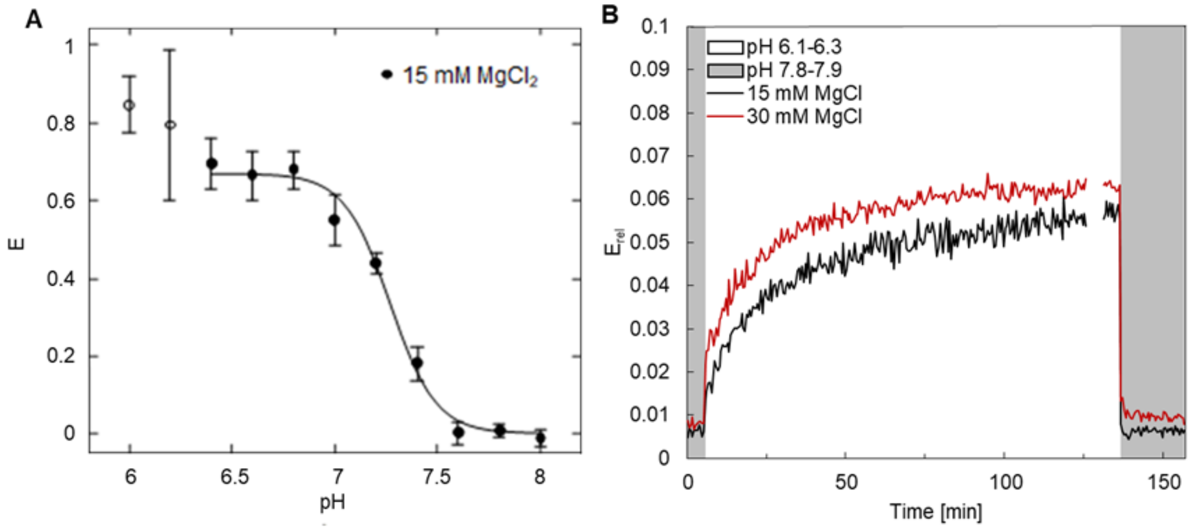

Figure S3. Aggregation of nanocapsules. (A) FRET efficiency of pHL nanocapsules with datapoints measured below pH 6.4. The increase of FRET efficiency at pH < 6.4 (uncolored data points) was accounted for sample aggregation and/or instability at low pH. (B) Kinetic measurement of the closing of pH-latch samples at 15 mM $MgCl_2$ and 30 mM $MgCl_2$. Curves show that the FRET efficiency values or closing kinetics are not significantly different between the 30 mM sample and the 15 mM sample, but the fluorescence intensity recordings of the 30 mM sample (raw data, not shown) show heavy fluctuation of the signal linked to scattering produced by large aggregates.

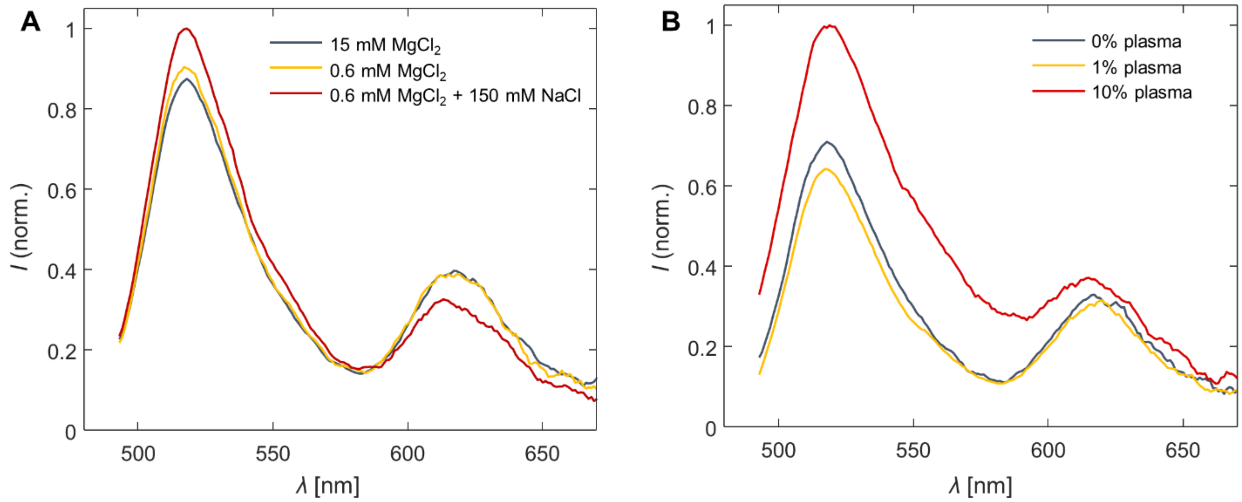

Figure S4. Effect of Mg^{2+} depletion and blood plasma on closed pHL nanocapsules at pH 6.4 studied with FRET. (A) Emission spectra of pHL nanocapsules in 1× FOB pH 6.4 (containing 15 mM $MgCl_2$, 5 mM NaCl), after buffer exchange to low-Mg^{2+} buffer (0.6 mM $MgCl_2$, 0.2 mM NaCl, pH 6.4), and after addition of 150 mM NaCl into theC low-Mg^{2+} sample. FRET efficiency decreases *ca.* 22% upon addition of NaCl. (B) Emission spectra of pHL nanocapsules in 1× FOB (pH 6.4) supplemented with different volumes of plasma. 10% plasma significantly obstructs the fluorescence measurement through increased scattering, preventing a reliable calculation of FRET efficiency from the spectrum. 10% plasma was also observed to increase sample pH (from 6.4 to above 7.0). All spectra in subfigures (A) and (B) have been scaled according to acceptor emission intensity at 616 nm after 560 nm direct excitation.

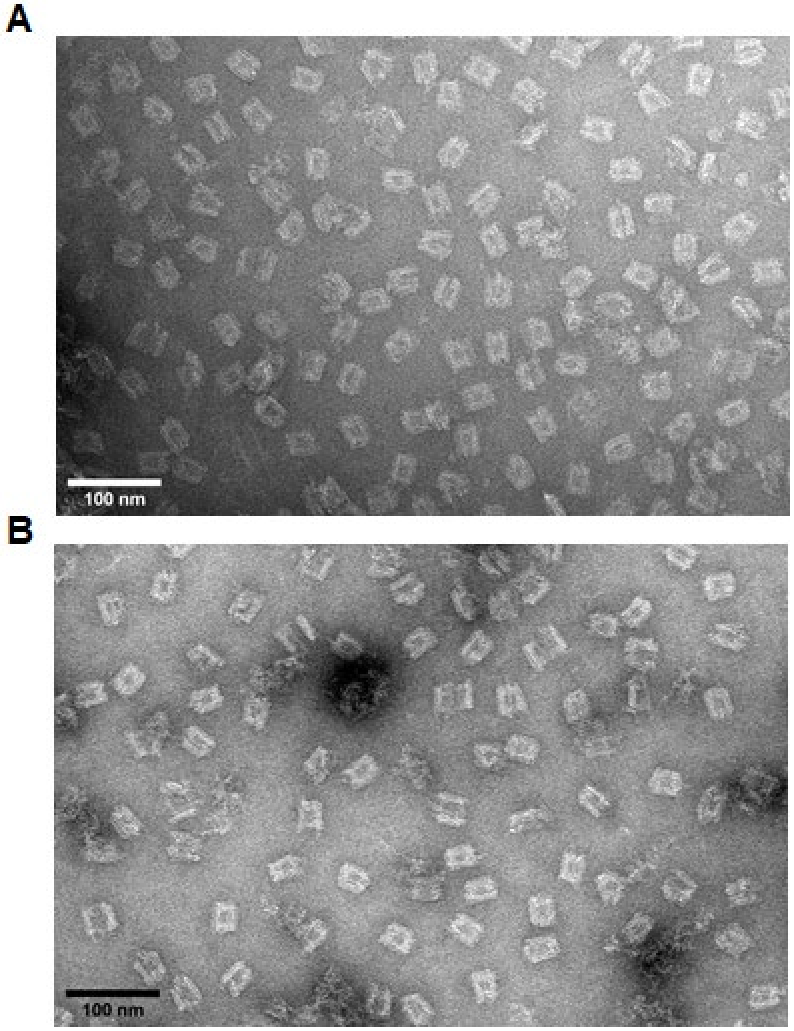

Figure S5. TEM verification of structural damage caused to the clC sample by repeated pH cycling. (A) clC sample at pH 6.3 after the first addition of acetic acid (pH decrease). (B) The same sample at pH 6.3, after changing the pH five times between 7.7 and 6.3 with additions of acetic acid or sodium hydroxide. It has been noted the $MgCl_2$ concentration decreases to ~11.8 mM due to the acid and base additions but is not considered as the main cause for the observed decrease of E_{REL} in the closed control, since the drops occur only with each NaOH addition.

