Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Polymerase-mediated synthesis of artificial RNA-DNA metal base pairs

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Materials and methods

All DNA and RNA containing oligonucleotides were purchased from Microsynth and purified by anion exchange HPLC. All DNA polymerases (Therminator, Vent (*exo*$^-$), Deep Vent, *Bst*, Taq, Dpo4, and the Klenow fragment of DNA polymerase I *exo*$^-$ (Kf *exo*$^-$)) were purchased from New England Biolabs as well as the natural dNTPs and rNTPs.

T_m melting experiments

The melting experiments were performed on an Agilent Cary UV-Vis 3500 Compact Peltier machine in 60 µl volume quartz cuvettes. Experiments were recorded in 5 mM MOPS, 3 mM $MgSO_4$, 150 mM $NaClO_4$, pH 6.8 and with 1 µM final duplex concentration in the presence or absence of metal cations (6 µM final concentration). Absorbance was monitored at 260 nm and the heating rate was set to 1°C/min. A heating-cooling cycle in the temperature range 20-90°C was applied and was repeated 3x per sample at least. The absorbance melting curves were converted to hyperchromicities, smoothed and the first derivative curves were obtained using the SigmaPlot software (version 14.5). The following salts were used: $AgNO_3$, $Hg(ClO_4)_2$, $MnSO_4$, $MgSO_4$.

General protocol for primer extension (PEX) reactions

The 5′-FAM-labelled primer (10 pmol) was annealed to the appropriate template (15 pmol) in H_2O by heating to 95 °C and then gradually cooling to room temperature (over 60 min). The appropriate DNA polymerase and metal cation solutions were then added to the annealed oligonucleotides mixture along with 1µL of 10× enzyme buffer (provided by the supplier of the DNA polymerase). Finally, natural dNTPs and/or NTPs were added for a total reaction volume of 10 µL. Following incubation at the optimal temperature for the enzyme, the reactions were quenched by adding stop solution (10 µL, formamide (70 %), ethylenediaminetetraacetic acid (EDTA, 50 mM), bromophenol (0.1 %), xylene cyanol (0.1 %)). The reaction mixtures were subjected to gel electrophoresis in denaturing polyacrylamide gel (20 %) containing trisborate–EDTA (TBE) 1× buffer (pH 8) and urea (7 M). Visualization was performed by fluorescence imaging by using a Typhoon Trio phosphorimager.

Protocol for the bypass experiments

After PEX reactions with UTP have been carried out as described above, 3 µL of EDTA 100 µM (30µM final concentration) were added to the reaction mixtures to chelate excess Hg^{II}. The reaction mixtures are then purified by running through Nucleospin columns. Following evaporation of the solvent in a speed-vac, appropriate polymerase buffers, polymerases, and dATP are added and the corresponding reaction mixtures are incubated at the adequate reaction temperature for 1h in a total reaction volume of 10 µL. The reactions were then quenched by adding stop solution (10 µL, formamide (70 %), EDTA 50 mM, bromophenol (0.1 %), xylene cyanol (0.1 %)). The reaction mixtures were subjected to gel electrophoresis in denaturing polyacrylamide gel (20 %) containing trisborate–EDTA (TBE) 1× buffer (pH 8) and urea (7 M). Visualization was performed by fluorescence imaging by using a Typhoon Trio phosphorimager.

Additional gel images:

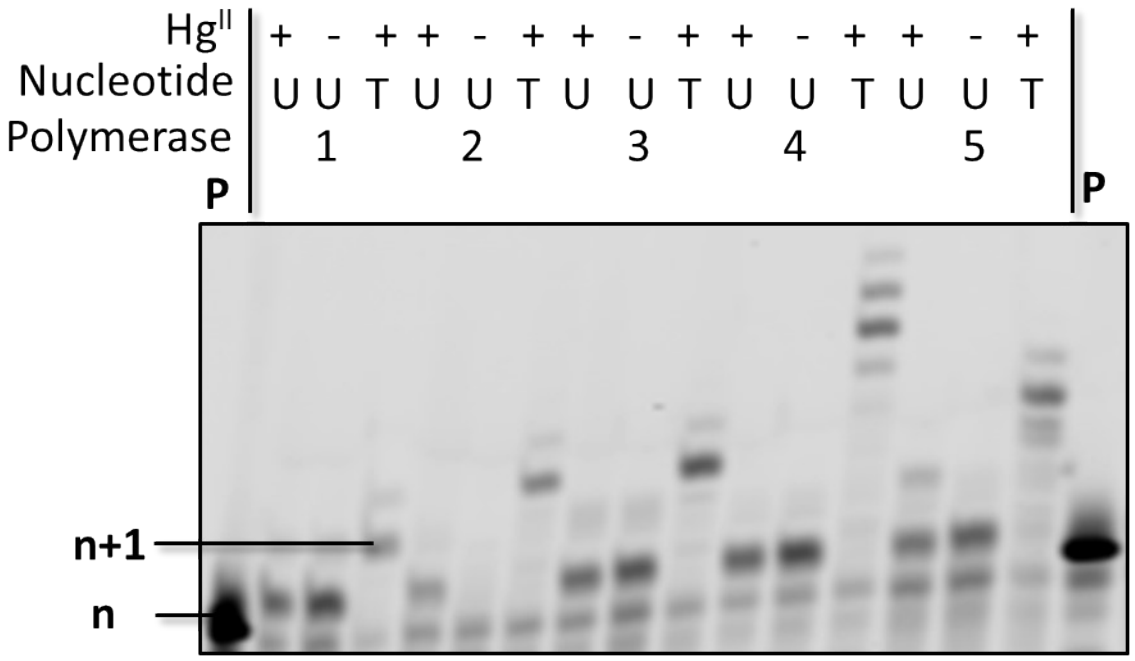

Figure S1. Gel image (PAGE 20%) of PEX reactions with primer P1 and template T1 and different polymerases in the presence or absence of Hg^{2+}. All reactions were carried out for 1h at the adequate temperature. Nucleotides were kept at 200 μM final concentration and [Hg^{2+}] was 30 μM in buffer 1. Polymerases used: 1. *Bst* (8U); 2. Taq (5U); 3. Vent (exo$^-$) (2U); 4. Dpo4 (2U); 5. Kf *exo*$^-$ (5U). U represents UTP, T represents dTTP, and **P** represents unreacted primer.

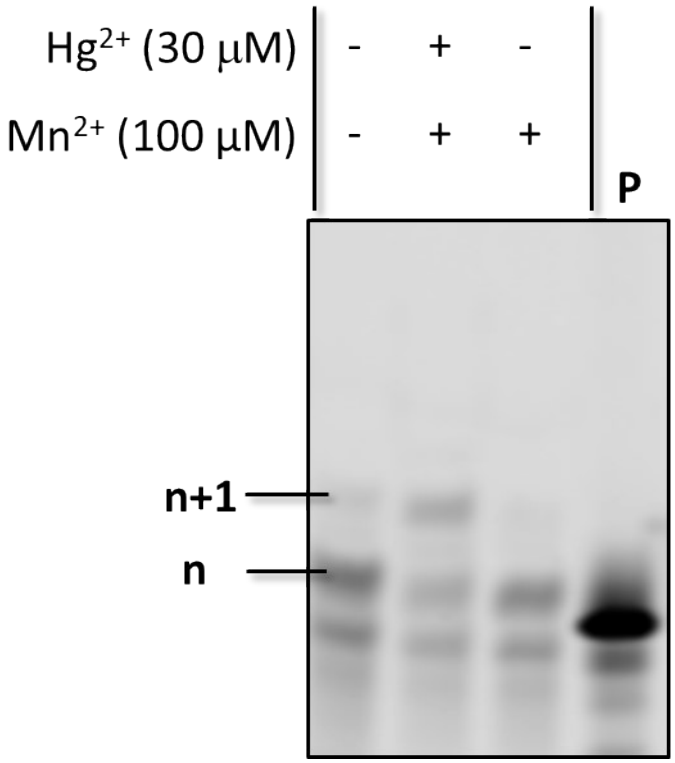

Figure S2. Gel image (PAGE 20%) of PEX reactions carried out with primer P1 and template T1 to evaluate the effect of Mn^{2+} on the formation of an rU-Hg^{II}-dT base pair. All reactions were run for 3h at 37°C with 5U of Kf *exo*$^{-}$ and 200 μM UTP. **P** represents unreacted primer.

