2-Dimensional, pH-responsive oligoglycine-based nanocarriers

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ABSTRACT: The nanocarrier capabilities of atomically smooth 2-dimensional sheets of a biantennary oligoglycine peptide $C_8H_{16}(-CH_2-NH-Gly_5)_2$ (also called tectomers) are demonstrated. We show that the pH-controlled, rapid and reversible assembly and disassembly of oligoglycine can be effectively used for controlled loading and release of the anticancer drug and fluorescent probe coralyne. The calculated partition coefficient in water is of the same order of magnitude or higher when compared to other nanocarriers such as liposomes and micelles, signifying the tectomer's impressive loading capabilities. Moreover, the loading of guest molecules in tectomers facilitates the protection from rapid photochemically-induced

degradation. Such efficient, pH-sensitive, stable and biocompatible nanocarriers are extremely attractive for biosensing, therapeutic and theranostic applications. Additionally, our results suggest that these planar self-assembled materials can also act as phase-transfer vehicles for hydrophobic cargoes further broadening their biomedical and technological applications.

1. INTRODUCTION

The development of new nanocarriers including micelles,^{1,2} liposomes,^{3,4} dendrimers,^{5,6} polymers,^{7,8} nanoparticles,^{9,10} functionalized carbon nanotubes (CNTs)¹¹⁻¹³ and graphene oxide (GO),^{14,15} has opened fascinating avenues in drug delivery, gene therapy and in a variety of other technological applications.¹⁶⁻²¹ In particular, nanocarriers that deliver drugs in response to specific stimuli (including temperature, redox potential, magnetic field, electrical field or pH) allow selective release of cargo and load, therefore offering enhanced performance.²²⁻²⁴ Most importantly, nanocarriers that combine efficient therapeutic and diagnostic functionalities have attracted considerable attention in the now flourishing area of theranostics.^{25,26}

Novel amino terminal oligoglycine assemblies, capable of spontaneous or surface-promoted formation of flat 2-dimensional (2D) structures have been recently reported.²⁷⁻²⁹ The stability of these assemblies is explained by the formation of a hydrogen bonded network, in polyglycine II (PGII) type architecture,³⁰ extended in two dimensions, and by the inaccessibility of water molecules to the internal regions of the assemblies (hydrophobic effect). This class of noncovalent assembly in a supramolecular compound has been termed tectomer²⁷⁻²⁹ with self-assembly and aggregation favoured when transitioned to a basic pH environment. Interestingly, though tectomer formation can take place in solution, it has been reported that the assembly

process proceeds two orders of magnitude more rapidly on a mica surface with the peptides capable of forming atomically smooth (2.6-4.3 nm in height) layers.³¹

Several biomedical applications for tectomers have already been suggested.^{29,32} For instance, enhanced antiviral activity could be achieved by multipoint binding of tectomers with viruses whereby the adsorption of the large glycopeptide sheet onto a viral surface may prevent eventual adhesion to cells. The absence of acute toxicity in mice has been reported for glycopeptide tectomers³³. More generally, tectomers capable of forming perfectly flat and rigid layers of a predetermined thickness appear promising for the design of new nanomaterials and as a platform for nanodevices.²⁸

In this report, we demonstrate for the first time that oligoglycine peptide self-assemblies can be used as pH-controlled nanocarriers. The chemical structure of the biantennary oligoglycine used is shown in Figure 1a. This oligoglycine has a $-(CH_2)_{10}-$ hydrophobic insertion in the center of the molecule, which determines its amphiphilic nature. For ease of discussion we use the term oligoglycine when referring to the chemical substance, and the term tectomer when referring to the 2D assembly (Figure 1b). In the literature oligoglycine-based bolaamphiphiles are described that have certain molecular structure similarity to our biantennary oligoglycine, but with dicarboxylic head groups at both ends.^{34,35} However, they assemble in a different fashion due to the electrostatic repulsion between ionized carboxylic groups, and they form microtubes and vesicles.

