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REPORT

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Quantitative analysis of oligonucleotide delivery into isolated mitochondria: a proof-of-concept method

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ABSTRACT

Mitochondria, with their own DNA, Represent a potential target for nucleic acid-based precision therapies. However, effective delivery of therapeutic oligonucleotides remains challenging due to the dual mitochondrial membranes and the localization of mitochondrial DNA within nucleoid complexes in the matrix. To understand the delivery process and assess the delivery efficiency of potential vectors, such as dendrimers, it is essential to effectively quantify the oligonucleotides that are successfully delivered to and remain within mitochondria. Currently, there are only limited yet inconvenient methods available for this purpose. Here, we describe a method for quantifying the delivery of fluorescent oligonucleotide cargos in isolated mitochondria using a microfiltration apparatus for reliable fluorescent analysis. By working within a range of dilutions, we are able to safeguard the concentration limits. The quantification protocol also enables the visualization of specific localization within mitochondria, allowing for the determination of whether delivery can occur across both membranes. This is particularly useful, as it offers a key insight into improving vectors as they must deliver the cargoes within the mitochondrial matrix. We validate this method in this proof-of-concept study, providing biological data to assess the difference between two amphiphilic dendrimer vectors for oligonucleotide delivery in mitochondria.

MULTIDISCIPLINARY ABSTRACT

Targeted delivery and localization of therapeutics represent key challenges within drug discovery and development processes, including the growing field of nucleic acid therapeutics such as oligonucleotides. Mitochondria harbor their own DNA, making them a potential, yet still challenging, therapeutic target. Concurrently, designing reliable assays to evaluate delivery efficiency remains challenging. Here, we utilized a slotted microfiltration apparatus to establish a protocol that enables reproducible relative quantification of mitochondrial delivery of oligonucleotides by dendrimers.

METHOD SUMMARY

Using a slotted microfiltration assay, we can quantify the efficiency of potential vectors in delivering small oligonucleotide cargos. In particular, the method specifies the delivery into isolated mitochondria and even sub-organelle localization.

ARTICLE HIGHLIGHTS

- In this paper, we introduce a quantitative microfiltration assay to measure the delivery of oligonucleotides into isolated mitochondria.
- This method allows sub-mitochondrial resolution, distinguishing intact mitochondria from mitoplasts and enabling analysis of matrix-specific localization.
- The protocol is versatile and adaptable, suitable for screening multiple vectors, mechanistic studies, and quantitative benchmarking, and can be modified for other organelles or whole-cell systems.
- Overall, this approach provides a practical platform to optimize nucleic acid delivery, facilitating the development of targeted mitochondrial therapies

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1. Introduction

Oligonucleotide (ON) therapeutics represent an ever-expanding field in the treatment of various diseases thanks to their ability to modulate gene expression by interacting with RNA via Watson-Crick base pairing [1]. Nevertheless, much work remains to be completed to overcome some of their challenges. One of the major difficulties yet to be overcome is the ability to produce consistent efficient delivery [2]. As of March 2024, only 20 ON therapies have been approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA), predominantly small-interfering RNAs (siRNAs) and antisense oligonucleotides (ASOs) [3]. Although the most commonly used strategies for oligonucleotide delivery include chemical modifications to improve 'drug-likeness' or covalent conjugation with cell-targeting or cell-penetrating moieties, it is expected that vector-based delivery will significantly enhance the delivery specificity and efficiency [2,4,5]. Several vectors have been proposed to enhance ONs delivery, including the use of polymeric vectors or nanoparticles [6–9], with only the lipid nanoparticle being approved for clinical use.

While much focus is given to deliver nucleic acid cargo to the cell in general, subcellular localization is a crucial consideration for understanding the effectiveness of delivery. A siRNA undertakes its role when delivered to the cytoplasm, while a DNA requires successful delivery into the nucleus to exert its function. Mitochondria, an organelle involved in a plethora of processes and essential to cells, are crucial within both health and disease, with key functions in cellular bioenergetics [10]. Mitochondria contain their own DNA (mtDNA) and RNA (mtRNA), whose damage has been implicated in a number of cancers [11] and diseases as well as in broad physiological changes such as aging [12–14], suggesting that they could be therapeutic targets to be exploited. Mitochondrial delivery is a less well-understood process than nuclear delivery, although several attempts have been made to select a vector candidate that can reach the mitochondria. Recently, nanoparticles, as polymeric vectors and lipid nanoparticles, have become an increasingly attractive approach to overcome these issues, thanks to their size and their ability to carry large amounts of suitable cargo, as well as their reduced cytotoxicity in cells [15]. Dendrimers, as a special family of polymers, can offer more advantages, thanks to their precise dendritic structure, cooperative multivalency confined within a nanoscale volume [16]. Alongside the classical polymer characteristics, a lipid-like system can further protect encapsulated cargo and prevent loss prior to mitochondrial delivery [17–22].

Confirmation of cargo delivery *ex vivo* is generally demonstrated through confocal microscopy co-localization with mitochondrial markers [17,23,24] or through PCR analysis of isolated mitochondria [25]. A limitation of both assays is related to the inability to determine whether a lack of co-localization is due to poor mitochondrial translocation or to the candidate vector's failure to cross the double mitochondrial membrane. Furthermore, mitochondrial localization itself doesn't prove specific mitochondrial matrix delivery either, meaning any cargo may remain 'stuck' within the Inter Membrane Space (IMS). Another restraint is related to the black-or-white nature of many assays: they either confirm or deny the presence of the cargo in a compartment, excluding the possibility of comparing and fine-tuning different delivery mechanisms.

