Microfluidics-Driven Assembly of Sonoresponsive Peptidic Nanoparticles for Ultrasound-Mediated Gene Delivery

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INTRODUCTION

Context – Building on S. Medina work,[1] Bracco previously explored nanopeptisomes, a novel class of peptide-based phase-change nanodroplets formulated with high-boiling-point perfluorocarbons (PFCs) for ultrasound-mediated gene delivery.[2,3]

Problematic – Extending this platform to low-boiling-point PFCs, which offer improved acoustic responsiveness allowing sonoporation at reduced acoustic pressure , remains challenging due to their high volatility and the need to preserve nanoparticle integrity during formulation.

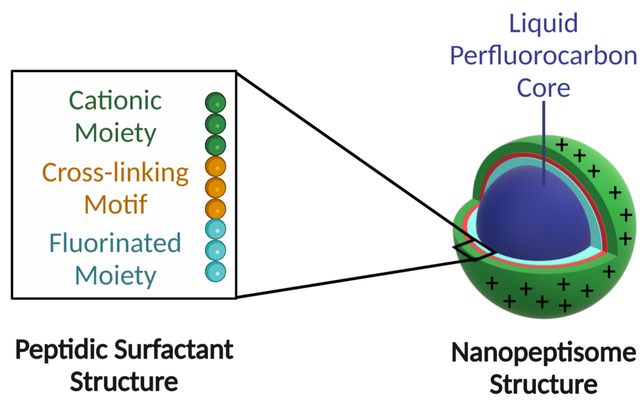
Solution – By combining **microfluidic assembly** with a **disulfiram-based** (DSF) **peptide cross-linking strategy**,[4] we successfully formulated stable low-boiling-point PFC Nanopeptisomes, overcoming the volatility-associated formulation challenges while maintaining ultrasound responsiveness.[5]

NANOPEPTISOME DESIGN

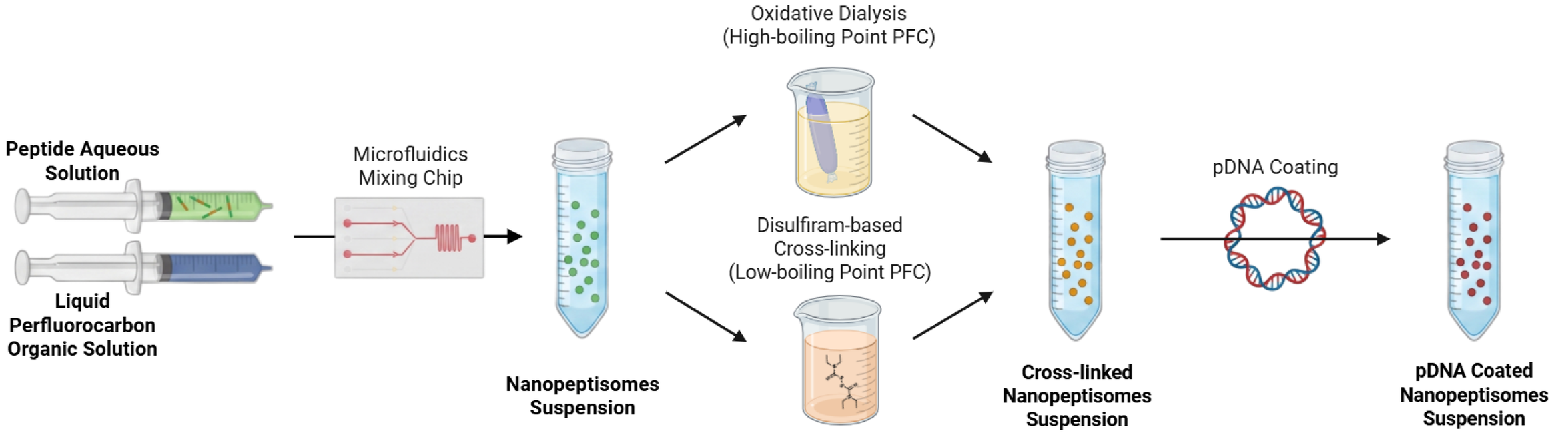
Hydrophilic cationic moiety using either Cell Penetrating Peptide (CPP) or Nuclear Localization Sequence (NLS) to bind anionic payload such as genetic material

Cross-linking region to enhance the NPP stability.

