Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

The Influence of G-Quadruplex Structure on DNA-Based Asymmetric Catalysis Using G-Quadruplex-Bound Cationic Porphyrine TMPyP4·Cu

*Michael Wilking and Ulrich Hennecke**

Dr. U. Hennecke
Organisch-Chemisches Institut
Westfäliche Wilhelms-Universität Münster
Corrensstraße 40, 48149 Münster (Germany)

E-mail: ulrich.hennecke@uni-muenster.de

Supporting Information

- Table of contents -

1. General Informations

^{1}H NMR spectra were recorded on a *Bruker DPX 300* (300 MHz) spectrometer. **CD** measurements were recorded on a J-815 (*Jasco*). **HPLC** measurements were performed on an *Agilent Technologies* 1200 Series HPLC using UV detection (230 nm). Separation was performed on a Chiralcel® OD-H (0.46 cm × 25 cm, *Daicel Chemical Industries, Ltd.*) column. All solvents were HPLC grade, purchased from Fisher Scientific and used as received.

2. Materials

DNA Oligonucleotides

ODN-1: 5‘-$AG_3(TTAGGG)_3$-3‘

ODN-2: 5‘-$G_3(TTAGGG)_3$-3‘

ODN-3: 5‘-$CG_3(TTAGGG)_3$-3‘

ODN-4: 5‘-$TG_3(TTAGGG)_3$-3‘

ODN-5: 5‘-$GG_3(TTAGGG)_3$-3‘

ODN-6: 5‘-$AAG_3(TTAGGG)_3$-3‘

ODN-7: 5‘-$ATG_3(TTAGGG)_3$-3‘

ODN-8: 5‘-$AG_3(TTAGGG)_2$TTTGGG-3‘

ODN-9: 5‘-$AG_3(TTAGGG)_2$TTCGGG-3‘

ODN-10: 5‘-AGGGTTAGGGTTTGGGTTAGGG-3‘

ODN-11: 5‘-AGGGTTTGGG$(TTAGGG)_2$-3‘

ODN-12: 5‘-$AG_3(TTAGGG)_3$AA-3‘

ODN-13: 5‘-$AAAG_3(TTAGGG)_3$AA-3‘

ODN-14: 5‘-AAAGGGTTAGGGTTTGGGTTAGGGA-3‘

ODN-15: 5‘-AAAGGGTTAGGGTTTGGGTTAGGG-3‘

ODN-16: 5’-TGAGGGTGGTGAGGGTGGGGAAGG-3’

ODN-17: 5’-TGAGGGTGGTGTGGGTGGGGAAGG-3’

ODN-18: 5’-AGGGTGGTGAGGGTGGGGAAGG-3’

ODN-19: 5’-GAGGGTGGTGAGGGTGGGGAAGG-3’

ODN-20: 5’-TAGGGTGGTGAGGGTGGGGAAGG-3’

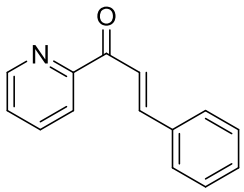
ODN-21: 5’-AGGGAGGGCGCTGGGAGGAGGG-3’

ODN-22a: 5’-CGCTATGCTGCATCGC-3’

ODN-22b: 3’-GCGATACGACGTAGCG-5’

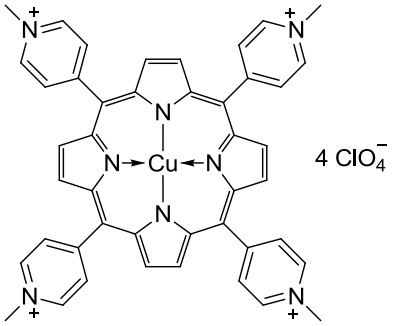
were purchased from *Metabion* (Martinsried, Germany).

The water used was purified and deionized using a *Elga Maxima* water purification system. 4,4′-Dimethyl-2,2′-dipyridyl, $Cu(NO_3)_2 \times 3\ H_2O$, 3-(*N*-morpholino)propanesulfonic acid (MOPS) and all other chemicals were obtained from commercial sources (*Sigma-Aldrich*, *Alfa Aesar*, *Fisher Scientific*) and used without further purification. 5,10,15,20-Tetrakis(1-methylpyridinium-3-yl)porphyrin was obtained from *Frontier Scientific* and was used without further purification. Cyclopentadiene **2** was freshly cracked before use. Dienophile **1** was prepared according to the literature procedure.[1]

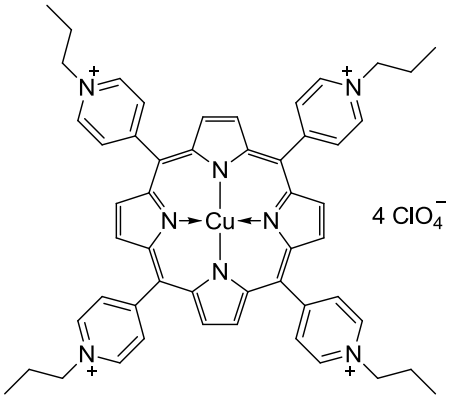


1

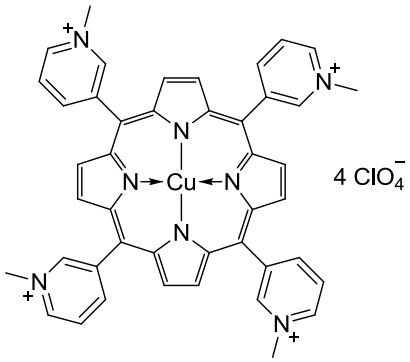
5,10,15,20-Tetrakis(1-methylpyridinium-4-yl)porphyrinatocopper(II) tetraperchlorate **4**, 5,10,15,20-Tetrakis(1-propylpyridinium-4-yl)porphyrinatocopper(II) tetraperchlorate **5**, 5,10,15,20-Tetrakis(1-methylpyridinium-3-yl)porphyrinatocopper(II) tetraperchlorate **7** and phthalocyanine **8** were synthesized according to literature procedures.[2]



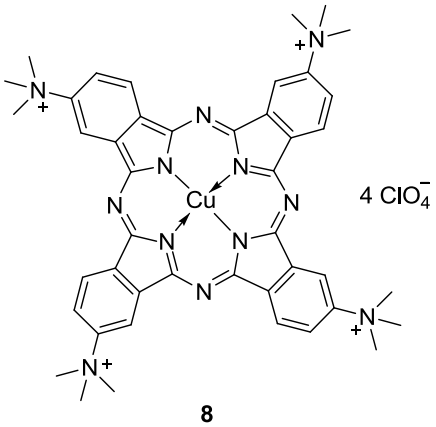
4



5



7



8

3. General Procedure for the *Diels- Alder*-Reactions

To an *Eppendorf* Vial (capacity 2 ml) containing MOPS buffer (0.5 mL, final conc. 10 mM, pH 6.5) and KCl (final conc. 50 mM) a solution of the corresponding Oligonucleotide (aq. 0.5 mM, 48 µL, 24 nmol final conc. 48 µM) was added. The solution was warmed to 95 °C for 3 minutes and then slowly cooled down over 2 hours to room temperature. After addition of a solution of the Cu-source (final conc. 40 µM), the reaction mixture was shaken for 30 minutes while cooling down to 5 °C.
Then, a solution of aza-chalcone **1** (20 mM in CH_3CN, 10 µL, 0.2 µmol) was added and the reaction was initiated by the addition of a solution of freshly distilled cyclopentadiene **2** (0.4 M in CH_3CN, 10 µL, 4.0 µmol, 20 equiv.). After shaking for 40 hours at 5 °C in a TMIX 220 (*Analytik Jena*), the reaction was stopped by the addition of Et_2O (1.5 mL). After separation of layers, the aqueous layer was extracted with Et_2O (2 × 1.5 mL) and the combined organic layers were filtered through a short pad of silica. After removal of the solvents, the crude product was dissolved in HPLC grade *n*-hexane/*i*-PrOH (90:10) and directly analyzed by HPLC. The conversion, diastereoselectivity (*endo*:*exo*) and enantiomeric excess (*ee*) were determined by chiral HPLC according to the literature procedure.[3]

HPLC conditions:
Product **3**: *Daicel* Chiralcel®-ODH, *n*-hexane/*i*-PrOH 98:2, 1 mL/min, 230 nm. Retention times:
6.4, 7.2 minutes (*exo* isomer); 8.2, 10.6 minutes (*endo* isomer).

4. Calculation the Conversion of 1

Conversions of **1** were calculated using the following formula [3]:

$$\text{Conversion } \mathbf{1}\ (\%) = \frac{PA_3}{PA_3 + PA_1 \times f}$$

Where PA_1 and PA_3 are the HPLC peak areas of **1** and **3**, respectively. And *f* is the correction factor determined to be 2.4807 from a fitting curve (Figure S1).

