Complete Real-Time Label-free in vitro assessment of DNA-based spherical nucleic acids for delivery of antisense TAU oligonucleotides in Tauopathy

Elena Scurti[1], Toni Laine[2], Jaakko Teppo[1], Pasi Virta[2], Tapani Viitala[1,3]

[1] Division of pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, Finland; [2] Department of Chemistry, University of Turku, Finland; [3] Faculty of Science and Engineering, Åbo Akademi, Turku, Finland

UNIVERSITY OF HELSINKI
FACULTY OF PHARMACY

elena.scurti@helsinki.fi
Pharmaceutical Biophysiscs group

Introduction

Spherical nucleic acids (SNAs) are emerging as promising nanocarriers for gene therapy due to their unique properties. They consist of three-dimensional structures with an inorganic core, such as Buckminsterfullerene, and oligonucleotides (ONs) dangling from the surface[1]. In this project, SNAs are multifunctionalized with 2'-deoxy oligoribonucleotides and hybridized with an antisense oligonucleotide sequence (AONs), which suppresses TAU protein, and tested for therapeutic purposes **(Fig 1)**.[2]

To assess a comprehensive analysis, from physicochemical characterization to protein corona formation and cell interactions[3], in a complete label-free and real-time approach, Surface Plasmon Resonance is employed as main platform to favor advancing research in nanotechnology-based gene therapy.

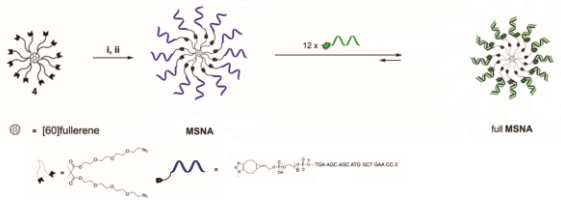
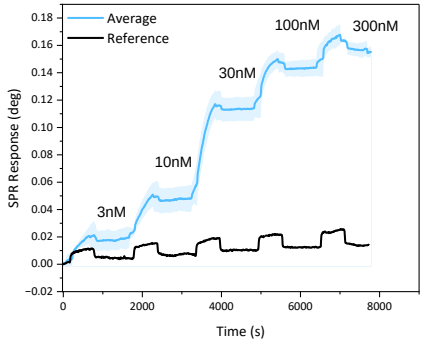


Figure 1. Synthesis and hybridization-mediated assembly of MSNAs. Conditions: i) ON 0.3eq/4,DMSO:H2O (9:1 v/v), overnight at room temperature; ii) ON 1.2 or 1.5 eq/azide arm of 4, 1.5 M or 1.75M NaCl (aq),3 days at room temperature.

Physicochemical characterization

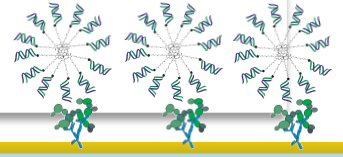
Biotinylated MSNAs (1:12 ONs branches), non-hybridized, are injected following a concentration series 3-300 nM **(Fig.2)**, then characterized in terms of size, refractive index (at λ 670 nm and 785 nm) and interaction kinetics (*ka*, *kd*, and KD) **(Table 1)**, with the avidin layer on the sensor. Size was further compared with previously calculated hydrodynamic sizes through Dynamic Light Scattering[1].

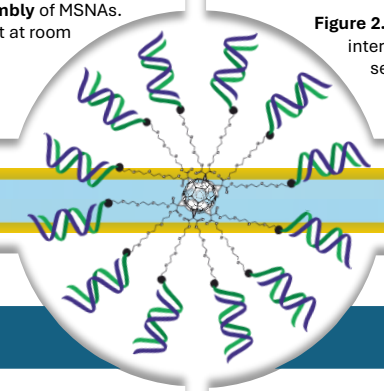
Figure 2. SPR sensorgrams of MSNAs interacting with avidin layer on the sensor (light blue), and with the biotinylated layer to check for aspecific interaction (black).



Parameter	Average (N=3 ± SD)
Size (nm)	12.25 nm ± 2.54
RI 670nm	1.345333 ± 0.0023
RI 785nm	1.338177 ± 0.00197
Association rate (1/M*s)	$1.70 \times 10^{5} \pm 2.31 \times 10^{4}$
Dissociation rate (1/s)	$9.81 \times 10^{-5} \pm 8.60 \times 10^{-6}$
Affinity (M)	$5.72 \times 10^{-10} \pm 9.19 \times 10^{-11}$

Table 1. Physicochemical parameters calculated from an SPR analysis.





