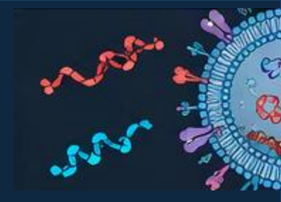
MECHANISTIC INSIGHTS INTO ANTIMICROBIAL PEPTIDE-EXTRACELLULAR VESICLE INTERACTIONS & THERAPEUTIC APPLICATIONS IN SEPSIS



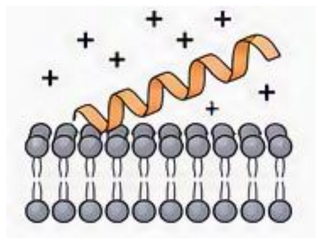
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INTRODUCITON



Membrane Active Peptides (MAPs)

- **High affinity towards lipid bilayers**
- **Disrupting cell membranes**
- **Modulating the innate immune response**

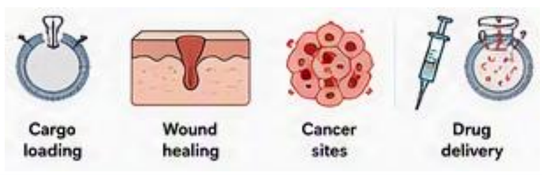
Two major classes of MAP

- **Antimicrobial peptides (AMPs)**
- **Cell-penetrating peptides (CPPs)**

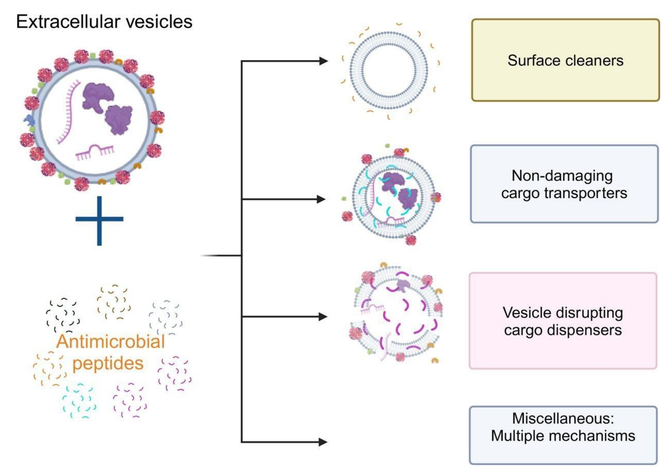
Extracellular vesicles (EVs)

- **Membrane-enclosed nanostructures**
- **Cargo carriers and cellular communication**
- **Present in biofluids: blood, urine, saliva, cerebrospinal fluid**
- **Phospholipid bilayer**
- **Protein corona on the surface: dynamic and reflects the immediate**

APPLICATIONS OF MAPS-EV SYSTEMS

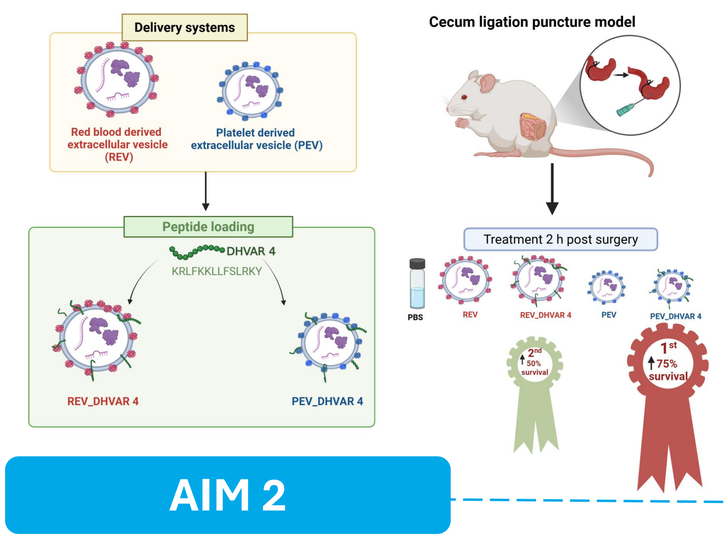


GRAPHICAL ABSTRACT



AIM 1

Characterize interactions between **26 membrane – active peptides(MAPs) and red blood cell-derived extracellular vesicles (REVs)**



