Electronic Supplementary Information

Structure-Guided Synthesis of a Protein-Based Fluorescent Sensor for Alkyl Halide

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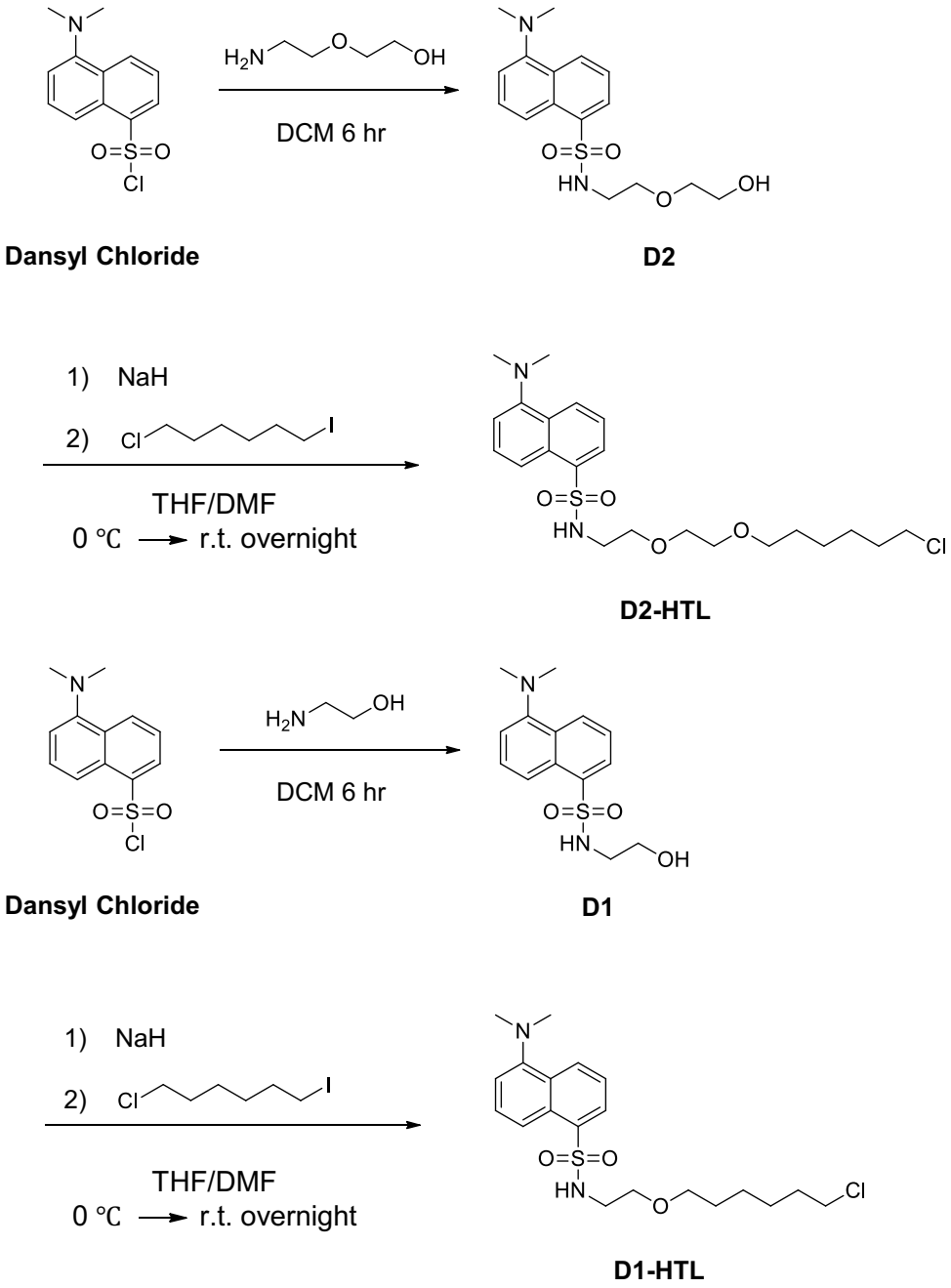
Materials and methods



Scheme S1. Synthesis of **D1-HTL** and **D2-HTL**

Probe synthesis To synthesize Dansyl-PEG2 (**D2**), 2-(2-aminoethoxy)ethanol (40 mg, 0.39 mmol, Sigma Aldrich) was added into dansyl chloride (100 mg, 0.37 mmol, Sigma Aldrich) dissolved in dichloromethane (anhydrous, Alfa aesar). The reaction was allowed to proceed at room temperature for 6 hours. After reaction, the crude product was dried by rotary evaporator and purified by silica gel column chromatography. Purified product was stored in -20℃ without any solvent. Yield: 122 mg (97%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.538 (d, J = 8.4 Hz, 1H), 8.290 (d, J = 8.8 Hz, 1H), 8.247 (d, J = 7.2 Hz, 1H), 7.561 (t, J = 8.0 Hz, 1H), 7.515 (t, J = 8.0 Hz, 1H), 7.185 (d, J = 7.6 Hz, 1H), 5.517 (t, J = 7.0 Hz, 1H), 3.568 (t, J = 4.4 Hz, 2H), 3.380 (t, J = 4.8 Hz, 2H), 3.309 (t, J = 4.4 Hz, 2H), 3.117 (q, J = 5.2 Hz, 2H), 2.881 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): 152.00, 134.90, 130.43, 129.89, 129.61, 129.44, 128.31, 123.18, 118.76, 115.22, 72.08, 69.09, 61.56, 45.40, 43.06. HR-ESI-MS: m/z calcd. for, 339.1373; measured: 339.1367.

To synthesize Dansyl-PEG2 HaloTag ligand (**D2-HTL**), Dansyl-PEG2 (D2, 95 mg) was added into dried NaH (10 mg, 0.336 mmol, Sigma Aldrich) dissolved in 2:1 dried THF and dried DMF at 0°C. After stirring at 0°C for 30 min, 1-Iodo-6-chlorohexane (103.5 mg, 0.42 mmol, Alfa aesar) was added to the solution. The reaction mixture was stirred overnight and was extracted with ethyl acetate (3 x 5ml), washed with cold water and brine. The combined organic layers were dried over Na_2SO_4 and concentrated. The crude product was purified by silica gel column chromatography. Purified product was stored in -80℃ in DMSO. Yield: 29.7 mg (23.2%).^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.534 (d, J = 8.4 Hz, 1H), 8.229 (d, J = 6.4 Hz, 1H), 8.210 (d, J = 9.2 Hz, 1H), 7.540 (t, J = 8.0 Hz, 1H), 7.512 (t, J = 8.0 Hz, 1H), 7.176 (d, J = 7.6 Hz, 1H), 3.642 (s, 2H), 3.588 (t, J = 7.2 Hz, 4H), 3.489 (t, J = 4.4 Hz, 2H), 3.343 (t, J = 6.4 Hz, 2H), 3.260 (t, J = 7.2 Hz, 2H), 2.878 (s, 6H), 1.488 (m, 2H), 1.417 (m, 2H), 1.150 (m, 2H), 0.990 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 151.78, 134.92, 130.37, 130.14, 129.99, 129.92, 127.95, 123.08, 119.46, 115.13, 72.39, 68.52, 61.66, 47.05, 45.66, 45.41, 44.73, 32.26, 27.45, 26.17, 25.54. HR-ESI-MS: m/z calcd. for, 457.1922; measured: 457.1919.

To synthesize Dansyl-PEG1 (**D1**), ethanol amine (67.8 mg, 1.11 mmol, Sigma Aldrich) was added into dansyl chloride (150 mg, 0.555 mmol, Sigma Aldrich) dissolved in dichloromethane (anhydrous, Alfa aesar). The reaction was allowed to proceed at room temperature for 6 hours. After reaction, the crude product was dried by rotary evaporator and purified by silica gel column chromatography. Purified product was stored in -20℃ without any solvent. Yield: 155.6 mg (95.5%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.550 (d, J = 8.4 Hz, 1H), 8.290 (d, J = 8.8 Hz, 1H), 8.250 (d, J = 7.2 Hz, 1H), 7.569 (t, J = 8.0 Hz, 1H), 7.522 (t, J = 8.0 Hz, 1H), 7.187 (d, J = 7.6 Hz, 1H), 5.314 (br, 1H), 3.607 (t, J = 4.8 Hz, 2H), 3.042 (q, J = 5.6 Hz, 2H).^{13}C NMR (100 MHz, $CDCl_3$): 147.32, 129.60, 125.89, 125.16, 124.97, 124.80, 123.80, 118.41, 113.77, 110.52. HR-ESI-MS: m/z calcd. for, 295.1111; measured: 295.1105

To synthesize Dansyl-PEG1 HaloTag ligand (**D1-HTL**), Dansyl-PEG1 (D1, 98 mg, 0.33 mmol) was added into dried NaH (11.9 mg, 0.4 mmol, Sigma Aldrich) dissolved in 2:1 dried THF and dried DMF at 0°C. After stirring at 0°C for 30 min, 1-Iodo-6-chlorohexane (123.2 mg, 0.5 mmol, Alfa aesar) was added to the solution. The reaction mixture was stirred overnight and was extracted with ethyl acetate (3 x 5ml), washed with cold water and brine. The combined organic layers were dried over Na_2SO_4 and concentrated. The crude product was purified by silica gel column chromatography. Purified product was stored in -80℃ in DMSO. Yield: 32.3 mg

(23.5%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.545 (d, J = 8.4 Hz, 1H), 8.280 (d, J = 8.8 Hz, 1H), 8.190 (d, J = 7.6 Hz, 1H), 7.538 (m, 2H), 7.182 (d, J = 7.6 Hz, 1H), 3.719 (t, J = 5.6 Hz, 2H), 3.420 (q, J = 6.4 Hz, 4H), 3.346 (t, J = 7.6 Hz, 2H), 1.621 (m, 2H), 1.528 (m, 2H), 1.301 (m, 2H), 1.167 (m, 2H).^{13}C NMR (100 MHz, $CDCl_3$): 151.89, 134.47, 130.56, 130.08, 129.83, 128.21, 123.11, 119.17, 115.25, 60.73, 49.42, 48.34, 45.39, 44.80, 32.30, 28.14, 26.28, 25.76, HR-ESI-MS: m/z calcd. for, 413.1660; measured: 413.1654.

