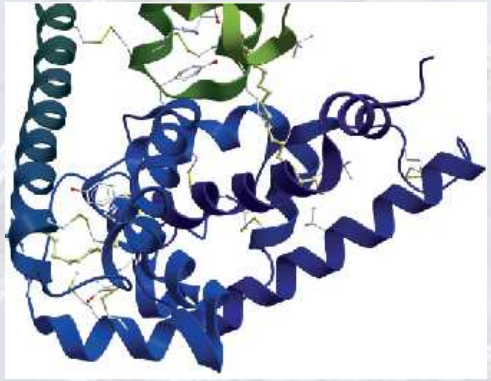
FORMAMP

INNOVATIVE NANOFORMULATION OF ANTIMICROBIAL PEPTIDES



Helena Bysell (Ph D, Project Manager)

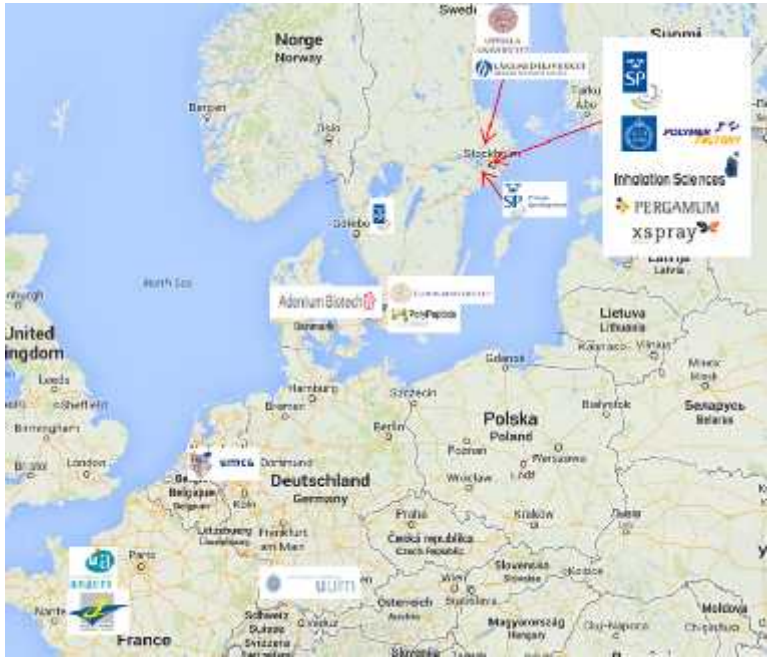
SP Chemistry, Materials and Surfaces- Life Science

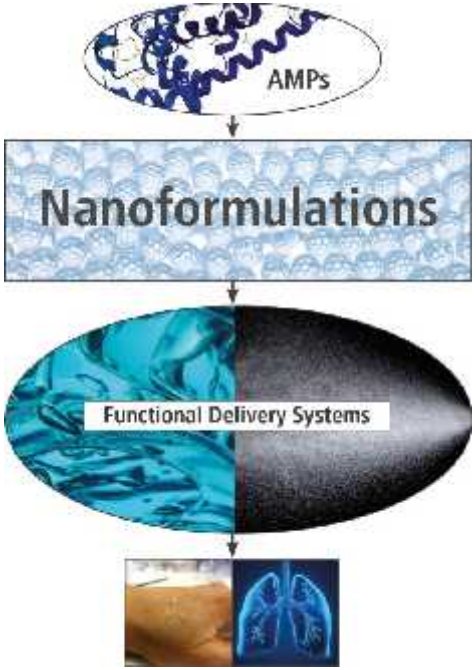
Early discovery of new antibiotics workshop

12-13 January 2017

FORMAMP Innovative Nanoformulation of Antimicrobial peptides

Vision: To reduce the alarming progress of multidrug-resistant bacteria
Mission: To develop new sustainable strategies for treatment of infectious diseases





Facts:
Project duration: 2013-2017
Budget: 10.5 MEuro,
EU contribution 8 MEuro
16 partners from 5 countries
Coordinator:
helena.bysell@sp.se
www.formampproject.com

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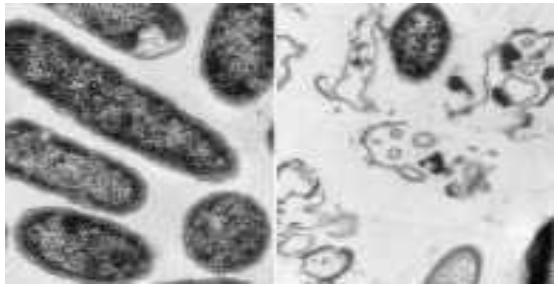
Antimicrobial peptides

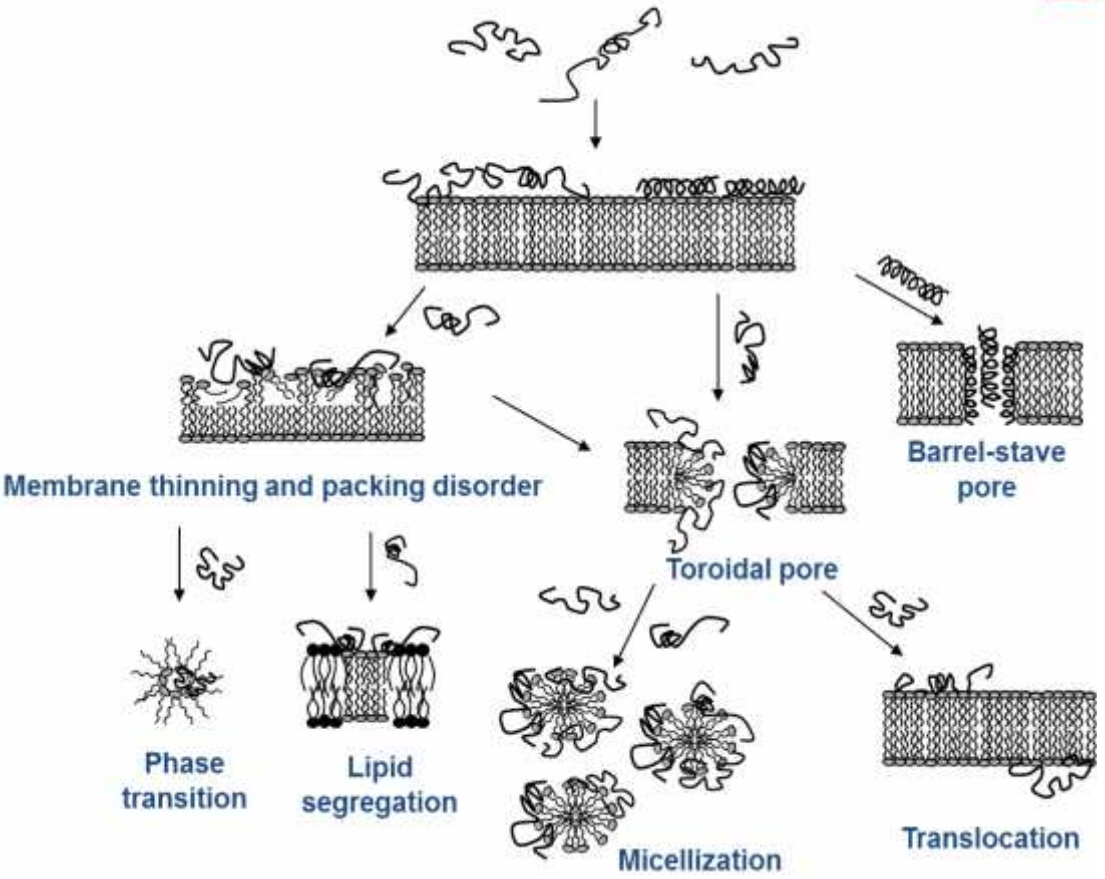


J Biol Chem , 2005 , 280, 34832

- Fast and non-specific mechanism of action
- Bacteria not as prone to develop high level resistance

Efficency and MoA influenced by

- Size
- Conformation
- Net charge
- Charge distribution
- Hydrophobicity



Ringstad, Uppsala University thesis, 2009

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AMPs in research and development