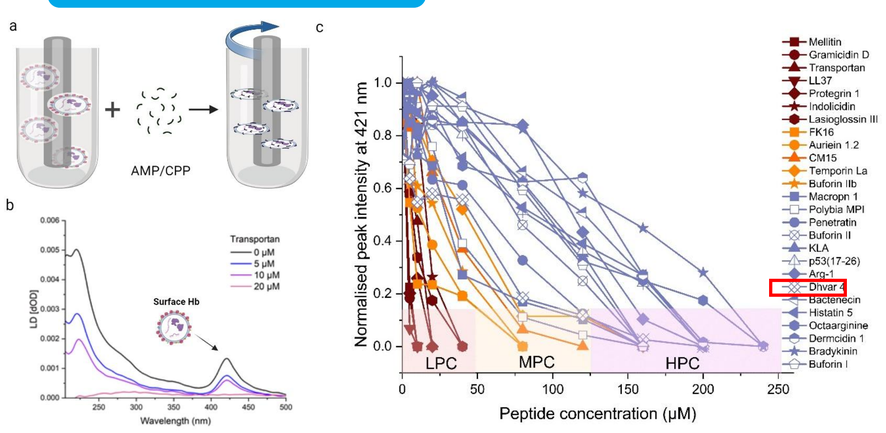
AIM 2

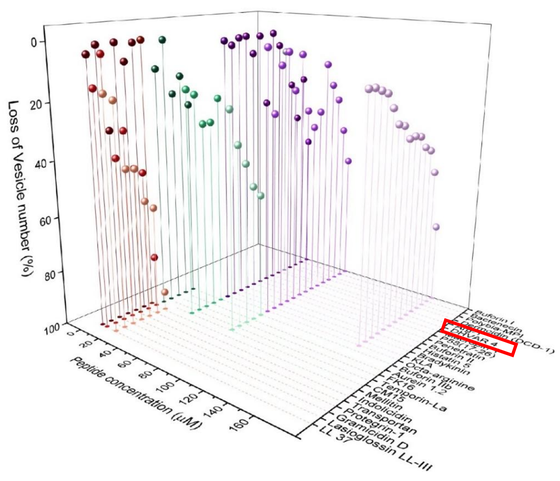
Evaluate the therapeutic efficacy of REV-DHVAR 4 and PEV-DHVAR 4 in **a cecum ligation puncture sepsis model**

