

Reductive methylation: An alternative to lysine→arginine mutagenesis

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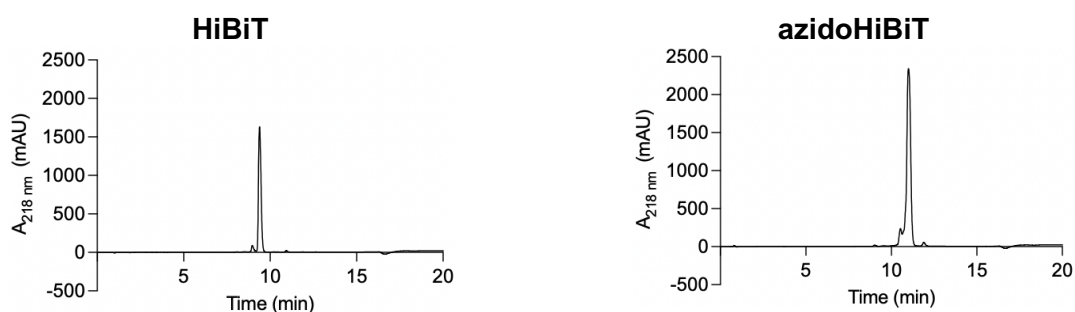
Table S1 | Abbreviations Used

Abbreviation	Definition
ACN	acetonitrile
BCA	bicinchoninic acid
BSA	bovine serum albumin
cLog P	calculated logarithm of the <i>n</i> -octanol/water partition coefficient
DBCO	dibenzocyclooctyne
DEF	2',7'-diethylfluorescein
DEFIA	2',7'-diethylfluorescein-5-iodoacetamide
DIC	<i>N,N'</i> -diisopropylcarbodiimide
DMEM	Dulbecco's modified Eagle's medium
DMF	<i>N,N</i> -dimethylformamide
DODT	2,2'-(ethylenedioxy)diethanethiol
DPBS	Dulbecco's phosphate-buffered saline
DTNB	5,5'-dithiobis(2-nitrobenzoic acid)
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
ESI	electrospray ionization
6-FAM	6-carboxyfluorescein
FBS	fetal bovine serum
Fmoc	fluorenylmethyloxycarbonyl
FPLC	fast protein liquid chromatography
HEK	human embryonic kidney
HiBiT	high-affinity NanoBiT peptide tag
HPLC	high-performance liquid chromatography
IC ₅₀	half-maximal inhibitory concentration
IMDM	Iscoe's modified Dulbecco's medium
IPTG	isopropyl- β -D-1-thiogalactopyranoside
K_d	equilibrium dissociation constant
k_{cat}/K_M	specificity constant
LC/MS	liquid chromatography/mass spectrometry
LgBiT	large NanoBiT subunit
MALDI-TOF	matrix-assisted laser desorption/ionization–time of flight
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2 <i>H</i> -tetrazolium
MWCO	molecular weight cutoff
NEDD8	neural precursor cell expressed developmentally downregulated protein 8
NTB	2-nitro-5-thiobenzoate

Abbreviation	Definition
OD	optical density
Oxyma	ethyl cyanohydroxyiminoacetate
PBS	phosphate-buffered saline
PEG	polyethylene glycol
PROTAC	proteolysis-targeting chimera
bioPROTAC	biological proteolysis-targeting chimera
Q-TOF	quadrupole time-of-flight
RI	ribonuclease inhibitor
RLU	relative luminescence units
RNase 1	ribonuclease 1 (EC 4.6.1.18; <i>Homo sapiens</i> : UniProtKB P07998)
RNase A	ribonuclease A (EC 4.6.1.18; <i>Bos taurus</i> : UniProtKB P61823)
RPMI	Roswell Park Memorial Institute
SD	standard deviation
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
SE	standard error
SUMO	small ubiquitin-like modifier
6-TAMRA	6-carboxytetramethylrhodamine
TB	terrific broth
TCEP	tris(2-carboxyethyl)phosphine
TFA	trifluoroacetic acid
T_m	temperature at the midpoint of thermal denaturation

Table S2 | Mass Spectrometry of Proteins and Peptides

	<i>m</i> , calc'd (Da)	<i>m</i> , obs'd (Da)	Δm (Da)	Comment
Unmodified Proteins				
RNase 1	14,566.24	14,596.47	+30.23	2 Met(O)
QBI-139	14,856.72	14,856.96	+0.24	
A19C/G88R RNase A	13,944.59	13,944.66	+0.07	
ribonuclease inhibitor	49,823.54	49,843.33	+19.79	1 Met(O)
Modified Proteins				
dmQBI-139	15,108.72	15,108.71	-0.01	
dmRNase 1	14,818.20	14,848.65	+30.45	2 Met(O)
C4R/P19C(NTB)/C118V QBI-139	15110.99	15111.29	+0.30	
A19C(NTB)/G88R RNase A	14,142.59	14,141.81	-0.78	
DEF-G88R RNase A	14,389.59	14,388.21	-1.38	
QBI-139-DBCO	15,588.59	15,588.76	+0.17	
QBI-139-HiBiT	17,335.54	17,336.76	+1.22	
dmQBI-139-DBCO	15,840.59	15,840.55	-0.04	
dmQBI-139-HiBiT	17,587.54	17,588.26	+0.72	
Peptides				
HiBiT	1,450.81	1,452.95	+2.15	
azidoHiBiT	1,746.95	1,749.10	+2.15	

**Figure S1** | Analytical HPLC chromatograms of synthetic peptides. Method: 0–40% v/v B over 15 min with A: H₂O/ACN/TFA 95/5/0.1; B: ACN/H₂O/TFA 95/5/0.1.