Hydrophobic fluorinated tail stabilize the PFC liquid core.



NANOPEPTISOME PREPARATION



Formulation

- Shell: Peptidic Surfactants
- Core:
  - C_6F_{14} Perfluorohexane PFH (b.p. 56°C)
  - C_5F_{12} Perfluoropentane PFP (b.p. 29°C)
  - C_4F_{10} Perfluorobutane PBP (b.p. - 2°C)

Microfluidics Process Settings

- **Flow Rate Ratio (FRR):** Volumetric ratio of the aqueous & organic phases → 2:1 (aqueous : organic)
- **Total Flow Rate (TFR):** Total speed at which both fluid streams are injected → 10 mL/min

Cross-linking

- **Oxydative Dialysis:**
  - Oxidation: Aqueous DMSO (2.5% v/v),
  - Purification: 5% Glucose dialysis
- **Disulfiram-based:**
  - Oxidation: 5% Glucose, 5 eq DSF, 4°C
  - Purification: Centrifugation

pDNA Coating

- **Plasmid:** pCPG-hCMVSCEP-LucSH
- **Solvent:** 5% Glucose
- **Incubation:** 15 min, r.t.
- **NPP Concentration:** 10^9 p/mL
- **pDNA Concentration:** 10 µg/mL

RESULTS

Optimization of Disulfiram-based cross-linking for Nanopeptisomes

Influence of Peptide/PFC molar ratio

Peptide/PFC Molar Ratio	Size (nm)	Polydispersity Index (PDI)	ζ-Potential (mV)	Conc. (p/mL)
0.08	847 ± 499	0.80 ± 0.18	1 ± 0.6	$4.9 \pm 0.1 \times 10^8$
0.02	**566 ± 38**	**0.58 ± 0.17**	**1 ± 2.5**	**$3.9 \pm 0.3 \times 10^8$**

- A **lower** peptide/PFC **molar ratio** (0.02) **reduced** particle **size** and **polydispersity**.

Influence of aqueous formulation phase

Aqueous Phase	Size (nm)	Polydispersity Index	ζ-Potential (mV)	Conc. (p/mL)
6M Gu.HCl	566 ± 38	0.58 ± 0.17	1 ± 2.5	$3.9 \pm 0.3 \times 10^8$
25 mM HEPES (pH 7.4)	> 1000	1	5.4 ± 1.3	$< 1 \times 10^8$
mQ H2O (pH 6.3)	492 ± 34	0.22 ± 0.04	18.4 ± 1.4	$1.2 \pm 0.1 \times 10^9$
Glucose 5% (pH 4.3)	**424 ± 4**	**0.25 ± 0.03**	**38.6 ± 1.6**	**$1.5 \pm 0.01 \times 10^9$**

- **5 eq. DSF** provided the **best overall compromise**, improving nanopeptisome monodispersity while maintaining a high particle concentration.

