New perspectives in the removal of protein corona: host defense peptides as useful tools in EV surface engineering

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INTRODUCTION

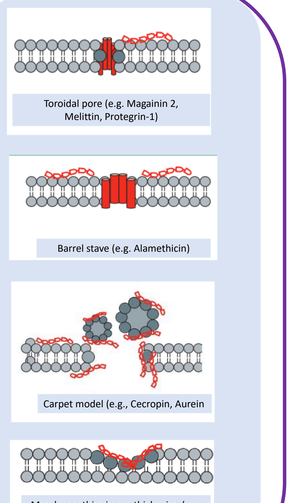
Red blood cell derived EVs

Membrane active peptides

EVs involved in :
Circulatory system
Infection sites
Cancer sites
Wounds

Interaction

Function/Application :
Cargo loading
Cancer sites
Infection sites
Wound healing
Nano- engineering



Membrane active peptides (MAPs) are promising biomaterials with antimicrobial and anticancer applications. They impart their biological applicability either by altering the cell membrane through perturbation, lysis, or by enabling targeted drug delivery.[1,2] There are several physiological instances where there is joint presence of EVs and MAPs where their interactions could be relevant. However, our knowledge here is scarce. Therein, we have selected 24 peptides and studied the interactions with red blood cell derived EV (REVs) to gain an overview on their interactions and on action mechanisms of the peptides.

Peptide Selection Strategy

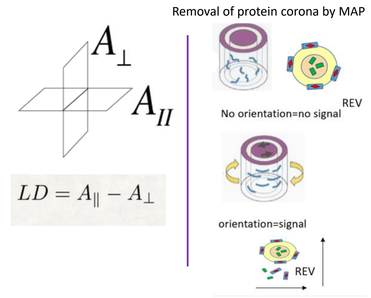
- Type of MAP
  - Cell penetrating peptide (CPP)
  - Antimicrobial peptide (AMP)
- Mode of interaction with model lipid membranes
- Structure: α helix, β sheet, random coil
- Sequential characteristic: enriched in tryptophan and histidine

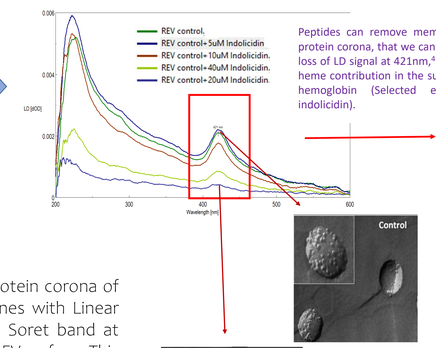
PEPTIDE	SEQUENCE	CHARGE	TYPE (MECHANISM)	ACTIVE CONFORMATION	EC_{max} (μM)
Mellitin	GIGAVLKVLTTGLAPA LISWIKRKRQQ	+5	AMP- Torroidal pore	α-helix	10
Gramicidin D	mixture of A B C	0	AMP-ion channel	Head to Head β Helix	10
LL 37	LLGDLLRKSKEKIGKEF KRIVQRIKDFLRNLVP RTES	+6	AMP-Membrane Permeabilisation	α-helix	10
Transportan	GWTLNSAGYLLGKIN LKALAALAKKIL	+4	CPP	Random coil	20
Protegrin-1	RGGRLCYCRRRFCVC VGR	+6	AMP-Pore formation	β Hairpin	20
Indolicidin	ILPWKWPWWPWRR	+4	AMP-Oxidised lipid targeting	Extended	40
Lasioglossin LL-III	VNWKKILGKIIKVVK RAGLQFPVGRLLRRLL	+6	AMP-Membrane Permeabilisation	α-helix	40
Buforin IIb	RRLLR	+7	AMP	Random coil	80
FK16	FKRIVQRIKDFLRNLV	+5	AMP	α-helix	80
Aurein 1.2	GLFDIIKKIAESF	+1	AMP-Carpet model	α-helix	80
CM15	KWKLFKKIGAVLKVL	+6	AMP- thinning with torroidal pore	α-helix	80
Temporin-La	LLRHVVKILEKYL	+3	CPP	α-helix	120
Macropin 1	GFGMALKLLKKVL	+4	AMP/ CPP	α-helix	160
Polybia-MPI	IDWKKLLDAAKQIL	+2	AMP	α-helix	160
Penitratin	RQIKIWFQNRRMKW KK	+7	CPP	α-helix/β-sheet (higher conc.)	160
Buforin II	TRSSRAGLQFPVGRV HRLLRK	+6	CPP	Random coil	160
KLA	KLAKLAKKLAKLAK	+6	AMP	α-helix/α &β-sheet (higher conc.)	160
PNC 28	ETFSDLWKLL	-1	CPP	Random coil	200
Arg-1	RQWRRWWQR	+5	CPP	α-helix	200
Bactenecin	RLCRIVVIRVCR	+4	AMP	β-sheet	200
DHVAR 4	KRLFRRWQWMKKY	+6	AMP	α-helix	200
Histatin 5	DSHAKRHHGYKRKFH EKHHSHRGY	+5	AMP	α-helix or β-sheet structures in hydrophobic solvents	240
Dermcidin (DCD-1)	SSLLEKGLDGAKKAVG GLGKLGKDA	+1	AMP-Barrel stave and AMP- Pore formation	α-helix	240
Octa-arginine	RRRRRRRR	+8	CPP	Random coil	240

