Smart Amino Acid-based Nanovectors For RNA Anti-viral Therapeutics: Design, Synthesis And Physicochemical / Biological Characterization

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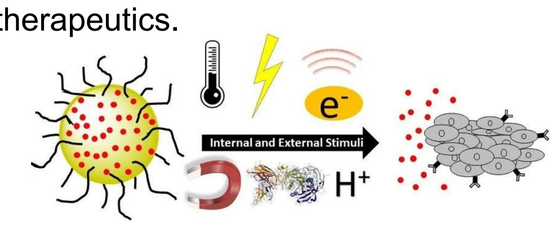
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1. Introduction

The development of effective therapeutics against viral diseases remains a major global health challenge. RNA-based strategies offer promising solutions by enabling the expression of therapeutic proteins or the silencing of pathological genes[1,2]. Their success, however, depends on nanocarriers capable of delivering RNA safely and efficiently. Gemini surfactants have emerged as versatile candidates for this purpose. When derived from natural building blocks, such as amino acids, peptides, or sugars, they also exhibit improved safety profiles, reinforcing their potential for biomedical applications[3].

2. Aim and Scope

Herein we present the work carried out at our laboratories, regarding the design and synthesis of versatile, stimuli-responsive, hence smart, amino acid and peptide-based gemini surfactants for RNA anti-viral therapeutics.