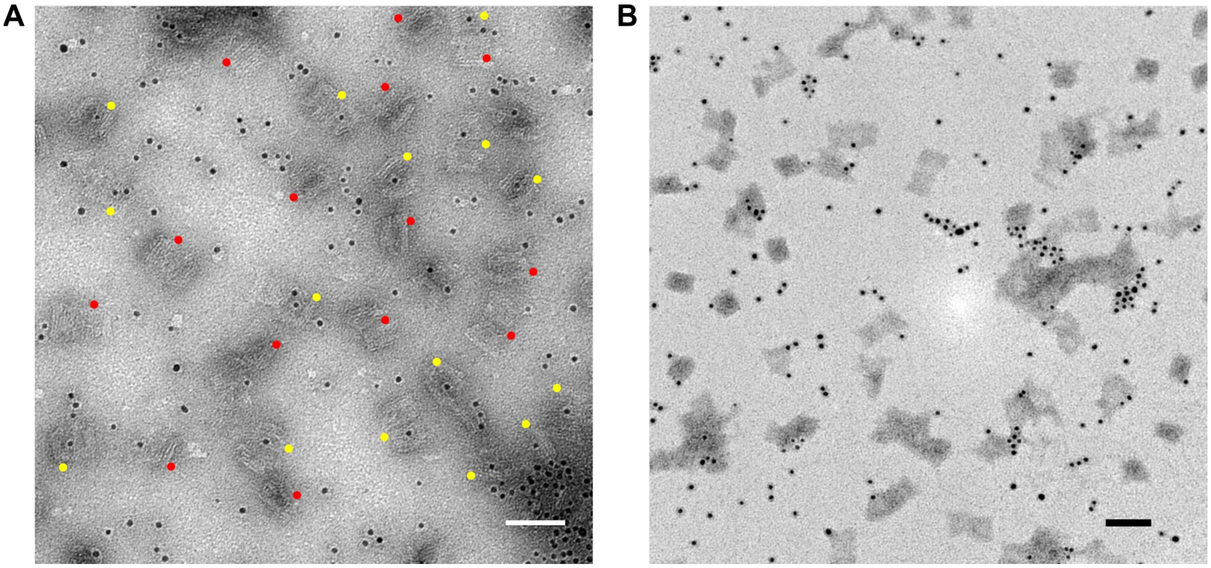

Figure S6. Additional TEM images of AuNP-loaded nanocapsules. (A) A typical sample of AuNP-loaded pHL nanocapsules in the open state used for loading yield estimation. Empty nanocapsules are marked with a red dot, and structures with a yellow dot contain a nanoparticle in the specified cargo-anchoring location. In this case, n = 29 and 52% of the capsules are loaded successfully. (B) When capsules are closed they cannot be loaded with AuNPs. Scale bars are 50 nm.

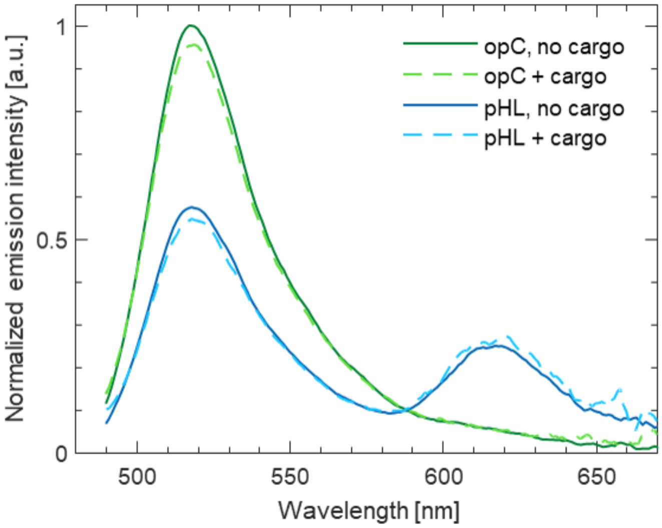

Figure S7. Emission spectra of phL and opC samples at pH 6.4 with and without HRP cargo. HRP loading does not change the emission properties of the nanocapsules labeled with a FRET pair. Most importantly, FRET efficiency in the pHL capsule does not decrease upon cargo loading indicating that cargo loading does not hinder the formation of the closed state. The spectra have been obtained with 460 nm excitation and normalized for concentration differences with the acceptor emission intensity collected after 560 nm excitation.

