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Cellular Internalization of Water-Soluble Helical Aromatic Amide Foldamers

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The intracellular transport of drugs and therapeutics represents one of the most exciting and challenging areas at the interface of chemistry, biology, and medicine. Most of the effort in this field so far has been devoted to the development of peptide-based delivery systems that can translocate therapeutic agents into their intracellular targets. More recently, the use of bio-inspired non-natural foldamers has resulted in the successful delivery of cargo molecules, which possess a wide range of sizes and physicochemical properties across the cell membrane. We report herein the synthesis of aromatic amide foldamers and their biological evaluation as cell-penetrating agents. By using a well-established synthetic route, a series of fluorescein-labeled cationic aryl amide conjugates has been constructed, and their cellular uptake into various human cell lines has been analyzed by flow cytometry and fluorescence microscopy. The assays revealed that longer oligomers achieve greater cellular translocation, with octamer **Q8** proving to be a remarkable vehicle for all three cell lines. Biological studies have also indicated that these helices are biocompatible, thus showing promise in their application as cell-penetrating agents and as vehicles to deliver biologically active molecules into cells.

Introduction

Great attention is currently being focused on the development of molecular transporters that can facilitate the entry of polar molecules such as proteins, peptides, or oligonucleotides through the otherwise impermeable cellular membrane. Although the successful delivery of various molecules with a wide range of molecular weight and physicochemical properties has already been achieved by using natural frameworks such as cell-penetrating peptides (CPPs),[1] CPPs' poor extra- and intracellular stability constitutes a limitation to this approach. For the past decade, a great deal of attention has been paid to non-natural oligomers and in particular to foldamers. The latter compounds fold into well-defined conformations in solution[2] and they adopt specific secondary or tertiary structures resembling those of naturally occurring biopolymers. This feature is relevant to biological activity in general and to cell penetration in particular to the extent that conformational control or conformational changes are often associated with peptide–membrane interactions.[3] Additionally, foldamers can be constructed from innumerable non-natural monomers; this results in a varied chemical composition, and they have been reported to show a fair stability towards proteolytic degradation.[4,5] All of these features make them excellent candidates as therapeutic biomimetics.

Among foldamers, aliphatic β-peptides and peptoids are the most closely related to α-peptides. However, the side chains of β-peptides and peptoids are appended to a different (carbon or nitrogen) atom than the α-carbon along the backbone. They have been reported to adopt conformations that closely mimic the folded structures of their natural counterparts[2,6] and, not surprisingly, they feature peptide-like biological activity.[2,7] For example, β-peptides and peptoids have been shown to possess antibacterial activity,[7d,8] to mediate translocation across the cell membrane,[9] or to recognize protein surfaces.[7a,10]

Contrary to aliphatic foldamers, the potential for biological applications of foldamers comprised of aromatic backbones seems less obvious. As shown by extensive structural studies, this relatively new subclass of foldamers could possess linear or helical structures that are very stable but considerably different from those of α-peptides.[11] Potential advantages of such oligomers lie in the availability of the precursor monomers, in the robustness and predictability of the oligomers' conformations, and in the resulting reliability of structure–activity relationships. Yet, despite their large structural differences from peptides, several aromatic foldamer families display interesting peptide-like biological activity. Examples of small synthetic aryl-based oligomers that adopt linear amphipathic conformations and exhibit potent antimicrobial activity have been reported.[12] Other oligomers have been shown to mimic the projection of side chains found in α-helical structures and to inhibit protein–protein interactions.[13]