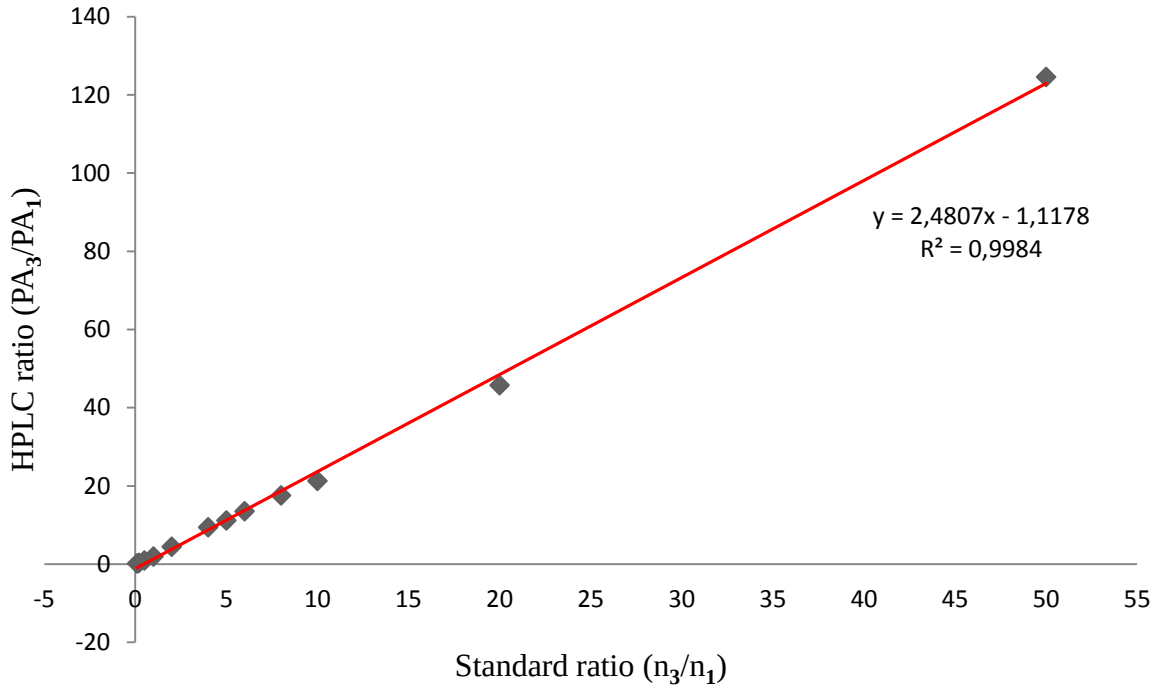


Figure S1: Determination of the correction factor *f*.

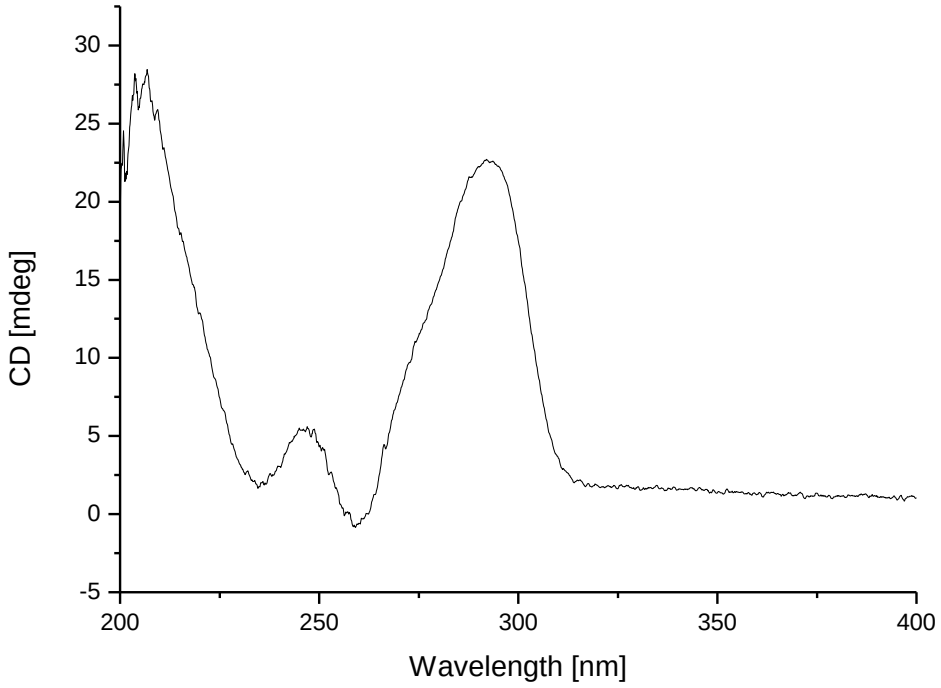
The HPLC ratios of peak areas (PA_3/PA_1) were determined with the standard molar ratios (n_3/n_1) of 1/10, 1/5, 1/2, 1, 2, 4, 5, 6, 8, 10, 20, 50. The correction factor (f = 2.4807) was estimated from the fitting curve (R^2 = 0.9984).

The HPLC chromatogram of the molar ratio (n_3/n_1) = 1 is shown in Chapter 9.

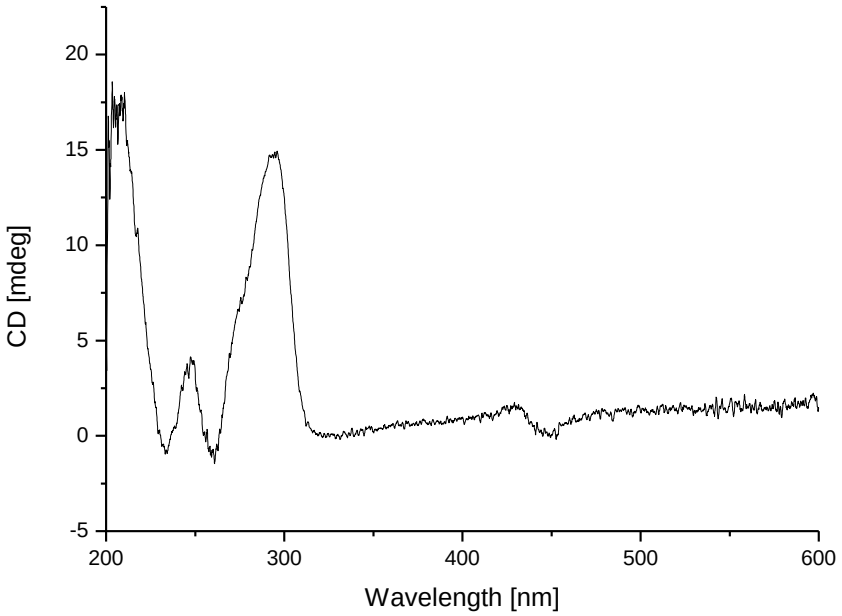
5. Circular Dichroism (CD) Measurements

Circular dichroism (CD) spectra were recorded on a Jasco J-815 CD spectrophotometer with a pathlength of 10 mm (1 ml quartz cell). ODNs (5 µM) were dissolved in MOPS buffer (10 mM) containing either potassium chloride (50 mM) or sodium chloride (50 mM). CD spectra were measured from 400 to 200 nm at a temperature of 20 °C and accumulated three times. A background spectrum of water was subtracted. CD spectra of ODN/TMPyP4CuCu **4** hybrids were measured under the same conditions except that TMPyP4·Cu **4** was added (final concentration: 4.2 µM) and the spectrum was measured from 600 nm to 200 nm.

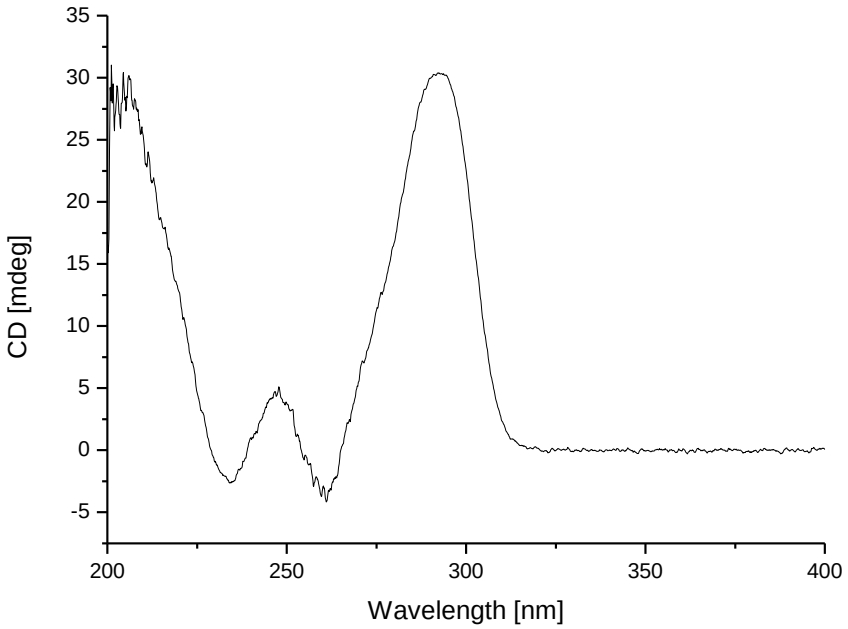
ODN-1 (5 µM ODN, 10 mM MOPS, 50 mM KCl)



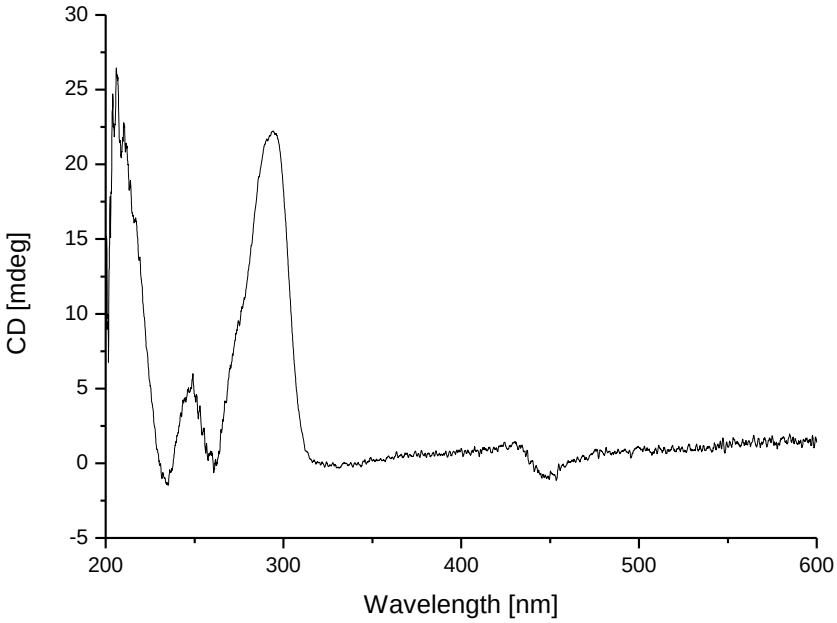
ODN-1 (5 µM ODN, 4.2µM TMPyP4Cu 4, 10 mM MOPS, 50 mM KCl)



