

Supplemental Information

Development of stabilized peptide-based PROTACs against estrogen receptor

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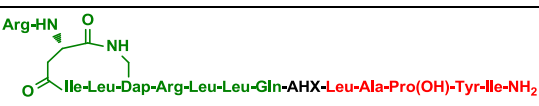
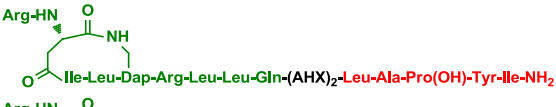
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1. Abbreviations of used chemicals

BSA, bovine serum albumin;
Dap, 2,3-diaminopropionic acid;
DAPI, 4',6-diamidino-2-phenylindole;
DCM, dichloromethane;
DIPEA, N,N-diisopropylethylamine;
DMEM, dulbecco's modified eagle medium;
DMF, N,N-dimethylformamide;
DTT, dithiothreitol;
EDTA, ethylenediaminetetraacetic acid; ER, estrogen receptor;
Et₂O, diethyl ether;
E2, estradiol;
FBS, fetal bovine serum;
FITC, fluorescein isothiocyanate;
Fmoc, 9-fluorenylmethyloxycarbonyl;
HCTU, O-(1H-6-chlorobenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate;
HOBt, 1-hydroxybenzotriazole;
HPLC, high-performance liquid chromatography;
LBD, ligand binding domain;
LC, liquid chromatography;
MBHA, 4-methylbenzylhydramine;
MS, mass spectrometry;
MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NMM, N-methyl morpholine;
OD, optical density;
PBS, phosphate buffer saline;
PERM, peptidomimetic estrogen receptor modulator;
PFA, paraformaldehyde;
PI, propidium iodide;
PMSF, phenylmethanesulfonyl fluoride;
PyBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate;
qPCR, quantitative polymerase chain reaction;
RIPA, radio immunoprecipitation assay;
RP-HPLC, reserved-phase high-performance liquid chromatography;
RPMI, Roswell Park Memorial Institute;
RT-PCR, reverse transcription-polymerase chain reaction;
SDS/PAGE, sodium dodecyl sulfate/polyacrylamide gel electrophoresis;
SEM, standard error of mean;
TCEP, tris(2-carboxyethyl)phosphine;
TFA, trifluoroacetic acid;
TIS, triisopropylsilane;
Tris, tris(hydroxymethyl)aminomethane;

2. Supporting tables and figures

Table S1. Structure of PROTACs with different linkers.

Peptide	Sequence
TD-PRTOAC	
TD-PRTOAC-1	
TD-PRTOAC-2	

For the peptides, the green part was the sequence targeting ER α , the black part was the linker group and the red part was the sequence binding VHL E3 ubiquitin ligase. Dap, 2,3-diaminopropionic acid. ABU, 4-aminobutyric acid. AHX, 6-aminohexanoic acid. Pro(OH), *trans*-4-hydroxy-L-proline.

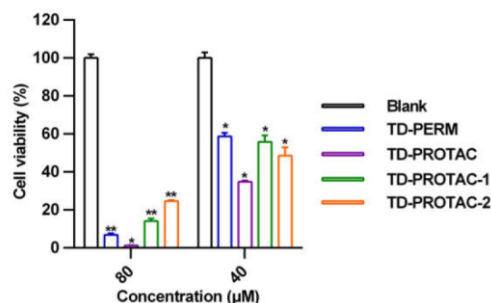


Figure S1. Antiproliferative effect of those peptides with different linkers in MCF-7 cells measured by MTT assay after 72 hours' treatment with different concentrations of peptides. *, $P < 0.05$. **, $P < 0.01$.

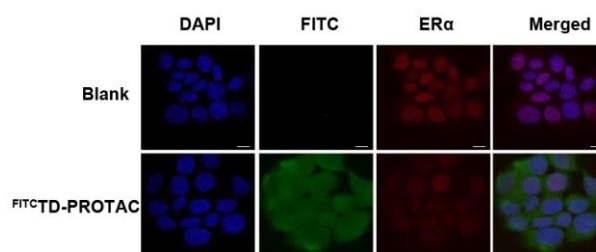


Figure S2. Confocal microscopy images of MCF-7 cells treated with 10 μM FITC-TD-PROTAC at 310 K for 12 hours and stained with ER α antibody. (Scale bar, 10 μm)

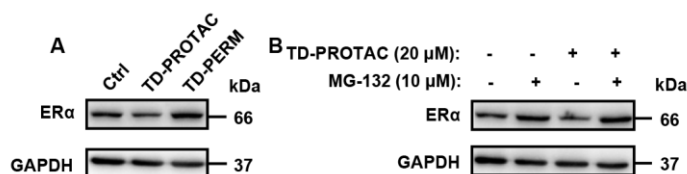


Figure S3. TD-PROTAC induces ER α degradation in MCF-7 cells. (A) ER α levels in MCF-7 cells after treatment with TD-PROTAC or TD-PERM with the concentration of 20 μM for 24 hours. (B) ER α levels in MCF-7 cells after treatment with 20 μM TD-PROTAC with or without 10 μM MG-132 for 6 hours.