Complexation-Decomplexation

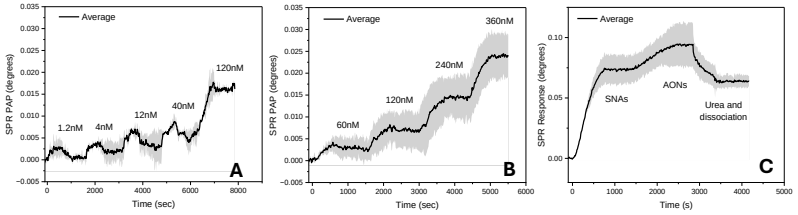


Figure 3. SPR sensorgrams of AONs at different concentrations interacting on a layer of MSNAs (30nM) below and above the stoichiometric concentration (120nM). **(A,B)** SPR sensorgram showing dissociation of AONs from SNAs after 10 mins 8M Urea injection **(C)**.

Parameter	Lower AONs conc	Higher AONs conc	Dissociation
Size increase (nm)	1.23 ± 0.16	2.97 ± 0.5	/
Association rate (1/M*s)	$4.39 \times 10^{3} \pm 9.0 \times 10^{1}$	$5.92 \times 10^{2} \pm 4.70 \times 10^{2}$	/
Dissociation rate (1/s)	$1.83 \times 10^{-4} \pm 1.65 \times 10^{-4}$	$2.34 \times 10^{-5} \pm 1.52 \times 10^{-5}$	$3.10 \times 10^{-3} \pm 2.25 \times 10^{-4}$
Affinity (M)	$3.89 \times 10^{-6} \pm 3.89 \times 10^{-6}$	$1.22 \times 10^{-7} \pm 1.06 \times 10^{-7}$	/

Table 2. Kinetic parameters calculated from the complexation and decomplexation steps.

Protein corona formation

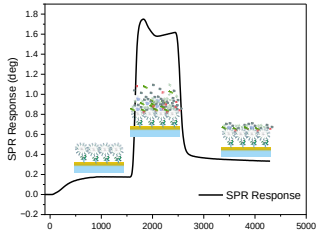


Fig 4. SPR sensorgrams of MSNAs interacting with human plasma

To allow the protein corona formation on top of the nanocarries, full biotinylated MSNAs were captured on the sensor and human plasma was injected for at least 15 minutes before flushing all the proteins that were not tightly bound on the MSNAs layer with runnig buffer. **(Fig 4)**. The eluate from SPR was processed and the proteins were labelled with TMT labels for proteomics analysis **(Fig 5)**.

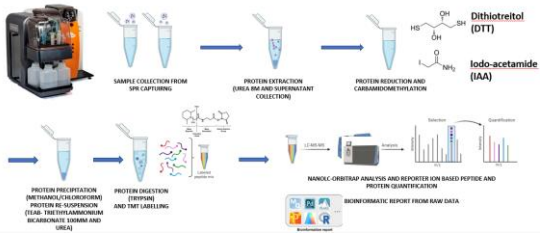


Figure 5. Schematic representation of an off-line SPR-MS sample processing for protein corona analysis.

References

1. Mirkin, C. A., Letsinger, R. L., Mucic, R. C., & Storhoff, J. J. (2020). A DNA-Based Method for Rationally Assembling Nanoparticles into Macroscopic Materials*. *Spherical Nucleic Acids*, *382*(August), 3–11.
2. Laine, T., Deshpande, P., Tähtinen, V., Coffey, E. T., & Virta, P. (2024). Chondroitin Sulfate-Coated Heteroduplex-Molecular Spherical Nucleic Acids. *ChemBioChem*, *202400908*, 1–9.
3. Kari, O. K., Rojalin, T., Salmaso, S., Barattin, M., Jarva, H., Meri, S., Yliperttula, M., Viitala, T., & Urtti, A. (2017). Multi-parametric surface plasmon resonance platform for studying liposome-serum interactions and protein corona formation. *Drug Delivery and Translational Research*, *7*(2), 228–240.

Conclusions

Several real-time, label-free SPR-based analysis platforms have been successfully used to characterize MSNAs. These methods provided precise physicochemical characteristics, detailed information on MSNAs interactions with AONs with plasma proteins, and more accurate dimensions compared to conventional methods. They also offered insights into complexation kinetics, ON binding strength, protein corona formation, allowing future analysis focusing on SNA-cell interaction kinetics and uptake.

Acknowledgements

MATRENA Doctoral Programme in Materials Research and Nanosciences
BUSINESS FINLAND
UNIVERSITY OF TURKU GRADUATE SCHOOL (UTUGS)
SUOMEN KULTTUURIRAHASTO
VAIKUTTAVUUS SÄÄTIÖ