As a test case, we use coralyne, a hydrophobic anticancer drug. Coralyne is a planar, crescent-shaped molecule with a dibenzoquinolizinium skeleton (Figure 1c). It has been shown to have anti-inflammatory, antisecretory, antimicrobial, antimalarial, and antileukemic activity,

Figure 1. Chemical structure of a) biantennary oligoglycine, with a schema of tectomer assembly in solution b), and c) coralyne cation.

2. EXPERIMENTAL SECTION

2.1. Materials. Biantennary oligoglycine peptide $C_8H_{16}(-CH_2-NH-Gly)_2 \cdot 2HCl$, purity > 95%, was supplied by PlasmaChem GmbH and used without further purification. Coralyne chloride (2,3,10,11-tetramethoxy-8-methylisoquino[3,2-a]isoquinolinium chloride, 38989-38-7 CAS) was purchased from Aldrich, and was purified by recrystallization with methanol (spectroscopic grade). Coralyne solutions were always kept in darkness and prepared prior for use. β -carotene ($\geq 97.5\%$) and citrate buffer pH 3.0 were purchased from Aldrich. Buffer pH 7.4 was prepared from Na_2HPO_4 and NaH_2PO_4 (both from Aldrich, $\geq 99.0\%$) solved in ultrapure water (Siemens LaboStar DI/UV 2 system, resistivity: $18.2\text{ M}\Omega\cdot\text{cm}$ at $25\text{ }^\circ\text{C}$).

Oligoglycine solutions were bath sonicated for 2 min and then probe sonicated in pulsed mode (1 s on, 3 s off, amplitude: 21%) for 6 min (6 min of effective "on" sonication). pH of ultrapure water, buffers and the prepared oligoglycine solutions were measured by means of a Crison Basic 20 pHmeter. In the case of ultrapure water, KCl was added to obtain a reliable pH measurement, due to the low electrical conductivity.

2.2. Electron microscopy characterization. Transmission electron microscopy (TEM, TECNAI T20 microscope) was used to characterize oligoglycine peptide self-assemblies at several pHs. Samples for TEM imaging were prepared as follows: a droplet of the oligoglycine peptide solution was deposited onto carbon-coated copper grids (previously treated in a UV-ozone chamber for 5 min). The samples were allowed to dry under ambient conditions after wicking away most of the solution. In the case of samples in buffer pH 3.0, samples were also characterized staining with phosphotungstic acid or uranyl acetate to distinguish vesicular structures.

Samples were also characterized by cryo-TEM to image peptide assemblies as they are in solution. The vitrification process required for cryo-TEM was achieved in an FEI Vitrobot. Samples for cryo-TEM were prepared by plunging the carbon coated copper grids, containing the freshly deposited droplet of oligoglycine peptide solution, into liquid ethane at -170 °C, and the vitrified samples were transferred to a Gatan TEM cryo transfer holder with liquid nitrogen protection. Since organic samples are highly beam-sensitive, FEI's Low-Dose software was used during cryo-TEM observation to control the electron dose and therefore prevent beam damage on the samples.

Scanning electron microscopy (SEM) characterization of dried oligoglycine solutions deposited on Al foil was performed with a Hitachi S-3400 N microscope. Samples were placed on a conductive amorphous carbon tape, and coated with a 8 to 12 nm thick gold layer by sputtering.

Environmental scanning electron microscopy (ESEM) was also used here as it offers the ability to image liquid samples by SEM without prior sample preparation (no conductive coating is required). A Quanta FEG 250 ESEM microscope (FEI Company) was used to collect micrographs of the prepared samples. The chamber pressure and sample temperature were set at 650 Pa and 1°C, respectively, which leads to a 100% relative humidity.