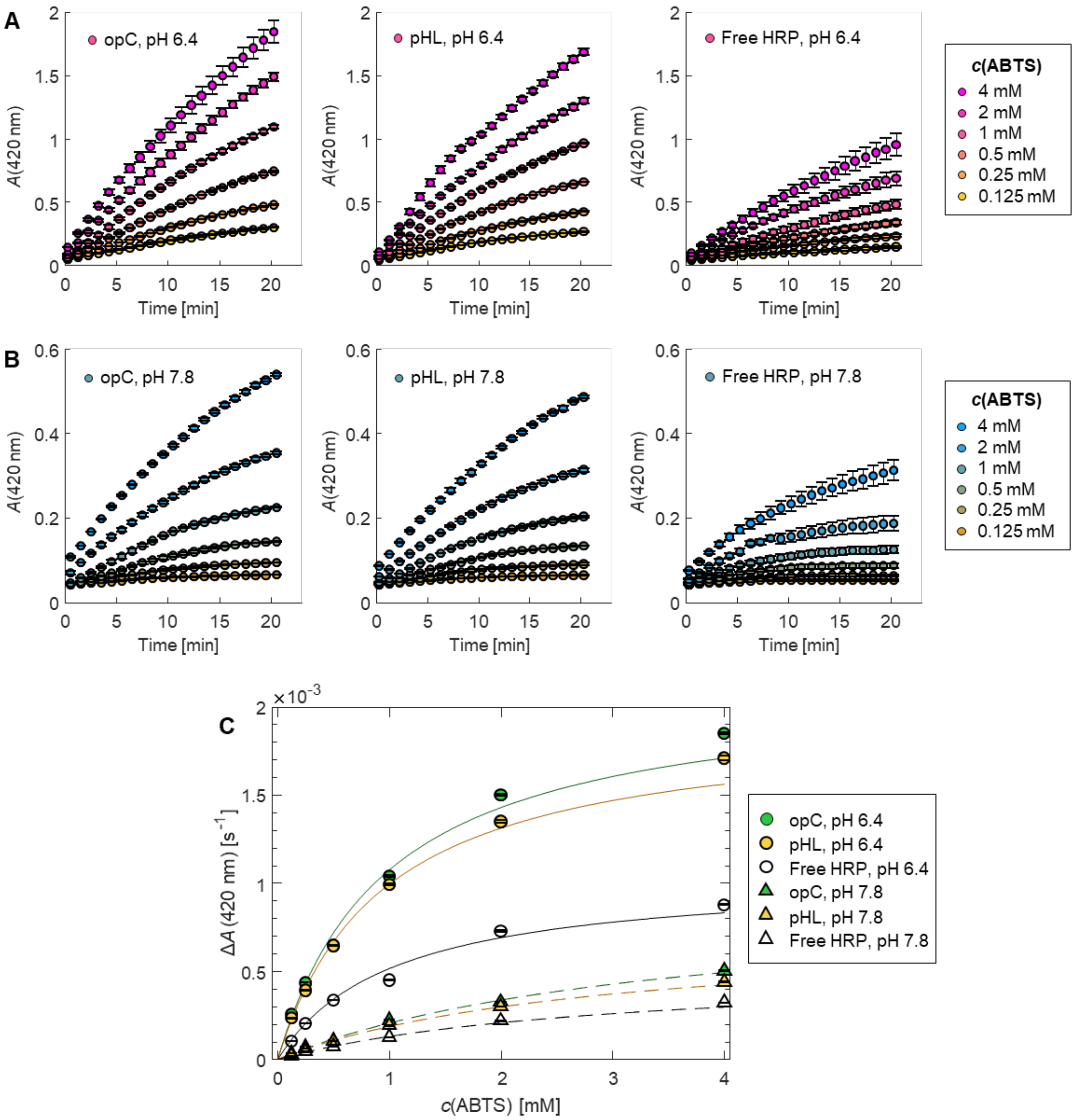

Figure S8. Analysis of the catalytic activity of HRP in HRP-capsule samples and free HRP in solution. (A) Formation of the oxidized product $ABTS^{\cdot+}$ catalyzed by HRP in pH 6.4 samples. The rate of ABTS conversion to $ABTS^{\cdot+}$ was followed according to the increase of absorption at 420 nm, measured in the presence of 0.125–4 mM ABTS and 4 mM H_2O_2. Nanocapsule concentration in opC and pHL samples was 2 nM, and concentration of HRP in the free HRP sample 2 nM. Initial catalytic rates for each ABTS concentration were determined from the slope or a linear equation fitted into the data points in the first 6 minutes. Error bars represent the standard error of the mean of three parallel samples. (B) Product formation in pH 7.8 samples. Apart from the higher pH, experimental conditions were identical to the pH 6.4 measurements. (C) Michaelis-Menten curves for determining V_{max} and K_m in each sample type, representing the relationship between ABTS concentration and the measured initial catalytic rates. The values for V_{max} and K_m are reported in the text.

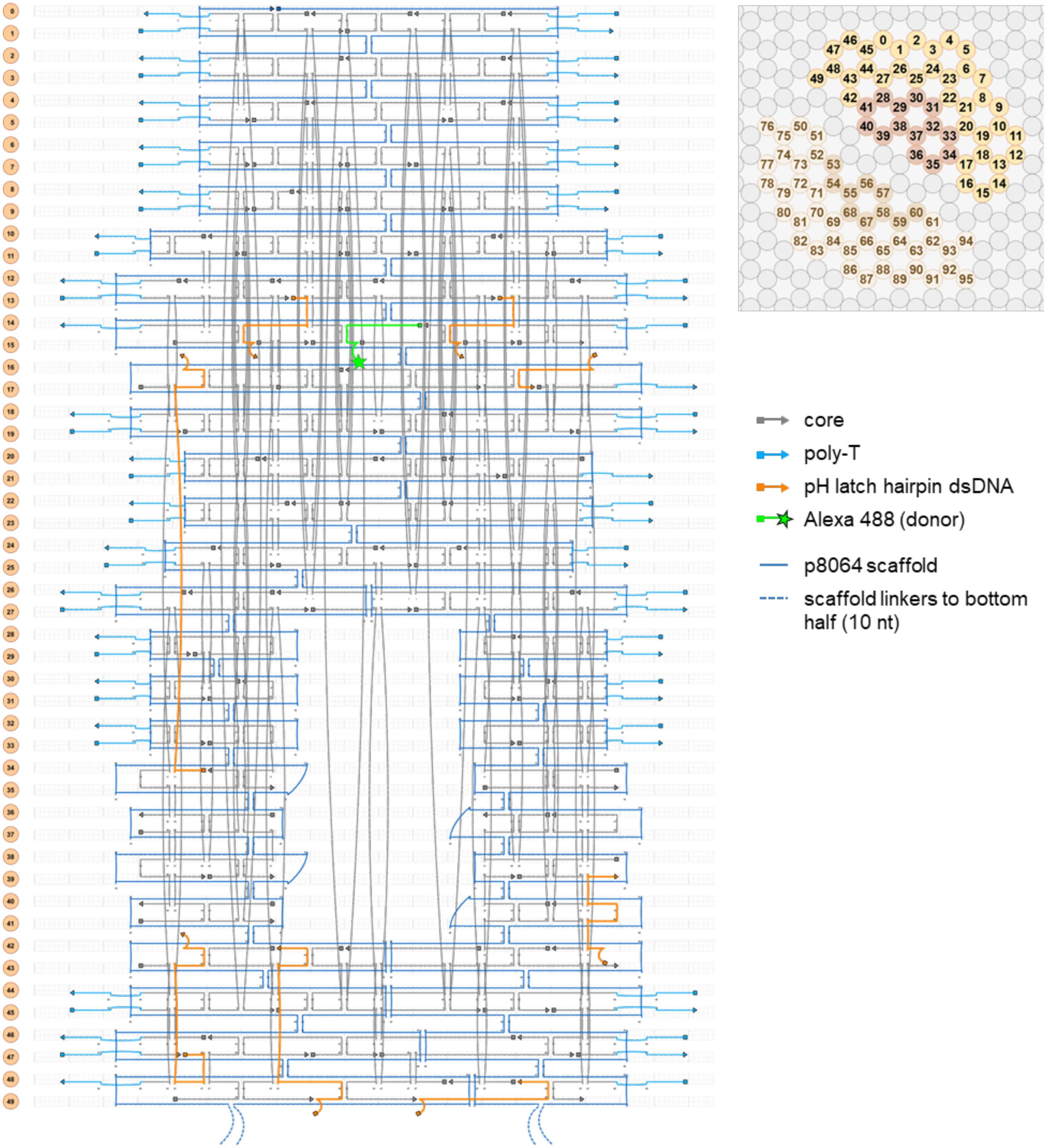

Figure S9. caDNAno blueprint of the nanocapsule top half. The top half of the capsule consists of helices 1-49 according to the helix map shown in the top-right part of the figure. Staple strands have been colored according to the strand type or function in the design. Locations of 3’ or 5’ extensions in pH latch strands and fluorophore-modified strand has been indicated, as well as location of the unhybridized scaffold regions (4 × 10 nt) linking the capsule halves together.