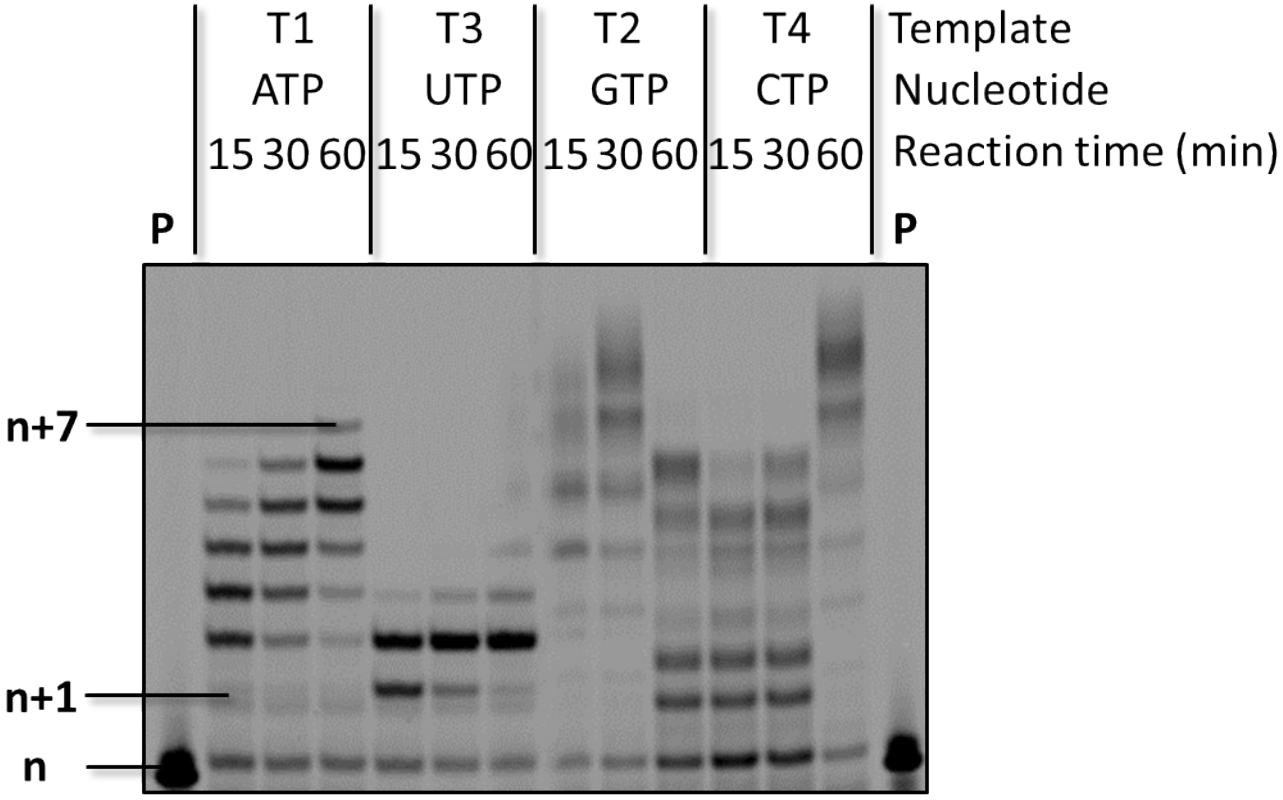

Figure S3. Gel image (PAGE 20%) of PEX reactions with templates T1-T4 and the corresponding, complementary NTPs. All reactions were run for 3h at 37°C with 5U of Kf *exo*$^{-}$ and 400 μM rNTP. **P** represents unreacted primer.

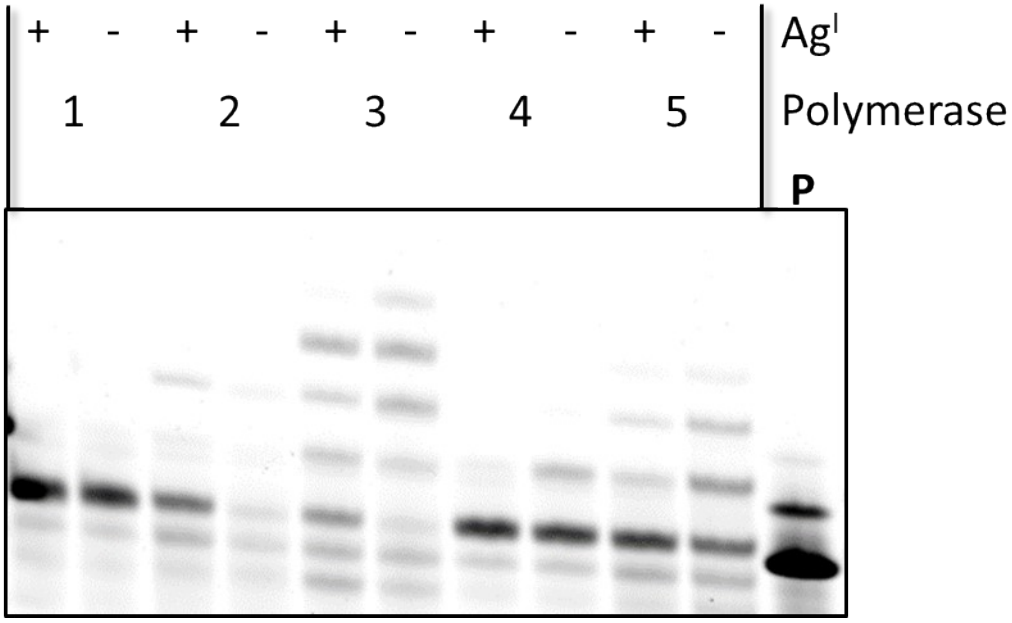

Figure S4. Gel image (PAGE 20%) of PEX reactions with CTP and primer P1/template T2. Effect of Ag^{I} (100 μM) on the reactions (1 h reaction time) catalyzed by different polymerases. All reaction mixtures contained 200 μM CTP and a reaction buffer devoid of Cl^{-}. 1. Polymerases used: 1. *Bst* (8U); 2. Taq (5U); 3. Vent (exo^{-}) (2U); 4. Dpo4 (2U); 5. Kf *exo*$^{-}$ (5U). **P** represents unreacted primer.

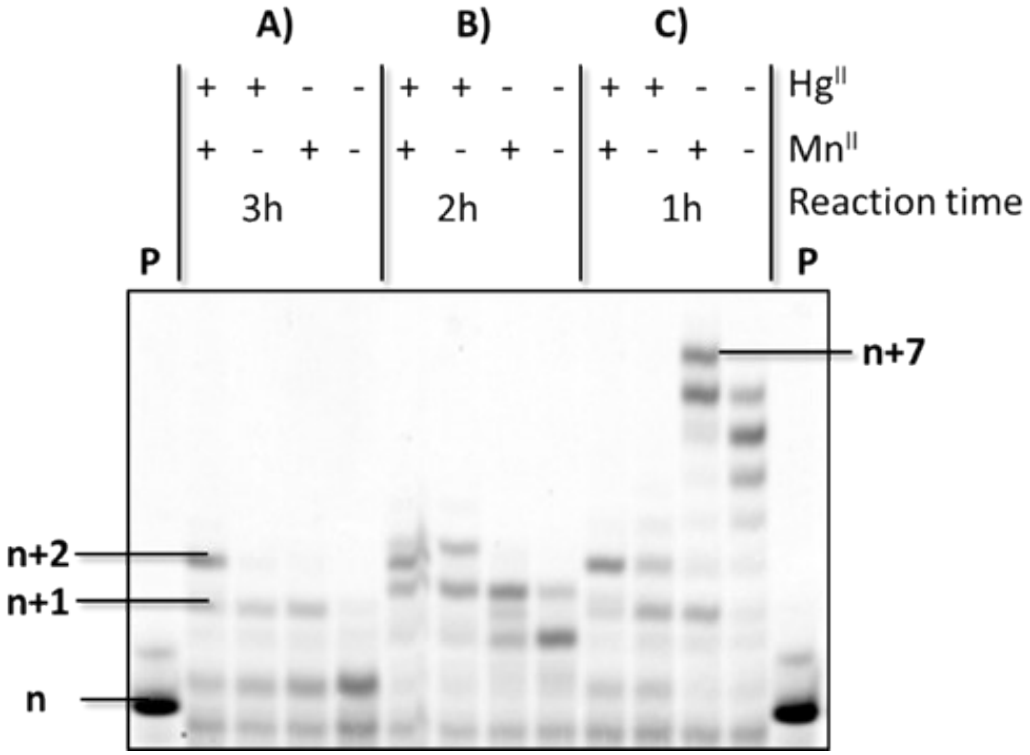

Figure S5. Gel analysis (PAGE 20%) of products from the bypass experiments carried out with the primer P1/ template T1 system. A) reactions with UTP (400 μM) and Kf *exo*$^{-}$ (5U) at 37°C; B) reaction with UTP (400 μM) and Kf *exo*$^{-}$ (5U) at 37°C for 3h followed by addition of dTTP (200 μM) and reaction 37°C for 2h; C) reaction with UTP (400 μM) and Kf *exo*$^{-}$ (5U) at 37°C for 3h followed by addition of dATP (200 μM) and reaction 37°C for 1h. **P** indicates unreacted primer.

