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# Innovative microfluidic preparation of monodisperse peptidic nanoparticles for ultrasound guided gene delivery

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## INTRODUCTION

**Context** – Gas-filled microbubbles (MB), clinically approved as ultrasound (US) contrast agents, have demonstrated efficacy in drug and gene delivery by exploiting the sonoporation phenomenon.[1]

**Problematic** – Intravascular microbubbles (MB) have a relatively short lifespan and are unable to accumulate in tissues due to their large size (1 to 10 µm), thus limiting their efficacy.

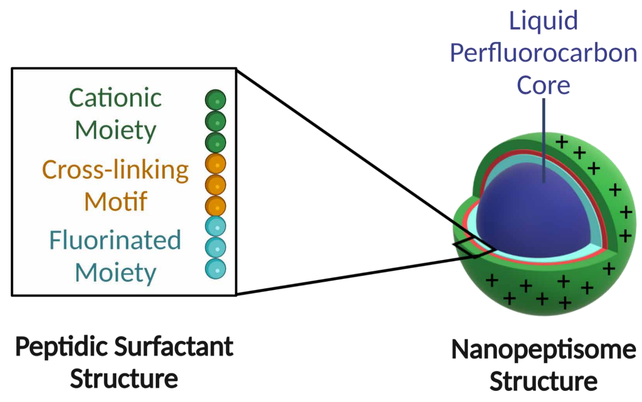
**Solution** – Perfluorocarbon nanodroplets (ND) could overcome those challenges due to their submicrometric size. Moreover, an external ultrasound stimulus can convert them into gaseous microbubbles by a phenomenon called acoustic droplet vaporization (ADV).[2] Nanopeptisomes (NPP) represents a new class of nanodroplets taking advantages of peptide versatility.[3]

## NANOPEPTISOME DESIGN

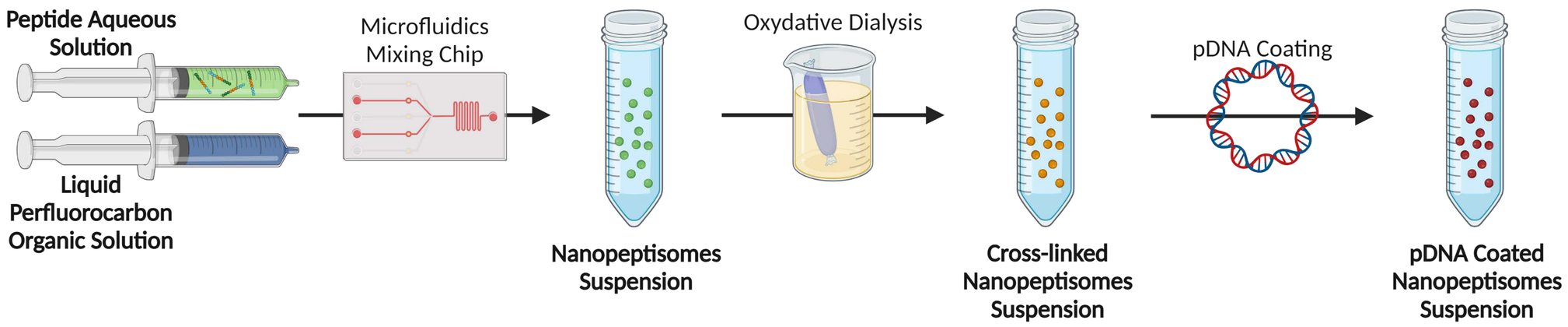
**Hydrophilic cationic moiety** using either Cell Penetrating Peptide (CPP) or Nuclear Localization Sequence (NLS) to bind anionic payload (PreS2, SV40, Pepfect14).