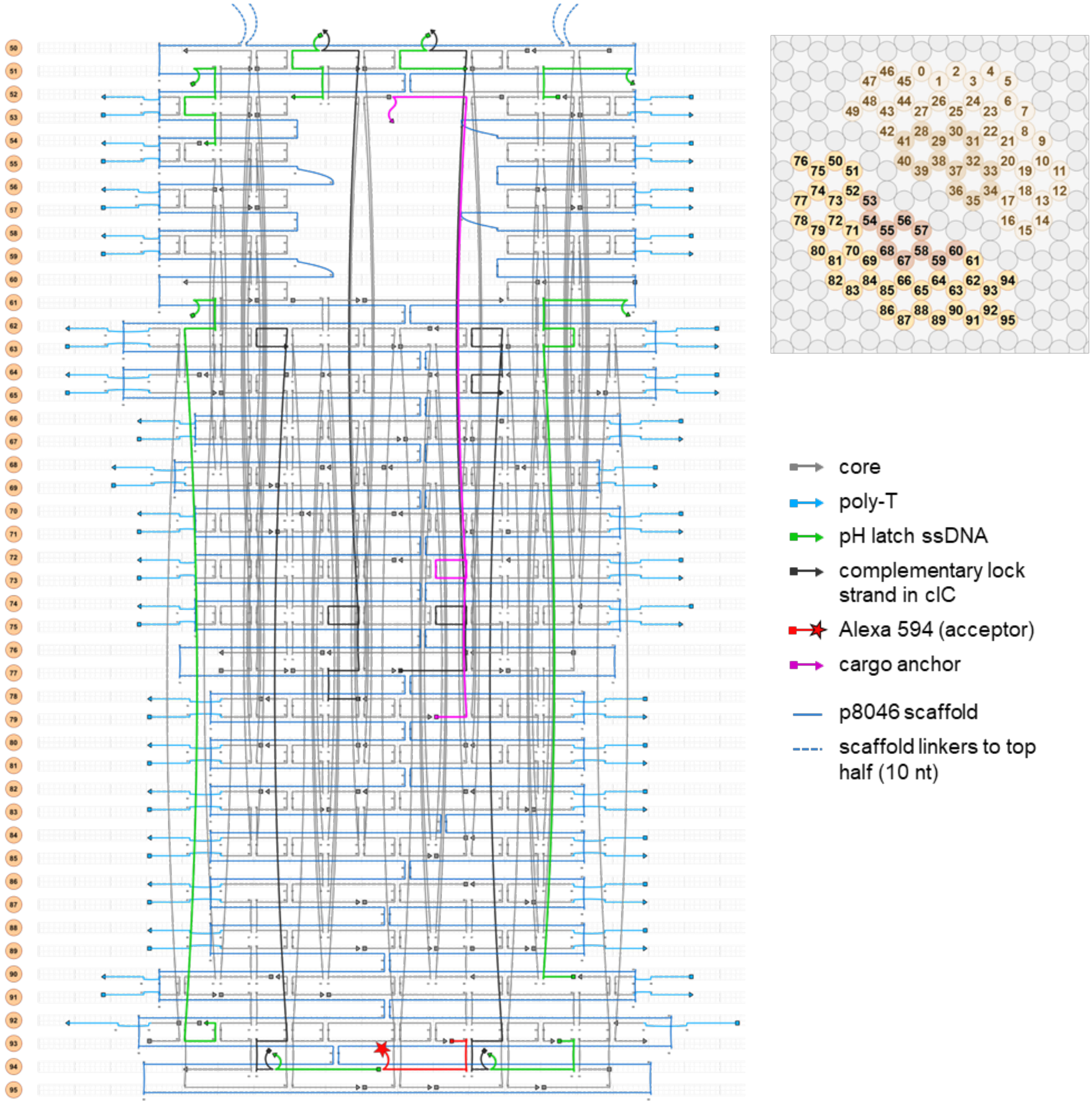

Figure S10. caDNAno blueprint of the nanocapsule bottom half. The bottom half of the capsule consists of helices 50-95 according to the helix map shown in the top-right part of the figure. Staple strands have been colored according to the strand type or function in the design. Locations of 3’ or 5’ extensions in pH latch strands, fluorophore-modified strand, and cargo anchoring strand has been indicated. To achieve correct strand direction for complementary lock strands in the clC design, the strands colored in black have been used for lock extensions instead of the adjacent pH latch strands.

List of all staple strands

All nanocapsules (pHL, clC, and opC) contain the staple strands listed in Tables S1 and S2. Depending on the nanocapsule type, these strands are combined with one set of staples making up the "lock region"; selected either from Table S3 (pH latch staples for pHL), Table S4 (complementary lock staples for clC), or Table S5 (staples with no lock extensions for opC). Start – end locations correspond to caDNAno design.

Table S1. Staple strands for the core structure and poly-T overhangs. Poly-T regions for passivation of the edges have been written in lowercase letters.

Functionality	Start - end	Sequence
core	0[55] - 26[49]	TCTATCAAATCAAGAAAAGAA
core	0[76] - 25[76]	ACGTCAACCCGAGAATCTACATTTAGTG
core	0[95] - 31[97]	TTAAAGTTCCAGGAGGGTATAAACGAGAACGGTCTAGCAT
core	1[63] - 47[69]	GGGTCAGAGGGCGAGTAACGCATGTGCTTATTACG
core	10[34] - 12[30]	GCCACCGTCTGTCCAACTA
core	10[93] - 18[84]	TAAGTTATCCGCTCGAATTCGTAAACGCGTGAAGGTTT
core	11[105] - 19[109]	ATTCCACAGCCGGACGCGG
core	11[42] - 20[46]	AAATTAATTATAATTCTAAAGTGG
core	11[63] - 19[69]	CTTCTTTCCAGAATACCTCAA
core	11[77] - 20[84]	TCCTGTGTGAAATTAGCCTGGTTTCGCAGCATCAG
core	12[109] - 36[105]	CCCGGCCGTGAGCCCTGCGTGTGTTCTTTCAACAATACTTTGTACCA
core	12[48] - 35[48]	AGTAGAACCATTGCTATTAACAAAACATAGATAGATACCCGG
core	12[69] - 16[63]	AGTAATATCATGGAAAACAGACCGAACGAACCACCCTACCTGAAAGCGT
core	15[28] - 11[41]	CCAGTAATAAAAGGATGGATTCCGCCAGGAACTCAATCACGC
core	15[45] - 10[35]	ATTCCAGACAATATTTTTTAGCCCTACCGCCTTGAAAAACAGTGAG
core	15[56] - 11[62]	CAGAGATTTTGACGAAAACGCACATCACAGCAATA
core	15[66] - 14[79]	ACCCTTCTGGCATCAGACGTCAT
core	15[87] - 17[90]	CCAGGGTGCCGGTGCCCCAGCTCGTCATAAAC
core	15[98] - 11[104]	TCACTGCATGCGGCTTCGCGTGTACCGAGCTCACA
core	16[62] - 9[62]	AAGAATAAAAAATAGGTGAGGTTGCTGACCTGAGATTAGACA
core	17[21] - 36[21]	AATGCGCATTTTCACAGATGAAAACAAT
core	17[49] - 15[55]	CGCCATTCGTGGCATGGCCAA
core	18[83] - 11[76]	CTTTGCACAGGCGCGGTGCCTAATGACGGACTGTT
core	19[70] - 12[70]	ATATCTGGAAGATAAAGTTTCTGCCAGCTCATGGTCATAGTT
core	2[62] - 22[52]	GCCGTAAAGCACTAAGCTTGACTTTGCCACAATTCCAAT
core	2[95] - 21[94]	GGCAAATCCTGTCGGTCCGAACGTGCTGGTCAGTCGG
core	20[111] - 40[105]	GCTTACGGGATAAAACCCTCAATAAAGCGGCAAAGAACATCCCAATTCT
core	20[45] - 39[48]	CAAACTCCGTGAGCTCTCTACCATGCAGCGCCAGAATCG
core	20[69] - 7[62]	CTCAATCAATATCTATCTTTATTTCCTCGCTTTGA
core	20[83] - 9[83]	CGGGGTCCAACGGCTGTCGTGCCAGCATTAGTGAGCTAACTC
core	21[95] - 13[93]	TGGGTGTCCACTCAATCATGGGTACCT
core	22[51] - 13[51]	AGACTAAAATGGTCAGTCATCACCCGGTCAGAAC