We describe a protocol that enables reliable quantification of delivered nucleic acid cargos and allows accurate comparison across different experimental conditions. Isolated mitochondria are incubated either with naked oligonucleotides (ONs) as a control (CTRL) or with ONs complexed to candidate delivery vectors with the ONs carrying fluorescent tags. Following incubation, a portion of the mitochondria is further fractionated to strip off the outer mitochondrial membrane (OMM) and IMS, leaving only the inner mitochondrial membrane (IMM) and the mitochondrial matrix. The obtained intact mitochondria and matrices are then diluted and transferred onto a nitrocellulose membrane via microfiltration. The fluorescent signal is quantified to assess overall uptake and sub-mitochondrial localization. The final analysis provides a higher-throughput comparison for screening delivery candidates and can be adapted to meet the user's requirements (Figure 1).

As a proof-of-concept, we tested mitochondrial delivery capabilities of two amphiphilic dendrimer vectors [16,26]: one with ionizable amine terminals (AD) [27,28] and the other with arginine terminals (AD-Arg) [29]. AD-Arg is expected to cross the mitochondrial membranes more efficiently as it bears multiple arginine terminals, in view to mimicking the cell penetration peptides poly(arginine) [19,20,30]. As a cargo, we used fluorescently tagged single- or double-stranded RNA sequences.

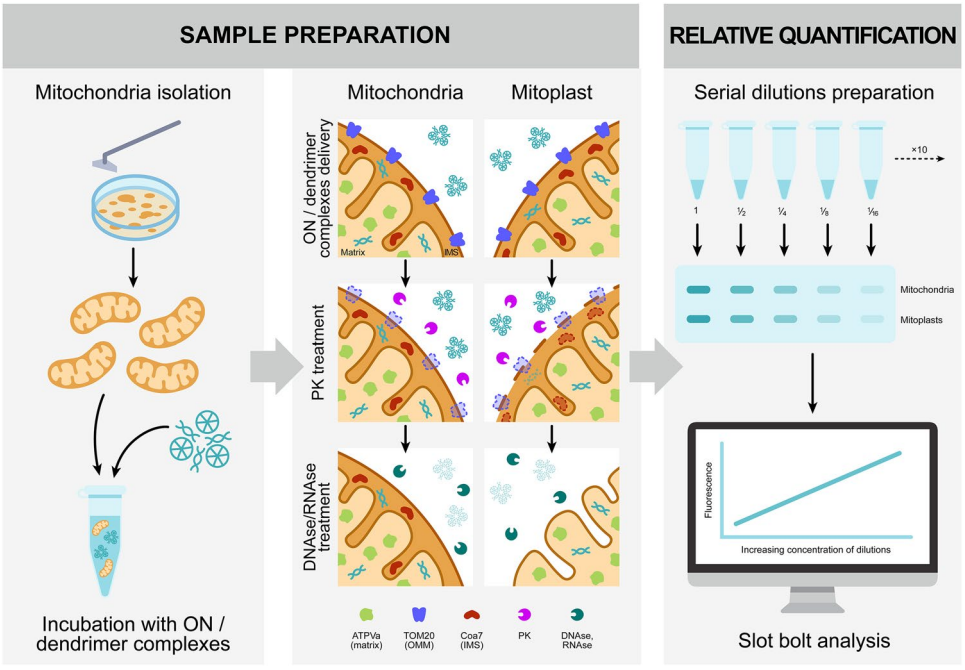

Figure 1. Schematic of the protocol. The graphical representation highlights the key steps of the procedure.

In summary, here we present a protocol that enables quantitative assessment of nucleic acid delivery and comparison of different vectors under defined experimental conditions. Using this approach, we demonstrate the proof-of-concept application for mitochondrial delivery and sub-mitochondrial localization of two amphiphilic dendrimer vectors, highlighting the potential of such methodologies to refine and optimize intracellular and organelle-targeted oligonucleotide therapies.

2. Methodology

2.1. Cell culture

HEK293 cells were grown at 37 °C with 5% CO2 in DMEM (Dulbecco's modified Eagle's medium), supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 10 µg/mL streptomycin.

2.2. Mitochondrial Isolation

Mitochondria were isolated as previously reported [11]. In brief, cells were mechanically homogenized in Mitochondrial Isolation Buffer (MIB) [20 mM Hepes pH7.6, 1 mM EDTA pH 7.4, 220 mM Mannitol, 70 mM Sucrose] supplemented with 2 mg/mL BSA and 2 mM PMSF, and then subjected to centrifugation at 650× g to remove intact whole cells and nuclei. The supernatant was pelleted at 14'000× g and the isolated mitochondria were washed, firstly with MIB supplemented with 1 M KCl, 2 mg/mL BSA, and 2 mM PMSF, and again in MIB alone. The final concentration of mitochondria was quantified by the Bradford Assay, with appropriate amounts set out for incubation with the complexes (see "*ON Delivery*").

For each control and experimental condition, the mitochondria were divided into two groups: intact mitochondria and 'mitoplasts', mitochondria processed to remove the outer mitochondrial membrane and the intermembrane space, as previously described [11]. Briefly, intact mitochondrial samples were resuspended in maintenance buffer [20 mM Hepes pH 7.4, 250 mM Sucrose] while mitoplast samples were resuspended in swelling buffer [20 mM Hepes pH 7.4, 5 mM Sucrose] and maintained on ice for 30 minutes. Mitoplast samples were then degraded with 10 µg/mL proteinase K (PK) (P6556, Sigma-Aldrich) for 10 minutes on ice, which was then inhibited with 2 mM PMSF. A final wash was completed with swelling buffer supplemented with 300 mM KCl and samples were centrifuged at 14'000× g for 10 min at 4 °C and resuspended in the desired volume.

