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Citric acid–γ-cyclodextrin crosslinked oligomers as carriers for Doxorubicin delivery

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Electronic Supplementary Information

GLOBAL ANALYSIS OF EQUILIBRIUM SPECTROSCOPIC DATA

The receptor-ligand complexation equilibria were investigated by performing spectroscopic titrations. The best complexation model and the association constants were determined by multivariate global analysis of multiwavelength data from a series of spectra (UV-vis absorption, CD or FL titration data) corresponding to different mixtures, using the commercial SPECFIT/32 (v.3.0.40, TgK Scientific) program.[1,2] Multiwavelength spectroscopic data sets (absorbances, ellipticities, fluorescence intensities) are arranged in matrix form **Y**, where a number Nw of

wavelengths and a number Nm of corresponding measured spectroscopic signals are ordered in columns, whereas ligand and receptor concentrations are inserted 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 a matrix (Nm×Ne) of concentration eigenvectors ($U^T \times U=1$, orthonormal) and **V** (Ne×Nw) is a matrix of spectroscopic eigenvectors ($V \times V^T$, orthonormal). This **Y'** matrix contains less noise than **Y** because the SVD 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 a number 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. The analysis relies mainly to absorption data, but also CD and fluorescence data may be analysed, in the latter case provided they are relevant to optically thin samples (linear dependence of fluorescence signal on concentration for all the species involved). Given the direct linearity between experimental signal and concentration and the relation that must exist between the concentrations of the various species in the postulated simultaneous equilibria, the program calculates the conditional association constants and the spectra of the complexes based on a non linear least square fit, using the Levenberg-Marquardt algorithm, to best reproduce the experimental data for all the explored wavelengths and ligand-receptor concentration couples. The quality of the fits was evaluated on the basis of their Durbin-Watson (DW) factor and the relative error of fit. 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 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 meaningful 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 test is ideal for the present type of data.

We applied this method to analyze UV-vis absorption, CD and FL titration experiments. Below, as an example, we describe the analysis of the titration of DOX with pγ-CyD with CD monitoring in the region 250-600 nm (data in Figure 6b of the main text). In the calculation the host concentration was expressed in γ-CyD units; the dimerization constant of DOX was fixed as $\log(K_{dim}/M^{-1})$= 4.8. and the spectra of DOx monomer and DOX dimer were also fixed. [3] The best fit corresponds to a complexation model with 1:1 and 1:2 pγ-CyD_unit:DOX complexes in equilibrium with free DOX monomer, DOX dimer and pγ-CyD units. The optimized parameters were the association constants expressed as $\log (K_{11}/M^{-1})$ and $\log(K_{12}/M^{-2})$. The extracted individual CD spectra of the complexes, the concentration profiles of all the DOX species in solution and the agreement between calculated and experimental ellipticity data at key wavelengths are reported in the main text in Figures 8a, 8b and 9a, respectively.

N. components = 2
N. measurements = 8
N. wavelengths = 351

[FACTOR ANALYSIS]
Tolerance = 1.000E-09
Max.Factors = 10
Num.Factors = 8
Significant = 3
Eigen Noise = 7.758E-01
Exp't Noise = 1.552E-01

#	Eigenvalue	Square Sum	Residual	Prediction
1	2.703E+05	1.417E+04	2.247E+00	Data Vector
2	8.370E+03	5.799E+03	1.438E+00	Data Vector
3	4.111E+03	1.688E+03	7.758E-01	Data Vector
4	6.295E+02	1.059E+03	6.144E-01	Probably Noise
5	4.098E+02	6.488E+02	4.811E-01	Probably Noise
6	2.613E+02	3.875E+02	3.719E-01	Probably Noise
7	2.126E+02	1.749E+02	2.499E-01	Probably Noise
8	1.749E+02	1.293E-06	2.149E-05	Probably Noise

[SPECIES]	[COLORED]	[FIXED]	[SPECTRUM]
1 0 0	False	False	
0 1 0	True	True	DOX Monomer. FIX
0 2 0	True	True	DOX Dimer. FIX
1 1 0	True	False	
1 2 0	True	False	

[SPECIES]	[FIXED]	[PARAMETER]		[ERROR]
1 0 0	True	0.00000E+00	+/-	0.00000E+00
0 1 0	True	0.00000E+00	+/-	0.00000E+00
0 2 0	True	4.84000E+00	+/-	0.00000E+00
1 1 0	False	3.34099E+00	+/-	2.22758E-01
1 2 0	False	8.65231E+00	+/-	3.32654E-01

[CONVERGENCE]

Iterations = 3

Convergence Limit = 1.000E-04

Convergence Found = 3.275E-11

Marquardt Parameter = 0.0

Sum(Y-y)^2 Residuals = 1.47072E+02

Std. Deviation of Fit(Y) = 2.28899E-01

[STATISTICS]

Experimental Noise = 1.552E-01

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Relative Error Of Fit = 12.3306%

Durbin-Watson Factor = 1.5702

Goodness Of Fit, Chi^2 = 2.176E+00

Durbin-Watson Factor (raw data) = 1.6708

[COVARIANCE]

4.491E-01	-1.026E-01
-1.026E-01	1.325E+00

[CORRELATION]

1.000E+00	-1.330E-01
-1.330E-01	1.000E+00

REFERENCES

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3. R. Anand, S. Ottani, F. Manoli, I. Manet and S. Monti, *RSC Adv.*, 2012, **2**, 2346 - 2357.