core	22[62] - 4[56]	GAGCCGTGACAACTAACCACCCAAGTGTAGCGGTCCGGCGAACGTGGCG
core	22[83] - 7[76]	CGCAAGACATCCTCTGGTTTTTCTTTGCGTTGCAT
core	24[69] - 1[62]	AGCCTTAGGCGGCCAAAATCCCGTAAAATTTTTTG
core	24[86] - 48[84]	TTCGTAAAGTGCTATTTCCTGAGACTTCCTGCCATCAAAGATTCA
core	25[49] - 2[63]	GAACAAGCCGCACATTAAATCCGGGGAAAGCCGCTATGAGGT
core	25[77] - 5[83]	ATGAAGGGTCTCGTCCCAGCACAGCAAGCGGTCCAACTCACCAGTGAGA
core	26[109] - 45[104]	ATTAAATGATATCTGCGAACGAGTGCCTGGAAG
core	27[56] - 49[77]	TCGACATAAAAAGGGTTTTCCCAAGCTTTGGGAAGGGAAACCAGGCAAAG
core	27[77] - 24[70]	GTCATTGTTGAGAGTAGGGTTTATAAATCAAAAGAGGTTTGCCGCTGGC
core	28[97] - 36[91]	GTCAACTAGAGAATAAACGTTTGTAAAATAAGCAA
core	29[32] - 34[35]	TATAAAATCGCTGATTGTCGGGAGATATACAGTAACAGCTGAAAT
core	30[41] - 24[37]	TCAAATTATTATCATATTA
core	35[91] - 15[97]	GTATATGACCCTGTGCCAAAAACAGGAAATCCCTTAGCCAGCCGCAGTG
core	36[104] - 33[97]	AAAACATTTAAGCATATCATATGTACCCTCAGAAA
core	36[48] - 33[34]	CCAGTCCTTTTACACTTTGAAACTATCAAAATTATAACAGAA
core	37[21] - 40[21]	GATTCGCCGCAGAGCATTTCACATCAAG
core	38[118] - 35[118]	CAGGCAACTCAGAGAATCGGTTTGCGGG
core	39[35] - 41[48]	GAGCAAAAAGTTACACCTTAAATTTCTGGCCAGCAATACCTC
core	39[98] - 42[105]	TAGCATTAATTAGCCGGGTAAAGATTCAAAAGGCCTCATTTGGTCAATA
core	4[55] - 8[53]	AGAAAGGGCGCTGGACACCCGTATGGTTGTT
core	4[95] - 10[94]	CTGAGCAGCTGAATTGGGCACGCGCGCCAGTCGTTGCGTTAGTG
core	40[104] - 28[98]	ACTAATATATATTTGGAGACA
core	40[48] - 29[31]	GAAACAAGATGATGTTTAACAGTATCAATATAATCGGAT
core	41[21] - 39[34]	CAAAATTAATTACAAAACAAAATTACCT
core	42[104] - 49[111]	ACCTGTTTCAGATTTAGTTTGTGAATATTGCGGAT
core	42[48] - 49[56]	GGATATTCATTTGACAATATATGTGAAACCTTTTTTCCGGCACCGCTTCT
core	42[62] - 0[56]	GCCGCCACCAGTGCCAGTCACAAGTTGGAAAACCG
core	42[90] - 42[63]	AAATCAGCTCATTTTTTAAAGAGGTGGA
core	45[105] - 47[109]	TTTCATTGCAACTACTGTA
core	45[49] - 27[55]	GAAAATTGACGTTGGTGTACA
core	46[34] - 26[31]	AAGACGCTAATTTTGCTTCTGTGATGGCAATTCTGGAAGGAGCGGA
core	46[97] - 0[77]	GTACAACCCGTCGGATTCTGAAAGGGGGCAATCAACATTAAAGACTCCA
core	47[56] - 47[55]	TCTTCGCGCAAGGCGACATAGCGATAGCCTGAGAGACGGGCC
core	47[70] - 27[76]	CCAGCTGCAACTGTTCCCAATAGGAACGTAGCCAGCTTTCGGCTATCAG
core	48[83] - 46[98]	GGCTGCGGCCCGTGGGAACAAACGGCGGATTGATGAAGTACG
core	48[97] - 39[97]	ATGGGATAAATAATTATTGTTAATTTGCGTTTAAATTTTGAG
core	49[28] - 46[35]	ATGCTGATGCAATTAACCTCCCATAGGTTTAGATT
core	5[44] - 45[48]	CTAGGAAGGGAAGATTTAGAATCGGATCACCCAGGGCGATAATCCTT
core	5[84] - 45[90]	CGGGCAAAGAGTTGGGCGAAAAATCCCTGAGTGTTGAACGTGTGTGAGC
core	50[95] - 68[94]	CGTGCGGTCAGGTAATTCGCAAAAAGAAGCAAAACCATAAGCTC
core	51[42] - 76[35]	TTTAGTTAGATCGCAAGGAAACTCCTTATTACGCA
core	51[63] - 77[69]	ACCTAAAACGACAGTAATAACGGAATAAAATATCTTACCGAATATAAAA
core	51[84] - 76[84]	TCCAACAATCTGCCCTTTGACCAAAAGA
core	52[68] - 72[70]	TGGTTTAATAGCAATAGCATAAGGGTA

core	52[90] - 77[90]	AGCTTCAAAATCCGTATCATCGGGTAAA
core	53[42] - 77[48]	TACTAGAGTGATAAAATAAGAAAGTAAGACATACA
core	56[34] - 64[32]	ATCGCCACGCCAACTAATAAGGTACCGAGCC
core	56[97] - 64[91]	AAACGAGATAAATACAATACTTTTGCCAAACGAAC
core	57[42] - 71[45]	TTAGGCAGGGCTTAAGCCAACGCCTGTTGAGA
core	60[97] - 95[97]	GCAAAAGTCGTTTACAAAAGGAGGTTTACACCCTCGAGGCTG
core	61[42] - 69[45]	TTTATCAACGACGATAAAGTACATTTTCCAATTTTGTTACAAATAA
core	61[49] - 95[62]	ACAATAGCCTAATTGGAGTGTAGAATGGGCCTATTTCGGAAC
core	61[63] - 61[90]	CTGAACAAGAGCAACACTATCATAACCC
core	62[76] - 95[76]	GTAAGAAAGGTGTACAAGCCCTCTGAAA
core	63[70] - 93[79]	GAACATTTAACGATGGAACCCATGTACCGGGATAGTCA
core	63[91] - 93[100]	TCATCAGAGACAGCTGAGTTTCGTCACCGAGCCACGTA
core	64[31] - 91[34]	CAATCATTCCAGACAGGAGGTCAGA
core	64[48] - 82[44]	GGAATCATAAGAACTCAAGATAGCGTCATAGCGACGCACCATATTAG
core	64[55] - 91[55]	CATCGTAACCAAGTACCAGAGATTCACA
core	64[76] - 89[76]	TAAGCAAGCCGTTTCGAACCTCCCGACTACAGTTTAGTTTTG
core	65[35] - 60[28]	GGTATTCTTACCGCCAAAAGGCAATAAACAACATG
core	66[90] - 84[84]	AATCATTAGGAGCCAGGTGAA
core	67[71] - 82[63]	ACGACCACCTTGCGGGAGGTTTCATCGCGATAGCCCGGAAAACCGACT
core	67[84] - 80[84]	GAAACACCAGTGAAAACCGGACTTCATCCTGAGGCCGTCACC
core	68[55] - 89[62]	TTTGCCAATCCTGACCTTAAAGCGAGGCCCTCAGACACCACCCTCAGAG
core	68[67] - 85[76]	TCCAGAACGTCAAAAATCTAAACCATGCAAAGG
core	68[93] - 89[95]	ATTCAGAACGCAACTTTAACTGGCGATTTTGGTTAG
core	69[46] - 80[44]	GAATAACATAAAATTATAGGGA
core	7[44] - 25[48]	CGTACCCGCGCTTTTACAACGAACGTTTTTGCG
core	7[63] - 22[63]	CGAGCACTGCGCGTCGCCGGCCAGAGCAATACTAATAGATTA
core	7[77] - 24[87]	TAATGAATCGGCCAGCCAGGGATAACGGTTTT
core	70[51] - 87[62]	GAAACGATTTTAATCAGGACTGTACCCTTATCCGGAACCAGAGCC
core	70[69] - 80[63]	TAGCAGCTTATCACAGGGCGA
core	71[46] - 78[44]	ATTGATAACCAATTCATGTTTA
core	71[84] - 79[76]	TTTGAAATCATAAGAACGAGGAGACTTTTTCATGAGGAAGAACAAGACA
core	71[91] - 87[95]	GAGGACAGGCTGACTATTCATGCTTTCGTTTAATTTTTTTCAAACTA
core	72[69] - 78[63]	ATTGAGCCCAAAGACGCAAAG
core	76[104] - 72[91]	TAAAACGAAAGAGGCCCCAGCAGATTTGCGACCTGCGGTCAA
core	76[55] - 70[52]	ATTAAGACCGAGGATTAAGAAGCAAGAATCAGAGAAACTGAAGAGA
core	76[83] - 76[56]	ATACACTAAACCCAAAAGAACTGGCATG
core	77[35] - 50[28]	AGAAAATCAGATAGTTACCAGACAAGACAAAGAAC
core	77[49] - 83[55]	TAAAGGTGGAATAAATGGTTTCCGATTGTCATTAATTTGGGATACCATT
core	77[91] - 83[95]	ATACGTAGGACTAAGTAGCAAGCGGGATTTGCAGGAACAACCTACCG
core	8[52] - 12[49]	AGAAAGGGATTAGTGTTTCCGTTGTTTGCCTG
core	80[62] - 51[62]	CATTCAAACCAGCGGCTAATAACAATGAGAAATACTCTTCTG
core	80[83] - 71[83]	CTCAGCAGCGAAAACGTATATTCGGTCGAAGAGTAGACCAAC
core	82[62] - 70[70]	TGAGCCAAGGTGAACTTTACACACCCTGAACAAAGTCAGACTATGAAAA
core	82[83] - 82[84]	ACGCATAACCGATCCGTCACCAATAAACAGCTTGAATCGCCC