2.3. Mitochondrial Quality control

Indicated amounts of the validation control samples suspended in 1X Laemmli were separated in a 12% SDS-PAGE gel for electrophoresis before transferring to a nitrocellulose membrane. Membranes were then blocked for 1 hour at RT in 5% milk in TBS buffer with 0.1% Tween-20 (TBS-T) and incubated separately overnight at 4 °C with the following primary antibodies: anti-ATPVa monoclonal 1:250 (ab14748; Abcam, Cambridge, UK; RRID:AB_301447), anti-TOMM20 monoclonal 1:250 (ab186734; Abcam; RRID:AB_2716623), or anti-COA7 polyclonal 1:500 (HPA029926; Atlas Antibodies, Stockholm, Sweden; RRID:AB_10602173). Membranes were washed three times with TBS-T and then incubated with the secondary antibodies anti-Mouse IR800 (#926-32210; Li-Cor Biosciences, Lincoln USA; RRID:AB_621842) and anti-Rabbit IR800 (#926-32211; Li-Cor Biosciences; RRID:AB_621843) at RT for 2 hours, before imaging with the Odyssey DLx system and analyzing with Image Studio™ Lite (Li-Cor Biosciences).

2.4. Oligonucleotide sequences

Oligonucleotide sequences were purchased from Integrated DNA Technologies (Coralville, Iowa, USA). The respective sequences are reported: Double-stranded RNA ON: sense 5′-CCAUGAGGUCAGCAUGGUCUGdT-3′, antisense 5′-/5IRD800CWN/CAGACCAUGCUGACCUCAUGGdT-3′; single-stranded RNA ON: 5′-/5IRD800CWN/CAGACCAUGCUGACCUCAUGGdT-3′.

2.5. Amphiphilic dendrimer nanoparticles

Amphiphilic dendrimers AD and AD-Arg were synthesized according to the reported protocols [27,29], lyophilized, and resuspended in MilliQ H_2O to a concentration of 2 mM for long-term storage at −80 °C. Further dilutions were made as required and stored at −20 °C. Structures of the amphiphilic dendrimers AD and AD-Arg are reported in Figure 3A. Both dendrimers are generation 3 dendrimers with 8 terminal ends.

2.6. ON/dendrimer preparation

The ON/dendrimer complexes were prepared, based on the number of terminal positive charges within the amphiphilic dendrimer and the phosphate groups in the nucleic acid cargo, as described in reference [27]. Using the equation below, the final volume of the total incubation, and the desired concentration of the ON cargo to be delivered, it is possible to calculate the required dendrimer concentration. The N/P ratio is defined as the total positive charges of dendrimer terminals versus the total number of phosphate groups in ON. An N/P ratio of 10 was maintained for short ON cargos.

$$N/P\,ratio = \frac{mol\,of\,dendrimer \times number\,of\,terminal\,positive\,charges}{mol\,of\,nucleic\,acid\,cargo \times number\,of\,phosphate\,groups}$$

2.7. ON delivery

Mitochondria were centrifuged and resuspended in a 10 µg/µL concentration in the required condition: experimental samples in complexed ON/dendrimer solution, control samples in uncomplexed ON solution, and samples for Quality Control in MIB solution. Samples were then incubated under the delivery conditions as previously optimized [28]. For short single- and double-stranded ONs complexed with dendrimer vectors, we incubated the samples for 5 hours at 37 °C with gentle agitation to prevent sedimentation. After incubations, all samples were washed and resuspended with RNase A (R5125, Sigma-Aldrich, Burlington, USA) as per the manufacturer's instructions. All samples were washed again before being divided into two equal samples per condition.

One half, with intact mitochondria, was retained, while the other was processed to remove the outer mitochondrial membrane (OMM) and the intermembrane space (IMS) (see "*Mitochondrial Isolation*"). Once

again, all samples were subject to RNase A degradation to ensure the final removal of unspecific ON delivery before final washes.

Final experimental and control samples were used to prepare dilutions. Intact mitochondrial or mitoplast pellets were resuspended in 200 µL of MIB, which was then subject to serial dilution by half. Validation control samples were resuspended in 20 µL of Laemmli 1X sample buffer (for more details see "*Mitochondrial Quality Control*").

2.8. Transfer and visualization

For the delivery of a 50 nM concentration of ON cargo, dilutions ranging from 1/16 to 1/512 were used. The microfiltration apparatus Bio-Dot® *SF* (Bio-Rad, Hercules, USA) was assembled with Whatmann papers soaked in Tris Buffered Saline (TBS) [20 mM Tris, 140 mM NaCl pH 7.6] above the bottom cassette, and a TBS-soaked 0.45 µM nitrocellulose membrane on top. Each layer was carefully rolled to remove potential bubbles. The top cassette was then added, and a vacuum was applied at – 40 kPa, followed by further tightening. Firstly, each well was flushed with TBS and drained by applying a vacuum. Then, the prepared dilutions were loaded into the apparatus, with each sample's dilution loaded horizontally. Afterward, the membranes were placed on Whatmann paper and dried at 37 °C for 3 hours, protected from light. The membranes were imaged with the Odyssey DLx system (Li-Cor Biosciences) at the 800 nm wavelength. The resulting images were quantified using the Image Studio™ Lite (Li-Cor Biosciences) software, and the data were exported for analysis in Microsoft Excel.

