Through this process, they identified GNF-2 as a highly selective inhibitor of Bcr-abl–dependent cell proliferation that, importantly, maintains potency against many, though not all, clinically relevant imatinib-resistant Bcr-abl mutants. The investigators propose that this new compound class inhibits Bcr-abl kinase activity in an allosteric, non-ATP-competitive manner. Interestingly, GNF-2 was shown to be inactive *in vitro* against the catalytic domain of c-abl kinase. Like imatinib, GNF-2 selectively inhibits proliferation and induces apoptosis of Bcr-abl transformed cells. However, unlike imatinib, GNF-2 is inactive in cells containing a mutation in the myristoyl-binding cleft of Bcr-abl. This binding pocket has been shown to function as an auto-regulatory site for c-abl kinase, where binding of a myristoylated residue on the N terminus results in stabilization of the protein in an inactive form[8]. Loss of this regulatory mechanism accompanies the formation of the Bcr-abl fusion protein. Therefore, Adrian *et al.* speculate that by virtue of its binding to the c-abl myristoyl pocket, GNF-2 stabilizes the kinase in an inactive state (**Fig. 1**).

The differential cytotoxicity approach used by Adrián *et al.*[2] represents a nontraditional screening paradigm that, from the outset, improved the likelihood of discovering new Bcr-abl inhibitors that were highly selective. An important benefit of this experimental approach lies in its ability to aid in the identification of inhibitors that have activity against an enzymatic conformation that exists only in the intracellular milieu. In contrast, historical screening programs directed toward protein kinase targets have often involved recombinant catalytic kinase domain substrates. The use of low ATP concentrations in high-throughput screens further increases the probability that hits will prove to be ATP-competitive in nature. It is therefore not surprising that re-identification of the same, highly promiscuous chemical matter has plagued many of these screens[9].

GNF-2 is an extremely useful tool and potentially may represent a breakthrough in the development of effective agents targeting CML. Further improvements in potency will be required before a member of this chemical class evolves into a viable drug candidate. Unfortunately, improvement of the pharmaceutical profile of GNF-2 analogs is not straightforward, especially in light of their inactivity against *in vitro* enzyme preparations. Traditional kinase drug-discovery programs often require significant medicinal chemistry efforts specifically directed toward improving target potency and selectivity *in vitro*. As GNF-2 targets a conformation of Bcr-abl that does not occur *in vitro*, the use of structure-guided drug design may prove difficult, forcing researchers to rely on cell-based functional testing as a means to elucidate structure-activity relationships. The cell-based strategy adopted by Adrián *et al.* provided a head start in obtaining the desired high degree of selectivity against Bcr-abl. However, it may prove challenging to now optimize potency when the cellular readout cannot distinguish enhanced permeability, altered metabolic inactivation or a variety of other factors from enhanced affinity for the target kinase.

Crystallographic studies will be required to confirm the hypothesis put forth by Adrián *et al.*, that inhibition of Bcr-abl by GNF-2 is attained by virtue of its binding to the c-abl myristoyl pocket. Although the precise mechanism of its activity and specificity has not been fully elucidated, GNF-2 represents a new class of putative allosteric kinase inhibitor. From a clinical standpoint, this compound is very interesting because of its high degree of specificity for Bcr-abl, which raises the possibility of obtaining a truly leukemia-specific drug with no expected off-target activity. Thus GNF-2, as the prototype for this compound class, points to a new region of Bcr-abl that may be exploitable for the development of improved therapies against CML.

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1. Druker, B.J. *et al. Nat. Med.* **2**, 561–566 (1996).
2. Adrian, F.J. *et al. Nat. Chem. Biol.* **2**, 95–102 (2006).
3. Schindler, T. *et al. Science* **289**, 1938–1942 (2000).
4. Goldman, J.M. & Melo, J.V. *N. Engl. J. Med.* **349**, 1451–1464 (2003).
5. Branford, S. *et al. Blood* **102**, 276–283 (2003).
6. Azam, M., Latek, R.R. & Daley, G.Q. *Cell* **112**, 831–843 (2003).
7. Carter, T.A. *et al. Proc. Natl. Acad. Sci. USA* **102**, 11011–11016 (2005).
8. Nagar, B. *et al. Cell* **112**, 859–871 (2003).
9. Posner, B.A. *Curr. Opin. Drug Discov.* **8**, 487–494 (2005).