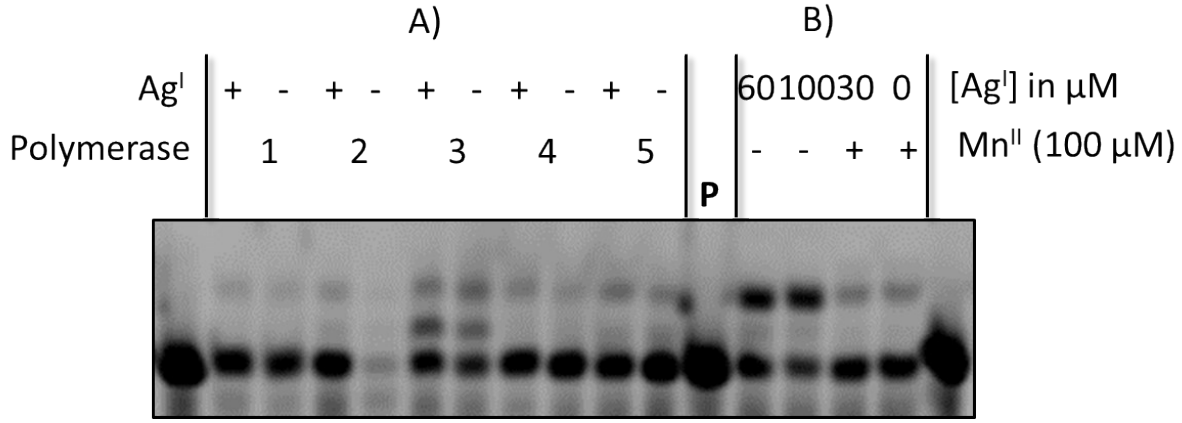

Figure S6. Gel image (PAGE 20%) of PEX reactions with CTP and primer P1/template T3. A) effect of Ag^{I} (30 μM) on the reactions (1 h reaction time) catalyzed by different polymerases and B) effect of Ag^{I} concentration and presence of Mn^{II} on the outcome of the reactions catalyzed by Kf *exo*$^{-}$. All reaction mixtures contained 200 μM CTP, 2 U of polymerases, and a reaction buffer devoid of Cl^{-}. Polymerases used: 1. *Bst*; 2. Taq; 3. Vent (exo^{-}); 4. Dpo4; 5. Kf *exo*$^{-}$. **P** represents unreacted primer.

Representative melting curves:

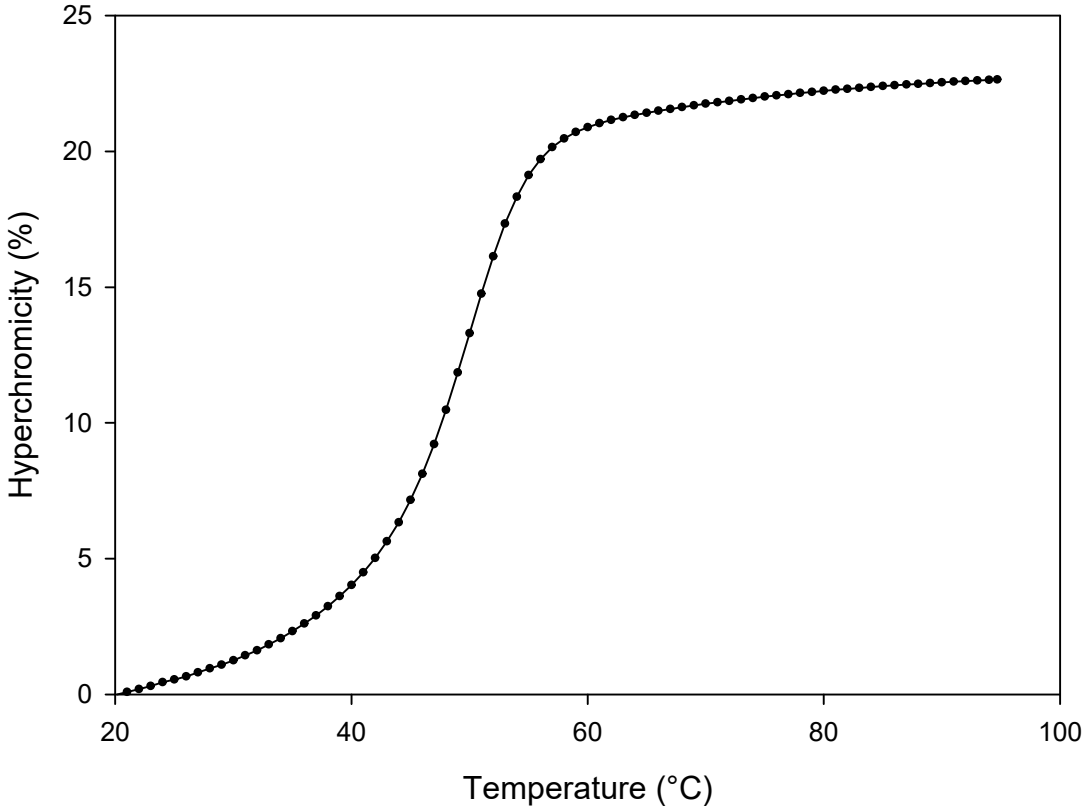

Figure S7 Denaturation of duplex 1 as determined UV-spectroscopically in the absence of metal cations.

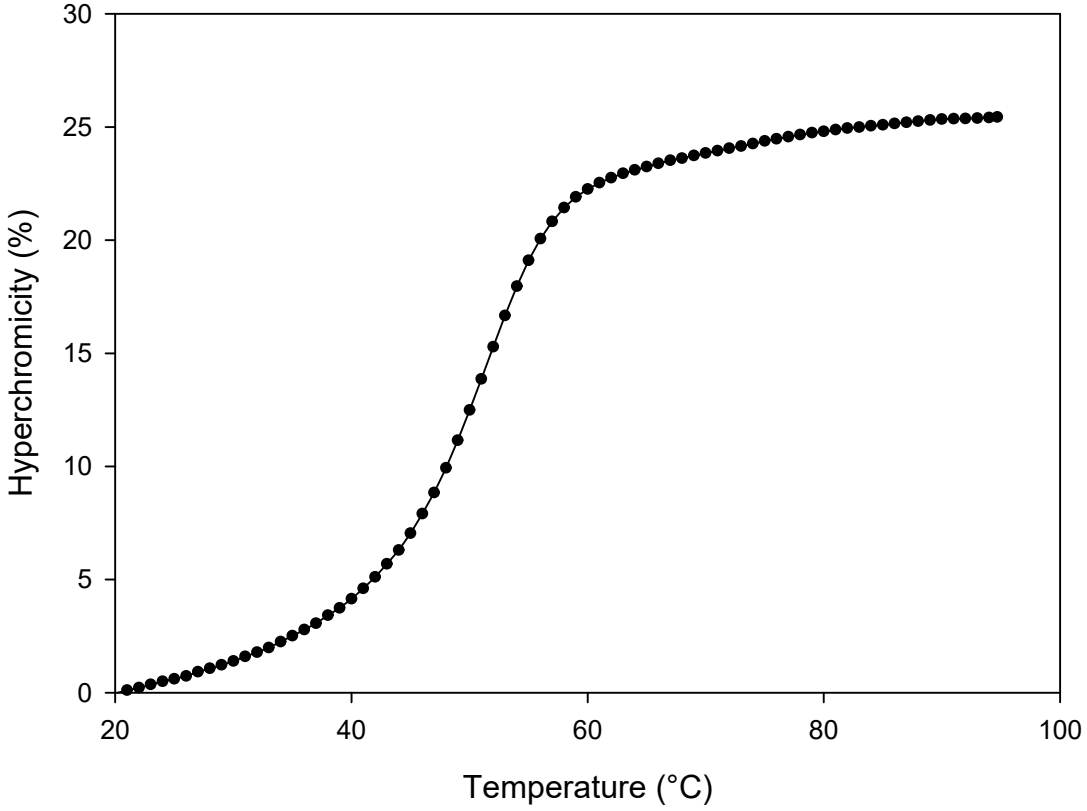

Figure S8. Denaturation of duplex 1 as determined UV-spectroscopically in the presence of Ag^{I}.