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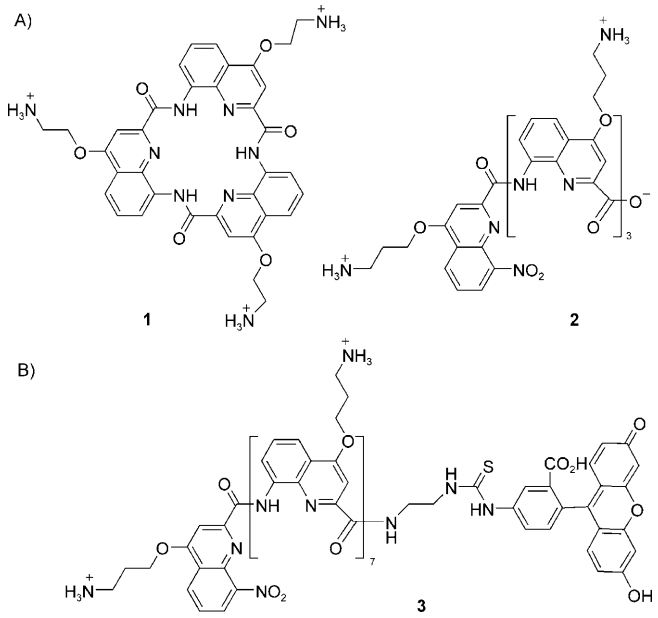
Oligomers of 8-amino-2-quinolinecarboxylic acid have been thoroughly studied by our group. They form helices that are wider, with a smaller helical pitch and of much greater stability than those of peptides.[14] It has been recently proven that despite the high hydrophobicity of their aromatic backbones, these oligoamides can be made highly water soluble by appending cationic side chains.[5,15] This provides important possibilities for their biological application, for example, recent experiments have shown that the planar cyclic trimer **1** and helical tetramer **2**, both bearing aminopropoxy side chains, display very high affinity for G-quadruplex DNA (Scheme 1).[15]



Scheme 1. Water soluble quinoline oligoamide foldamers. A) Oligomers with affinity for G-quadruplex DNA. B) Helical octamer with potential membrane translocation ability.

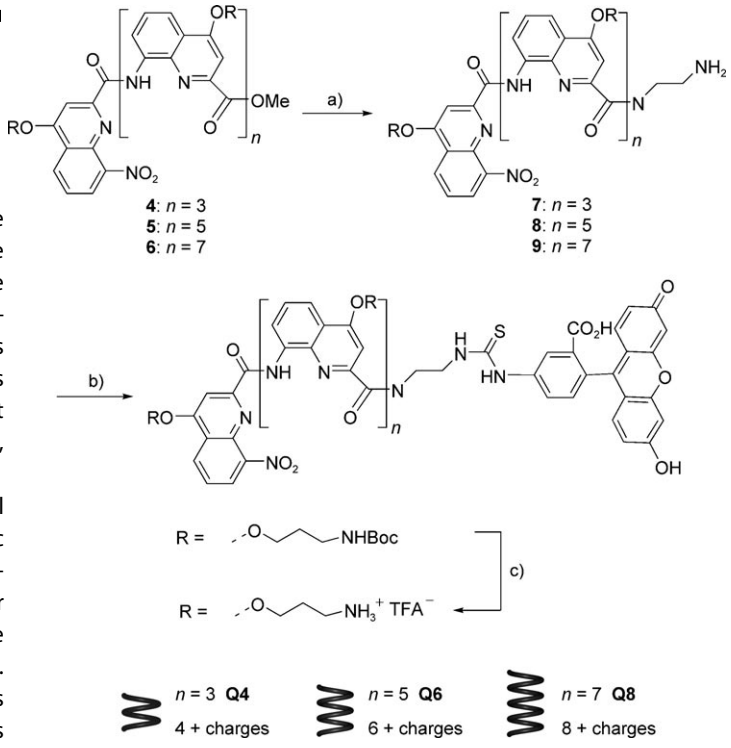
As cationic side chains are known to assist in membrane permeation, some preliminary cell-penetration studies were carried out on helical octameric oligomer **3**. The results were very encouraging, indicating that **3** readily crosses the membrane of HeLa cells, that it is biocompatible, and that it resists degradation by proteases.[5] Helical aromatic oligoamides thus appear to be a new class of biologically active substances that differ considerably from classical drug-like molecules, peptides, and peptidomimetics based on aliphatic backbones.

With the prospect that foldamers with an ability to cross cell membranes could be used as transporters to carry therapeutic agents into cells, we report here the synthesis and characterization of fluorescein-labeled oligomers and their cellular uptake in various human cell lines of epithelial or immune origin (HeLa cells, Jurkat lymphocytes, and Huh-7 hepatocytes). In particular, usually hard-to-transfect Jurkat cells provide us with a good model system to test the feasibility of our helices to translocate the cell membrane. The studies were targeted to the effect of structural features such as the length and therefore the number of charges on the oligomers' ability to cross the membrane of various cell lines. The longest octamer **3** displays an extraordinary ability to cross the plasma membrane in all three cases, and at concentrations as low as 10 μM. Cell uptake experiments in which endocytosis is inhibited were also performed and provide us with insights into the mechanism of translocation. The results suggested that the helices enter the cells and are transported to the cytoplasm by endosomes.