2.3. Atomic force microscopy. Atomic force microscopy (AFM) study was performed using a NTEGRA AFM microscope (NT-MDT), operating in semi-contact mode. AFM probes (Nanosensors™) with a force constant ranging from 10 N·m⁻¹ to 130 N·m⁻¹ were used. All images were corrected for the tilt of the sample and background subtraction was employed. One drop of the prepared oligoglycine peptide solutions was spin-coated onto a freshly cleaved mica

substrate (at 3000 rpm for 7 seconds). After 5 seconds of sample deposition, substrate was rotated twice again at the same speed and time, to remove excess of material.

2.4. Zeta-potential characterization. Zeta-potential measurements of oligoglycine self-assemblies aqueous solutions, without and with coralyne ($10 \mu\text{g}\cdot\text{mL}^{-1}$), were performed using Zetasiser Nano ZS equipment (Malvern), based on electrophoretic light scattering technology. The refractive index and viscosity of the solvent were properly taken into account within the instrument software manager.

2.5. Absorption and fluorescence spectra. Absorption UV-vis spectra were recorded on a Shimadzu UV-1800 spectrophotometer. In these experiments coralyne concentration used was $3 \mu\text{g}\cdot\text{mL}^{-1}$.

The interaction between the fluorophore and oligoglycine assemblies was quantified by measuring the changes in coralyne fluorescent optical properties. Steady-state fluorescence measurements were performed with a Yobin Ivon FluoroMax-P spectrophotometer (Horiba). The option S/R was used to correct lamp profile or temporal fluctuations (R is a reference detector included in the instrument). The excitation wavelength was set to 410 nm and the emission covered the spectra from 400 to 600 nm (maximum emission 476 nm). Slits were set at 2 nm for the excitation and emission. In this kind of studies, the interaction of a compound with assemblies is usually followed by adding a stock solution successively to an aqueous solution of the compound in study and, after each addition, the spectroscopic signal is collected to monitor the interaction. However, due to the high susceptibility of coralyne to photochemical degradation, here several dilutions of a stock solution of oligoglycine peptide were prepared in different samples. Then, a constant volume from coralyne stock solution was added to each of

these samples in which tectomer assemblies were already formed. After an incubation time of 30 min in the dark and at room temperature 22°C, the fluorescence signal of each sample was monitored as a function of the oligoglycine peptide concentration up to 1 mg·mL⁻¹. Fluorophore concentration was kept constant as low as 0.1 µg·mL⁻¹ in all samples to minimize dimer formation, which would complicate partition coefficient calculation. Background dispersion intensities in coralyne-free samples were subtracted from each recording of fluorescence intensity. On the other hand, in order to obtain enhanced loading efficiency, a second sample preparation procedure was tried: different amounts of oligoglycine were dissolved in a coralyne solution in the same incubation and concentration conditions as mentioned above, thus formation of tectomers took place in the presence of coralyne.

These studies were performed in ultrapure water and in buffer pH 3.0. When using pH 7.4 buffered solutions, as the oligoglycine is not soluble in these conditions, the oligoglycine peptide was first dissolved in a solution of coralyne in water and then the buffer was added to the solution to reach pH 7.4. After allowing the dispersion to settle for 5 hours, supernatant and sediment were characterized.

2.6. Time-resolved fluorescence measurements. Time-resolved fluorescence measurements were performed with an Edinburgh Instruments FLS920 fluorescence lifetime spectrometer using the time-correlated single-photon counting technique. The excitation lamp was a picosecond EPL-375 Laser, with emission wavelength 374.6 nm. The resulting signal was detected using a R928P photomultiplier tube. Fluorescence intensity decay curves were analyzed by nonlinear least-squares regression, fitting the data to a sum of exponentials as expressed by Equation 1:

$$I(t) = \sum_i \alpha_i \exp(-t/\tau_i) \quad (1)$$

where α_i is the normalized amplitude ($\sum_i \alpha_i = 1$) of the i th component associated with fluorescence lifetime τ_i . The fractional intensity, f_i , of the i th decay component is given by Equation 2:

$$f_i = \frac{\alpha_i \tau_i}{\sum_i \alpha_i \tau_i} \quad (2)$$

Tail-fitting using either the mono- or biexponential model was applied, using FAST (fluorescence analysis software technology) decay analysis software.