2.9. Statistical analysis

Statistical Analysis was conducted with Microsoft Excel. To compare vectors normalized to the control, a one-tailed one-sample *t*-test was used, while to compare normalized vectors to one another, a one-tailed paired t-test was used. Student's *t*-test for two-groups comparisons. *p*-values of less than 0.05 were considered significant (*), while *p*-values of less than 0.01 were considered very significant (**).

3. Results and discussion

The evaluation of the cellular, subcellular, or suborganelle localization of any delivered cargo by a potential vector remains challenging in many aspects. In particular, few assays are available to quantify the amount of cargo delivered to the desired compartments. Most widely used protocols are qualitative, which limits the possibility of objectively comparing different delivery systems. In this work, we focused on developing a protocol for the relative quantification of the amount of cargo delivered to the different subcompartments of mitochondria. As a cargo model, we used RNA oligonucleotides labeled with a fluorophore emitting at 800 nm. These RNA ONs were then complexed with the amphiphilic dendrimer vector (Figure 1). As a proof of concept, we evaluated the ability of two different dendrimers, AD and AD-Arg (Figure 3A), to deliver RNA into isolated mitochondria. We anticipated that the modified amphiphilic dendrimer AD-Arg, would be a more specific and reliable carrier thanks to inclusion of Arginine moieties at the terminals of the dendrimer. In *de novo* mitochondrial translocation, mitochondria-destined proteins harbor so-called 'Mitochondrial Targeting Sequences', which contain repeating positively-charged and often Arginine residues [30], which we envision this dendrimer mimicking. Previous work with Arginine-containing amphiphilic dendrimers has also shown improved siRNA delivery to cells, hypothesized to relate to their mimicry of Arginine-rich cell penetrating peptides positive charge and hence an improved membrane penetration [19].

To compare the capacity of these two dendrimers to cross both the OMM and IMM, we isolated mitochondria before incubation with the ON/dendrimer complexes (Figure 2A). Following the incubation of the isolated mitochondria with the ON/dendrimer complex, a step of digestion with the appropriate enzyme (RNase) ensured the complete degradation of the cargo that was not delivered inside the mitochondria, with the imported cargo being protected by the mitochondrial membranes. The obtained mitochondria were then divided into two portions, one portion contained intact mitochondria while the other underwent sub-fractionation to collect the mitochondrial matrices (mitoplasts). The RNase

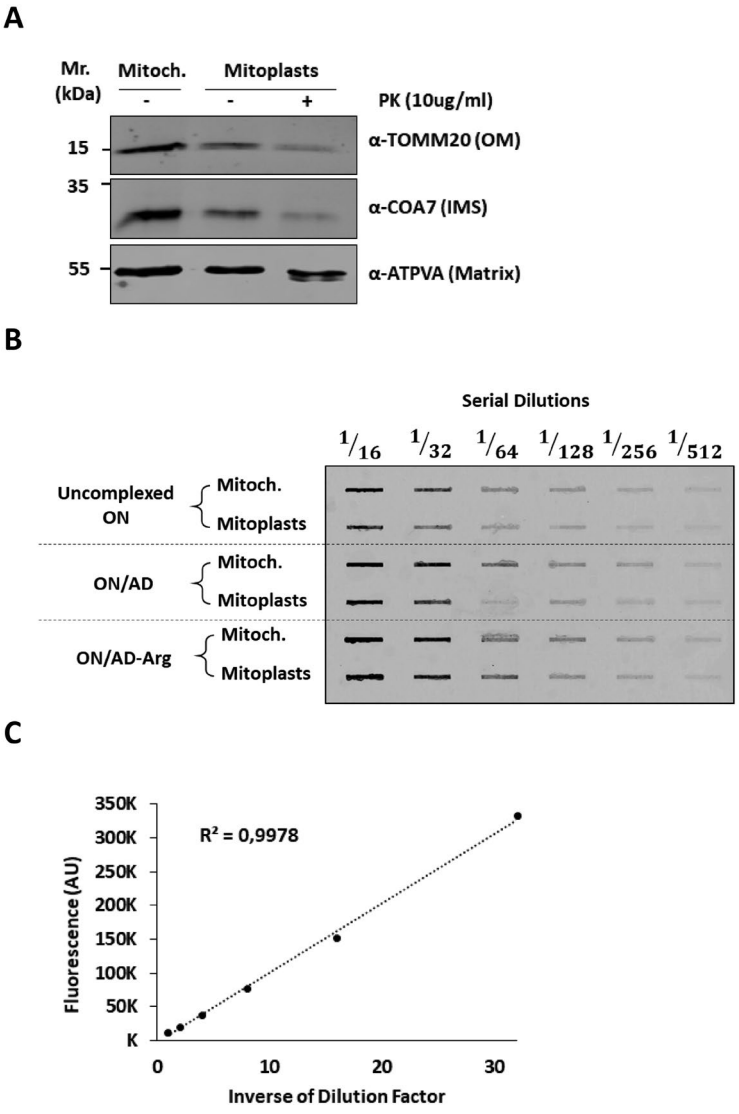

Figure 2. Protocol Validation and Assessment. **A.** Western blot analysis of intact mitochondria and mitoplasts treated with or without proteinase K (PK). Antibodies against markers for the outer mitochondrial membrane (TOMM20), intermembrane space (COA7), and the matrix (ATPVa) were used to confirm the integrity of mitochondria and mitoplasts. **B.** Representative loading pattern of the serial dilutions of the analyzed samples. Dilution values shown are indicative for 50 nM delivered cargo. **C.** Representative graph of mitochondrial fluorescence values derived from the quantification of the membrane in B *vs* inverse dilutions. IMS = Intermembrane Space; OMM = Outer Mitochondrial Membrane; AU = Arbitrary Units.

incubation step was repeated in both intact mitochondria and mitoplast samples to ensure once more the reliability of the signal detection only in the desired compartment.

