Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

Metal-Chelated Polymer Nanodiscs for NMR Studies

*Nathaniel Z. Hardin[+], Vojč Kocman[+], Giacomo M. Di Mauro, Thirupathi Ravula, and Ayyalusamy Ramamoorthy**

ange_201910118_sm_miscellaneous_information.pdf

Supporting Information

Materials and methods

Poly(Styrene-co-Maleic Anhydride) cumene terminated ~1.3:1 styrene-to-maleic anhydride molar ratio (SMAnh, M_n ~1600 g/mol), Triethylamine (Et_3N), potassium chloride (KCl), potassium phosphate monobasic (KH_2PO_4) and potassium phosphate dibasic (K_2HPO_4) , sodium chloride (NaCl), hydrochloric acid (HCl), sodium hydroxide (NaOH), trifluoracetic acid (TFA), diethyl ether (Ether), N-methyl-2-pyrrolidone (NMP) were purchased from Sigma-Aldrich®. 2-Aminoethyl-mono-amide-DOTA-tris(t-Butyl ester) was purchased from Macrocyclics®, 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) was purchased from Avanti Lipids Polar, Inc®, DNA was purchased from Integrated DNA Technologies, Inc.

Synthesis of SMA-EA-DOTA

1 g of SMAnh was dissolved in 40 mL of NMP then 435 mg (1 eq per chain) of 2-Aminoethyl-mono-amide-DOTA-tris(t-Bu ester) was added to the solution, then 1 mL of trimethylamine was added. The reaction mixture was then stirred at 80 °C for 2 hours. Then 0.4 mL of ethanolamine and another 1 mL trimethylamine was added to the solution and the solution was heated at 80 °C for 2 hours. The solution was cooled to room temperature, precipitated with HCl and washed 3x with water. The resulting compound was deprotected using 30 ml TFA for 2 hours at room temperature and precipitated using diethyl ether. The precipitate was washed 3 times with diethyl ether and dried under vacuum.

Nanodiscs preparation and purification

20 mg DMPC was suspended in 10 mM potassium phosphate, 100 mM potassium chloride buffer (pH 7.4). 60 mg SMA-EA-DOTA was added and the solution was diluted to 3 mL and incubated overnight at 32 °C. The nanodiscs solution was then purified using self-packed Superdex 200, 10/600 GL column operated on an AKTA purifier (GE Healthcare, Freiburg, Germany). Samples were monitored at 254 nm and the first peak was collected (Figure 1a) and concentrated to 1 mL for use in NMR studies.

Static light scattering (SLS)

SLS experiments were performed by observing scattered radiation at 90° through a 1 cm quartz cuvette using an excitation wavelength of 400 nm and emission wavelength of 404 nm at 25 °C. All SLS experiments were obtained on a FluoroMax-4® Spectrofluorometer from Horiba Scientific®.

pH stability measurements

A solution made by 1 mg/mL SMA-EA-DOTA:DMPC (1:1 w/w) nanodiscs was titrated both with 1M HCl and 1M NaOH and results were monitored with SLS . Results are shown in Figure S2.

SLS metal ion titrations

Nanodiscs stability was tested by titrating a 1 mg sample of 1:1 w/w nanodiscs in pH 7.4, 10 mM HEPES buffer with 2 M $CuCl_2$.

Transmission Electron Microscopy (TEM)

TEM data was acquired on a Technai® T - 20® machine (FEI®, Netherlands) using an operating voltage of 80 kV.

Dynamic Light Scattering (DLS)

DLS was performed using a Wyatt Technology® DynaPro® NanoStar® using a 1 µL quartz MicroCuvette.

Fourier-Transform Infrared (FT-IR) Spectroscopy

The FT-IR spectra from 4000 to 800 cm^{-1} were recorded using a Thermos scientific ATR-FTIR instrument. Lyophilized powder samples of polymers were used to record the spectra SMA-EA was obtained as previously reported.[1]

CPMAS solid-state NMR experiments

Carbon-13 CPMAS experiments were carried out on a Bruker 500 MHz solid-state NMR spectrometer under 12 kHz MAS using a 3.2 mm triple-resonance MAS probe operating at 500.112 MHz and 125.721 MHz for ^{1}H and ^{13}C nuclei, respectively, and using a 3.2 mm zirconia (ZrO_2) rotor. The reported ^{13}C CPMAS spectra were acquired using 3 µs 90° pulse, 2 ms CP contact time, 20 ms acquisition time, 3072 scans, 3 s recycle delay and a 58 kHz radio-frequency decoupling of protons during acquisition. ^{13}C chemical shifts were calibrated using adamantine.

Spin Inversion Recovery ^{1}H NMR

NMR spectra were recorded using a 500 MHz NMR spectrometer equipped with a Bruker TXI probe. A basic inversion recovery experiment using an excitation sculpting employing soft selective pulses as described elsewhere.[2] Experiments were run using 16 scans per FID for nanodiscs samples and 256 scans for samples containing DNA, and between 11 and 15 FIDs were used for a single T_1 experiment. Spectral width was ~6,000 Hz for nanodiscs without DNA and ~10,000 Hz for all DNA containing samples. 90° pulses were set between 9 and 11 µs with a RF power level of ~15 W. The transmitter frequency was set to the bulk water-proton resonance.

NMR DNA sample preparation and purification

The oligonucleotide wtTel23 with the oligonucleotide sequence 5'-TAGGG(TTAGGG)$_3$-3' was purchased from Integrated DNA Technologies, Inc. Samples were purified and desalted with the use of a 3 kDa centrifugation filter to give a stock solution with the concentration of 0.6 mM per strand. An NMR sample with concentration of 0.1 mM per strand and in the presence of 100 mM KCl with a pH value of about 7 was prepared.