2.7. Confocal fluorescence and brightfield microscopy. 1 mg mL⁻¹ oligoglycine solutions containing 0.1 µg·mL⁻¹ fluorophore were analysed by optical microscopy. Confocal fluorescence and brightfield images were obtained using a Leica SP5 laser scanning confocal microscope equipped with spectral HyD detectors. Confocal acquisition using a 63x 1.4 NA HCX PL APO oil immersion objective allowed fluorescence to be captured as discrete ~600 nm optical sections at different focal planes. Adjustments to the spectral emission detection window allowed loaded fluorophore self-assemblies to be distinguished from background fluorescence in solution (laser excitation = 405 nm; fluorescence emission window = 441-555 nm). We confirmed that peptide samples in absence of fluorophore did not emit detectable levels of fluorescence under these conditions.

3. RESULTS AND DISCUSSION

3.1. The effect of pH on the oligoglycine assembly. As can be seen in the TEM images in Figure 2a and b, the formation of 2D assemblies takes place in water. The platelets formed can be several microns in size. Electron diffraction patterns collected during TEM imaging provide evidence of their crystalline nature (Figure 2c). TEM characterization was also performed on a sample corresponding to a liquid solution stored at room temperature for 2 months, and similar images were obtained to the fresh sample, indicating high stability of tectomers in solution with respect to aging. Cryo-TEM (Figure 2d) and ESEM (Figure 2e) studies additionally verify that the self-assembly already occurs in solution. Moreover, the drift of the baseline position in UV-vis spectra observed upon increasing the oligoglycine concentration (Figure S1) due to light scattering also supports this. It should be noted, that the pH values are dependent on oligoglycine concentration in water, as HCl is also present in the solution (HCl comes from the protocol of synthesis of oligoglycines, see Table S1 in the Supporting Information).

SEM provides additional evidence of the planar assembly of oligoglycine after drying on a substrate, as shown in Figure 2f. AFM was used to study the morphology of the platelets deposited on a mica substrate. As can be seen, these assemblies form multilayered stacks (Figure 2g,h) each of them ~ 5.6 nm high (Figure 2i). It is very interesting to note, that the 2D structure and size of these assemblies resembles that of graphene and inorganic layered materials exfoliated in solution.^{48,49} As one of its dimension (thickness) is in the nanoscale, tectomers can be therefore considered as nanomaterials.^{50,51}

Figure 2. a) and b): TEM micrographs of tectomers that result from $1 \text{ mg} \cdot \text{mL}^{-1}$ oligoglycine solutions in water (pH 5.6), and c) electron diffraction pattern of 2D assembly; d) cryo-TEM and e) ESEM micrographs of tectomers in $1 \text{ mg} \cdot \text{mL}^{-1}$ oligoglycine water solutions; f) SEM image of tectomer assemblies dried on Al foil; g-i) AFM characterization of tectomers prepared from $1 \text{ mg} \cdot \text{mL}^{-1}$ oligoglycine solutions in water deposited on mica: g) phase, h) topography images, and i) corresponding line profile showing $\sim 5.6 \text{ nm}$ thick individual tectomer platelets.

In acidic solutions (pH 3.0), the hydrophilic oligoglycine antennae are repulsed due to the positive charge on the abundant protonated amino groups, which hinders tectomer assembly or fully prevents it. Sufficiently large repulsive forces favour a curved surface over a planar one in which the headgroups are more closely spaced. The driving force for the formation of spontaneous vesicles would be the asymmetric distribution of charge between the inner and outer monolayer.⁵² TEM studies reveal that vesicular structures, 40-70 nm in diameter, instead of tectomers, are formed at pH 3.0 (Figure 3). Uranyl acetate or phosphotungstic acid were used as staining agents (Figure 3b and c). The shape of vesicles appeared distorted due to the alterations induced during the drying steps, possible artefacts due to the interaction between the sample and the stain, and exposition of samples to vacuum.⁵³ The thickness of the stained vesicular structure is ~ 4 nm.