Results and Discussion

Design and synthesis

In order to determine the effect of the backbone length and of the number of charges on cellular uptake, oligomers **4**, **5**, and **6**, which contain water-solubilizing ammonium side chains protected as *tert*-butyl carbamates (Boc), were prepared according to procedures described previously (Scheme 2).[5] The introduction of the fluorescein tag was carried out by treating esters **4**, **5** and **6** with ethylene diamine in the presence of 4-dimethylaminopyridine (DMAP) and subsequently with fluorescein 5-isothiocyanate (FITC; see Experimental Section). TFA-mediated removal of the Boc protecting groups afforded the desired water-soluble tetramer **Q4**, hexamer **Q6**, and octamer **Q8** (**3**), which were purified by reversed-phase HPLC and characterized by MALDI-TOF mass spectrometry.



Scheme 2. Synthetic scheme for the preparation of fluorescein-tagged foldamers. a) Ethylene diamine, DMAP, 50 °C, overnight; b) FITC, DMF, RT, overnight; c) TFA/CH_2Cl_2, RT, 2 h.

Effect of foldamer length on cell viability

To determine the toxicity of the molecules, the growth of human cervical carcinoma (HeLa) cells in the presence of helices **Q4**, **Q6** and **Q8** was evaluated by means of the MTS assay (see the Experimental Section for details). Results are reported in Figure 1. Whereas neither **Q4** nor **Q6** was toxic up to 200 μg mL^{-1}, **Q8** became highly toxic at this concentration, and reduced cell viability by 94%.

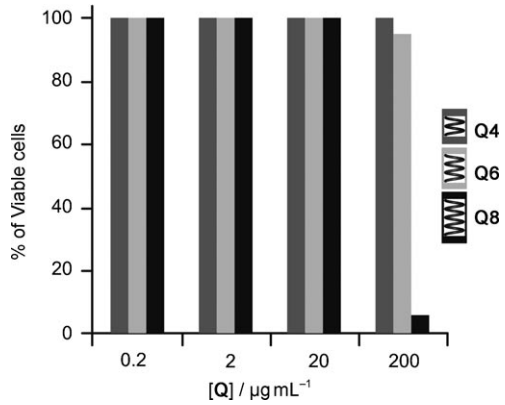


Figure 1. Effect of the cationic helices **Q4**, **Q6**, and **Q8** on cell viability. HeLa cells were treated with varying concentrations of the cationic helices and incubated for five days at 37 °C. Cell viability was assessed by the MTS method, with 100% viability assigned to control cells that had received no helices.

It is striking that toxicity differs to such an extent at 200 μg mL^{-1}, despite the apparent resemblance between the three compounds. It is possible that the increased relative toxicity of the longer oligomer results from the higher number of charges in the molecule. This outcome is in concordance with those reported in the literature, in which highly positively charged carrier entities, such as poly(arginine) or poly(L-lysine) oligomers, promote cell death as a result of the initial contact with the negatively charged extracellular matrix that coats the cellular membrane prior to penetration.[16] In any case, the toxicity values obtained for oligomer **Q8** are comparable to those of the Tat(48–60) protein transduction domain or other cell-penetrating agents,[17] and toxicity occurs at doses that largely exceed those normally used in vectorization.

Cell-penetrating activity of the foldamers

The ability of the fluorescein-tagged aromatic tetramer **Q4**, hexamer **Q6**, and octamer **Q8** to penetrate into cells was quantified by means of flow cytometry with HeLa cells, human hepatic Huh-7 cells and human T-lymphocyte Jurkat cells. The cells were incubated in their corresponding medium for 1 h at 37 °C with a 30 μM concentration of **Q4**, **Q6**, or **Q8**. Jurkat cells, which grow in suspension, were analyzed directly by flow cytometry, whereas HeLa and Huh-7 cells, which all grow as adherent monolayers, were detached and dissociated by trypsin/EDTA before flow cytometry. Untreated cells and cells incubated with FITC alone were tested as negative controls, and no fluorescence was detected in these cases (data not shown).