NMR Data analysis

NMR data were analyzed and plotted using Bruker® Topspin® version 3.5 pl 6 and Mestrelab Research® S.L. MestReNova® version 12.0.4-22023.

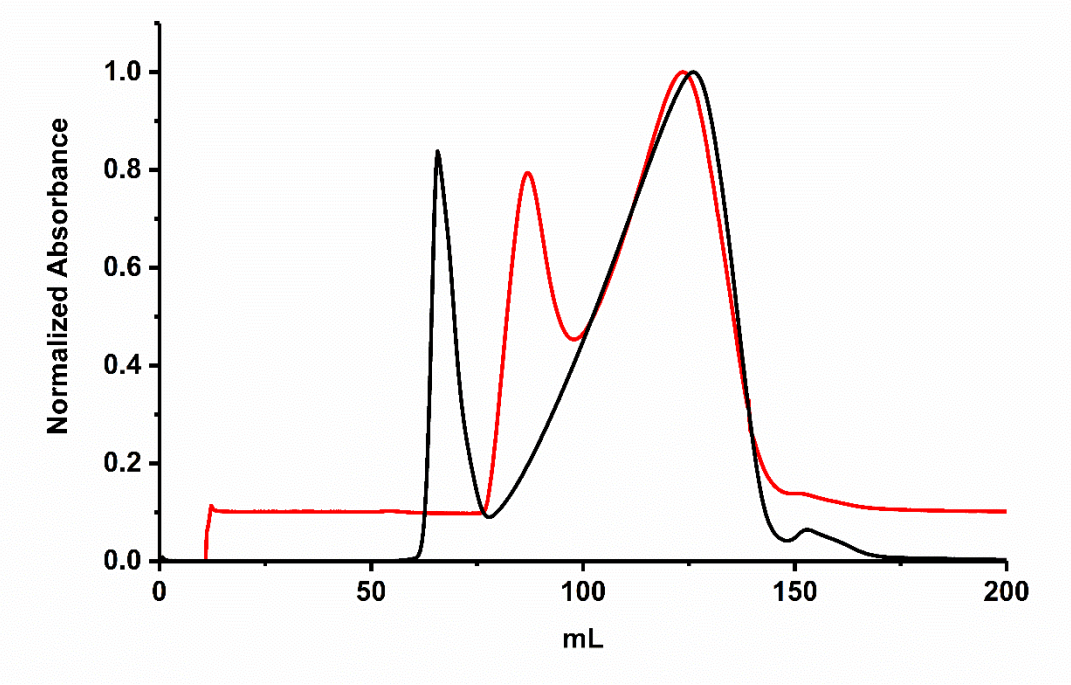

Figure S1: Size exclusion profiles of SMA-EA-DOTA nanodiscs. Black line represents nanodiscs prepared with a polymer:DMPC weight ratio of 1:1, red line is nanodiscs prepared with a polymer:DMPC weight ratio of 3:1.

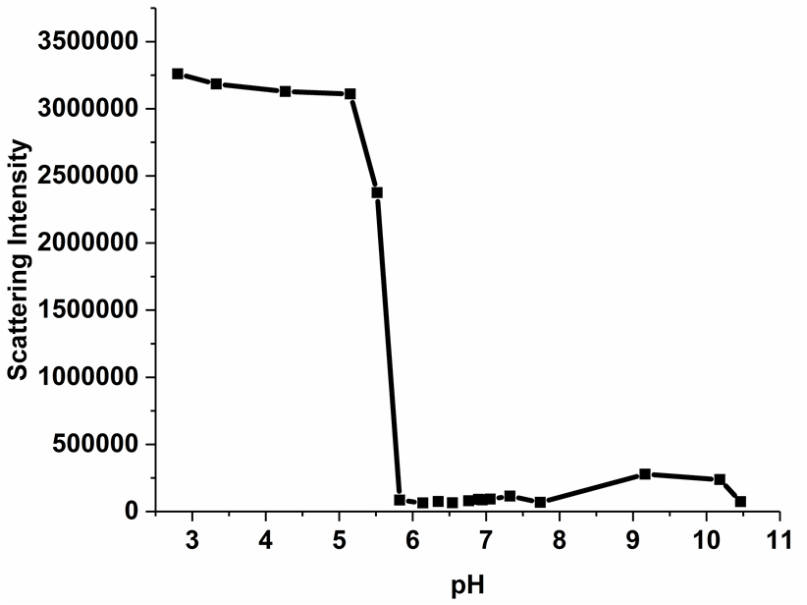

Figure S2: Scattering intensity of a SMA-EA-DOTA nanodiscs at different pH values.

