EXTENDED PDF FORMAT
SPONSORED BY
Travel Grants to the 15th International Congress of Immunology Apply Now!
www.rndsystems.com

As Good as Chocolate

Krzysztof Palczewski and Philip D. Kiser

Science **340**, 562 (2013);
DOI: 10.1126/science.1238521

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by clicking here.

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines here.

The following resources related to this article are available online at www.sciencemag.org (this information is current as of May 2, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:
http://www.sciencemag.org/content/340/6132/562.full.html

A list of selected additional articles on the Science Web sites **related to this article** can be found at:
http://www.sciencemag.org/content/340/6132/562.full.html#related

This article **cites 15 articles**, 7 of which can be accessed free:
http://www.sciencemag.org/content/340/6132/562.full.html#ref-list-1

This article appears in the following **subject collections:**
Biochemistry
http://www.sciencemag.org/cgi/collection/biochem

Downloaded from www.sciencemag.org on May 2, 2013

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. Copyright 2013 by the American Association for the Advancement of Science; all rights reserved. The title *Science* is a registered trademark of AAAS.

ces. Second, the three-fold symmetry of the building blocks is established by the central hub, which is made up of a three helix bundle to which the interaction helices are projected by means of a disulfide bond. Third, the wedge shape of the building blocks in cross section results from charge repulsion at the N-termini of the central hub and the interaction helices.

Coiled-coil binding between α-helices has been used in other assembling molecular systems. However, designs for nanoscale peptide assemblies often result in discrete structure in one or two dimensions but are polydisperse overall because they aggregate continuously in a third dimension. This lack of control over uniformity is often due to design principles that fail to take into account competing, but desired processes to limit assembly. For example, one design combines heterodimeric coiled coils with overhanging "sticky ends." These peptides assemble into extended fibers of variable length (*10*). The value of coiled-coil binding to Fletcher *et al*.'s design is underlined by the fact that a subtle single amino acid change at the molecular interface results in a shift to an alternate, but still discrete, structure (see the figure). Presumably the mutation enhances binding affinity between the building blocks, resulting in an increase in the rate of closure.

Establishing symmetry in building blocks, a strategy which is incorporated into Fletcher *et al*.'s second design rule, has previously been shown to be key for the generation of other closed, hollow nanostructures, albeit small ones, using designed proteins. For example, King *et al*. induced natively symmetric multicomponent proteins to form monodisperse, hollow protein cages by using the protein structures as hubs upon which protein-protein interactions between the hubs were grafted (*11*).

The wedge shape of Fletcher *et al*.'s building block is also an essential design rule. Not unlike the size of a lipid head group influencing the convex shape of a bilayer (*12*), or the angle of the keystone affecting the diameter of an architectural arch (*13*), the skew of the wedge shape defines the curvature of the resulting enclosed nanostructure. Equally important, the wedge shape provides a means to limit the assembly as it forms. The other two design rules allow symmetric assembly but could result in the generation of extended sheets of undefined size. Curving these sheets increases the likelihood that the high-energy edges of the sheets meet, thus generating closed and highly monodisperse structures.

The three simple design rules defining the interactions in Fletcher *et al*.'s system result in the formation of a robustly controlled morphology (see the figure). Because the system is so precisely defined from the molecular to the nanoscale level, it provides easily testable mechanistic hypotheses and predictions for the controlled manipulation of the rules to generate new morphologies (*14*). It could provide a robust model system to understand how nanoscale properties emerge from molecular components. Moreover, the structures could be used for protein delivery or as biomimetic mini-organelles to sequester and transport enzymatic activity.

References

1. S. Johnson, *Emergence: The Connected Lives of Ants, Brains, Cities, and Software* (Scribner, New York, 2001).
2. J. M. Fletcher *et al*., *Science* **340**, 595 (2013); 10.1126/science.1233936.
3. E. H. C. Bromley *et al*., *ACS Chem. Biol.* **3**, 38 (2008).
4. W. C. Ruder, T. Lu, J. J. Collins, *Science* **333**, 1248 (2011).
5. E. Winfree, F. Liu, L. A. Wenzler, N. C. Seeman, *Nature* **394**, 539 (1998).
6. C. J. Reedy, B. R. Gibney, *Chem. Rev.* **104**, 617 (2004).
7. J. Kim *et al*., *J. Am. Chem. Soc.* **134**, 20573 (2012).
8. M. R. Ghadiri, J. R. Granja, R. A. Milligan, D. E. McRee, N. Khazanovich, *Nature* **366**, 324 (1993).
9. E. K. O'Shea, R. Rutkowski, P. S. Kim, *Science* **243**, 538 (1989).
10. M. J. Pandya *et al*., *Biochemistry* **39**, 8728 (2000).
11. N. P. King *et al*., *Science* **336**, 1171 (2012).
12. J. N. Israelachvili, D. J. Mitchell, B. W. Ninham, *Faraday Transac.* **2**, 1525 (1976).
13. M. S. Ardejani, X. L. Chok, C. J. Foo, B. P. Orner, *Chem. Commun.* **49**, 3528 (2013).
14. C. Tarabout *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **108**, 7679 (2011).

10.1126/science.1237708

BIOCHEMISTRY

As Good as Chocolate

Structural details of how ligands bind to serotonin receptors should guide the development of pharmaceuticals with fewer side effects.