A comparison of the uptake of the three compounds in the three cell lines is shown in Figure 2A. Octamer **Q8** displays a

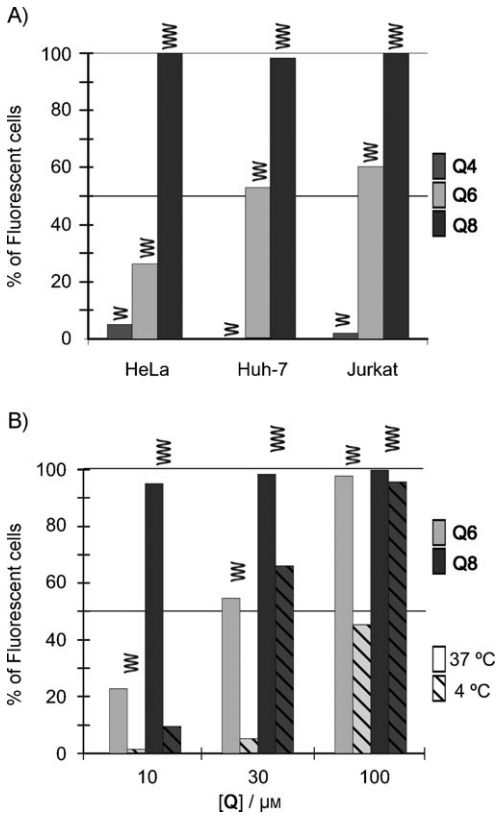


Figure 2. A) Percentage of fluorescent HeLa, Huh-7 and Jurkat cells after incubation with 30 μM concentration of Q4, Q6, and Q8. B) Percentage of fluorescent Huh-7 cells after incubation with various concentrations of **Q6** and **Q8**, at 37 °C (plain columns) and 4 °C (hatched columns).

remarkable ability to associate to any cell line, labeling 100% of cells after 1 h at 37 °C. Hexamer **Q6** on the other hand has half the affinity of **Q8** for Huh-7 and Jurkat cells, and its cellular uptake is reduced to about 26% in HeLa cells. The uptake of tetramer **Q4** by any cell line appears to be rather insignificant, if one assumes that the absence of fluorescence is not due to a selective quenching by intracellular biomacromolecules. The increasing uptake efficiency with oligomer size might point to the role of the number of positive charges carried by **Q6** and **Q8**, as observed in other studies on the penetrability of cationic peptide and peptoid hybrids. Indeed, it is well known that the specific regions that allow the effective translocation of proteins such as HIV-TAT or antennapedia contain a large number of lysine and arginine residues, and many effective CPPs that have been developed after these findings consist entirely of positively charged amino acids.[18] In the case of **Q4**, **Q6**, and **Q8**, the side chains operate cooperatively: when cellular uptake is plotted not as a function of the concentration of oligomer, but as a function of the concentration

of side chain, **Q8** and **Q6** are still much better than **Q4**. For example, the uptake of **Q4** at 30 μM is much lower than that of **Q8** at 10 μM (see the Supporting Information).

To shed light on the cell uptake mechanism of **Q6** and **Q8**, the concentration and temperature dependence of the translocation process in Huh-7 cells was analyzed (Figure 2B). At 37 °C, **Q6** uptake was directly related to its concentration, with almost 100% of cells labeled at 100 μM. The longer oligomer **Q8** achieved a much more efficient penetration into Huh-7 cells, as 95% of the cells were already labeled at the lowest dose of 10 μM.

To assess whether cell uptake of these foldamers relies on an energy-dependent mechanism, we investigated this process at a low temperature (4 °C), aimed at inhibiting active membrane transport such as translocation by endocytotic pathways (Figure 2B, hatched bars). Huh-7 cells were incubated for 1 h at 4 °C with different concentrations of **Q6** or **Q8**. Lowering the temperature substantially reduced the percentage of labeled cells at 10 μM for **Q8** (the fraction of fluorescent cells is reduced from 95 to 10%) and at 30 μM for **Q6** (a reduction in fluorescence from 55% to 5%). Intriguingly, under endocytosis-inhibiting conditions 60 and 45% of cells were still labeled at 30 μM for **Q8** and at 100 μM for **Q6**, respectively. It is also worth pointing out that 100% of cells were labeled by **Q8** at 100 μM, whether they were incubated at 4 or at 37 °C. This suggests that mechanisms other than energy-dependent pathways might be involved, and this result prompted us to examine the cellular localization of the foldamers by fluorescence microscopy.