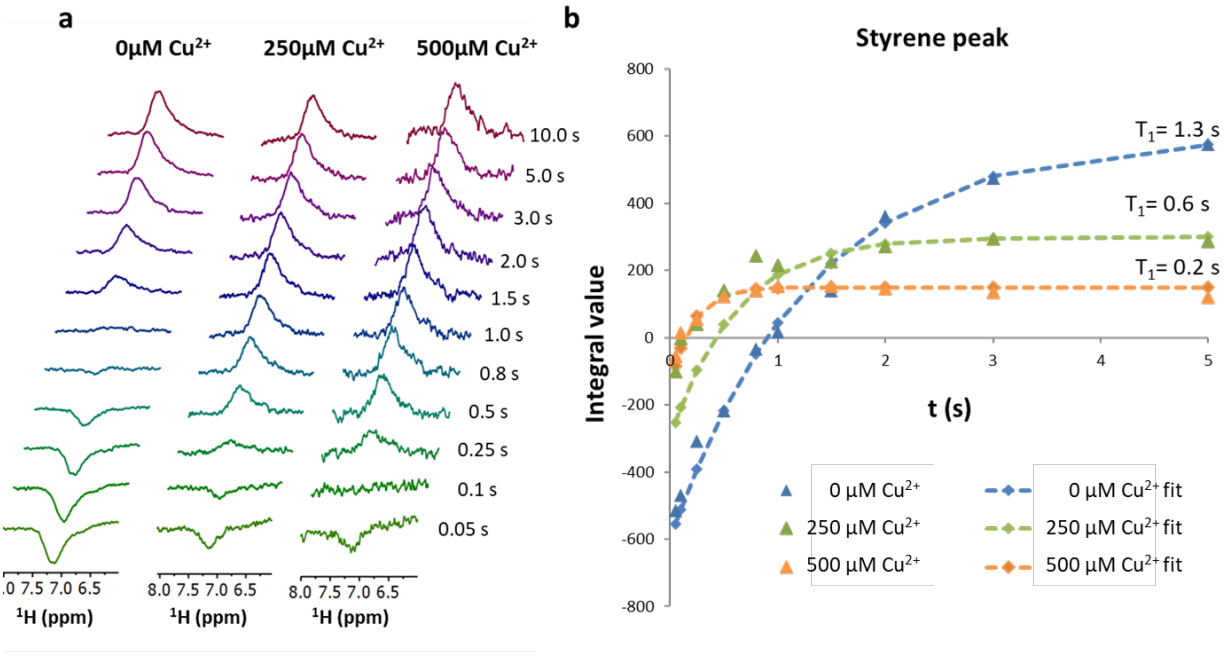

Figure S3: a) Inversion recovery experimental spectra obtained at different Cu^{2+} concentrations focused on the styrene peak. b) Fitting of the styrene peak inversion recovery experiment data and determination of T_1 times. Experiments were conducted at 0, 250 and 500 μM concentrations. The triangles represent the data and the dashed lines are the fits. The spectra were recorded on a 500 MHz NMR spectrometer at 25 °C.

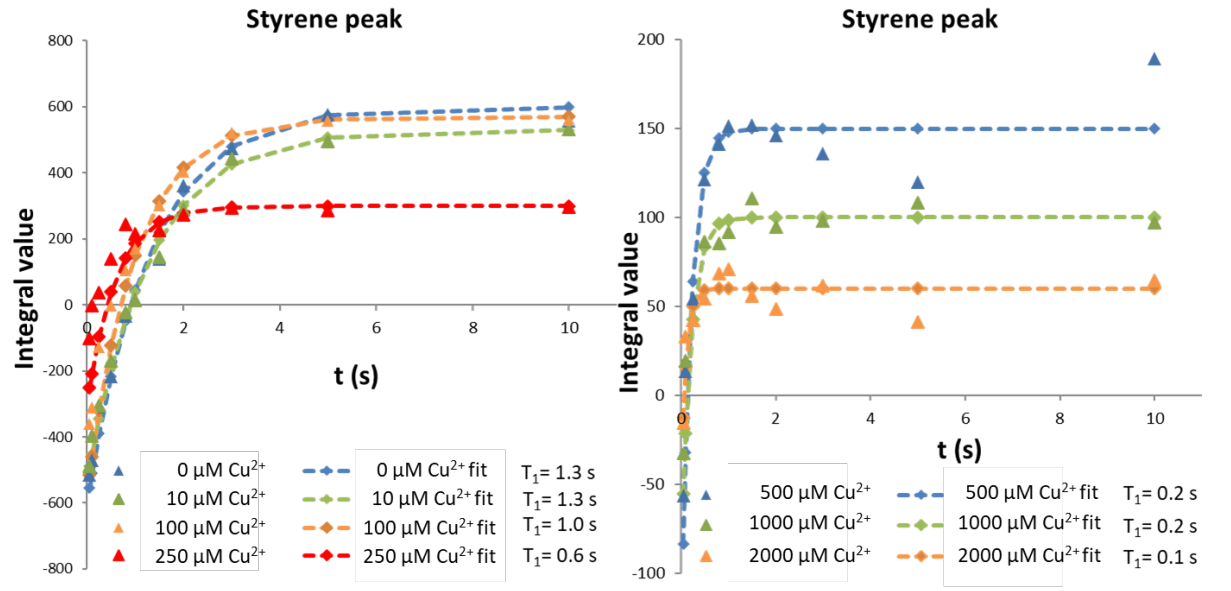

Figure S4: All the fitting for the styrene peak inversion recovery experiment data recorded on polymer nanodisc samples with concentrations of Cu^{2+} varying between 0 and 2000 μM. The triangles represent the data and the dashed lines are the fits. The spectra were recorded on a 500 MHz NMR spectrometer at 25 °C.