**Cross-linking region** is expected to enhance the NPP stability.

**Hydrophobic fluorinated tail** stabilize the PFC liquid core.




## NANOPEPTISOME PREPARATION[4,5]




### FORMULATION

- Shell: Peptidic Surfactants
- Core:
  - $C_6F_{14}$; Perfluorohexane PFH (b.p. 56°C)
  - $C_5F_{12}$ Perfluoropentane PFP (b.p. 29°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

### DIALYSIS

- **Solvent :**
  - Oxidation: Aqueous DMSO (2.5% v/v)
  - Purification: 5% Glucose
- **MWCO :** 10 kDa

### 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

## CHARACTERIZATION

### 1 Particle characterization

- **Methods:** DLS, NTA and Fluorometry
- **Properties:**
  - Size (nm),
  - PolyDispersity Index (PDI),
  - ζ-Potential (mV),
  - Concentration (p/mL),
  - DNA Coating (µg/mL or µg/p).

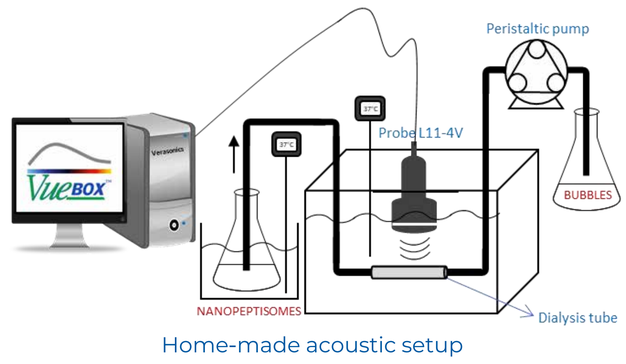
Zetasizer Nano **DLS**

NanoSight NS300 **NTA**

Qubit 4 Fluorometer **Fluorometry**

### 2 Acoustical characterization

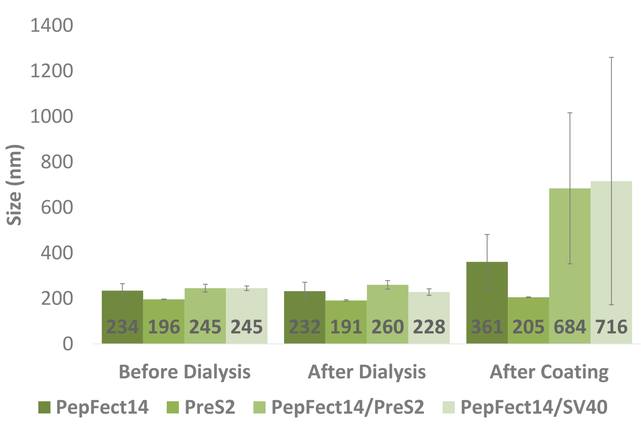
- Progressive increase of the acoustic pressure applied to the nanopeptisomes.
- **ADV threshold** defined as the minimum acoustic pressure required to convert liquid PFC non echogenic droplets into echogenic microbubbles.

