Electronic Supplementary Material (ESI) for Nanoscale.

Dendritic Nanoconjugates for Intracellular Delivery of Neutral Oligonucleotides

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Supporting information

Materials and Methods:

Preparation of fluorescent labelled nanoconjugates

To prepare fluorescent labelled nanoconjugates, the PMO functionalized with an additional amine at the 5′ position was custom synthesized by Gene Tools, LLC and was labelled with a fluorescent dye by reacting it with DyLight™ 650 NHS Ester (Life Technologies, Carlsbad, CA, USA) at a 1:3 molar ratio of PMO to dye in PBS (pH 7.5) for 30min at room temperature. The SSO-DL650 was purified by gel filtration in a PD-10 Desalting Column (GE Healthcare). After treatment with DTT, the labelled and unlabeled PMOs at the ratio of 1:4 were reacted with the SPDP modified dendrimer to prepare fluorescent labelled nanoconjugates.

RT-PCR of Mcl-1

Total RNA was isolated using TriReagent (Molecular Research Center). Total RNA was converted into first-strand cDNA using Enhanced Avian First Strand Synthesis Kit (Sigma). Mcl-1 cDNA was amplified by PCR using forward (5′- CCTGATGCCACCTTCTAGGTCC-3′) and reverse (5′- CTCTCGGTACCTTCGGGAGC -3′) primers. Cycles of PCR proceeded at 94°C for 30 s, 56°C for 30 s and 72°C for 60 s for 30 cycles. The PCR products were separated on agarose gels, and bands were visualized using a Gel Doc imaging system (Bio-Rad, Hercules, CA, USA).

Supporting figures:

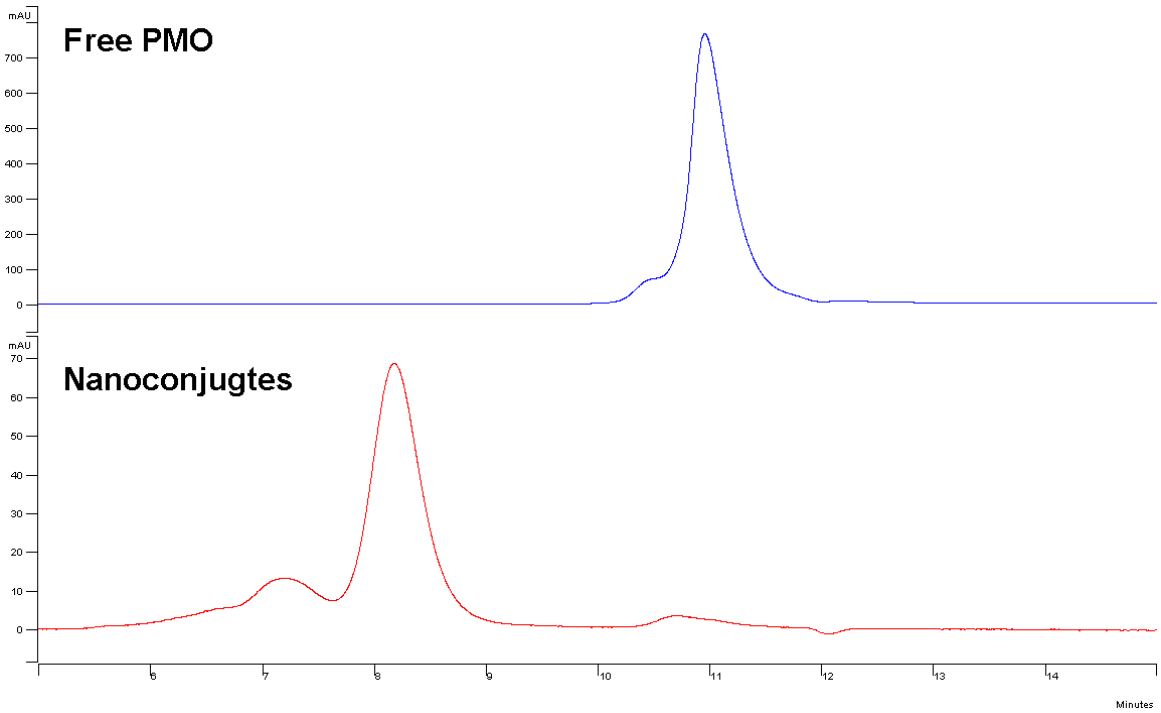

Fig. S1: Size-exclusion HPLC analysis. SEC-HPLC was performed using a Varian HPLC system (ProStar/Dynamax, Walnut Creek, CA) equipped with a Yarra SEC-3000 column (Phenomenex, Torrance, CA). The unconjugated dendrimer did not elute on the SEC column due to strong electrostatic interaction with the column material. The nanoconjugates eluted earlier than free PMOs in the column with the retention time of 8.2 and 10.1 min, respectively, indicating that the PMOs were successfully linked to the PAMAM dendrimers and the followed purification step using the Sephadex G-100 gel column removed the remaining free PMOs from the final product of nanoconjugates. The minor peak in front of the nanoconjugate chromatography peak represents aggregates of the nanoconjugates.

