Supplementary Information

Affinity of the anthracycline drugs Doxorubicin and Sabarubicin for human telomeric G-quadruplex structures

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SI-1. Global analysis of equilibrium spectroscopic data with Singolar Value Decomposition and non linear regression modelling.

This application was performed using the commercial SPECFIT/32^{TM} program, based on the publications of A. Zuberbühler at the University of Basel, Switzerland (see refs. RS1 and RS2). Multiwavelength spectroscopic data sets are arranged in matrix form **Y**, where a number Nw of wavelengths and a number Nm of corresponding measured spectroscopic signals are in columns, whereas ligand and receptor concentrations are in rows. Thus each element of the data matrix Y_{ij} corresponds to a wavelength j and an experimental quantity (absorbance, circular dichroism, fluorescence intensity) for a given couple of concentrations i of ligand and receptor (typically in our experiments one of them is kept constant). A least square best estimator **Y'** of the original data **Y** is reconstructed as the eigenvector representation **Y'** = **U×S×V**, where **S** is a vector that contains the relative weights of the significant eigenvectors (Ne, number of significant eigenvectors), **U** is an matrix (Nm×Ne) of concentration eigenvectors ($U^T \times U=1$, orthonormal) and **V** (Ne×Nw) is an matrix of spectroscopic eigenvectors ($V \times V^T$, orthonormal). This **Y'** matrix contains less noise than **Y** because the SVD decomposition procedure can factor random noise from the principal components. This reconstructed data matrix **Y'** is utilized in the global fitting instead of the original data matrix **Y**. Complexation equilibria are solved assuming a complexation model (i.e. contemporary presence of complexes of given stoichiometries in equilibrium with free species in solution) and optimizing the numeric combination of all the spectroscopic contributions to best reproduce the **Y'** signals. Optimization is performed by the least square method, using the Levenberg-Marquardt algorithm, for all the explored wavelengths and ligand-receptor concentration couples. The optimized parameters are the association constants. The spectra of the equilibrium components are also extracted.

We applied this method to the analysis of fluorescence data for both **1** and **2** titration with 21-mer, represented in Figure 3a and 3b in the manuscript. The SVD analysis of the data of the **1** fluorescence titration resulted to be the following:

[FACTOR ANALYSIS]

Tolerance = 1.000E-09

Max. Factors = 10

Num. Factors = 6

Significant = 2

Eigen Noise = 6.259E+02

Experimental Noise = 3.130E+02

#	Eigenvalue	Square Sum	Residual	Prediction
1	1.181E+13	5.030E+09	1.180E+03	Data Vector
2	3.616E+09	1.414E+09	6.259E+02	Data Vector
3	3.277E+08	1.087E+09	5.487E+02	Probably Noise
4	2.648E+08	8.219E+08	4.773E+02	Probably Noise
5	1.959E+08	6.259E+08	4.166E+02	Probably Noise
6	1.662E+08	4.598E+08	3.571E+02	Probably Noise

The SVD analysis of the data of the **2** fluorescence titration resulted to be the following:

[FACTOR ANALYSIS]

Tolerance = 1.000E-09

Max. Factors = 10

Num. Factors = 6

Significant = 1

Eigen Noise = 9.827E+02

Experimental Noise = 4.913E+02

#	Eigenvalue	Square Sum	Residual	Prediction
1	3.438E+13	3.196E+09	9.827E+02	Data Vector
2	1.318E+09	1.878E+09	7.533E+02	Possibly Data
3	6.091E+08	1.269E+09	6.193E+02	Probably Noise
4	3.477E+08	9.210E+08	5.277E+02	Probably Noise
5	2.493E+08	6.717E+08	4.508E+02	Probably Noise
6	1.848E+08	4.870E+08	3.838E+02	Probably Noise