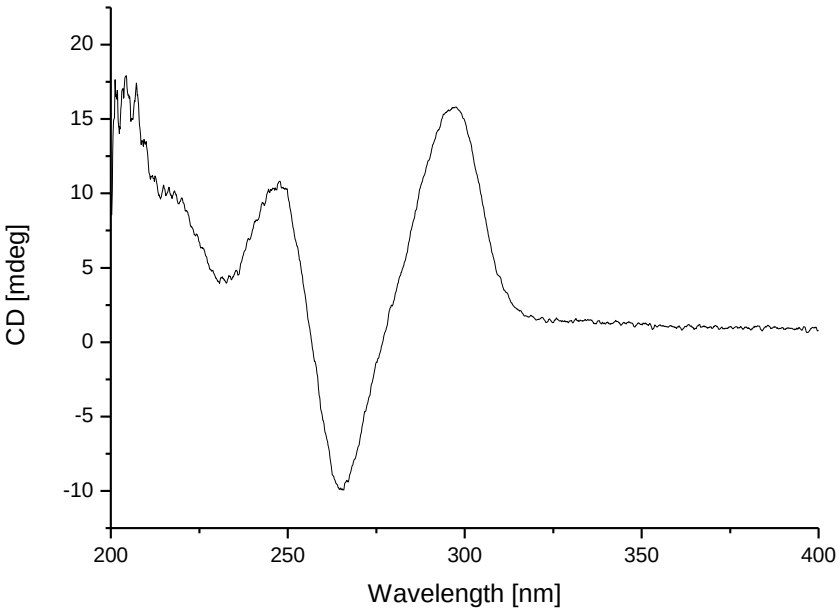
ODN-2 (5 µM ODN, 10 mM MOPS, 50 mM KCl)



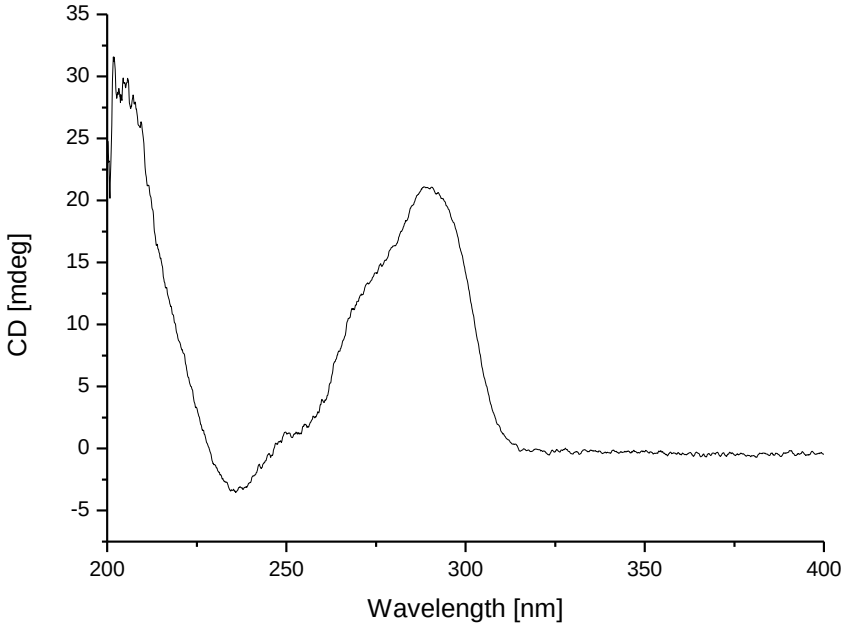
ODN-2 (5 µM ODN, 4.2µM TMPyP4Cu 4, 10 mM MOPS, 50 mM KCl)



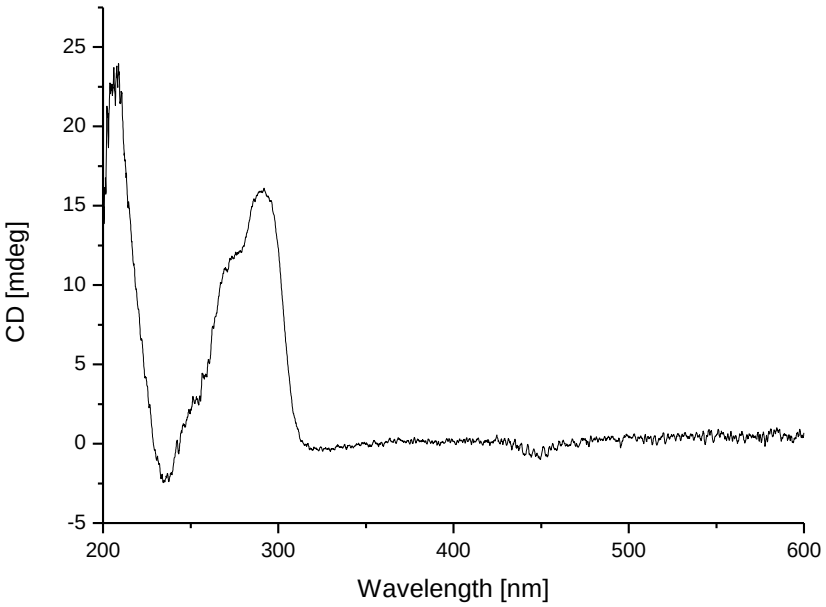
ODN-2 (5 µM ODN, 10 mM MOPS, 50 mM NaCl)



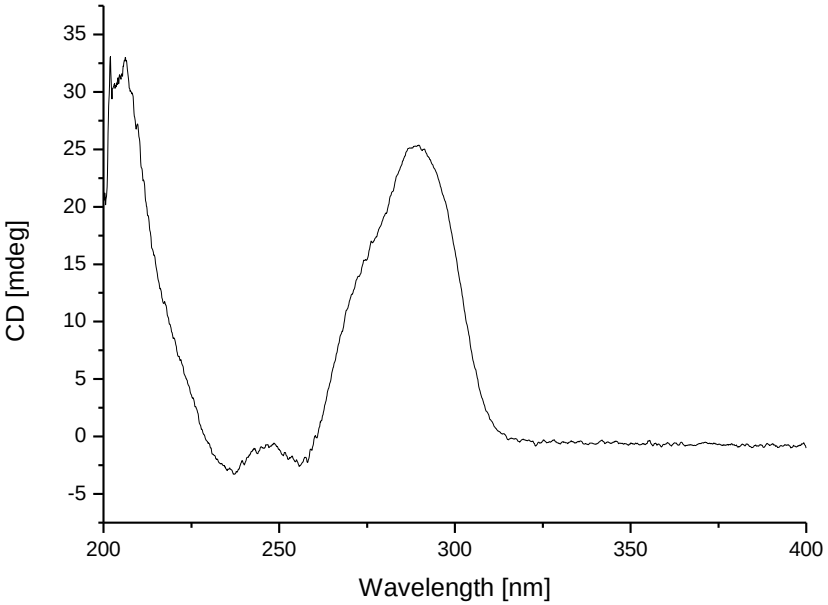
ODN-5 (5 µM ODN, 10 mM MOPS, 50 mM KCl)



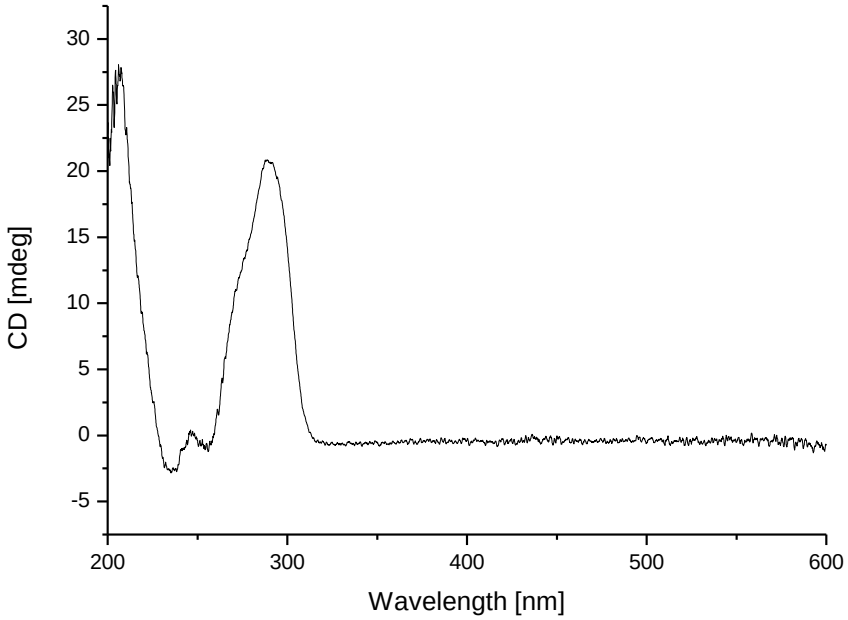
ODN-5 (5 µM ODN, 4.2µM TMPyP4Cu 4, 10 mM MOPS, 50 mM KCl)



ODN-8 (5 µM ODN, 10 mM MOPS, 50 mM KCl)



ODN-8 (5 µM ODN, 4.2µM TMPyP4Cu 4, 10 mM MOPS, 50 mM KCl)

