Supporting Information

Tailored Transition-Metal Coordination Environments in Imidazole-Modified DNA G-Quadruplexes

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1 Synthesis

All chemicals were obtained from commercial sources and used without further purification. Gel permeation chromatography (GPC) purification of ligands was performed on a LC-9210 II NEXT system with CHCl$_3$ (HPLC grade) as eluent. NMR measurements were conducted at 298 K on Avance-600 and Avance-700 instruments from Bruker and on 500 MHz Bruker Avance neo NMR. Chemical shifts for $^1$H and $^{13}$C are reported in ppm on the $\delta$ scale; $^1$H and $^{13}$C signals were referenced to the residual solvent peak. The following abbreviations are used to describe signal multiplicity for $^1$H-NMR spectra: s: singlet, d: doublet, t: triplet, dd: doublet of doublets; dt: doublet of triplets; m: multiplet, br: broad. High resolution electrospray ionization mass spectrometry (ESI HRMS) was performed on Bruker Apex IV FTICR, Bruker compact and Bruker timsTOF ESI mass spectrometers.

DMT-protected glycidol 2

DMT-protected glycidol 2 was synthesized enantiomerically pure according to a modified literature procedure. To a solution of (R or S)-glycidol (1.0 mL, 15.1 mmol, 1 equiv.) and Et$_3$N (5.4 mL, 40.7 mmol, 2.7 equiv.) in CH$_2$Cl$_2$ (34 mL) DMT-Cl (4,4'-Dimethoxytrityl chloride; 6.45 g, 19 mmol, 1.26 equiv.) was added and the mixture stirred at 20 °C for 16 h. The reaction mixture was washed with half saturated aq. NaHCO$_3$ (50 mL) and extracted with CH$_2$Cl$_2$ (2 x 30 mL). The combined organic layers were dried over MgSO$_4$ and the solvent was removed under reduced pressure to afford a dark red oil. The product was purified by column chromatography (n-pentane/EtOAc, 98/2 → n-pentane/EtOAc, 90/10) to afford the DMT-protected glycidol 2 as a viscous colourless oil.

$^1$H-NMR (600 MHz, CDCl$_3$): $\sigma$ (/ppm) = 2.63 (dd, 1H), 2.78 (dd, 1H), 3.13 (dd, 2H), 3.32 (dd, 1H), 3.79 (s, 6H), 6.82 – 6.85 (m, 4H), 7.20 – 7.23 (m, 1H), 7.28 – 7.31 (m, 2H), 7.34 – 7.37 (m, 4H), 7.46 – 7.48 (m, 2H).

$^{13}$C-NMR (150 MHz): $\sigma$ (/ppm) = 45.0, 51.5, 55.6, 64.9, 86.5, 113.5, 127.1, 128.2, 128.5, 130., 136.4, 145.1, 158.8.

Chemical formula: $C_{24}H_{24}O_4$

Molecular weight: 376.45 g mol$^{-1}$

Yield: 5.1 g, 13.55 mmol, 90 %
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Glycol-based imidazole nucleoside 3

Glycol-based imidazole nucleoside 3 was prepared according to a previous report.\(^1\) To a solution of imidazole (0.54 g, 8 mmol, 2 equiv.) in 1,4-dioxane (7.5 mL) a solution of 2 (R or S) in 1,4-dioxane (2.5 mL) was added. The reaction was stirred at 80 °C for 21 h until all starting material was consumed. The reaction mixture was washed with half saturated aq. NaHCO\(_3\) (40 mL) and extracted with CH\(_2\)Cl\(_2\) (2 × 30 mL). The combined organic layers were dried over MgSO\(_4\) and the solvent was removed under reduced pressure to afford a yellow oil. The product was purified by flash chromatography (CH\(_2\)Cl\(_2\)/MeOH, 100/0 → CH\(_2\)Cl\(_2\)/MeOH, 90/10) followed by gel permeation chromatography (CHCl\(_3\)) to give the product (3) as a white foamy solid.

\(^1\)H-NMR (600 MHz, CDCl\(_3\)): \(\sigma /\text{ppm} = 3.14\) (dd, 1H); 3.22 (dd, 1H); 3.6 (s, 1H); 3.79 (s, 6H); 3.91 – 3.95 (m, 1H), 3.95 (dd, 1H); 4.08 (dd, 1H); 6.82 – 6.86 (m, 6H); 7.22-7.24 (m, 1H); 7.29-7.32 (m, 7H); 7.41-7.43 (m, 2H).

\(^{13}\)C-NMR (150 MHz): \(\sigma /\text{ppm} = 50.6, 55.6, 64.8, 70.5, 68.8, 113.6, 120.0, 127.3, 128.3, 128.4, 129.3, 130.3, 136.0, 144.8, 159.0\).

**Chemical formula:** C\(_{27}\)H\(_{28}\)N\(_2\)O\(_4\)

**Molecular weight:** 444.53 g mol\(^{-1}\)

**Yield:** 1.16 g, 2.61 mmol, 66 %

**HRMS (ESI, pos., Calc. [M+H]\(^+\) : 445.2122**

ACN/water, 1/1, 0.1% TFA) Found: m/z = 303.1365 [DMT]+, 445.2109 [M+H]+

Phosphoramidite 4 (R/S)

A solution of compound 3 (0.4 g, 0.82 mmol, 1 equiv.) and DIPEA (857 µL, 4.92 mmol, 6 equiv.) in dry CH\(_2\)Cl\(_2\) was stirred under argon atmosphere. To this solution, 2-cyanoethyl N,N-diisopropyl chlorophosphoramidite (233 mg, 220 µL, 0.984 mmol, 1.2 equiv.) was added dropwise and the reaction mixture stirred for 90 minutes. After completion, the solvent was removed under reduced pressure to afford the crude phosphoramidite 4 as a slightly yellow oil with an estimated yield of 80 %. The compound was used for solid phase synthesis without further purification.
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Chemical formula: \( C_{36}H_{45}N_{4}O_{5}P \)

Molecular weight: 644.75 g mol\(^{-1}\)

\(^{31}\)P-NMR (242 MHz, CDCl\(_3\)): \( \sigma /\text{ppm} = 149.0, 149.8 \).