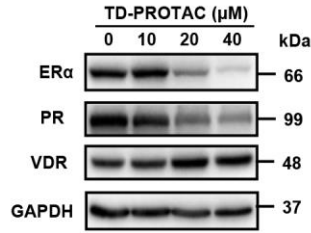


Figure S4. The effect of **TD-PROTAC** on the levels of PR and VDR protein. ER α , PR and VDR levels in T47D cells after treatment with **TD-PROTAC** at the indicated concentrations for 24 hours.

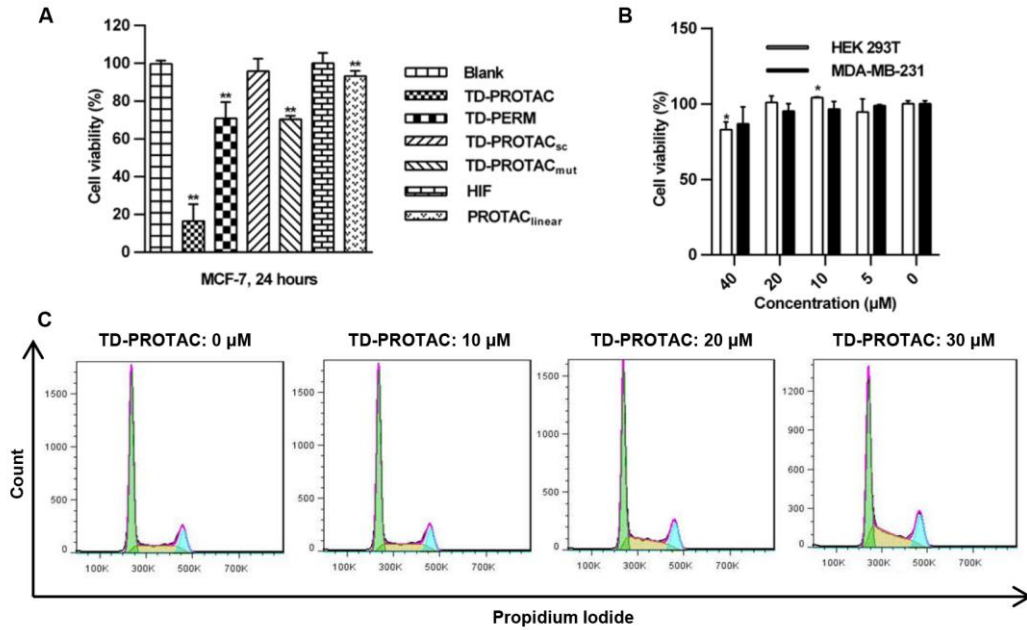


Figure S5. The effect of peptides on cell proliferation of different cell lines and cell cycle effect of **TD-PROTAC** in T47D cells. (A) Antiproliferative effect of 40 μ M different peptides in MCF-7 cells measured by MTT assay after 24 hours' treatment. (B) Antiproliferative effect of **TD-PROTAC** with different concentrations in MDA-MB-231 and HEK 293T cells using MTT assay after 24 hours' treatment. (C) Representative Propidium Iodide versus cell counts histogram of **TD-PROTAC**-treated T47D cells. The cell cycle of T47D cells was analyzed by PI staining combining flow cytometry after treatment with different concentrations of **TD-PROTAC** for 12 hours with DEAN-JETT-FOX algorithm in FlowJo 7.6.1 software. Data are presented as means $\pm$ SEM of triplicate experiments. *, $P < 0.05$. **, $P < 0.01$.