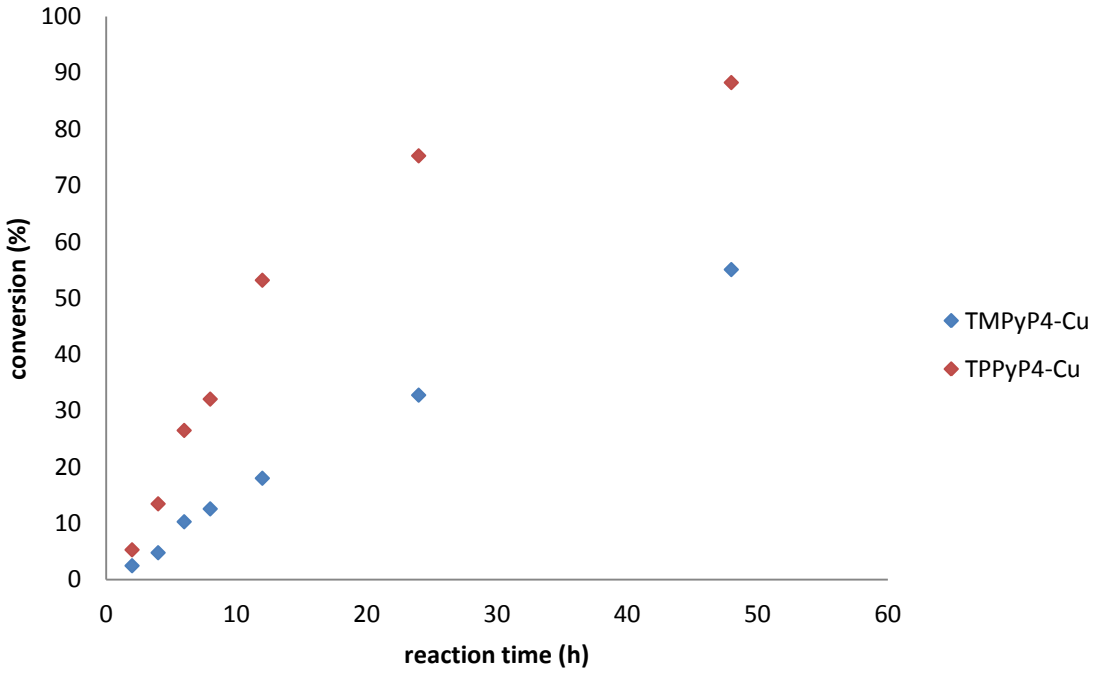


6. Kinetic Measurements

To an *Eppendorf* Vial (capacity 2 ml) containing MOPS buffer (0.5 mL, final conc. 10 mM, pH 6.5) and KCl (final conc. 50 mM) a solution of the corresponding Cu-source (final conc. 40 µM) was added and the reaction mixture was shaken for 30 minutes while cooling down to 5 °C.

Then, a solution of aza-chalcone **1** (20 mM in CH_3CN, 10 µL, 0.2 µmol) was added and the reaction was initiated by the addition of a solution of freshly distilled cyclopentadiene **2** (0.4 M in CH_3CN, 10 µL, 4.0 µmol, 20 equiv.). After shaking for a specific time at 5 °C in a TMIX 220 (*Analytik Jena*), the reaction was ended by adding Et_2O (1.5 mL). After separation of the layers, the aqueous layer was extracted with Et_2O (2 × 1.5 mL) and the combined organic layers were filtered through a short pad of silica. After removal of the solvents, the crude product was dissolved in HPLC grade *n*-hexane/*i*-PrOH (90:10) and the conversion was directly analyzed by HPLC as already described above.

entry	Reaction time (h)	Conversion (%) of **1** using TMPyP4Cu-Cu **4** as Catalyst	Conversion (%) of **1** using TPPyP4Cu **5** as Catalyst
1	2	2	5
2	4	5	13
3	6	10	27
4	8	13	32
5	12	18	53
6	24	33	75
7	48	55	88



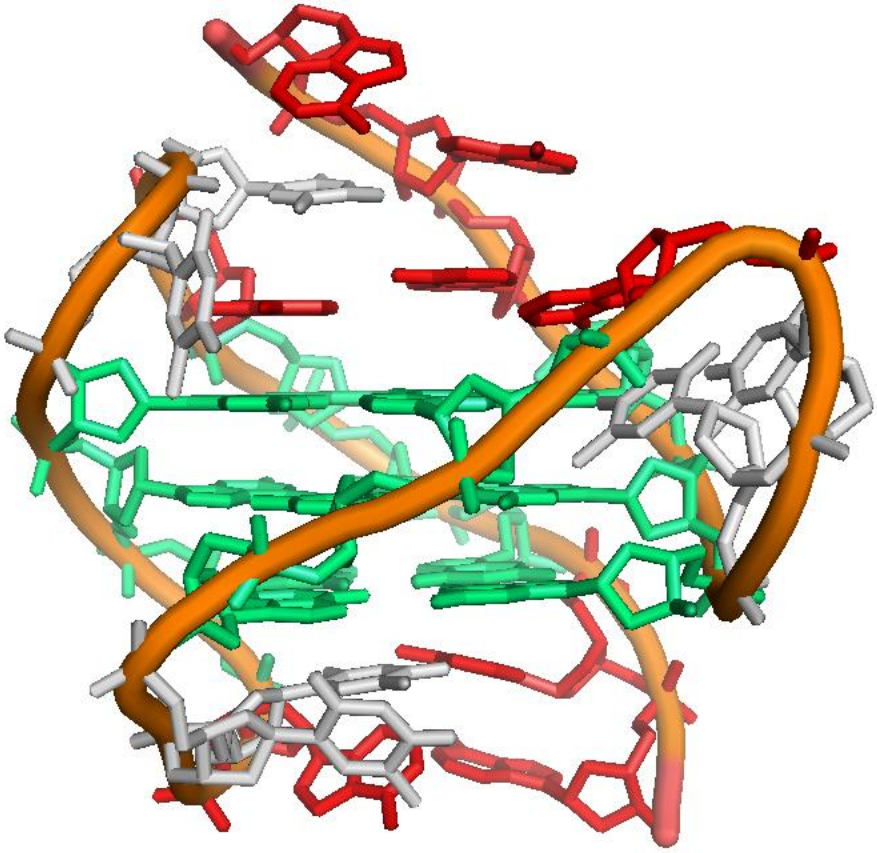
7. Structual Drawings of G-Quadruplex-Structures

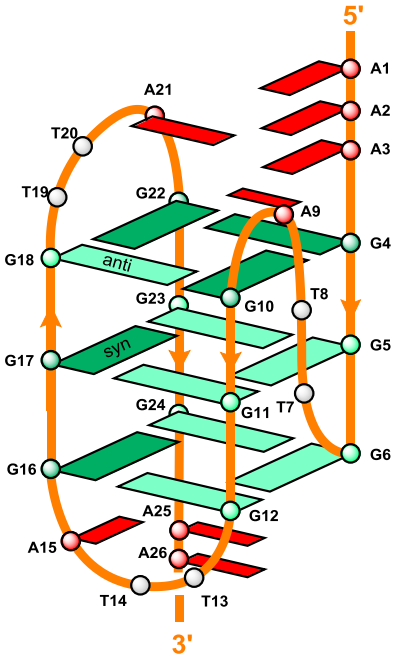
H-Tel- G-Quadruplex Structure:

pdb: 2HY9 - J. Dai, C. Punchihewa, A. Ambrus, D. Chen, R. A. Jones, D. Yang, *Nucleic Acids Res.* **2007**, *35*, 2440.

5‘-AAAGGGTTAGGGTTAGGGTTAGGGAA-3‘ - h-tel- Sequence

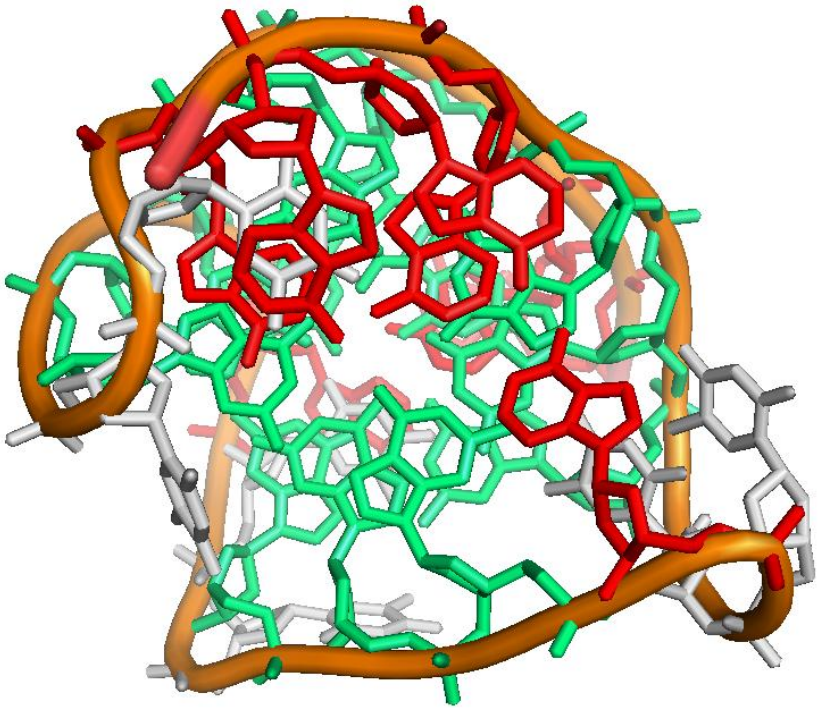
5‘-AAAGGGTTAGGGTTAGGGTTAGGGAA-3‘ - Structure





In green: guanines, in red: nucleobases overlooking the 5’ or 3’-guanine tetrad.

Red nucleobase were deleted or substituted in **ODN-1** to **ODN-15**.