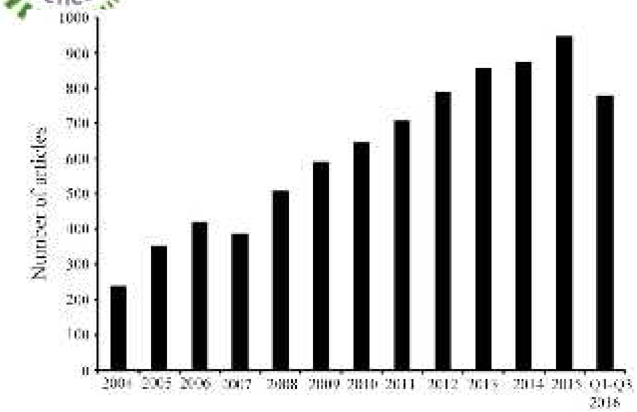


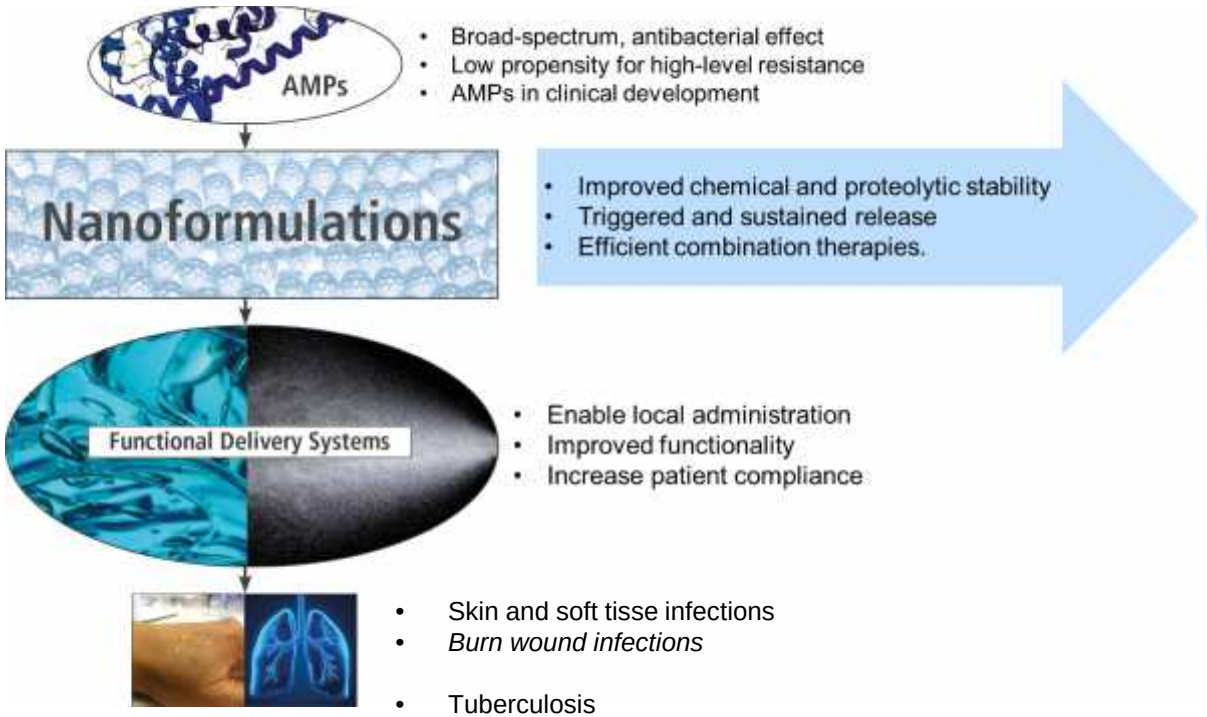
TABLE 1 | Selected AMPs in clinical phase of development.

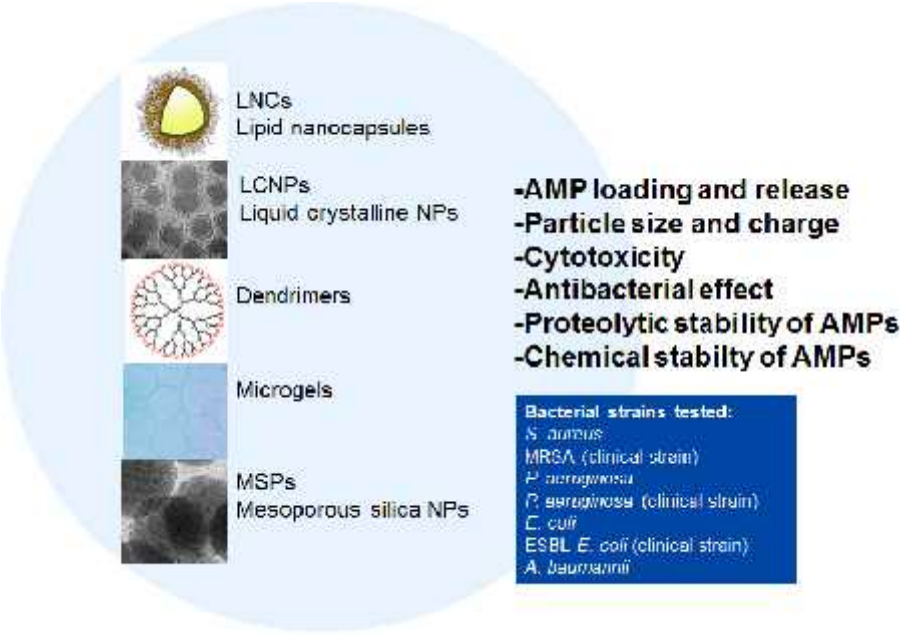
AMP	Description	Phase	Indication	Administration	Clinical trial identifier if available
Pexiganan (MSI-78)	Analog of magainin (skin of African clawed frog)	Phase III	Infected diabetic foot ulcers	Topical cream	NCT00563394, NCT00563433
Omiganan	Derived from indolicidin (bovine)	Phase III	Catheter infections and rosacea	Topical gel	NCT00231153, NCT01784133
Lytixar (LTX 109)	Synthetic antimicrobial peptidomimetic	Phase I/II	Uncomplicated Gram-positive skin infections, impetigo, and nasal colonization with S. aureus	Topical hydrogel	NCT01223222, NCT01803035, NCT01158235
hLF1-11	Derived from lactoferricin (human)	Phase I/II	Bacteraemia and fungal infections in immunocompromized haematopoetic stem cell transplant recipients	Intravenous treatment (in saline)	NCT00509938
Novexatin (NP-213)	Derived from α-defensins (human)	Phase II	Onychomycosis (fungal nail infection)	Topical brush-on treatment	
CZEN-002	Dimeric octamer derived from α-MSH (human)	Phase IIb	Vaginal candidiasis	Vaginal gel	
LL-37	LL-37 (human)	Phase I/II	Hard-to-heal venous leg ulcers	Polyvinyl alcohol-based solution for administration in the wound bed	
PXL01	Derived from lactoferricin (human)	Phase II	Prevention of post-surgical adhesion formation in tendon surgery	Hyaluronic acid-based hydrogel for administration at the surgical site	NCT01022242
Iseganan (IB-367)	Derived from protegrin 1 (porcine leukocytes)	Phase III	Oral mucositis in patients receiving radiotherapy for head and neck malignancy	Oral solution	NCT00022373
	... histatin 3	Phase II	Oral candidiasis in HIV seropositive patients	Mouthrinse	NCT00659971