core	83[56] - 68[56]	AGCAAGGAGCACCGTTTGTTTAGCCTAA
core	84[83] - 68[68]	TTTCTTGTGACAAGTAAGGCTTGGCGTCTT
core	85[77] - 67[83]	CTCCAAAGTGAATTCTGACGA
core	86[83] - 64[77]	AATCTCCAAAAAATGGAGTGAGAATAGAACTTTCATATGCGAACAACAT
core	87[63] - 67[70]	ACCACGCTTTCGGTCATAGCCGCGCGTTTTTGAAGATCTTACCAACGCTA
core	89[63] - 64[56]	CCGCCAACACGGAACCGCCTCGTTTTAGTTATTTT
core	89[77] - 62[77]	TCGTCTTGTTAGCGACAGGTAAGGCATA
core	89[84] - 86[84]	TCCAGACCTAAACAAAGGAACCGTTGAA
core	9[44] - 30[42]	ATTAATCAGAGCAGGTTATTAATACAAAAAAGACAGCGGA
core	9[63] - 20[70]	GGAACGGAACGTGCGGAGCACTAACAGCATAAACC
core	9[84] - 22[84]	ACATTAAGGAAACCAGCACCGCAGCAAC
core	91[35] - 94[28]	CGATTGGAGTCTCTCTTTTGACGGGGTCAGTGCCT
core	91[56] - 61[62]	AACAAATAAATCTACTACCAGAACCACCACCGCACATCCCATATAAGTC
core	92[31] - 90[46]	TTTATATAAACAGTTAATGCCCCCTAAAGCGCCCTTGATCCG
core	94[111] - 65[109]	GGGGTTTCACCCTCCAGATACTTAGGAAATCTACGACCAG
core	95[63] - 63[69]	CTATTATAACTCATTAAAGCCACATAGCCCGGAATAAATAATTCATCGA
core	95[77] - 90[84]	CATGAAAGTATTAAATTTTCAGTAACACCCTCATA
core	95[98] - 90[105]	AGACTCCTCAAGAGACCCTCAAGTACAACTGTAGC
core + poly-T	0[118] - 0[96]	tttttttttAGTCCACTA
core + poly-T	1[21] - 2[21]	tttttttttTGAACCAACCCTAAttttttttt
core + poly-T	10[127] - 11[127]	tttttttttATACGACAACttttttttt
core + poly-T	11[12] - 10[12]	tttttttttAAGAGAGTAAttttttttt
core + poly-T	12[132] - 12[110]	tttttttttTTGAGGATC
core + poly-T	12[29] - 12[5]	TCGGCCTTGttttttttt
core + poly-T	13[5] - 18[7]	tttttttttCCAGAACTGCCACGttttttttt
core + poly-T	14[132] - 15[111]	tttttttttGTGCTGCGGCCAGAGCGCCTG
core + poly-T	17[102] - 9[116]	TGGGCTGGTACGCCGGGAGCATAAGCGCTCAttttttt
core + poly-T	18[134] - 13[132]	tttttttttACTGTTGCCTCCTCttttttttt
core + poly-T	19[110] - 19[134]	TTGCGGTATttttttttt
core + poly-T	19[30] - 14[5]	GCAAAGCAACAGAATATTAATTTACATTGGCAGttttttttt
core + poly-T	19[7] - 19[29]	tttttttttGAGCCAGCA
core + poly-T	2[118] - 2[96]	tttttttttTCCGAAATC
core + poly-T	21[19] - 20[19]	tttttttttAGGAATTGAAttttttttt
core + poly-T	22[125] - 30[105]	tttttttttGCGTGGTTTTTAAAGAGTAAT
core + poly-T	23[19] - 22[19]	tttttttttGAAGTATTTAttttttttt
core + poly-T	24[120] - 25[120]	tttttttttTTCCGTGCCGttttttttt
core + poly-T	24[36] - 24[14]	ATTTTAAAAttttttttt
core + poly-T	25[14] - 31[34]	tttttttttGAGTAACCTGAATATAGAACC
core + poly-T	26[132] - 26[110]	tttttttttAGCTGATAA
core + poly-T	26[30] - 26[5]	ATTATCATCAttttttttt
core + poly-T	26[48] - 0[22]	ACCACCAGGCGGTTTAGTGAATAACCTTCCCTTAGGGCCCACtttttttt
core + poly-T	27[5] - 44[7]	tttttttttTATCAGATAAATCGttttttttt
core + poly-T	28[127] - 29[127]	tttttttttGTGAGAAAGGttttttttt
core + poly-T	29[12] - 28[12]	tttttttttTGTTTCTGATttttttttt

core + poly-T	3[21] - 4[21]	tttttttttAGCCCCCGAAAGCGttttttttt
core + poly-T	30[104] - 3[118]	GTGTAATTGCTGATGCAAACGTTGATGGttttttttt
core + poly-T	30[127] - 31[127]	tttttttttTGCCTTGCAAttttttttt
core + poly-T	31[12] - 30[12]	tttttttttAGGGTATGGAttttttttt
core + poly-T	31[35] - 6[21]	TACCAGGTTTGAGGATTAGACTAATGCGttttttttt
core + poly-T	31[98] - 23[125]	GTCAATAGCTGGTCCGGACTTGTAGAACttttttttt
core + poly-T	32[127] - 33[127]	tttttttttTTAGAAATTTttttttttt
core + poly-T	33[12] - 32[12]	tttttttttCGTAATTGCAttttttttt
core + poly-T	33[35] - 8[21]	ATAAATTTCAACAGTTGAGGAGGGAGCTttttttttt
core + poly-T	33[98] - 21[125]	AGCCCAAGCTGGAGTGCCATCCCACGCAttttttttt
core + poly-T	34[118] - 17[134]	GCCTTTAAGCAAATttttttttt
core + poly-T	4[118] - 4[96]	tttttttttCGCCTGGCC
core + poly-T	43[21] - 48[5]	AACAGTAGTTGGGTttttttttt
core + poly-T	44[134] - 27[132]	tttttttttCCCAATTTCAACCGttttttttt
core + poly-T	45[7] - 46[5]	tttttttttTATTAATTGAGAAGttttttttt
core + poly-T	45[91] - 1[117]	GAGTAGTGTCTGGCGTATCACCATCAATTGCCGGATTTGGAAtttttttt
core + poly-T	46[132] - 45[134]	tttttttttTAAATATCCATATAttttttttt
core + poly-T	47[110] - 47[132]	GCTCAACATttttttttt
core + poly-T	47[5] - 47[29]	tttttttttGAATTTATC
core + poly-T	48[132] - 43[118]	tttttttttTAATTGCACCATTA
core + poly-T	5[21] - 5[43]	tttttttttGAGCGGGCG
core + poly-T	50[111] - 75[125]	CTTTTGACCAAGCGttttttttt
core + poly-T	52[127] - 53[127]	tttttttttGACTTCGAAttttttttt
core + poly-T	53[105] - 73[125]	GGAAGCCCAAATATTTACTTAttttttttt
core + poly-T	53[12] - 52[12]	tttttttttTAAACAAGAAttttttttt
core + poly-T	54[127] - 55[127]	tttttttttCCCTGCTTTAttttttttt
core + poly-T	54[31] - 68[14]	TGCGTCTTACCAGCCATAttttttttt
core + poly-T	55[12] - 54[12]	tttttttttCAAATTTATAttttttttt
core + poly-T	56[127] - 57[127]	tttttttttATGCTCTCAAttttttttt
core + poly-T	57[105] - 71[125]	AATCCCCTTAAACAATCAGGTACTATTAGGTGTACttttttttt
core + poly-T	57[12] - 56[12]	tttttttttACAATATTttttttttt
core + poly-T	58[127] - 59[127]	tttttttttGTTTAAAAATttttttttt
core + poly-T	59[12] - 58[12]	tttttttttATAAAAGAATttttttttt
core + poly-T	6[118] - 5[118]	tttttttttTTTGCGTTTGCCCTttttttttt
core + poly-T	60[116] - 67[125]	AAAATAGCGAGATAATAGTGACTGGAAATTGGGttttttttt
core + poly-T	62[132] - 93[118]	tttttttttACTAATGAGAACCG
core + poly-T	63[5] - 64[5]	tttttttttTCCTTATAGCAAGCttttttttt
core + poly-T	64[132] - 63[132]	tttttttttAAGAAAATACCACAttttttttt
core + poly-T	65[110] - 65[132]	TCAGGACGTttttttttt
core + poly-T	65[5] - 65[34]	tttttttttTAGAAGGCTTATCC
core + poly-T	66[125] - 57[104]	tttttttttAGATGGTTTAATTTAGTAGTATAGCGTCTTCATTG
core + poly-T	67[19] - 66[19]	tttttttttTGCACTATTTttttttttt
core + poly-T	68[120] - 69[120]	tttttttttCGTAAATCAAttttttttt
core + poly-T	69[14] - 56[35]	tttttttttTTATCCCAATCCAAAATAAACAGTATAAATTGAGA