Glycol-based imidazole nucleoside 5

A suspension of NaH (60 % in mineral oil; 200 mg, 5 mmol, 4 equiv.) in DMF (2 mL) was degassed three times before a solution of \( N \)-hydroxyethyl imidazole (0.3 g, 2.66 mmol, 2 equiv.) in DMF (4 mL) was dropwise added under argon at 0 °C. The suspension was allowed to reach 20 °C and was stirred for 60 min until no \( \text{H}_2 \) formation was observed. Then a solution of compound 2 (\( R \) or \( S \) enantiomer) (0.5 g, 1.33 mmol, 1 equiv.) in DMF (4 mL) was dropwise added and the reaction was heated up to 40 °C for 16 h. After all starting material was consumed, the reaction mixture was quenched with an aq. solution of \( \text{NaHCO}_3 \) (50 mL) at 0°C. The reaction mixture was extracted with CH\(_2\)Cl\(_2\) (3 × 50 mL) and the combined organic layers were dried over MgSO\(_4\). The solvent was removed under reduced pressure to afford a yellow oil. The product was purified by flash chromatography (CH\(_2\)Cl\(_2\)/MeOH, 100/0 \( \rightarrow \) CH\(_2\)Cl\(_2\)/MeOH, 90/10) followed by gel permeation chromatography (CHCl\(_3\)) to give the product (5) as a foamy solid.

\(^1\)H-NMR (700 MHz, CDCl\(_3\)): \( \sigma /\text{ppm} = 2.34 \) (broad, 1H, OH); 3.15 - 3.19 (m, 2H); 3.47 (dd, 1H); 3.51 - 3.53 (d, 1H); 3.66 - 3.71 (m, 2H); 3.79 (s, 6H); 3.90 - 3.93 (m, 1H); 4.03 - 4.07 (m, 2H); 6.82 - 6.84 (m, 4H); 6.89 (broad, 1H); 7.01 (broad, 1H); 7.2 - 7.23 (m, 1H); 7.28 - 7.30 (m, 2H); 7.29 - 7.31 (m, 4H); 7.41 - 7.42 (m, 2H); 7.46 (broad, 1H).

\(^{13}\)C-NMR (175 MHz, CDCl\(_3\)): \( \sigma /\text{ppm} = 47.3, 55.6, 64.7, 70.2, 71.0, 73.1, 86.5, 113.5, 119.7, 127.2, 128.2, 128.4, 129.6, 130.4, 136.22, 137.7, 145.0, 158.9 \).

Chemical formula: \( C_{29}H_{32}N_{2}O_{5} \)

Molecular weight: 488.58 g mol\(^{-1}\)

Yield: 0.48 g, 0.98 mmol, 74 %

MS (ESI, pos.): Calc. [M+H]\(^+\): 489.2384

ACN/water, 1/1, 0.1% TFA): Found: \( m/z = 303.136 \) [DMT]\(^+\), 489.2381 [M+H]\(^+\)
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Phosphoramidite 6 (R/S)

A solution of compound 5 (0.4 g, 0.82 mmol, 1 equiv.) and DIPEA (857 µL, 4.92 mmol, 6 equiv.) in dry CH₂Cl₂ was stirred under argon atmosphere. To the solution 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (233 mg, 220 µL, 0.984 mmol, 1.2 equiv.) was added dropwise and the reaction mixture stirred for 90 minutes. After completion, the solvent was removed under reduced pressure to afford the crude phosphoramidite 6 as a slightly yellow oil with an estimated yield of 80 %. The compound was used for solid phase synthesis without further purification.

Chemical formula: C₃₈H₄₉N₄O₆P
Molecular weight: 688.80 g mol⁻¹

³¹P-NMR (242 MHz, CDCl₃):  δ/ppm =149.2, 149.4.

2 Solid phase DNA synthesis

Solid phase DNA synthesis was carried out on a K&A Laborgerte GbR H-8 synthesizer using the standard phosphoramidite method on a 1 µmol scale. Cartridges (Biosearch Technologies or Link Technologies) for solid phase synthesis were self-packed with CPG (1000 Å, 33 µmol/g) purchased from Sigma Aldrich. First, the cartridge was treated three times with 3 % DCA (dichloroacetic acid in CH₂Cl₂). Second, the coupling step was performed by mixing activator (5-(benzylthio)-1H-tetrazole, BTT) and phosphoramidite in a 1:1 ratio. The incubation time for the standard canonical nucleotides was 0.5 min whereas for artificial nucleotides the coupling time was extended to 3.5 min. Next, unreacted 5'-OH groups were acetylated with a 1:1 mixture of capping solutions Cap A and Cap B followed by a final oxidation step (see table 1). After each step, the cartridge was washed with acetonitrile followed by a drying step using argon gas. The described cycle was repeated for every incorporated base.

Apart from the oxidizer solution, all solvents were bought in anhydrous quality. Standard 4,4'-dimethoxytrityl (DMT) and cyanoethyl (CE) protected phosphoramidites (DMTdT-CEP, DMT-dG(iBu)-CEP, DMT-dA(Bz)-CEP) were purchased from Sigma Aldrich and were dissolved for DNA synthesis in dry acetonitrile (0.1 M).
Table 1. Solutions used in DNA solid phase synthesis. All solutions were prepared under argon 16 h hours before solid phase synthesis and stored at 4°C. Activator and capping solutions were stored over molecular sieves (3 Å).

| Step          | Composition                                      |
|---------------|--------------------------------------------------|
| Detritylation | 3 % DCA in CH₂Cl₂                                 |
| Activation    | 0.3 M BTT in ACN                                  |
| Cap A         | THF/lutidine/acetic anhydride, 8/1/1              |
| Cap B         | 10 % (w/v) N-methyl imidazole in THF              |
| Oxidation     | 50 mM I₂ in THF/pyridine/water, 78/20/2          |

After DNA solid phase synthesis, the oligonucleotide was cleaved from the solid support using concentrated NH₄OH at 55 °C for 16 h. The sample was filtered through VWR Centrifugal filters. Solvent volume was reduced in a S-Concentrator BA-VC-300H from H. Saur Laborgeräte to ~300 µL and purified by reversed phase HPLC. After purification, the DMT-protection group was removed and the sample desalted on SepPak C₁₈ cartridges. Desalted oligonucleotides were lyophilized and stored at -20 °C until use.