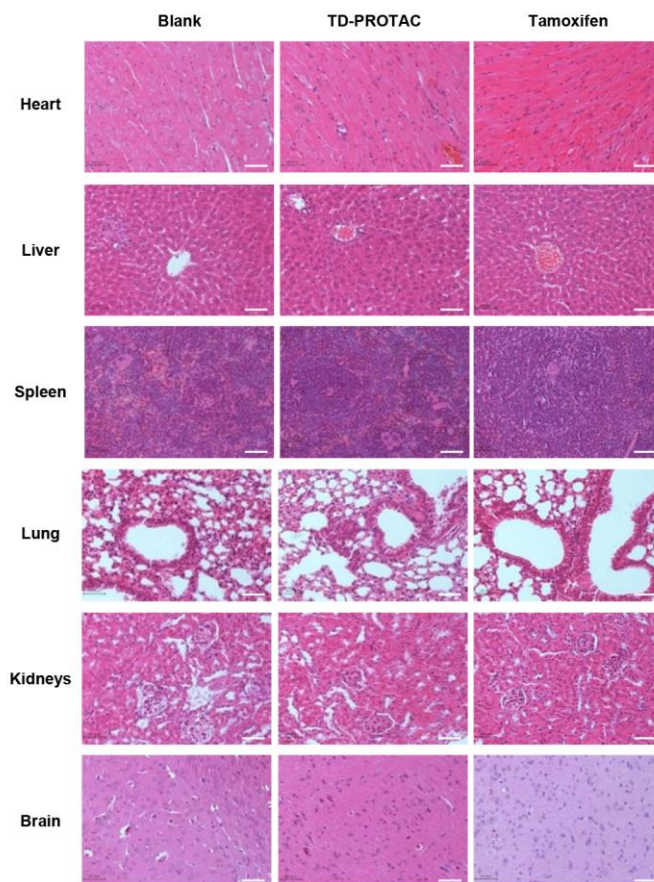


Figure S6. Hematoxylin and eosin (H&E) staining of organs collected from different groups of mice after 42 days-post treatment. Scale bar, 50 μ m. MicroSpot Focusing Objective, 20X.

Table S2. Sequences of used primers for qRT-PCR

Primer	Forward (5' – 3')	Reverse (5' – 3')
pS2	CATGGAGAACAAGGTGATCTG	CAGAAGCGTGTCTGAGGTGTC
GAPDH	GGAGCGAGATCCCTCCAAAT	GGCTGTTGTCATACTTCTCATGG

Table S3. Peptides Characterization. Calculated and found m/z are presented as $[M + 1H]^+ / [M + 1Na]^+ / [M + 2H]/2^+ / [M + 3H]/3^+$

Entry	Chemical formula	Calculated m/z	Found m/z	Synthetic yield
TD-PERM	$C_{62}H_{104}N_{20}O_{15}$	1368.8	685.7	10.1%
HIF	$C_{31}H_{48}N_6O_8$	632.3	633.4, 655.3	15%
TD-PROTAC	$C_{83}H_{142}N_{24}O_{19}$	1779.1	594.4, 891.0	9.5%
TD-PROTAC-1	$C_{89}H_{153}N_{25}O_{20}$	1892.17	631.1, 947.50	9.0%
TD-PROTAC-2	$C_{93}H_{160}N_{26}O_{21}$	1977.22	660.6, 990.20	8.5%
TD-PROTAC_{sc}	$C_{83}H_{142}N_{24}O_{19}$	1779.1	594.4, 890.9	10.0%
TD-PROTAC_{mut}	$C_{78}H_{134}N_{24}O_{18}$	1695.0	848.9	9.8%
PROTAC_{linear}	$C_{88}H_{147}N_{25}O_{18}$	1842.1	615.4, 922.6	14.1%

