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Characterisation of small molecule binding to DNA using a quartz crystal resonant sensor

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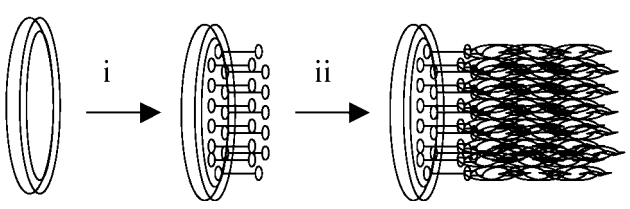
A new development in quartz crystal resonant sensor (QCRS) technology allows real-time analysis of binding by small molecules to DNA and has been tested using superoxide dismutase mimics.

Low molecular weight compounds interact with DNA in a variety of ways, from intercalation by π-stacking and hydrogen bonding to covalent bond formation.[1] The strength of the interaction is a good indication of each compound's binding mode with DNA and may be a direct indication of mutagenicity. We have developed a test based on the quartz crystal resonant sensor (QCRS) to measure DNA–analyte binding directly in real time. The QCRS is a robust sensor for which applications as diverse as volatile organic compound detection,[2] monitoring of antibody–antigen agglutination[3] and gene sensing have been found.[4] The technique uses surface modified piezoelectric quartz crystals to register the presence of chemical and biological species in the liquid phase *via* the change in resonant frequency as a function of analyte adhesion with time. Our method allows the determination of the type of interaction between DNA and small molecules, together with direct observation of any destructive effects such as DNA scission.† Briefly, thin film gold electrodes on the quartz crystals are coated with a bifunctional binder (NeutrAvidin™) followed by suitably functionalised DNA (Scheme 1). Solutions of small molecule analytes are then passed over the sensor in a flow-through cell and the frequency changes resulting from binding, due to the concomittant mass increase of the sensor, are monitored. The duration of the experiment makes the technique ideally suited to rapid screening of small molecule–DNA interactions. To confirm that the binding observed was indeed to DNA and not to one of the substrates on the QCRS, test runs were performed on partially modified sensors. Solutions of compounds were passed over crystals which had NeutrAvidin™, but no DNA, attached. Any observed frequency changes were short lived and final deviations from the base frequency were approximately 5% of the deviation shown when DNA was present. To ensure consistency all tests were carried out at the same flow rate (5 μl min^{-1}).

Analytes expected to bind to DNA in a variety of different ways were chosen to test the technique. Cisplatin **1** was chosen to probe strong, irreversible binding.[5] 5-Aminoacridine **2** and 9-amylaminoacridine **3**[6] were chosen to represent varying degrees of intercalation. Bleomycin **4** was included as an example of a well known DNA cleaving agent.[7]

To complement the behaviour of these analytes we also tested manganese(III) salen chloride **5**[8] and its 3,3′-methoxy analogue **6**.[9] These compounds act as successful superoxide dismutase mimics *in vivo* and have recently been shown to extend average lifespan in nematode worms, *Caenorhabditis elegans*.[10] Observation of the binding, if any, to DNA may reveal how they work and will represent a true trial of the system.

Fig. 1 shows that **1** took less than two minutes from injection to saturate the sensor and exhibited a drop in resonant frequency of 68 Hz. As expected, **2** bound slowly, taking about 8 min to saturate binding sites, and gave an overall frequency change of 8 Hz. The binding profile for **3** has a similar pattern to that of **2** but a greater frequency change (32 Hz) was observed due to the increased hydrogen bonding available to this compound. Compound **4** showed initial binding followed by a plateau which we speculate is due to a rearrangement process. The frequency then dropped again before finally rising after a further 8 minutes. This behaviour is consistent with cleavage of DNA and its subsequent removal from the surface of the sensor. Initial association rates calculated for each compound show a greater than 10-fold difference between cisplatin and 9-amylaminoacridine, and an additional 75-fold difference when compared with 5-aminoacridine (Table 1). Early literature studies involving acridines suggest that more than one site is involved in the intercalation process.[11] A mechanism published for proflavin suggested that its mechanism was distinct from either intercalation or electrostatic attraction.[12] It is thought unlikely that our observed responses are due solely to the binding of low molecular weight compounds to DNA. In particular, a degree of reorganisation is likely within the structure of the DNA that may cause frequency shifts due to viscoelastic interfacial changes additive to the initial mass change. Similar phenomena have been observed with ligands binding to RNA.[13,14]



Scheme 1 Sensor preparation: i, surface modification of gold covered quartz crystal with NutrAvidin™; ii, addition of biotinylated DNA.