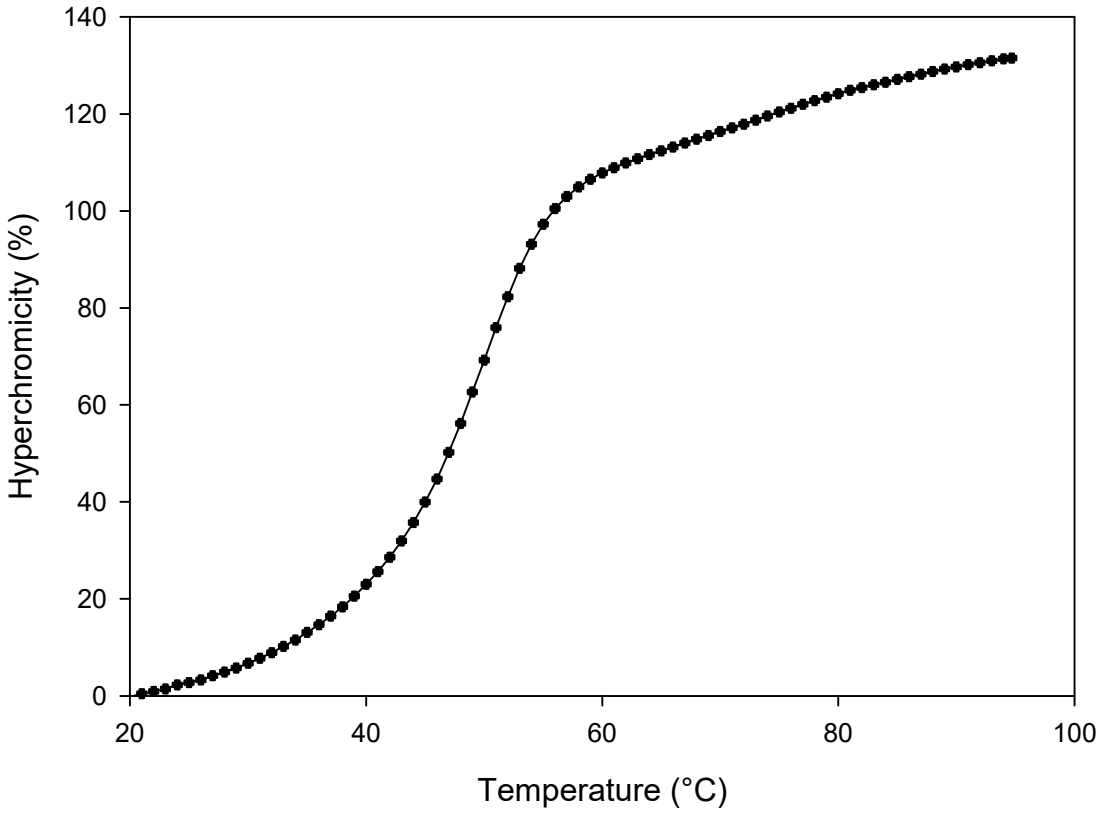

Figure S9. Denaturation of duplex 1 as determined UV-spectroscopically in the presence of Hg^{II}.

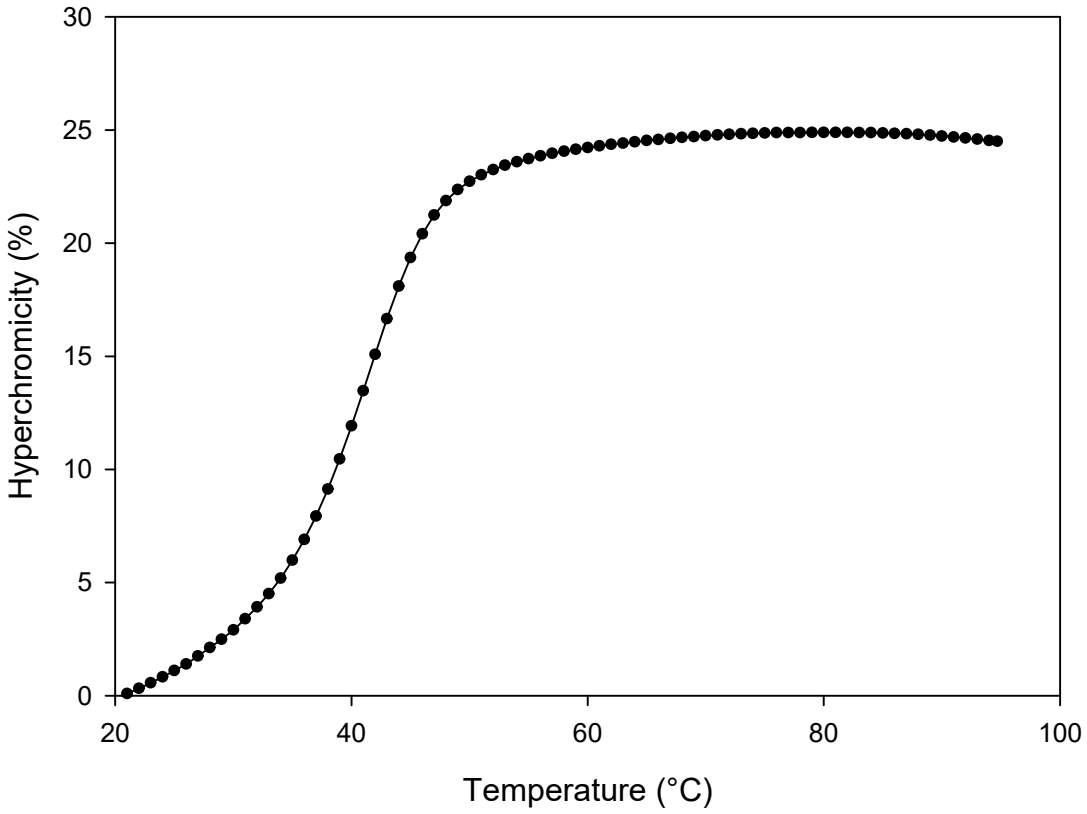

Figure S10. Denaturation of duplex 2 as determined UV-spectroscopically in the absence of metal cations.

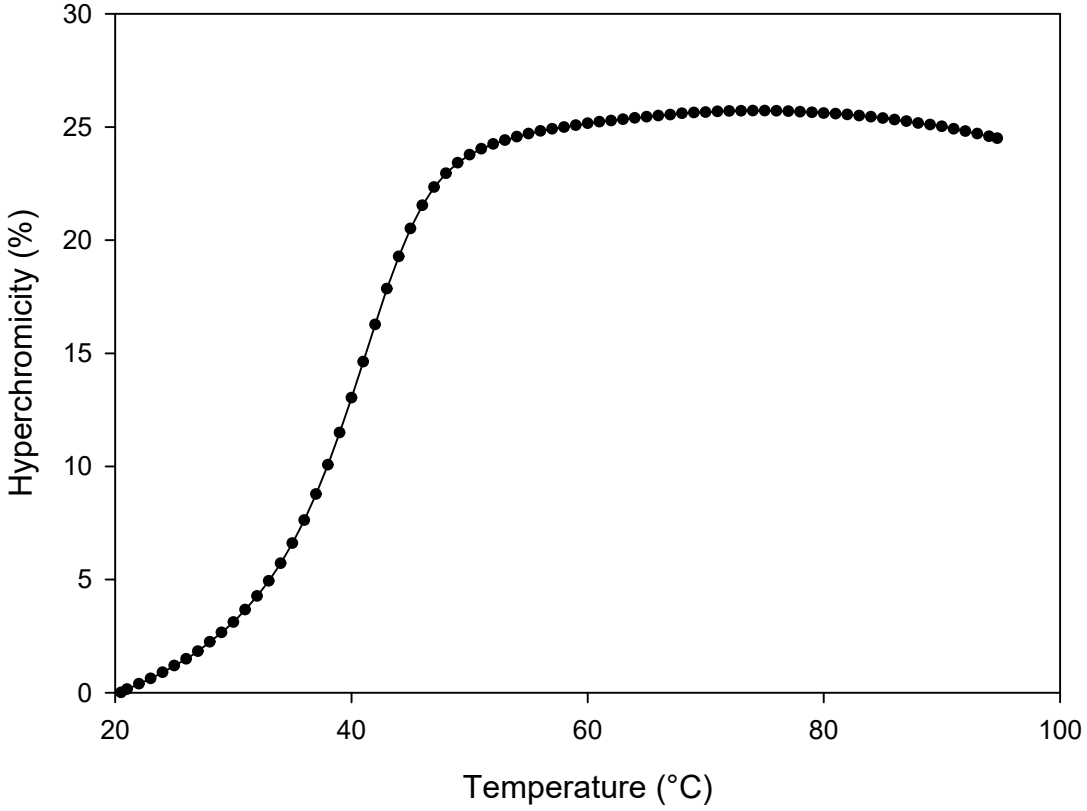

Figure S11. Denaturation of duplex 2 as determined UV-spectroscopically in the presence of Mn^{II}.