Cloning and Protein Production. The DNA fragments encoding the residues 2–297 of wild type HaloTag were amplified by PCR, and cloned into a modified pET-Duet vector including a TEV protease cleavage site. The constructs used in this study are listed in supplementary table 1. For protein production, the plasmids were transformed into *Escherichia coli* BL21 (DE3) cells. The cells grown at 37˚C to an OD_{600} 0.7, then shifted to 18˚C, and proteins were induced by adding 0.2 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG). Cells were harvested using centrifugation after 20 hours post induction, and resuspended in a lysis buffer comprising 25 mM sodium phosphate pH 7.8, 400 mM sodium chloride, and 10 mM imidazole. After sonication, lysed cells were clarified using centrifugation for 60 min at 32,000 g. The supernatant was applied onto Ni^{2+}-IMAC affinity column equilibrated with the binding buffer consisting of 25 mM sodium phosphate pH 7.8, 400 mM NaCl, and 10 mM imidazole. The proteins were eluted with binding buffer supplemented with 400 mM imidazole. Histidine tags were removed using TEV protease, followed by dialysis against 25 mM Tris pH 7.8, 150 mM NaCl, and 5 mM β-mercaptoethanol. The dialyzed proteins were passed through Ni^{2+} affinity column again to remove histag and TEV protease. The proteins were further purified by gel filtration (Superdex 200 16/600 column, GE Healthcare), eluted in buffer with 25 mM Tris pH 7.5, 150 mM NaCl, and 5 mM DTT. For HaloTag-D2-HTL ligand complex, the purified proteins were mixed with D2-HTL ligand in a 1:3 molar ratio (protein:ligand) for 5 hours, and subjected to another round of size-exclusion purification under same condition to remove extra D2-HTL. The complex was concentrated to approximately 15 mg/ml and flash frozen in liquid nitrogen for storage. All mutants were generated using a PCR based site directed mutagenesis and the mutations were confirmed by DNA sequencing.

Crystallization and Structure Determination. Crystals of apo-^{M175C}HaloTag were obtained by the hanging-drop method in the well solution containing 25% PEG 20K, 100 mM Tris pH 8.2, 200 mM magnesium chloride, and 5% tertiary butanol at 20°C. The initial crystals of ^{M175C}HaloTag conjugated with D2-HTL ligand were grown in the crystallization solution comprising 1.8 M ammonium sulfate and 100 mM citric acid pH 5.5 at room temperature. Because the crystal of holo-^{M175C}HaloTag were grown as a needle cluster, the micro-seeding method was performed to obtain the diffraction quality crystals. Microseeds generated by Seed Bead Kit (HR2-320, Hampton Research) were added to crystallization drop comprising protein and reservoir solution. By microseeding, the plate shape crystals were obtained in the improved reservoir solution containing 1.3 M ammonium sulfate and 100 mM citric acid pH 5.5 at room temperature. For X-ray diffraction experiments, the crystals of apo-^{M175C}HaloTag and holo-^{M175C}HaloTag were transferred to cryo-solutions consisting of crystallization solution plus 20% glycerol and then flash-frozen in liquid nitrogen. Diffraction data were collected at beamline 7A of the Pohang Accelerator Laboratory (PAL) and processed using HKL2000[1]. Space groups of the apo-^{M175C}HaloTag and holo-^{M175C}HaloTag are $P4_32_12$ (a, b=62.642, c=164.192 Å), and $P2_12_12_1$ (a=44.393, b=72.355, c=174.942 Å), respectively. The crystal structures were determined by molecular replacement with the Phaser program[2], using the apo-HaloTag structure (PDB ID: 4KAF) as a reference model. The model building and refinement were performed by Coot[3] and Phenix[4] program, respectively. All structural figures were generated by Pymol (https://www.pymol.org). Crystallographic data were summarized in Table S2.

Spectral Measurements of Alkyl Halides Assay. 200 μM D1-HTL, D2-HTL, HaloTag protein and alkyl halides were prepared as a stock solution. Without alkyl halides reaction, 1 μl of D1-HTL or D2-HTL was added into 96-well black plate (SPL, Korea). 198 μl PBS (pH 7.4) and 1ul HaloTag protein were added sequentially. For alkyl halides assay, 1 μl HaloTag protein and 1 μl alkyl halide were reacted in 197 μl of PBS solution in the black plate for 10 min. After 10 min reaction, 1 μl of D1-HTL or D2-HTL was added into the solution. Alkyl halides assay was analyzed by Spectramax i3x (Molecular Devices).

Alkyl Halides Assay in Human Serum. 1 μl of 75mM alkyl halides were mixed with 74 μl human serum (Sigma Aldrich, H4522). Every 10 min, 1 μl human serum mixtures were added with 1:1000 diultion into 1 μM ^{M175P}HaloTag solution in PBS and incubated for 10 min. Then, 1 μl of 1 mM D1-HTL was added into mixtures. After 5 min, D1-HTL fluorescence was obtained by Spectramax i3x (Molecular Devices).

Mass Sample Preparation. 1 mM 1-Bromohexane and 1-Bromoheptane was incubated in human serum (containing 2mg serum protein) for 1 hr. 100 μg of alkyl halide-treated human serum was loaded on 10% SDS-PAGE gel and run by 15 mA current for 1 hr. Gels were cut into 1mm x 1mm size by knife and washed with water 3 x 5 min. Then water was replaced by 100 mM ammonium bicarbonate (ABC, Sigma Aldrich, A6141) buffer and incubated for 5 min. Next 1:1 of 100 mM ABC and acetonitilie was incubated 2 x 5 min and it's replace by 100% acetonitile and incubated for 5 min. 100 mM ABC, 1:1 solution, and 100% acetonitlile was again incubated for 5 min, respectively. After removing of 100% acetonitlie, gels were dried by speed-vac for 10 min. 10 mM of dithiothreitol (DTT) in 100 mM ABC solution was added to dried gel and incubated at 56 ℃ for 1 hr. DTT solution was replaced by 55 mM iodoacetamide (IAM, Sigma Aldrich, I1149) in 100 mM ABC solution for 30 min with dark condition. After alkylation by IAM, gels were washed by 100 mM ABC, 1:1 solution, and 100% acetonitlile 2 x 5 min, respectively. Gels were again dried by speed-vac and 25 ng/μl concentration of trypsin was incubated overnight. Peptides were eluted by 1:2 mixture of 5% formic acid and 100% acetonitrile solution. Eluted peptides were dired with speed-vac and again eluted with 0.1% formic acid for purification by desalting step.

Desalting and LC MS/MS A home-made column was used for purification of eluted sample. The end of a 200-μL Eppendorf tip was blocked with a 3M Empore C8 disk (3M Bioanalytical Technologies, 2214) and ~10mg of activated charcoal (Sigma Aldrich, C5510) was added. The column was activated by sequential centrifugation at 2000 × g with 150 μL of 3 % acetonitrile/0.1 % formic acid (v/v), 150 μL of 100 % acetonitrile, and 150 μL of 3 % acetonitrile/0.1 % formic acid (v/v). Then, the samples in the 0.1 % formic acid were added to the column. The column was washed using 150 μL of 0.1 % formic acid (v/v) two times. The column was then eluted with 140 μL of 70 % acetonitrile/0.1 % formic acid (v/v) twice and then with 200 μL of 100 % acetonitrile. The eluted fraction was dried in a speed-vac and peptides were analyzed on LTQ-Orbitrap Elite mass spectrometer (Thermo Scientific) equipped with a nanoelectrospray ion source. A C18 reverse-phase HPLC column (500 mm × 75 μm i.d.) was used to separate the peptide mixture using a 2.4–17.6% acetonitrile/0.1% formic acid gradient for 120 min at a flow rate of 300 nL/min. For MS/MS analysis, precursor ion scan MS spectra (m/z 400 - 2000) were acquired using the Orbitrap spectrometer at a resolution of 60 K at 400 m/z with an internal lock mass. The 20 most intensive ions were isolated and fragmented in the linear ion trap by collisionally induced dissociation (CID).

Table S1. Photophysical properties of Dansyl HaloTag ligands

entry	λ_{ex}[a]	λ_{em}[b]	Solvent	Second order rate constant ($M^{-1}s^{-1}$)	Quantum Yield[c]	Extinction Coefficient ($M^{-1}cm^{-1}$)
D1-HTL	316	568	PBS	N/A	0.0015	429
D2-HTL	328	572	PBS	N/A	0.0011	411
D2-HTL+^{WT}HaloTag	275	508	PBS	0.053×10^4	0.14	11550
D2-HTL+^{M175P}HaloTag	274	507	PBS	0.175×10^4	0.17	13200
D1-HTL+^{WT}HaloTag	276	508	PBS	0.10×10^4	0.23	14850
D1-HTL+^{M175P}HaloTag	274	507	PBS	1.14×10^4	0.41	15000

[a] excitation wavelength (nm)

[b] emission wavelength (nm)

[c] Quantum yields were measured by comparison of quantum yield of dansyl chloride[5]

Table S2. Plasmids Information for HaloTag mutants.

Name (expected size)	Features	Promotor/ Vector	Details
HaloTag wild type (34.1kDa)	Histag-TEV-*BamHI*-HaloTag7(wild type) -Stop codon-*HindIII*	T7/ pET-duet	HaloTag7 (PDB ID: 4KAF): 2-297 aa; Histag: HHHHHH TEV: ENLYFQS
HaloTag (M175C) (34.1kDa)	Histag-TEV-*BamHI*-HaloTag7(M175C)-Stop codon-*HindIII*	T7/ pET-duet	HaloTag7 (PDB ID: 4KAF): 2-297 aa; Histag: HHHHHH TEV: ENLYFQS
HaloTag (M175H) (34.1kDa)	Histag-TEV-*BamHI*-HaloTag7(M175H)-Stop codon-*HindIII*	T7/ pET-duet	HaloTag7 (PDB ID: 4KAF): 2-297 aa; Histag: HHHHHH TEV: ENLYFQS
HaloTag (M175K) (34.1kDa)	Histag-TEV-*BamHI*-HaloTag7(M175K)-Stop codon-*HindIII*	T7/ pET-duet	HaloTag7 (PDB ID: 4KAF): 2-297 aa; Histag: HHHHHH TEV: ENLYFQS
HaloTag (M175L) (34.1kDa)	Histag-TEV-*BamHI*-HaloTag7(M175L)-Stop codon-*HindIII*	T7/ pET-duet	HaloTag7 (PDB ID: 4KAF): 2-297 aa; Histag: HHHHHH TEV: ENLYFQS
HaloTag (M175N) (34.1kDa)	Histag-TEV-*BamHI*-HaloTag7(M175N)-Stop codon-*HindIII*	T7/ pET-duet	HaloTag7 (PDB ID: 4KAF): 2-297 aa; Histag: HHHHHH TEV: ENLYFQS
HaloTag (M175P) (34.1kDa)	Histag-TEV-*BamHI*-HaloTag7(M175P)-Stop codon-*HindIII*	T7/ pET-duet	HaloTag7 (PDB ID: 4KAF): 2-297 aa; Histag: HHHHHH TEV: ENLYFQS
HaloTag (M175T) (34.1kDa)	Histag-TEV-*BamHI*-HaloTag7(M175T)-Stop codon-*HindIII*	T7/ pET-duet	HaloTag7 (PDB ID: 4KAF): 2-297 aa; Histag: HHHHHH TEV: ENLYFQS
HaloTag (M175W) (34.1kDa)	Histag-TEV-*BamHI*-HaloTag7(M175W)-Stop codon-*HindIII*	T7/ pET-duet	HaloTag7 (PDB ID: 4KAF): 2-297 aa; Histag: HHHHHH TEV: ENLYFQS
HaloTag (M175Y) (34.1kDa)	Histag-TEV-*BamHI*-HaloTag7(M175Y)-Stop codon-*HindIII*	T7/ pET-duet	HaloTag7 (PDB ID: 4KAF): 2-297 aa; Histag: HHHHHH TEV: ENLYFQS