core + poly-T	7[21] - 7[43]	tttttttttTACAGGGCG
core + poly-T	70[125] - 53[104]	tttttttttCAGGCGCATAGGCTGATGAACTAGTCAGATTAAGA
core + poly-T	71[19] - 70[19]	tttttttttCGCATGGAAGttttttttt
core + poly-T	72[125] - 52[102]	tttttttttGAACGAGGCGCAGACTCCATGCGC
core + poly-T	73[19] - 72[19]	tttttttttGTTAAATTGAttttttttt
core + poly-T	74[127] - 74[105]	tttttttttttACAAAGT
core + poly-T	75[19] - 74[19]	tttttttttCAAAGCCGAAttttttttt
core + poly-T	78[118] - 50[96]	tttttttttGCTTTGAATGCCACACAACGGGATTATATATCGTAAC
core + poly-T	78[43] - 78[21]	TTTTGTCACttttttttt
core + poly-T	79[21] - 51[41]	tttttttttAATAGAACACAAGAGCCCAATATAAGGCAATATAT
core + poly-T	8[118] - 7[118]	tttttttttCCGCTTTGGGAGAGttttttttt
core + poly-T	80[118] - 79[118]	tttttttttCGCTTTTCGGCTACttttttttt
core + poly-T	80[43] - 80[21]	GGGAAGGTAttttttttt
core + poly-T	81[21] - 53[41]	tttttttttTTGACGGAAAACAGTAGACGGTAGTATCTCATAAT
core + poly-T	82[118] - 81[118]	tttttttttCAATGACGAGTTAAttttttttt
core + poly-T	82[43] - 82[21]	AGCCAGCAAttttttttt
core + poly-T	83[21] - 84[21]	tttttttttACCAGTAAGAATCAttttttttt
core + poly-T	83[96] - 83[118]	ATAGTTGCGttttttttt
core + poly-T	84[118] - 56[98]	tttttttttTCAGCTTTACCCAACAAAGCTATCAAAAGTTCAGA
core + poly-T	85[21] - 57[41]	tttttttttTGCCTTTTAGTTGCCCAGCTAGAGCCAGATGTAAT
core + poly-T	86[118] - 85[118]	tttttttttTAATAATGTATCGGttttttttt
core + poly-T	87[21] - 88[21]	tttttttttCATAATCCCCTCAGttttttttt
core + poly-T	87[96] - 87[118]	AAGGAATTGttttttttt
core + poly-T	88[118] - 60[98]	tttttttttTGTATGGTCATTATTTAATAAGAGGGGGGGCTTTT
core + poly-T	89[21] - 61[41]	tttttttttGCCACCCCAGCATTAGAACGGATGTAGAGCGCCTG
core + poly-T	89[96] - 89[118]	TAAATGAATttttttttt
core + poly-T	9[21] - 9[43]	tttttttttAGGAGGCCG
core + poly-T	90[127] - 91[127]	tttttttttAACGCACTACttttttttt
core + poly-T	90[45] - 86[21]	CCGCTCAGAGCGCCGCCAAAAATCATAGCGTTTGCCATCttttttttt
core + poly-T	91[12] - 90[12]	tttttttttAGGCAGGTTGttttttttt
core + poly-T	92[136] - 92[112]	tttttttttttAACCGCC
core + poly-T	92[27] - 92[5]	CCGTTCCttttttttttt
core + poly-T	93[21] - 62[5]	AGCGTCACAATAATttttttttt

Table S2. Staple strands for FRET and cargo anchoring.

Functionality	**Start - end**	**Sequence**
Alexa Fluor 488 3', top half FRET	14[78] - 15[65]	ACCGGGGTACCTACATAGAtt/3AlexF488N/
Alexa Fluor 594 3', bottom half FRET	93[80] - 94[67]	CCGTGTGCCGTCGAGAGGGTTtt/3AlexF594N/
Cargo anchoring strand	79[77] - 52[69]	GCATCGGGGAACCGTGTGTCGAAGCGAACCAGATAAtttAAAGAAGAAAGAAAAA

+ 5’ thiol modified strand for cargo molecule - DNA conjugates, complementary to the cargo anchoring strand: /5ThioMC6-D/TTTTTCTTTCTTCTTT

Table S3. Staple strand set for pH latch functionalization of pHL nanocapsules. Hairpin-forming extensions have been colored orange, and ssDNA counterparts green. dsDNA regions and ssDNA sequences with the same set number contain matching polypurine - polypurine sequences for Hoogsten triplex formation. 3×T spacers between the latch and the core structure, as well as 4×T hairpin loops have been marked with lowercase letters.

Functionality	**Start - end**	**Sequence**
latch 15/1 - pHL set 1 hairpin	13[52] - 15[44]	AGGACTCAATCGTCTGAAGACtttGAGAGAGAAGAAGAAGAAAGttttCTTTCTTCTTCTTCTCTCTC
latch 15/2 - pHL set 2 hairpin	13[94] - 15[86]	GTTCGGGCCGTTTTCACGGATtttAAAGGAAGAGAGAAGAAAGGttttCCTTTCTTCTCTCTTCCTTT
latch 16/1 - pHL set 3 hairpin	34[34] - 16[30]	TGCGTAGGAACTGAGAATGtttCCTTTCTTTCTTTCTTCCTCttttGAGGAAGAAAGAAAGAAAGG
latch 16/2 - pHL set 4 hairpin	16[114] - 17[101]	AGAGAAGAAAAGAGGAAGGAttttTCCTTCCTCTTTTCTTCTCTtttGATGCCGGGTTACCTGCACAC
latch 50/1 - pHL set 5 hairpin	50[54] - 52[49]	CTTTTCTCTTCTTCCCTTTCttttGAAAGGGAAGAAGAGAAAAGtttTCAGGAAATTTCACGACCGT
latch 50/2 - pHL set 6 hairpin	50[75] - 51[83]	TTTCTTTCCTTTCCCTCTCTttttAGAGAGGGAAAGGAAAGAAAtttGGGACGTTCCGGAAGCAAAC
latch 42/1 - pHL set 7 hairpin	47[30] - 42[30]	AAAATGGCTTAGCATAAATATTACtttCCCCCTTTCTTTTTTCTTCTttttAGAAGAAAAAAGAAAGGGGG
latch 42/2 - pHL set 8 hairpin	42[115] - 39[118]	AAAAGGGAGAAGAAAAGAGGttttCCTCTTTTCTTCTCCCTTTTtttAATGGGGCGCGGTGGCATAATAAAT
latch 94/1 - pHL set 1 ssDNA	94[66] - 94[46]	GATATAAGTTGGTAATAAGTTtttCTCTCTCTTCTTCTTCTTTC
latch 94/2 - pHL set 2 ssDNA	93[101] - 94[88]	CCGCTGCTCAGTACCAGGCGGtttTTTCCTTCTCTCTTCTTTCC
latch 61/1 - pHL set 3 ssDNA	61[30] - 92[32]	CTCCTTCTTTCTTTCTTTCCtttAGAACAACCAATTACATGGGAA
latch 61/2 - pHL set 4 ssDNA	90[104] - 61[114]	ATTCCACTTGAGATATAACGCCCAGACGACGATAAAAAtttTCTCTTCTTTTCTCCTTCCT
latch 49/1 - pHL set 5 ssDNA	49[57] - 42[49]	CTTTCCCTTCTTCTCTTTTCtttGGTGCCGGCGATCGGTGCTAACGACGGCGGGAAC
latch 49/2 - pHL set 6 ssDNA	49[78] - 48[98]	TCTCTCCCTTTCCTTTCTTTtttCGCCATTCGCCGTCACGTTGGTGTATTAACCGTA
latch 51/1 - pHL set 7 ssDNA	50[54] - 52[49]	TCTTCTTTTTTCTTTCCCCCTTtttTCAGTTAAATACCGGAAATA
latch 51/2 - pHL set 8 ssDNA	50[75] - 51[83]	GTTTATTAGAGAGTACCTTTAAtttTTTTCCCTCTTCTTTTCTCC
core	77[70] - 50[76]	GATTTCCATTAAACGCCTGATCACTCATAGTTTGAG
core	78[62] - 50[55]	ACACCACGGCAACAGCCCTTTAACGCAATATCGGCC
core	94[45] - 63[48]	TTAATGATACATACGAGCGTATTAA
core	94[87] - 65[90]	ATAAACTCAGGAATTACGGAAAGATTAACGGATTTTAAG