Concentrations were individually determined at 260 nm using a Thermo Scientific Nanodrop One instrument. The absorption of the imidazole modifications at 260 nm could be neglected.

Reversed phase HPLC

Reversed-phase HPLC was performed on Agilent Technologies 1260 Infinity I or II HPLC systems equipped with Agilent Zorbax 300 SB-C18 4.5/10 x 250 mm or Macherey-Nagel EC 250 x 4.6 NUCLEODUR 100-5 C18ec columns. The flow rate was set to 0.75/1 (analytical) or 2.5 mL min⁻¹ (semi preparative). Samples were filtered through VWR Centrifugal filters to ensure clear solutions and the column was equilibrated against buffer A (0.05 M TEAA pH 7 (triethylammonium acetate)). After equilibration, samples were analysed using a standard gradient starting with buffer A (100 %) going to buffer B (80 %) over 30 min. Typical sample volumes were 10 µL with DNA concentrations ranging from 100 to 200 µM. Buffer A: Aqueous solution of 0.05 M TEAA (triethylammonium acetate) adjusted to pH 7; buffer B: 30 % v/v 0.05 M TEAA pH 7 in 70% v/v acetonitrile.

Table 2. G-quadruplex forming sequences

| Sequence 5' → 3' |
|------------------|
| G₄L              | AGG GG      |
| G₃L              | AGG GGG     |
| htel             | AGG GTT AGG GTT AGG GTT AGG G |
| htelL₂            | AGG TTT AGG GTT AGG GTT ATG G |
| htelL₃            | AGG GTT AGG GTT AGG GTT ATG G |
| htelL₂B           | AGG GTG TGG GTT AGG GTG TGG G |
| htelL₂B           | AGG GTG TGG GTT AGG GTG TGG G |
| htelL₄            | AGG GTT AGG GTT AGG GTT AGG G |
| htelL₅            | AGG GTT AGG GTT AGG GTT AGG G |
| htelL₆            | AGG GTT AGG GTT AGG GTT AGG G |
| htelL₇            | AGG GTT AGG GTG TAG GTG TAG G |
| htelL₈            | AGG GTT AGG GTG TAG GTG TAG G |
| htelL₉            | AGG GTT AGG GTG TAG GTG TAG G |
| htelL₁₀           | AGG GTT AGG GTG TAG GTG TAG G |
| htelL₁₁           | AGG GTT AGG GTG TAG GTG TAG G |
| htelL₁₂           | AGG GTT AGG GTG TAG GTG TAG G |
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3 UV/VIS spectroscopy

All UV/VIS based measurements were carried out on Jasco V-650 and Jasco V-750 absorption spectrometers in quartz glass cuvettes. Samples were prepared with 3.75 (tetramolecular) and 1.875 µM (unimolecular) ssDNA in 100 mM NaCl (tetramolecular) or KCl (unimolecular) unless stated differently, 10 mM LiCaCo pH 7.2 and, if present, 1 equiv. transition metal cations (refers to 0.94 µM for tetramolecular and 1.88 µM for unimolecular G-quadruplexes). As transition metal sources CuSO₄, NiSO₄, ZnI₂ and Co(NO₃)₂ were used. To form the G-quadruplexes, solutions were heated to 85 °C and cooled down to 4 °C at 0.5 °C min⁻¹. Tetramolecular samples were then frozen at –20 °C for one hour to ensure full G-quadruplex formation.³¹ Thermal difference spectra (TDS) were recorded from 220 to 350 nm by subtracting the low temperature spectrum (4 °C) from the high temperature spectrum (85 °C). The absorption at 350 nm was then set to 0.

Thermal denaturation experiments were carried out to determine the thermal stability expressed in the melting temperature Tₘ. Therefore, the change in absorbance, as the temperature was increased with 0.5 °C min⁻¹, was followed at 295 nm indicative for G-quadruplex denaturation and at 350 nm as control. Note that for unimolecular G-quadruplexes at every temperature an equilibrium between folded and unfolded species is given. This is not true for tetramolecular G-quadruplexes which is the reason why the melting temperature for tetramolecular G-quadruplexes depends on the heating rate. The spectrometer bandwidth was set to 2 nm. To control the temperature, a cuvette containing water was equipped with a temperature probe connected to the spectrometer. Water evaporation from the samples was avoided by addition of small amounts of silicon oil. To ensure equal heating rates between different experiments, always five samples were measured at a time. The measured absorbance was plotted against the temperature after the absorbance at 350 nm was subtracted to remove background absorption. Spectra were normalized to fraction folded values between 1 and 0 corresponding to fully folded or unfolded G-quadruplexes, respectively.

Thermal difference spectra (TDS)
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Figure 1. TDS of G₄L₂S recorded from 220 to 350 nm: a) without metal addition, after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 2. TDS of G₄L²R recorded from 220 to 350 nm: a) without metal addition, after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 3. TDS of htelR₄ recorded from 220 to 350 nm: a) without metal addition, after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
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Figure 4. TDS of hteL1S recorded from 220 to 350 nm: a) without metal addition, after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 5. TDS of hteL2S recorded from 220 to 350 nm: a) without metal addition, after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 8. TDS of htel\(L_2R_2\) recorded from 220 to 350 nm: a) without metal addition, after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 6. TDS of htel\(L_2R_3\) recorded from 220 to 350 nm: a) without metal addition, after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 7. TDS of htel\textsubscript{L}\textsubscript{S}\textsubscript{3} recorded from 220 to 350 nm: a) without metal addition, after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 8. TDS of htel\textsubscript{L}\textsubscript{S}\textsubscript{4} recorded from 220 to 350 nm: a) without metal addition, after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 9. TDS of htel\(L_2^S\) recorded from 220 to 350 nm: a) without metal addition, after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 10. TDS of htel\(L_2^R\) recorded from 220 to 350 nm: a) without metal addition, after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 11. TDS of htel$_2$L$_5$ recorded from 220 to 350 nm: a) without metal addition, after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a-e).