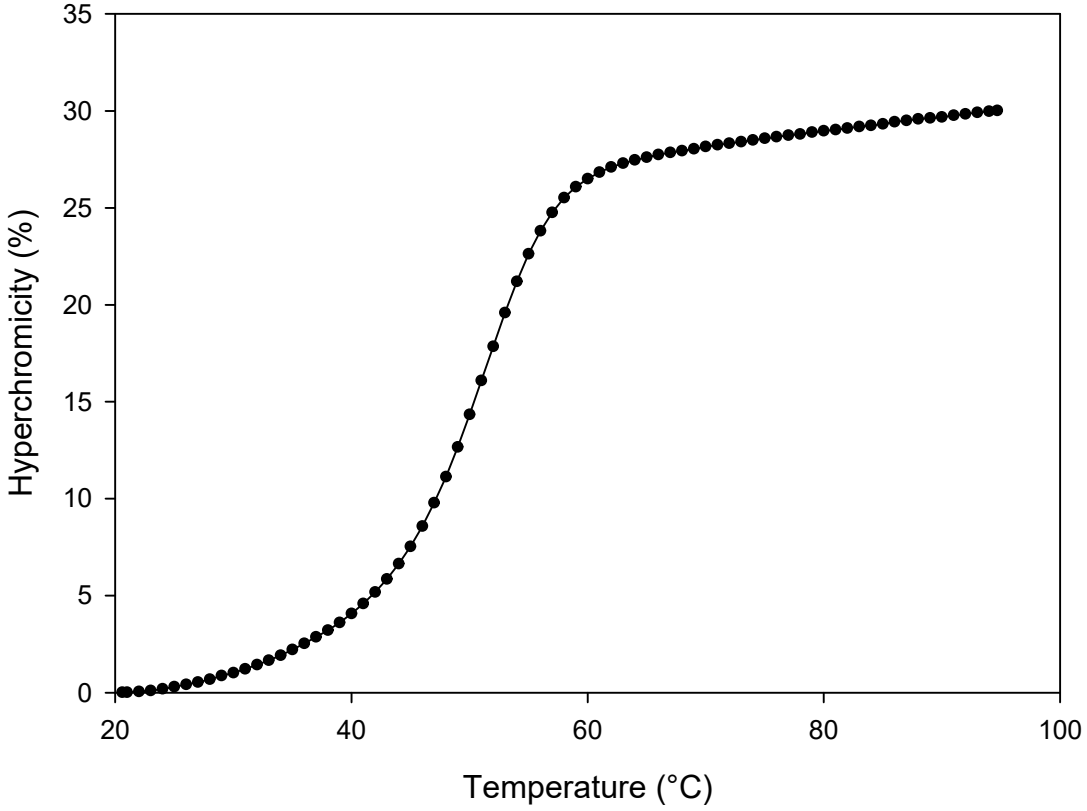

Figure S12. Denaturation of duplex 2 as determined UV-spectroscopically in the presence of Hg^{II}.

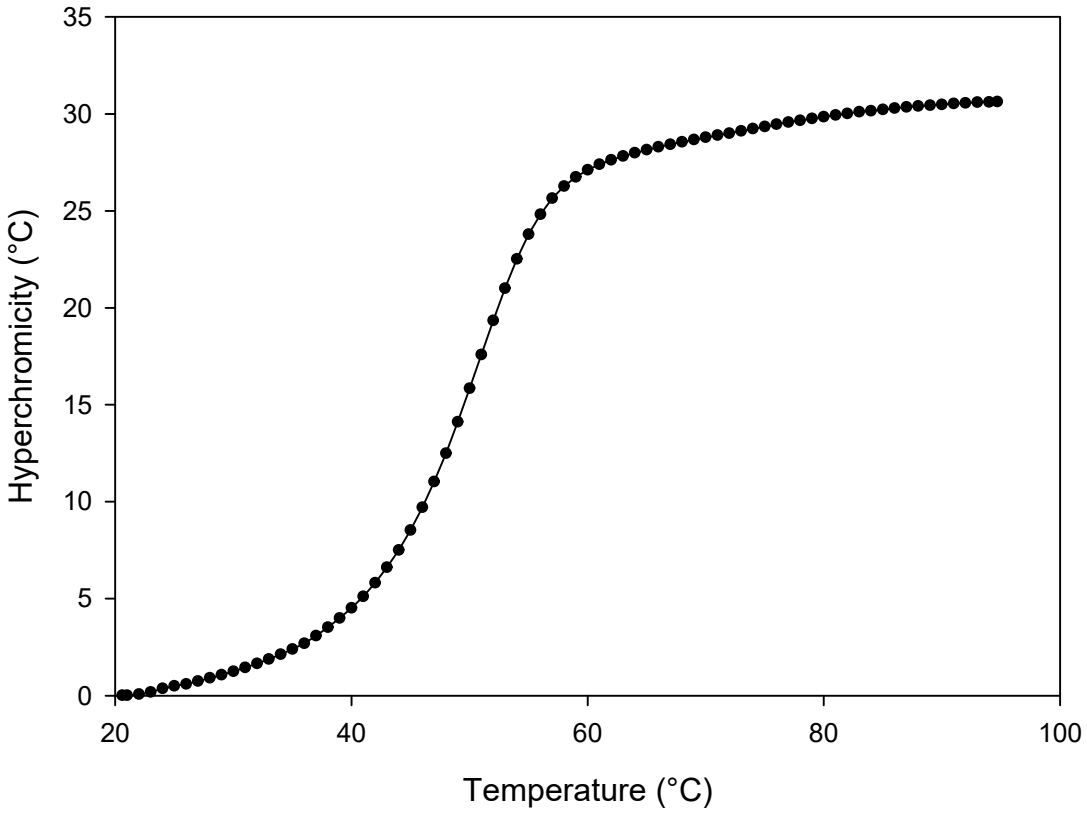

Figure S13. Denaturation of duplex 2 as determined UV-spectroscopically in the presence of Mn^{II} and Hg^{II}.

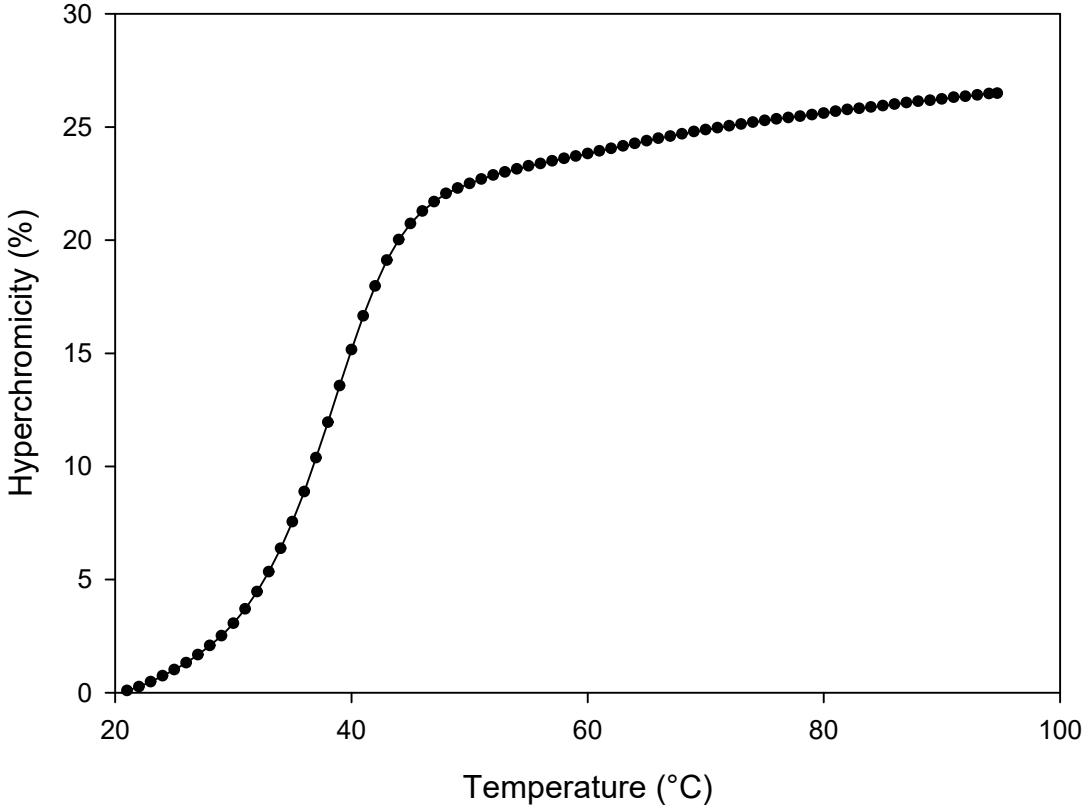

Figure S14. Denaturation of duplex 3 as determined UV-spectroscopically in the absence of metal cations.