Figure 3. Low resolution a), and high resolution b) TEM characterization of $1 \text{ mg} \cdot \text{mL}^{-1}$ oligoglycine solutions at pH 3.0, that shows the presence of vesicular structures, using uranyl acetate b) or phosphotungstic acid c), as staining agents.

Figure 4 shows UV-vis spectra of oligoglycine solution in buffer pH 7.4. The pH shifting of the oligoglycine solutions towards alkaline triggers massive aggregation due to deprotonation

of terminal amino groups. In this environment, the solution immediately becomes cloudy and tectomers eventually precipitate out of solution (Figure 4b). It was determined by UV-vis spectroscopy that the remaining oligoglycine concentration in the supernatant after 5 hours was only 2% of the initial one. It is worth noting that the UV-vis spectra of the supernatant obtained after 5 hours and that collected after 24 hours were coincident, which indicates that precipitation is almost complete after 5 hours. Very interestingly, oligoglycine self-assembly and disassembly is fully and immediately reversible by changing the pH (Movie S1).

Figure 4. a) $1 \text{ mg} \cdot \text{mL}^{-1}$ oligoglycine solution in buffer pH 7.4. TEM images of the supernatant A and of the sediment B that settles out after 5 hours are shown in the insets. b) UV-vis spectrum of the resulting supernatant is compared to those of oligoglycine solutions in water (a= $1 \text{ mg} \cdot \text{mL}^{-1}$, b= $0.4 \text{ mg} \cdot \text{mL}^{-1}$, c= $0.1 \text{ mg} \cdot \text{mL}^{-1}$, d= $0.07 \text{ mg} \cdot \text{mL}^{-1}$, e= $0.05 \text{ mg} \cdot \text{mL}^{-1}$, f= $0.04 \text{ mg} \cdot \text{mL}^{-1}$, g= $0.02 \text{ mg} \cdot \text{mL}^{-1}$, h= $0.01 \text{ mg} \cdot \text{mL}^{-1}$), which allows to estimate that only 2% of the starting oligoglycine concentration remains in the supernatant at pH 7.4.

3.2. pH-controlled coralyne loading and release. Due to the amphiphilic nature of the biantennary oligoglycine used in this study, as well as to its pH-sensitive assembly and disassembly, we then explored if tectomers could be efficiently used as nanocarriers of the hydrophobic anticancer drug coralyne. Coralyne, as shown in Figure 1c, is a quaternary ammonium salt that bears a permanent positive charge at all pH values. Coralyne is significantly hydrophobic in character due to the conjugated aromatic ring system. As a consequence, non-electrostatic interactions are essential^{44-46,54-57}. Thus, coralyne, whose positive charge is spread over a conjugated ring system⁵⁸, will have a tendency to penetrate into organized assemblies with hydrophobic cores such as the biantennary oligoglycine. The hydrophobic effect together with van der Waals interactions will play a major role as driving force for coralyne loading in biantennary oligoglycine tectomers.

Zeta-potential measurements provide valuable information relating to the surface electrical charge of particles⁵⁹. 1 mg·mL⁻¹ oligoglycine solutions in water led to a zeta-potential value of +16 mV, which can be explained by partial protonation of the amine groups. The zeta-potential increases up to +30 mV upon addition of coralyne at 15 µg·mL⁻¹ which is indicative of the successful incorporation of coralyne in the tectomer structure.

The effective coralyne loading within the tectomers is evidenced by confocal fluorescence microscopy (Figure 5). Bright field images depicted in Figure 5a demonstrate that oligoglycine self-assembly into tectomers occurs in solution and images in Figure 5b provide further evidence of effective coralyne loading within the tectomers.

Figure 5. Bright field microscopy a, c) and confocal fluorescence microscopy images of tectomer b, d) corresponding to $1 \text{ mg} \cdot \text{mL}^{-1}$ oligoglycine solution in water, with coralyne at $0.1 \text{ } \mu\text{g} \cdot \text{mL}^{-1}$.