Figure 12. TDS of hteL$_2$L$_6$ recorded from 220 to 350 nm: a) without metal addition, after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a-e).
Figure 13. TDS of htel$^{L_2}$ recorded from 220 to 350 nm: a) without metal addition, after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 14. TDS of htel$^{L_2}$ recorded from 220 to 350 nm: a) without metal addition, after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 15. TDS of htel\textsuperscript{L2}, recorded from 220 to 350 nm: a) without metal addition, after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 16. TDS of htel\textsuperscript{L2,B} recorded from 220 to 350 nm: a) without metal addition, after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 17. TDS of htelL2S3B recorded from 220 to 350 nm: a) without metal addition, after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 18. TDS of htelL2R2B recorded from 220 to 350 nm: a) without metal addition, after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 19. TDS of htelL2,B recorded from 220 to 350 nm: a) without metal addition, after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

4 Thermal denaturation experiments

Table 3. Melting temperatures $T_m$ and stabilizations ($\Delta T_m$) in absence and after addition of Cu(II), Zn(II), Ni(II) and Co(II)

|         | No metal | Cu(II) | Ni(II) | Zn(II) | Co(II) |
|---------|----------|--------|--------|--------|--------|
| htelL1S | 31       | 79 (+48)| 77 (+46)| 54 (+23)| 64 (+33)|
| G3L1S   | 32       | 83 (+51)| 81 (+49)| 61 (+29)| 73 (+41)|
| G3L4S   | 36       | 76 (+40)| 73 (+37)| 52 (+16)| 63 (+27)|
| G3L8S   | 34       | 74 (+40)| 68 (+34)| 46 (+12)| 54 (+20)|
| htelL1S | 37       | 36 (-1)| 36 (-1)| 36 (-1)| 37 (+0)|
| htelL1S | 29       | 29 (+0)| 28 (-1)| 29 (+0)| 29 (+0)|
| htelL2S | 27       | 28 (+1)| 27 (+0)| 27 (+0)| 26 (-1)|
| htelL2S | 53       | 55 (+2)| 53 (+0)| 54 (+1)| 53 (+0)|
| htelL2S | 29       | 29 (+0)| 29 (+0)| 29 (+0)| 28 (-1)|
| htelL2S | 52       | 52 (+0)| 49 (-3)| 52 (+0)| 52 (+0)|
| htelL2S | 28       | 31 (+3)| 28 (+0)| 28 (+0)| 27 (-1)|
| htelL2S | 50       | 56 (+6)| 51 (+1)| 52 (+2)| 50 (+0)|
| htelL2S | 29       | 31 (+2)| 28 (-1)| 29 (+0)| 30 (+1)|
| htelL2S | 45       | 49 (+4)| 46 (+1)| 49 (+4)| 46 (+1)|
| htelL2S | 33       | 56 (+23)| 45 (+12)| 36 (+3)| 35 (+2)|
| htelL2S | 33       | 53 (+20)| 45 (+12)| 35 (+2)| 35 (+2)|
| htelL2S | 37       | 60 (+23)| n.d. | 56 (+19)| n.d. |
| htelL2S | 42       | 63 (+21)| n.d. | 63 (+21)| n.d. |
| htelL2S | 34       | 56 (+22)| 45 (+11)| 36 (+2)| 36 (+2)|
| htelL2S | 34       | 51 (+17)| 45 (+11)| 37 (+3)| 32 (-2)|
| htelL2S | 36       | 62 (+28)| n.d. | 52 (+16)| n.d. |
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|   | htelL^{28}_{ri}C | 43 | 66 (+23) | n.d. | 55 (+12) | n.d. |
|---|-----------------|----|----------|------|----------|------|
|   | htelL^{28}_{ri} | 33 | 54 (+21) | 55 (+22) | 37 (+4) | 37 (+4) |
|   | htelL^{28}_{ri}, 2 equiv. Metal | 33 | 46 (+13) | 55 (+22) | 38 (+5) | 36 (+3) |
|   | htelL^{28}_{ri} | 34 | 50 (+16) | 47 (+13) | 36 (+2) | 35 (+1) |
|   | htelL^{28}_{ri}, 2 equiv. Metal | 34 | 47 (+13) | 46 (+12) | 37 (+3) | 36 (+2) |
|   | htelL^{28}_{ri} | 36 | 54 (+18) | 59 (+23) | 44 (+8) | 44 (+8) |
|   | htelL^{28}_{ri}, 2 equiv. Metal | 34 | 52 (+18) | 52 (+18) | 42 (+8) | 36 (+2) |
|   | htelL^{28}_{ri} | 35 | 50 (+15) | 51 (+16) | 42 (+7) | 41 (+6) |
|   | htelL^{28}_{ri}, 2 equiv. Metal | 35 | 51 (+16) | 50 (+15) | 44 (+9) | 43 (+8) |
|   | htelL^{28}_{ri} | 28 | 43 (+15) | 46 (+18) | 36 (+8) | 36 (+8) |
|   | htelL^{28}_{ri}, 2 equiv. Metal | 28 | 46 (+18) | 46 (+18) | 38 (+10) | 39 (+11) |
|   | htelL^{28}_{ri} | 29 | 43 (+14) | 44 (+15) | 36 (+7) | 30 (+1) |
|   | htelL^{28}_{ri}, 2 equiv. Metal | 29 | 46 (+17) | 44 (+15) | 39 (+10) | 32 (+3) |

