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DNA binding and cytotoxicity of fluorescent curcumin-based Zn(II) complexes

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1) Figure S1 - Absorption spectra in DMSO

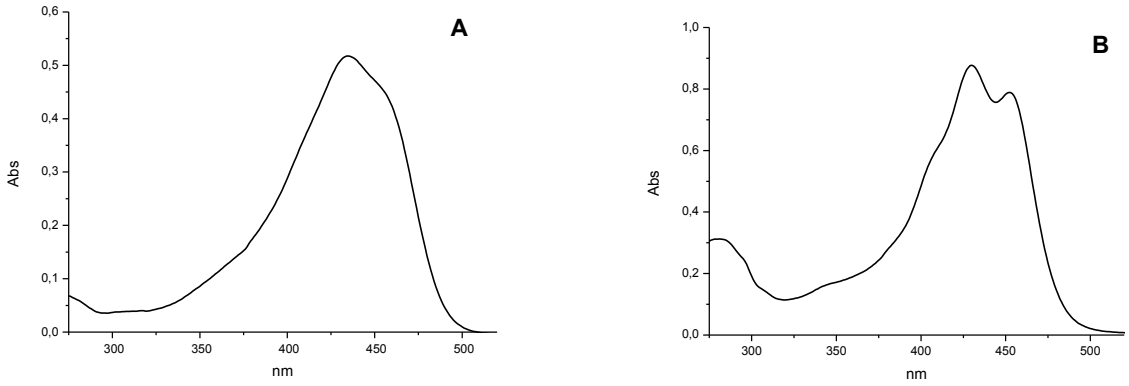

Figure S1: Absorption spectra in DMSO solution at room temperature of curcumin (curc) (A) and of complex **1** (B)

2) Relation between the degree of dye ordering and the measured fluorescence anisotropy

Both fluctuations of dyes around their binding position (wobbling)[1] and fluctuations of helices around their average direction within liquid crystalline (LC) phases contribute to the depolarization of the static fluorescence signal. Therefore, the contrast of the observed fluorescence modulations can yield information about the overall degree of alignment of dyes within DNA helices and of helices within the LC phase. To estimate these contributions and to judge the quality of curc binding, we combine the signals obtained from V and H polarized incident light into an anisotropy parameter A:

$$A = \frac{I_V - I_H}{I_V + I_H}$$

Then, we can obtain a relation between A and $\langle P_2 \rangle = \int P(\vartheta) \frac{3\cos^2 \vartheta - 1}{2} d\vartheta$, an order parameter which provides an estimate of the width of the angular distribution *P(ϑ)* of molecules around their average direction, i.e. how well ordered are the molecules (dye *and* helices together).

To this aim, we describe the orientation of the dye/DNA complex (assuming intercalation) according to the usual choice for the coordinates of an ordered LC system[2], as illustrated below:

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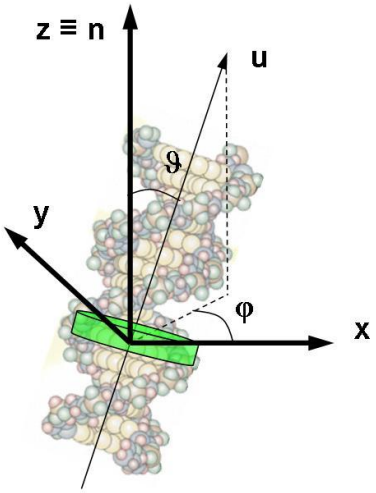

- z: coordinate along the average direction of helices **n** (usually the nematic director, here the columnar main axis);
- ϑ: angle between the molecular axis (**u**) and the z-axis: $\mathbf{u}\cdot\mathbf{n}=\cos\vartheta$;
- φ: angle between the x-axis and the x-y projection of **u** .

Based on the experimental procedure (we only choose LC domains with helices in a planar arrangement), we make the reasonable assumption that **n** lies in the plane of the cell and the resulting description is that of a 2D system. Dye molecules are approximated to have disk symmetry: they are excited proportionally to the component of the optical field in the plane of the disk.

A - Polarization of incident light along z

Each molecule absorbs and emits proportionally to $\sin^2\vartheta$. The total fluorescence intensity I_z is in this case

$$I_z=\int P(\vartheta)\sin^2\vartheta d\vartheta=\frac{2}{3}\left(1-\langle P_2\rangle\right)$$

where $P(\vartheta)$ is the angular distribution of the molecules (normalized to give $\int P(\vartheta)d\vartheta=1$) and

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$$\langle P_2 \rangle = \int P(\vartheta) \frac{3\cos^2 \vartheta - 1}{2} d\vartheta$$

is the order parameter, providing a measure of the degree of alignment of the molecules: it is 1 for perfectly aligned molecules and 0 for isotropic arrangement.

