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Repurposing ubiquitination for innovative antibody conjugation

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Repurposing ubiquitination for innovative antibody conjugation

Angela F. el Hebieshy

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Repurposing ubiquitination for innovative antibody conjugation

Proefschrift

ter verkrijging van
de graad van doctor aan de Universiteit Leiden,
op gezag van rector magnificus prof.dr.ir. H. Bijl,
volgens besluit van het college voor promoties
te verdedigen op donderdag 16 oktober 2025
klokke 13.00 uur

door

Angela Fouad el Hebieshy

geboren te Hulst

in 1989

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klokke 13.00 uur

door

Angela Fouad el Hebieshy

geboren te Hulst

in 1989

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In cherished memory of prof. dr. Huib Ovaa (1973-2020).

In his mentorship, he encouraged to connect the disconnected and to innovate.

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Abbreviations

Acm	Acetamidomethyl
ADCs	Antibody-drug conjugates
BiTEs	Bispecific T-cell engagers
BLI	Bio-layer interferometry
BMDCs	Bone marrow-derived dendritic cells
Boc	<i>tert</i> -Butyloxycarbonyl
CD	Circular dichroism
CDR	Complementarity-determining region
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CTV	Cell trace violet
CuAAC	Copper(I)-catalyzed azide-alkyne cycloaddition
Cy5	Cyanine dye 5
DCM	Dichloromethane
DCs	Dendritic cells
DIC	N,N'-Diisopropylcarbodiimide
DIPEA	N,N-diisopropylethylamine
DMEM	Dulbecco's Modified Eagle Medium
DMF	Dimethylformamide
DOTA-GA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-glutamic acid
DTP	2,2'-Dithio-dipyridin
DTT	Dithiothreitol
DUBs	Deubiquitinating enzymes
DVD-Ig	Dual variable domain immunoglobulin
E1	Ubiquitin-activating enzyme
E2	Ubiquitin-conjugating enzyme
E3	Ubiquitin-ligating enzyme
EDT	Ethanedithiol
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ESI-TOF	Electrospray ionization-time of flight
Fab	Fragment antigen-binding
Fc	Fragment crystallizable region
FCS	Fetal calf serum
FGE	Formylglycine generating enzyme

FITC	Fluorescein isothiocyanate
Fmoc	Fluorenylmethyloxycarbonyl
FPLC	Fast protein liquid chromatography
FR	Framework region
GFP	Green fluorescent protein
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HDR	Homology Directed Repair
IMDM	Iscove's Modified Dulbecco's Medium
<i>i</i> Pr ₃ SiH	Triisopropylsilane
IPTG	Isopropyl β-D-1-thiogalactopyranoside
K48	Lysine 48
LC-MS	Liquid chromatography-mass spectrometry
LDH	Lactate dehydrogenase
LPS	Lipopolysaccharide
mAb	Monoclonal antibody
MeCN	Acetonitrile
MES	2-(N-morpholino)ethanesulfonic acid
MESNa	Sodium 2-mercaptoethanesulfonate
MHC	Major histocompatibility complex
moDCs	Monocyte-derived dendritic cells
MOPS	3-(N-morpholino)propanesulfonic acid
MPAA	4-Mercaptophenylacetic acid
mTG	Microbial transglutaminase
Nbs	Nanobodies
NCL	Native chemical ligation
NHS	N-hydroxysuccinimide
NleL	Non-LEE-encoded effector ligase
NMP	N-Methyl-2-pyrrolidone
OTUB1	OTU deubiquitinase 1
OVAP	Ovalbumin peptide
PBS	Phosphate-buffered saline
PEG	Polyethylene glycol
PG	Protective group
PyBOP	Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
Rho	Rhodamine

RP-HPLC	Reverse phase high performance liquid chromatography
RT	Room temperature
ScFv	Single-chain variable fragment
SD	Standard deviation
sdAb	Single-domain antibody
SPPS	Solid-phase peptide synthesis
Srt	Sortase
TCEP	Tris(2-carboxyethyl)phosphine
TCR	T-cell receptor
TFA	Trifluoroacetic acid
THPTA	Tris(hydroxypropyltriazolylmethyl)amine
TIPS	Triisopropylsilane
Ub	Ubiquitin
Ub ₂	Di-ubiquitin
Ub ^{acc}	Acceptor Ubi-tag
Ubch7	Ubiquitin-conjugating enzyme H7
Ub ^{don}	Donor Ubi-tag
UBE1	Ubiquitin-activating enzyme E1
Ube2g2	Ubiquitin-conjugating enzyme E2G 2
Ub-PA	Ubiquitin-propargylamide
Ub ^{WT}	Wild-type ubiquitin
UCHL3	Ubiquitin carboxyl-terminal hydrolase L3
UPLC	Ultra performance liquid chromatography
VHH	Variable heavy domain of heavy chain
WT	Wild type
β-ME	Beta-mercaptoethanol