SELF-ASSEMBLED LACTOFERRIN NANOPARTICLES FOR GENE DELIVERY VIA SINGLE STEP FORMULATION

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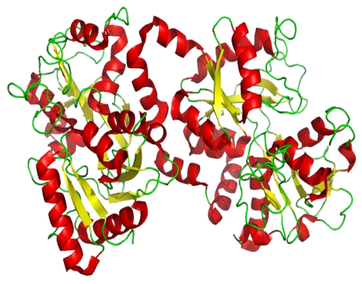
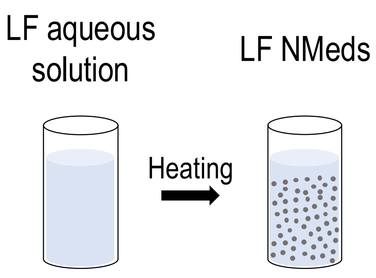
Lactoferrin Self-Assembling Nanomedicines (LF NMeds) for siRNA Complexation, Stabilization, and Delivery





Lactoferrin (LF)

- Biological Multifunctional Glycoprotein
- Cationic → siRNA complexation
- Heat-Induced Self-Assembly

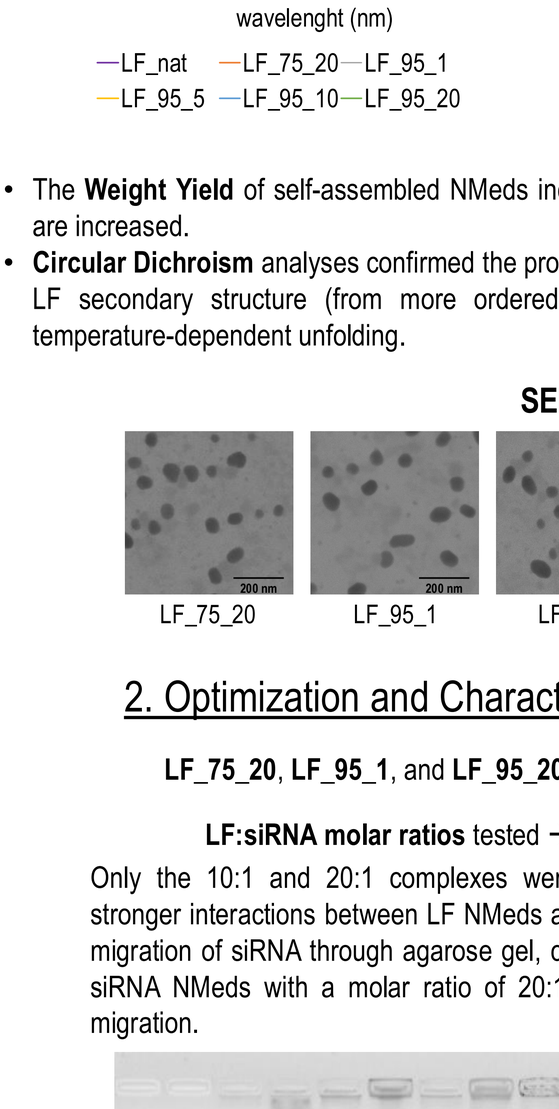
Heat-Induced Denaturation of LF

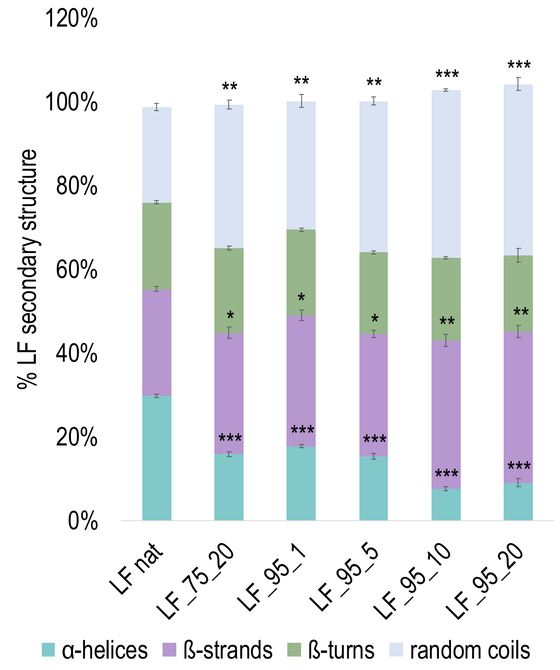
- Self-Assembling NMeds
- Control and Reproducibility
- Rapid and Sustainable Method

Optimization: Parameters Involved and Variables Tested

LF Concentration	10 mg/mL		
pH	5.5 (natural)	7	
Temperature	55°C	75°C	95°C
Time	30 s – 2 min	5 – 10 min	20 min
	Very Short	*Short*	*Medium*

Circular Dichroism





*p <0.05, **p <0.01, and ***p <0.001, Student's t-test, n=3.
All the statistics refer to LF_nat used as a control.