**Figure 20.** Thermal denaturation experiments of G4L_{ri} monitored at λ = 295 nm and converted to fraction folded: a) in absence of transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
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Figure 21. Thermal denaturation experiments of G4L2 monitored at $\lambda = 295$ nm and converted to fraction folded a) in absence of transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 22. Thermal denaturation experiments of htelL4 monitored at $\lambda = 295$ nm and converted to fraction folded a) in absence of transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 23. Thermal denaturation experiments of htel\textsuperscript{L1S4} monitored at \( \lambda = 295 \) nm and converted to fraction folded a) in absence of transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 24. Thermal denaturation experiments of htel\textsuperscript{L2R2} monitored at \( \lambda = 295 \) nm and converted to fraction folded a) in absence of transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 25. Thermal denaturation experiments of htelL2S2 monitored at $\lambda = 295$ nm and converted to fraction folded a) in absence of transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 26. Thermal denaturation experiments of htelL2S2B in NaCl monitored at $\lambda = 295$ nm and converted to fraction folded a) in absence of transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 27. Thermal denaturation experiments of htelL2R2B in NaCl monitored at λ = 295 nm and converted to fraction folded a) in absence of transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 28. Thermal denaturation experiments of htelL2R3B monitored at λ = 295 nm and converted to fraction folded a) in absence of transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 29. Thermal denaturation experiments of htelL2S3 monitored at λ = 295 nm and converted to fraction folded a) in absence of transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 30. Thermal denaturation experiments of htelL2S3B in NaCl monitored at λ = 295 nm and converted to fraction folded a) in absence of transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 31. Thermal denaturation experiments of htelL2R3B in NaCl monitored at \( \lambda = 295 \) nm and converted to fraction folded a) in absence of transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 32. Thermal denaturation experiments of htelL2R4 monitored at \( \lambda = 295 \) nm and converted to fraction folded a) in absence of transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 33. Thermal denaturation experiments of htelL2S4 monitored at $\lambda = 295$ nm and converted to fraction folded a) in absence of transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 34. Thermal denaturation experiments of htelL2R5 monitored at $\lambda = 295$ nm and converted to fraction folded a) in absence of transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 35. Thermal denaturation experiments of htel\textsuperscript{L}S\textsubscript{5} monitored at $\lambda = 295$ nm and converted to fraction folded a) in absence of transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
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Figure 36. Thermal denaturation experiments of htelL2R monitored at $\lambda = 295$ nm and converted to fraction folded a) in absence of transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 37. Thermal denaturation experiments of htelL2S monitored at $\lambda = 295$ nm and converted to fraction folded a) in absence of transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 38. Thermal denaturation experiments of htelL2S monitored at $\lambda = 295$ nm and converted to fraction folded a) in absence of transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 39. Thermal denaturation experiments of htel^{L2R7} monitored at $\lambda = 295$ nm and converted to fraction folded a) in absence of transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 40. Thermal denaturation experiments of htel^{L2R4}B and htel^{L2R4}C monitored at $\lambda = 295$ nm and converted to fraction folded: a) htel^{L2R4}B without transition metal and with 1 equiv. b) Cu(II), c) Zn(II). d) htel^{L2R4}C without transition metal and with 1 equiv. e) Cu(II), f) Zn(II).
Figure 41. Thermal denaturation experiments of htel$^{L_2S_4}B$ and htel$^{L_2S_4}C$ monitored at $\lambda = 295$ nm and converted to fraction folded: a) htel$^{L_2S_4}B$ without transition metal and with 1 equiv. b) Cu(II), c) Zn(II). d) htel$^{L_2S_4}C$ without transition metal and with 1 equiv. e) Cu(II), f) Zn(II).
5 Circular dichroism (CD) measurements

CD measurements were carried out on a Chirascan qCD spectrometer in black quartz cuvettes. Samples were prepared as described under UV/VIS spectroscopy. Temperature was controlled using a Quantum Northwest temperature control attached to a sample probe. Spectra were recorded from 205 to 350 nm (120 nm min⁻¹) with a 1 nm interval and 0.5 nm bandwidth three times and averaged using the built-in software. The averaged spectra were smoothed (adjacent averaging) with a factor of 5 and the smoothed background (sample containing only buffer, smooth factor 10) was subtracted. The background was recorded once from 205 to 350 nm with the same settings as for the samples in the same cuvette.

Figure 42. CD spectra of the folded G-quadruplex G₄L₂ at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 43. CD spectra of the folded G-quadruplex G₄L₂S at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 44. CD spectra of the folded G-quadruplex hteIL₁₄ at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 45. CD spectra of the folded G-quadruplex htelL1S at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 46. CD spectra of the folded G-quadruplex htelL2R at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
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Figure 47. CD spectra of the folded G-quadruplex htelL2S2 at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 48. CD spectra of the folded G-quadruplex htelL2R2B in NaCl at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 49. CD spectra of the folded G-quadruplex htelL25R2 in KCl at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 50. CD spectra of the folded G-quadruplex htelL25S2B in NaCl at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
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Figure 51. CD spectra of the folded G-quadruplex htelL2S2B in KCl at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 52. CD spectra of the folded G-quadruplex htelL3R3 at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure S3. CD spectra of the folded G-quadruplex htelL2S3 at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure S4. CD spectra of the folded G-quadruplex htelL2S3B in NaCl at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 55. CD spectra of the folded G-quadruplex htelL253B in KCl at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a-e).

Figure 56. CD spectra of the folded G-quadruplex htelL253B in NaCl at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a-e).
Figure 57. CD spectra of the folded G-quadruplex htelL2R3B in KCl at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 58. CD spectra of the folded G-quadruplex htelL4 at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 59. CD spectra of the folded G-quadruplex hetel^L2 at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 60. CD spectra of the folded G-quadruplex hetel^R5 at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 61. CD spectra of the folded G-quadruplex htelL2 at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 62. CD spectra of the folded G-quadruplex htelL2 at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 63. C CD spectra of the folded G-quadruplex htelL2S at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 64. CD spectra of the folded G-quadruplex htelL2S at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).
Figure 65. CD spectra of the folded G-quadruplex htelL2R7 at 4 °C with ellipticities given in milli-degree: a) without transition metals and after addition of 1 equiv. b) Cu(II), c) Ni(II), d) Zn(II) and e) Co(II); f) superposition of a)-e).

Figure 66. CD spectra of the folded G-quadruplex htelL2R4 and htelL2R4 at 4 °C with ellipticities given in milli-degree: a) htelL2R4 without transition metal and with 1 equiv. b) Cu(II), c) Zn(II). d) htelL2R4 without transition metal and with 1 equiv. e) Cu(II), f) Zn(II).
Figure 67. CD spectra of the folded G-quadruplex htelL²S₄B and htelL²S₄C at 4 °C with ellipticities given in milli-degree: a) htelL²S₄B without transition metal and with 1 equiv. b) Cu(II), c) Zn(II). d) htelL²S₄C without transition metal and with 1 equiv. e) Cu(II), f) Zn(II).