Several binding models were tested. The fits were evaluated on the basis of their Durbin-Watson (DW) factors. The DW test is very useful to check for the presence of auto-correlation in the residuals. This method is recommended for systematic misfit errors that can arise in titration experiments. It examines the tendency of successive residual errors to be correlated. The Durbin-Watson statistics ranges from 0.0 to 4.0, with an optimal mid-point value of 2.0 for uncorrelated residuals (i.e., no systematic misfit). In practice fits with $1.6 < DW < 2.4$ can be acceptable. In contrast to the χ^2 (Chi-squared) statistics, which requires the noise in the experimental data is random and normally distributed, the DW factor is meanigful even when the noise level in the data set is low. Since the factorized data usually have a significantly lower noise level than the original data, DW factor is ideal for the present type of data. We tried to fit with exclusive presence of a 1:1 or a 2:1 complex or the contemporary presence of 1:1 and 2:1 complexes and, on the basis of the factor analysis above, we admitted for **1** that in addition to the free drug fluorescence we could have also fluorescence from one of the two complexes (either the 1:1 or the 2:1) . The best complexation model on the basis of the DW factor resulted to be that with 1:1 and 2:1 drug:21-mer complexes with binding constants $\log(K_{11}/M^{-1}) = 5.98\pm0.08$ and $\log(K_{21}/M^{-2}) = 10.93\pm0.08$ (Durbin Watson factor = 2.0, relative error of fit 4.0%) for **1** and $\log(K_{11}/M^{-1}) = 5.78\pm0.12$ and $\log(K_{21}/M^{-2}) = 11.48\pm0.05$ (Durbin Watson factor = 1.6) for **2**, and both 1:1 and 2:1 complexes for both **1** and **2**

being non emissive. Inclusion in the calculation of a contribution to the total fluorescence from one complexed species for **1** (upon the SVD analysis) led to negligibly changed association constants, slightly decreasing the relative error of fit from 4.0% to ~3.7% but increasing the DW factor from 2.0 to ~ 2.3. Therefore we inferred both complexes were substantially non emissive for both drugs. An example of the quality of the agreement between the experimental data and the best fits is shown in Figure S1a and S1b.

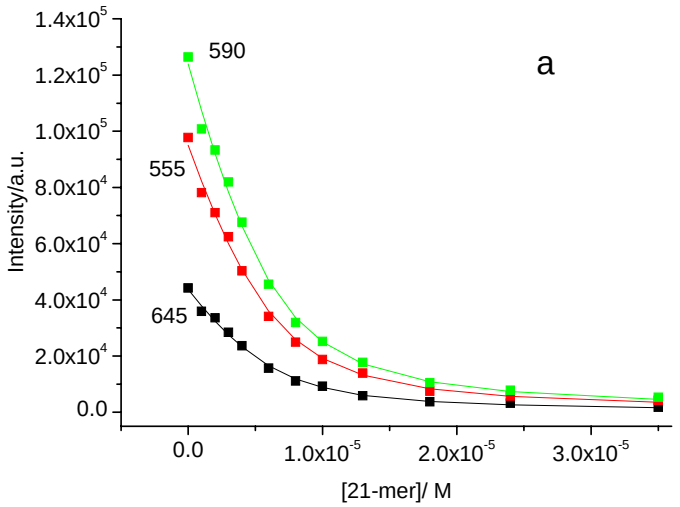

Figure S1. (a) Fluorescence intensity at key wavelengths for excitation at 485 nm of a 1×10^{-5} M **1** solution titrated with 21-mer in TRIS/EDTA/KCl buffer, pH 7.4. Symbols, experimental values; lines, calculated values with $\log(K_{11}/M^{-1})$ = 5.98±0.08 and $\log(K_{21}/M^{-2})$ =10.93±0.08.

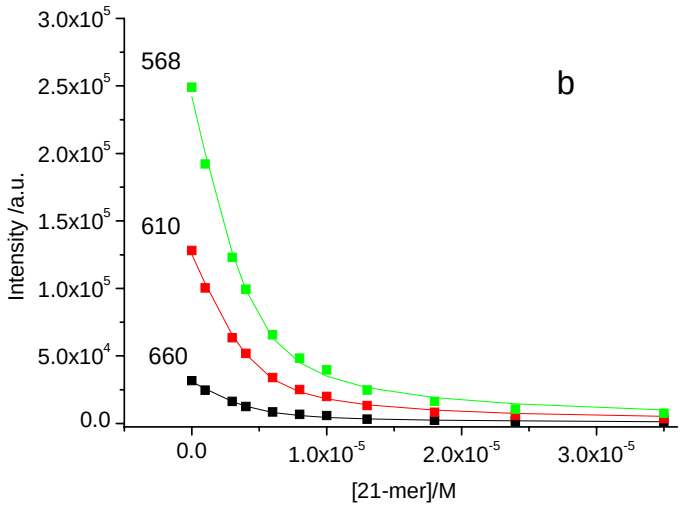

Figure S1. (b) Fluorescence intensity at key wavelengths for excitation at 485 nm of a 1×10^{-5} M **2** solution titrated with 21-mer in TRIS/EDTA/KCl buffer, pH 7.4. Symbols, experimental values; lines, calculated values with $\log(K_{11}/M^{-1})$ = 5.78±0.12 and $\log(K_{21}/M^{-2})$ =11.48±0.05.