Table S3. X-ray Data Collection and Refinement Statistics

	^{M175C}HaloTag	^{M175C}HaloTag/D2-HTL
Data set:	Native	Native
PDB accession #:	5Y2X	5Y2Y
X-ray source	Beamline 7A, PAL	Beamline 7A, PAL
Temperature (K)	100	100
Space group:	$P4_32_12$	$P2_12_12_1$
Cell parameters a, b, c (Å)	62.642, 62.642, 164.192	44.393,72.355,174.942
α, β, γ (°)	90.00, 90.00, 90.00	90.00, 90.00, 90.00
Data processing		
Wavelength (Å)	1.00000	1.00000
Resolution (Å)	50-2.02	50-2.27
R_{merge} (%)[a]	9.4 (33.4)	5.6 (7.3)
I/σ	26.4 (5.4)	56.1 (38.6)
Completeness (%)	99.5 (90.8)	98.4 (88.0)
Redundancy	6.1 (5.5)	10.4 (9.6)
Measured reflections	423819	275846
Unique reflections	22255	49251
Refinement statistics		
Data range (Å)	34.44-2.02	34.73-2.27
Reflections	22178	26291
Nonhydrogen atoms	2366	4730
Water molecules	259	318
R.m.s. Δ bonds (Å)[b]	0.005	0.012
R.m.s. Δ angles (°)[b]	1.022	1.367
R-factor (%)[c]	16.3	15.8
R_{free} (%)[c, d]	19.2	21.5
Ramachandran plot, residues in		
Most favored regions (%)	95.9	95.7
allowed regions (%)	4.1	4.3
Disallowed regions (%)	0	0

*Highest resolution shell is shown in parenthesis.

[a] $R_{merge} = 100 \times \sum_h \sum_i | I_i(h) - \langle I(h) \rangle | / \sum_h \langle I(h) \rangle$, where $I_i(h)$ is the ith measurement and $\langle I(h) \rangle$ is the weighted mean of all measurement of $I(h)$ for Miller indices h.

[b] Root-mean-squared deviation (r.m.s. Δ) from target geometries.

[c] R-factor = $100 \times \sum |F_P - F_{P(calc)}| / \sum F_P$.

[d] R_{free} was calculated with 5% of the data.

Supplementary Results

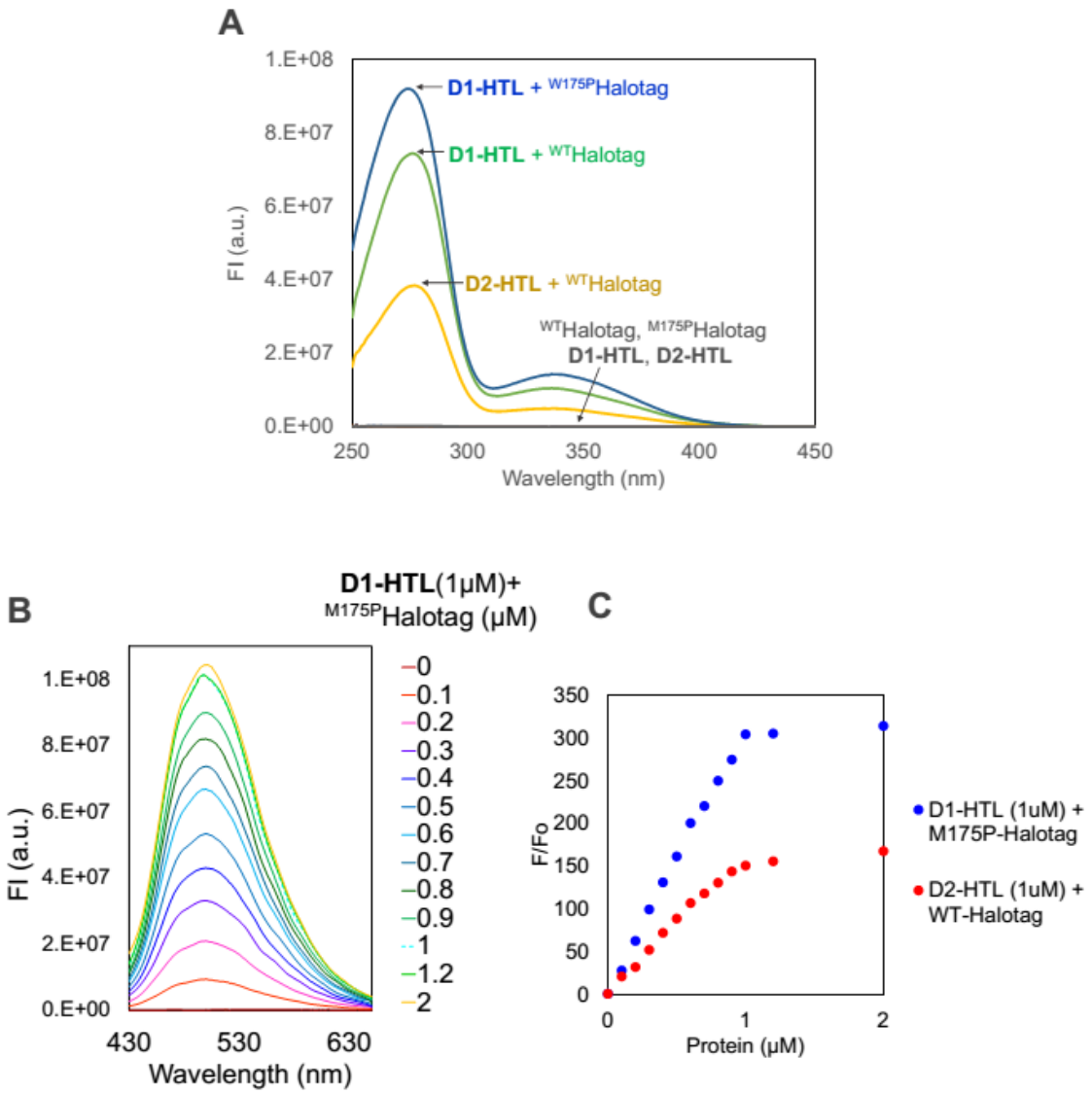


Figure S1. Fluorescence Excitation and Emission Spectra of D1-HTL. (A) Fluorescence excitation spectra of **D1-HTL** and **D2-HTL** (1 μM) before and after addition of wild-type (WT) HaloTag or ^{W175P}HaloTag (1 μM). Fluorescence emission wavelength was fixed at 520 nm. Maximum fluorescence excitation wavelength was observed at 280nm. (B) Fluorescence emission spectra of **D1-HTL**(1 μM) upon addition of ^{W175P}HaloTag (from 0 to 2 μM). The fluorescence was measured after 30min incubation. (C) Fluorescence intensity change (F/Fo) of **D1-HTL** and **D2-HTL** at 515 nm (excitation at 280 nm) upon addition of ^{W175P}HaloTag and ^{WT}HaloTag, respectively. F_o value indicates fluorescence emission intensity of **D1-HTL** or **D2-HTL** without addition of HaloTag proteins.

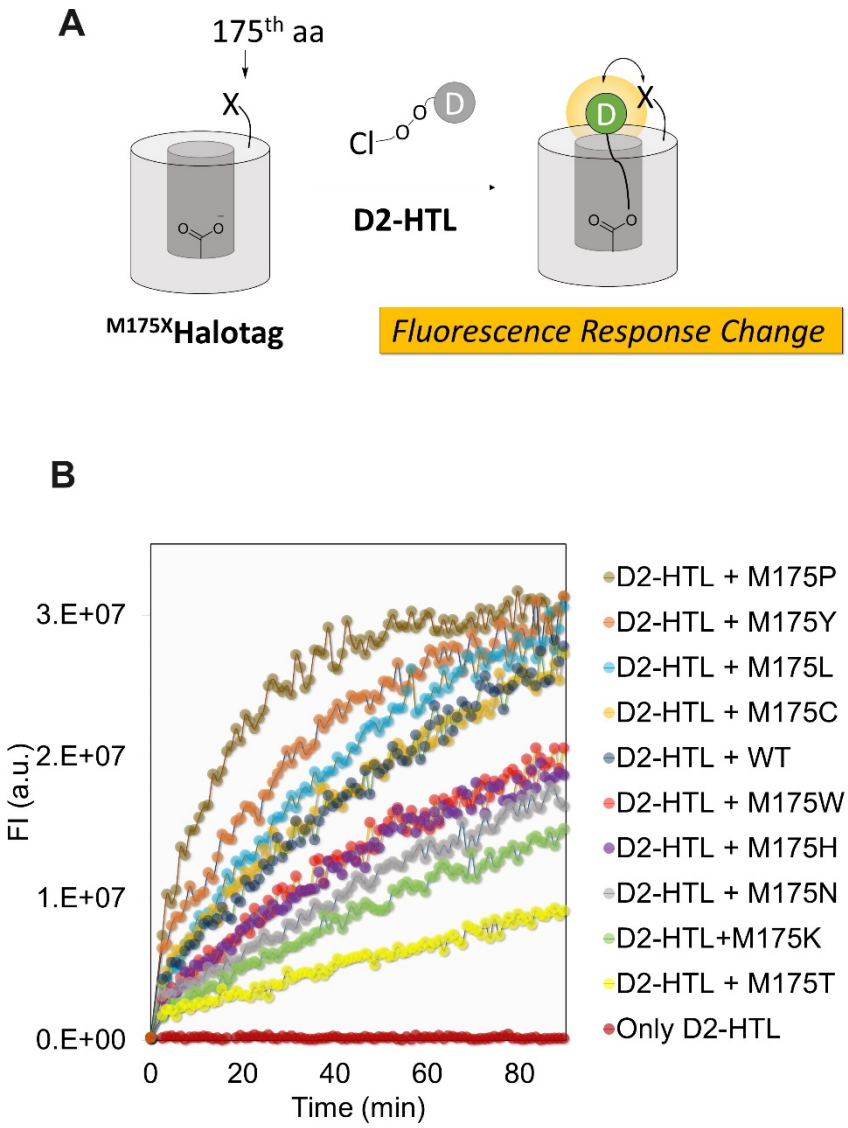


Figure S2. Time-course measurement of D2-HTL with HaloTag Mutants. (A) scheme of HaloTag mutants (M175X) screening assay with D2-HTL. (B) Time-course measurement of the fluorescence response of D2-HTL (1 μM) by the addition of each HaloTag mutant (1 μM); The excitation wavelength is 280 nm and emission wavelength is 515 nm.