6 Native ESI-MS

In native ESI-MS, the secondary structure is kept intact. To differentiate between folded and unfolded G-quadruplexes in the gas phase, two phenomena are most instructive. First, if tetramolecular G-quadruplexes are investigated and the structure is denatured, single-stranded DNA instead of a tetramer would be observed and second, valid for tetra- and unimolecular G-quadruplexes, ESI mass spectrometry from electrolyte-containing solutions always gives rise to series of unspecific adducts with sodium or potassium cations. For fully denatured species, a statistical distribution of adducts starting with zero cations would be observed and for a native, folded species a distribution is observed starting with n-1 explicitly bound cations where n is the number of G-tetrads.

Ion mobility measurements were performed on a Bruker timsTOF instrument combining a trapped ion mobility spectrometer (TIMS) with a time-of-flight (TOF) mass spectrometer in one instrument.

In contrast to the conventional drift tube method to determine mobility data, where ions are carried by an electric field through a stationary drift gas, the TIMS method is based on an electric field ramp to hold ions in place against a carrier gas pushing them in the direction of the analyzer. Consequently, larger sized ions that experience more carrier gas impacts leave the TIMS units first and smaller ions elute later. This method offers a much higher mobility resolution despite a smaller device size.

Measurement: After the generation of ions by electrospray ionisation (ESI, analyte concentration: 12.5 µM G-quadruplex, 0.5 mM KCl and 50 mM TMAA (trimethyl ammonium acetate) pH 6.8, solvent: acetonitrile-water, 1:1, capillary voltage: 4500 V, end plate offset voltage: 500 nebulizer gas pressure: 0.4/0.6 bar, dry gas flow rate: 6/9 l min⁻¹, dry temperature: 303 K), the desired ions were orthogonally deflected into the TIMS cell consisting of an entrance funnel and the TIMS analyser (carrier gas: N₂, temperature: 305 K, entrance pressure: 2.55 mbar, exit pressure: 0.89 mbar, IMS imeX ramp end: 1.66 1/K0, IMS...
imeX ramp start: 0.51 1/K0). As a result, the ions are stationary trapped. After accumulation (accumulation time: 70/200 ms), a stepwise reduction of the electric field strength leads to a release of ion packages separated by their mobility. After a subsequent focusing, the separated ions are transferred to the TOF-analyser.\(^{[28-30]}\)

The ion mobility \(K\) was directly calculated from the trapping electric field strength \(E\) and the velocity of the carrier gas stream \(v_g\) via

\[
K = \frac{v_g}{E} = \frac{A}{U_{\text{release}} - U_{\text{out}}}
\]

(1)

where \(A\) is a calibration constant (based on calibration standards), \(U_{\text{release}}\) is the voltage at which the ions are released from the analyser and \(U_{\text{out}}\) is the voltage applied to the exit of the tube. The ion mobility is corrected to standard gas density via

\[
K_0 = K \frac{P}{1013 \text{ hPa}} \frac{237 \text{ } K}{T}
\]

(2)

to obtain the reduced mobility \(K_0\), where \(P\) is the pressure and \(T\) is the temperature. By using the Mason-Schamp equation, the collisional cross-section \(\Omega\) can be calculated:

\[
\Omega = \frac{18 \pi}{16} \frac{z e}{(k_B T)^2} \left( \frac{1}{K_0} \right)^{1/2} \frac{1}{N_0}
\]

(3)

where \(z e\) is the ion charge, \(k_B\) is the Boltzmann constant, \(\mu\) is the reduced mass of analyte and carrier gas and \(N_0\) is the number density of the neutral gas.\(^{[28-30]}\)

For calibration of both the TIMS and TOF analysers, commercially available Agilen ESI tuning mix was used. The instrument was calibrated before each measurement, including each change in the ion mobility resolution mode (“imeX” settings: survey, detect or ultra).

Figure 68. Native ESI-MS coupled with trapped ion mobility of Gal\(^{25}\) with 1 equiv. Cu(II): a) ion mobility distribution, b) measured mass spectrum and c) mobility-extracted mass spectrum.
Figure 69. Native ESI-MS coupled with trapped Ion Mobility of GaL²⁺ with 1 equiv. Cu(II). In a) ion mobility distribution, b) measured mass spectrum and c) mobility-extracted mass spectrum.

Figure 70. Native ESI-MS of htelL²⁻B with 1 equiv. Cu(II).
Figure 71. Native ESI-MS of htel$^{2+}$B in absence of transition metals.

Figure 72. Native ESI-MS of htel$^{2+}$B with 1 equiv. Cu(II).
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Figure 73. Native ESI-MS of htelL24B in absence of transition metals.

Figure 74. Native ESI-MS of htelL24 in absence of any transition metal.
Figure 75. Native ESI-MS of htelL2L4 with 1 equiv. Cu(II).

Figure 76. Native ESI-MS of htelL2L4 with 1 equiv. Ni(II).
Figure 77. Native ESI-MS of htel\textsuperscript{4+} with 1 equiv. Zn(II).

Figure 78. Native ESI-MS of htel\textsuperscript{4+} with 1 equiv. Co(II).
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Figure 79. Native ESI-MS of htel$^{2+}$ with 1 equiv. Cu(II).

Figure 80. Native ESI-MS of htel$^{2+}$ with 1 equiv. Ni(II).
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Figure 81. Native ESI-MS of htel\(_{22}L_6\) with 1 equiv. Zn(II).