- The **Weight Yield** of self-assembled NMeds increases when the heating temperature and duration are increased.
- **Circular Dichroism** analyses confirmed the protein denaturation process by highlighting a change in LF secondary structure (from more ordered to more disordered) and revealed a time and temperature-dependent unfolding.

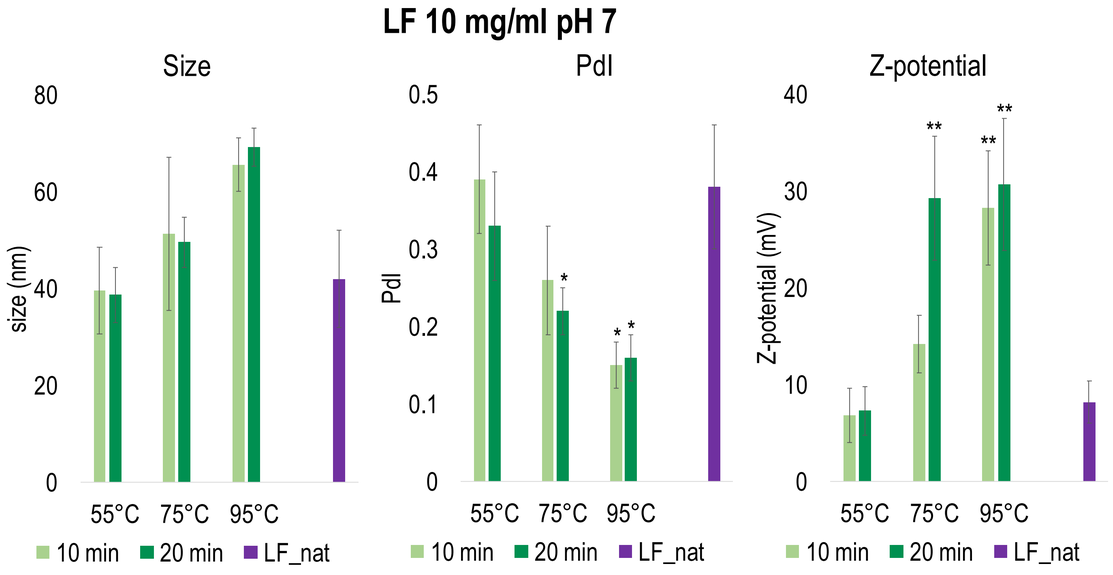
RESULTS

1. Optimization and Characterization of LF NMed Self-Assembly

LF 10 mg/ml pH 5.5

Investigations conducted on LF solution (10 mg/ml) at its natural **pH (~5.5)** revealed **a high polydispersity (PdI > 0.4)** and **low reproducibility** of the formulations.

LF 10 mg/ml pH 7



	95°C	
	1 min	**5 min**
Size (nm)	66.3 ± 6.7	64.6 ± 4.4
PdI	0.14 ± 0.02*	0.15 ± 0.04*
Z-pot. (mV)	28.7 ± 6.1**	29.1 ± 5.7**

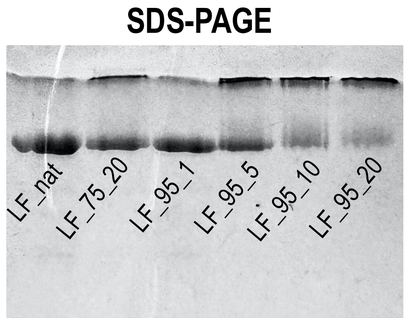
Investigations conducted on LF solution (10 mg/ml) at **pH 7** revealed that heating **at 75°C for at least 20 min and at 95°C regardless of the heating duration** leads to the assembly of homogeneous (**PdI <0.2**) and positively surface-charged NMeds (**Z-potential ~30 mV**).

*p <0.05, **p <0.01, and ***p <0.001, Student's t-test, n=3. All the statistics refer to LF_nat used as a control.