On our opinion the model with contemporary presence of 1:1 and 2:1 associates in solution describes reasonably well the system. Therefore we applied it to analyse the absorption data of Figure 4a and 5a in the manuscript. The number of colored species in this case is 4 (a tail up to 350 nm for the free 21-mer, the free drug, the 1:1 and the 2:1 complexes).The spectra of the free species were introduced in the calculation as known data. We fixed the 1:1 association constant (K_{11}) to facilitate convergence. The best fit for **1** corresponded to $\log(K_{21}/M^{-2}) = 10.56\pm0.60$ (Durbin Watson factor = 1.71) with $\log(K_{11}/M^{-1}) = 5.73$, the average value from fluorescence and ITC analysis, for **2** to $\log(K_{21}/M^{-2}) = 10.91\pm0.49$ (Durbin Watson factor = 1.34) with $\log(K_{11}/M^{-1}) = 5.78$, the value obtained from both fluorescence and ITC analysis. The spectra reported in Figure 4b and 5b were extracted. In the following Figures S2 and S3 the quality of the agreement between experimental and calculated absorbances at key wavelengths is shown.

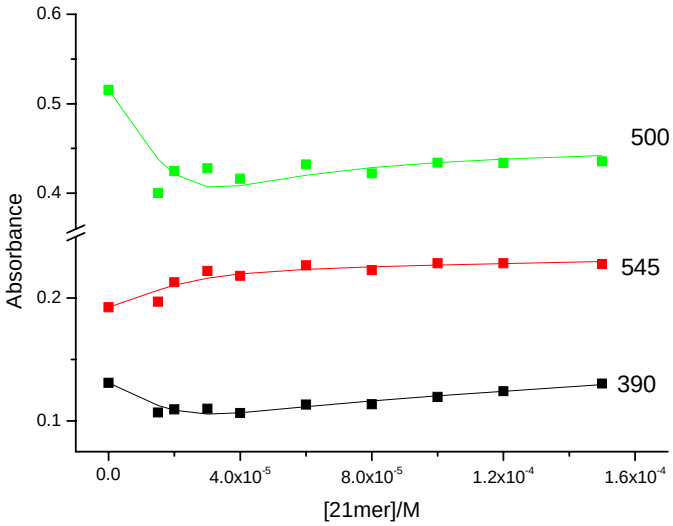

Figure S2. Absorbance ay key wavelength of compound **1**, 5×10^{-5} M, titrated with 21-mer in TRIS/EDTA/KCl buffer at pH 7.4, d=1.0 cm. Symbol, experimental values; line, calculated values with $\log(K_{11}/M^{-1}) = 5.73$ fixed and $\log(K_{21}/M^{-2}) = 10.56\pm0.60$.

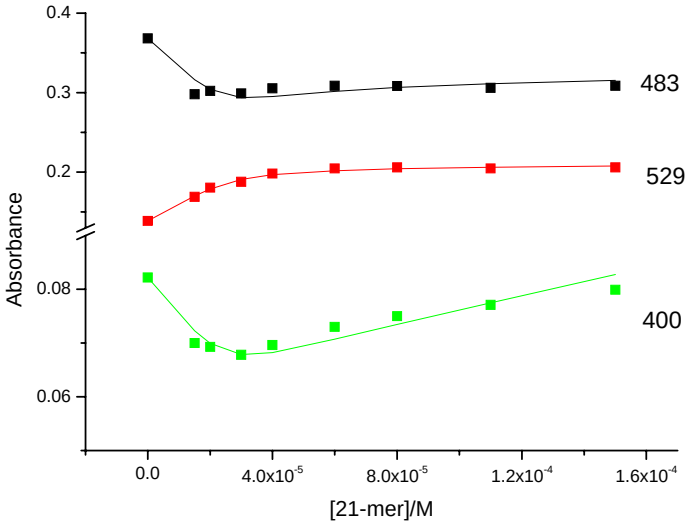

Figure S3. Absorbance ay key wavelength of compound **2**, 5×10^{-5} M, titrated with 21-mer in TRIS/EDTA/KCl buffer at pH 7.4, d=1.0 cm. Symbol, experimental values; line, calculated values with $\log(K_{11}/M^{-1}) = 5.78$ fixed and $\log(K_{21}/M^{-2}) = 10.91\pm0.49$.