Figure 82. Native ESI-MS of htel\(_{22}L_6\) with 1 equiv. Co(II).
7 MD simulations

Simulations were performed using GROMACS 2016.1 and GROMACS 2019.2.[3-6] The AMBER force field ff99bsc1 was modified with new force field parameters regarding the imidazole ligand according to a previously published protocol.[10-14] Missing forcefield parameters for the imidazole ligand were based on analogy to existing force field parameters. For both enantiomers, the same set of parameters was used. Parameters regarding the Cu(II) and Zn(II) complex were derived using VFFDT (Visual Force Field Derivation Toolkit) with a Gaussian '09 geometry optimized structure (B3LYP/6-311+G(d,p)).[10,15-18]

To obtain a square-planar and tetrahedral coordination environment for Cu(II) and Zn(II), respectively, adjacent ligands were named differently, to avoid ambiguity in the parameterization. Improper dihedrals were used to maintain coplanarity between the imidazole plane and the imidazole – metal plane. The respective parameters for Cu(II) and Zn(II) were estimated based on literature values (Table 5).[17,18]

As starting structure, the crystal structure of a tetramolecular parallel stranded (pdb entry: 2O4F) and the NMR solution structure of an unimolecular antiparallel (143D, 1C35) G-quadruplex were used and modified.[19,20] Obtained starting structures where solvated in a rhombic bounding box with TIP3P water. Negative charges on the phosphates were neutralized with Na\(^+\) (tetramolecular) or K\(^+\) (unimolecular) cations and the concentration of Na/KCl was set to 100 mM. Prior to the MD simulation, the starting structure was subjected to three rounds of energy minimization and equilibrated at 298 K and 1 bar. The final MD was simulated for 100 ns in an NPT ensemble. For details see a previous publication.[10]

Partial charges (RESP)

Partial point charges were derived by RESP charge fitting. Therefore the imidazole ligands and the imidazole Cu(II) complex were fragmented according to the original capping scheme, structure optimized (B3LYP/6-311+G(d,p)) and submitted to the REDServer-Development (Table 4). Inter-molecular and intra-molecular charge constraints were used to maintain the correct total charge of the ligand. In all cases, the constrained charges were similar to the unconstrained values.[21-24]

For the Zn(II) complex a different procedure was applied. The complex was structure optimized (HF/6-31+G\(^*\)) and used as input for antechamber to calculate the RESP charges.[25]

Table 4. RESP charges for L in absence and presence of Cu(II) and Zn(II)

|       | Free ligand | Ligand in complex with Zn(II) | Ligand in complex with Cu(II) |
|-------|-------------|------------------------------|------------------------------|
| O3'   | -0.5155     | -0.5155                      | -0.5155                      |
| C1    | 0.1600      | 0.1600                       | 0.1600                       |
| H1    | 0.0978      | 0.0978                       | 0.0978                       |
| C2    | 0.0465      | 0.0465                       | 0.0465                       |
| H21   | 0.0578      | 0.0578                       | 0.0578                       |
| H22   | 0.0578      | 0.0578                       | 0.0578                       |
| O2    | -0.5103     | -0.5103                      | -0.5103                      |
| C3    | -0.0023     | -0.0023                      | -0.0023                      |
| H31   | 0.0851      | 0.0851                       | 0.0851                       |
| H32   | 0.0851      | 0.0851                       | 0.0851                       |
| O3    | -0.3230     | -0.361395                    | -0.3192                      |
| C4    | 0.0562      | 0.418549                     | 0.1006                       |
| H41   | 0.0694      | -0.033767                    | 0.0693                       |
| H42   | 0.0694      | -0.033767                    | 0.0693                       |
| C5    | -0.0772     | -0.294730                    | -0.0844                      |
Table 5: Forcefield parameters regarding the imidazole ligand L and the respective metal complexes with Cu and Zn

| Bond         | bond length \( b_0 \) [nm] | force constant \( k_0 \) [kJ mol\(^{-1}\) nm\(^{-2}\)] | comment            |
|--------------|-----------------------------|---------------------------------|-------------------|
| CT NB 1      | 0.1475                      | 282001.6                        | Same as CT N*     |
| CE CI 1      | 0.1526                      | 259408                          | Same as CE CT     |
| CR NX/Y 1    | 0.1335                      | 408358.4                        | Same as CR NB     |
| CV NB 1      | 0.1394                      | 343088                          | Same as CV NB     |
| CV CW 1      | 0.1375                      | 428441.6                        | Same as CC CV     |
| CW NX/Y 1    | 0.1394                      | 343088                          | Same as CV NB     |
| NX/Y CU 1    | 0.20219                     | 69965.4                         | VFFDT             |
| NX/Y ZN 1    | 0.209                       | 28325.7                         | VFFDT             |

| angle        | angle                       | force constant \( k_0 \) [kJ mol\(^{-1}\) nm\(^{-2}\)] | comment            |
|--------------|-----------------------------|---------------------------------|-------------------|
| OS CI CE H1  | 109.5                       | 418.4                           | Same as OS CE CT  |
| CT CI H1 CT  | 109.5                       | 418.4                           | Same as CT CT H1  |
| CI CT NB CT  | 109.5                       | 418.4                           | Same as CT CT N*  |
| CI CE OS CT  | 109.5                       | 418.4                           | Same as OS CE CT  |
| H1 CT NB CT  | 109.5                       | 418.4                           | Same as H1 CT N*  |
| CW CV H4 CW  | 120                         | 418.4                           | Same as CC CV H4  |
| CV CW H4 CW  | 120                         | 418.4                           | Same as CC CV H4  |
| CR NB CT CW  | 128.8                       | 585.76                          | Same as CK N* CT  |
| CV NB CT CW  | 128.8                       | 585.76                          | Same as CK N* CT  |
| CW CV NB CW  | 120                         | 585.76                          | Same as CC CW NB  |
| CR NX/Y CW   | 120                         | 585.76                          | Same as CR NA CW  |
| NX/Y CW CV   | 120                         | 585.76                          | Same as CC CW NA  |
| NB CR NX/Y/Z | 120                         | 585.76                          | Same as NA CR NA  |
| H4 CW NX/Y/Z | 120                         | 418.4                           | Same as H4 CW NA  |
| H5 CR NX/Y/Z | 120                         | 418.4                           | Same as H5 CR NA  |
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| NX/Y | CU | NY/X | 1 | 90 | 521.81 | estimated |
|------|----|------|---|----|--------|-----------|
| NX/Y | CU | NX/Y | 1 | 180 | 512.5 | estimated |
| CW   | NX/Y | CU | 1 | 126.8 | 890 | VFFDT |
| CR   | NX/Y | CU | 1 | 126.9 | 857.2 | VFFDT |
| CW   | NX/Y | ZN | 1 | 126.6 | 202.1 | VFFDT |
| CR   | NX/Y | ZN | 1 | 126.6 | 199.6 | VFFDT |
| NX/Y | ZN | NY/X | 1 | 109.5 | 134.7 | VFFDT |
| NX/Y | ZN | NX/Y | 1 | 109.5 | 134.7 | VFFDT |

