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# **Functional and Structural Characterisation of Staphylococcal Superantigen-Like Protein 10 (SSL10)**

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A thesis submitted in fulfilment of the requirements for the degree of Doctor  
of Philosophy

Department of Molecular Medicine and Pathology  
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# Abstract

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*Staphylococcus aureus* is a highly versatile gram-positive bacterium that owes its success to the remarkable range of pathogenic factors that it has at its disposal. Staphylococcal superantigen-like protein 10 (SSL10) is a tightly regulated, highly conserved protein exclusive to the arsenal of *S.aureus*. It is located on the genomic island *vSaa* alongside ten other related *ssl* genes. The 2.75 Å crystal structure of SSL10 displays strong structural homology to the superantigen toxins with an N-terminal OB-fold domain linked to a C-terminal  $\beta$ -grasp domain; however they are functionally distinct.

SSL10 binds to human IgG and displays striking specificity for the Fc domain of the  $\gamma$ 1 subclass. Its strong affinity for IgG only extends to primate species. Kinetic analysis of SSL10 binding to human IgG1 suggests a dissociation constant in the high nanomolar range through a single site 1:1 stoichiometry interaction. Truncation of SSL10 into its C-terminal  $\beta$ -grasp domain abolished its ability to bind IgG1 suggesting the presence of key residues in the N-terminal OB-fold domain. SSL10 competes for binding to IgG1 with cell surface Fc $\gamma$ Rs on monocytes, and consequently interferes with the phagocytosis of IgG1-opsonised bacteria by neutrophils. This is a key survival strategy for *S.aureus* given that phagocytic cells play a crucial role in the immune clearance of gram positive bacteria.

This small 24 kDa protein displays strong functional diversity through its C-terminal  $\beta$ -grasp domain, disrupting the proteolytic cascades of complement and coagulation. It targets complement C4 and inhibits the classical and mannan-binding lectin pathways of complement. SSL10 also affinity purifies prothrombin, fibrinogen, fibronectin and plasminogen from plasma; key factors involved in the clotting process. It potently delays coagulation prior to thrombin activation suggesting an alternative role for fibrinogen and fibronectin in anchoring SSL10 at the site of infection where it would be most effective. It is clear these bacterial proteins are strategically designed to accommodate a number of circumstances. This study illustrates the incredible complexity of host-pathogen interactions and highlights the need for a comprehensive understanding of this bacterium if we are to successfully develop alternative therapeutics in the future.

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# Abbreviations

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°C	Degrees celsius
amp	Ampicillin
ATCC	American type cell culture collection
BaCl	Barium chloride
bp	Base pair
BSA	Bovine serum albumin
CH	Heavy chain constant region
CHIPS	Chemotaxis inhibitory protein of <i>Staphylococcus aureus</i>
C-terminal	Carboxy terminus
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DTT	Dithiothreitol
Eap	Extracellular adherence protein
ECM	Extracellular matrix
Ecb	Extracellular complement binding protein
Efb	Extracellular fibrinogen binding protein
Fab	Immunoglobulin antigen binding fragment
FACS	Fluorescence activated cell sorter
Fc	Immunoglobulin crystallisable fragment
FcγR	Immunoglobulin G Fc receptor
FCS	Fetal calf serum
Fig	Figure
fMLP	N-formyl-methionyl-leucyl-phenylalanine
FPLC	Fast performance liquid chromatography
FSC	Forward scatter
g	Gram
GlcNAc	N-acetylglucosamine
hr	Hour
HrtA	Heme transporter A
IDA	Iminodiacetic acid
IEC	Immune evasion cluster
Ig	Immunoglobulin
IPTG	Isopropyl β-D-thiogalactopyranoside
ITC	Isothermal titration calorimetry
kDa	Kilodalton
L	Litre

LB	Luria-Bertani broth
LPS	Lipopolysaccharide
M	Molar
mA	Milliampere
MFI	Mean fluorescence intensity
mg	Milligram
MHC	Major histocompatibility complex
min	Minute(s)
μL	Microlitre
mL	Milliliter
μM	Micromolar
mM	Millimolar
Mr	Molecular weight
ng	Nanogram
NMS	Normal mouse serum
NRS	Normal rabbit serum
NTA	Nitrilotriacetic acid
N-terminus	Amino-terminus
OB-fold	Oligosaccharide/oligonucleotide binding fold
O/N	Overnight
PBL	Peripheral blood lymphocyte
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PE	Phycoerythrin
pg	Picogram
PHA	Phytohemagglutinin
pI	Isoelectric point
PMSF	Phenylmethylsulfonyl fluoride
PNGase F	Peptide-N-glycosidase F
RBC	Red blood cell
rpm	Revolutions per minute
RPMI	Roswell Park Memorial Institute Medium - 1640
RT	Room temperature
s	Second(s)
Sag	Superantigen
Sbi	<i>S. aureus</i> IgG-binding protein
SCIN	Staphylococcal complement inhibitor protein
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SET	Staphylococcal exotoxin-like
SpA	Staphylococcal Protein A



SPR	Surface plasmon resonance
SSC	Side scatter
TBS	Tris buffered saline
TCA	Trichloroacetic acid
TCR	T-cell receptor
TEMED	N,N,N,N – tetramethylethylenediamine
TNF $\alpha$	Tumor necrosis factor alpha
Trx	Thioredoxin
TSST-1	Toxic shock syndrome toxin-1
V	Volts
VH	Heavy chain variable region
VL	Light chain variable region
w/w	Weight per weight
w/v	Weight per volume
v/v	Volume per volume