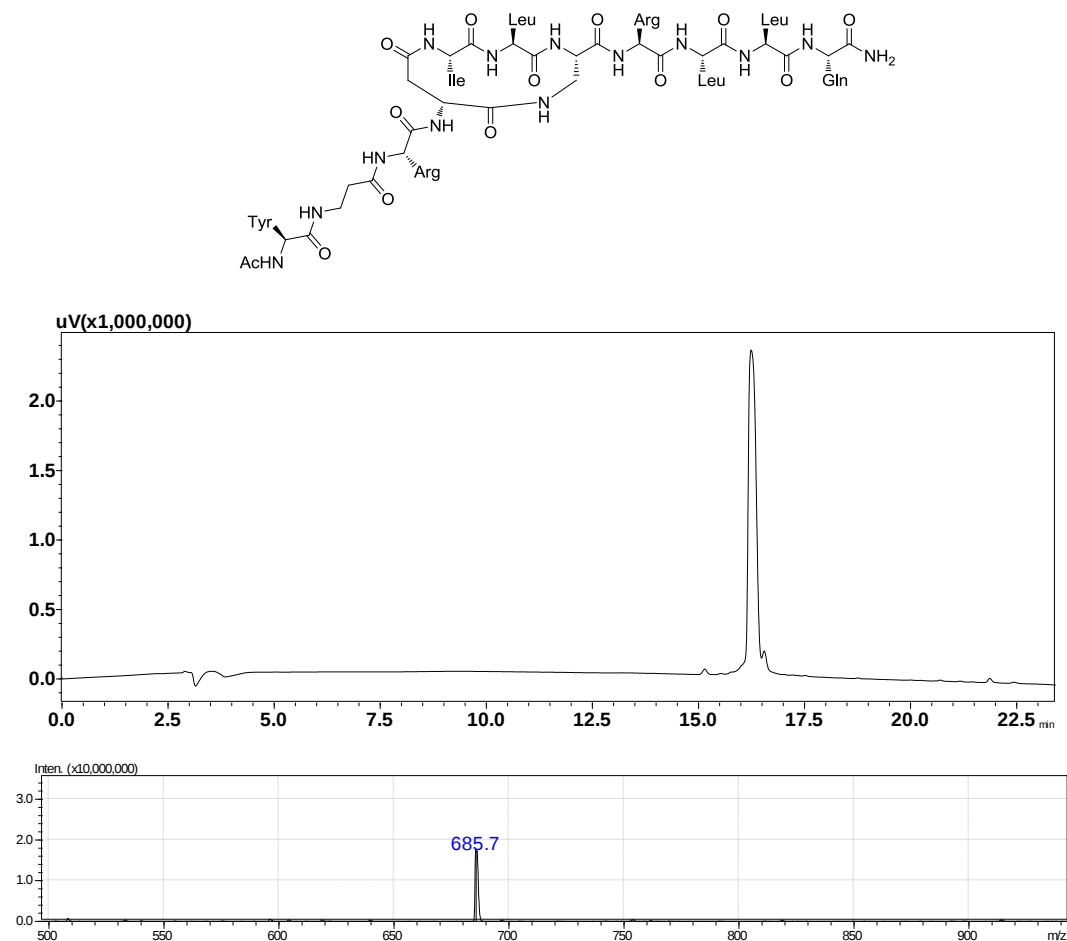
^{Fluorescent} TD-PERM	C ₇₂ H ₁₀₄ N ₂₀ O ₁₇ S	1552.7	518.8, 777.7	12%
^{Fluorescent} HIF	C ₅₃ H ₆₂ N ₈ O ₁₃ S	1050.47	526.5, 1051.5	15.3%
^{Fluorescent} TD-PROTAC	C ₁₀₇ H ₁₅₇ N ₂₅ O ₂₆ S	2239.2	747.8, 1121.3	12%
^{Fluorescent} TD-PROTAC _{sc}	C ₁₀₇ H ₁₅₇ N ₂₅ O ₂₆ S	2239.2	747.8, 1121.3	9.3%
^{Fluorescent} TD-PROTAC _{mut}	C ₁₀₂ H ₁₅₀ N ₂₆ O ₂₄ S	2155.1	719.6, 1079.1	8.9%
^{Fluorescent} PROTAC _{linear}	C ₁₁₂ H ₁₆₃ N ₂₇ O ₂₄ S	2302.2	577.1, 768.8, 1152.6	11.9%

3. Appendix

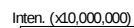
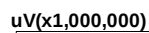
Peptides were analyzed and purified by HPLC (SHIMAZU Prominence LC-20AT) using a C18 analytic column (Agilent ZORBAX SB-Aq, 4.6 × 250 mm, 5 μm, flow rate 1.0 mL/min) and a C18 semi-preparative column (Agilent Eclipse XDB-C18, 9.4 × 250 mm, 5 μm, flow rate 5 mL/min). H₂O (containing 0.1% TFA) and pure acetonitrile were used as solvents in linear gradient mixtures. LC-MS spectra were carried out on SHIMAZU LC-MS 8030 (ESI-MS).

HPLC Traces and MS Spectra

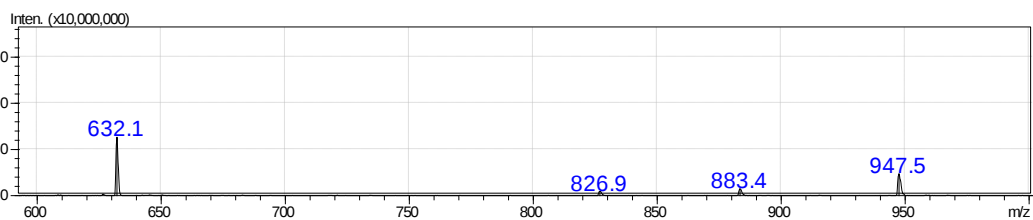
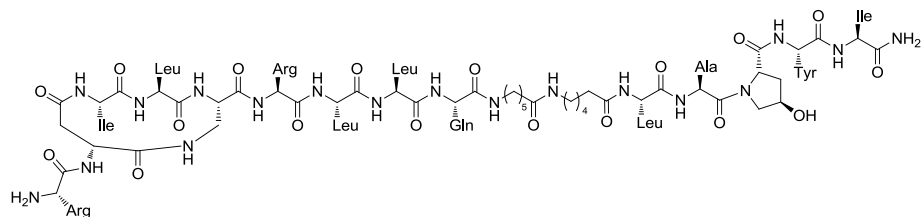
TD-PERM