# Getting under wraps: alkylating DNA in the nucleosome

Kent S Gates

**DNA in the nucleus of mammalian cells is extensively associated with proteins. Potential anticancer agents can access, recognize and alkylate nucleosomal DNA, even at sites that seem completely occluded by association with histone proteins.**

DNA inside cells is wrapped around a complex of histone proteins to yield higher-order structures known as nucleosomes and chromatin[1] (**Fig. 1**). Inspection of the nucleosome structure[2] might lead to the suspicion that substantial regions of DNA that face the histone core will be protected from reaction with small molecules such as anticancer drugs and mutagens. Contrary to that expectation, on page 79 of this issue, Boger and coworkers present data showing that the natural products yatakemycin and duocarmycin SA alkylate nucleosomal DNA, even at 'difficult' regions where target sites on the DNA face the protein core, with efficiencies and sequence specificities nearly identical to those seen for their reactions with 'naked' (protein-free) DNA[3]. This work offers a striking new demonstration that the dynamic nature of histone-DNA interactions can allow small molecules (with a molecular mass of less than 1,000 Da) to access target sites on the double helix that seem to be completely masked in the static structure of the nucleosome.

Accurate 'readout' (transcription) and copying (replication) of DNA is absolutely required for the normal operation of cells. It is not unexpected, then, that chemical modification of cellular DNA has profound biological consequences and represents an important area of study in the fields of medicinal chemistry, toxicology and biotechnology[4,5]. In studies aimed at elucidating the chemical processes underlying the biological activity of

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DNA-damaging drugs and mutagens, it is common to use isolated DNA fragments to model the cellular substrate[6–8].

Of course, DNA inside the nucleus of mammalian cells differs from naked DNA, in that it is associated with proteins to yield higher-order structures. The first level of this higher-order organization is the nucleosome core particle, in which a 147–base pair (bp) stretch of DNA is wrapped around a disc-shaped protein complex consisting of four pairs of histone proteins (eight total proteins). The 1.7 turns of left-handed superhelix imposed on the DNA when it is incorporated into a nucleosome causes considerable curvature of about 45° per helical turn and yields grooves on the double helix that are deep and narrow facing toward the protein core and wide and flattened facing away[2]. There is overwinding of the helix in the central three turns and underwinding in the remainder of the DNA. Proteins in the histone core make close contact with 14 segments of the DNA minor groove and insert an arginine residue into the groove at each of these sites. In the cell nucleus, nucleosome core particles are connected by 20- to 60-bp regions of linker DNA and, in partnership with the linker histone H1, condense to form chromatin[1] (**Fig. 1**). In this way, 2 meters of genomic DNA is compacted into a nuclear space less than 1 picoliter ($1 \times 10^{-12}$ liters) in volume.

Inspection of the static X-ray structure of the nucleosome[2] might lead to the expectation that substantial regions of nuclear DNA will be completely protected from reaction with electrophilic alkylating agents such as anticancer drugs or mutagens. Notably, this is usually not the case. In fact, reactions of alkylating agents with nucleosomal DNA typically mirror those seen with naked DNA, showing very similar sequence preferences and only modestly (2- to 3-fold) decreased adduct yields[9–11].

Yatakemycin and duocarmycin SA are potential anticancer agents with extremely potent biological activity. These natural products associate noncovalently with AT tracts in duplex DNA and alkylate at the N3 position of adenine residues. Both noncovalent binding and alkylation are highly dependent on the size and shape of the DNA minor groove at the target sites, making it especially interesting to investigate whether these compounds can recognize and covalently modify the rather distorted DNA found in the nucleosome.

In this issue, Boger and coworkers[3] report that alkylation of DNA in a nucleosome core particle by yatakemycin and duocarmycin SA closely parallels their reactions with naked DNA. In this case, the sequence preferences are essentially identical, and alkylation yields decrease by a factor of only 1.4 to 1.8 in nucleosomal DNA versus naked DNA. The compounds efficiently alkylate 'difficult' sites that seem to be completely obscured by association with histone proteins in the X-ray structure of the nucleosome core particle[2]. Efficient reaction at these sites is especially remarkable given that, prior to DNA alkylation, yatakemycin and duocarmycin SA must 'find' and then noncovalently associate with fairly large (approximately 5-bp) binding sites. Access to 'difficult', protein-bound sites in nucleosomal DNA may be especially favored for alkylating agents whose unproductive decomposition reactions (such as hydrolysis) are slow relative to the rates of histone-DNA dissociation and DNA alkylation[10]. In essence, such agents have the ability to 'wait around' for the transient dissociation of the DNA-histone complexes that may allow them to recognize and alkylate 'normal' B-form DNA. Alternatively, the reaction could proceed via an induced-fit mechanism in which the alkylating agent induces distortion of DNA in the histone-DNA complex into a B-form-like structure, with the wider, shallower minor groove required for DNA modification by this class of agents. Notably, additional experiments show that the alkylation of nucleosomal DNA by these potential anticancer agents does not significantly disrupt the structural integrity of the nucleosome core particle, despite the likelihood[12] that the DNA at these alkylation target sites is considerably distorted relative to that in the native nucleosome core particle.

