

paper could open a new era for truly living radical polymerization.

1. Matyjaszewski, K., and Tsarevsky, N.V. (2014). *J. Am. Chem. Soc.* **136**, 6513–6533.
2. Ouchi, M., Terashima, T., and Sawamoto, M. (2009). *Chem. Rev.* **109**, 4963–5050.
3. Moad, G., Rizzardo, E., and Thang, S.H. (2008). *Acc. Chem. Res.* **41**, 1133–1142.
4. Jenkins, A.D., Jones, R.G., and Moad, G. (2010). *Pure Appl. Chem.* **82**, 483–491.
5. Goto, A., and Fukuda, T. (2004). *Prog. Polym. Sci.* **29**, 329–385.
6. Chen, M., Zhong, M., and Johnson, J.A. (2016). *Chem. Rev.* **116**, 10167–10211.
7. Dadashi-Silab, S., Doran, S., and Yagci, Y. (2016). *Chem. Rev.* **116**, 10212–10275.
8. McKenzie, T.G., Fu, Q., Uchiyama, M., Satoh, K., Xu, J., Boyer, C., Kamigaito, M., and Qiao, G.G. (2016). *Adv. Sci.* **3**, 1500394.
9. Carmean, R.N., Becker, T.E., Sims, M.B., and Sumerlin, B.S. (2017). *Chem* **2**, this issue, 93–101.
10. Otsu, T. (2000). *J. Polym. Sci. A Polym. Chem.* **38**, 2121–2136.

Preview

Understanding the Binding of *cis* and *trans* Isomers of Combretastatin to Tubulin

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In this issue of *Chem*, collaborative efforts from the Cavalli and Steinmetz groups have resulted in a more cogent understanding of the binding of *cis*- and *trans*-combretastatin isomers to tubulin by means of structural and computational studies.

Combretastatin analog CA-4 and its derivatives show potent anticancer activity in the nanomolar range. It has been proposed that these compounds exert their activity by destabilizing microtubules as a result of their binding to the colchicine site of tubulins and thus inhibiting assembly and dynamics.¹

Designing a drug requires exploration of large regions of chemical space to uncover candidate molecules with the desired biological activity. Structure-based drug design is a robust process that requires knowledge of the actual 3D structure of a validated complex formed between a macromolecular target and a drug to elucidate the exact binding mode of the drug and, hence, design new and more efficient derivatives.² In the particular case of combretastatin analogs, even though important structure-activity in-

formation is known (i.e., the *cis*-CA-4 isomer is more potent than the *trans* isomer, indicating a different binding mode to tubulin), the lack of 3D structural information has handicapped both the full understanding of the interactions responsible for the activity and the rational design of new CA-4 analogs.

In this issue of *Chem*, the Cavalli and Steinmetz groups³ report a high-resolution crystal structure of the tubulin-*cis*-CA-4 complex and show that *cis*-CA-4 binds to the colchicine site of tubulin. This structure presents interactions both similar and unrelated to those of the well-known anti-tubulin agent colchicine. By comparing the tubulin-*cis*-CA-4 and tubulin structures (bound and unbound states), the authors found interactions similar to those reported between other colchicine-site ligands and both β - and

α -tubulin. They also compared *cis*-CA-4 and colchicine binding modes by superimposing both tubulin complexes and found that the equivalent ring A in both systems was buried deeper in the pocket in the case of *cis*-CA-4. Even though the authors do not mention it explicitly, this is probably simply because *cis*-CA-4 is slightly more elongated than colchicine (Figure 1). Thus, in order to fit into the pocket, cycle A has to move deeper into the pocket to displace a water molecule present in the tubulin-colchicine complex and involved in two intermolecular hydrogen bonds between the drug and target.

Much more interesting are the authors' findings about the rationale for the inhibitory activity of tubulin-*cis*-CA-4. Microtubules exist in cells in a dynamic equilibrium between their polymerized form and $\alpha\beta$ -tubulin dimers. They undergo cycles of elongation of the microtubule polymer (by adding $\alpha\beta$ -tubulin dimers) and catastrophe (by releasing $\alpha\beta$ -tubulin dimers). Thus, antitubulin drugs can act as stabilizers of microtubules (e.g., taxanes) or as microtubule-depolymerizing agents (e.g., vinka alkaloids). It is well known that whereas free tubulin has a *curved*