Huh-7 cells were therefore incubated with the FITC-labeled **Q6** and **Q8** derivatives and FITC alone as a negative control, at 37 °C and 4 °C. The extraordinary cell-penetrating ability of foldamers **Q6** and **Q8** in Huh-7 cells was verified by the images obtained. At 37 °C (Figure 3A and B), cells showed an efficient cellular uptake when exposed to either **Q8** or **Q6** oligomers, and the punctuated fluorescence pattern of the cells indicates that both foldamers had accumulated in vesicles, characteristic of an endocytotic mechanism for internalization. In the case of Huh-7 cells, it is not clear to what extent oligomers escape endosomes and are released in the cytoplasm, as the punctuated fluorescence is quite persistent. However, confocal microscopy observations previously made for **Q8** in HeLa cells showed a substantially homogeneous fluorescence in the cytoplasm, which suggests that, once inside the cell, the foldamer-FITC conjugate is released from the endosome (Supporting Information).[5] The stronger fluorescence observed for **Q8** compared to **Q6** is in agreement with quantitative flow cytometry data. It is also evident from Figure 3D and E (taken after incubation at 4 °C), that intracellular fluorescence is largely reduced when cells are incubated under conditions that inhibit endocytosis. It is worthwhile noting that in cells incubated at 4 °C with 100 μM **Q8**, the helices appear to bind strongly to the plasma membrane (presumably its external surface), and remain associated with the cells; this accounts for the high percentage of labeled cells obtained in flow cytometry analysis, which in this case does not reflect penetration. This is perhaps unsurprising, considering the highly cationic nature of the compounds and the overall negative charge of the cell surface. In the case of T-lymphocyte Jurkat cells (phase contrast images in the Supporting Information), due to the very large nuclear/cell ratio of the cells, the discrimination between intracellular and membrane fluorescence is not straightforward. We believe, however, that a very localized fluorescence pattern at 37 °C for both **Q8** and **Q6** provides a strong indication of membrane translocation. Furthermore, the homogeneous fluorescent pattern around the cell at 4 °C agrees with a membrane association of the helices rather than internalization.

The cell-membrane association of the **Q8** helices at low temperature suggests that internalization is dependant on nonspecific, electrostatic interactions of oligomers with the membrane. The higher positive charge of **Q8** could promote a stronger initial attachment of the cationic side chains to the membrane compared with **Q6** or **Q4**; this results in a more efficient cell penetration for the longest oligomers. Whether cell-surface receptors are involved in the endocytosis has not yet been ascertained. In fact, although extensive studies on the mechanism of transduction have been made, it has not been fully elucidated, and controversy remains.[19]

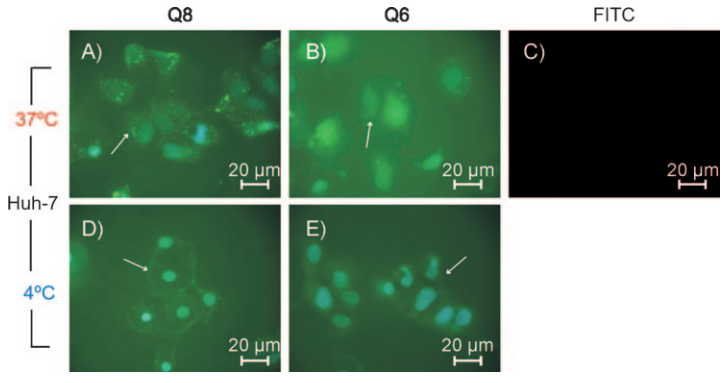


Figure 3. Fluorescence microscopy images of Huh-7 cells incubated for 1 h with **Q6**, **Q8**, and FITC at 100 μM concentration. Images A)–C) were taken following incubation at 37 °C, whereas images D) and E) were taken following incubation at 4 °C. The nucleus was counter-stained in blue by using 4′,6-diamidino-2-phenylindole (DAPI). White arrows indicate endosomal fluorescence (A and B) and fluorescence arising from association to the membrane (D and E).

Conclusions

In summary, as part of our studies on the potential use of aromatic, oligoamide foldamers as drug carriers, we have investigated the effects that altering structural features, such as length and number of charges, have on the cell translocation activity of the foldamers. The results indicate that a minimum length and number of positive charges is necessary for an efficient cell-translocation, with the octamer exhibiting excellent penetration abilities in HeLa, Huh-7, and more importantly in hard-to-transfect Jurkat cell-lines. We have also demonstrated that these compounds display practical advantages, including good water solubility and nontoxicity, up to

high concentrations. The experiments carried out at lower temperatures have provided an insight into the mechanism of the process and suggest that the uptake of **Q4**, **Q6**, and **Q8** follows an endocytic pathway. These results have been obtained with foldamers containing simple aminopropoxy side chains. Chemical modification at this position provides a ready source of diversity and the possibility to optimize the ability of aromatic foldamers to translocate the plasma membrane. Further efforts will focus on studying the feasibility of these compounds to deliver bioactive cargo molecules into cells.