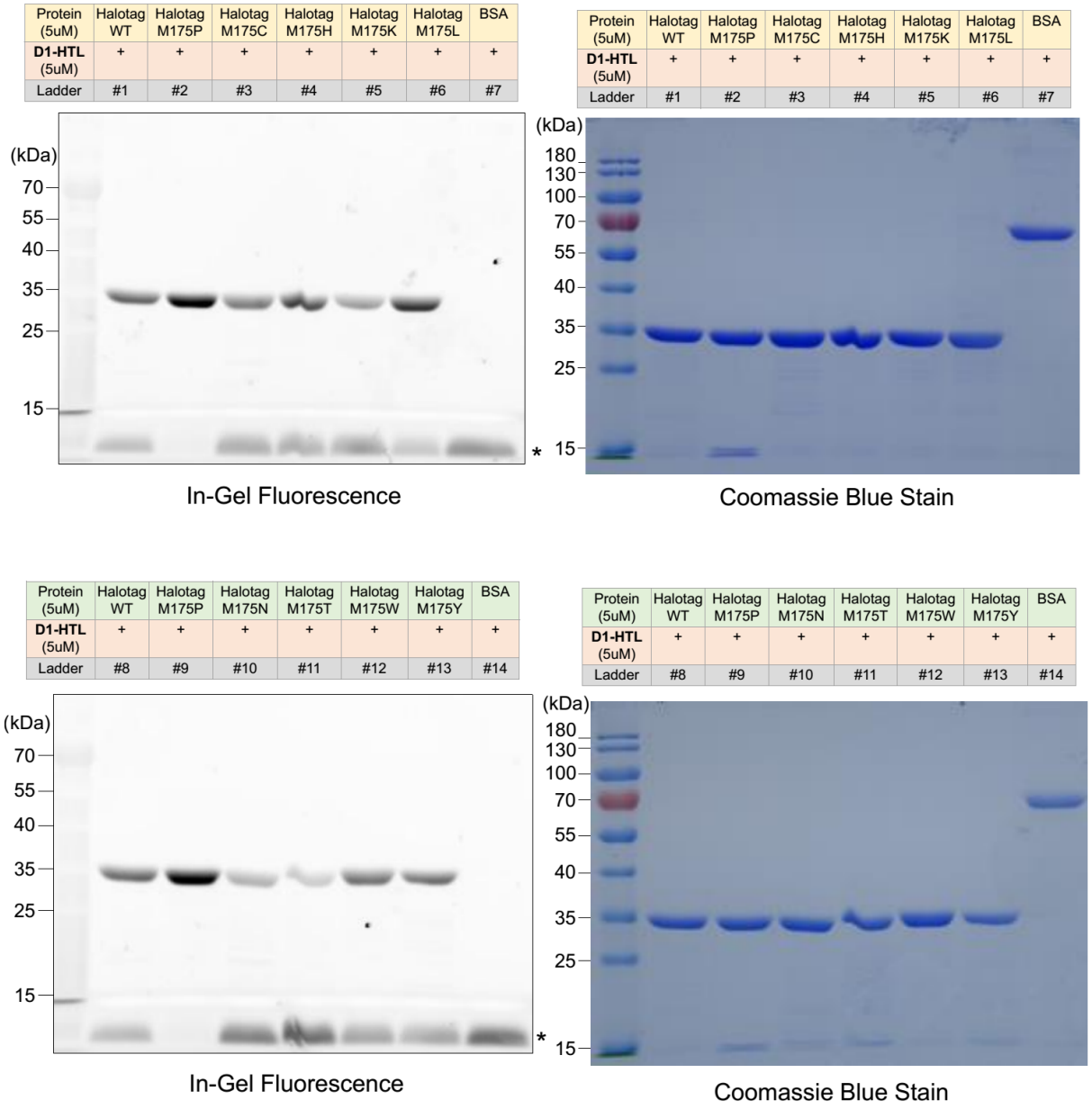


Figure S3: In-gel fluorescence image of HaloTag mutants with D1-HTL. HaloTag mutants proteins and **D1-HTL** (each 50 pmol) were incubated for 10min at RT and the protein-ligand complex were analyzed by 10% SDS-PAGE. Wild-type, M175P, M175C, M175H, M175K, M175L-HaloTag (upper) and Wild-type, M175P, M175N, M175T, M175W, M175Y (below) were analyzed separately. In-gel fluorescence was analyzed by AF430 channel in Chemidoc (Syngene, UK). Aesterisk marks indicates unbound D1-HTL. Same amount of protein addition was validated by Coomassie Blue stain (right) with the same gel.

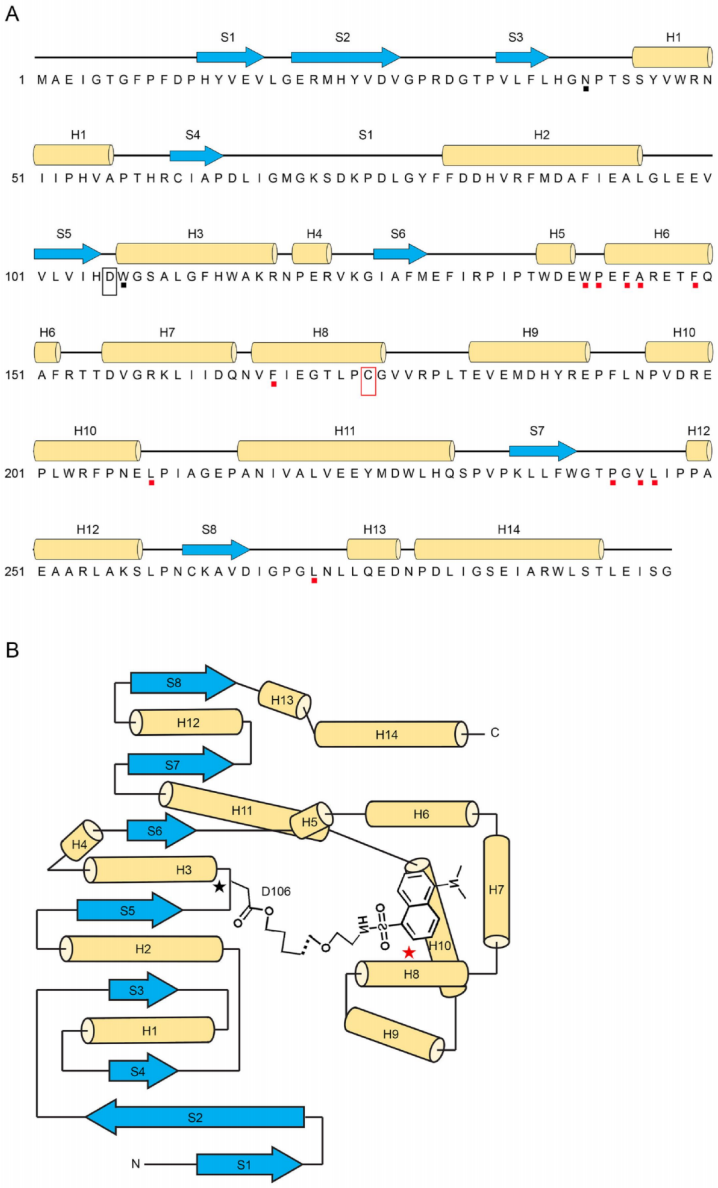


Figure S4. Primary and secondary structure of HaloTag. (A) The sequences of ^{M175C}HaloTag are represented with the secondary structure elements determined by crystal structure. Blue arrows and yellow cylinders indicate the 🚲-strands and ✔-helices, respectively. The residue highlighted by red and black box indicates the mutation site (M175C) and conjugation site (D106) by **D2-HTL**. The red and black dots represent residues that are involved in the interaction with **D2-HTL** ligand, and residues coordinating chloride ion, respectively. (B) Figure shows the topology diagrams of the holo-^{M175C}HaloTag with helices and strands represented as cylinders and arrows, respectively. The HaloTag protein consists of fourteen ✔-helices and eight 🚲-strands. The black and red star indicate the conjugating residues of **D2-HTL** ligand and mutation site (M175) used in this study, respectively.

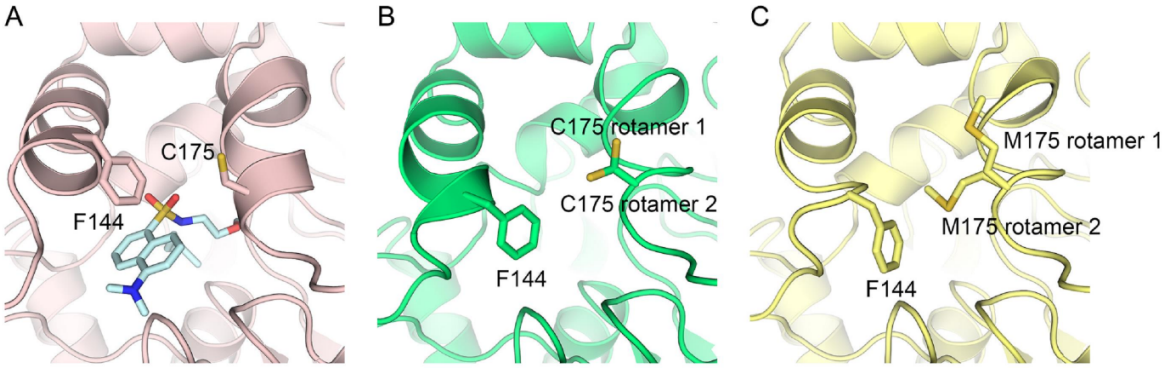


Figure S5: F144 and M175 of HaloTag act as a gate for D2-HTL conjugation. Figure shows the molecular diagrams of holo-^{M175C}HaloTag (A), apo-^{M175C}HaloTag (B), and wild-type HaloTag (C) to compare the structural differences at dansyl ring binding sites among three proteins. The D2-HTL coordinated by ^{M175C}HaloTag is shown cyan stick model in (A). The two structural variations for C175 and M175 in apo-HaloTag are indicated in (B) and (C), respectively.