Problems with:
-St
-T
-High cost

Formulation

FORMAMP concept





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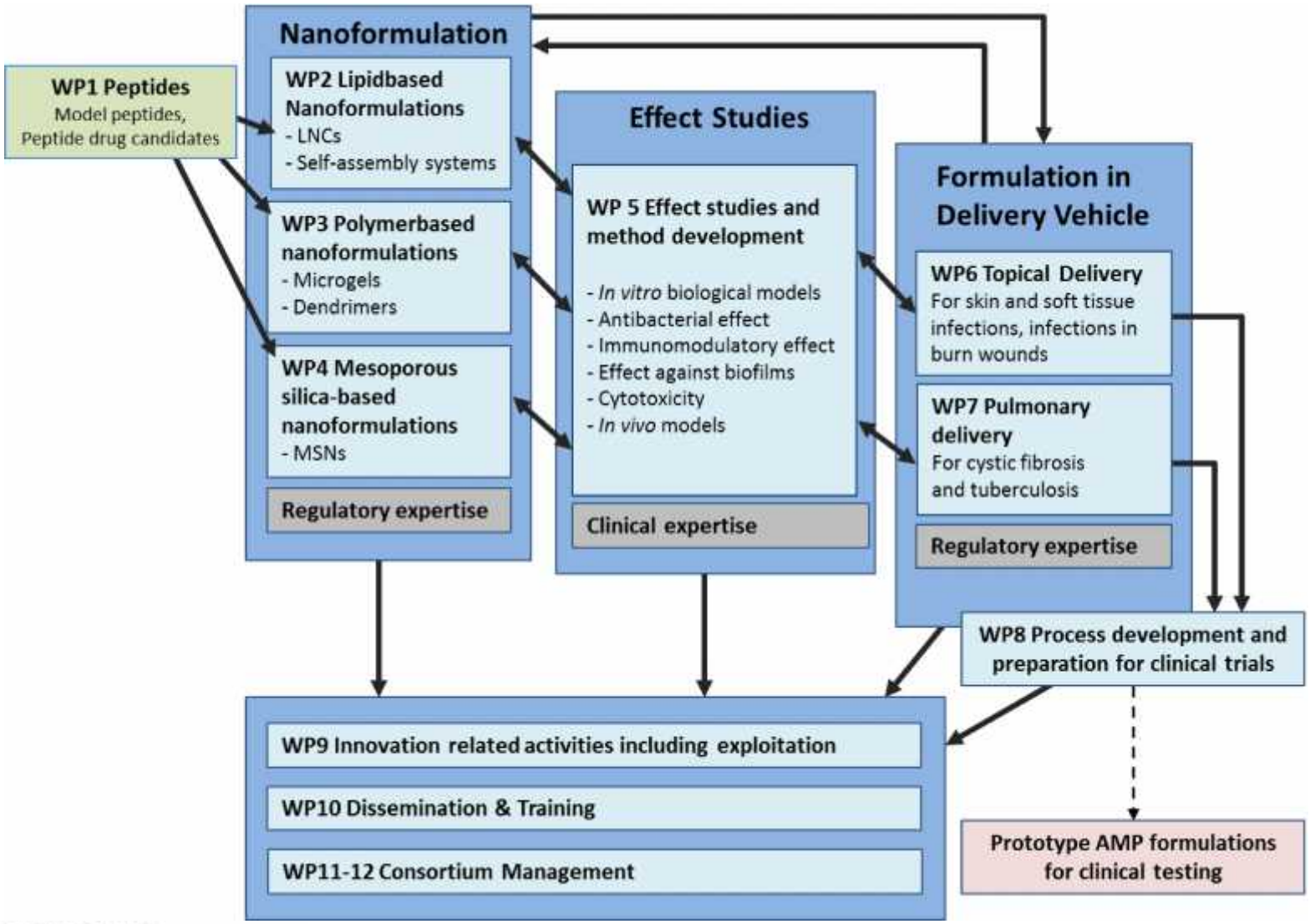
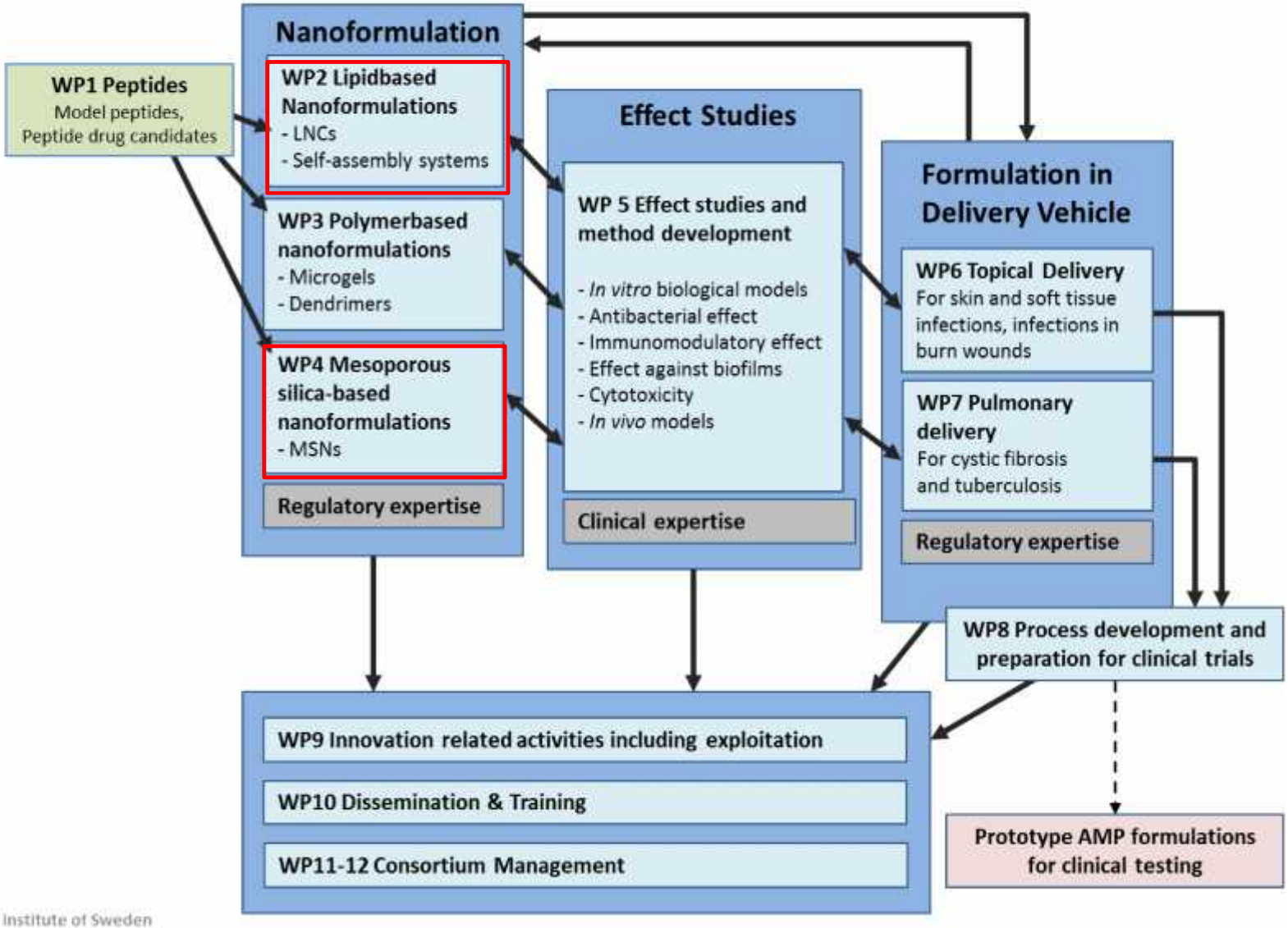

Nanoformulation
WP1 Peptides
Model peptides,
Peptide drug candidates
WP2 Lipidbased Nanoformulations
- LNCs
- Self-assembly systems
WP3 Polymerbased nanoformulations
- Microgels
- Dendrimers
WP4 Mesoporous silica-based nanoformulations
- MSNs
Regulatory expertise
Effect Studies
WP 5 Effect studies and method development
- In vitro biological models
- Antibacterial effect
- Immunomodulatory effect
- Effect against biofilms
- Cytotoxicity
- In vivo models
Clinical expertise
Formulation in Delivery Vehicle
WP6 Topical Delivery
For skin and soft tissue infections, infections in burn wounds
WP7 Pulmonary delivery
For cystic fibrosis and tuberculosis
Regulatory expertise
WP8 Process development and preparation for clinical trials
WP9 Innovation related activities including exploitation
WP10 Dissemination & Training
WP11-12 Consortium Management
Prototype AMP formulations for clinical testing

FORMAMP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Model peptides,
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For skin and soft tissue infections, infections in burn wounds
WP7 Pulmonary delivery
For cystic fibrosis and tuberculosis
Regulatory expertise
WP8 Process development and preparation for clinical trials
WP9 Innovation related activities including exploitation
WP10 Dissemination & Training
WP11-12 Consortium Management
Prototype AMP formulations for clinical testing