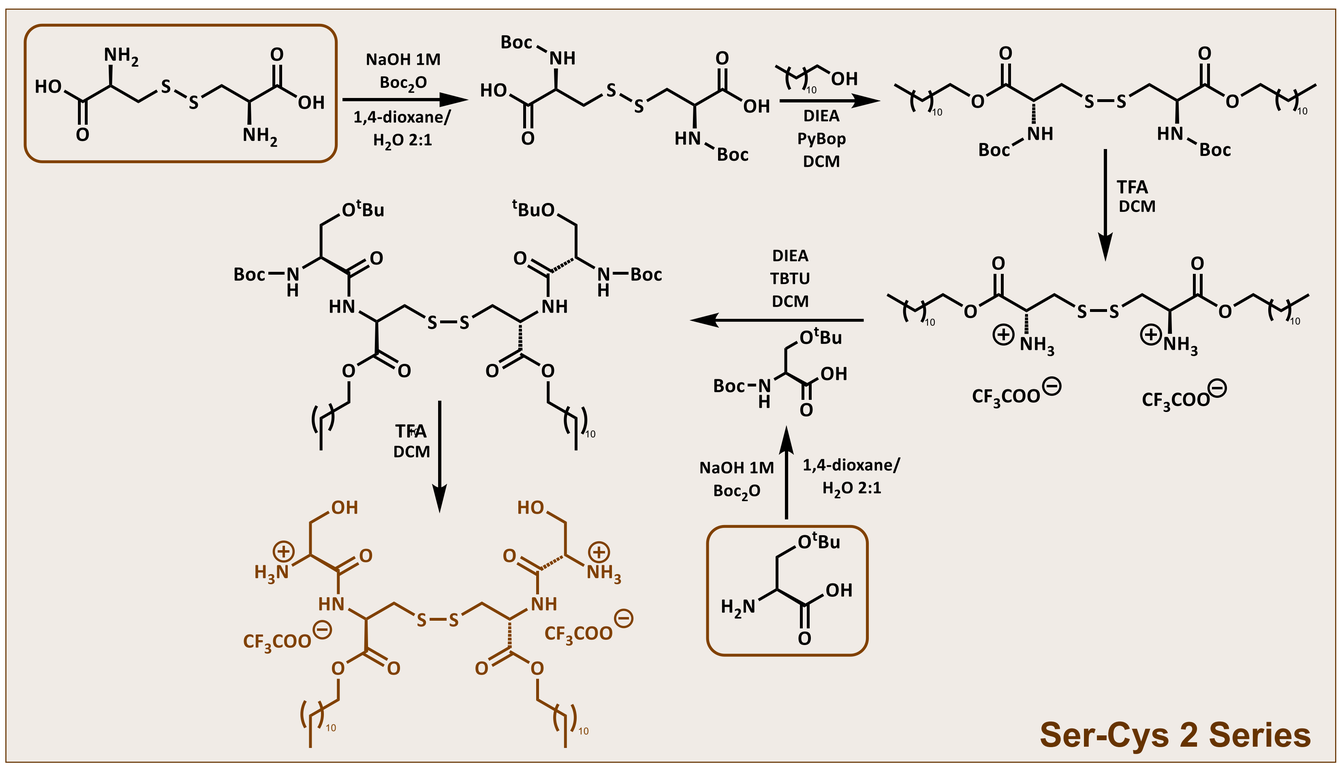


3. Results

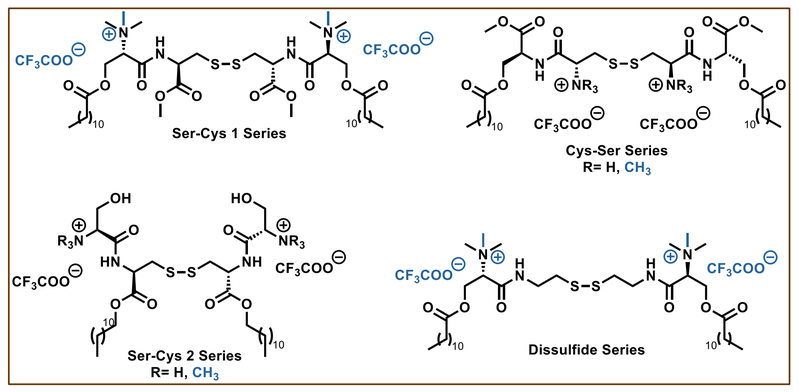
Design of amino acid-based gemini surfactants, **responsive to local pH and redox environment**, as nanovectors for viral infections – **Project SMART4VIR**.

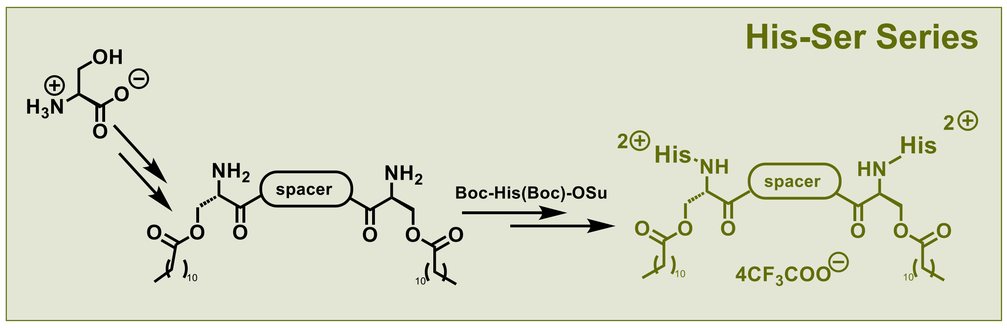
Surfactant-based RNA nanocarriers will change their properties along the delivery path to be effective in the different critical steps.

Design and Synthesis



Surfactants based on **serine** and **cystine** synthesized





Physical Chemistry

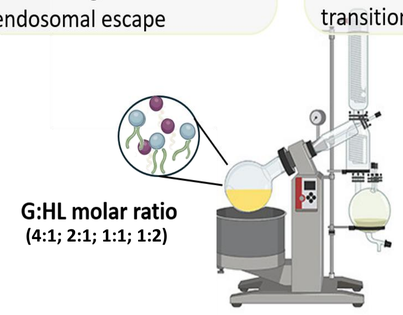
Development of surfactant / lipid vesicles

Novel Serine-derived gemini surfactants (G)

Positive charge
Ester linkages to facilitate endosomal escape

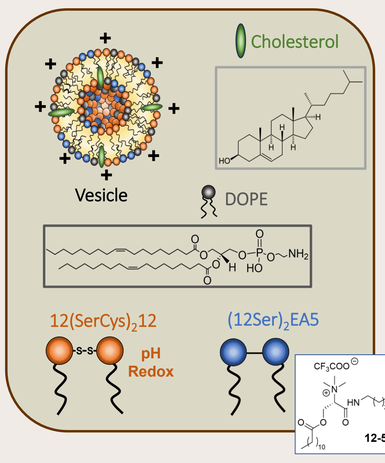
Helper lipid (HL)

Enhances bilayer fluidity and Promotes inverted phase transitions

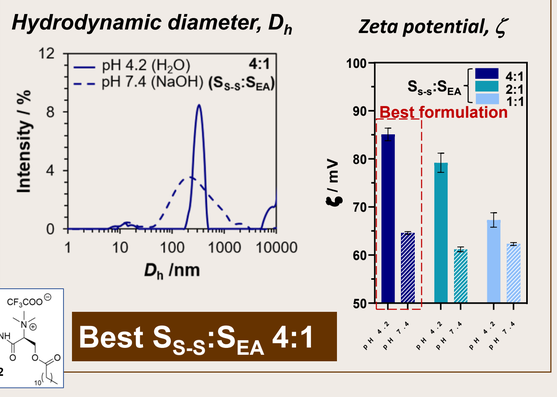


Developed systems show potential for stimuli-responsive intracellular gene delivery.