The samples were finally collected, and a range of serial dilutions was generated to ensure the linearity of the fluorescent signal (Figure S1 and S2). The appropriate dilutions were loaded into the assembled microfiltration apparatus (*Bio-Dot*® SF), first with the uncomplexed ON and then with the ON/dendrimer complexes. The apparatus vacuum was then applied in order to transfer the samples onto the nitrocellulose membrane, which was imaged at the appropriate wavelength and quantified. After visualization, the bands obtained were quantified by calculating the mean intensity (Figure 2B), and a graph was plotted comparing the mean intensity with the dilution factor (Figure 2C). To determine the average value of the cargo delivered, the intensity of each band was subsequently divided by the corresponding dilution factor to obtain a mean value for the sample, which was then normalized to the control. The obtained results were then plotted as a bar graph (Figure 3B and 3C), while in parallel the integrity of mitochondria and mitoplasts was confirmed via Western blot (Figure 2A).

As a proof-of-concept, we applied the protocol to analyze the delivery to isolated mitochondria of both single- (ssON) and double-stranded RNA (dsON) oligonucleotides conjugated to an infrared (IR)

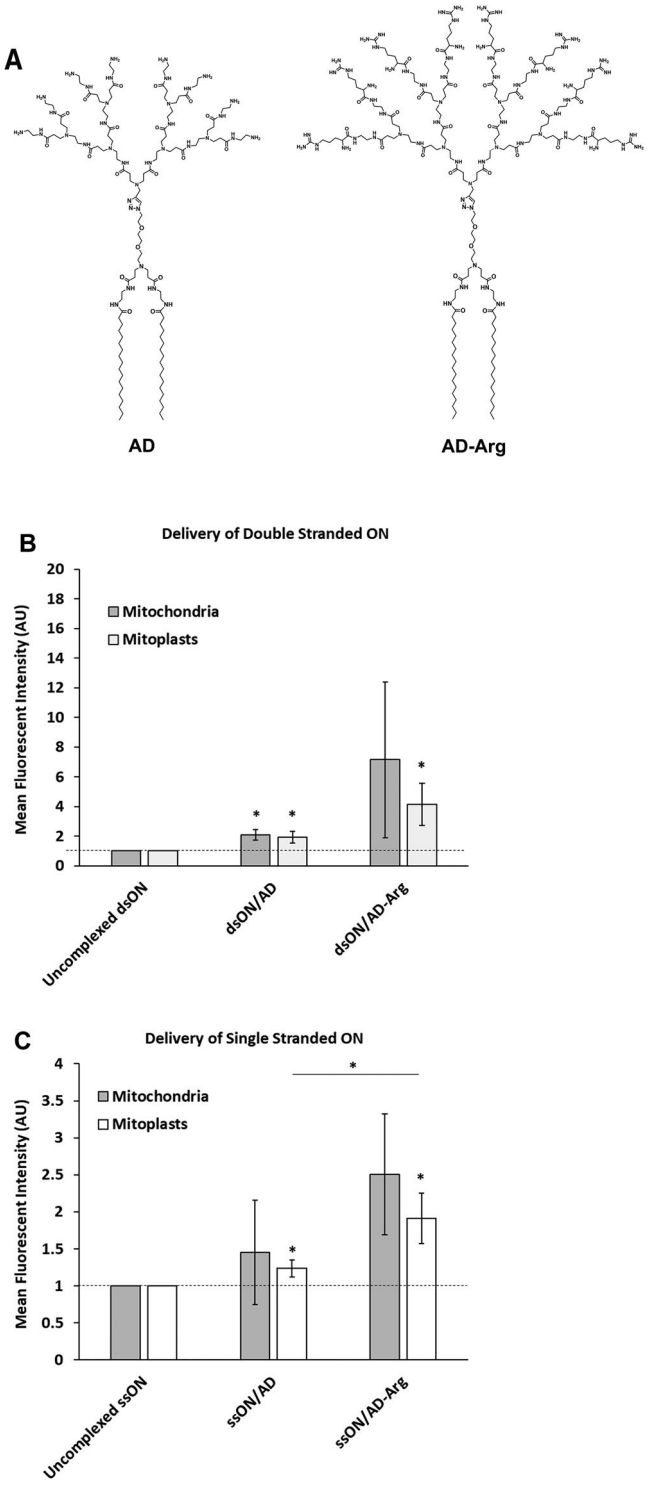

Figure 3. Biological Validation. **A.** Relevant chemical structures of the unmodified amphiphilic dendrimer AD (*left*) and the modified amphiphilic dendrimer AD-Arg (*right*). The two dendrimers were used for the delivery of single-stranded (ssON) or double-stranded (dsON) Oligonucleotides, as reported in the manuscript. **B** Histogram showing the fluorescent signal following delivery of dsON using the dendrimers AD and AD-Arg. Bars represent Mean +/- SD, normalized to the uncomplexed dsON control. Statistical significance was assessed against the control with a one-sample one-tailed t-test or assessed between AD and AD-Arg with a paired one-tailed t-test. Gray bars show delivery in whole mitochondria, while white bars show specific mitoplast delivery. n = 3; * = $p < 0.05$. **C** Histogram showing the fluorescent signal following delivery of ssON using the dendrimers AD and AD-Arg. Bars represent Mean +/- SD, normalized to the uncomplexed dsON control. Statistical significance was assessed against the control with a one-sample one-tailed t-test or assessed between AD and AD-Arg with a paired one-tailed t-test. Gray bars show delivery in whole mitochondria, while white bars show specific mitoplast delivery. n = 3; * = $p < 0.05$.

