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Exploring and exploiting bacterial protein glycosylation systems

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Exploring and exploiting
bacterial protein glycosylation systems

Liubov Yakovlieva

Liubov Yakovlieva

PhD thesis

Rijksuniversiteit Groningen

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and in accordance with
the decision by the College of Deans.

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by

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“I must not fear.
Fear is the mind-killer.
Fear is the little-death that brings total obliteration.
I will face my fear.
I will permit it to pass over me and through me.
And when it has gone past I will turn the inner eye to see its path.
Where the fear has gone there will be nothing.
Only I will remain.”

Litany against Fear

Frank Herbert, “Dune” (1965)

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List of abbreviations

AA	amino acid	DCM	dichloromethane
Ab	antibody	dd	doublet of doublets
Ac	acetyl	δ	chemical shift, ppm
ABC	ammonium bicarbonate	DIC	<i>N,N</i> -diisopropylcarbodiimide
ACN	acetonitrile	DIPEA	<i>N,N</i> -diisopropylethylamine
ADEM	acute disseminated encephalomyelitis	DMF	<i>N,N</i> -dimethylformamide
AE	anion exchange	DMSO	dimethyl sulfoxide
AIDA	adhesin involved in diffused adherence	DNA	deoxyribonucleic acid
Ala	alanine	DOSY	diffusion ordered NMR spectroscopy
APC	antigen-presenting cell	DP	degree of polymerization
APDC	ammonium pyrrolidine dithiocarbamate	EDTA	ethylenediaminetetraacetic acid
Ara	arabinose	EF-P	elongation factor P
Arg	arginine	EG	early glycoforms
Asn	asparagine	ELISA	enzyme-linked immunosorbent assay
Asp	aspartic acid	eq	molar equivalents
ATP	adenosine triphosphate	ESI	electrospray ionization
AzGlc	azidoglucose	ESKAPE	<i>Enterococcus faecium</i> , <i>Staphylococcus aureus</i> , <i>Klebsiella pneumoniae</i> , <i>Acinetobacter baumannii</i> , <i>Pseudomonas aeruginosa</i> , <i>Enterobacter spp.</i>
Boc	<i>tert</i> -butoxycarbonyl	Et	ethyl
BOP	(benzotriazol-1- yloxy)tris(dimethylamino) phosphonium hexafluorophosphate	FA	formic acid
BQ	benzoquinone	FF	fast flow
BSA	bovine serum albumin	Fmoc	fluorenylmethoxycarbonyl
Bz	benzoyl	FPLC	fast protein liquid chromatography
cat.	catalyst	Galf	galactofuranose
CAZy	Carbohydrate-Active enZymes	GalNAc	<i>N</i> -acetylgalactosamine
CDP	cytidine diphosphate	Galp	galactopyranose
CeS	cellulose synthase complex	GDP	guanine diphosphate
CLIPS	Chemical Linkage of Peptides onto Scaffolds	Glc	glucose
CMP	cytidine monophosphate	GlcA	glucuronic acid
CNS	central nervous system	Gln	glutamine
CPS	capsular polysaccharide	GlcNAc	<i>N</i> -acetylglucosamine
Cryo-EM	cryogenic electron microscopy	Glu	glutamic acid
CV	column volume	Gly	glycine
Cys	cysteine	GT	glycosyltransferase
d	doublet	h	hours
DBU	1,8-diazabicycloundec-7- ene	HATU	(1[bis(dimethylamino)methylene]-1H- 1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate
DC-SIGN	dendritic cell - specific intracellular adhesion molecule 3-grabbing non- integrin	HEPES	4-(2-hydroxyethyl)-1- piperazineethanesulfonic acid
DCE	1,2-dichloroethane	Hex	hexose
		HFIP	1,1,1,3,3,3-hexafluoro-2-propanol
		His	histidine
		HMW1ct	C-terminal fragment of high molecular weight adhesin
		HSQC	heteronuclear single quantum correlation
		Hz	hertz