The increase in fluorescence intensity for coralyne in the presence of tectomers is shown in Figure 6. As coralyne is loaded in a more rigid and hydrophobic microenvironment, hindering alternative relaxation mechanisms that occur in polar solvent, the non-radiative decay constant will be decreased. It has also been suggested by Toptygin⁶⁰ that the radiative decay rate can be increased due to increases in the local refractive index. Both contributions may explain the increased fluorescence emission intensities obtained for coralyne in the presence of tectomers.

Our previous research on fluorophores showed that increases in fluorescence emission intensity were induced by a wide number of compounds with long aliphatic chains.^{61,62} We have used this for the detection and quantification of alkanes (ng) and other nonabsorbing molecules

in real matrices (*e.g.*, lipids) by thin-layer chromatography (TLC) using fluorophore-impregnated silica gel plates.^{63,64}

Upon repeating the collection of fluorescence emission spectra in water several times, it was observed that photochemically-induced damage was significantly lower for solutions in which coralyne was in the presence of oligoglycine compared to coralyne-only (Figure S2), therefore indicating that coralyne molecules are incorporated and protected in tectomer assemblies.

The partition coefficient of the fluorescent molecule between self-assembly and aqueous phases is defined as

$$K_p = \frac{[C]_a}{[C]_w} \quad (3)$$

where $[C]_a$ and $[C]_w$ are the coralyne molar concentrations in the assemblies and free in the aqueous solution, respectively. Fluorescence emission intensity measurements can be used to calculate K_p by means of the following equation^{65,66}

$$\Delta I = \frac{\Delta I_{\max} [A]}{1/(K_p \gamma) + [A]} \quad (4)$$

In this equation, $\Delta I = I - I_0$ stands for the difference between the fluorescence intensity of coralyne measured in the presence (I) and in the absence of oligoglycine (I_0); $\Delta I_{\max} = I_{\infty} - I_0$ is the maximum value of ΔI , as I_{∞} is the limiting value of I which would be obtained if all the partitioning molecules were loaded in the assembly; $[A]$ is the oligoglycine concentration and γ is the molar volume of the self-assembly phase; $\Delta I_{\max}$ and K_p can be calculated by fitting the ΔI vs. $[A]$ plot by a nonlinear regression routine.

Figure 6. Fluorescence emission spectra and the corresponding I_0/I plots at $\lambda_{\text{max}} = 476$ nm of coralyne/oligoglycine solutions in water, following two different procedures: a, b) addition of coralyne to oligoglycine solutions at increasing concentrations (pre-formed assemblies), and c, d) increasing amounts of oligoglycine solved in coralyne solutions, so that tectomers form in the presence of coralyne. Coralyne concentration was $0.1 \mu\text{g} \cdot \text{mL}^{-1}$ in all samples. Fitting experimental data using Equation 4 allows partition coefficient calculation.

Changes in fluorescence intensity as a function of oligoglycine concentration in water are shown in Figure 6a,b. A series of fluorophore-oligoglycine solutions containing different amounts of oligoglycine peptide has been prepared, keeping the coralyne concentration constant at $0.1 \mu\text{g} \cdot \text{mL}^{-1}$ (see Experimental Section), so that coralyne internally incorporates to the pre-