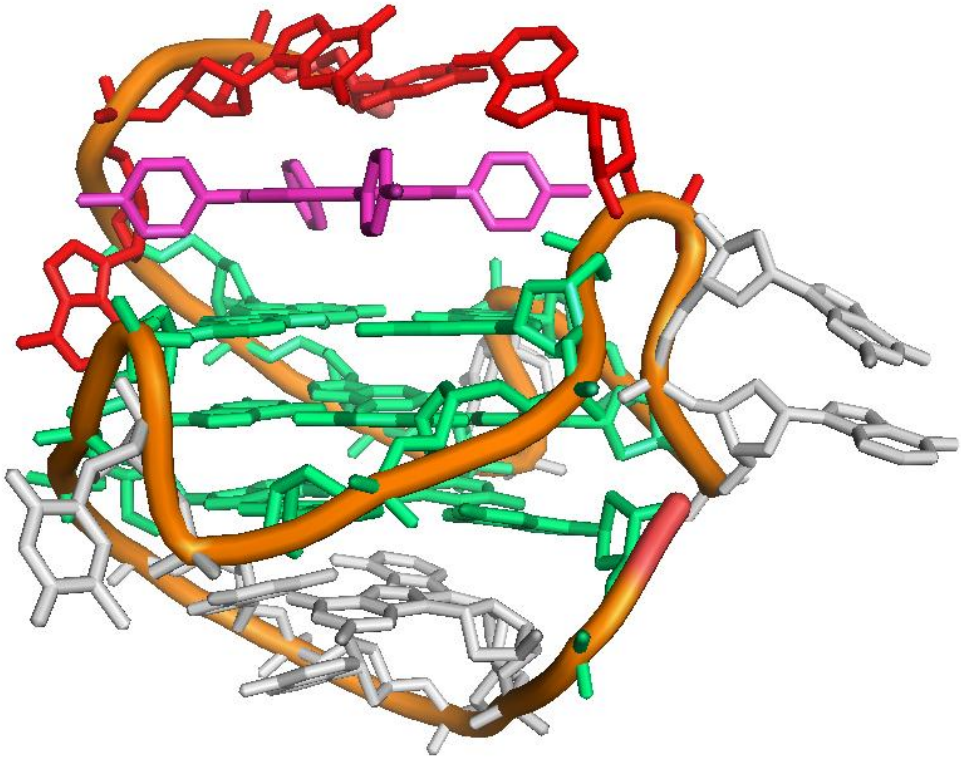


C-Myc- G-Quadruplex Structure:

Pdb 2A5R - A. T. Phan, V. Kuryavyi, H. Y. Gaw, D. J. Patel, *Nat. Chem. Biol.* **2005**, *1*, 167.

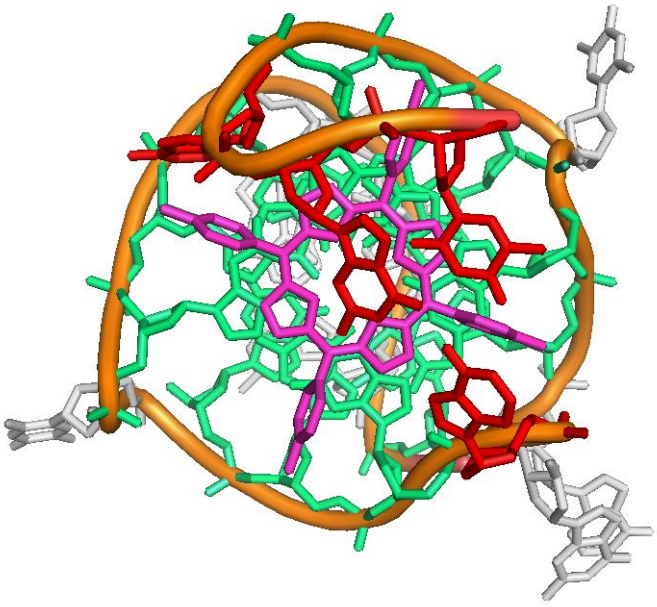
5’-TGAGGGTGGTGAGGGTGGGGAAGG-3’ - c-myc- Sequence

5’-TGAGGGTGGIGAGGGTGGGGAAGG-3’ - Structure

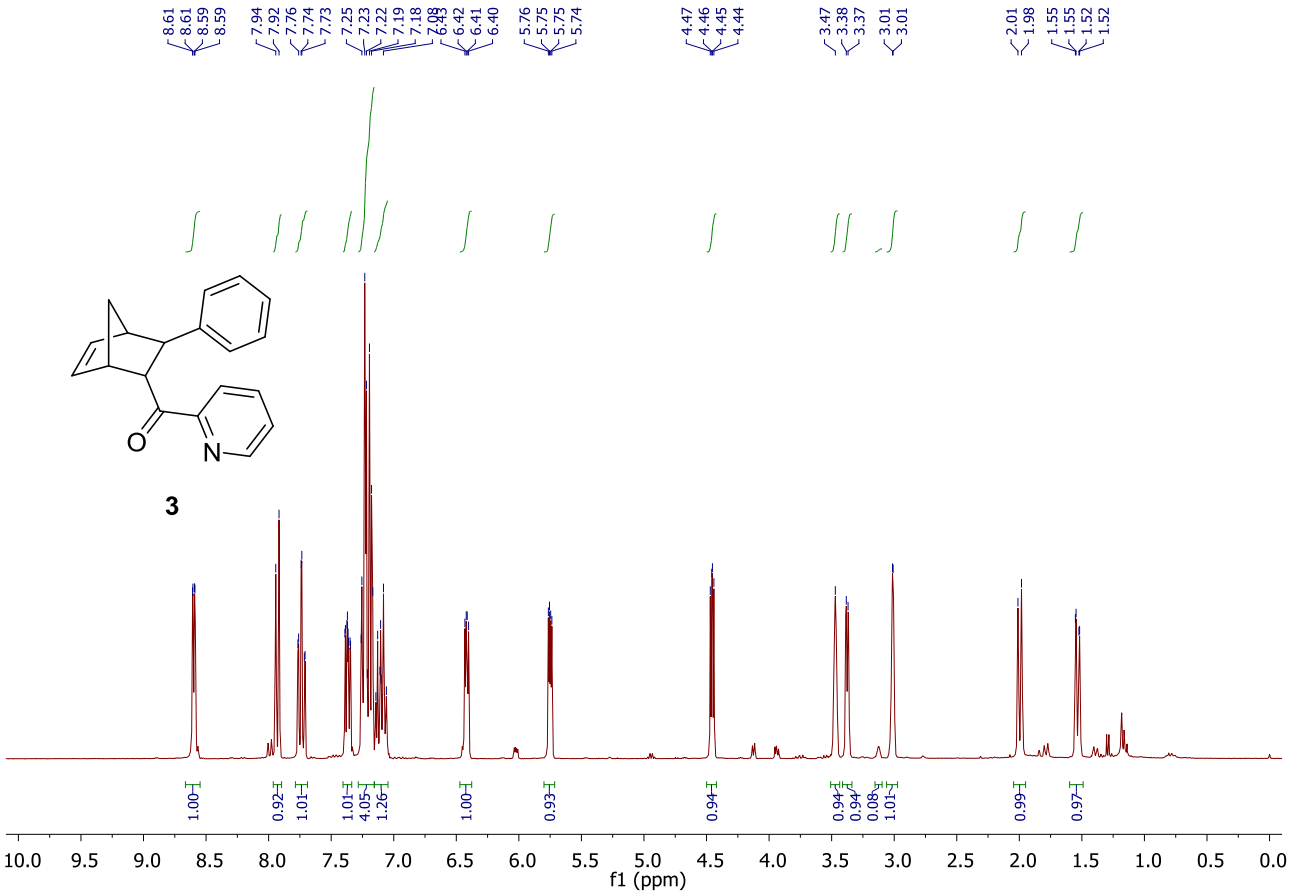


In green: guanines, in red: nucleobases overlooking the 5’-guanine tetrad.

Red nucleobase were deleted or substituted in **ODN-16** to **ODN-21**.



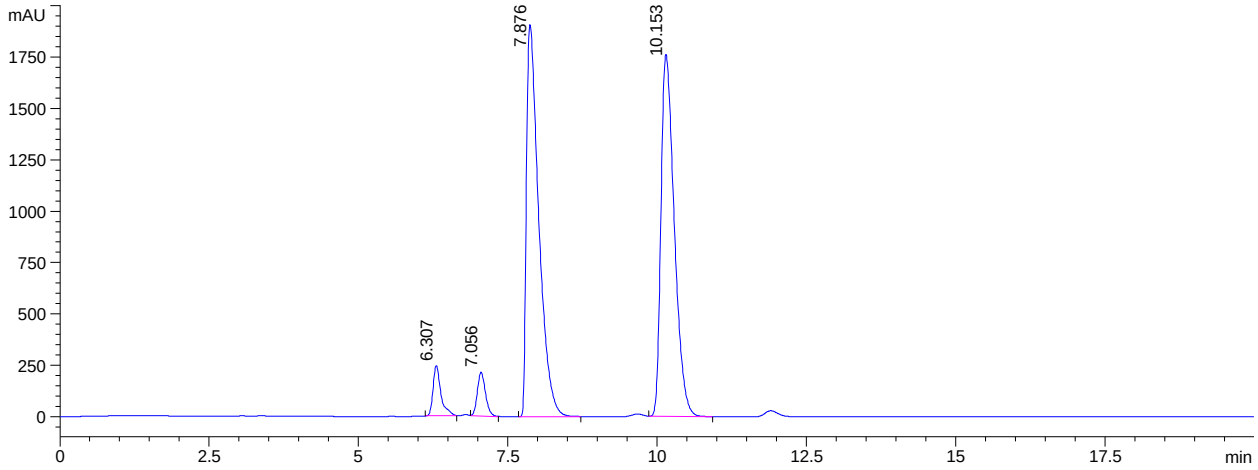
8. ^{1}H NMR Spektrum of 3



9. Selected HPLC traces

rac-3

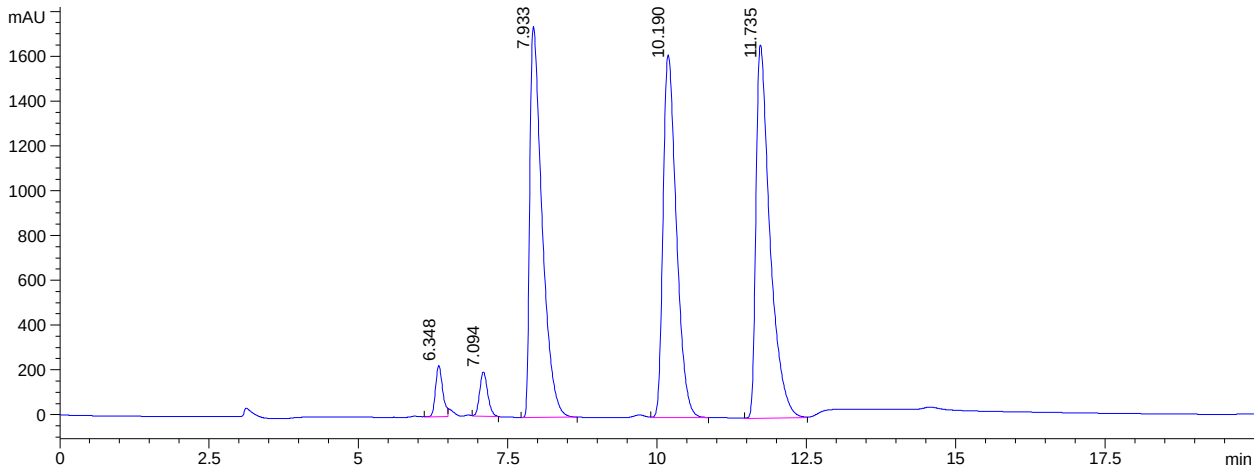
OD-H, Hex:iPrOH 98:02, 1 mL/min
racemic mixture of 3