NMed formulations selected for further investigations:

- LF denatured at 75°C for 20 min (**LF_75_20**)
- LF denatured at 95°C for 1 min (**LF_95_1**)
- LF denatured at 95°C for 5 min (**LF_95_5**)
- LF denatured at 95°C for 10 min (**LF_95_10**)
- LF denatured at 95°C for 20 min (**LF_95_20**)

SDS-PAGE

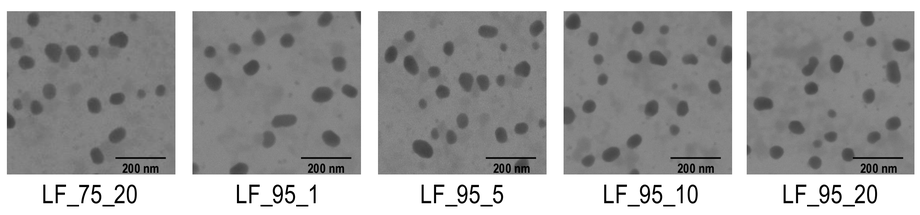


Weight Yields %

- LF_75_20 → 34 ± 8%
- LF_95_1 → 23 ± 6%
- LF_95_5 → 40 ± 7%
- LF_95_10 → 60 ± 8%
- LF_95_20 → 74 ± 8%

*Calculated after ImageJ quantifications of SDS-PAGE gels (expressed as mean ± sd, n=3)

SEMFEG

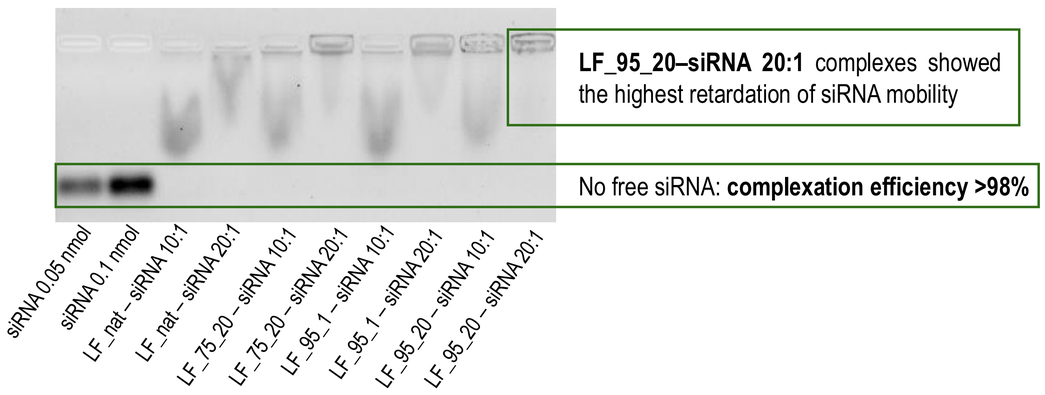


2. Optimization and Characterization of LF-siRNA NMeds

LF_75_20, **LF_95_1**, and **LF_95_20** were selected for siRNA complexation

LF:siRNA molar ratios tested → **2:1**, **5:1**, **10:1**, and **20:1** mol:mol

Only the 10:1 and 20:1 complexes were further characterized, as they exhibited stronger interactions between LF NMeds and siRNA, and a greater ability to retard the migration of siRNA through agarose gel, compared to the 2:1 and 5:1 complexes. LF-siRNA NMeds with a molar ratio of 20:1 showed the highest retardation of siRNA migration.



LF_95_20–siRNA 20:1 complexes showed the highest retardation of siRNA mobility

No free siRNA: **complexation efficiency >98%**

- The siRNA complexation did not impact on the physicochemical properties of LF NMeds, resulting in **LF-siRNA NMeds** showing a **particle size** ranging from **50 to 70 nm**, narrow size distribution (**PdI <0.2**), and **Z-potential** of approximately **+30 mV.**
- **Storage stability** studies showed that all LF-siRNA NMeds are highly stable under three different storage conditions (**liquid at 4°C**, **frozen at -20°C**, and **lyophilized at -20°C**) for **14 days**, as both the physicochemical properties and the complexation efficiency remained unaltered.

siRNA Protection Efficiency (%) from Ribonuclease (RNase) degrading enzymes

naked siRNA (negative ctrl)	*LF_75_20-siRNA (10:1)*	*LF_75_20-siRNA (20:1)*	*LF_95_1-siRNA (10:1)*	*LF_95_1-siRNA (20:1)*	*LF_95_20-siRNA (10:1)*	*LF_95_20-siRNA (20:1)*
0%	50 ± 6%	43 ± 3%	54 ± 2%	38 ± 7%	66 ± 2%	40 ± 3%

ONGOING EXPERIMENTS

***In vitro* biosafety and bioefficacy**, particularly focusing on the **anti-cancer effect** on three different cell lines: glioblastoma, pancreatic carcinoma, and metastatic melanoma.