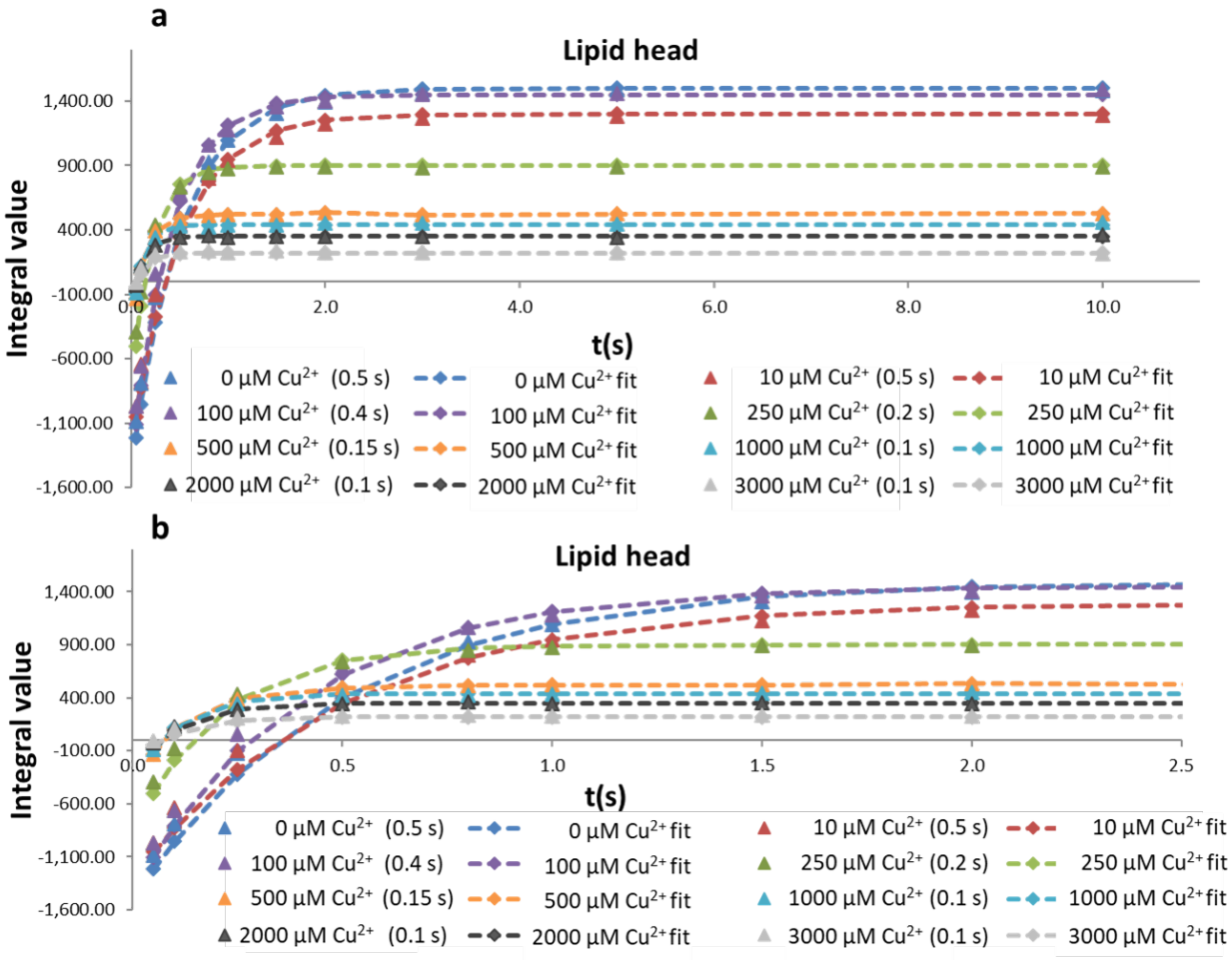

Figure S5: a) All the fitting for the styrene peak inversion recovery experiment data recorded on polymer nanodisc samples with concentrations of Cu^{2+} varying between 0 and 3000 μM. The triangles represent the data and the dashed lines are the fits. b) A close-up focus on the data between 0 and 2.5 s so the fitting can be more clearly seen. The spectra were recorded on a 500 MHz NMR spectrometer at 25 °C.

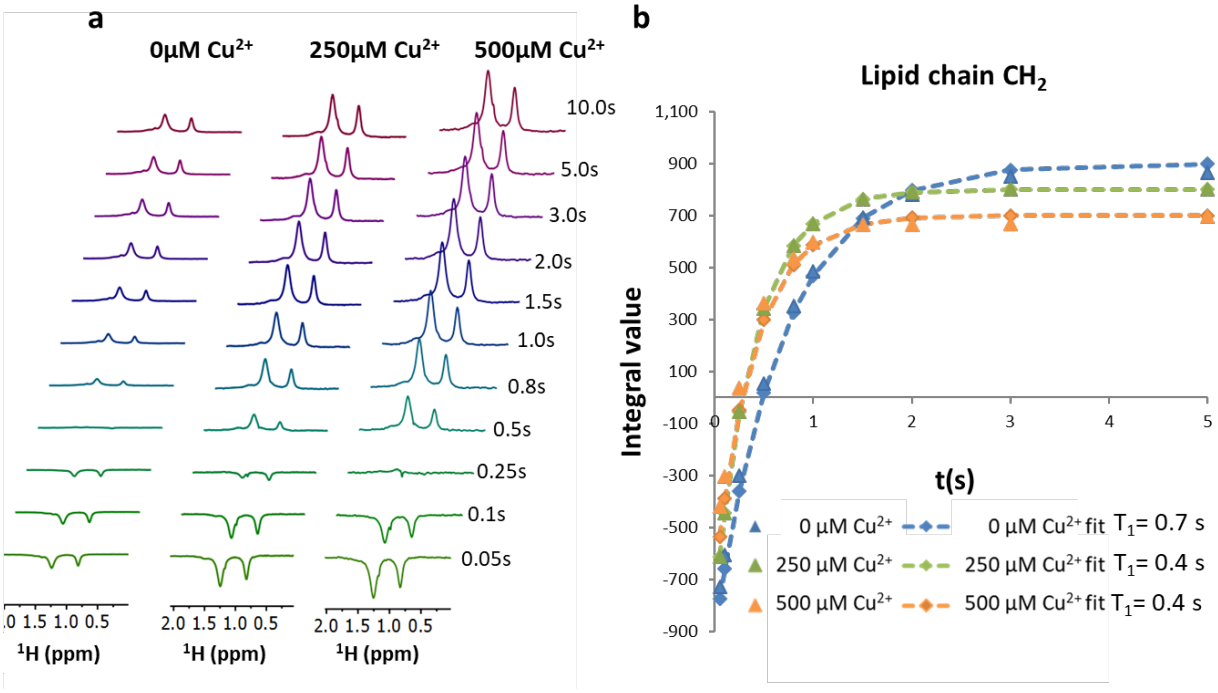

Figure S6: a) Inversion recovery experiments at different Cu^{2+} concentrations focused on the lipid chain CH_2 peak. b) Fitting of the lipid chain CH_2 peak inversion recovery experiment data and determination of the T_1 times. The experiments were conducted using 0, 250 and 500 μM Cu^{2+} concentrations. The triangles represent the data and the dashed lines are the fits. The spectra were recorded on a 500 MHz NMR spectrometer at 25 °C.