Ser-Cys 2 Series

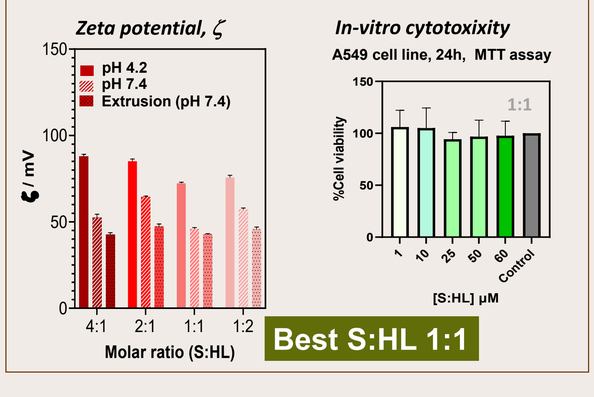


Optimization of the $S_{S\text{-}S}$:S_{EA} ratio



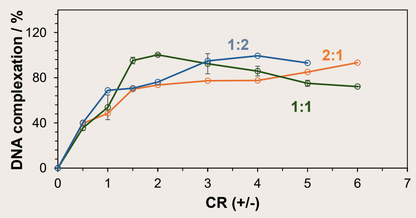
Best $S_{S\text{-}S}$:S_{EA} 4:1

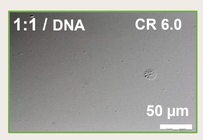
Optimization of the S:HL ratio, $S_{S\text{-}S}$:S_{EA} fixed (4:1)



Best S:HL 1:1

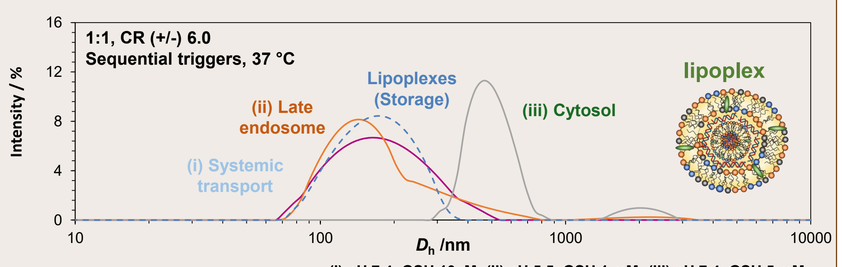
DNA complexation → lipoplexes





Light micrography of vesicles / DNA interactions

DNA release under cytosol mimicking conditions



(i) pH 7.4; GSH 10µM; (ii) pH 5.5; GSH 1 mM; (iii) pH 7.4; GSH 5 mM.

From D_h determinations

Mix 1:1(S:HL) successfully generates small, monodisperse lipoplexes

4. Conclusions

Amongst the surfactants based on serine and cystine/disulfide, the **Ser-Cys 2** derivative presented the best profile:

- The 1:1 (S:HL) formulation was identified as the optimal composition, combining efficient DNA complexation, high surface charge, and favourable size distribution.
- Lipoplexes exhibit high sequential stimuli-triggered destabilization, releasing the DNA under cytosol-mimicking conditions.

Preliminary studies highlight the promising profile of the **His-Ser** derivative. This surfactant exhibits pH- and temperature-dependent self-assembly, spontaneously forming vesicles at near-natural pH. Upon acidification, a transition to tubular aggregates occurs, leading to solution gelation. Moreover, it is non-cytotoxic up to 100 µM and promotes cell proliferation.

References

1. R.M. Meganck, R.S. Baric, *Nat. Med.,* **2021**, *27*, 401-410.
2. C. Wang, Y. Zhang, Y. Dong, *Acc. Chem. Res.*, **2021**, *54*, 4283-4293.
3. C. Costa, I.S. Oliveira, J.P.N. Silva, S.G. Silva, C. Botelho, M.L.C. do Vale, M. Oliveira, A.C. Gomes, E.F. Marques, *J. Colloid Interface Sci.*, **2021**, *584*, 34-44.

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