Table S4. Staple strand set for complementary lock functionalization of clC nanocapsules. Duplex-forming extensions have been colored dark green. Extensions with the same set number contain complementary sequences for duplex formation.

Functionality	**Start - end**	**Sequence**
latch 15/1 - clC pair 1 ssDNA	13[52] - 15[44]	AGGACTCAATCGTCTGAAGACtttCTCTCTCTTCAAGAAGAAAG
latch 15/2 - clC pair 2 ssDNA	13[94] - 15[86]	GTTCGGGCCGTTTTCACGGATtttAAAGGAAGAGTCTTCTTTCC
latch 16/1 - clC pair 3 ssDNA	34[34] - 16[30]	TGCGTAGGAACTGAGAATGtttCCTTTCTTTCAAAGAAGGAG
latch 16/2 - clC pair 4 ssDNA	16[114] - 17[101]	TCCTTCCTCTAAAGAAGAGAtttGATGCCGGGTTACCTGCACAC
latch 50/1 - clC pair 5 ssDNA (*)	78[62] - 50[55]	ACACCACGGCAACAGCCCtttAACGCAATATCGGCCTTTGAAAAGAGAAGAAGGGAAAG
latch 50/2 - clC pair 6 ssDNA (*)	77[70] - 50[76]	GATTTCCATTAAACGCCTGATCACTCATAGTTTGAGtttAAAGAAAGGAAAGGGAGAGA
latch 42/1 - clC pair 7 ssDNA	47[30] - 42[30]	AAAATGGCTTAGCATAAATATTACtttCCCCCTTTCTAAAAAGAAGA
latch 42/2 - clC pair 8 ssDNA	42[115] - 39[118]	CCTCTTTTCTAGAGGGAAAAtttAATGGGGCGCGGTGGCATAATAAAT
latch 94/1 - clC pair 1 ssDNA (*)	94[45] - 63[48]	CTTTCTTCTTGAAGAGAGAGtttTTAATGATACATACGAGCGTATTAA
latch 94/2 - clC pair 2 ssDNA (*)	94[87] - 65[90]	GGAAAGAAGACTCTTCCTTTtttATAAACTCAGGAATTACGGAAAGATTAACGGATTTTAAG
latch 61/1 - clC pair 3 ssDNA	61[30] - 92[32]	CTCCTTCTTTGAAAGAAAGGtttAGAACAACCAATTACATGGGAA
latch 61/2 - clC pair 4 ssDNA	90[104] - 61[114]	ATTCCACTTGAGATATAACGCCCAGACGACGATAAAAAtttTCTCTTCTTTAGAGGAAGGA
latch 49/1 - clC pair 5 ssDNA	49[57] - 42[49]	CTTTCCCTTCTTCTCTTTTCtttGGTGCCGGCGATCGGTGCTAACGACGGCGGGAAC
latch 49/2 - clC pair 6 ssDNA	49[78] - 48[98]	TCTCTCCCTTTCCTTTCTTTtttCGCCATTCGCCGTCACGTTGGTGTATTAACCGTA
latch 51/1 - clC pair 7 ssDNA	50[54] - 52[49]	TCTTCTTTTTAGAAAGGGGGTTtttTCAGTTAAATACCGGAAATA
latch 51/2 - clC pair 8 ssDNA	50[75] - 51[83]	GTTTATTAGAGAGTACCTTTAAtttTTTTCCCTCTAGAAAAGAGG
latch 50/1 - clC no extension	50[54] - 52[49]	TCAGGAAATTTCACGACCGT
latch 50/2 - clC no extension	50[75] - 51[83]	GGGACGTTCCGGAAGCAAAC
latch 94/1 - clC no extension	94[66] - 94[46]	GATATAAGTTGGTAATAAGTT
latch 94/2 - clC no extension	93[101] - 94[88]	CCGCTGCTCAGTACCAGGCGG

* the strands marked with an asterisk are core strands adjacent to the corresponding latch strands in the pHL and opC samples. The strands have been exchanged to achieve correct strand directionality for DNA hybridization (see black strands in Figure S10).

Table S5. Staple strand set without strand extensions for opC nanocapsules.

Functionality	Start - end	Sequence
latch 15/1 - opC (no extension)	13[52] - 15[44]	AGGACTCAATCGTCTGAAGAC
latch 15/2 - opC (no extension)	13[94] - 15[86]	GTTCGGGCCGTTTTCACGGAT
latch 16/1 - opC (no extension)	34[34] - 16[30]	TGCGTAGGAACTGAGAATG
latch 16/2 - opC (no extension)	16[114] - 17[101]	GATGCCGGGTTACCTGCACAC
latch 42/1 - opC (no extension)	47[30] - 42[30]	AAAATGGCTTAGCATAAATATTAC
latch 42/2 - opC (no extension)	42[115] - 39[118]	AATGGGGCGCGGTGGCATAATAAAT
latch 49/1 - opC (no extension)	49[57] - 42[49]	GGTGCCGGCGATCGGTGCTAACGACGGCGGGAAC
latch 49/2 - opC (no extension)	49[78] - 48[98]	CGCCATTCGCCGTCACGTTGGTGTATTAACCGTA
latch 50/1 - opC (no extension)	50[54] - 52[49]	TCAGGAAATTTCACGACCGT
latch 50/2 - opC (no extension)	50[75] - 51[83]	GGGACGTTCCGGAAGCAAAC
latch 51/1 - opC (no extension)	51[30] - 54[32]	TTTCAGTTAAATACCGGAAATA
latch 51/2 - opC (no extension)	52[101] - 51[115]	GTTTATTAGAGAGTACCTTTAA
latch 61/1 - opC (no extension)	61[30] - 92[32]	AGAACAACCAATTACATGGGAA
latch 61/2 - opC (no extension)	90[104] - 61[114]	ATTCCACTTGAGATATAACGCCCAGACGACGATAAAAA
latch 94/1 - opC (no extension)	94[66] - 94[46]	GATATAAGTTGGTAATAAGTT
latch 94/2 - opC (no extension)	93[101] - 94[88]	CCGCTGCTCAGTACCAGGCGG
core	77[70] - 50[76]	GATTTCCATTAAACGCCTGATCACTCATAGTTTGAG
core	78[62] - 50[55]	ACACCACGGCAACAGCCCTTTAACGCAATATCGGCC
core	94[45] - 63[48]	TTAATGATACATACGAGCGTATTAA
core	94[87] - 65[90]	ATAAACTCAGGAATTACGGAAAGATTAACGGATTTTAAG