EC_{max}: Maximum Efficient hemoglobin removing Capacity

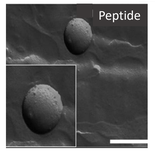
Removing Protein Corona by Membrane Active Peptides

Polarised Light Spectroscopy (Linear Dichroism)

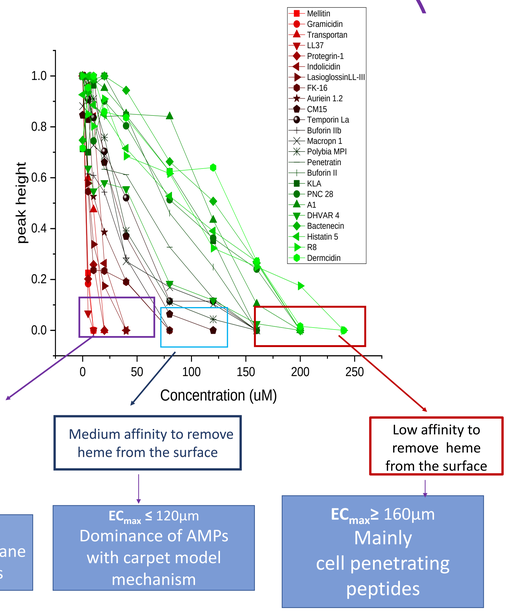




Peptides can remove members of the protein corona, that we can track on the loss of LD signal at 421nm,[4] which is the heme contribution in the surface-bound hemoglobin (Selected example on indolicidin).



We aimed to address the interaction of MAPs with the protein corona of EVs. For this our group has earlier studied REV membranes with Linear Dichroism.[3,4,5] As it can be seen by the decrease of the Soret band at 421nm we can track the removal of hemoglobin from the REV surface. This gives insight into possible mechanism of peptides as it influences the removal of protein corona. Low affinity towards removal of heme along with the protein corona is exhibited by cell penetrating peptides (CPP) as they enter the cell without disrupting the surface of the lipid membrane. On the other hand, membrane disrupting peptides show high affinity to remove heme from the surface. While carpet model peptide shows medium heme removing capacity.



High affinity to remove heme on the surface

EC_{max} ≤ 40μm
Dominance of membrane disrupting peptides

Medium affinity to remove heme from the surface

EC_{max} ≤ 120μm
Dominance of AMPs with carpet model mechanism

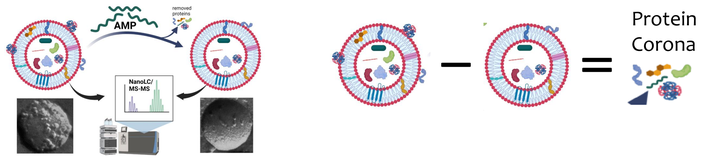
Low affinity to remove heme from the surface

EC_{max}≥ 160μm
Mainly cell penetrating peptides

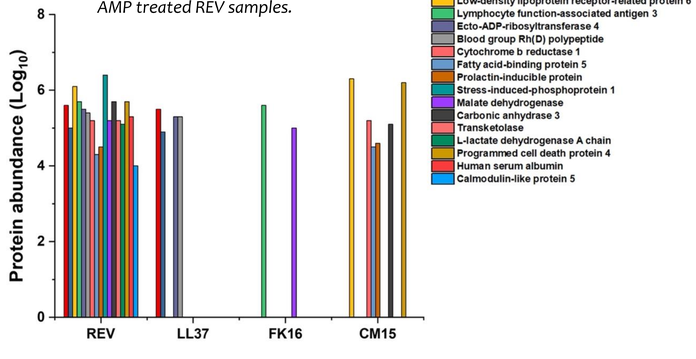
EC_{max}: Maximum Efficient hemoglobin removing Capacity

Subtractive Proteomics for identifying Protein Corona Members

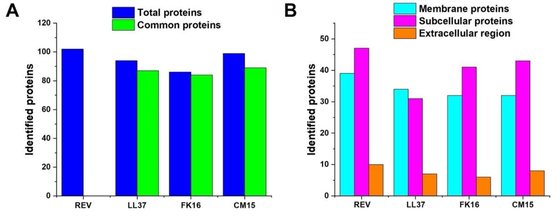
Employment of AMPs and a subtractive approach enabled indirect identification of protein corona on a REV model[6]



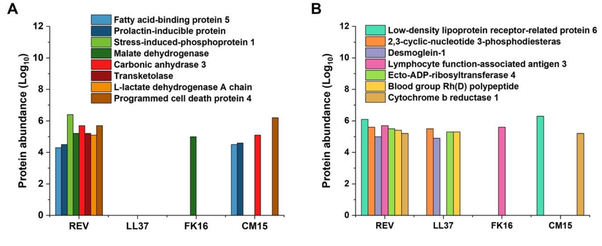
Protein abundance of identified protein corona members in REV control and AMP treated REV samples.



Total and common identified proteins (A) and classification of the identified proteins for each sample (B).



Proteomic differences in protein corona members of control and AMP-treated REV samples based on their cellular localization (A) soluble proteins and (B) membrane proteins.



CONCLUSION

- These results give an insight into the surface interactions of membrane active peptides and REVs. This helps us to attain a broad perspective on the molecular level interactions which could, in turn, provide vital information on engineering the surface and the interior of EVs with short MAPs.
- All peptides were effective in removing the protein corona however efficacy varies based on the interactive mechanism the particular peptides adopt.
- Using proteomics with the surface protein removal ability of MAPs we demonstrated on REV models how protein corona members could be determined.

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