Application note Short summary

Formulation of Peptide-Based Nanoparticles Using TAMARA

Peptide-based nanoparticles (PBNs) are promising non-viral carriers for nucleic acid delivery, offering an alternative to conventional lipid- and polymer-based systems. Among these, **WRAP5**, a cationic amphipathic cell-penetrating peptide, spontaneously assembles with negatively charged nucleic acids through electrostatic interactions to form nanoparticles.

Controlled formulation is essential for achieving reproducible nanoparticle physicochemical properties and biological performance. **Microfluidic mixing using TAMARA** provides precise control over key process and formulation parameters while allowing WRAP5 PBNs to be produced through **fully aqueous-to-aqueous mixing**, without an organic phase or solvent-exchange step.

TAMARA microfluidic formulation system was evaluated for the formulation of **WRAP5 PBNs carrying either siRNA or pDNA**. The effects of **FRR, TFR, mixer geometry, and formulation volume** were investigated on nanoparticle size and polydispersity, alongside storage stability and *in vitro* biological activity.

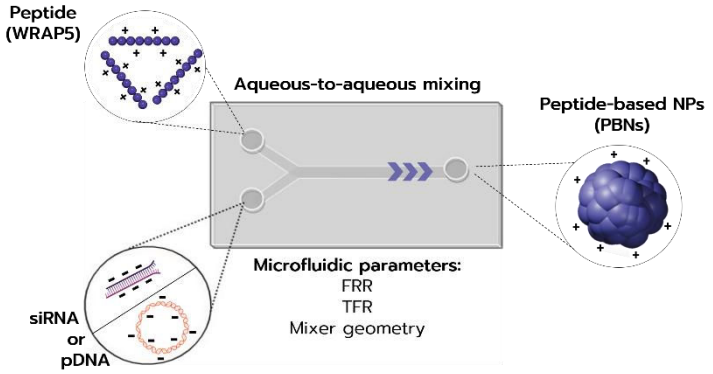


***Figure 1**. Microfluidic formulation of WRAP5 PBNs.*

Stable and biologically active WRAP5 PBNs carrying siRNA or pDNA were reproducibly produced using TAMARA across diverse microfluidic conditions.

This work was carried out by Thania Hammoum, Karidia Konate, Sébastien Deshayes, Eric Vivès, and Prisca Boisguérin (PhyMedExp, Inserm U1046, CNRS UMR 9214, University of Montpellier), with support from Yannick Mousli, Sezen Gul, and Audrey Nsamela (Inside Therapeutics). The full study was published in the Journal of Peptide Science by Hammoum et al., 2026 (doi: 10.1002/psc.70107).

Microfluidic production of WRAP5 PBNs using TAMARA

WRAP5 PBNs encapsulating siRNA or pDNA were formulated using TAMARA across different FRRs (1:1, 3:1, 5:1), TFRs (1.5, 5, 8 mL/min), mixer geometries (herringbone or baffle), and formulation volumes (0.5 or 1.5 mL), then characterized by DLS to assess particle size and PDI. Selected formulations were further evaluated for storage stability at 4°C.

Key findings

- Across 72 formulations, WRAP5:siRNA and WRAP5:pDNA PBNs showed **small particle sizes (~50–70 nm) and low PDI values (<0.22), independent of FRR, TFR, or mixer geometry**.
- Increasing the formulation volume from **0.5 to 1.5 mL maintained PBN physicochemical properties**, which were comparable to manually prepared formulations.
- During storage at 4°C, siRNA-loaded PBNs showed a moderate increase in particle size, whereas **pDNA-loaded PBNs maintained high colloidal stability for up to 70 days**.

TAMARA enabled robust WRAP5 PBN production across a broad microfluidic operating window, with sustained colloidal stability during storage.

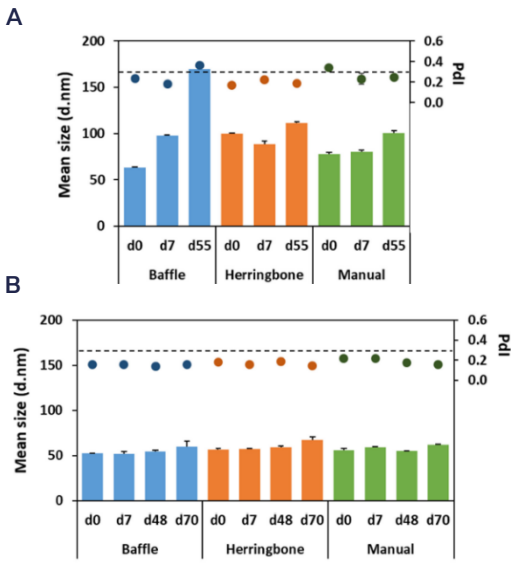
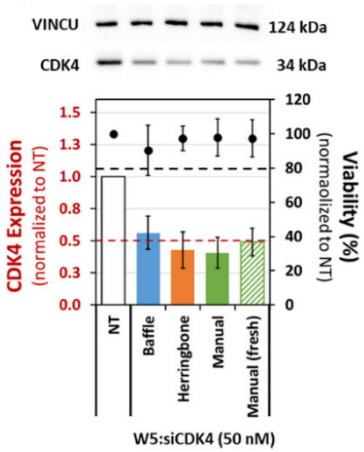


***Figure 2**. Storage stability of WRAP5 PBNs at 4°C. **(A)** WRAP5:siRNA and **(B)** WRAP5:pDNA PBNs produced using TAMARA with the baffle or herringbone mixer, or by manual formulation.*

Target protein knockdown by WRAP5:siRNA PBNs

The biological activity of WRAP5:siRNA PBNs was evaluated in GIST-T1 cells using an siRNA targeting CDK4. Target protein knockdown was assessed by Western blot after 24 h at 50 nM siRNA, alongside cell viability, across different FRRs, mixer geometries, and storage conditions.