fluorophore. The results confirm the ability of both dendrimer to deliver ONs, with the arginine-terminated dendrimer AD-Arg showing a significantly better delivery of RNA ON cargos as compared to the amine-terminated AD. For dsON cargo, AD induced a small but significant increase in accumulation within whole mitochondria and in matrix-specific delivery, whereas AD-Arg achieved a more pronounced enrichment in both mitochondrial and matrix fractions (Figure 3B, Supplementary Table S1). Similarly, for ssON cargo, both dendrimers followed the same overall trend observed for dsON delivery, with AD-Arg consistently outperforming AD across all tested conditions (Figure 3C, Supplementary Table S1).

Together, these results validate our protocol as a robust and reliable method for comparing multiple delivery candidates for mitochondrial targeting of RNA oligonucleotide cargos.

4. Conclusion

The delivery of ONs to different cellular compartments remains a challenging and open field. Among the many difficulties that researchers need to overcome (i.e., optimal ON complex formation and specific targeting to an organelle), a significant limitation is the absence of reliable protocols to compare the delivery efficiency of different vectors. While quantitative PCR may be applied to analyze specific samples, such as whole cells, additional complex stem-loop primers and proprietary equipment are required [31]. Other protocols under consideration, including some fluorometric assays, show promise but have limitations, such as compatibility only with specific nucleic acid sequences [32,33], focus solely on downstream effects of delivery [34], or being tested only in bacterial models [35]. Additional approaches, such as flow cytometry, are limited in their scope, preventing sub-cellular and sub-mitochondrial localization. Here, we present a protocol for assessing amphiphilic dendrimer vectors for on-demand delivery of ONs into isolated mitochondria, and the specific localization into the mitochondrial matrix. It provides quantification with reduced variability and improved practicality. Additionally, this assay can be easily applied to evaluate the delivery efficiency of a panel of other potential vectors, as well as adjusted to compare the efficiency of vectors from the same family carrying modifications aimed at improving delivery efficiency. The final analysis provides a comparison for screening delivery of multiple candidates and can be adapted to meet the user's requirements.

Requiring only an ultrafiltration apparatus to perform the transfer of the samples to a membrane, the protocol can be easily implemented in any laboratory, and it can be modified to cover the specific experimental requirements (i.e., the fluorophore conjugated to the ONs can be substituted with any chromogenic tag). While we have validated the protocol using RNA-based oligonucleotide sequences, this procedure is also suitable for DNA-based sequences, requiring only a change to a suitable digestion enzyme. It would also be possible to adapt the protocol for other desired organelles, which must only be similarly isolated through existing optimized protocols, such as for cytoplasm [36–38], perinuclear region [39], nuclei [11,37,39], Golgi [40,41], Endoplasmic Reticulum [42,43], or simply collected whole cells.

In conclusion, our work introduces a novel method for quantitatively evaluating the delivery of ONs into mitochondria, enabling the potential application in several other scenarios with the ultimate goal of providing scientists with a new tool to bridge the technical gap in the field of subcellular ON delivery. A step-by-step protocol is available within the Supplementary Data as well as available on protocols.io (DOI: dx.doi.org/10.17504/protocols.io.4r3l29qj3v1y/v1) [44].

5. Future perspective

The protocol presented here is a highly adaptable methodology and can be applied in several scenarios, from relative screening of multiple delivery vectors to mechanistic studies or even quantitative benchmarking of mitochondrial delivery. Its flexibility and accessibility make it a valuable tool for advancing the understanding and development of nucleic acid therapeutics and their efficient delivery to mitochondria.

Author contributions

Joshua McHale and Carlo Vascotto conceived the idea, designed the experiments, analyzed the data, and prepared the manuscript. Joshua McHale and Veronica Bazzania conducted the experiments and analyzed the data.

Abdechakour Elkihel and Jing Wu synthesized and evaluated the dendrimers. Ling Peng analyzed the data and provided crucial support. All authors reviewed and edited the manuscript.

Disclosure statement

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Data availability statement

Raw data are available on Zenodo (https://doi.org/10.5281/zenodo.17300597). Step-by-step protocol available on protocol.io (dx.doi.org/10.17504/protocols.io.4r3l29qj3v1y/v1).