| improper dihedral | angle | force constant $k_\phi$ [kJ mol$^{-1}$ nm$^{-2}$] | comment |
|-------------------|-------|---------------------------------|---------|
| CW                | CR    | NX               | CU       | 4     | 180  | 30 | estimated |
| CW                | CR    | NX               | ZN       | 4     | 180  | 30 | estimated |

| proper dihedral | angle | force constant $k_\phi$ [kJ mol$^{-1}$ nm$^{-2}$] | comment |
|-----------------|-------|---------------------------------|---------|
| X               | CV    | CW               | X        | 9     | 180  | 6.276 | Same as X CC CW X |
| X               | CT    | NB               | X        | 9     | 0    | 0     | Same as X CT N* X |
| X               | CW    | NX/Y             | X        | 9     | 180  | 9.7278 | Same as X CW NA X |
| X               | NX/Y  | CR               | X        | 9     | 180  | 9.7278 | Same as X CW NA X |
| X               | CE    | CI               | X        | 9     | 0    | 0.65084 | same as X CT CT X |
| P               | OS    | CI               | CE       | 9     | 297.765 | 4.518 | same as P OS CE CT |
| CT              | CE    | CI               | OS       | 9     | 348.09 | 4.0284 | same as OS CI CT CT |

Figure 83. Representative MD-snapshot of G$_{4}$L$^{28}$. Ligands were preferentially found to interact with the 5'-G-tetrad or the groove regions.
Figure 84. Representative MD-snapshot of G₅L₂R in complex with Cu(II). Coordination of L to Cu(II) led to a decreased RMSD compared to unbound L (Fig. 79).

Figure 85. Representative MD-snapshot of G₅L₂R in the gas phase. L was mostly located in the groove regions of the G-quadruplex rather than interacting with the 5’-G-tetrad.

Figure 86. Representative MD-snapshot of G₅L₂R in complex with Cu(II) in the gas phase. L is coordinating Cu(II) in a propeller shaped square planar geometry and is not able to back fold into the groove regions (compare Fig. 81). This might cause the slight CCS increase after Cu(II) complexation.
Figure 87. Representative MD-snapshot of htelL<sub>2</sub>₄. L was preferentially found to interact by π-π-stacking with the G-tetrad or nucleobases from the loop regions.

Figure 88. Representative MD-snapshot of htelL<sub>2</sub>₄ in complex with Cu(II). Complexation of Cu(II) leads to a decreased movement of L compared to the Cu(II) free sample as expressed by lower RMSD values.

Figure 89. Representative MD-snapshot of htelL<sub>2</sub>₄ in complex with Zn(II). In contrast to Cu(II) for the Zn(II) complex five ligands were found coordinating to Zn(II) in a trigonal bipyramidal geometry. As additional ligand nucleobases from the loop regions as well as water were found. In this figure the carbonyl oxygen from thymine is coordinating to Zn(II).
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Figure 90. Representative MD-snapshot of htelL₂₄₆. L was preferentially found to interact via π-π-stacking with the G-tetrad or nucleobases from the loop regions.

Figure 91. Representative MD-snapshot of htelL₂₄₆ in complex with Cu(II). Complexation of Cu(II) leads to a decreased movement of L compared to the Cu(II) free sample as it is expressed in lower RMSD values.

Figure 92. Representative MD-snapshot of htelL₂₄₆ in complex with Zn(II). In contrast to Cu(II) for the Zn(II) complex five ligands were found coordinating to Zn(II) in a trigonal bipyramidal geometry. One of the two nonbonded imidazole ligands was usually involved in coordination.
Figure 93. Representative MD-snapshot of hteL^{25}C. L was preferentially found to interact via π-π-stacking with the G-tetrad or nucleobases from the loop regions.

Figure 94. Representative MD-snapshot of hteL^{25}C in complex with Cu(II). Complexation of Cu(II) leads to a decreased movement of L compared to the Cu(II) free sample as it is expressed in lower RMSD values.

Figure 95. Representative MD-snapshot of hteL^{25}C in complex with Zn(II). In contrast to Cu(II) for the Zn(II) complex five ligands were found coordinating to Zn(II) in a trigonal bipyramidal geometry. As additional ligand nucleobases from the loop regions as well as water were found. In this figure water is coordinating to Zn(II).
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Figure 96. Analytical data for G4L2R

Figure 97. Analytical data for G4L2S
Figure 98. Analytical data for htelL154

Figure 99. Analytical data for htelL184

Sequence (5’→ 3’):
AGG LTT ALG GTT
AGG LTT ALG G
Figure 100. Analytical data for htelL24

Figure 101. Analytical data for htelL24
Figure 102. Analytical data for htel^{225}

Figure 103. Analytical data for htel^{235}
Supporting Information

Figure 104. Analytical data for htel\textsuperscript{L6}

Sequence (5' → 3')
AGG LTL TLG GTT
AGG LTL TLG G

Analytical RP-HPLC

Figure 105. Analytical data for htel\textsuperscript{A6}

Sequence (5' → 3')
AGG LTL TLG GTT
AGG LTL TLG G

Analytical RP-HPLC
Supporting Information

Figure 106. Analytical data for htelL^{R}

Figure 107. Analytical data for htelL^{S}
Supporting Information

Figure 108. Analytical data for htelL²₃

Figure 109. Analytical data for htelL²₃
Figure 110. Analytical data for hteL$_{2}^{34}$

Figure 111. Analytical data for hteL$_{2}^{34}$
Supporting Information

Figure 112. Analytical data for htelL24jB

Figure 113. Analytical data for htelL24jB
Figure 114. Analytical data for htelL2R3B

Figure 115. Analytical data for htelL2S3B
Figure 116. Analytical data for htelL²S₄B

Figure 117. Analytical data for htelL²R₄B
Figure 118. Analytical data for htel24C

Sequence (5→3):
AGG TLT LGG TTA
GTT LTL GG

Analytical RP-HPLC

Figure 119. Analytical data for htel24C

Sequence (5→3):
AGG TLT LGG TTA
GTT LTL GG

Analytical RP-HPLC
9 Literature

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