I-TASSER	Iterative Threading ASSEmblY Refinement	MS	multiple sclerosis
IC ₅₀	inhibitor concentration that results in half-maximal enzyme activity	MTBE	methyl tert-butyl ether
Ile	isoleucine	MurNAc	N-acetylmuramic acid
IPTG	isopropyl β-thiogalactopyranoside	Mw	molecular weight
IR	infrared spectroscopy	MW-SPPS	microwave assisted solid-phase synthesis
ITC	isothermal titration calorimetry	MWCO	molecular weight cutoff
<i>J</i>	coupling constant	n.a.	not available
<i>k</i> _{cat}	enzyme turnover number	n.d.	not determined
<i>K</i> _D	dissociation constant	NADH	nicotinamide adenine dinucleotide
Kdo	3-deoxy-D-manno-octulosonic acid	NAG	N-acetylglucosamine
<i>K</i> _m	Michaelis constant	NAM	N-acetylmuramic acid
<i>k</i> _{off}	rate constant for the dissociation of the protein-ligand complex	NCAM	neural cell adhesion molecule
<i>k</i> _{on}	rate constant for the association of the protein-ligand complex	NDP	nucleotide diphosphate
KOW	Kyrpides-Woese-Ouzounis	NGT	N-glycosyltransferase
LC-MS	liquid chromatography-coupled mass spectrometry	NMR	nuclear magnetic resonance
Leu	leucine	NOE	Nuclear Overhauser Effect
LPS	lipopolysaccharide	NOESY	Nuclear Overhauser Effect Spectroscopy
LS	light scattering	NTA	nitrilotriacetic acid
Lys	lysine	NTHi	nontypeable <i>Haemophilus influenzae</i>
m	multiplet	OD	optical density
m/z	mass over charge ratio	OGT	O-GlcNAc transferase
MALDI-TOF	matrix-assisted laser desorption ionization time of flight	ON	overnight
MALLS	multi-angle light laser scattering	OST	oligosaccharyltransferase
Man	mannose	Oxyma	ethyl cyanohydroxyiminoacetate
ManNAc	N-acetylmannose	PAGE	polyacrylamide gel electrophoresis
MBP	myelin basic protein	Pbf	2,2,4,6,7-pentamethyldihydrobenzo furan-5-sulfonyl
MD	molecular dynamics	Pd	palladium
Me	methyl	PDB	protein data bank
MHC	major histocompatibility complex	PG	peptidoglycan
min	minutes	Phe	phenylalanine
MOE	metabolic oligosaccharide engineering	<i>P_{intr}</i>	intrinsic processivity
MOG	myelin oligodendrocyte glycoprotein	Pls	plasmin-sensitive protein
MOPS	3-(N-morpholino) propanesulfonic acid	<i>P_n</i>	processivity factor
MRSA	methicillin-resistant <i>Staphylococcus aureus</i>	ppm	parts per million
		Pro	proline
		PST	polysialyltransferase
		q	quartet
		qNMR	quantitative NMR
		Rha	rhamnose
		RI	refractive index
		RIF	relative ionization factor
		RNA	ribonucleic acid
		RP-LC	reverse-phase liquid chromatography
		rpm	rotations per minute
		RT	room temperature
		s	singlet
		SAAT	self-associating autotransporters

SDR	serine-aspartate repeats
SDS	sodium dodecyl sulphate
SEC	size-exclusion chromatography
SEDS	Shape, Elongation, Division and Sporulation family of proteins
Ser	serine
SH	SUMO-His ₆
Sia	sialic acid
SPE	solid phase extraction
SPPS	solid-phase peptide synthesis
SPR	surface plasmon resonance
SPS	solvent purification system
SRRP	serine-rich repeat protein
STD-NMR	Saturation transfer difference Nuclear Magnetic Resonance
t	triplet
TB	terrific broth
TBA	tetrabutylammonium
TBAOH	tetrabutylammonium hydroxide
TBS	Tris buffer saline
^t Bu	<i>tert</i> -butyl
^t BuOH	<i>tert</i> -butanol
TCEP	tris (2-carboxyethyl) phosphine
TDP	thymidine diphosphate
TEAA	triethylammonium acetate
Tf	triflate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
Thr	threonine
TIS	triisopropylsilane
TLC	thin-layer chromatography
TOCSY	total correlation spectroscopy
TOF	time of flight
TON	turnover number
TPR	tetratrico peptide repeat
Tris	tris(hydroxymethyl)aminomethane
TR-NOE	transfer NOE
Trp	tryptophan
Trt	trityl
Tyr	tyrosine
UDP	uridine diphosphate
UMP	uridine monophosphate
Und	undecaprenol
UniDec	Universal deconvolution
UV	ultraviolet
V ₀	initial velocity
Val	valine
WTA	wall teichoic acid
Xyl	xylose