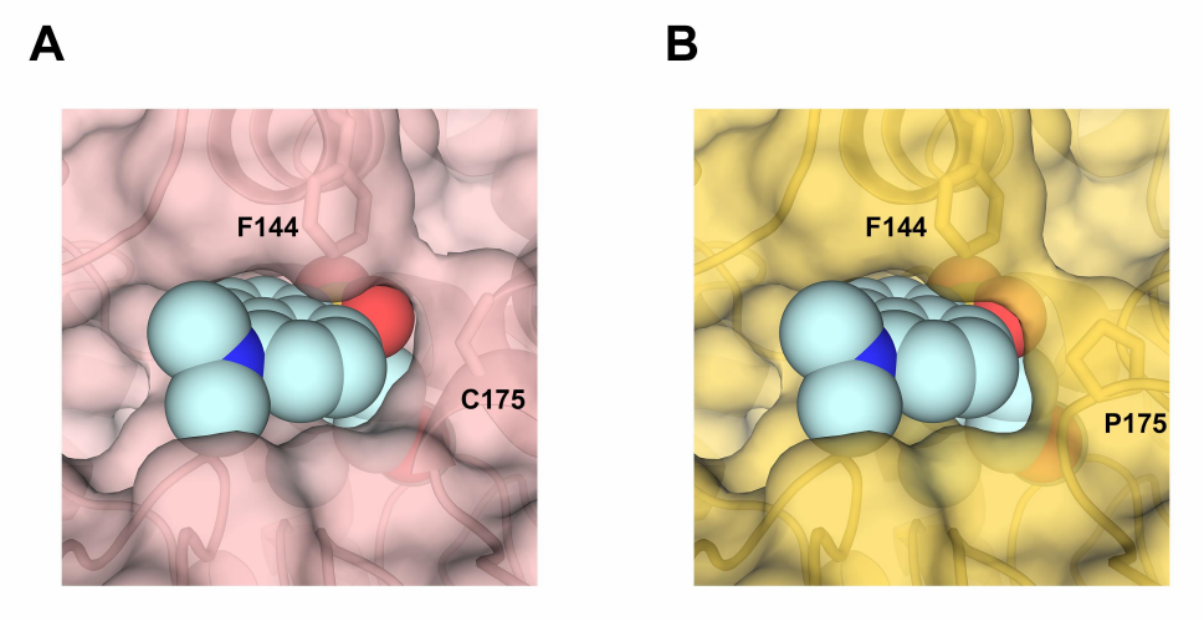


Figure S6. Surface filling model of holo-HaloTag complex. This surface filling model highlighting the van der waals interaction between dansyl ring of D2-HTL (cyan) and the side chains of F144, C175 (A) or P175 (B) in HaloTag pocket. Note that the structure of P175 is from putative modeling based on the crystal structure of holo-Halo tag

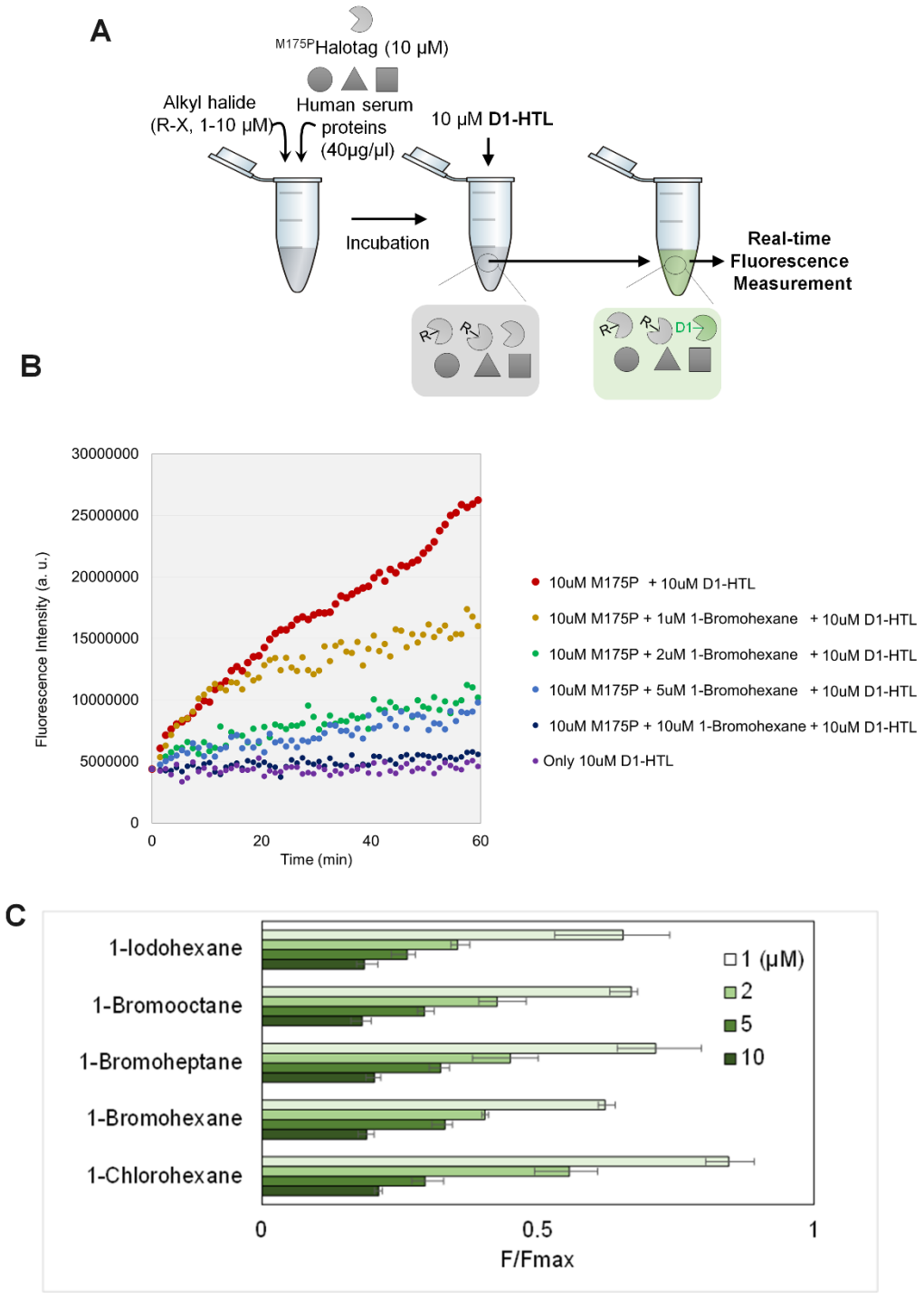
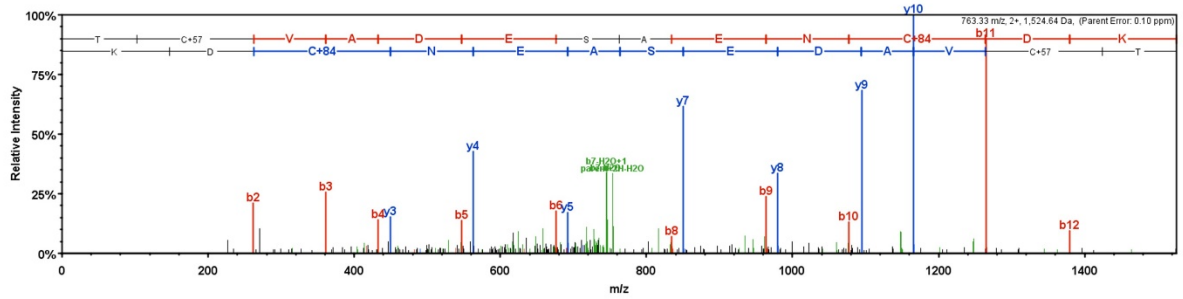
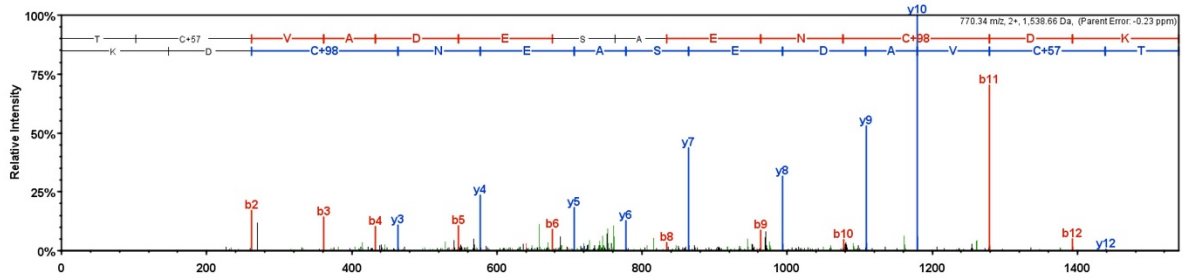


Figure S7. Alkyl halides detection in human serum by using D1-HTL and ^{M175P} Haltoag. (A) Scheme of fluorescent measurement of alkyl halide in human serum by using our hybrid sensor (B) Time-course detection of 1-bromohexane (1-10 μM) in human serum using D1-HTL (10 μM) and ^{M175P}HaloTag (10 μM); excitation at 280 nm/emission at 510 nm. (C) Detection of other alkyl halides (1-10 μM) in human serum using ^{M175P}HaloTag and D1-HTL hybrid sensor (each 10 μM).

(1-Bromohexane)_TCVADESAENC+84.094DK Human Serum Albumin
Charge; 2+ Mass; 1524.64



(1-Bromoheptane)_TCVADESAENC+98.110DK Human Serum Albumin
Charge; 2+ Mass; 1538.66



(1-Bromoheptane)_AAFTEC+98.110CQAADK Human Serum Albumin
Charge; 2+ Mass; 1411.65

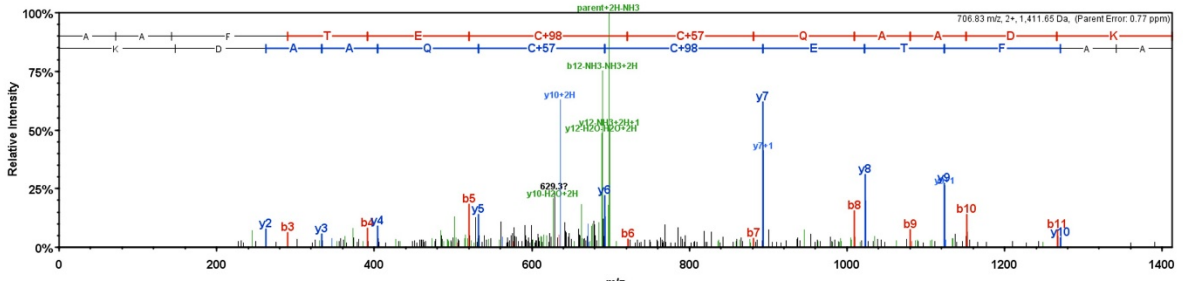


Figure S8. MS/MS spectra of alkylated peptides. MS/MS spectra of alkylated peptides of human serum albumin (HAS) by 1-bromohexane and 1-bromoheptane were shown.