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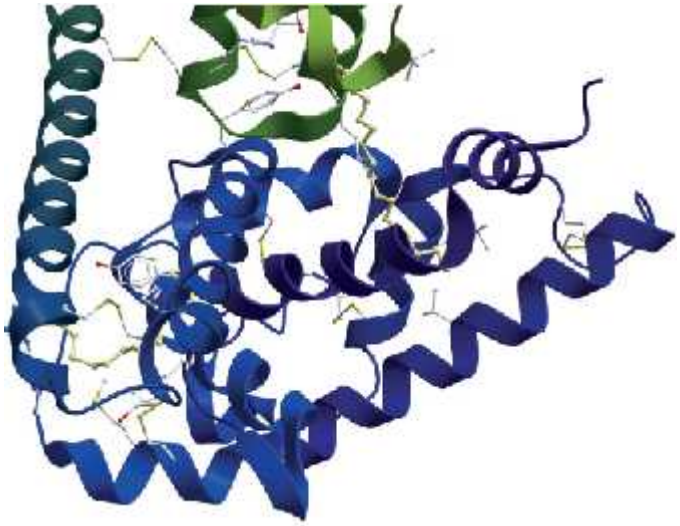
Results -Peptides



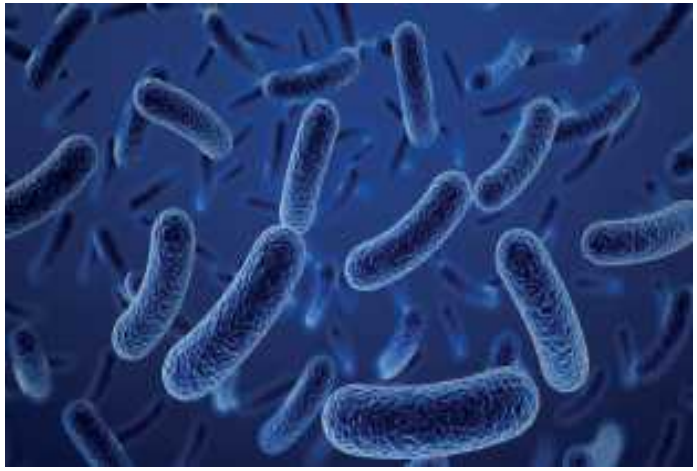
-5 clinical AMP candidates have been selected and synthesized in mg-g-scale.

-A peptide effective against *Mycobacterium tuberculosis*, both intracellular and extracellular, has been identified, synthesized and is currently evaluated *in vivo.*

-Target product profiles (TPPs) for the different peptides have been developed.

-Analytical methods have been developed and optimized

Results –AMP and nanocarriers



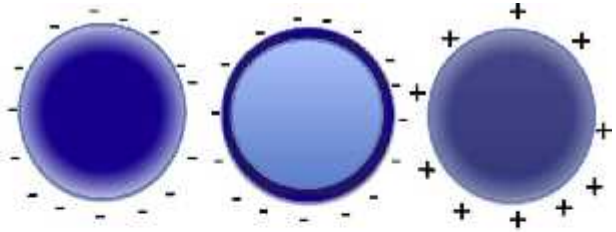
-AMPs can be successfully loaded into the different nanocarrier systems and strategies for optimizing the loading and release are being evaluated.

-More than 400 peptide-carrier combinations have been evaluated *in vitro*. Results show that the antibacterial effect is preserved in 80% of the cases for encapsulated AMPs and also enhanced for 10% of the peptide-carrier combinations.

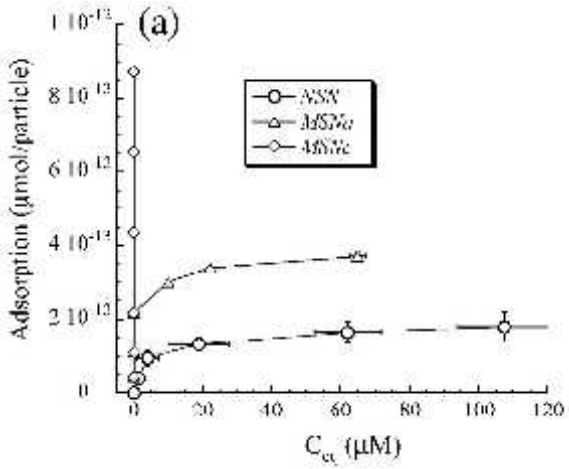
-AMPs can be protected against proteolytic degradation in selected nanocarriers.

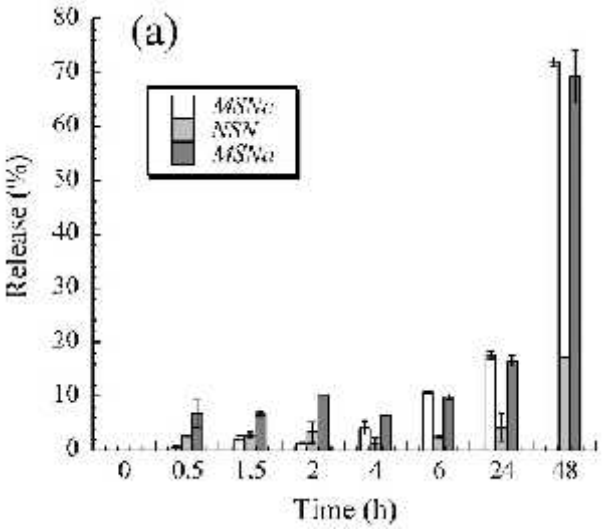
-The majority of AMP-loaded particles display good results with respect to cytotoxicity.

Formulation of AMPs in mesoporous silica



	MSNc (-)	NSN (-)	MSNa (+)
Peptide load	+++++	+	+
Location	Pore	Surface	Pore
Antimicrobial entity	Peptide	Particle	Particle
Proteolytic stability	+	-	-
Toxicity	-	-	+





-Surface charge and surface area strongly influences loading and release of LL-37 and thereby membrane interactions and antimicrobial effect
-Peptide localization correlates to proteolytic stability

Malmsten and Linden et al. Journal of Colloid and Interface Science 475 (2016) 161–170

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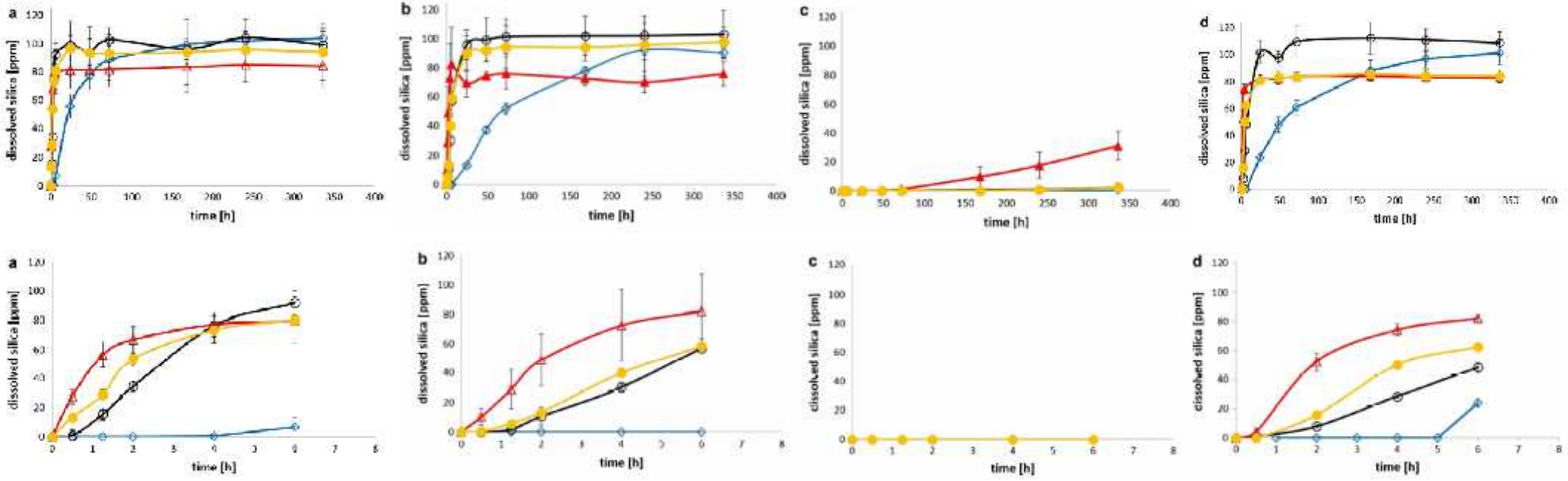
Formulation of AMPs in mesoporous silica

Dissolution kinetics of mesoporous silica nanoparticles in different simulated body fluids