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

[1] Zamecnik PC, Stephenson ML. Inhibition of Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucleotide. Proc Natl Acad Sci U S A. 1978;75(1):280–284. doi: 10.1073/pnas.75.1.280[75545]
[2] Hammond SM, Aartsma-Rus A, Alves S, et al. Delivery of oligonucleotide-based therapeutics: challenges and opportunities. EMBO Mol Med. 2021;13(4):e13243. doi: 10.15252/emmm.202013243[33821570]
[3] Vinjamuri BP, Pan J, Peng P. A review on commercial oligonucleotide drug products. J Pharm Sci. 2024;113(7):1749–1768. doi: 10.1016/j.xphs.2024.04.021[38679232]
[4] Roberts TC, Langer R, Wood MJA. Advances in oligonucleotide drug delivery. Nat Rev Drug Discov. 2020;19(10):673–694. doi: 10.1038/s41573-020-0075-7[32782413]
[5] Androsavich JR. Frameworks for transformational breakthroughs in RNA-based medicines. Nat Rev Drug Discov. 2024;23(6):421–444. doi: 10.1038/s41573-024-00943-2[38740953]
[6] Lapshinov NE, Pridvorova SM, Zherdev AV, et al. Freeze-driven adsorption of oligonucleotides with polyA-anchors on Au@Pt nanozyme. Int J Mol Sci. 2024;25(18):10108. doi: 10.3390/ijms251810108[39337597]
[7] Yang L, Ma F, Liu F, et al. Efficient delivery of antisense oligonucleotides using bioreducible lipid nanoparticles in vitro and in vivo. Mol Ther Nucleic Acids. 2020;19:1357–1367. doi: 10.1016/j.omtn.2020.01.018[32160706]
[8] Wang Y, Miao L, Satterlee A, et al. Delivery of oligonucleotides with lipid nanoparticles. Adv Drug Deliv Rev. 2015;87:68–80. doi: 10.1016/j.addr.2015.02.007[25733311]
[9] Tanaka H, Takata N, Sakurai Y, et al. Delivery of oligonucleotides using a self-degradable lipid-like material. Pharmaceutics. 2021;13(4):544. doi: 10.3390/pharmaceutics13040544[33924589]

[10] San-Millán I. The key role of mitochondrial function in health and disease. Antioxidants (Basel). 2023;12(4):782. doi: 10.3390/antiox12040782[37107158]
[11] Bazzani V, Barchiesi A, Radecka D, et al. Mitochondrial apurinic/apyrimidinic endonuclease 1 enhances mtDNA repair contributing to cell proliferation and mitochondrial integrity in early stages of hepatocellular carcinoma. BMC Cancer. 2020;20(1):969. doi: 10.1186/s12885-020-07258-6[33028238]
[12] Picca A, Guerra F, Calvani R, et al. The contribution of mitochondrial DNA alterations to aging, cancer, and neurodegeneration. Exp Gerontol. 2023;178:112203. doi: 10.1016/j.exger.2023.112203[37172915]** This article provides the strongest justification for the importance and potential of mtDNA/RNA as therapeutic targets.
[13] Amorim JA, Coppotelli G, Rolo AP, et al. Mitochondrial and metabolic dysfunction in ageing and age-related diseases. Nat Rev Endocrinol. 2022;18(4):243–258. doi: 10.1038/s41574-021-00626-7
[14] Chocron ES, Munkácsy E, Pickering AM. Cause or casualty: the role of mitochondrial DNA in aging and age-associated disease. Biochim Biophys Acta Mol Basis Dis. 2019;1865(2):285–297. doi: 10.1016/j.bbadis.2018.09.035[30419337]
[15] Auth T. Polymeric and polymer-functionalized drug delivery vectors: from molecular architecture and elasticity to cellular uptake. Polymers (Basel). 2025;17(16):2243. doi: 10.3390/polym17162243[40871191]
[16] Ni Q, Wu J, Galanakou C, et al. Dendrimer engineering to overcome delivery challenges of nucleic acids. Nat Rev Bioeng. 2025;3(10):806–807. doi: 10.1038/s44222-025-00347-w
[17] Faria R, Boisguérin P, Sousa Â, et al. Delivery systems for mitochondrial gene therapy: a review. Pharmaceutics. 2023;15(2):572. doi: 10.3390/pharmaceutics15020572[36839894]* This review contextualizes our work and highlights the gap that we are trying to fill in our manuscript.
[18] Liew SS, Qin X, Zhou J, et al. Smart design of nanomaterials for mitochondria-targeted nanotherapeutics. Angew Chem Int Ed Engl. 2021;60(5):2232–2256. doi: 10.1002/anie.201915826[32128948]
[19] Liu X, Liu C, Zhou J, et al. Promoting siRNA delivery via enhanced cellular uptake using an arginine-decorated amphiphilic dendrimer. Nanoscale. 2015;7(9):3867–3875. doi: 10.1039/c4nr04759a[25283447]
[20] Yamada Y, Akita H, Kamiya H, et al. MITO-Porter: a liposome-based carrier system for delivery of macromolecules into mitochondria via membrane fusion. Biochim Biophys Acta. 2008;1778(2):423–432. doi: 10.1016/j.bbamem.2007.11.002[18054323]
[21] Buchke S, Sharma M, Bora A, et al. Mitochondria-targeted, nanoparticle-based drug-delivery systems: therapeutics for mitochondrial disorders. Life (Basel). 2022;12(5):657. doi: 10.3390/life12050657[35629325]
[22] Wang X, Shao N, Zhang Q, et al. Mitochondrial targeting dendrimer allows efficient and safe gene delivery. J Mater Chem B. 2014;2(17):2546–2553. doi: 10.1039/c3tb21348j[32261422]
[23] Bae Y, Jung MK, Lee S, et al. Dequalinium-based functional nanosomes show increased mitochondria targeting and anticancer effect. Eur J Pharm Biopharm. 2018;124:104–115. doi: 10.1016/j.ejpb.2017.12.013[29305141]
[24] Mallick S, Song SJ, Bae Y, et al. Self-assembled nanoparticles composed of glycol chitosan-dequalinium for mitochondria-targeted drug delivery. Int J Biol Macromol. 2019;132:451–460. doi: 10.1016/j.ijbiomac.2019.03.215[30930268]
[25] Yasuzaki Y, Yamada Y, Ishikawa T, et al. Validation of mitochondrial gene delivery in liver and skeletal muscle via hydrodynamic injection using an artificial mitochondrial reporter DNA vector. Mol Pharm. 2015;12(12):4311–4320. doi: 10.1021/acs.molpharmaceut.5b00511[26567847]
[26] Chen J, Zhu D, Liu X, et al. Amphiphilic dendrimer vectors for RNA delivery: state-of-the-art and future perspective. Acc Mater Res. 2022;3(5):484–497. doi: 10.1021/accountsmr.1c00272[35782755]** This article reviews the direct importance and potential of amphiphilic dendrimers as nucleic acid delivery systems, and is important for the consideration of dendrimer selection.
[27] Chen J, Ellert-Miklaszewska A, Garofalo S, et al. Synthesis and use of an amphiphilic dendrimer for siRNA delivery into primary immune cells. Nat Protoc. 2021;16(1):327–351. doi: 10.1038/s41596-020-00418-9[33277630]* This article provides proof of amphiphilic dendrimers as novel and important candidates for a reproducible delivery platform.
[28] Zhang P, Li Z, Cao W, et al. A PD-L1 antibody-conjugated PAMAM dendrimer nanosystem for simultaneously inhibiting glycolysis and promoting immune response in fighting breast cancer. Adv Mater. 2023;35(41):e2305215. doi: 10.1002/adma.202305215[37522451]
[29] Chen J. Amphiphilic dendrimers for nucleic acid delivery Aix-Marseille University; 2021 Doctoral thesis. ;.
[30] Boob AG, Tan S-I, Zaidi A, et al. Design of diverse, functional mitochondrial targeting sequences across eukaryotic organisms using variational autoencoder. Nat Commun. 2025;16(1):4151. doi: 10.1038/s41467-025-59499-3[40320395]
[31] Hurley J, Roberts D, Bond A, et al. Stem-loop RT-qPCR for microRNA expression profiling. Fan J-B,Next-Generation MicroRNA Expression Profiling Technology: Methods and Protocols. Totowa, NJ Humana Press; 2012.33–52. In: ed. : ; :[22144190]
[32] He Q, Luo H, Chen L, et al. Nanographite-based fluorescent biosensor for detecting microRNA using duplex-specific nuclease-assisted recycling. Luminescence. 2020;35(3):347–354. doi: 10.1002/bio.3733[31840880]
[33] Zhao X, Jiang S, Yan J, et al. Photocaged fluorescent probes for spatiotemporally monitoring G-quadruplex DNA in live cells. Bioorg Chem. 2025;160:108446. doi: 10.1016/j.bioorg.2025.108446[40209353]
[34] Deng Z, Ding J, Bu J, et al. Fluorophore label-free light-up near infrared deoxyribonucleic acid nanosensor for monitoring extracellular potassium levels. Anal Chem. 2024;96(10):4023–4030. doi: 10.1021/acs.analchem.3c03881[38412242]