Influence of disulfiram stoechiometry

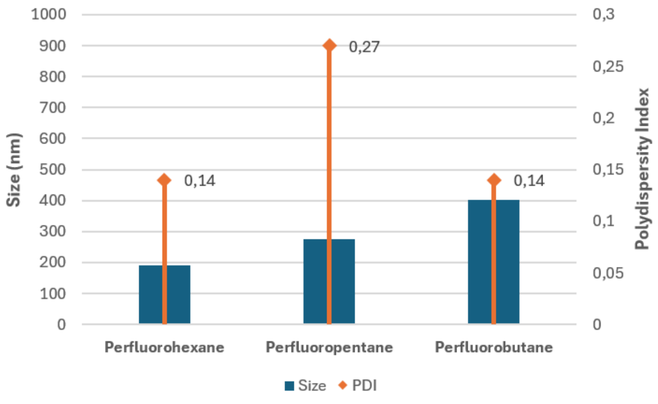
Stoechiometry (eq. DSF)	Size (nm)	Polydispersity Index	ζ-Potential (mV)	Conc. (p/mL)
0.5	431 ± 8	0.09 ± 0.03	48.2 ± 2.3	$4.5 \pm 0.4 \times 10^9$
2.5	403 ± 10	0.17 ± 0.04	42.6 ± 0.7	$2.4 \pm 0.2 \times 10^9$
5	**401 ± 10**	**0.14 ± 0.04**	**39.9 ± 2.5**	**$3.9 \pm 0.1 \times 10^9$**
7.5	398 ± 15	0.18 ± 0.03	30.8 ± 5.6	$2.2 \pm 0.1 \times 10^9$
10	424 ± 4	0.25 ± 0.03	38.6 ± 1.9	$1.5 \pm 0.01 \times 10^9$

- A **low-pH 5% glucose** aqueous phase **improved particle size**, monodispersity, concentration and ζ-potential, **suggesting enhanced colloidal stability**.

Mechanistic Hypothesis: Optimized pH and DSF stoichiometry may limit DDTC-anion accumulation and the associated destabilizing neutralization of the nanopeptisomes surface charge.

Influence of the Perfluorocarbon Core on Nanopeptisome Properties

Physico-Chemical Properties

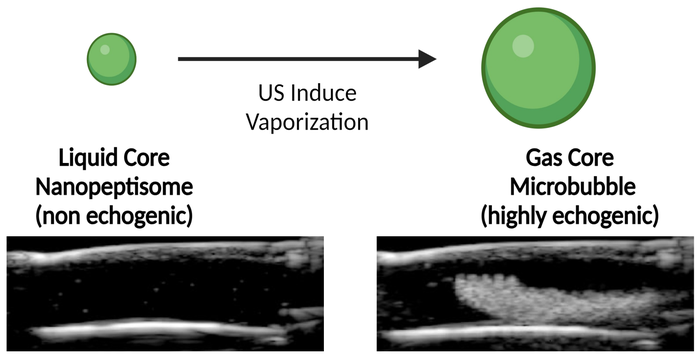


PFH and PFP Nanopeptisomes were prepared by oxidative dialysis, whereas PFB required the newly developed disulfiram cross-linking strategy.

- Nanopeptisome size increase with decreasing PFC boiling point.
- PDI seems uncorrelated to PFC.
- All formulations achieved concentration > 1×10^9 p/mL.

Trend likely to reflects a combined influence of **PFC** physicochemical **properties** and **peptide-shell organization**, which together **determine interfacial stabilization** during assembly.

Acoustic Response



Gas	ADV Threshold (MPa)	Mechanical Index
C_6F_{14}	~ 10,20	~ 4,16
C_5F_{12}	~ 7,64	~ 3,13
C_4F_{10}	~ 4,14	~ 1,69

- **Acoustic properties mainly driven** by the **perfluorocarbon liquid core**.
- **Lowering the boiling point** led to **reduced mechanical index (MI).**
- **Mechanical Index** suitable **only** for **therapeutic applications**.

CONCLUSIONS

- An optimized **disulfiram-mediated cross-linking strategy enabled the stable formulation of low-boiling-point nanopeptisomes**, overcoming a key limitation of previous nanopeptisome formulations.
- **Expanding** the **nanopeptisome** platform to both high- and low-boiling-point PFCs **increases** formulation **tunability**, notably low-boiling-point cores enable NPP activation at lower acoustic pressures, potentially reducing sonoporation-induced cellular stress.
- The **optimized** low-boiling-point **nanopeptisome platform** is **compatible** with our **previously** established **genetic** cargo **loading** strategy.

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