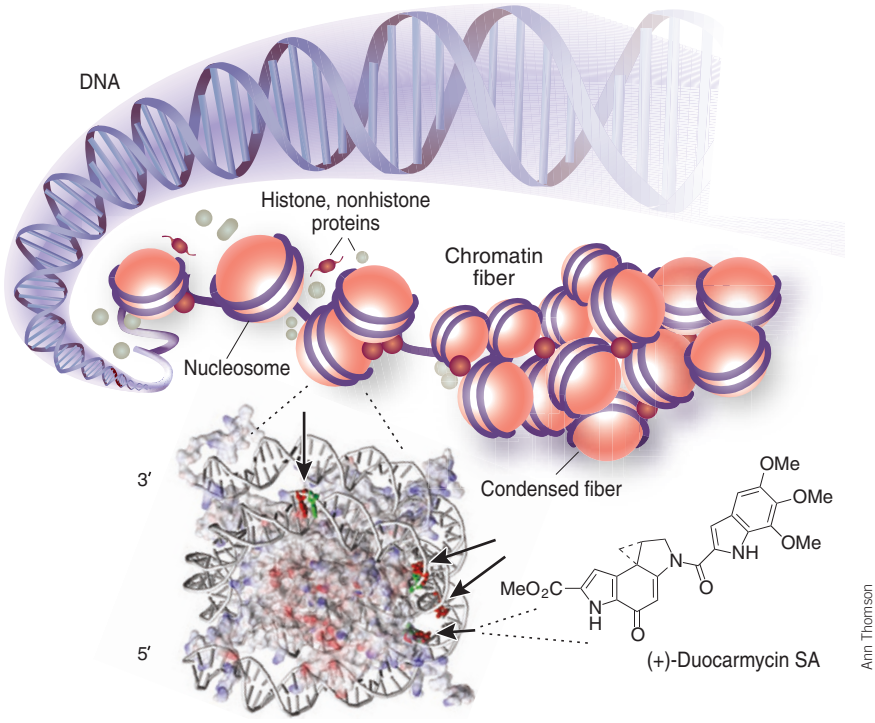


**Figure 1** DNA in cells is associated with histone proteins to yield higher-order structures known as the nucleosome core particle and chromatin. Natural products such as duocarmycin can access, recognize and alkylate protein-associated sites in the nucleosome core particle.

Overall, these studies highlight the plasticity of the DNA-protein interactions in the nucleosomal core particle and show that the natural products yatakemycin and duocarmycin SA can access, recognize and alkylate large (5-bp), protein-associated binding sites in the nucleosome core particle. The core particle evidently can then adjust its structure to accommodate the chemically modified DNA. From a practical perspective, the authors correctly note that the similarities between the reactions observed for nucleosomal and naked DNA seen in these studies affirm the longstanding idea that *in vitro* experiments using naked (protein-free) DNA represent a valid and indispensable approach for predicting the site and structure of DNA damage that will be caused by small organic molecules *in vivo*. Such studies are a central part of important efforts directed at understanding the relationships between the chemical structure of DNA damage and the resulting biological response.

1. Schalch, T., Duda, W., Sargent, D.F. & Richmond, T.J. *Nature* **436**, 138–141 (2005).
2. Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F. & Richmond, T.J. *Nature* **389**, 251–260 (1997).
3. Trzupek J.D., Gottesfeld, J.M. & Boger, D.L. *Nat. Chem. Biol.* **2**, 79-82 (2006).
4. Gates, K.S. in *Comprehensive Natural Products Chemistry* (ed. Kool, E.T.) 491–552 (Pergamon, New

York, 1999).
5. Gates, K.S., Nooner, T. & Dutta, S. *Chem. Res. Toxicol.* **17**, 839–856 (2004).
6. Lawley, P.D. & Brookes, P. *Biochem. J.* **89**, 127–138 (1963).
7. Takeshita, M., Grollman, A.P., Ohtsubo, E. & Ohtsubo, H. *Proc. Natl. Acad. Sci. USA* **75**, 5983-5987 (1978).
8. Zang, H. & Gates, K.S. *Chem. Res. Toxicol.* **16**, 1539–1546 (2003).
9. MacLeod, M.C. *Carcinogenesis* **16**, 2009–2014 (1995).
10. Millard, J.T., Spencer, R.J. & Hopkins, P.B. *Biochemistry* **37**, 5211–5219 (1998).
11. Smith, B.L., Bauer, G.B. & Povirk, L.F. *J. Biol. Chem.* **269**, 30587–30594 (1994).
12. Suto, R.K. *et al. J. Mol. Biol.* **3326**, 371–380 (2003).