The UV circular dichroism variations for 21-mer titrated with **1** and **2** in Figures 6a and 7a, respectively, were also analysed using the same approach. The number of colored species is 4 (the free 21-mer, the free drug, the 1:1 and the 2:1 complexes). The best fit binding constants were $\log(K_{21}/M^{-2}) = 11.62\pm0.34$ (Durbin Watson factor = 1.48) with $\log(K_{11}/M^{-1}) = 5.73$ (fixed) for **1** and $\log(K_{21}/M^{-2}) = 11.96\pm0.30$ (Durbin Watson factor = 1.74) with $\log(K_{11}/M^{-1}) = 5.78$ (fixed) for **2**. The spectra of Figure 6b and 7b were extracted. Examples of the quality of CD data reproduction for **1** and **2** are in Figure S4 and S5.

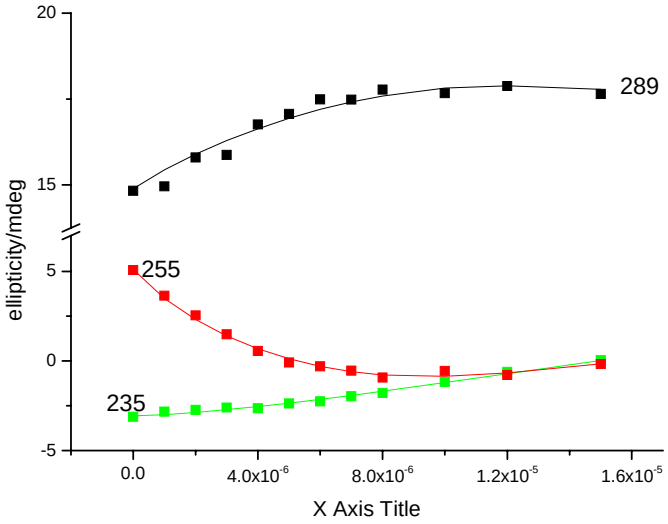

Figure S4. Ellipticity ay key wavelength of compound **1**, 5×10^{-5} M titrated with 21-mer in TRIS/EDTA/KCl buffer at pH 7.4, d=1.0 cm. Symbol, experimental values; line, calculated values with $\log(K_{11}/M^{-1}) = 5.73$ fixed and $\log(K_{21}/M^{-2}) = 11.62\pm0.34$.

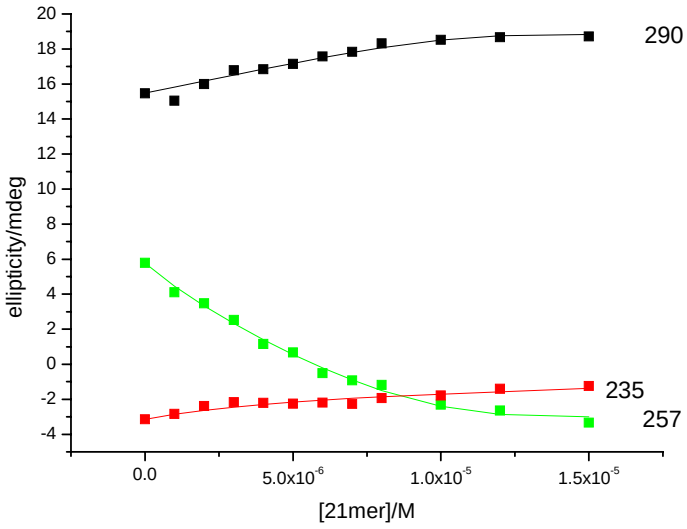

Figure S5. Ellipticity ay key wavelength of compound **2**, 5×10^{-5} M titrated with 21-mer in TRIS/EDTA/KCl buffer at pH 7.4, d=1.0 cm. Symbol, experimental values; line, calculated values with $\log(K_{11}/M^{-1}) = 5.78$ fixed and $\log(K_{21}/M^{-2}) = 11.96\pm0.30$.

References

RS1. Harald Gampp, Marcel Maeder, Charles J. Meyer, and Andreas D. Zuberbühler "Calculation Of Equilibrium Constants From Multiwavelength Spectroscopic Data I. Mathematical Considerations", Talanta, 1985, 32, 95-101.

RS2. Harald Gampp, Marcel Maeder, Charles J. Meyer, and Andreas D. Zuberbühler "Calculation Of Equilibrium Constants From Multiwavelength Spectroscopic Data II. SPECFIT: Two User Friendly Programs In BASIC And Standard FORTRAN 77", Talanta, 1985, 32, 257-264.