B - Polarization of incident light along x

Each molecule absorbs and emits proportionally to $\sin^2\gamma$, γ being the angle between **u** and $\mathbf{u}_x$, the unit vector along the x-axis. The total fluorescence intensity I_x is in this case:

$$I_x = \int P(\vartheta) \sin^2 \gamma d\vartheta = \int P(\vartheta)\left(1 - (\mathbf{u} \cdot \mathbf{u}_x)^2\right) d\vartheta = \int P(\vartheta)\left(1 - \sin^2 \vartheta \cos^2 \varphi\right) d\vartheta$$

Since the distribution is flat in φ, we can average on it and obtain

$$I_x = \int P(\vartheta)\left(1 - \sin^2 \vartheta/2\right) d\vartheta = \frac{1}{3}\left(2 + \langle P_2 \rangle\right)$$

From the experiment we can determine the ratio $A = \frac{I_x - I_z}{I_x + I_z}$ (for vertical and horizontal polarization incident on vertical and horizontal helices). From the equations before we obtain:

$$I_x - I_z = \langle P_2 \rangle$$

from which follows

$$|\mathrm{A}|_{\max} = \frac{3\langle P_2 \rangle}{4 - \langle P_2 \rangle},$$

thus establishing a simple relationship between the measured anisotropy of fluorescence and the order parameter of the ensemble of dye molecules:

$$\langle P_2 \rangle = \frac{4|A|_{\max}}{3 + |A|_{\max}}$$

Should the dye molecules not disks but intercalant rods or intercalant disks with transition dipole along one specific diameter of the disk, the amplitude of absorption and consequent emission would

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also depend on the azimuthal angle φ. Since we don't expect any anisotropy in φ (there is no preferential azimuthal orientation either of the helices in the LC phase or of the dye molecules within the helices), both I_x and I_z would be reduced by the average of $\cos^2 \varphi$, that is they would be reduced to half their value evaluated before for symmetric disks. Therefore, even in this case, the final equation would be the same.

3) Estimate of the amplitude of the dye fluctuations

Once $\langle P_2 \rangle$ is known, we can estimate the angular spread of the molecules by assuming a functional form for $P(\vartheta)$, typically a bell-shaped curve centered on **n**. For example, following Onsager[3], we can pose:

$$P(\vartheta) = const * \cosh(\alpha \cos \vartheta)$$

where the parameter α determines the width of the distribution, and *const* is a normalization pre-factor. In this case the half-width of the distribution is $1/\sqrt{\alpha}$.

From the measured values of $|A|_{max}$ for EB, curc and its complexes, we have extracted $\langle P_2 \rangle$ and the corresponding angular spread, as reported in Table S1.

Compound	$\|A\|_{max}$ (±0.05)	$\langle P_2 \rangle$ (±0.04)	Angular spread (±2°)
EB	0.65	0.71	19
curc	0.45	0.52	26
1	0.5	0.57	24
2	0.51	0.58	24

Table S1: Experimental values of the contrast of fluorescence anisotropy for the investigated compounds, and of the associated degree of ordering.

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The fluctuations of EB intercalated within DNA base pairs have been estimated between 7° and 15° [4]. If we assume $\langle P_2 \rangle = \langle P_2 \rangle_{LC} \cdot \langle P_2 \rangle_{dye}$, we obtain $\langle P_2 \rangle_{LC} = 0.82 \pm 0.08$, which corresponds to a significant degree of order, consistent with the columnar phase. Since the quality of the LC order is not changing among the investigated samples, we determine $\langle P_2 \rangle_{curc} = 0.64 \pm 0.06$, corresponding to 21° fluctuations, and $\langle P_2 \rangle_{1,2} = 0.70 \pm 0.06$, corresponding to 19° fluctuations.

We may want to independently verify this value from the measured optical birefringence Δn of the LC phases, properly rescaled for concentration:

$$\Delta n = \Delta n_{sat} / c \cdot \langle P_2 \rangle_{LC} \cdot c$$

where Δn_{sat} is the saturated birefringence of perfectly aligned molecules[5]. We find Δn to be between 0.025 and 0.03 in all the samples, but since literature values for $\Delta n_{sat}/c$ of DNA[6] span a factor of 3, they do not allow to extract an accurate value for $\langle P_2 \rangle_{LC}$ from birefringence. On the other side, direct experiments on aligned oligonucleotides are prevented by the extremely high magnetic fields required to orient LCs of short DNA.

In principle, we cannot exclude that the observed angular modulation of the fluorescence may come from groove-binders, since depending on their average orientation relative to helix axis, the main fluorescence contribution of groove-binders can be either parallel of perpendicular to helix axis. However, the usual values of tilt angles assigned to groove-binding molecules are between 20° and 45° [7] (curiously different from the inclination of the groove itself in usual B-DNA, which is close to 60° [8]). For such values, the dominant contribution, parallel to helices, would lead to a fluorescence modulation opposite to the one experimentally observed, and for any angle a lower contrast of fluorescence anisotropy would be found.

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