Lung Fluid (SLF) | Body Fluid (SBF) | Gastric Fluid (SGF) | PBS



non-porous 200 nm
mesoporous 80 nm
mesoporous 200 nm
mesoporous 1500 nm

Braun et al. J Sol-Gel Sci Technol, 2016

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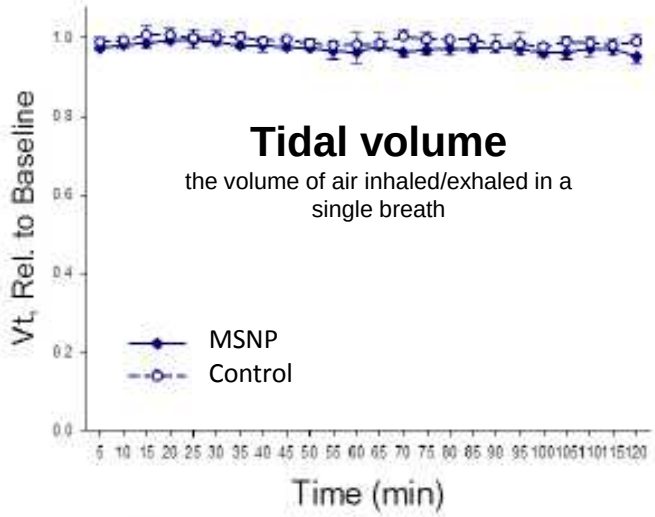
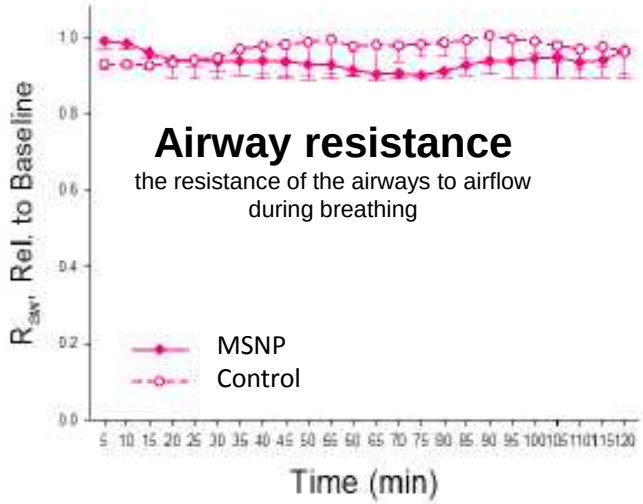
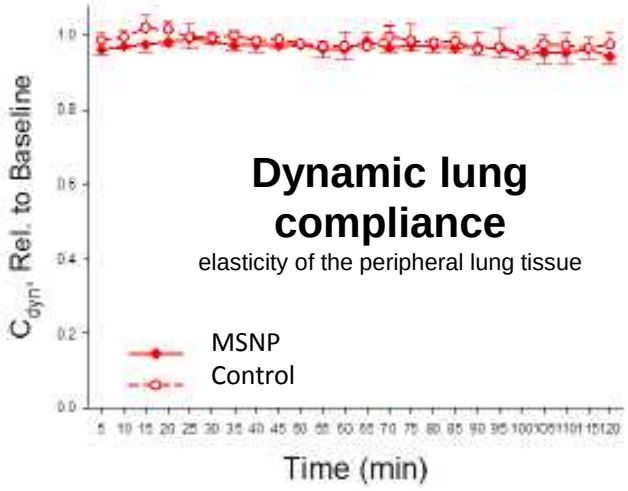
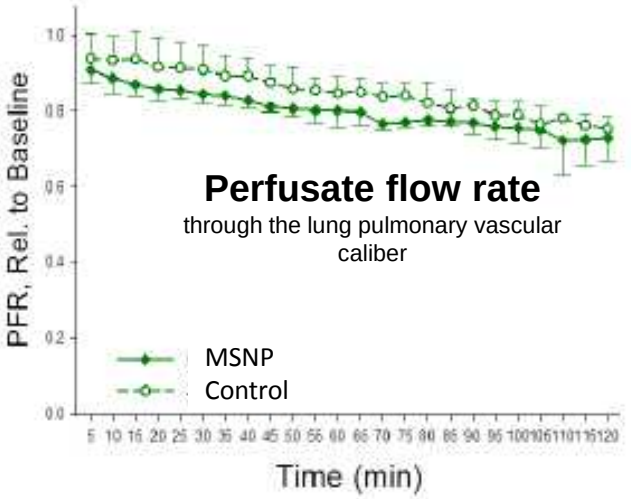
Formulation of AMPs in mesoporous silica

Ex vivo safety- physiological lung-function variables









No acute lung toxicity was thus observed in the rat lungs exposed ex vivo to a high dose of particles

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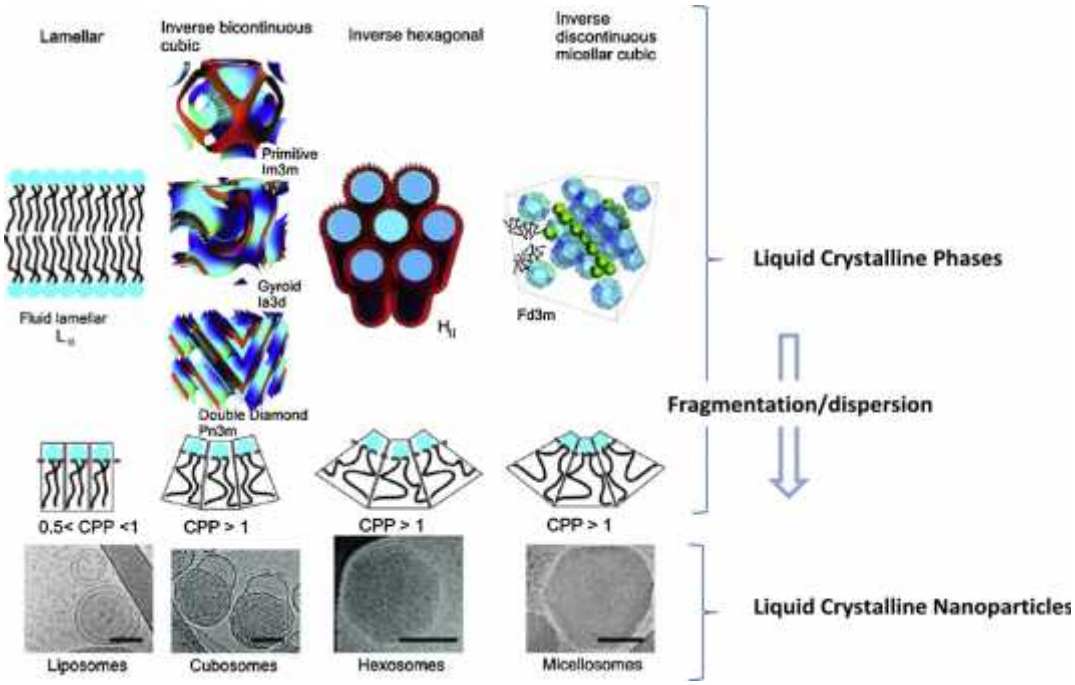
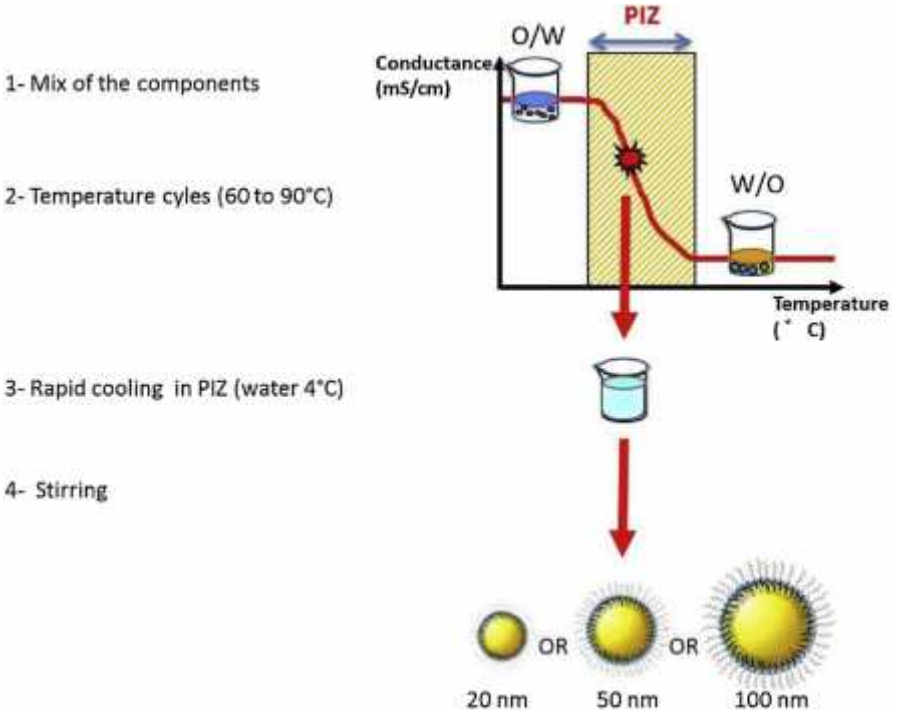
Formulation of AMPs in lipid nanoformulations