SI-2. Melting experiments

We have performed melting experiments monitoring absorption changes for 21-mer (3×10^{-6} M) and mixtures of 21-mer (3×10^{-6} M) with drug (1×10^{-5} M). The first derivative dA(T)/dT yielded a melting temperature of 61°C for 21-mer and 68-70°C for the mixtures in agreement with the CD results. See Figure S6 below.

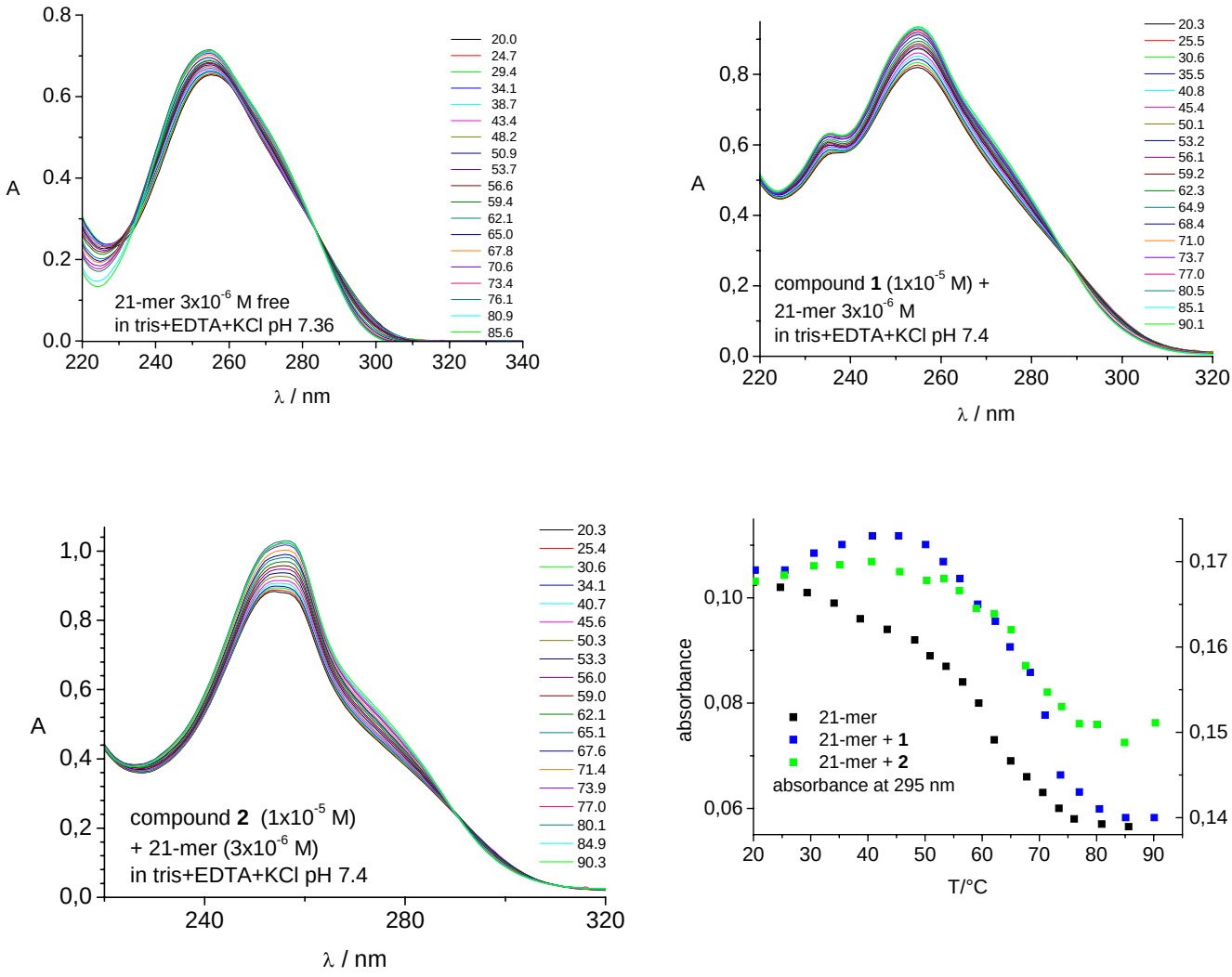

Figure S6. Temperature dependent absorption spectra for 21-mer (3×10^{-6} M) and mixtures of 21-mer (3×10^{-6} M) with drug **1** and **2** (1×10^{-5} M) and plot of absorbance vs. T at 295 nm. Cell path 1 cm.