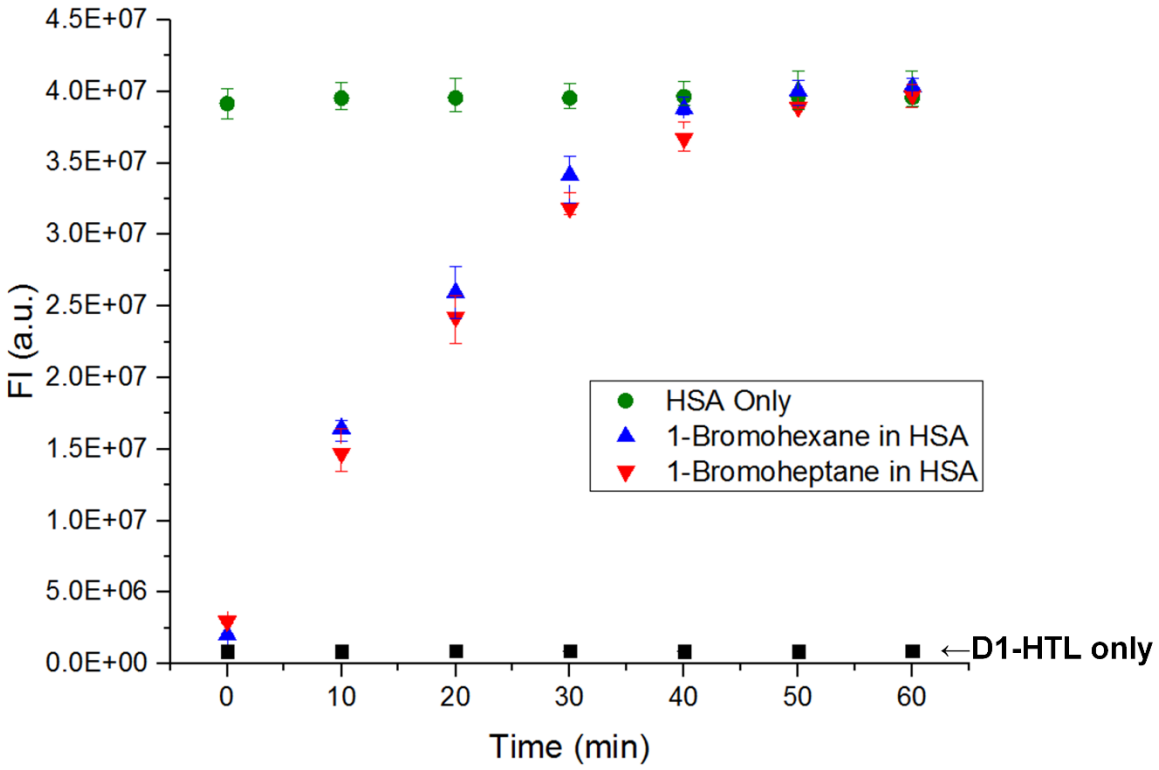


Figure S9. Time-course detection of consumed alkyl halides (e.g. 1-bromohexane, 1-bromoheptane) by human serum albumin (HSA, 20 μg/μl) using D1-HTL (1 μM) and ^{M175P}HaloTag (1 μM). 1mM alkyl halides were pre-incubated with 20 μg/μl human serum albumin and the pre-incubated solution (0-60 min) was 1000x diluted in hybrid probe solution of D1-HTL (1 μM) and ^{M175P}HaloTag (1 μM). The dansyl fluorescence was measured after 10min incubation at room temperature (excitation at 280 nm/emission at 510 nm).

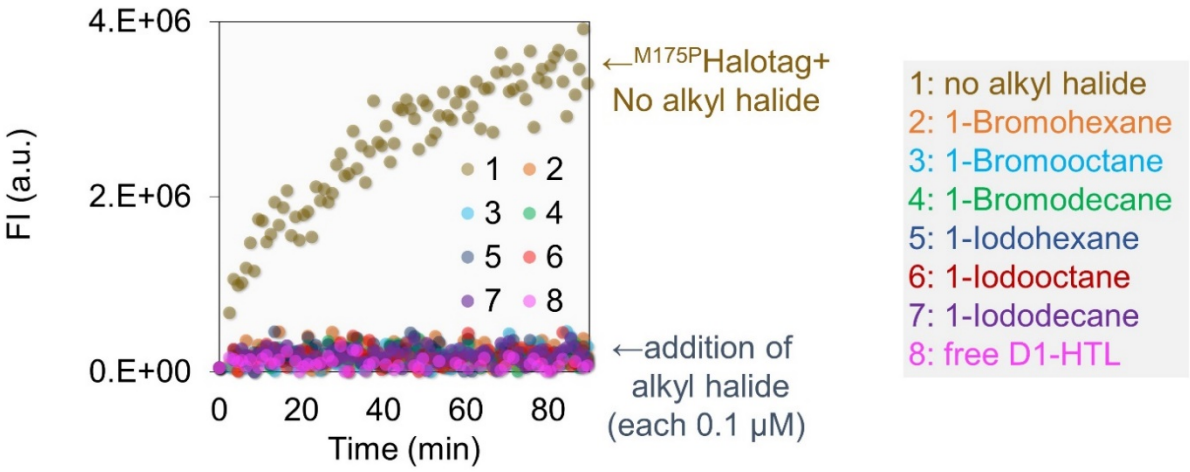


Figure S10: Alkyl halides detection in tap water. Time course measurement of fluorogenic response of D1-HTL (0.1 µM) upon addition of ^{M175P}HaloTag or a premix of M175PHaloTag and various alkyl halide (0.1 µM each) in tap water. The excitation wavelength is 280 nm and the emission wavelength is 515 nm.