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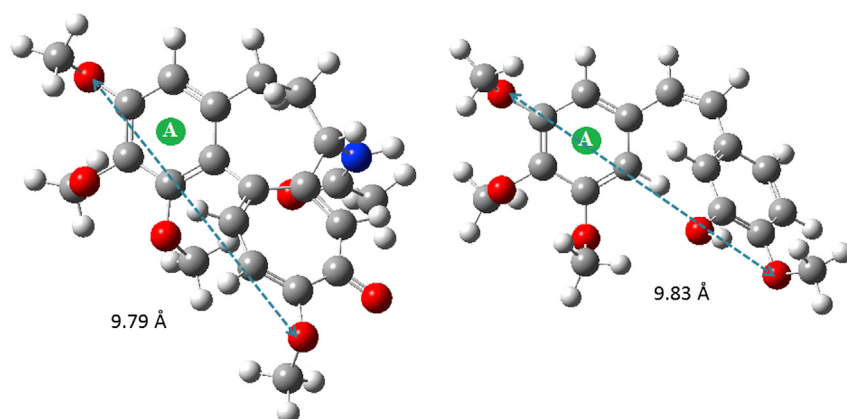


Figure 1. Structures of Colchicine, on the Left, and *cis*-CA-4, on the Right, Indicating the A Cycle and Approximate Length

structure, tubulin dimers in microtubules adopt a *straight* conformation.⁴ The results presented by Cavalli, Steinmetz, and colleagues suggest that *cis*-C4-A inhibits tubulin not because it binds to the colchicine site in microtubules but rather because it inhibits the tubulin transition from curved to straight. This is significant not only because it agrees with the hypothesis that combretastatin analogs inhibit tubulin polymerization but also because it has recently been reported that ERG expression confers resistance to taxane chemotherapy and enhances sensitivity to microtubule destabilizing drugs,⁵ and thus *cis*-C4-A analogs can be seen as potential therapies for treating ERG⁺ cancers.

The observed difference in activity between the *cis* and *trans* isomers of C4-A can be explained only by the less efficient binding to tubulin of the *trans* form. An elegant way to demonstrate this is by studying the conformational change (*cis* to *trans*) within the actual colchicine binding site of tubulin by starting with the crystal structure of the tubulin-*cis*-C4-A complex and using metadynamics. This particular computational tool, developed by Laio and Parrinello in 2002, enhances sampling in molecular-dynamics simulations and con-

structs the free-energy surface as a function of a few collective variables (CVs). Metadynamics has found many successful applications in several domains of science and strongly depends on the selection of the appropriate set of CVs.⁶ During the metadynamics simulation, the location of the system (molecule) in the space, as determined by the chosen CVs, is calculated, and a positive Gaussian potential is added to the actual energy of the system, disallowing it to return to the previous point. Throughout the simulation, more Gaussians add up, discouraging (even further) the system from moving back to its previous steps until the system explores the conformational space. When the modified free energy becomes a constant as a function of the CVs, the energy landscape can be recovered as the opposite of the sum of all Gaussians without an initial estimate of the energy.

In this particular case, the Cavalli and Steinmetz groups³ used metadynamics to explore the conformational space of the CA-4 derivative within the colchicine site as a function of two particular CVs responsible for the *cis-trans* isomerization: CV1 was defined as the rotation around the C=C bond, and CV2 was defined as the combination of the two C-C tor-

sions around the C=C bond. These CVs allowed exploration of the whole conformational space. The “poses” obtained for the *cis* isomer are in agreement with the crystal structure and indicate that *cis*-C4-A is able to occupy a wide space within the colchicine site of tubulin. On the contrary, the minima obtained for the *trans* conformation indicate the loss of key drug-target interactions.

Metadynamics studies provide important information on the structure of the optimal drug-target complexes: the poses. However, for an accurate assessment of binding energy, other approaches should be considered. Among those available, a computational tool widely used to provide information on the strength of drug-target interactions is to calculate relative binding free energies within a thermodynamic cycle. In the work highlighted here, Cavalli, Steinmetz, and colleagues carried out a thermodynamic cycle to evaluate the difference in binding free energy between the *cis*- and *trans*-C4-A isomers “unbound” and “bound” to tubulin. As the unbound state for both isomers, they used a solvation model with water, whereas the bound states were the tubulin complexes obtained from the metadynamics study. The resulting free-energy landscape indicates that tubulin stabilizes *cis*-CA-4 more than the *trans* conformation, suggesting that the tubulin-*cis*-CA-4 complex is more stable than the tubulin-*trans*-CA-4 one by 4.5 kcal·mol⁻¹. This energy difference is equivalent to a ~20,000-fold increase in tubulin-binding affinity and accounts for the experimental differential activity observed between *cis*-CA-4 and *trans*-CA-4.