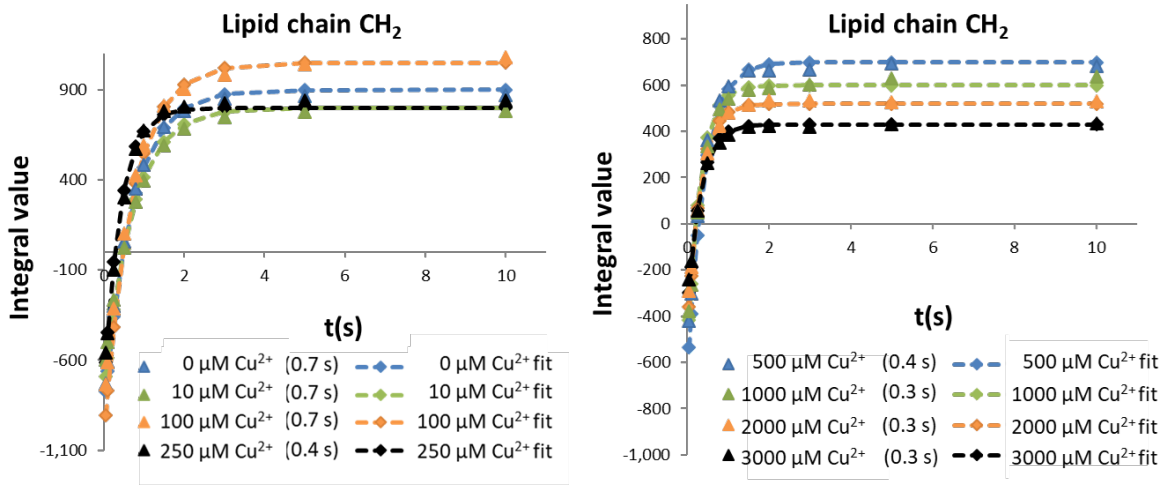

Figure S7: All the fitting for the lipid chain CH_2 peak inversion recovery experiment data recorded on polymer nanodisc samples with concentrations of Cu^{2+} varying between 0 and 3000 μM. The triangles represent the data and the dashed lines are the fits. The spectra were recorded on a 500 MHz NMR spectrometer at 25 °C.

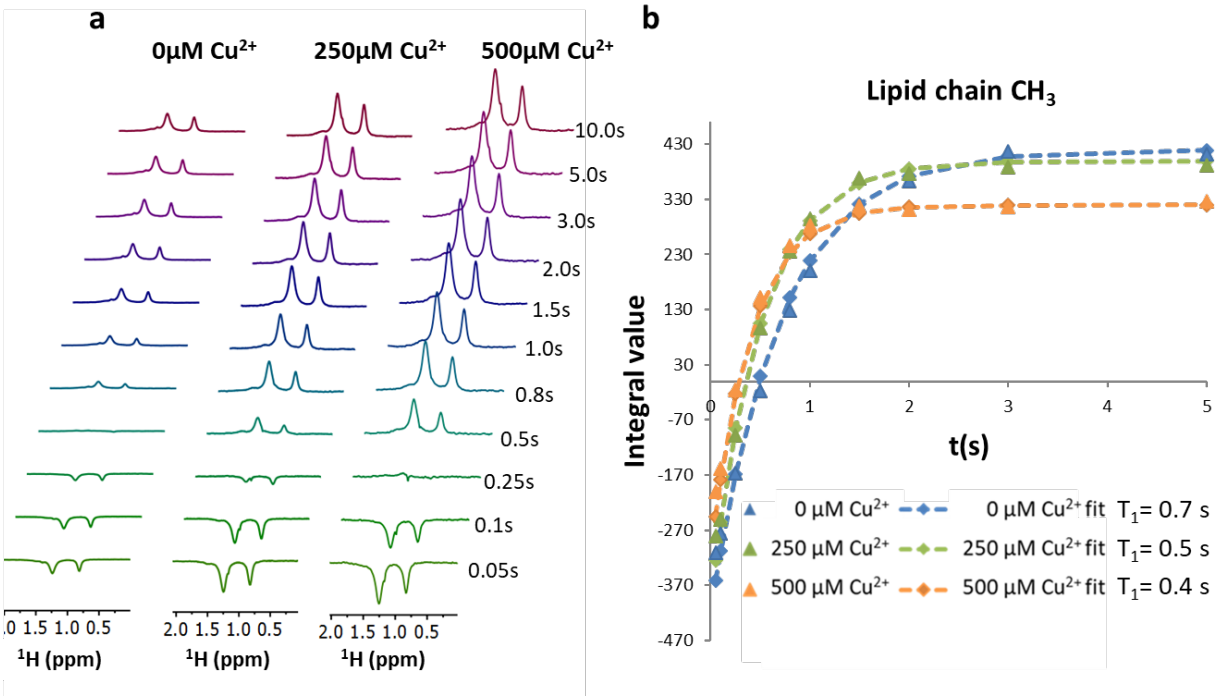

Figure S8: a) Inversion recovery experiments at different [Cu^{2+}] concentrations focused on the lipid chain CH_3 peak. b) Fitting of the lipid chain CH_3 peak inversion recovery experiment data and determination of the T_1 times. The experiments were conducted at 0, 250 and 500 μM concentrations. The triangles represent the data and the dashed lines are the fits. The spectra were recorded on a 500 MHz NMR spectrometer at 25 °C.