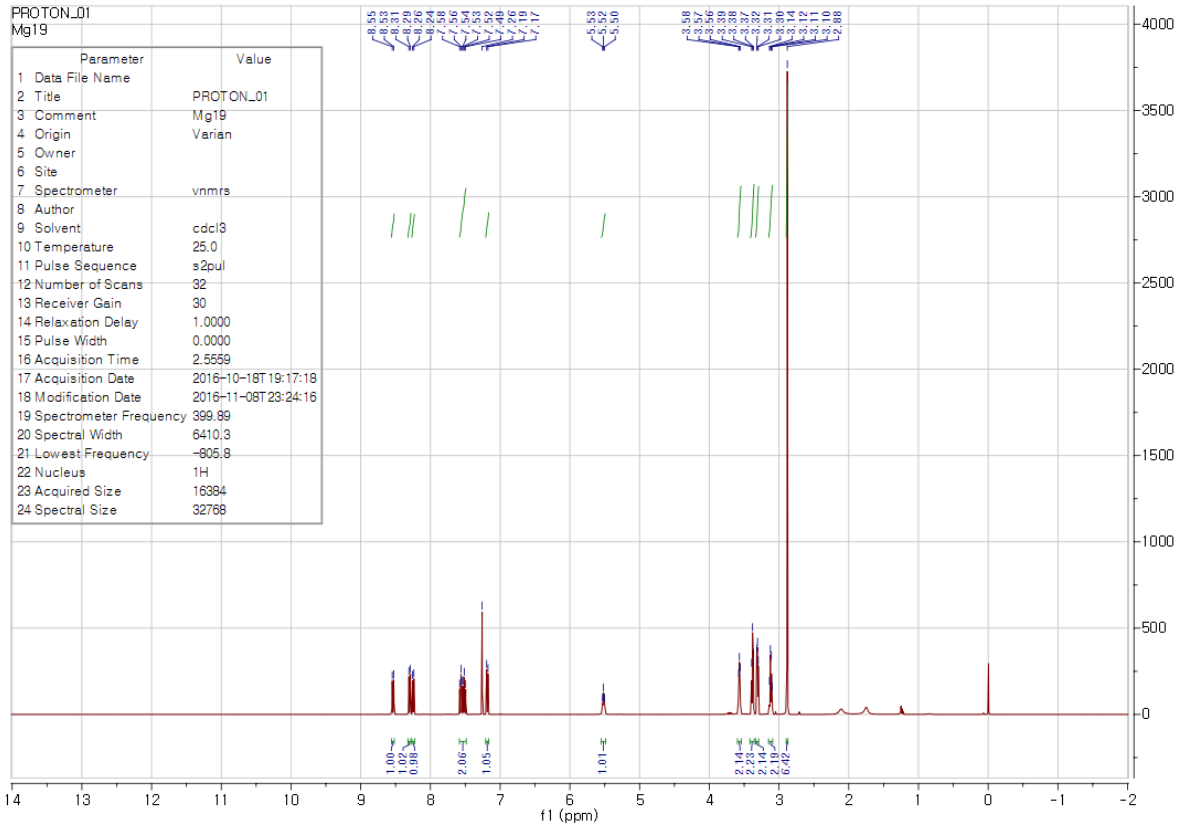


Figure S11: ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **D2**

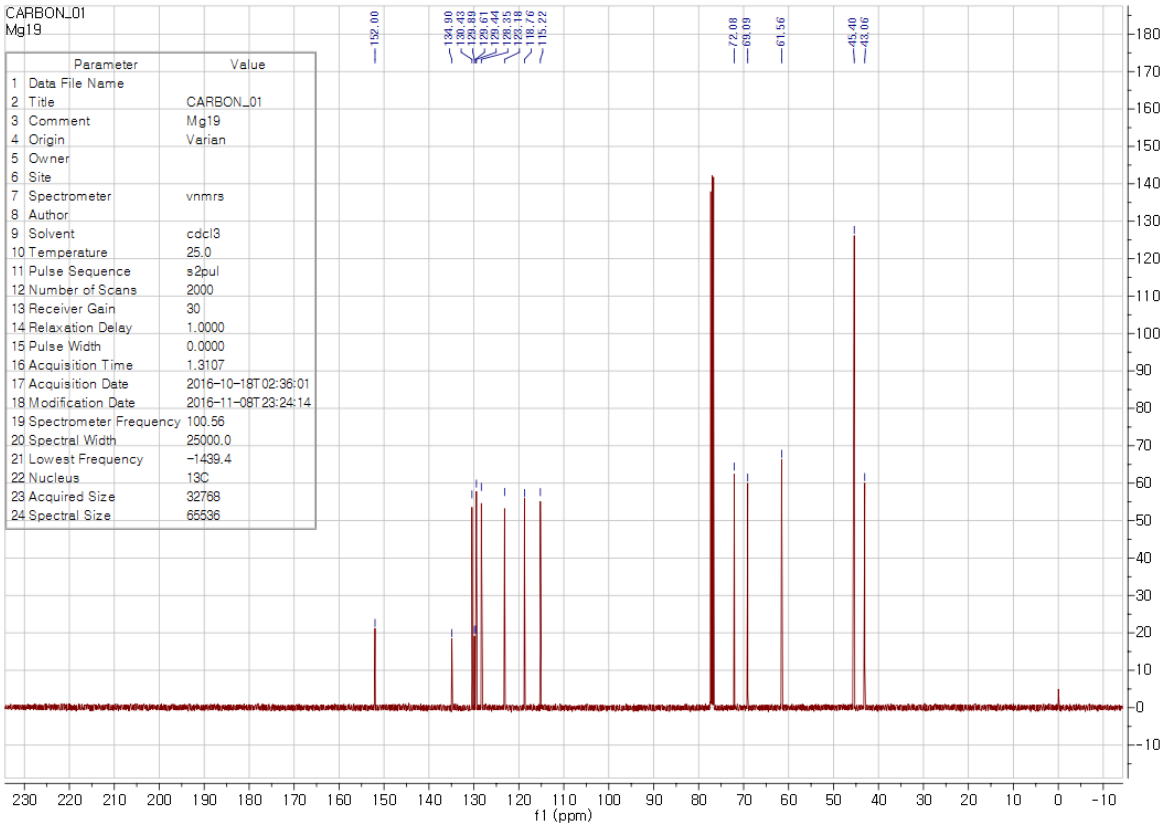


Figure S12: ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **D2**

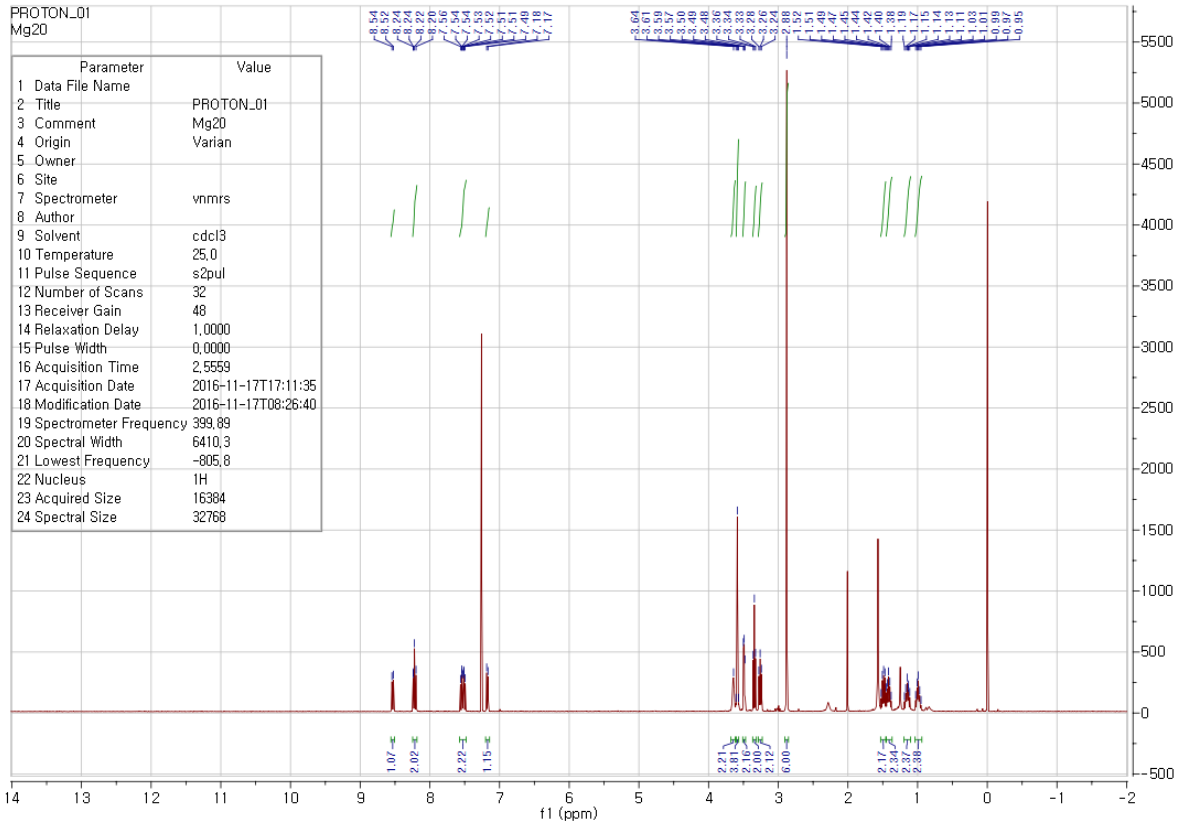


Figure S13: ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **D2-HTL**

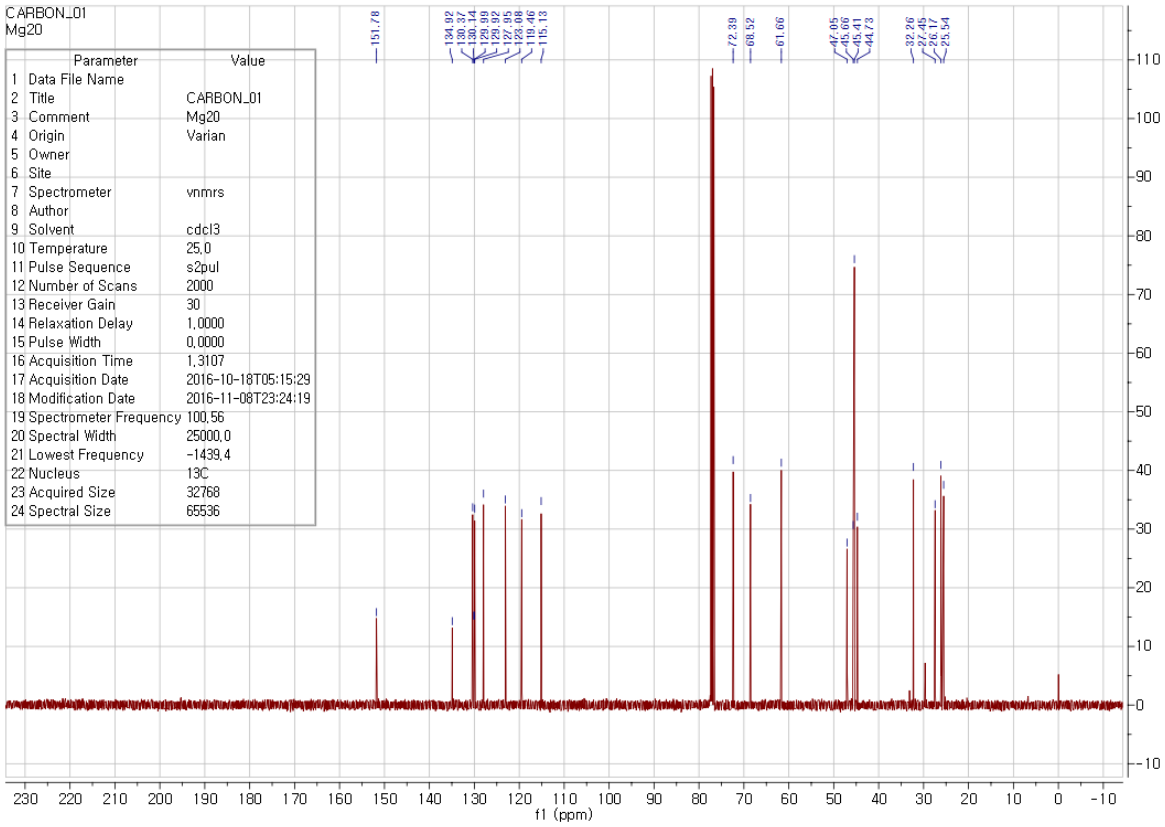


Figure S14: ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **D2-HTL**

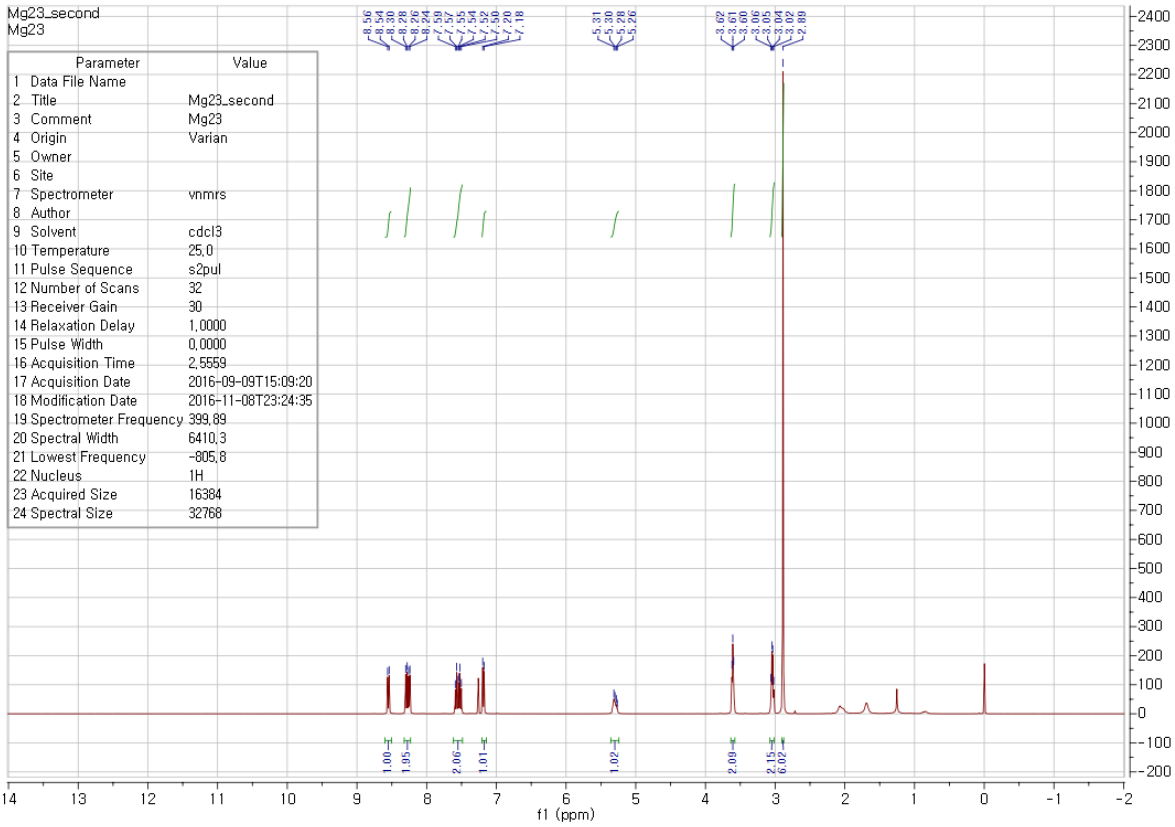


Figure S15: ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **D1**

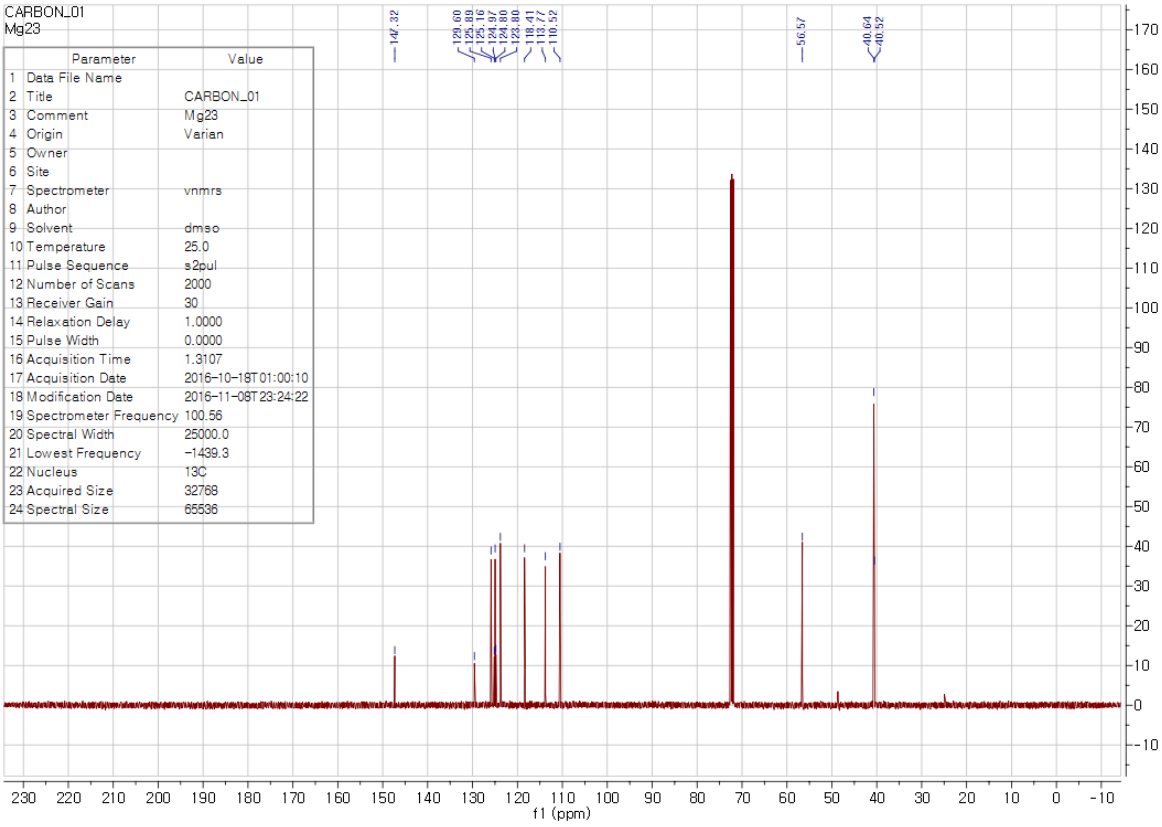


Figure S16: ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **D1**

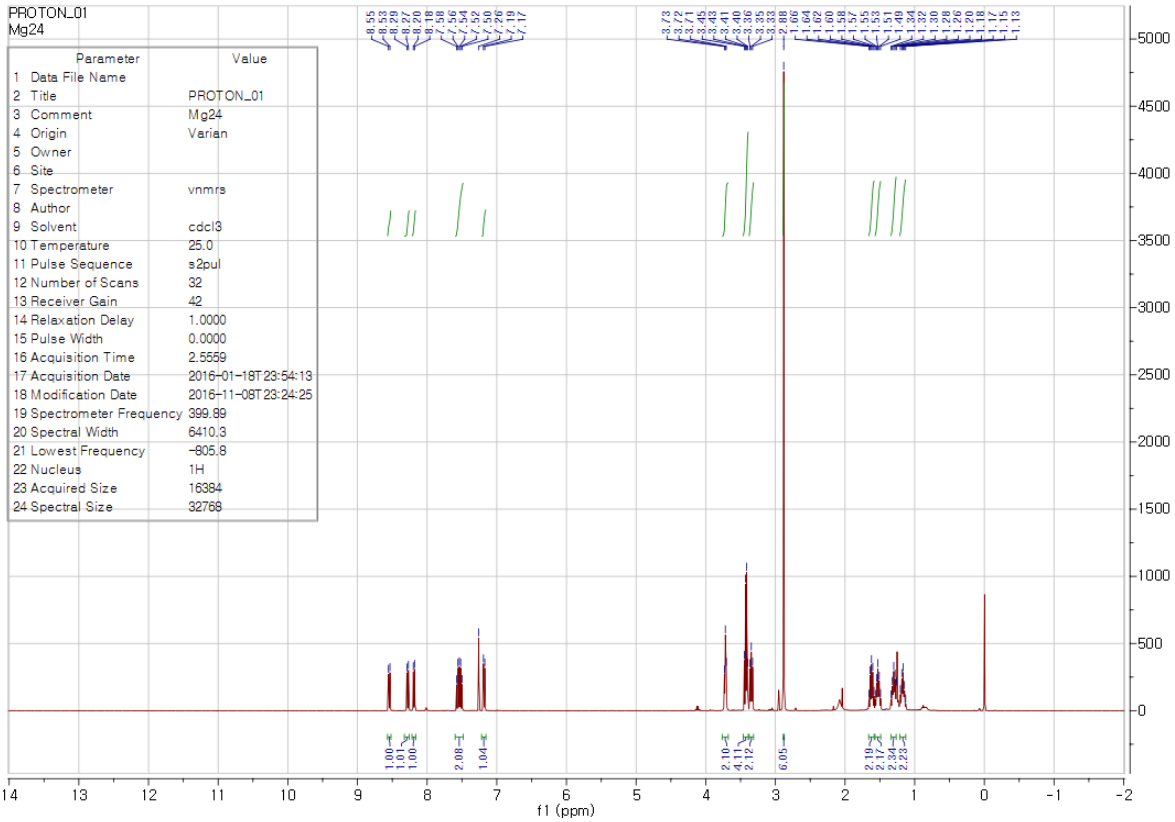


Figure S17: ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **D1-HTL**

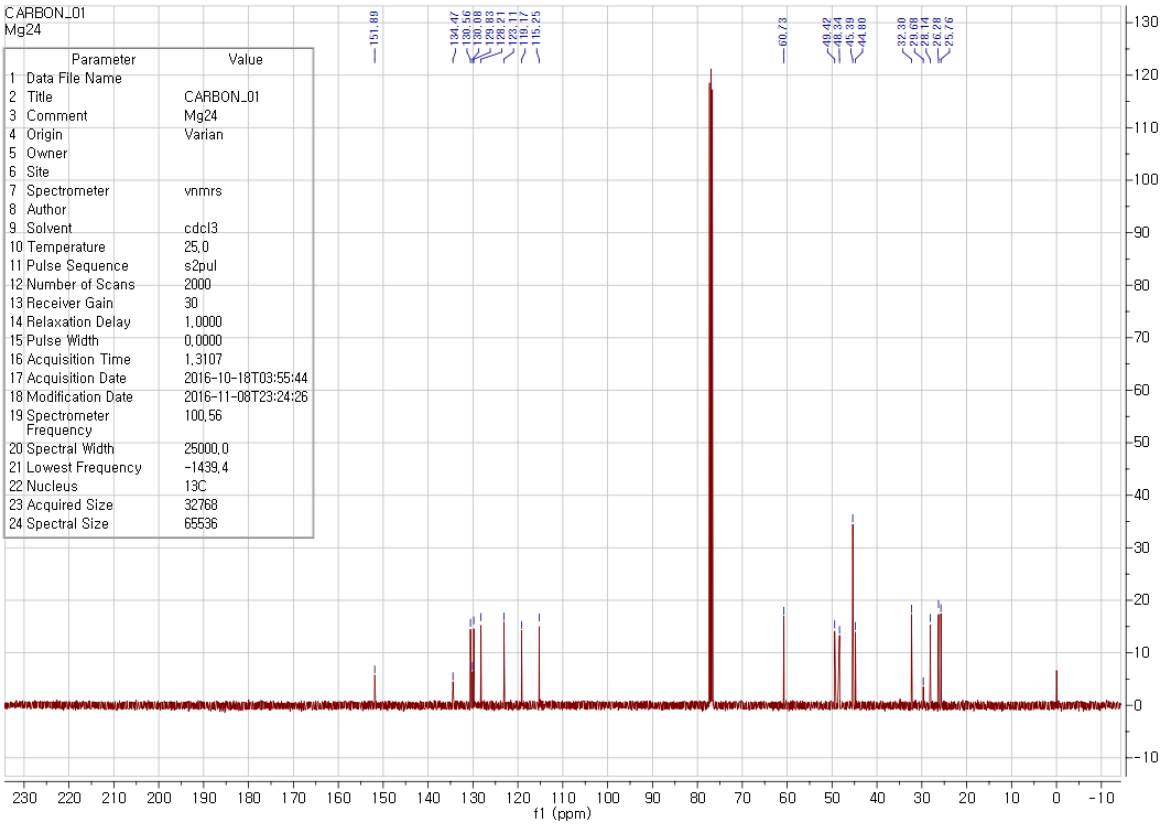