RESULTS

1. MAP- EV interactions

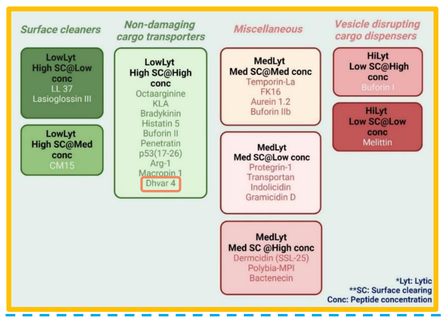
Peptide name	Sequence	Net charge*	Length	Origin	Known mechanism, active conformation
Arg-1	RQWRRWWQR	+4	9		AMP
Aurein 1.2	GLFDIIKKIAESF	+1	13	Litoria frog skin secretions	AMP, helical
Bactenecin	RLCRIVVIRVCR	+5	12	bovine neutrophils	AMP
Bradykinin	RPPGFSPFR	+2	9	Produced during inflammation	Membrane active peptide
Buforin I	AGRGKQGGKVRAKAKTRSSRAGLQFPVGRVHRLLRKGNY	+13	39	Stomach tissue of the Asian toad bufo bufo gargarizans	AMP/CPP, helical
Buforin II	TRSSRAGLQFPVGRVHRLLRK	+6	17	Derived from Buforin I	AMP/CPP, helical
Buforin IIb	RAGLQFPVGRLLRRLLRRLLR	+7	21	Derived from Buforin II	AMP/CPP, helical
CM15	KWKLFKKIGAVLKVL	+6	15	Cecropin-melittin derivative	AMP, carpet, helical
Dermcidin (SSL-25)	SSLLEKGLDGAKKAVGGLGKLGKDA	+3	25	Human sweat	AMP-Pore and ion channel formation, helical
Dhvar 4	**KRLFKKLLFSLRKY**	**+7**	**14**	**Oral host defence peptides**	**AMP, helical**
FK16	FKRIVQRIKD FLRNLV	+5	16	Derived from LL37	AMP, carpet, helical
Gramicidin	Mix of A ,B ,C			Bacillus aneurinolyticus	AMP
Histatin 5	DSHAKRHHGYKRKFHEKHHSHRGY	+4	24	Saliva	AMP, helical
Indolicidin	ILPWKWPWWPWRR	+4	13	Cattle, granules of bovine neutrophil	AMP-oxidised lipid targeting,
KLA	KLAKLAKKLAKLAK	+6	14	Synthetic	AMP, helical
Lasioglossin III (LL-III)	VNWKKILGKIIKVVK	+6	16	Venom, eusocial bee Lasioglossum laticeps	AMP, membrane permeabilization, helical
LL37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	+7	37	Homo sapiens, skin, and intestine	AMP, carpet, helical
Macropin 1	GFGMALKLLKKVL	+4	15	skin secretions of the frog species Litoria genimaculata	AMP/CPP, membrane disruption
Melittin	GIGAVLKVLTTGLPALISWIKRKRQQ	+6	26	Honey bee venom	AMP,toroidal pore, helical
Octa-arginine	RRRRRRRR	+9	8	Arginine derivative	CPP
Penetratin	RQIKIWFQNRRMKWKK	+7	16	Drosophila Antennapedia homeodomain	**CPP α-helical/β-sheet conformations depend on phospholipid composition and peptide–lipid ratio**
Polybia-MPI	IDWKKLLDAAKQIL	+1	14	Social wasp venom	AMP,pore formation, helical
Protegrin-1	RGGRLCYCRRRFCVCVGR	+6	18	Pigs	AMP-Pore formation, sheet
p53 (17-26)	ETFSDLWKLL	0	10		ACP
Temporin-La	LLRHVVKILEKYL	+3	13	Anuran skin, Rana Temporia	AMP helical
Transportan	GWTLNSAGYLLGKINLKALAALAKKIL	+4	27	Galanin-mastoparan Conjugatem linked by lysine	CPP , helical





Characterization of surface protein by Linearly polarized light spectroscopy (LD)

- Peptides classified into **low (LPC), medium (MPC), and high (HPC)** groups based on the effective peptide concentration (**ec_{max}**) required for complete signal loss.
- These groups were used to assess **protein corona removal efficiency**.



Correlative flow cytometry analysis of lytic trends in REVs at various peptide concentrations

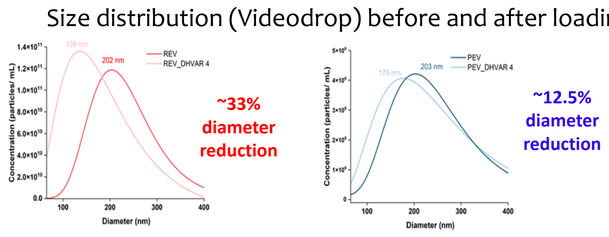
- Vesicle counts measured at three EC_{max} levels.
- Color gradient = increasing concentration.
- Lower vesicle count = higher lytic activity.