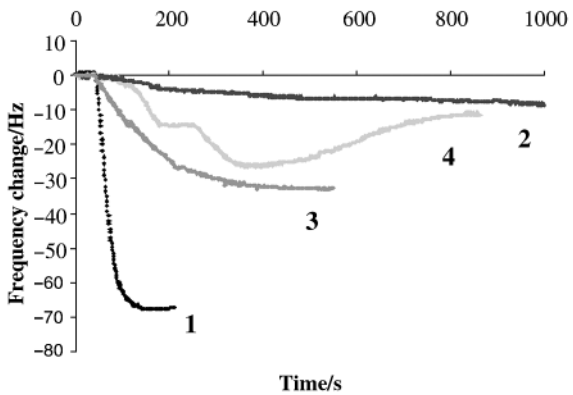


Fig. 1 Binding curves for compounds **1**, **2**, **3** and **4**.

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Table 1 Mass changes and initial binding rates for analytes

Compound	Mass change upon addition to sensor/μg[a]	Initial association rate/s^{-1}
Cisplatin **1**	0.60	1.54
5-Aminoacridine **2**	0.50	0.02
9-Amylaminoacridine **3**	0.60	0.14
Bleomycin **4**	0.56	0.20
[Mn(III)(salen)]$^+$ **5**	0.70	0.25
[Mn(III)(3,3′-methoxysalen)]$^+$ **6**	0.71	0.03

[a] Calculations assume saturation of compounds on DNA.

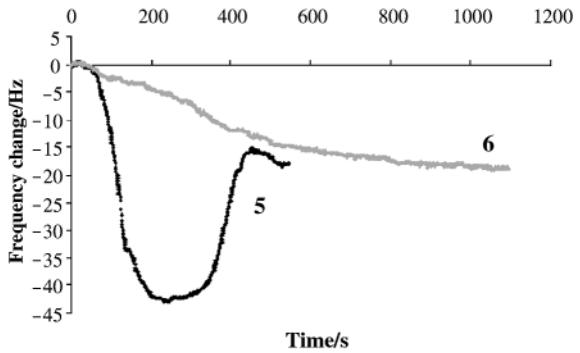

Fig. 2 Binding curves for compounds **5** and **6**.

It can be seen from Fig. 2 that **5** has a similar profile to that of **4** indicating initial strong binding to DNA followed by cleavage. To confirm this interpretation we stained the QCRS-tethered DNA with acridine orange. Fluorescence microscopy of the QCRS prior to addition of **5** showed a spatially homogeneous distribution of stained DNA across the sensor surface. Further microscopy of the eluent, following addition of **5**, showed that it contained acridine orange-stained DNA, confirming that **5** had pro-oxidant activity towards free DNA. Conversely the 3,3′-methoxy analogue, **6**, appeared to intercalate weakly, with a similar profile to the acridine derivatives, giving a maximum frequency drop of 20 Hz. Staining DNA with acridine orange prior to addition of **6** and examination of the eluent post addition indicated no significant cleavage. After 8 min the binding profile of **5** follows that of **6** indicating that they both act as weak intercalators in the long term.

Melov *et al*. have shown that both **5** and **6** extend lifespan of *C*. *elegans*; however, only **6** gives rise to re-emergent fertility in longer lived worms.[10] We conclude that the lack of pro-oxidant activity of **6**, as indicated by the QCRS, is responsible in part for its greater benefits.

It appears that both the magnitude of the frequency change and, more importantly, the binding profile due to each analyte are directly related to the type of analyte–DNA interaction. The QCRS is therefore an ideal technique to study the interactions of a wide variety of small molecules with DNA and other biological macromolecules.

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Notes and references

† *Experimental conditions*: 8.6 mm diameter, 10 MHz AT-cut quartz crystals (Hi-Q International, Cambridge, UK) were furnished with 100 nm thick gold electrodes (19.5 mm^2 area), placed into an in-house fabricated flow-through liquid cell (20 μl volume) and connected to a novel gain control oscillator[15] designed specifically to work in liquid environments of high viscosity and density. Output from the crystals was monitored using a Fluka-6689 frequency counter capable of sub-single hertz resolution, data being collected once per second *via* Fluka Timeview software. Crystals were washed with HPLC grade ethanol prior to deposition of Neutr-Avidin™ (Pierce, IL, USA). The electrodes were coated with Neutr-Avidin™ by a single 20 μl injection of the binding agent (2 mg ml^{-1} in Sorensen's phosphate buffer) *via* an all PEEK™ Rheodyne MMB six-way valve into the running buffer until a stable baseline with noise below 0.2 Hz was observed. Once a stable signal had been obtained for the modified crystal, it was washed with biotinylated DNA (b-DNA, Vector Laboratories Ltd, Peterborough, UK) at 50 μg ml^{-1} in Sorensen's phosphate buffer (20 μl injection loop, 1 μg per injection). Washes were repeated until no further frequency change was observed in order to ensure that the sensor surface was saturated (*ca*. 2.5 μg of DNA on the surface). The b-DNA contained a range of lengths from 125 to 23 100 base pairs to ensure variation in potential binding sites for the analytes. Modified electrodes were exposed to solutions of the DNA binding agents (500 μM in phosphate buffer solution, Table 1, flow rate 5 μl min^{-1}, 22 ± 0.5 °C) and resultant frequency changes monitored for up to 20 min. Run-to-run reproducibility was better than 5% (N = 3) in all cases.

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