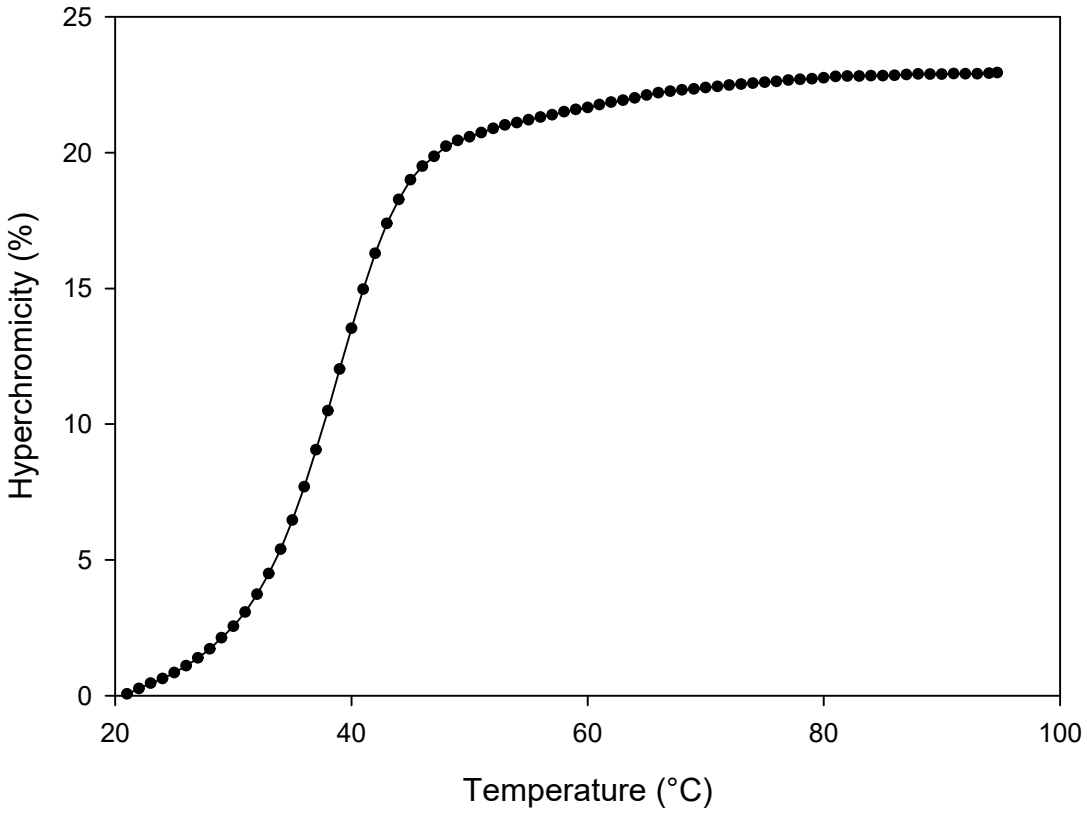

Figure S15. Denaturation of duplex 3 as determined UV-spectroscopically in the presence of Mn^{II}.

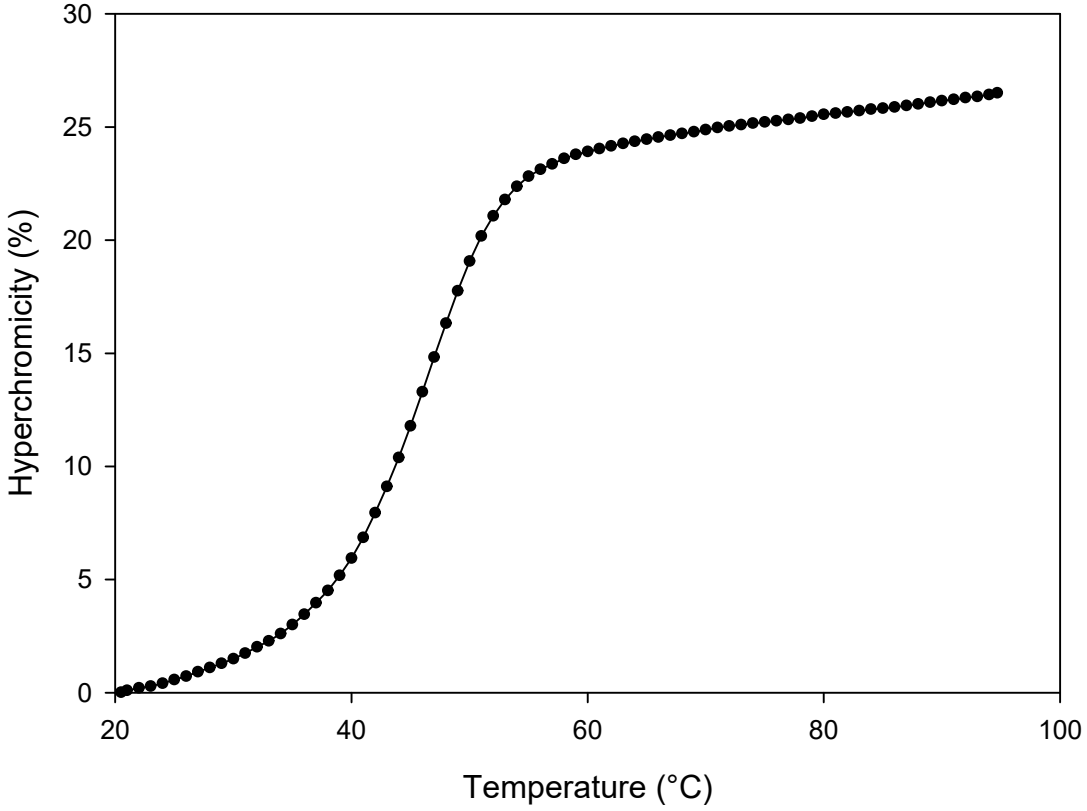

Figure S16. Denaturation of duplex 3 as determined UV-spectroscopically in the presence of Hg^{II}.

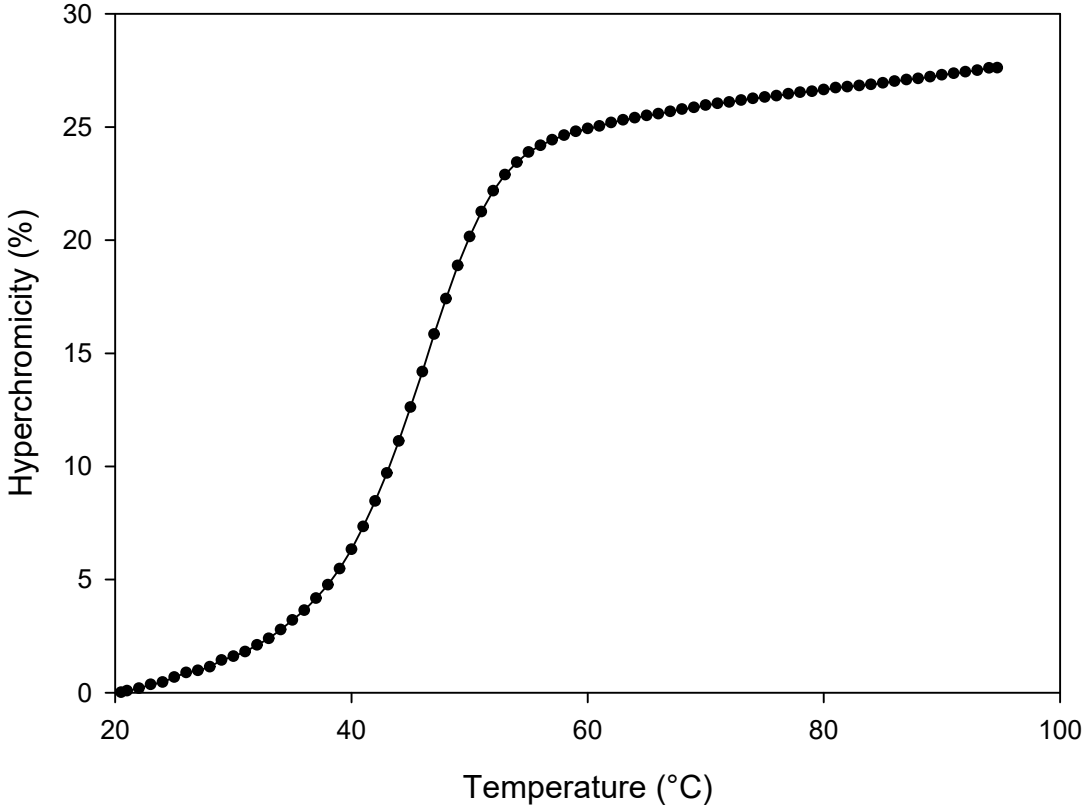

Figure S17. Denaturation of duplex 3 as determined UV-spectroscopically in the presence of Mn^{II} and Hg^{II}.