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3 formed tectomer. A partition coefficient value $K_p = 5 \times 10^3$ has been calculated from data at $\lambda_{\max}$
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6 = 476 nm. This partition coefficient value is the same order of magnitude calculated for coralyne
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8 incorporation into 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) liposomes ($K_p = 6 \times 10^3$)
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10 and more than one order of magnitude higher than incorporation to n-dodecylphosphocholine (DPC)
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12 micelles ($K_p = 3 \times 10^2$).⁵⁵ Interestingly, if the tectomers are formed directly in the presence of
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14 coralyne considerably enhanced loading efficiency is achieved ($K_p = 1 \times 10^4$) (Figure 6c,d).
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17 Moreover, the loading of coralyne is more effective at pH 7.4 (Figure 7) due to the extended
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19 oligoglycine assembly as well as the fact that the deprotonation of tectomers leads to diminished
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21 electrostatic repulsion relative to the coralyne. Note that under these conditions, tectomers
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23 rapidly sediment out of solution. On the other hand, at pH 3.0, the positive surface of protonated
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25 amino groups in oligoglycine vesicular assemblies prevents the incorporation of coralyne, and it
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27 was observed that the fluorescence of coralyne was not altered by the addition of oligoglycine at
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29 pH 3.0 (Figure S3).
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34 As explained in the Experimental Section, the fluorophore concentration was kept
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36 constant at as low as $0.1 \mu\text{g} \cdot \text{mL}^{-1}$ in all samples to minimize dimer formation, which would
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38 complicate partition coefficient calculations. It is known that coralyne, even at low
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40 concentrations, undergoes self-aggregation in aqueous solutions by stacking interactions^{56,67} and
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42 the fluorescence quantum yield of the dimer is lower than that of the monomer.⁶⁸ Fluorescence
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44 time-resolved experimental results confirm this and are shown in the Supporting Information
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46 (Figure S4).
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51 The high affinity of coralyne for the tectomer coupled with the reversible assembly and
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53 disassembly capabilities of the latter allow for controlled loading and release of coralyne which
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55 is easily stimulated by change of pH (Figure 7). We performed two experiments in parallel. In (I)
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we added deionized water and in (II) we added a buffer solution pH 7.4 respectively to a solution of coralyne ($3 \mu\text{g}\cdot\text{mL}^{-1}$) and oligoglycine ($2 \text{ mg}\cdot\text{mL}^{-1}$) in water pH 5.2. The addition of the buffer solution caused immediate precipitation of tectomers loaded with coralyne (II). After 5 hours, the UV-vis spectrum clearly shows a reduced amount of coralyne in the supernatant compared to (I). Finally, pH 2.2 buffer solution was added to both (A) and (B) to reach a final pH 3.0 (solution A' and B'). In the case of (B) the oligoglycine tectomers immediately disassemble, releasing coralyne back into the solution. Figure 7c shows that the absorbance intensity of solution B' is coincident to that of solution A'. The amino terminal groups of oligoglycine at pH 3.0 are strongly protonated leading to complete drug release. At pH 7.4, drug-loaded tectomers could be isolated for example by lyophilization from solution or could be prepared by any of the well-known pharmaceutical methods. These results clearly point out that tectomers offer promise for their use as carriers for pH-controlled drug delivery applications.

Figure 7. pH-controlled loading and release of coralyne a), monitored by absorption UV-vis spectra b, c). Pictures show that, starting from coralyne/oligoglycine solution in water, if pH is increased to 7.4, massive oligoglycine aggregation occurs and tectomers finally fell out of solution (B). The sediment was then redissolved (B') upon addition of pH 2.2 buffer.

3.3. Tectomers as phase transfer vehicles for β -carotene. Oligoglycine self-assemblies can also be used as phase-transfer vehicles to improve solubility of highly hydrophobic compounds in aqueous media opening new and attractive avenues for a range of applications. Figure 8 shows the oligoglycine-assisted transfer of β -carotene from a nonpolar, organic phase to an aqueous phase. In this experiment, β -carotene was dissolved in dichloromethane at $1.5 \text{ mg} \cdot \text{mL}^{-1}$. Even after agitation, no transfer to the upper water phase occurred (Figure 8a). However if oligoglycine was present in the aqueous phase at $1 \text{ mg} \cdot \text{mL}^{-1}$, an amount of β -carotene is transferred from the organic phase (Figure 8b). As β -carotene is hydrophobic, it has a high affinity for the hydrophobic core of the tectomer thus facilitating transfer from the organic to the aqueous phase. The upper phase was separated and split into two vials, to which buffer solutions were added. Upon addition of buffer pH 3.0, no β -carotene precipitates (Figure 8c), indicating the load remains unreleased. On the other hand, if pH 7.4 buffer solution is added (Figure 8d), β -carotene loaded tectomers sediment out.