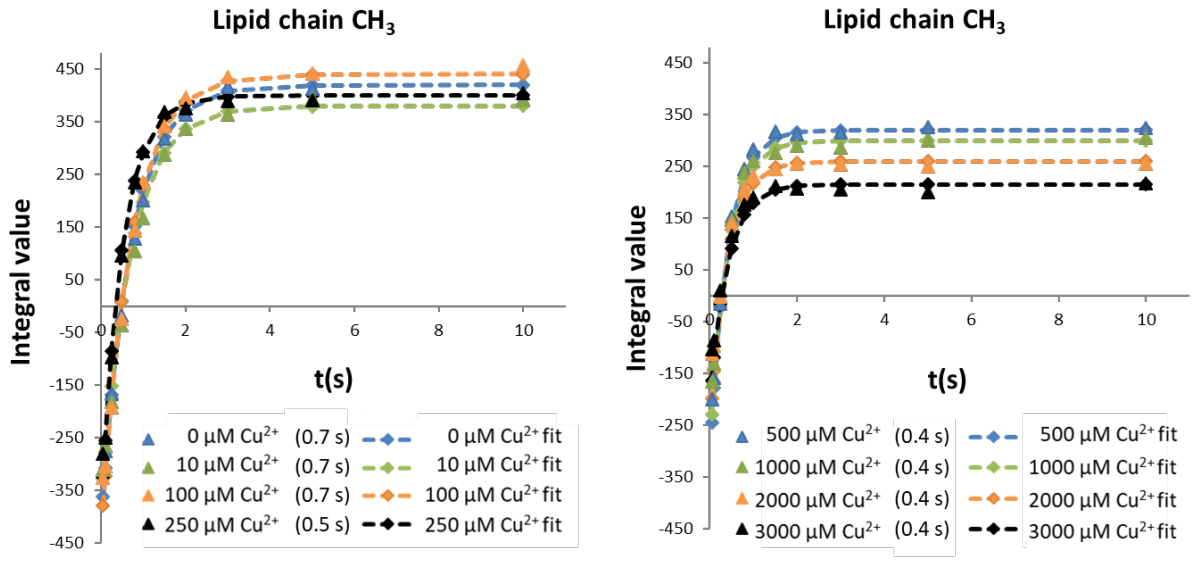

Figure S9: All the fitting for the lipid chain CH_3 peak inversion recovery experiment data recorded on polymer nanodisc samples with concentrations of Cu^{2+} varying between 0 and 3000 μM. The triangles represent the data and the dashed lines are the fits. The spectra were recorded on a 500 MHz NMR spectrometer at 25 °C.

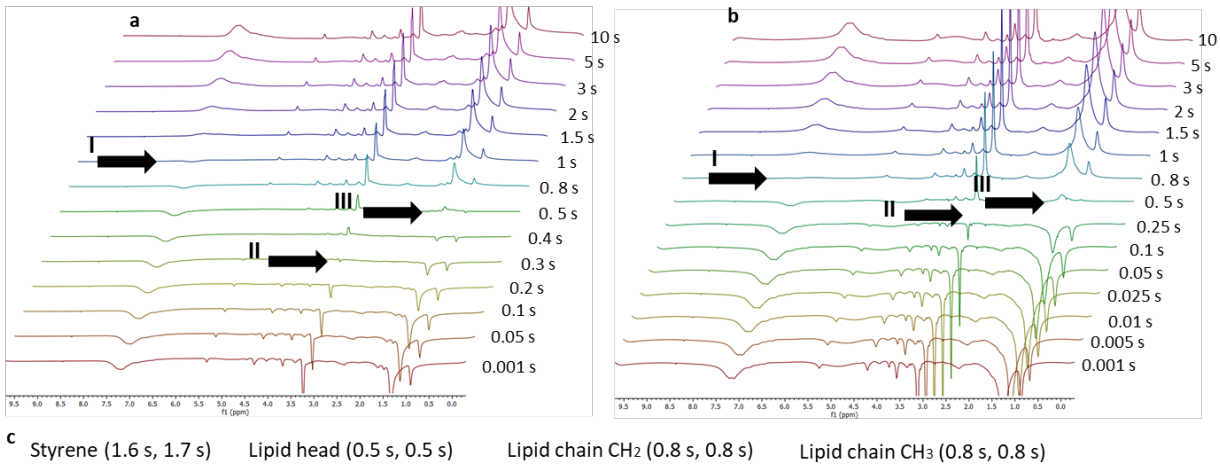

Figure S10: SMA EA nanodisc without the addition of the DOTA chelator in absence and presence of Cu^{2+} ions. a) The SMA-EA nanodiscs in absence of Cu^{2+} ions b) The SMA-EA nanodiscs in presence of 500 µM Cu^{2+} ions. c) The T_1 values for styrene, lipid head, lipid chain CH_2 and lipid chain CH_3 signals. The first number is the value for the nanodisc in the absence of Cu^{2+} ions. The second number is the value for the SMA-EA nanodiscs in presence of 500 µM Cu^{2+} ions. Roman numerals I, II and III indicate where the intensities of styrene, lipid head and lipid chain peaks, respectively are close to or zero. The spectra were recorded on a 500 MHz NMR spectrometer at 25 °C.