DHVAR 4, selected for therapeutic efficacy evaluation with REV and PEV

T. Sonallya et al., *Journal of Colloid and Interface Science*, Feb 2025

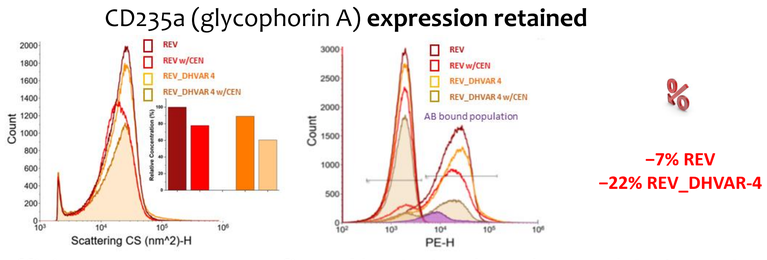
2. Effects of DHVAR 4 loading and washing on EV properties

Size distribution (Videodrop) before and after loading



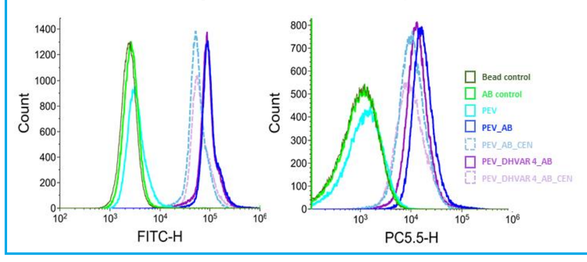
Overall vesicle integrity retained

CD235a (glycophorin A) **expression retained**



−7% REV
−22% REV_DHVAR-4

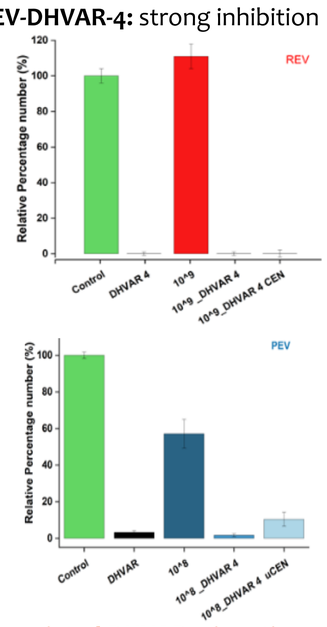
Efficient PEV capture confirmed by CD63-AlexaFluor and CD61-PerCy5.5 -positive fluorescence



	CD63	CD61
PEV %	23%	32%
PEV_DHVAR 4 %	45%	37%

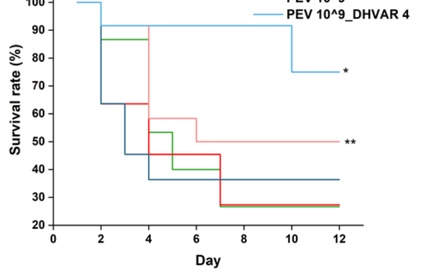
3. Antimicrobial activity against *E.coli*

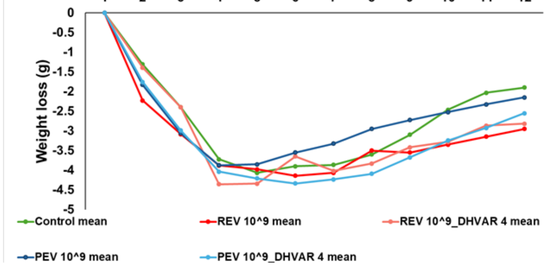
EVs alone: no inhibition of *E. coli*
REV-DHVAR-4: complete inhibition at low bacterial load
PEV-DHVAR-4: strong inhibition



EV-associated DHVAR-4 is active even after centrifugation

4. Therapeutic evaluation of treatment on the CLP sepsis model





PEV_DHVAR 4 ACHIEVES 75% SURVIVAL RATE

CONCLUSIONS

- Eight distinct MAP-EV interaction mechanism identified
- REVs and PEVs retain structural integrity and surface markers
- EV-loaded DHVAR 4 shows potent antimicrobial activity with minimal toxicity
- **Platelet derived EV (PEV)** DHVAR 4 are promising platform for targeted AMP delivery and sepsis therapy.

ACKNOWLEDGEMENT

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