Figure 8. Solubilization of β -carotene in oligoglycine aqueous solution (a, b: bottom layer, dichloromethane; upper layer, water). a) Aqueous layer without oligoglycine, and b) with oligoglycine at $1 \text{ mg} \cdot \text{mL}^{-1}$, in which it can be observed β -carotene transferred from organic to aqueous phase after 24 hour. Then, the upper layer containing oligoglycine and β -carotene in b) was removed and split into two different vials, to which c) pH 3.0-, or d) pH 7.4-buffer solutions were added.

3.4. Discussion. As assembly/disassembly of the tectomer is highly sensitive to pH modulation, these nanocarriers are promising candidates for site-specific controlled drug delivery. The loading of guest molecules may be achieved at neutral pH, meaning increased solubility coupled to enhanced protection from rapid degradation. Drug release would occur in an acidic pH environment, for instance in the stomach ($\text{pH} < 3$). Also it is known that some pathological tissues (tumors, inflamed and infected tissue) have a more acidic environment compared to normal tissues.⁶⁹ As hydrophobicity has been an obstacle that hinders the practical use of many anticancer drugs, the use of oligoglycine peptides as effective biocompatible delivery system for hydrophobic therapeutic anticancer drugs is of interest (to name a few examples, coralyne, nitidine, fagaronine, camptotecine, flavopereirine, ellipticine, acridine and derivatives). Also, as the tectomer/coralyne system reported here provides both therapeutic and sensor functionalities, these pH-sensitive nanocarriers may be attractive in theranostics. However, much work, involving *in vitro* and *in vivo* studies, still needs to be conducted to fully evaluate their potential biomedical applications.

The effect of particle shape on drug carrier performance has recently gained more attention because of its influence on tissue biodistribution and endocytic uptake.⁷⁰⁻⁷² The planar

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3 shape of tectomers creates new opportunities for therapeutic and technological applications. Also
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5 tectomer stability with respect to aging in solution is very high, in contrast to liposomes, which
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7 tend to aggregate and fuse, which results in leakage, and methods are needed to improve
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9 stabilization with respect to aging.^{73,74} Contrary to hydrogels, whose most significant weakness is
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11 that their response time is too slow,²¹ tectomer assemblies are rapidly formed or destroyed. The
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13 performance might be further improved by exploiting the chemical functionalization of the
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15 amino termini of the oligoglycines both as the monomer stage or as assembled tectomer,²⁹ to
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17 target certain tissues or surfaces.
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27 4. CONCLUSIONS

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30 2-Dimensional oligoglycine assemblies act as efficient, pH-responsive nanocarriers. The pH-
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32 controlled rapid and reversible assembly and disassembly of oligoglycine has been used for the
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34 controlled loading and release of the anticancer drug and fluorescent probe coralyne. The
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36 associated partition coefficient values are similar or higher in magnitude compared to other
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38 nanocarriers, such as liposomes and micelles, suggesting that these assemblies have competitive
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40 loading capabilities. Moreover, tectomers in solution exhibit remarkable stability with respect to
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42 aging. The planar shape of tectomers may be advantageous in the fields of drug delivery and
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44 material science as contact is maximized with cells and surfaces. A whole plethora of biomedical
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46 and technological applications can be envisaged for these nanocarriers that can also act as phase-
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ASSOCIATED CONTENT

Supporting Information.

This material is available free of charge via the Internet at <http://pubs.acs.org>.

pH measurements of oligoglycine solutions; absorption spectra of coralyne at increasing oligoglycine concentrations; photochemically-induced damage study; fluorescence emission spectra in buffer pH 3.0; time-resolved fluorescence measurements; movie showing rapid assembly or disassembly of tectomers as a function of pH.

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