Figure S18: ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **D1-HTL**

Reference in Supporting Information

(1) Otwinowski, Z.; Minor, W. In *Methods Enzymol.*; Charles W. Carter, Jr., Ed.; Academic Press: 1997; Vol. Volume 276, p 307.
(2) McCoy, A. J.; Grosse-Kunstleve, R. W.; Adams, P. D.; Winn, M. D.; Storoni, L. C.; Read, R. J. *J. Appl. Crystallogr.* **2007**, *40*, 658.
(3) Emsley, P.; Lohkamp, B.; Scott, W. G.; Cowtan, K. *Acta Crystallogr. D* **2010**, *66*, 486.
(4) Adams, P. D.; Afonine, P. V.; Bunkoczi, G.; Chen, V. B.; Davis, I. W.; Echols, N.; Headd, J. J.; Hung, L.-W.; Kapral, G. J.; Grosse-Kunstleve, R. W.; McCoy, A. J.; Moriarty, N. W.; Oeffner, R.; Read, R. J.; Richardson, D. C.; Richardson, J. S.; Terwilliger, T. C.; Zwart, P. H. *Acta Crystallogr. D* **2010**, *66*, 213.
(5) Keyur, D. B.; Hrishikesh, S. G.; Bharat, A. M.; Disha, J. V/; Debdepp, M.; Vinod K. J. *J Fluoresc*. 2012, 22, 1493