[35] Liu S, Chen S, Tian L, et al. A graphene-oxide-based fluorometric assay for norA gene transcription in MRSA using Nb.BbvCI-assisted target recycling and T7 exonuclease-triggered cascade dual recycling signal amplification. Talanta. 2023;259:124549. doi: 10.1016/j.talanta.2023.124549[37062089]
[36] Senichkin VV, Prokhorova EA, Zhivotovsky B, et al. Simple and efficient protocol for subcellular fractionation of normal and apoptotic cells. Cells. 2021;10(4):852. doi: 10.3390/cells10040852[33918601]
[37] Suzuki K, Bose P, Leong-Quong RY, et al. REAP: a two minute cell fractionation method. BMC Res Notes. 2010;3(1):294. doi: 10.1186/1756-0500-3-294[21067583]
[38] Huynh HT, Shcherbinina E, Huang H-C, et al. Biochemical separation of cytoplasmic and nuclear fraction for downstream molecular analysis. Curr Protoc. 2024;4(5):e1042. doi: 10.1002/cpz1.1042[38767195]
[39] Shaiken TE, Opekun AR. Dissecting the cell to nucleus, perinucleus and cytosol. Sci Rep. 2014;4(1):4923. doi: 10.1038/srep04923[24815916]
[40] Tang D, Wang Y. Golgi isolation. Cold Spring Harb Protoc. 2015;2015(6):562–567. doi: 10.1101/pdb.prot075911[26034300]
[41] Graham JM. Isolation of Golgi membranes from tissues and cells by differential and density gradient centrifugation. Curr Protoc Cell Biol. 2001;Chapter 3:Unit 3.9. doi: 10.1002/0471143030.cb0309s10[18228361]
[42] Wieckowski MR, Giorgi C, Lebiedzinska M, et al. Isolation of mitochondria-associated membranes and mitochondria from animal tissues and cells. Nat Protoc. 2009;4(11):1582–1590. doi: 10.1038/nprot.2009.151[19816421]
[43] Leiro M, Ventura R, Rojo-Querol N, et al. Endoplasmic reticulum isolation: an optimized approach into cells and mouse liver fractionation. Bio Protoc. 2023;13(17):e4803. doi: 10.21769/BioProtoc.4803[37719073]
[44] McHale J, Bazzani V, Elkihel A, et al. Quantitative Analysis of Oligonucleotide Delivery into Isolated Mitochondria. protocols.io. 2026;. doi: 10.17504/protocols.io.4r3l29qj3v1y/v1