#	Meas. Ret. Time	Height	Height %	Area	Area %
1	6.307	244.045	5.916	2241.731	3.803
2	7.056	213.123	5.166	2010.808	3.411
3	7.876	1907.456	46.238	27339.621	46.375
4	10.153	1760.711	42.680	27361.793	46.412

1:1 mixture of aza-chalcone 1 and products 3

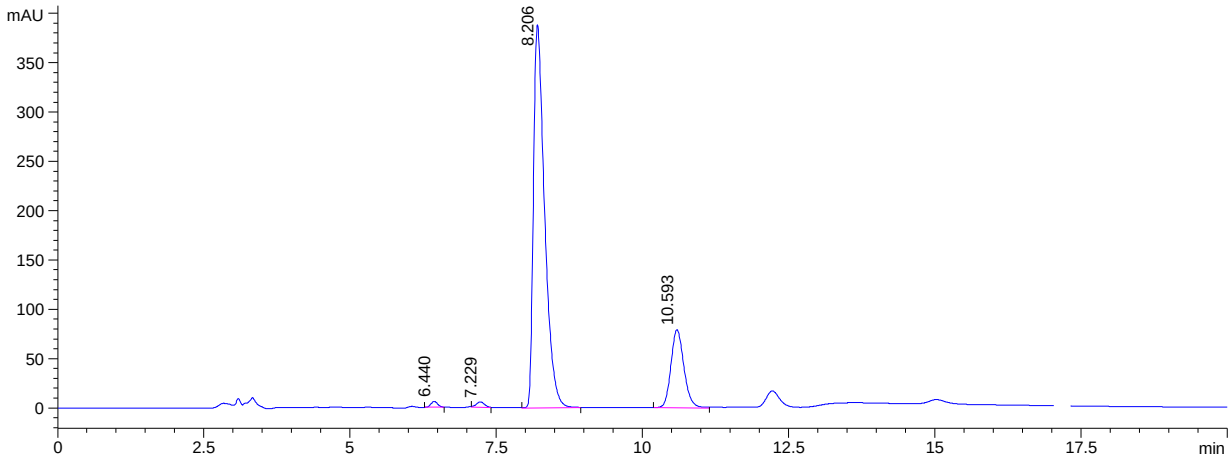
OD-H, Hex:iPrOH 98:02, 1 mL/min
Calibration curve for the conversion of 1: molar ratio (n3/n1) = 1/1



#	Meas. Ret. Time	Height	Height %	Area	Area %
1	6.348	227.274	4.163	1902.487	2.345
2	7.094	197.757	3.622	1850.982	2.281
3	7.933	1746.407	31.988	25025.025	30.845
4	10.190	1621.458	29.699	25139.738	30.986
5	11.735	1666.757	30.529	27213.104	33.542

ODN-1, Porphyrine 4, 50 mM KCl (table 1, entry 1)

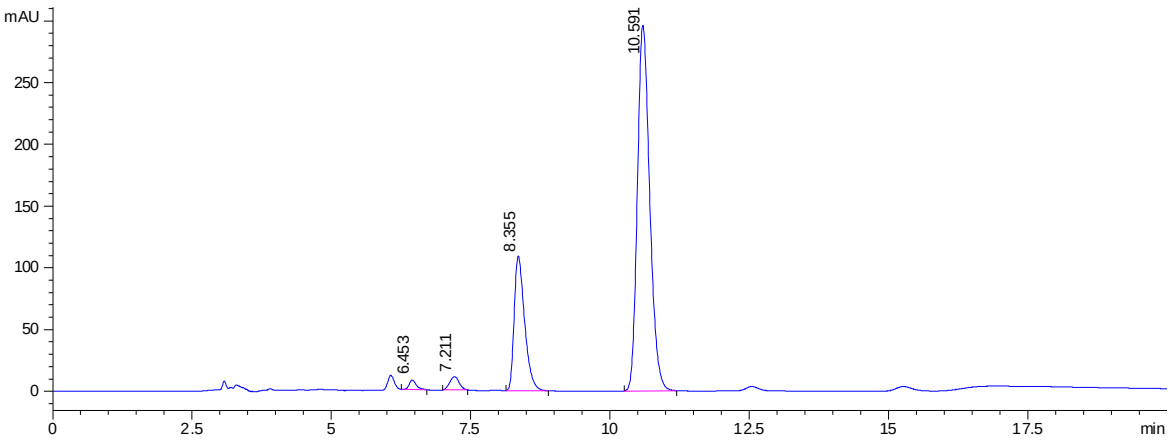
OD-H, Hex:iPrOH 98:02, 1 mL/min
G-Quadruplex DNA - h-tel - 12 mol%, MOPS 10mM, KCl 50mM, TMePyP-Cu-ClO4 - 10 mol%, 5 °C, 40 h



#	Meas. Ret. Time	Height	Height %	Area	Area %
1	6.440	5.953	1.242	46.923	0.723
2	7.229	5.423	1.132	53.650	0.827
3	8.206	388.583	81.103	5164.930	79.595
4	10.593	79.167	16.523	1223.513	18.855

ODN-2, $Cu(NO_3)_2$, 50 mM NaCl (table 1, entry 3)

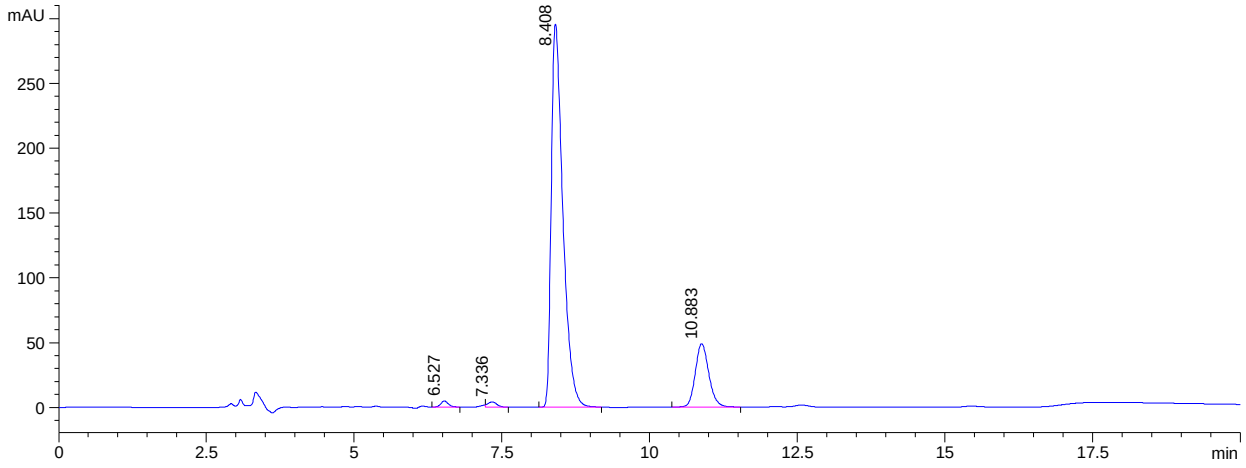
OD-H, Hex:iPrOH 98:02, 1 mL/min
G-Quadrupholex DNA - h-tel-mod01 - 12 mol%, MOPS 10 mM, NaCl 50 mM, Cu(NO3)2 * 3H2O, 5 °C, 40 h
without dna hybridization



#	Meas. Ret. Time	Height	Height %	Area	Area %
1	6.453	7.858	1.854	70.670	1.141
2	7.211	10.827	2.554	127.572	2.060
3	8.355	108.893	25.691	1422.076	22.961
4	10.591	296.283	69.901	4573.074	73.838

ODN-4, Porphyrine 4, 50 mM KCl (table 1, entry 5)

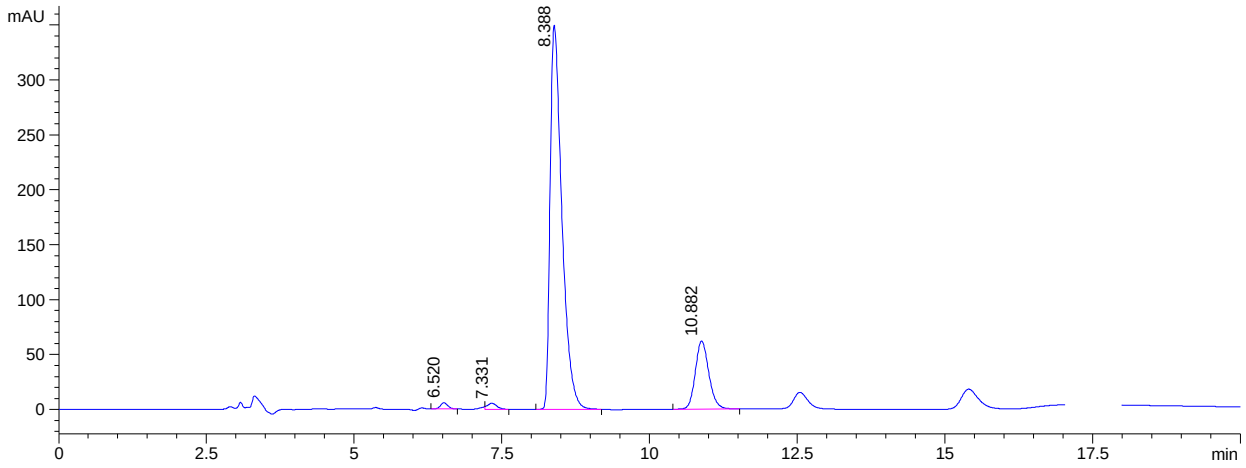
OD-H, Hex:iPrOH 98:02, 1 mL/min
G-Quadruplex DNA - h-tel-mod03 - 12 mol%, MOPS 10mM, KCl 50mM, TMePyP-Cu-ClO4 - 10 mol%, 5 °C, 40 h