Key findings

- → **CDK4 expression was reduced by approximately 50%** across all tested FRRs and both mixer geometries.
- → **Biological activity was retained after storage at 4°C for up to 55 days**, with comparable knockdown for TAMARA and manually prepared formulations.
- → **Cell viability remained comparable to non-treated cells** across the evaluated conditions.

***Figure 3**. CDK4 knockdown and cell viability following treatment with WRAP5:siRNA PBNs in GIST-T1 cells. PBNs produced using the baffle or herringbone mixer (FRR 1:1, TFR 5 mL/min) and stored for 55 days at 4°C were compared with stored and freshly prepared manual formulations.*

TAMARA-formulated WRAP5:siRNA PBNs maintained consistent target protein knockdown across diverse process conditions and following prolonged storage, without affecting cell viability.

Reporter gene expression by WRAP5:pDNA PBNs

The biological activity of WRAP5:pDNA PBNs was evaluated in HeLa cells using an mCHERRY-encoding plasmid with a nuclear localization sequence (NLS). PBNs produced using either mixer (FRR 1:1, TFR 1.5 mL/min), and stored at 4°C for 48 days, were compared with manually prepared PBNs. mCHERRY expression was assessed by confocal microscopy 24 h after transfection with 1 µg pDNA.

Key findings

- → **Nuclear mCHERRY expression was detected in >35% of HeLa cells across** both TAMARA mixer geometries and the manual formulation.
- → **Comparable expression levels** were observed across baffle, herringbone, and manual formulations.
- → mCHERRY fluorescence in the cytoplasm suggests that overall transfection efficiency has been underestimated.

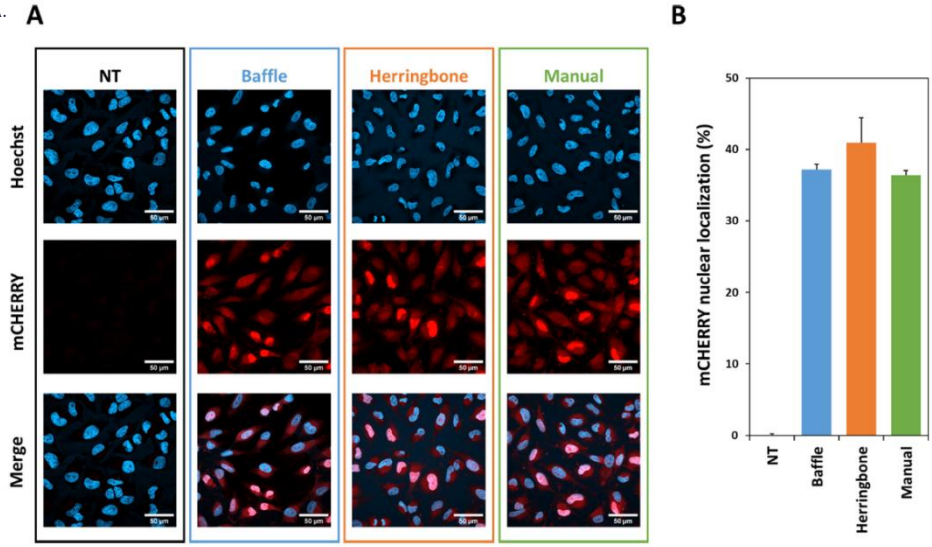


***Figure 4**. mCHERRY expression mediated by WRAP5:pDNA PBNs in HeLa cells. **(A)** Representative confocal images and **(B)** quantification of nuclear mCHERRY expression following treatment with TAMARA or manually formulated PBNs stored for 48 days at 4°C. Blue: Hoechst; red: mCHERRY.*

TAMARA-formulated WRAP5:pDNA PBNs retained reporter gene expression after 48 days of storage at 4°C, with activity comparable to manually prepared formulations.

Conclusion

This application note demonstrates how TAMARA enables **controlled microfluidic formulation of PBNs through fully aqueous-to-aqueous mixing**, providing:

- → **Consistent PBN physicochemical properties** across different FRRs, TFRs, and mixer geometries, supporting a broad microfluidic operating window.
- → **Reproducible PBN production** across formulation volumes.
- → **Sustained stability and biological activity** for siRNA- and pDNA-loaded PBNs.

TAMARA provides a robust and reproducible platform for aqueous microfluidic formulation of PBNs for both siRNA and pDNA delivery.

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