This significant structural and computational study by the Cavalli and Steinmetz groups sheds light on the high microtubule-destabilizing activity observed for the *cis*-C4-A

combretastatin derivative as a result of its binding to the colchicine binding site of tubulin. Additionally, it explains the lower activity of *trans*-C4-A because its shape does not complement the binding pocket. More importantly, in having resolved this crystallographic structure, the authors provide an essential tool for the future structure-based

design of new combretastatin analogs as potential anticancer agents.

1. Dong, M., Liu, F., Zhou, H., Zhai, S., and Yan, B. (2016). *Molecules* 21, 1375.
2. Blundell, T.L. (1996). *Nature* 384 (6604, Suppl), 23–26.
3. Gaspari, R., Prota, A.E., Bargsten, K., Cavalli, A., and Steinmetz, M.O. (2017). *Chem* 2, this issue, 102–113.

4. Alushin, G.M., Lander, G.C., Kellogg, E.H., Zhang, R., Baker, D., and Nogales, E. (2014). *Cell* 157, 1117–1129.
5. Galletti, G., Matov, A., Beltran, H., Fontugne, J., Miguel Mosquera, J., Cheung, C., MacDonald, T.Y., Sung, M., O'Toole, S., Kench, J.G., et al. (2014). *Nat. Commun.* 5, 5548.
6. Barducci, A., Bonomi, M., and Parrinello, M. (2011). *WIREs Comput. Mol. Sci.* 1, 826–843.

Preview

Colloids Can “See” the Light

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Synthetic active matter is changing the way we think about and engineer new functional materials. In a recent work published in *Nature Nanotechnology*, Tang and coworkers engineered an army of microswimmers that mimic the collective phototactic behavior of algae in solution.

While looking through the lens of his microscope at the motion of pollen grains suspended in water, Robert Brown wrote in his lab journal, “These motions were such as to satisfy me, after frequently repeated observation, that they arose neither from currents in the fluid, nor from its gradual evaporation, but belonged to the particle itself.”¹ Brown was deeply fascinated by what appeared to him as tiny particles mysteriously animated by a perpetual source of energy. Today, despite our knowledge that these jittery movements are simply the manifestation of random collisions between colloidal particles and the molecules of the fluid they are suspended in, Brownian motion still inspires a great deal of modern scientific research.

Through Brownian motion, colloidal particles autonomously explore their environment and interact with each other by constantly probing the available energy landscape. The careful engineering of particle features such as shape and surface chemistry can aid

the rational design of this landscape to guide the self-organization of the particles into supracolloidal architectures and ultimately new functional materials.

There is, however, a fundamental difference between this special form of bottom-up microfabrication and the self-assembly processes that are responsible for the dynamical complexity of living organisms. In fact, whereas classical colloidal self-assembly is strictly regulated by the rules of equilibrium thermodynamics, living systems can harness flows of matter and energy to operate out of the equilibrium and perform complex functions such as self-propulsion, sensing, repairing, and even self-replication.

Over the past decade, a lot of research effort has been invested in characterizing and controlling colloidal matter outside of thermodynamic equilibrium with the intent of bringing such advanced functionalities to artificial systems.²

Energized by external fields^{3,4} or by the presence of a chemical fuel,⁵ new generations of active colloidal systems are rapidly evolving. The simplest examples of active colloids are the so-called microswimmers—particles (generally Janus-like spheres) equipped with catalytic “engines” capable of propelling them at speeds of a few micrometers per second. Other than self-propulsion, however, active colloids can exhibit a broad range of new functionalities from artificial rheotaxis⁶ to active cargo transportation.⁷ Most importantly, they are opening new and exciting self-assembly pathways that more closely resemble those observed in living systems.^{3,8}

Complex self-assembly in microswimmers is intimately linked to our ability to direct their motion. In their work published in *Nature Nanotechnology*,⁹ Tang and coworkers introduce a new class of artificial microswimmers whose unique structure and surface chemistry grant unprecedented guidance capabilities, whereby light is used for navigating particles through complex microenvironments.

Using some clever microfabrication, the authors first prepared a 2D array of

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