Experimental Section

General procedures and materials: Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. CH_2Cl_2 and diisopropylethylamine (DIEA) were distilled from CaH_2 prior to use. Chemical shifts are calibrated against residual solvent signals of $CDCl_3$ ($\delta = 7.26$, 77.2) or CD_3OH ($\delta = 3.31$, 49.1). Chromatography on silica gel was performed by using Merck Kieselgel Si 60. RP-HPLC analyses were performed on a Thermo system by using a Chromolith performance RP-18e column (4.6×100 mm, 5 μm) with P1000 XR pumps. The mobile phase was 0.1% TFA/H_2O (*v/v*), unless otherwise noted, at a flow rate of 3 mL min^{-1}. Column effluent was monitored by UV detection at 214 and 254 nm with a Thermo UV 6000 LP diode array detector. Semipreparative purifications of the compounds were performed on a Varian PrepStar system with SD-1 Dynamax pumps and a Microsorb C18 column (21.4 mm×250 mm, 100 Å pore size, 5 μm). The mobile phase was the same as that for the analytic system, unless otherwise noted, at a flow rate of 20 mL min^{-1}. Column effluent was monitored by UV detection at 214 and 254 nm by using a Varian UV/Vis Prostar 325 diode array detector. Electrospray ionization (ESI) mass spectra were obtained in the positive ion mode and MALDI-TOF mass spectra were obtained in positive ion mode by using α-cyanohydroxycinnamic acid as a matrix. Dulbecco's modified eagle medium (DMEM) and Roswell Park Memorial Institute (RPMI) medium for cell culture were purchased from Invitrogen. Cell viability was assessed by using a spectrophotometric microplate Berthold Appolol B911 reader at 490 nm. Fluorescence microscopy experiments were accomplished on a ZEISS axiovert 200. The oligonucleotide quantification by UV absorbance at 260 nm were performed on a NanoDrop ND-1000 spectrophotometer. Flow cytometry assays were carried out on a COULTER EPICS XL.

Synthesis

*Amine functionalized tetramer **7***: DMAP (catalytic) was added to tetramer **4** (0.05 g, 34.12 μmol, 1 equiv) in ethylene diamine (5.0 mL, excess), and the reaction mixture was stirred at 50 °C overnight. Water was then added (5.0 mL), and the product was precipitated from the aqueous layer. It was filtered off and purified by flash column chromatography on silica gel (EtOAc/cyclohexane 8:2) to obtain the corresponding product (0.04 g, 72% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 1.34$–1.46 (m, 27H), 1.49 (s, 9H), 2.15–2.29 (m, 8H), 3.21–3.33 (m, 2H), 3.42–3.56 (m, 10H), 4.13–4.26 (m, 6H), 4.46–4.64 (m, 2H), 4.73 (brs, 1H), 4.92 (brs, 1H), 5.10 (brs, 1H), 5.58 (brs, 1H), 5.96 (brs, 2H), 6.75 (s, 1H), 6.83 (s, 1H), 7.29 (t, $J = 7.5$ Hz, 1H), 7.40 (t, $J = 7.9$ Hz, 1H), 7.51–7.68 (m, 4H), 7.70–7.84 (m, 2H), 7.85–8.00 (m, 3H), 8.04 (d, $J = 7.5$ Hz, 1H), 8.51 (d, $J = 7.5$ Hz, 2H), 8.98 (d, $J = 7.5$ Hz, 1H), 11.64 (s, 2H), 12.30 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 28.7$, 29.1, 29.2, 29.4, 29.5, 37.4, 37.5, 38.0, 38.2, 52.8, 65.5, 66.6, 66.8, 67.2, 67.9, 79.4, 79.5, 79.7, 79.8, 97.6, 98.9, 100.3, 100.7, 116.0, 116.3, 116.7, 116.8, 116.9, 117.1, 118.0, 121.9, 121.9, 122.0, 123.7, 124.7, 125.9, 127.1, 127.7, 127.9, 128.6, 133.8, 134.1, 135.4, 138.4, 139.1, 139.2, 145.5, 148.8, 151.1, 153.9, 156.2, 156.4, 156.5, 160.8, 161.4, 162.2, 162.9, 163.1, 163.6, 164.5; MS (ESI): *m/z* 1485 $[M+Na]^+$, 1463 $[M+H]^+$.