Liquid crystalline nanoparticles (LCNPs)



Mulet et al., 2013

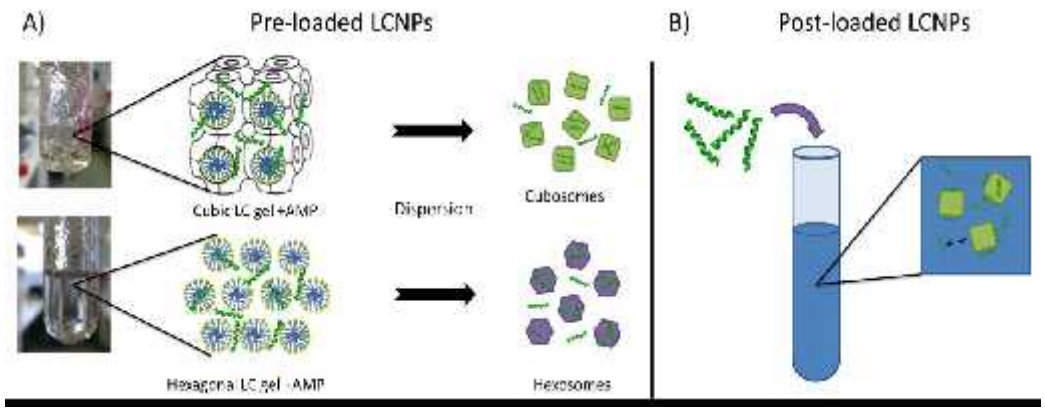
Lipidic nanocapsules (LNCs)



Matougui et al, International Journal of Pharmaceutics 502 (2016) 80–97

Formulation of AMPs in lipid nanoformulations

Liquid crystalline nanoparticles (LCNPs)



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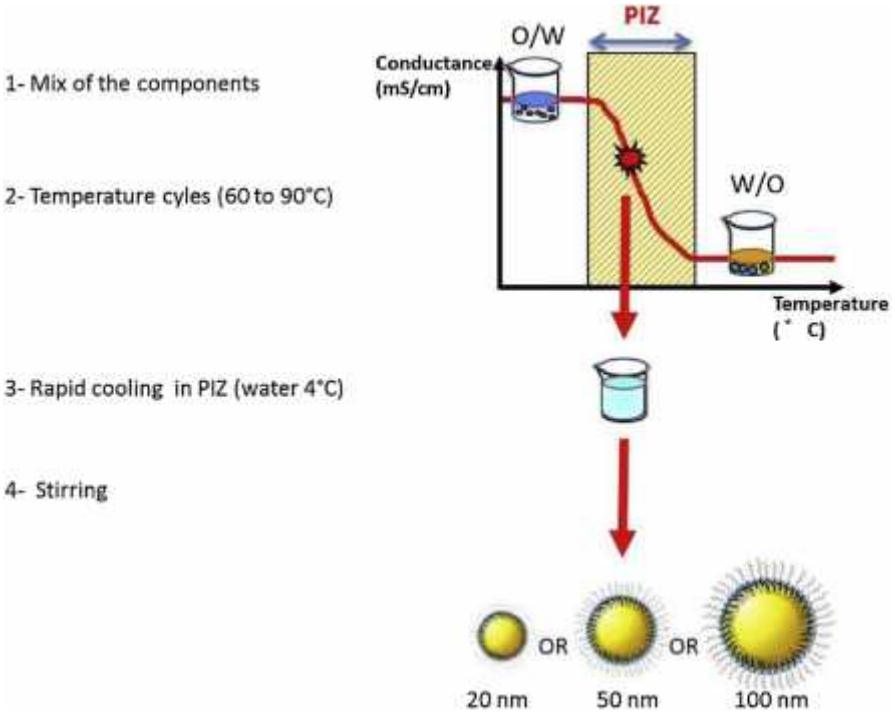
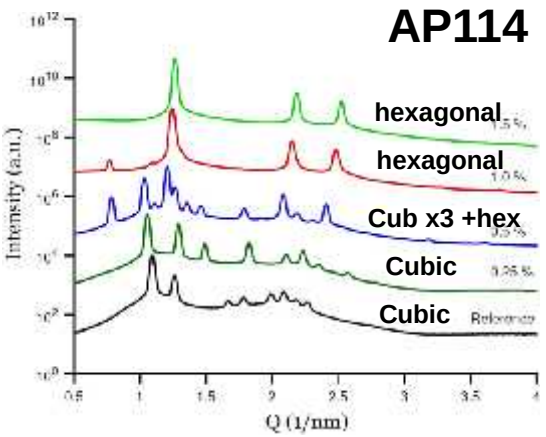
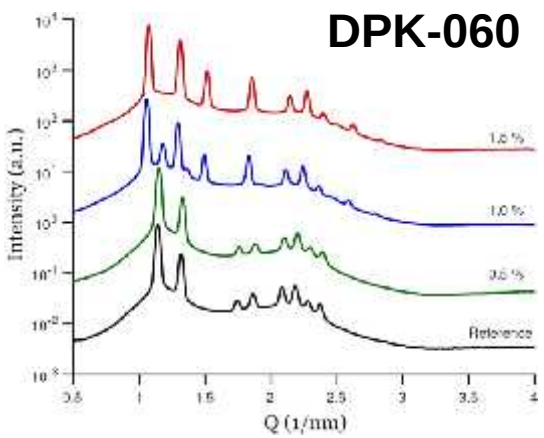


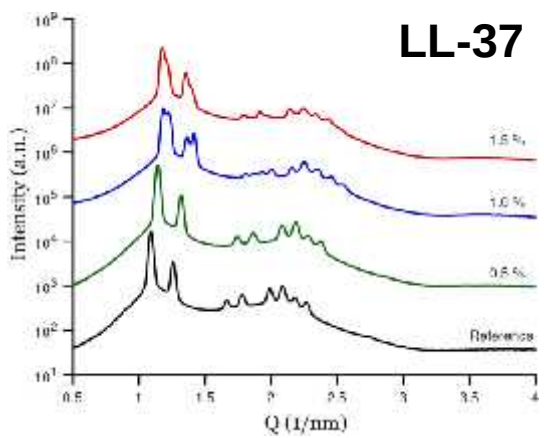
Matougui et al, International Journal of Pharmaceutics 502 (2016) 80–97

Formulation of AMPs in lipid nanoformulations

-Physicochemical characterization- phase behavior







- **Cubic → hexagonal**
- **Increased negative curvature (Ia3d→Hex)**

- **Cubic → new cubic**
- **Decreased negative curvature (Ia3d→Pn3m)**

- **Cubic → same cubic**
- **No change in curvature (Ia3d)**

No phase transitions for hexagonal gels

Hydrophobicity	AP114 > LL-37 > DPK-060
Net charge	DPK-060 > LL-37 > AP114
Molecular weight	LL-37 ~ AP114 > DPK-060