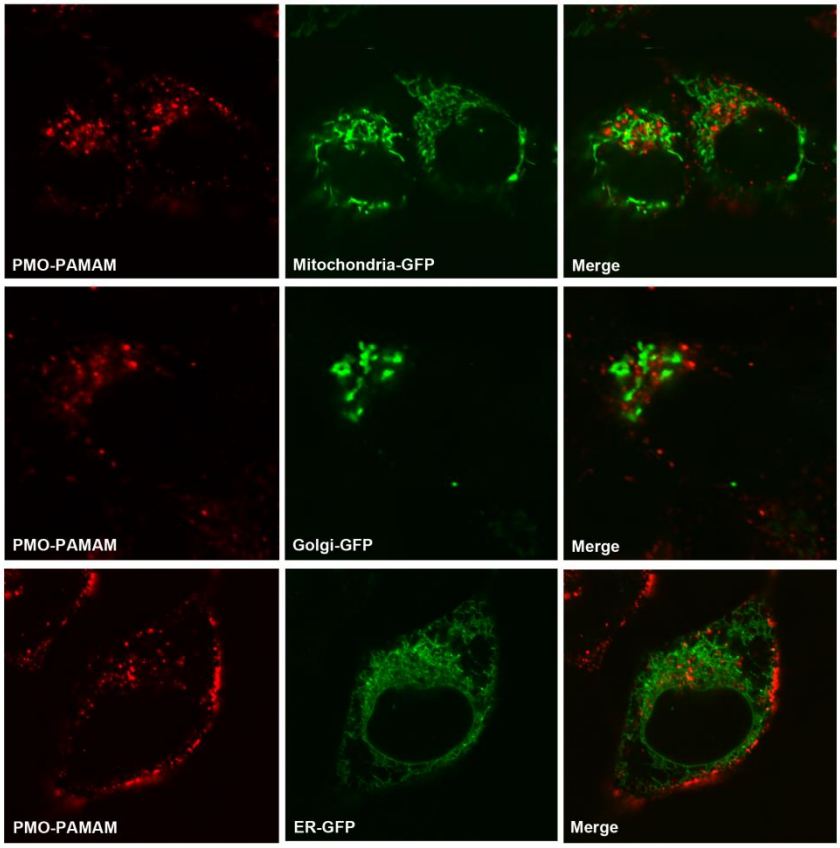

Fig. S2: Subcellular localization of the nanoconjugates. A375 cells were transfected with GFP chimeras that serve as markers for mitochondria, Golgi network, and ER. Thereafter, cells were incubated with the fluorescent nanoconjugates (100nM) for 4h. Live cells were observed by confocal microscopy.

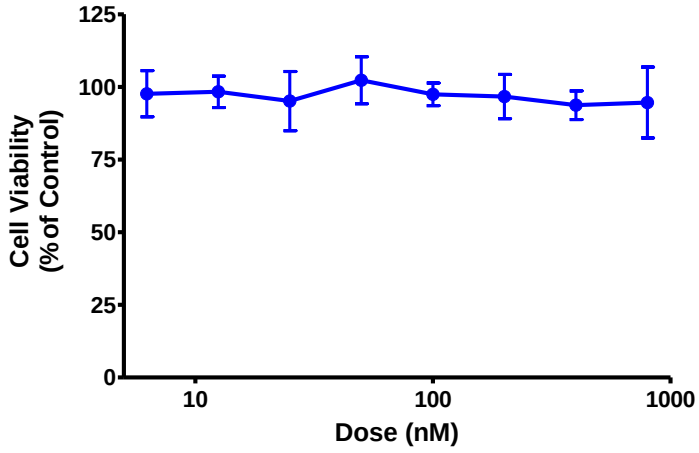

Fig. S3: Cytotoxicity of the dendritic nanoconjugates carrying SSO623. A375 cells were treated with the nanoconjugates carrying SSO623 at various concentrations for 4h. Cell viability was measured in the treated cells after 72-h culture using the Alamar Blue assay.