*Amine functionalized hexamer **8**:* DMAP (catalytic) was added to hexamer **5** (0.02 g, 9.34 μmol, 1 equiv) in ethylene diamine (4.0 mL, excess), and the reaction mixture was stirred at 50 °C overnight. Water (5.0 mL) was then added, and the product was precipitated from the aqueous phase. It was filtered off and purified by flash column chromatography on silica gel (EtOAc 100%) to obtain the corresponding amine functionalized hexamer (0.01 g, 50% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 1.35$–1.65 (m, 54H), 2.11–2.45 (m, 12H), 3.05–3.33 (m, 4H), 3.36–3.78 (m, 12H), 4.03–4.59 (m, 12H), 4.73 (brs, 1H), 4.95 (brs, 1H), 5.12 (brs, 1H), 5.58 (brs, 1H), 5.83 (brs, 2H), 6.05 (brs, 1H), 6.28 (s, 1H), 6.57 (s, 1H), 6.59 (s, 1H), 6.72 (s, 1H), 6.78 (s, 1H), 7.07–7.48 (m, 5H), 7.54 (s, 1H), 7.55 (d, $J = 7.2$ Hz, 1H), 7.66–7.82 (m, 3H), 7.82–7.97 (m, 4H), 8.02 (d, $J = 8.4$ Hz, 1H), 8.08 (d, $J = 7.3$ Hz, 1H), 8.21 (d, $J = 6.6$ Hz, 1H), 8.32 (d, $J = 6.4$ Hz, 1H), 8.38 (d, $J = 8.8$ Hz, 1H), 8.48 (d, $J = 7.3$ Hz, 1H), 11.21 (s, 1H), 11.39 (s, 2H), 11.6 (d, 1H), 11.82 (d, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 28.4$, 29.1, 29.5, 37.4, 38.2, 52.5, 63.5, 66.2, 66.6, 66.9, 67.3, 79.0, 79.3, 79.4, 79.5, 97.4, 97.7, 98.0, 97.0, 99.7, 100.2, 115.7, 116.1, 116.3, 116.7, 116.9, 117.0, 121.4, 121.7, 121.9, 122.3, 123.4, 124.3, 126.0, 126.3, 126.6, 127.2, 127.5, 127.9, 132.6, 133.4, 133.5, 133.7, 134.1, 137.5, 138.0, 138.2, 138.3, 138.7, 138.8, 144.9, 145.0, 148.5, 148.8, 149.0, 150.6, 153.1, 156.2, 156.3, 156.5, 159.7, 160.3, 160.5, 161.0, 161.2, 161.8, 162.5, 162.8, 162.9, 163.0, 163.4, 164.1; MS (ESI): *m/z*: 2172 $[M+Na]^+$, 2150 $[M+H]^+$.

*Amine functionalized octamer **9***: Experimental procedure and spectroscopic data previously published.[5]

*Fluorescein functionalized tetramer **Q4***: Fluorescein 5-isothiocyanate (0.01 g, 0.02 mmol, 2 equiv) was added to a solution of amine functionalized tetramer **7** (0.02 g, 13.74 μmol, 1 equiv) in DMF (1.0 mL), and the reaction mixture was stirred at room temperature overnight. The DMF was evaporated in vacuo, and the resulting residue was dissolved in TFA/CH_2Cl_2 (1:1, 2.0 mL) and stirred at room temperature for 4 h. The solvents were evaporated to provide the crude product, which was purified by semipreparative HPLC on a C18 column and a water/acetonitrile gradient with 0.1% TFA to provide **Q4** (0.01 g, 52% yield) as a yellow solid. ^{1}H NMR (CD_3OH, 300 MHz): $\delta = 2.35$–2.50 (m, 4H), 2.52–2.62 (m, 4H), 3.25–3.42 (m, 6H), 3.46–3.59 (m, 6H), 4.20–4.35 (m, 4H), 4.47–4.67 (m, 4H), 6.67 (s, 2H), 7.34 (t, $J = 6.4$ Hz, 1H), 7.42 (s, 1H), 7.51–7.77 (m, 10H), 7.78–8.02 (m, 9H), 8.36 (d, $J = 8.1$ Hz, 1H), 8.63 (d, $J = 8.8$ Hz, 1H), 8.88 (d, $J = 7.1$ Hz, 1H), 11.46 (s, 1H), 11.61 (s, 2H), 11.24 (s, 1H); MS (MALDI): *m/z*: 1454 $[M+H]^+$.