Boge et al. Langmuir, 2016, 32 (17), pp 4217–4228

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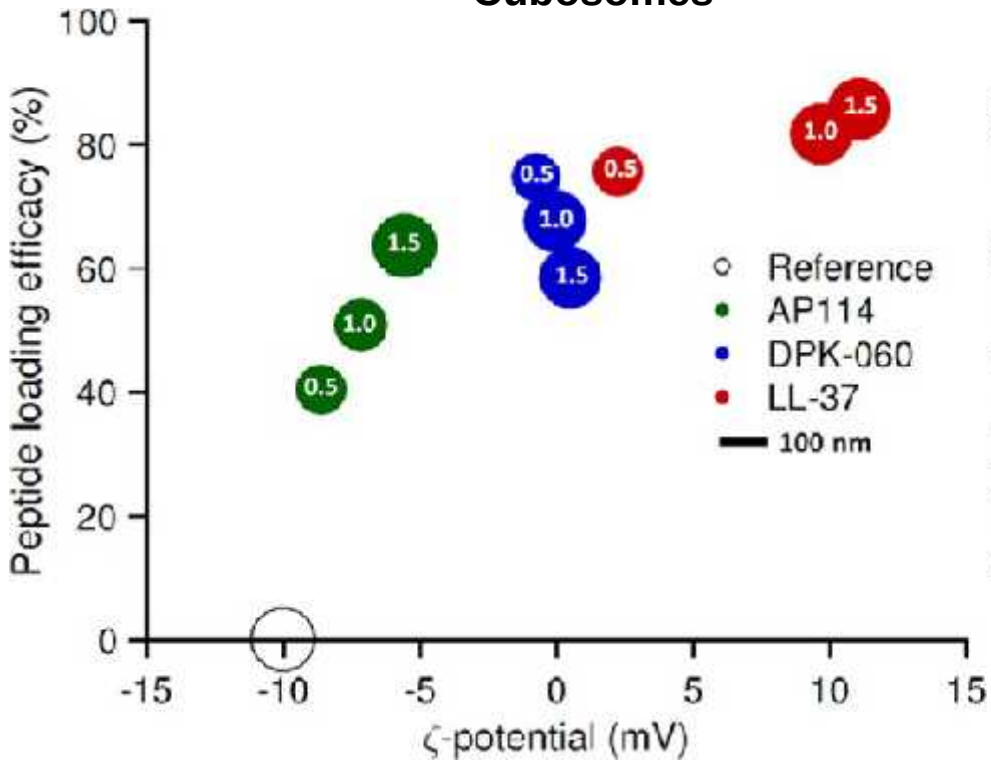
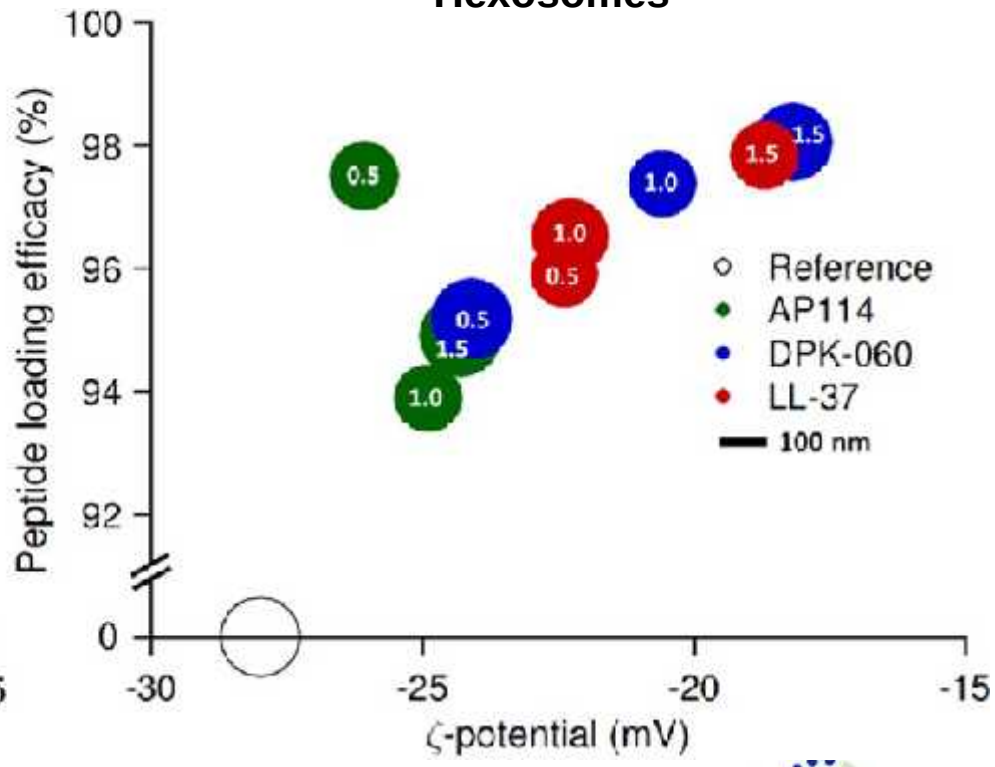
Formulation of AMPs in lipid nanoformulations

-Physicochemical characterization- particle size and charge, peptide loading

Cubosomes



Hexosomes



Boge et al. Langmuir, 2016, 32 (17), pp 4217–4228

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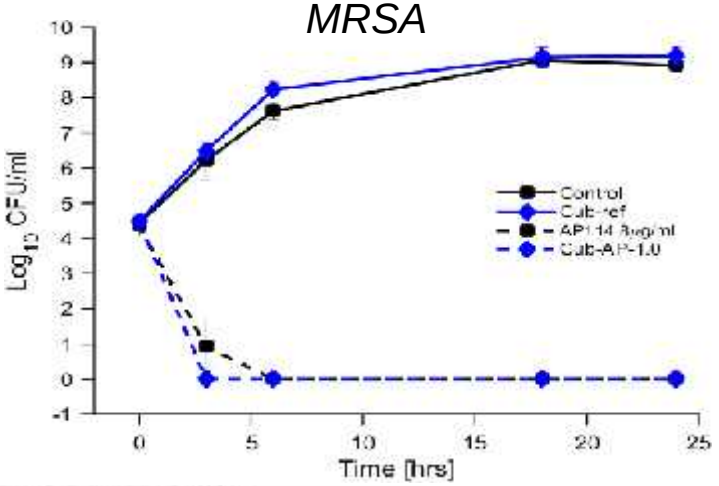
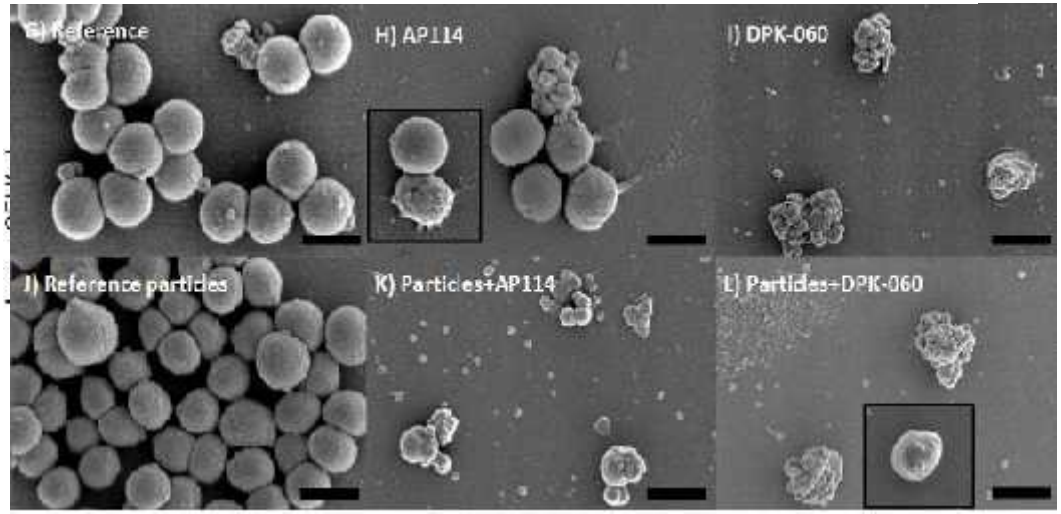
Formulation of AMPs in lipid nanoformulations

-Antibacterial effect

MIC values (µg/mL)

Bacterial strain	AP114			DPK-060			LL-37		
	UF	Pre (GMO)	Post (GMO)	UF	Pre (GMO)	Post (GMO)	UF	Pre (GMO)	Post (GMO)
S. aureus	8	8	8-16	4	2-4	1-2	8-16	>16	>16
MRSA	4	4	4	4	2-4	2	8-16	>16	>16
P. aeruginosa	-	-	-	8	16	8-16	8-16	>16	8-16
P. aeruginosa clinical strain	-	-	-	16	8-16	8	8-16	8	8-16
E. coli	-	-	-	8	2-4	4	16	16	8-16
ESBL *E. coli* clinical strain	-	-	-	4-8	2-4	2-4	16	>16	≥16
A. Baumannii	-	-	-	4-8	16	16	16	>16	≥16