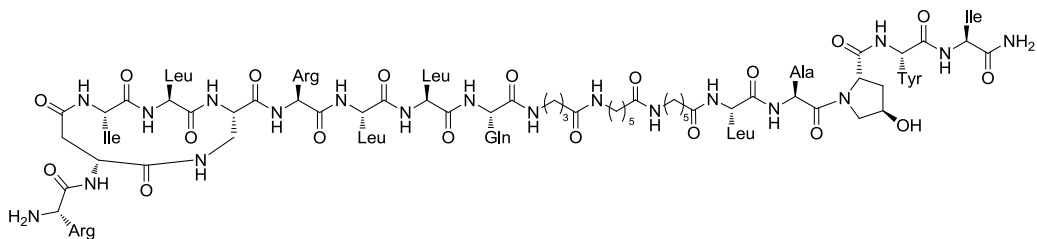
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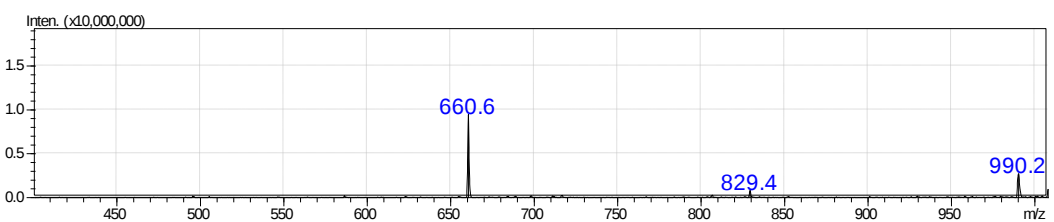