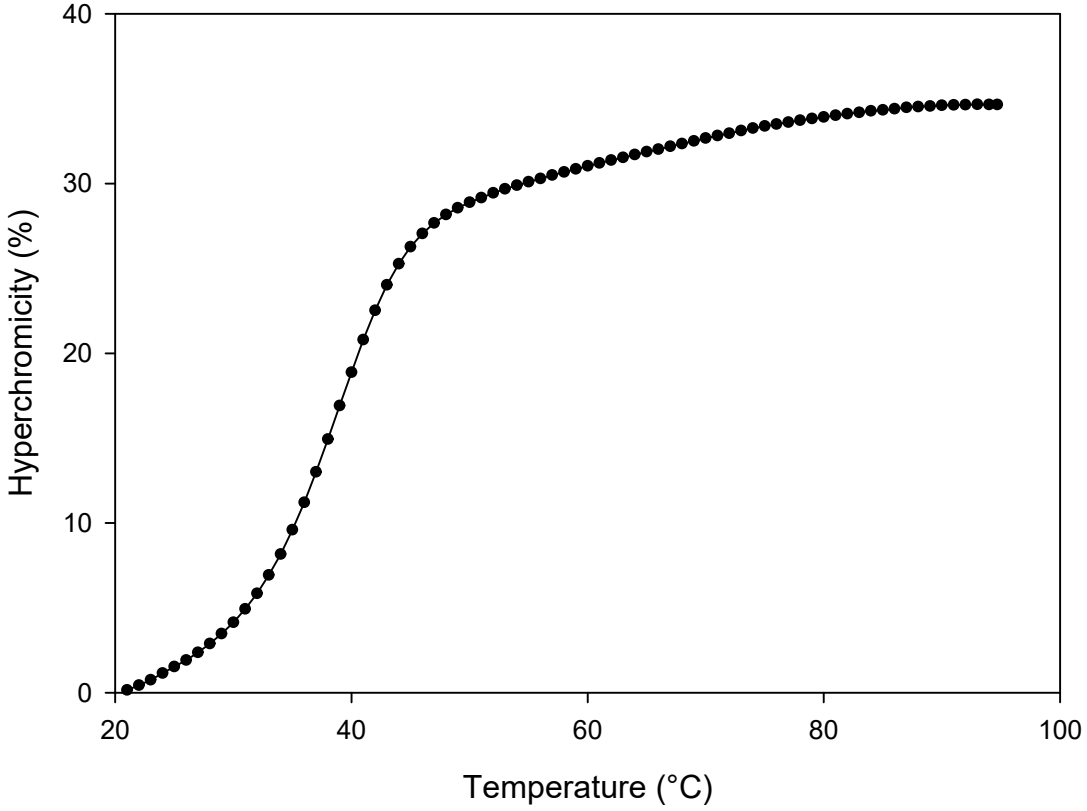

Figure S18. Denaturation of duplex 4 as determined UV-spectroscopically in the absence of metal cations.

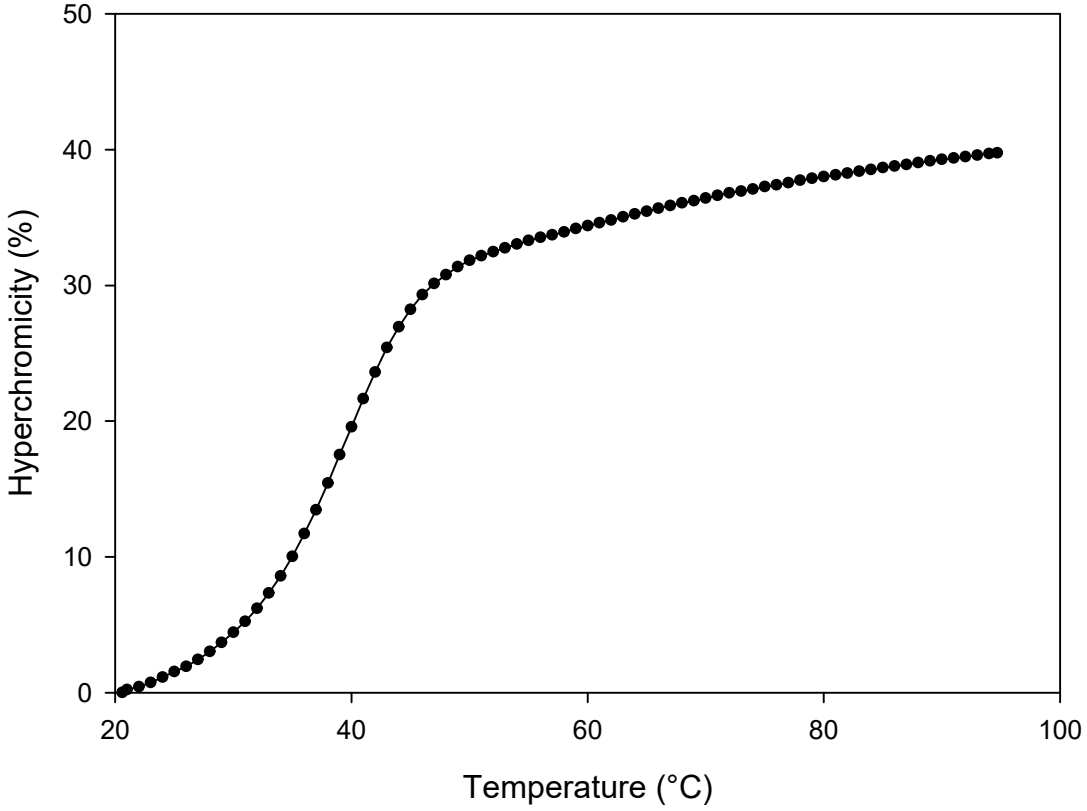

Figure S19. Denaturation of duplex 4 as determined UV-spectroscopically in the presence of Ag^{I}.

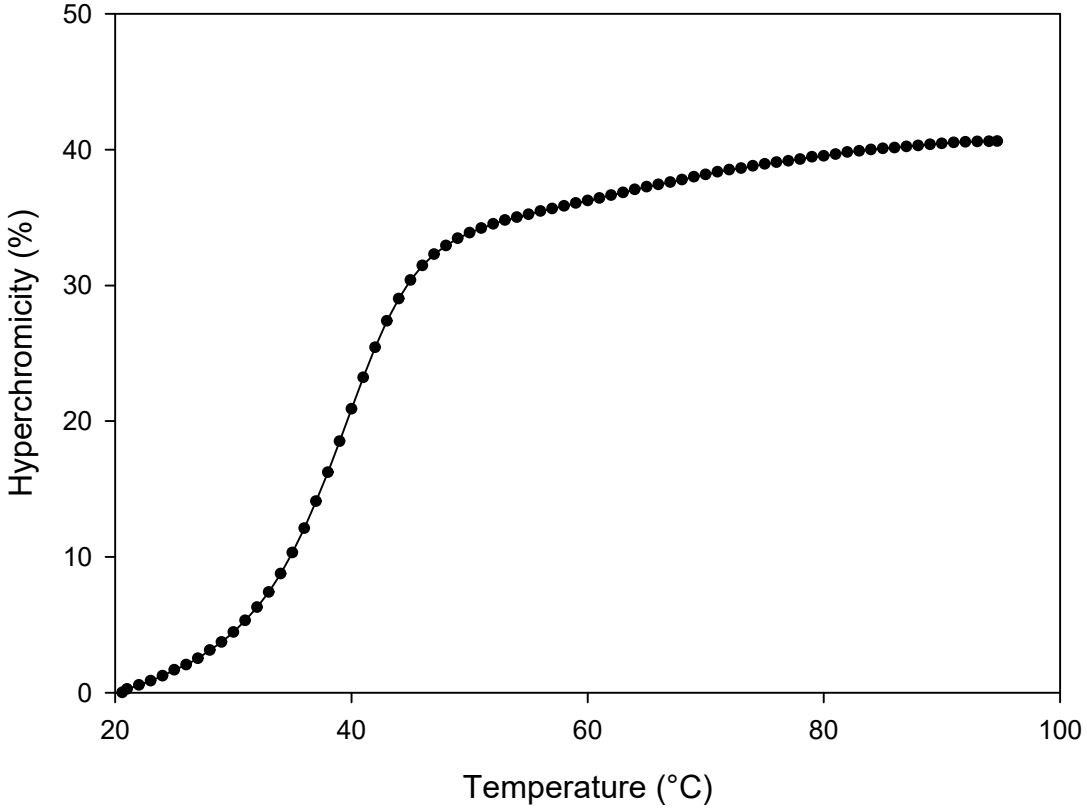

Figure S20. Denaturation of duplex 5 as determined UV-spectroscopically in the absence of metal cations.