# Michaelis-Menten is dead, long live Michaelis-Menten!

Nils G Walter

**Modern single-molecule tools, when applied to enzymes, challenge fundamental concepts of catalysis by uncovering mechanistic pathways, intermediates and heterogeneities hidden in the ensemble average. It is thus reassuring that the Michaelis-Menten formalism, a pillar of enzymology, is upheld, if reinterpreted, even when visualizing single turnover events with a microscope focus.**



The seminal 1913 discovery of Leonor Michaelis and Maud Menten[1] arguably represents the beginning of enzyme kinetics as a systematic field and remains a pillar of enzymology. Thousands of enzymes have been characterized using the Michaelis-Menten formalism, which describes the rate of multiple enzymatic turnovers as a function of substrate concentration (**Fig. 1**). Close to a hundred years of successful, largely undisputed use does not imply, however, that modern insight cannot add exciting new twists. In this issue of *Nature Chemical Biology*, Sunney Xie and co-workers at Harvard[2] have employed creative single-molecule microscopy to ask the simple question of whether the Michaelis-Menten formalism is upheld even when visualizing catalysis one substrate molecule at a time. The answer is yes, but the microscopic interpretation changes in light of enzymatic heterogeneity.

Michaelis and Menten showed that invertase (now known as β-fructofuranosidase), a yeast enzyme central to sugar metabolism that catalyzes the hydrolysis of sucrose into an optically distinct mixture of glucose and fructose ('invert sugar'), has a characteristic hyperbolic dependence on substrate concentration. Two regimes can be distinguished under conditions where substrate is in excess over enzyme (multiple-turnover conditions) (**Fig. 1**). At limiting (low) substrate concentration, the measured rate constant increases linearly with substrate concentration, indicating that reversible substrate binding ($k_{on}$, $k_{off}$) is mostly rate limiting. At saturating (high) substrate concentration, the measured rate constant is independent of substrate concentration and the catalytic turnover ($k_{cat}$) becomes fully rate limiting.

Applying single-molecule microscopy to β-galactosidase, a bacterial enzyme essential for sugar utilization and a modern enzymological work horse, the Harvard group has now taken a closer look at these two regimes (**Fig. 1**). A derivative of the enzyme's lactose substrate yields brief fluorescent bursts upon hydrolytic turnover before the fluorescent product diffuses out of a laser focus. A succession of turnovers by a single immobilized enzyme thus yields a meteor shower of fluorescence bursts. Kinetic information on multiple turnovers by a single enzyme is derived from a large number of waiting times between two successive fluorescence bursts. The authors find that the average waiting time of a single enzyme molecule plotted against the inverse of the substrate concentration recapitulates the linear Lineweaver-Burke relationship observed in an ensemble measurement. This demonstrates that the Michaelis-Menten equation holds even at the single-molecule level. The average waiting time from a large number of single enzyme molecules then is related to the macroscopic turnover rate constant.

At low substrate concentration, Xie and co-workers find that a single time constant characterizes the waiting times between substrate turnovers of a single enzyme. This implies that the limiting rate constants under these conditions, $k_{on}$ and $k_{off}$, are uniform over long periods of time (**Fig. 1**). By contrast, at high substrate concentration the waiting times between substrate turnovers show an asymmetric probability distribution. This implies that the limiting rate constant under these conditions—the catalytic turnover rate constant, $k_{cat}$—varies over time for an individual enzyme (**Fig. 1**). Although the molecular basis for such catalytic heterogeneity is unclear, Xie and co-workers propose that conformational isomers of the enzyme are the cause. The broad distribution of $k_{cat}$ values (referred to as $\chi^2$ in the context of a single-molecule observation) (**Fig. 1**) suggests that large numbers of such conformers with highly variable catalytic powers exist for a single enzyme, and interconvert only slowly (as compared to the catalytic turnover rate). Such slow interconversion is also referred to as a 'memory effect', in the sense that each enzyme molecule has a memory of its conformational state and retains it for some time longer than the turnover time; such single enzymes are described as showing dynamic disorder, indicating that they display various conformational states that are not static but slowly interconvert. From the autocorrelation function of the fluorescence bursts observed for single enzymes at high substrate concentration (that is, the correlation of the fluorescence time trace against a time-shifted version of itself), Xie and co-workers were able to extract time constants for these conformational isomerizations, which themselves show a broad distribution ranging from milliseconds to tens of seconds. The good agreement between this distribution and the known range of timescales of conformational fluctuations in proteins[3] further supports the notion that catalytic heterogeneity is caused by (dynamic) conformational heterogeneity.

What consequences does all this heterogeneity at the single-enzyme level have for the Michaelis-Menten formalism? The good news is that the Michaelis-Menten equation as a phenomenological description still holds. Yet our interpretation of the extracted $k_{cat}$ rate constant has to be significantly revised. More specifically, the $k_{cat}$ (or, formally, $\chi^2$) value derived at saturating substrate concentration turns out to be the weighted harmonic mean of the different catalytic turnover rate constants represented in the single enzyme over time. Consequently, the Michaelis constant

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