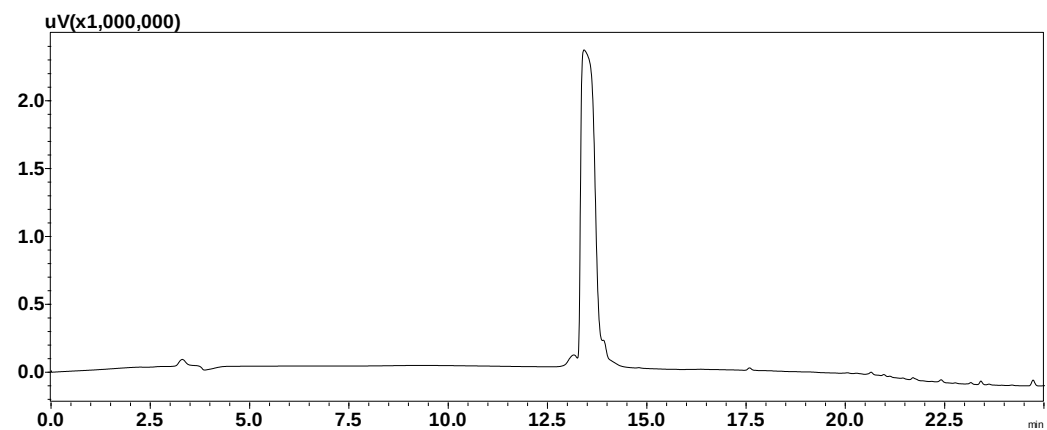
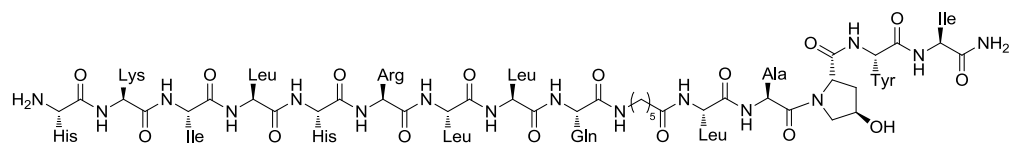
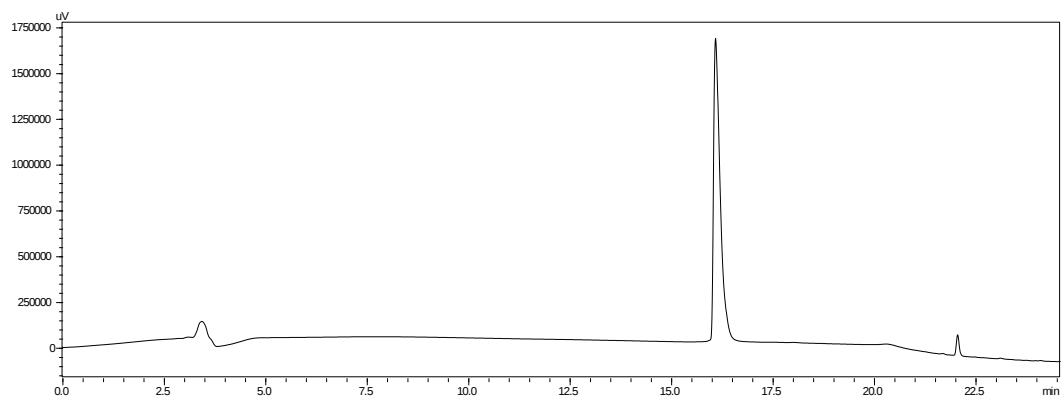
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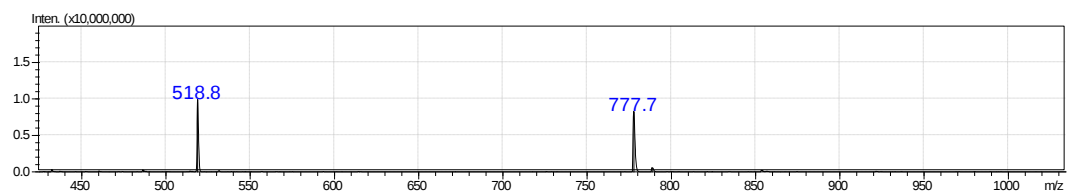
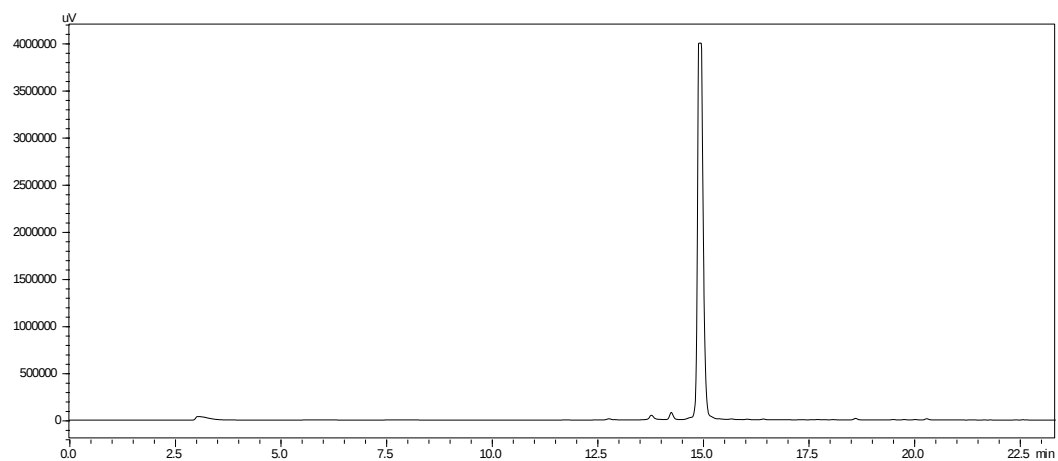
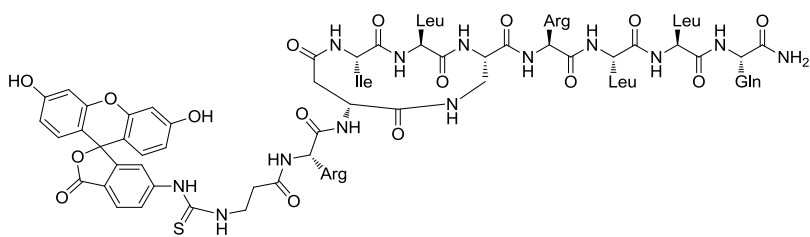
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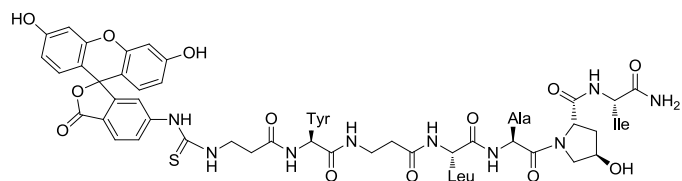
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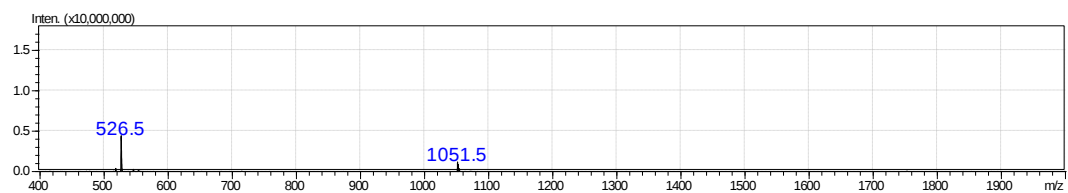
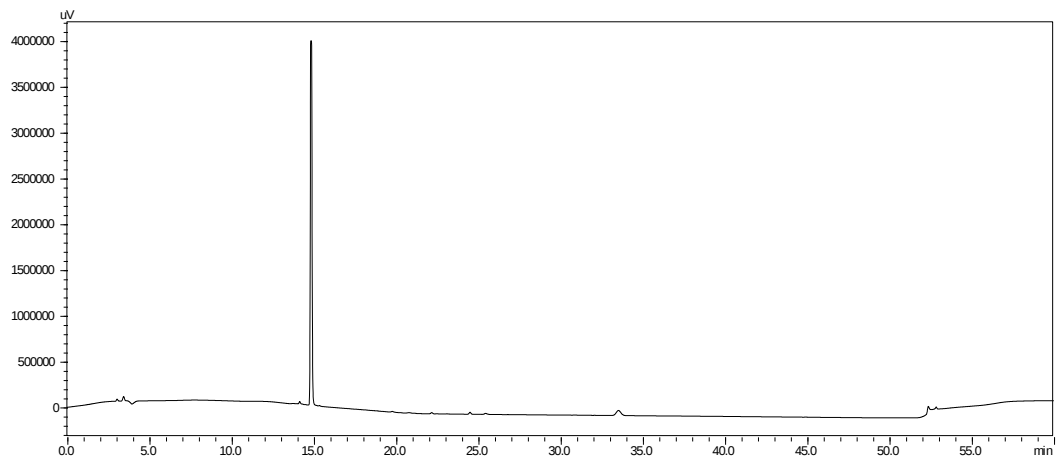