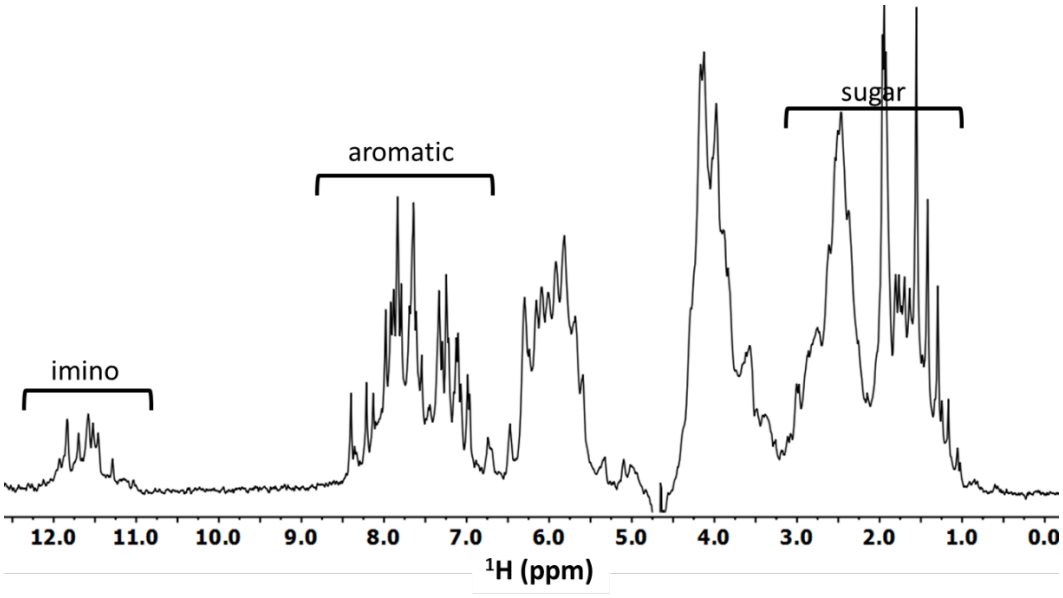

Figure S11: 1D ^{1}H NMR spectrum of the wtTel23 G-quadruplex. The spectrum was recorded at 0.1 mM oligonucleotide concentration per strand, 100 mM KCl, pH~7.0 and 25 °C on a 500 MHz spectrometer. The amino, aromatic, and sugar (H2'/H2'') regions are indicated in the above spectra.

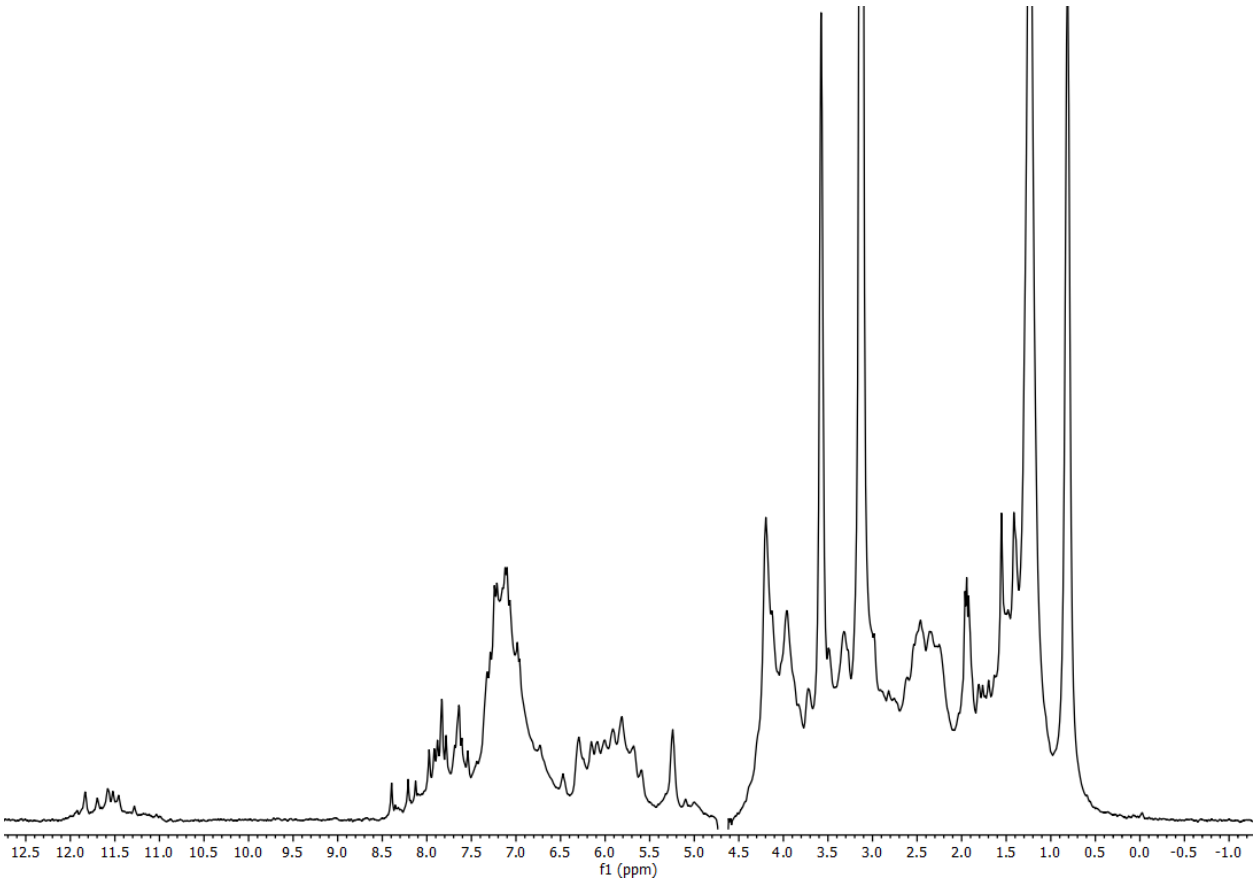

Figure S 12: 1D ^{1}H NMR spectrum of the wtTel23 G-quadruplex in the presence of SMA-EA-DOTA nanodisc. The spectrum was recorded at 0.1 mM oligonucleotide concentration per strand, 100 mM KCl, pH~7.0 and 25 °C on a 500 MHz NMR spectrometer.