#	Meas. Ret. Time	Height	Height %	Area	Area %
1	6.527	4.736	1.340	41.596	0.849
2	7.336	4.098	1.159	44.179	0.902
3	8.408	295.662	83.659	4035.945	82.381
4	10.883	48.918	13.842	777.376	15.868

ODN-6, Porphyrine 4, 50 mM KCl (table 1, entry 7)

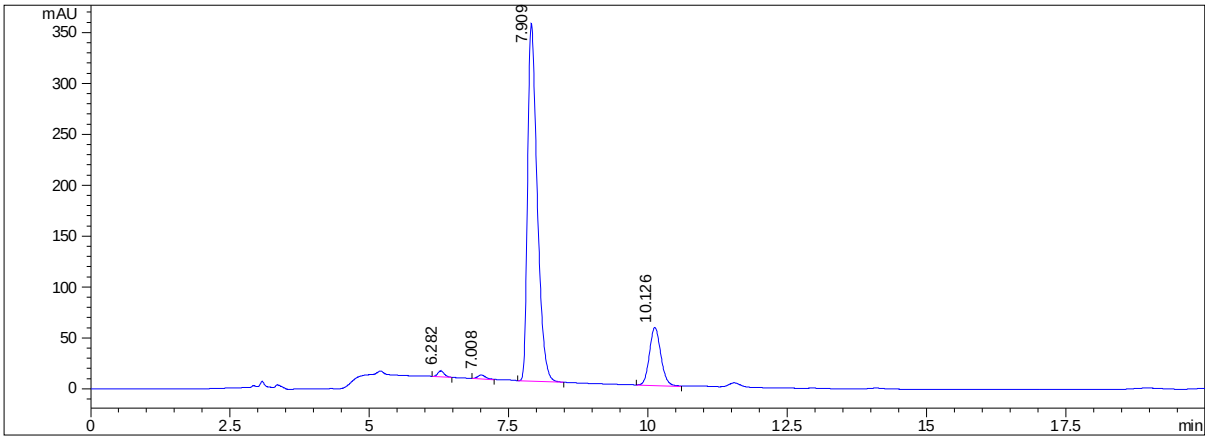
OD-H, Hex:iPrOH 98:02, 1 mL/min
G-Quadruplex DNA - h-tel-mod06 - 12 mol%, MOPS 10mM, KCl 50mM, TMePyP-Cu-ClO4 - 10 mol%, 5 °C, 40 h



#	Meas. Ret. Time	Height	Height %	Area	Area %
1	6.520	5.730	1.355	51.371	0.867
2	7.331	5.394	1.276	58.026	0.979
3	8.388	349.834	82.720	4828.303	81.471
4	10.882	61.955	14.649	988.708	16.683

ODN-10, Porphyrine 4, 50 mM KCl (table 1, entry 11)

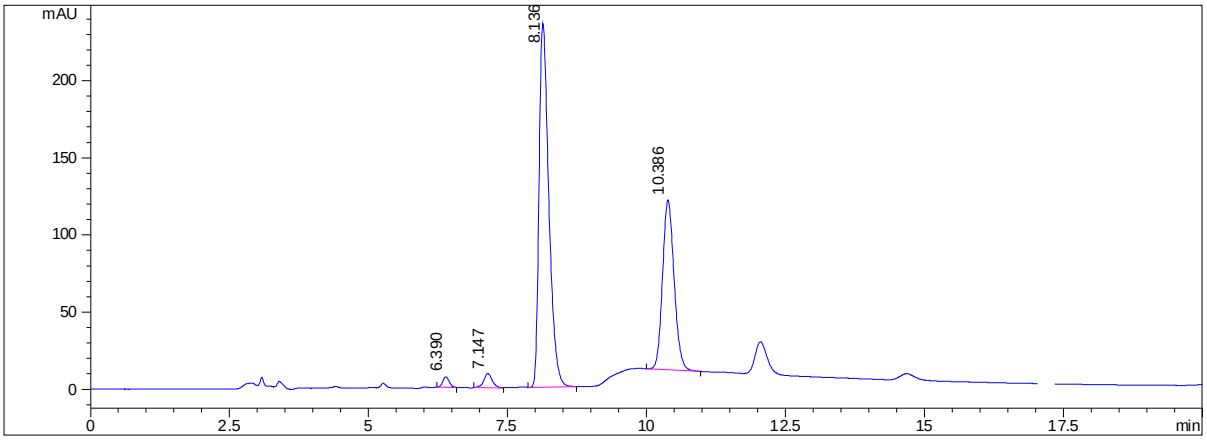
OD-H, Hex:iPrOH 98:02, 1 mL/min
G-Quadruplex DNA - h-tel-mod08 - 12 mol%, MOPS 10mM, KCl 50mM, TMePyP4Cu - 10 mol%, 5 °C, 40 h



#	Meas. Ret. Time	Height	Height %	Area	Area %
1	6.282	5.899	1.409	47.805	0.934
2	7.008	3.970	0.948	39.651	0.775
3	7.909	351.818	84.004	4218.265	82.439
4	10.126	57.123	13.639	811.082	15.851

ODN-12, Porphyrine 4, 50 mM KCl (table 1, entry 13)

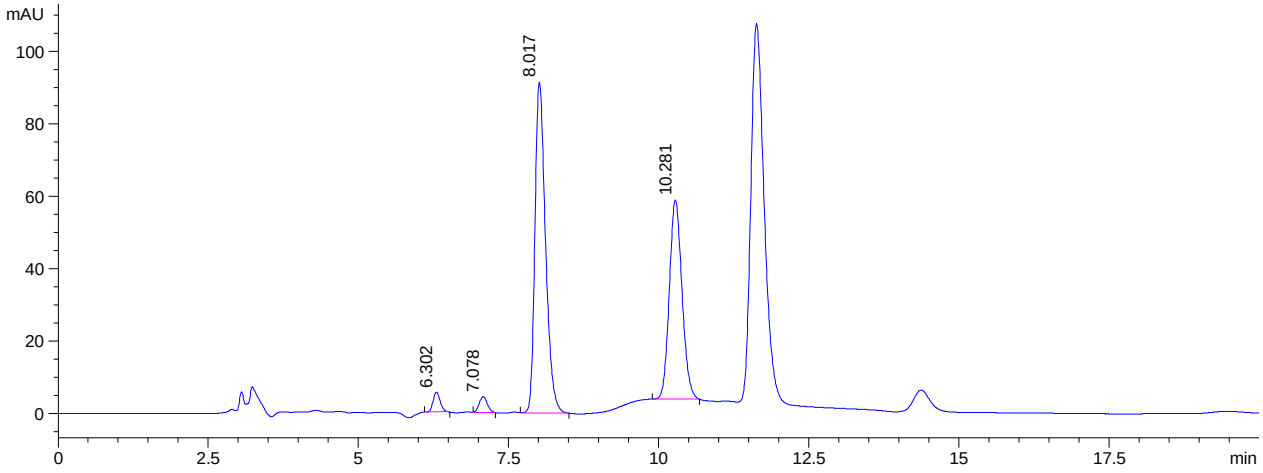
OD-H, Hex:iPrOH 98:02, 1 mL/min
G-Quadruplex DNA - h-tel-mod10 - 12 mol%, MOPS 10 mM, Kcl 50 mM, TMePyP4Cu 10 mol%, 5 °C, 40 h



#	Meas. Ret. Time	Height	Height %	Area	Area %
1	6.390	6.739	1.861	52.747	1.140
2	7.147	9.388	2.592	101.398	2.192
3	8.136	235.938	65.146	2879.270	62.231
4	10.386	110.104	30.401	1593.356	34.438

ODN-16, Porphyrine 4, 50 mM KCl (table 1, entry 17)

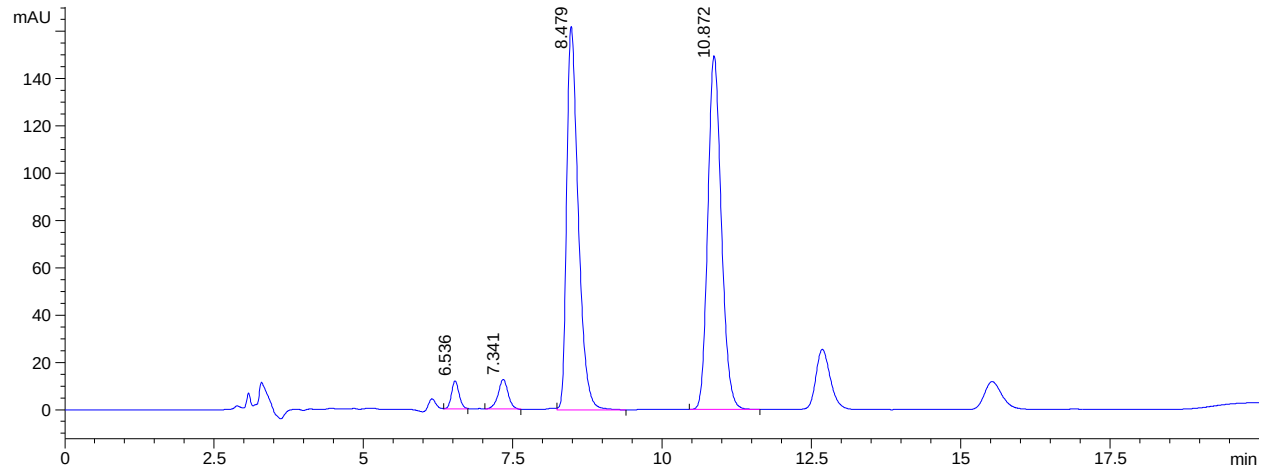
OD-H, Hex:iPrOH 98:02, 1 mL/min
G-Quadruplex DNA - c-myc - 12 mol%, MOPS 10mM, KCl 50mM, TMePyP-Cu-ClO4 - 10 mol%, 5 °C, 40 h