FITC **TD-PERM**

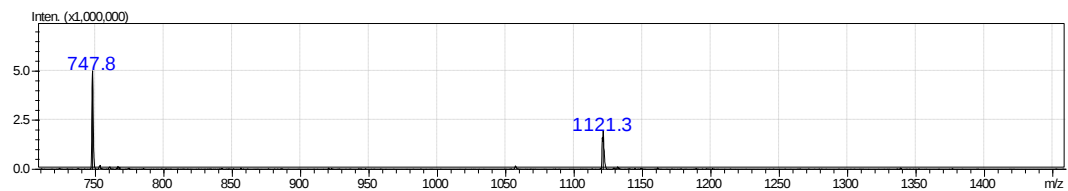
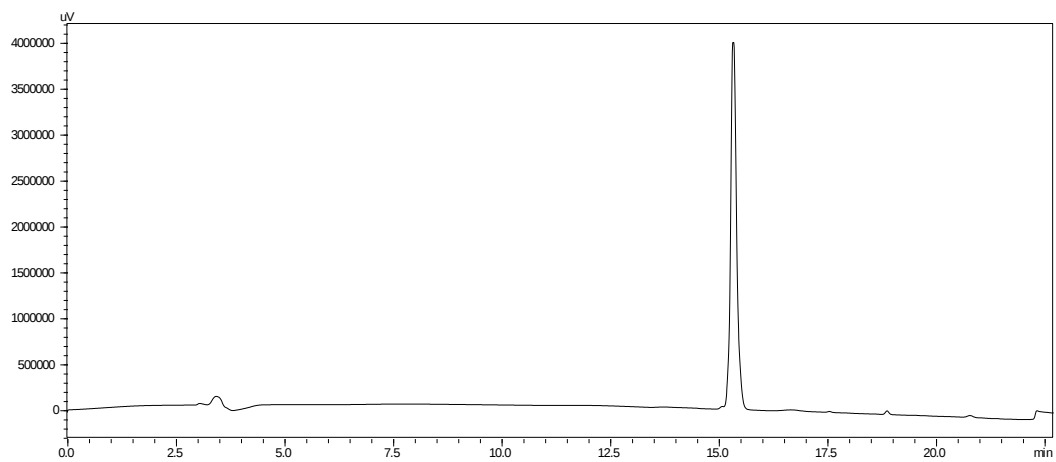
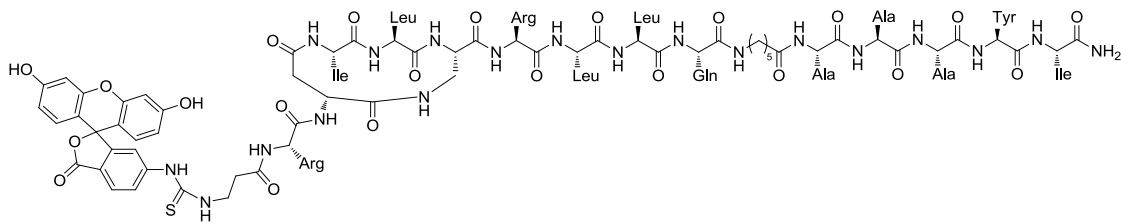