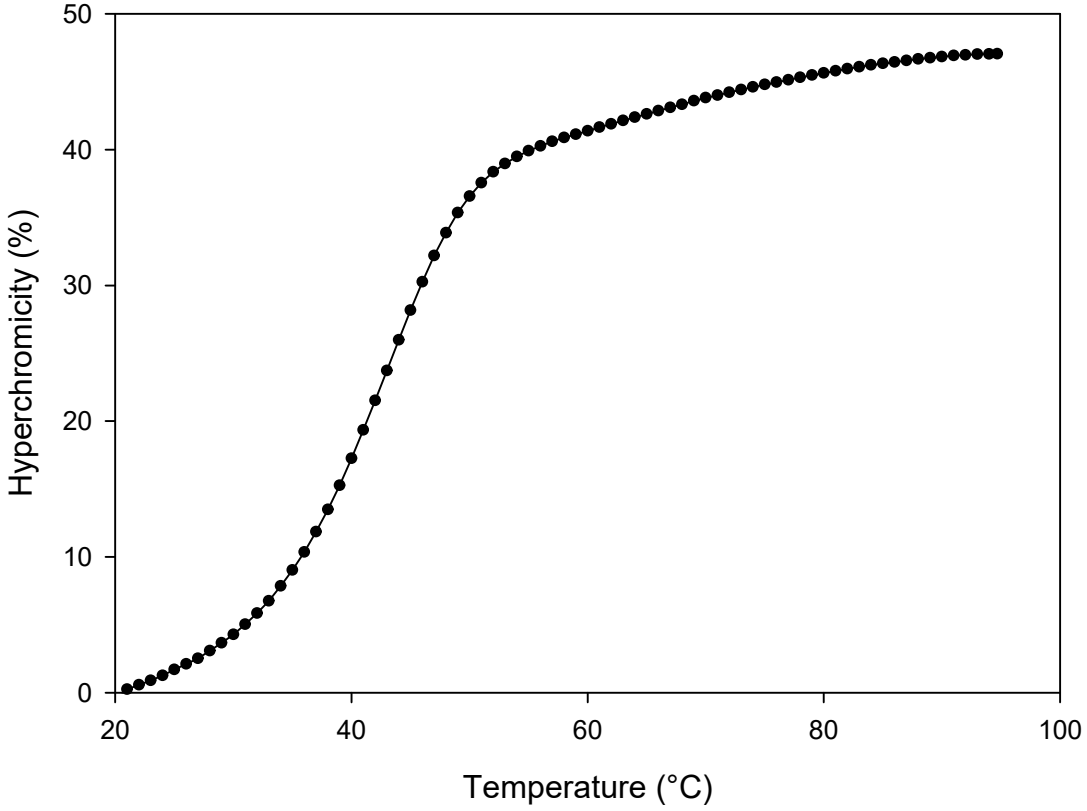

Figure S21. Denaturation of duplex 5 as determined UV-spectroscopically in the presence of Ag^{I}.

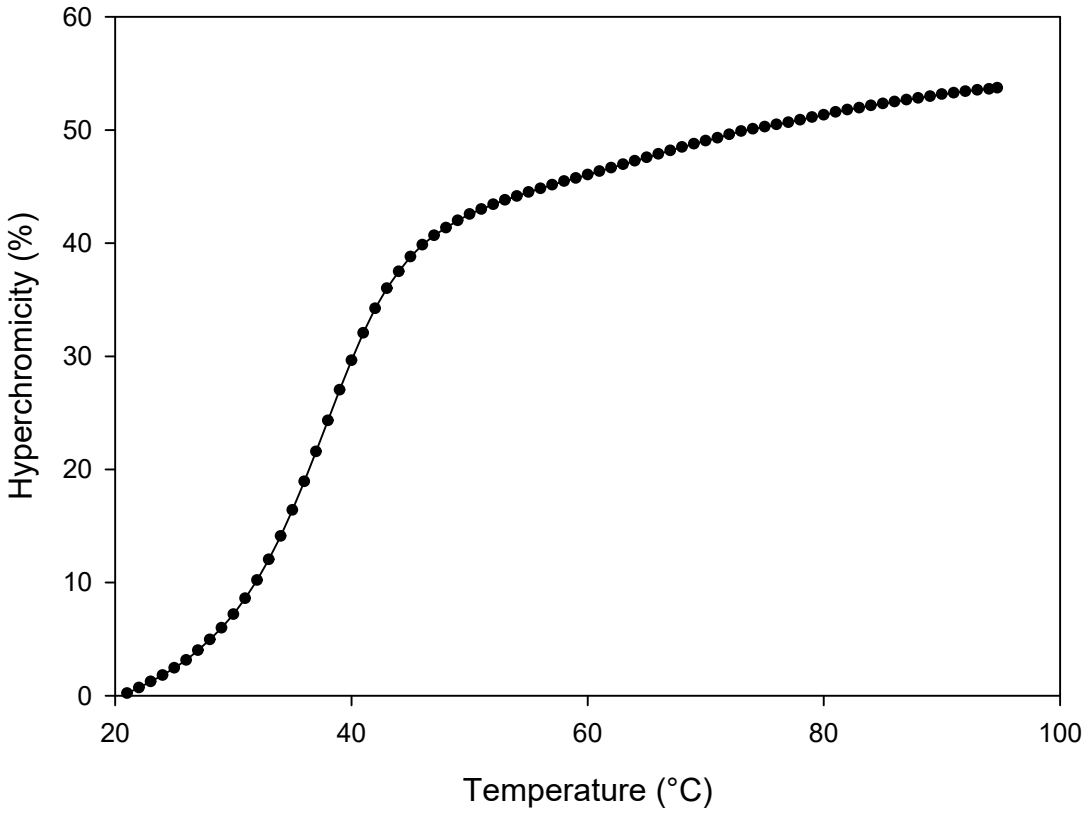

Figure S22. Denaturation of duplex 6 as determined UV-spectroscopically in the absence of metal cations.

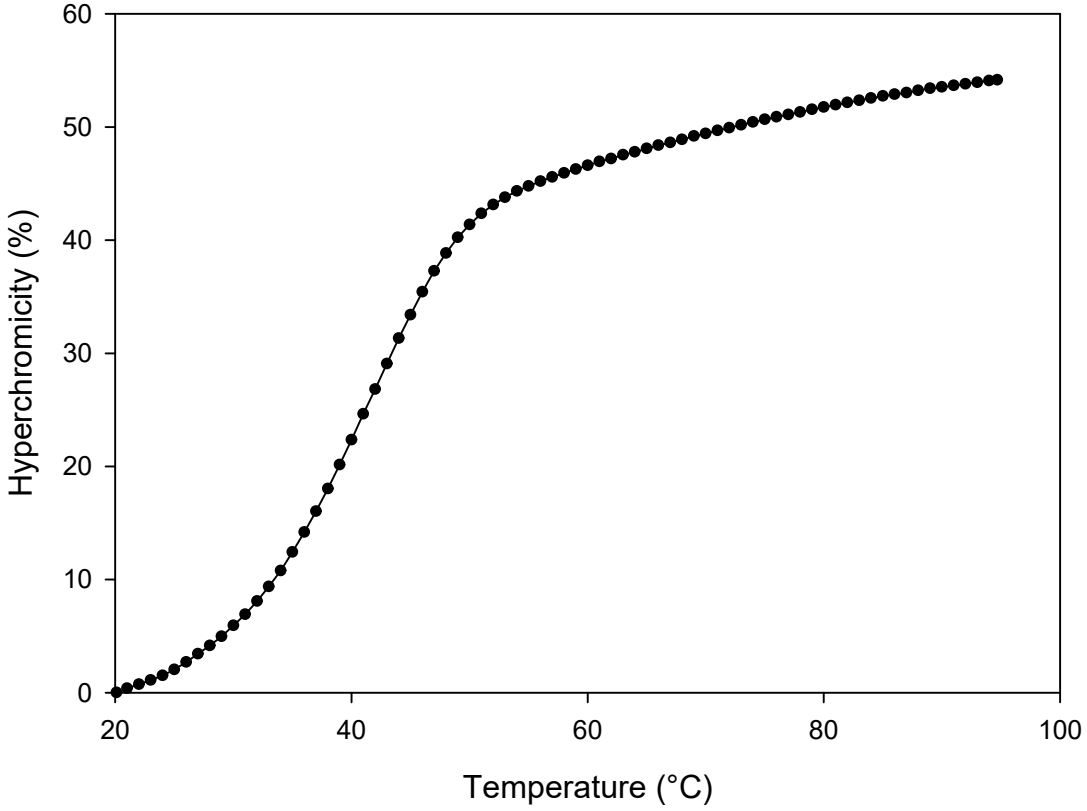

Figure S23. Denaturation of duplex 6 as determined UV-spectroscopically in the presence of Ag^{I}.