#	Meas. Ret. Time	Height	Height %	Area	Area %
1	6.302	5.474	3.498	44.317	2.196
2	7.078	4.444	2.840	42.715	2.116
3	8.017	91.550	58.500	1121.716	55.575
4	10.281	55.028	35.162	809.629	40.113

ODN-21, Porphyrine 4, 50 mM KCl (table 1, entry 22)

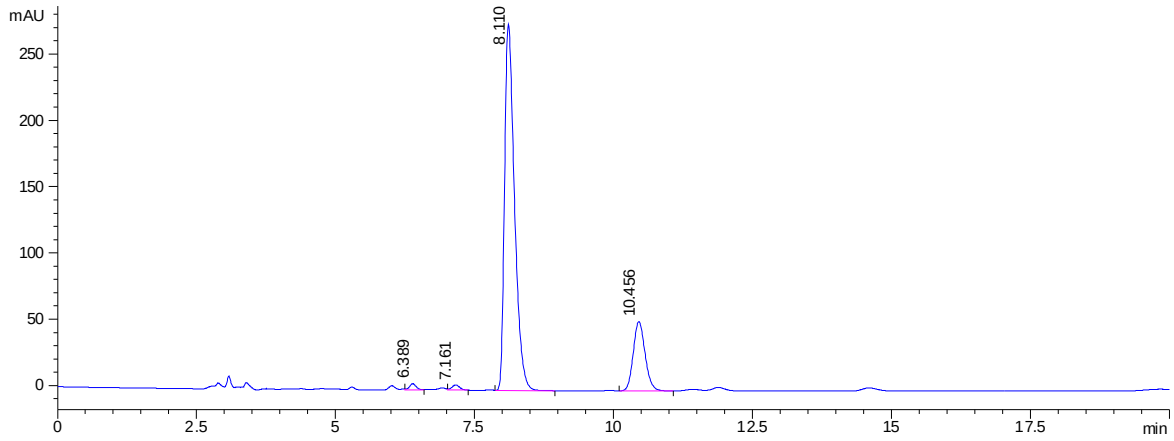
OD-H, Hex:iPrOH 98:02, 1 mL/min
G-Quadruplex DNA - c-kit - 12 mol%, MOPS 10mM, KCl 50mM, TMePyP-Cu-ClO4 - 10 mol%, 5 °C, 40 h



#	Meas. Ret. Time	Height	Height %	Area	Area %
1	6.536	11.866	3.528	105.181	2.194
2	7.341	12.537	3.727	143.304	2.989
3	8.479	162.259	48.240	2188.305	45.637
4	10.872	149.694	44.505	2358.250	49.181

ODN-1, Porphyrine 5, 50 mM KCl (table 2, entry 1)

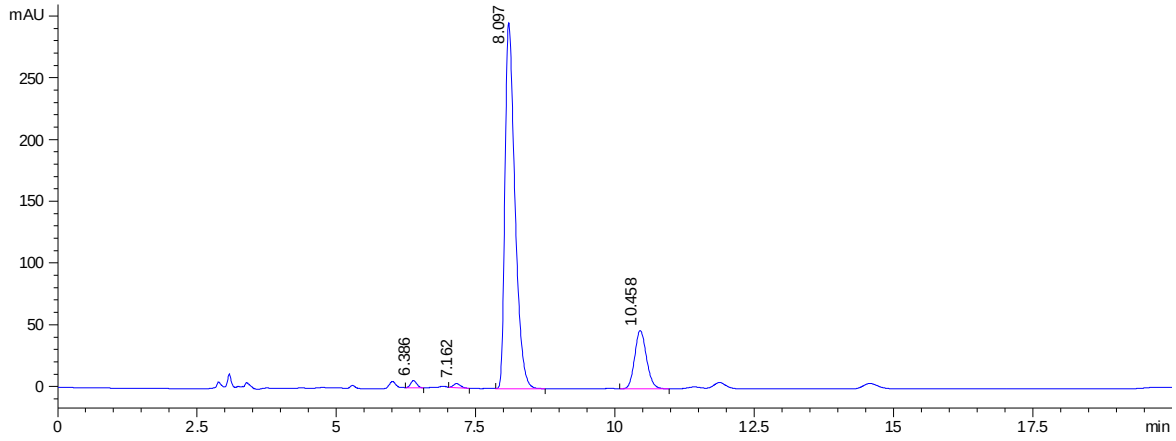
OD-H, Hex:iPrOH 98:02, 1 mL/min
G-Quadruplex DNA - h-tel - 12 mol%, MOPS 10mM, KCl 50mM, TPrPyP4Cu - 10 mol%, 5 °C, 40 h



#	Meas. Ret. Time	Height	Height %	Area	Area %
1	6.389	4.519	1.341	36.998	0.862
2	7.161	3.721	1.104	35.421	0.826
3	8.110	276.446	82.032	3444.494	80.281
4	10.456	52.311	15.523	773.627	18.031

ODN-10, Porphyrine 5, 50 mM KCl (table 2, entry 3)

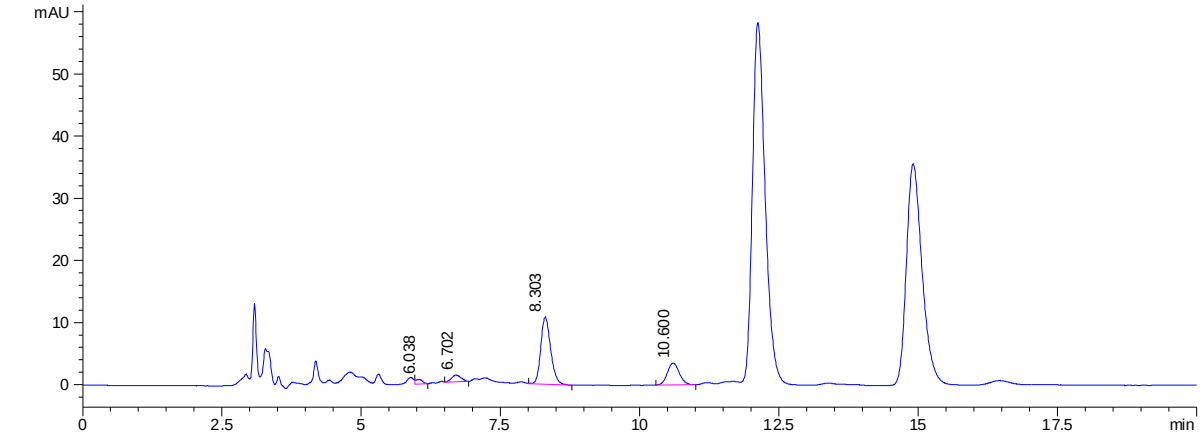
OD-H, Hex:iPrOH 98:02, 1 mL/min
G-Quadruplex DNA - h-tel-mod08 - 12 mol%, MOPS 10mM, KCl 50mM, TPrPyP4Cu - 10 mol%, 5 °C, 40 h



#	Meas. Ret. Time	Height	Height %	Area	Area %
1	6.386	5.864	1.660	45.546	1.018
2	7.162	3.335	0.944	29.258	0.654
3	8.097	296.643	83.987	3697.226	82.620
4	10.458	47.358	13.408	702.962	15.709

ODN-1, Porphyrine 7, 50 mM KCl (table 2, entry 1)

OD-H, Hex:iPrOH 98:02, 1 mL/min
G-Quadruplex DNA - h-tel - 12 mol%, MOPS 10mM, KCl 50mM, TMePyP3-Cu-ClO4 - 10 mol%, 5 °C, 40 h



#	Meas. Ret. Time	Height	Height %	Area	Area %
1	6.038	0.724	4.489	5.808	2.848
2	6.702	1.093	6.779	12.809	6.282
3	8.303	10.773	66.842	132.689	65.076
4	10.600	3.528	21.891	52.592	25.793

10. Literature

[1] S. Otto, F. Bertoncin, J. B. F. N. Engberts, *J. Am. Chem. Soc.* **1996**, *118*, 7702-7707.

[2] a) A. D. Adler, F. R. Longo, J. D. Finarelli, J. Assour, L. Korsakoff, *J. Org. Chem.* **1967**, *32*, 476-476; b) S.-Q. Liu, H.-R. Sun, Z.-T. Sun, J.-Q. Xu, D.-M. Li, *Synth. Commun.* **2000**, *30*, 2009-2017; c) K. Groves, A. J. Wilson, A. D. Hamilton, *J. Am. Chem. Soc.* **2004**, *126*, 12833-12842; d) F.-Di Cong, B. Ning, X.-G. Du, C.-Y. Ma, H.-F. Yu, B. Chen, *Dyes and Pigments*, **2005**, *66*, 149-154; e) J. Alzeer, P. J. C. Roth, N. W. Luedtke, *Chem. Commun.*, **2009**, *45*, 1970.1971; f) C. Romera, L. Sabater, A. Garofalo, I. M. Dixon, G. Pratviel, *Inorg. Chem.* **2010**, *49*, 8558-8567.

[3] N. S. Oltra, G. Roelfes, *Chem. Commun.* **2008**, *44*, 6039-6041.