FITC **HIF**

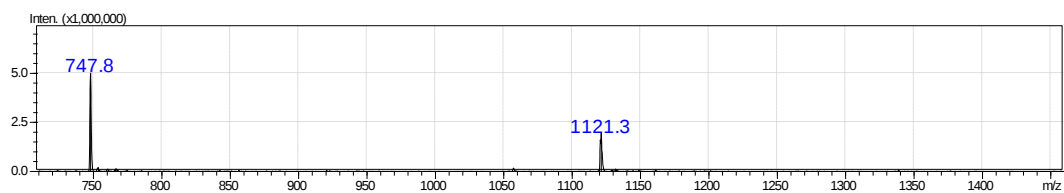
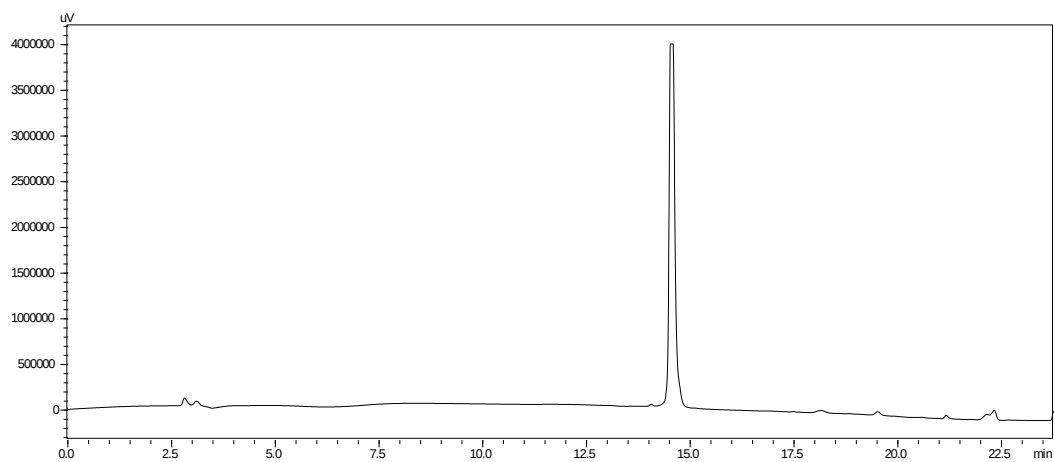
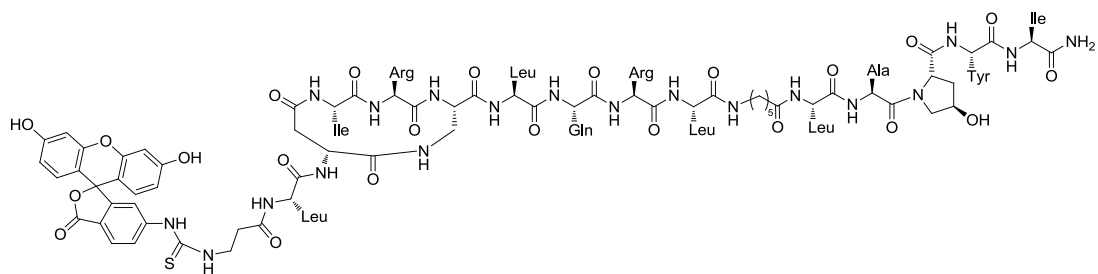




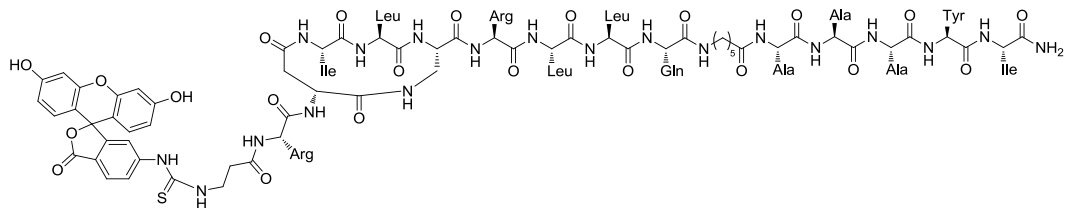
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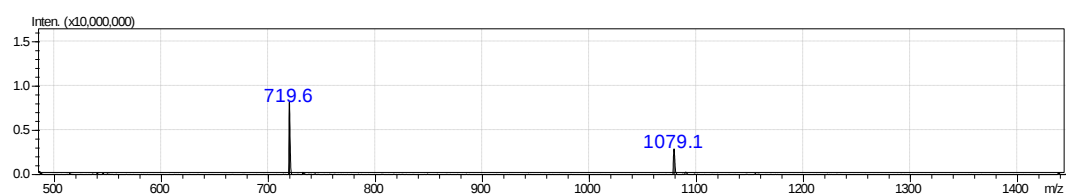


FITC-TD-PROTAC_{sc}



FITC-TD-PROTAC_{mut}





FITC**PROTAC**_{linear}