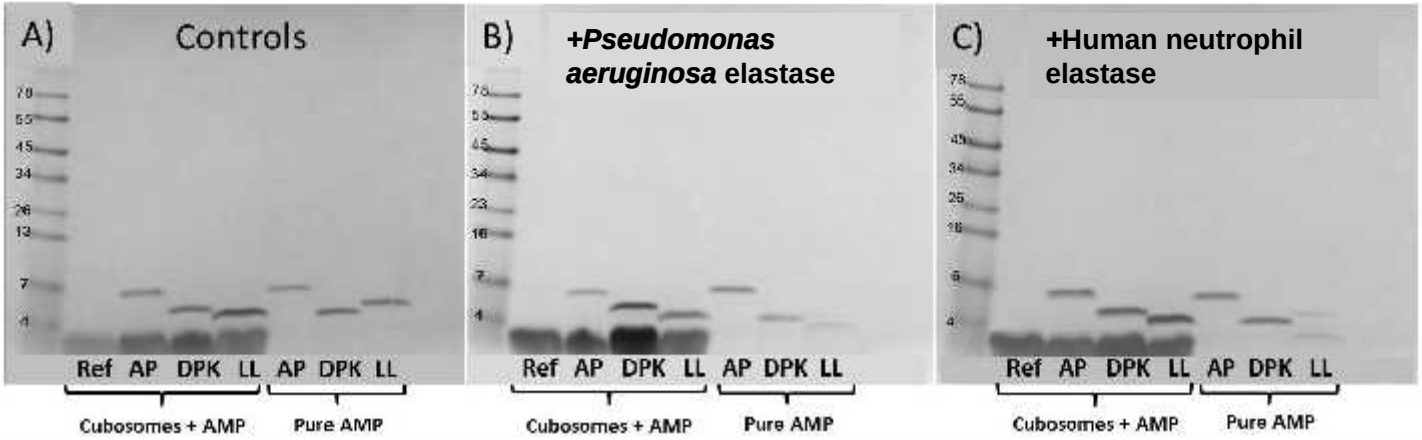


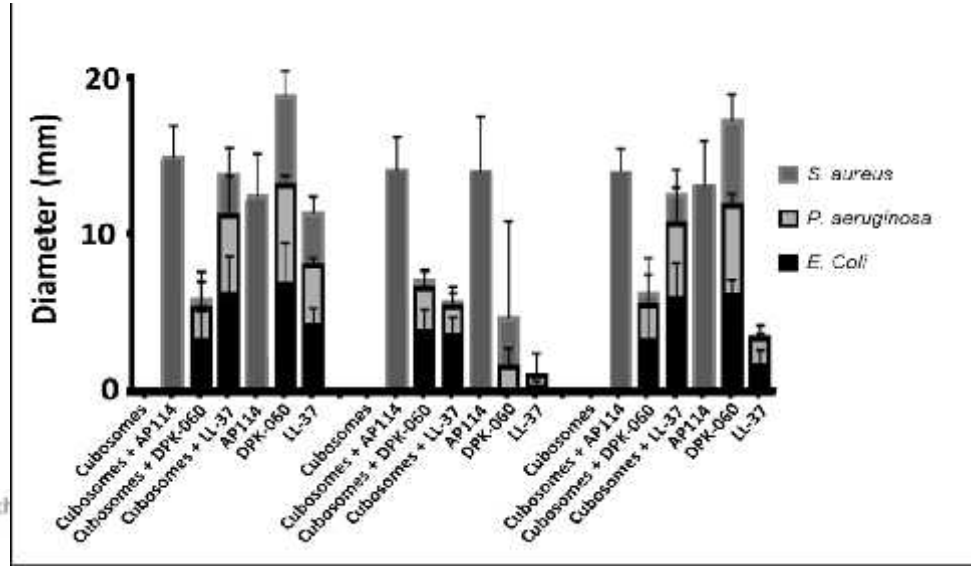
Boge et al. Langmuir, 2016, 32 (17), pp 4217–4228

Boge et al. In Manuscript

Formulation of AMPs in lipid nanoformulations

-Proteolytic stability



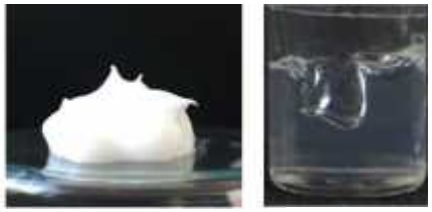


LL37 can be protected from proteolytic degradation when associated to cubosomes

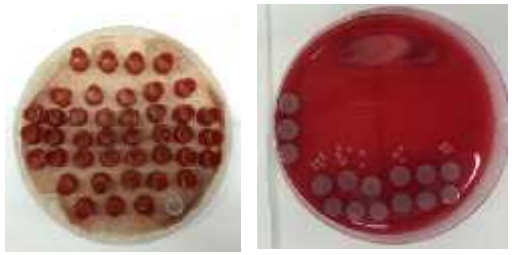
Boge et al. In manuscript

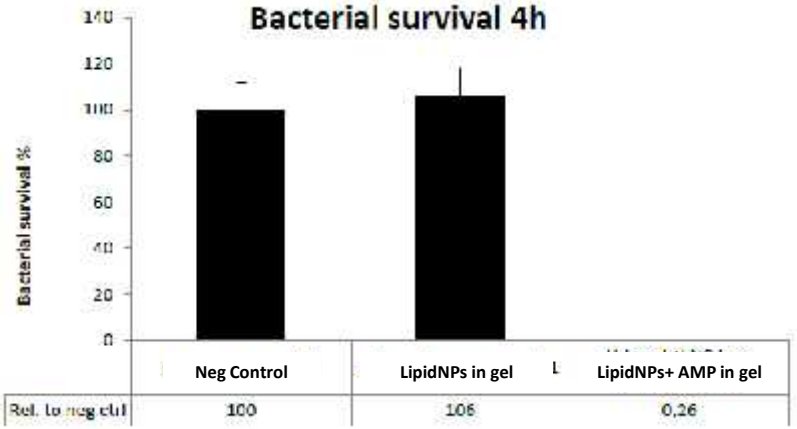
Formulation of AMPs in lipid nanoformulations

-*Ex vivo* antibacterial effect and chemical stability of prototype formulations

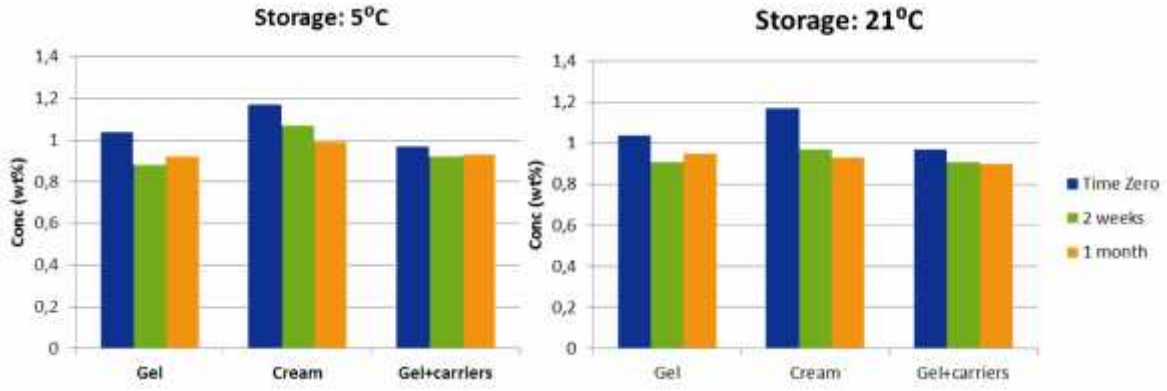


Effect





Stability



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Next steps

-Understand the mechanisms-synegistic effects
-Formulation strategies for biofilms
-Further evaluation of effect and toxicity on refined models (*ex vivo, in vivo*).
-Stability studies

Finding funding for follow up projects!!!

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Thank you!

www.formampproject.com

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The research in FORMAMP receives funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 604182. http://ec.europa.eu/research

Martin Andersson CHALMERS

MAX Lab MAX IV

SP Technical Research Institute of Sweden

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