*Fluorescein functionalized hexamer **Q6***: Fluorescein 5-isothiocyanate (0.04 g, 9.20 μmol, 2 equiv) was added to a solution of amine functionalized hexamer **8** (0.01 g, 4.60 μmol, 1 equiv) in DMF (1.0 mL), and the reaction mixture was stirred at room temperature overnight. The DMF was evaporated in vacuo, then the resulting product was dissolved in TFA/CH_2Cl_2 (1:1, 2.0 mL), and the solution was stirred at room temperature for 2 h. The solvents were evaporated to provide a crude residue that was purified by semipreparative HPLC on a C18 column by using a water/acetonitrile gradient with 0.1% TFA to provide **Q6** as a yellow solid (5.0 mg, 46% yield). ^{1}H NMR (CD_3OH, 300 MHz): $\delta = 2.35$–2.70 (m, 12H), 3.20–3.58 (m, 16H), 4.11–4.43 (m, 6H), 4.46–4.63 (m, 6H), 6.42 (s, 1H), 6.45 (s, 1H), 6.59 (s, 1H), 6.64 (s, 1H), 6.71 (s, 1H), 6.85 (t, $J = 8.1$ Hz, 1H), 7.13 (d, $J = 8.1$, 1H), 7.19 (s, 1H), 7.28 (d, $J = 7.5$ Hz, 1H), 7.31–7.65

(m, 10H), 7.77–7.86 (m, 3H), 7.92–8.12 (m, 7H), 8.19–8.32 (m, 3H), 8.49 (d, $J=7.1$ Hz, 1H), 8.59 (d, $J=6.8$ Hz, 1H), 11.44 (s, 1H), 11.48 (s, 2H), 11.72 (s, 1H), 12.05 (s, 1H); MS (MALDI): *m/z*: 1940 $[M+H]^+$.

Fluorescein functionalized octamer ***Q8***: The experimental procedure and spectroscopic data have been previously published.[5]

Biological assays

Cell culture: Human HeLa (cervix carcinoma), Jurkat (T-lymphoma) and Huh-7 (hepatocarcinoma) cells were grown in DMEM, RPMI and DMEM, respectively, supplemented with 10% decomplemented fetal calf serum, L-glutamine (2 mM), and 1% of nonessential amino acids at 37 °C in a 5% CO_2 atmosphere. All culture reagents were purchased from Invitrogen.

Cytotoxicity assay: HeLa cells were plated into a 96-well plate at a density of 2000 cells per well in their growth medium, and solutions of the cationic helices at varying concentrations (0.2, 2, 20, and 200 $\mu g\,mL^{-1}$) in DMEM were added; this provided a final volume of 100 μL per well. Following incubation in the presence of the helices for five days at 37 °C, (5% CO_2), Cell Titer960 Aqueous One Solution (Promega) was added to each well (20 μL), and the cells were incubated for 1 h. The absorbance at 490 nm was read on a plate reader. The tests were conducted in triplicate for each concentration. Optical densities measured for wells containing cells that received no helices were considered to represent 100% growth. MTS stands for 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium).

Fluorescence microscopy: Cells were plated into a 24-well plate containing a glass coverslip at densities of 100000 HeLa, 80000 Huh-7, or 200000 Jurkat cells per well. After two days of culture, fluorescein-labeled cationic helices (100 μM) were added for 1 h at 37 °C or 4 °C in the growth medium. The cells, either attached on glass coverslips (HeLa and Huh-7) or in suspension (Jurkat), were then rinsed twice with phosphate buffered saline (PBS), fixed in 3% formaldehyde in PBS for 15 min at room temperature, and mounted onto glass slides by using a Vectashield (Vector Laboratories, Burlingame, CA, USA) mounting solution. The slides were observed with an Axiovert 200 fluorescent microscope (Zeiss).

Flow cytometry: Cells were plated into a 24-well plate and incubated with fluorescein-labeled foldamers at 10, 30 and 100 μM under the same conditions as for fluorescent microscopy. The cells were then rinsed twice with PBS, and HeLa and Huh-7 cells were detached from the plates with 0.05% (*w/v*) trypsin/EDTA (Invitrogen; 50 μL per well) at 37 °C. The cells were suspended in ice-cold PBS (250 μL). The percentage of fluorescent cells as well as the mean fluorescent intensity were analyzed in a COULTER EPICS XL flow cytometer. Cells that had not been incubated with fluorescein-labeled foldamers were used as a negative control. Cells incubated with fluorescein alone were also verified to be negative.

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