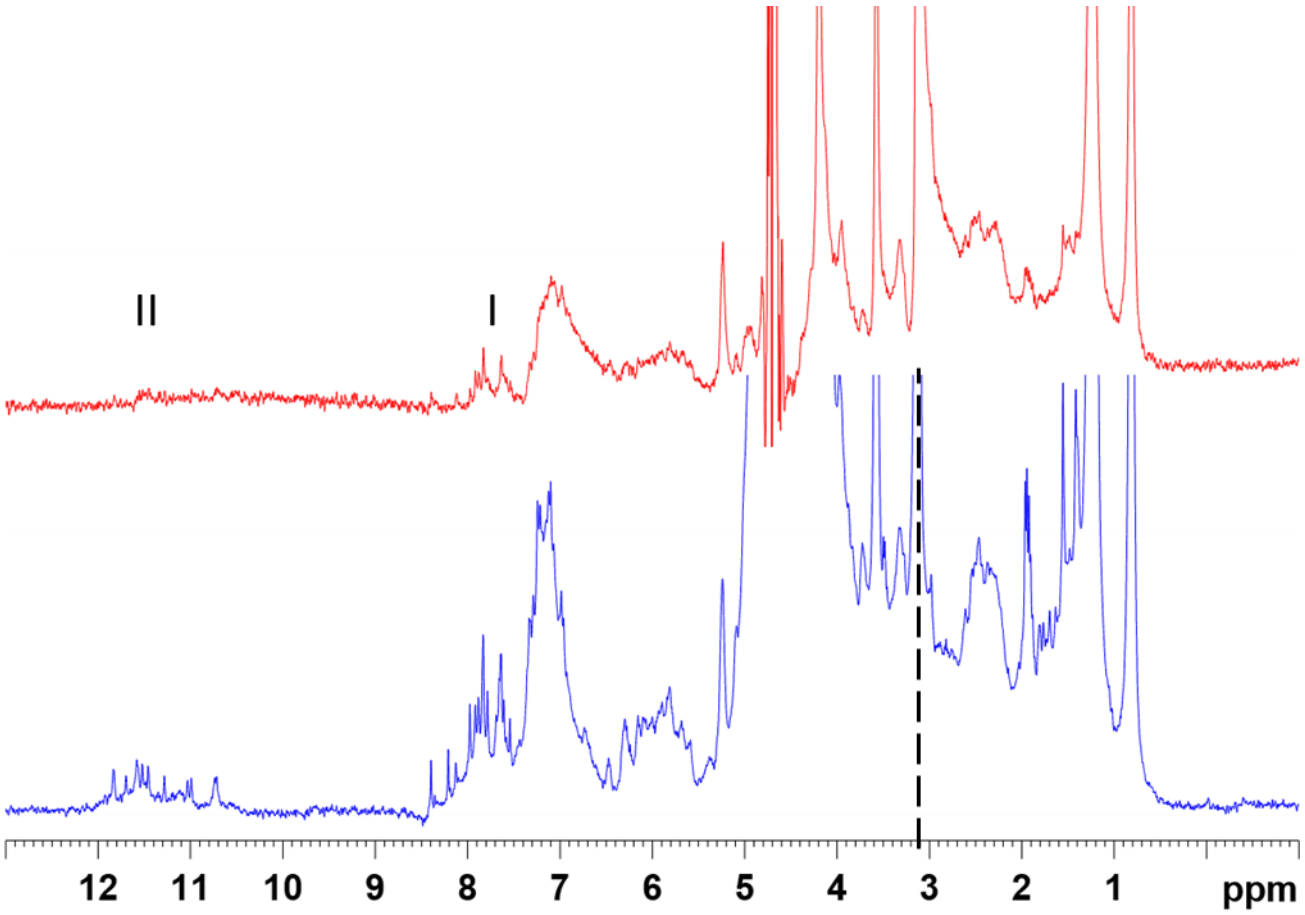

Figure S13: STD (Saturation-Transfer Difference) NMR spectrum of the wtTel23 G-quadruplex in the presence of the polymer nanodisc. The reference spectrum is shown in blue and the saturation transfer difference spectrum is shown in red. The dotted line indicates the lipid head CH_3 signal that was saturated. The roman numeral I indicates the aromatic signals and the roman numeral II indicates the imino proton signals. The spectrum was recorded at 0.1 mM oligonucleotide concentration per strand, 100 mM KCl, pH~7.0 and 25 °C on a 500 MHz NMR spectrometer. Number of scans = 256, Sweep width = 10000 Hz, offset = 2348.61, 90° pulse = 7.9, Saturation time = 3 s, on resonance excitation = 3.128 ppm, off resonance excitation 40.0 ppm.

References

[1] T. Ravula, S. K. Ramadugu, G. M. Di Mauro, A. Ramamoorthy, *Angew. Chemie Int. Ed.* **2017**, *56*, 11466–11470.

[2] M. M. Hoffmann, H. S. Sobstyl, S. J. Seedhouse, *Magn. Reson. Chem.* **2008**, *46*, 660–666.