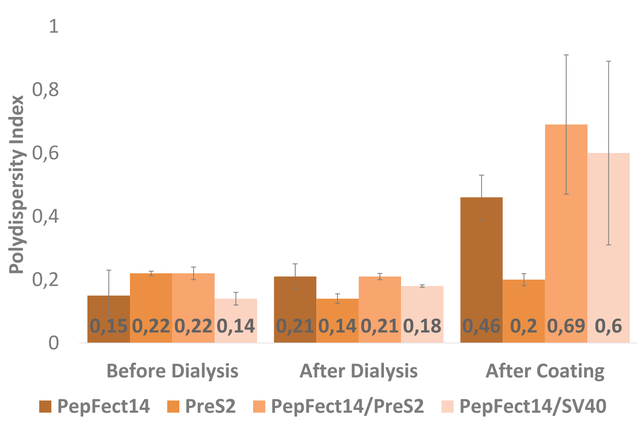



Home-made acoustic setup

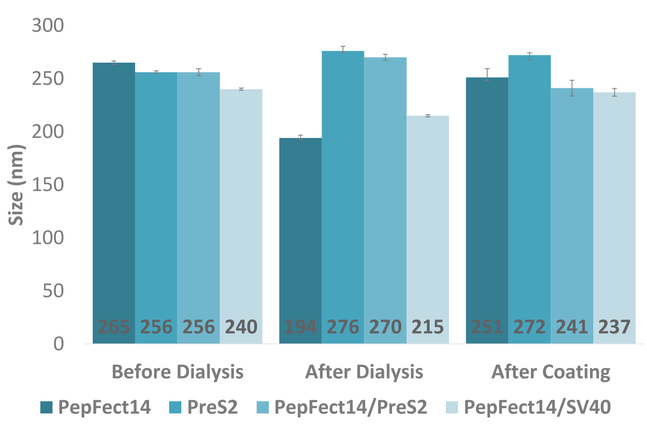
## RESULTS

### Perfluorohexane Core NPP Characterization




### Perfluoropentane Core NPP Characterization




| Peptide | Concentration (p/mL) Before Dialysis | After Dialysis | |
|---|---|---|---|
| PepFect14 | 2,5x$10^{10}$ ± 0,2 | 7,6x$10^{9}$ ± 0,04 | ↘ |
| PreS2 | * | * | |
| PepFect14/PreS2 | 3,5x$10^{10}$ ± 0,3 | 1,0x$10^{10}$ ± 0,4 | ↘ |
| PepFect14/SV40 | 2,6x$10^{10}$ ± 0,2 | 1,6x$10^{10}$ ± 0,03 | ↘ |

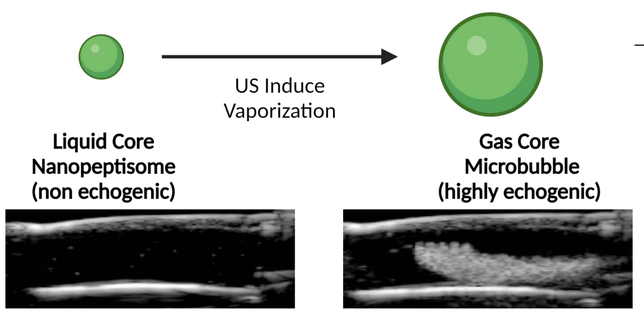
* On going analysis.

| Peptide | ζ-Potential (mV) Before Coating | After Coating |
|---|---|---|
| PepFect14 | 58,9 ± 9,1 | -1,1 ± 3,3 |
| PreS2 | 24,7 ± 0,7 | -32 ± 1,1 |
| PepFect14/PreS2 | 66,8 ± 3,5 | -17,8 ± 4,6 |
| PepFect14/SV40 | 60,8 ± 4,0 | -18,6 ± 3,0 |

| Peptide | ζ-Potential (mV) Before Coating | After Coating |
|---|---|---|
| PepFect14 | 31,5 ± 6,1 | 27,1 ± 1,0 |
| PreS2 | 42,0 ± 0,4 | 33,1 ± 1,9 |
| PepFect14/PreS2 | 53,6 ± 5,95 | 35,8 ± 3,5 |
| PepFect14/SV40 | 33,1 ± 10,1 | 31,7 ± 2,6 |

- Successful formulation of **monodispersed NPP** (PDI ≤ 0,2).
- **Oxidative dialysis** cause a **decrease** of NPP **concentration**.
- **Coating induce aggregation** (↗ Size, PDI and ↘ ζ-Potential) by **diminishing electrostatic repulsion** between NPP.
- Similar trend to perfluorohexane NPP relative to the concentration evolution.
- **No aggregation observed** (= Size, PDI), ζ-Potential still highly positive, thus **maintaining electrostatic repulsion** between NPP.

### Acoustic Response Study




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

- **Acoustic properties only driven** by the **perfluorocarbon liquid core**.
- **Mechanical Index** suitable **only** for **therapeutic applications**.

## CONCLUSIONS

- **pDNA coated nanopeptisomes** offer an **innovative nanometric support** to **deliver genetic material** through sonoporation combined with echographic visualization.
- **Peptidic composition** of the shell display **little influence** on the **particle properties** (size, PDI, concentration and ζ-Potential) offering a **vast choice** of peptide sequence **depending on the target**.
- This **study underlines** the **importance** to **control** both **dialysis** and **coating steps** to ensure the best NPP properties.

## REFERENCES

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