Krzysztof Palczewski and Philip D. Kiser

No one could have imagined how important the 1948 discovery of the vasoconstrictor serotonin (5-hydroxytryptamine or 5-HT) would be to the field of human physiology (*1*). Elucidation of the 5-HT structure (*2*) and synthesis of the molecule with the expected biological activity (*3*) soon followed. This monoamine is a ligand for 15 receptors, and drugs that target 5-HT receptors are widely used to treat conditions including migraine headache, depression, anxiety, nausea, vomiting, and irritable bowel syndrome, reflecting the wide diversity of physiological and pathophysiological processes in which 5-HT is involved (*4*). On page 615 and 610 in this issue, Wacker *et al*. (*5*) and Wang *et al*. (*6*), respectively, report the crystal structure of human 5-HT_{2B} bound to the antimigraine agent ergotamine and compare it with the 5-HT_{1B}-ergotamine structure. Together with biochemical and computational data, these structures reveal molecular mechanisms responsible for divergent signaling patterns of ergotamine, serotonin, and the psychedelic drug lysergic acid diethylamide (LSD).

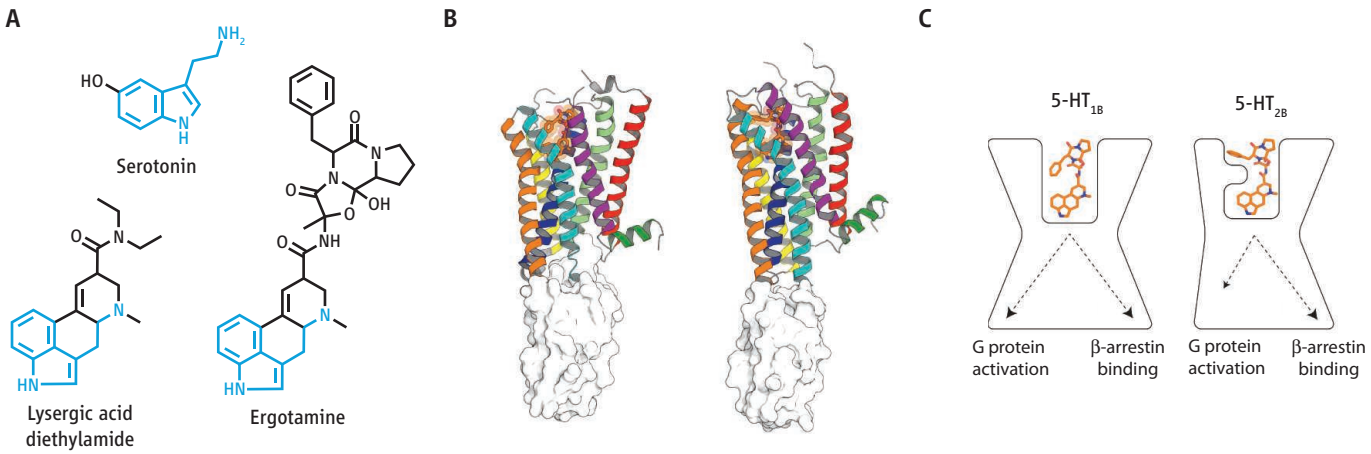
The structures were obtained by fusing either receptor to a thermally stabilized bacterial protein [apocytochrome b562RIL (BRIL)]. This approach stabilizes the receptor to promote crystallization but does not alter ligand-binding properties. The structural information, together with computational ligand-docking experiments, reveal similar binding modes for ergotamine, 5-HT, and LSD to the ligand-binding pocket formed by residues conserved in the 5-HT receptor family, thereby clarifying the family-wide agonist activity of 5-HT. However, there are some key differences between the two receptors (see the figure). In both structures, an accessory binding pocket adjacent to the binding site for the natural ligand (5-HT) can accommodate chemical groups located distal to the core indoleamine moiety in a differential manner, which possibly could control signaling. The 5-HT_{1B} receptor displays a 3 Å outward shift at the extracellular end of helix V relative to the 5-HT_{2B} receptor, resulting in a more open, extended pocket that explains receptor subtype selectivity for ligands.

5-HT receptor subtypes are classified according to their ligand-binding preferences, sequence homology, and signaling mechanisms. With the exception of the type 3 recep-

Department of Pharmacology, School of Medicine, Case Western Reserve University, 2109 Adelbert Road, Cleveland, OH 44106–4965, USA. E-mail: kxp65@case.edu; pdk7@case.edu

Downloaded from www.sciencemag.org on May 2, 2013

Published by AAAS



Uncovering bias in serotonin receptor signaling. (**A**) Natural (serotonin/5-HT) and synthetic (ergotamine and LSD) serotonin receptor ligands have a common indole amine structure (blue) at their core. (**B**) Crystallographic structures of the human 5-HT_{1B} (left) and 5-HT_{2B} (right) receptors in a complex with ergotamine (orange). The BRIL fusion protein used to facilitate crystallization is shown as a white surface. (**C**) Ergotamine stabilizes distinct conformations in the two 5-HT receptors, providing a structural explanation for the biochemically observed phenomenon of biased signaling.

tor, which is a ligand-gated cation channel, all 5-HT receptors are seven-transmembrane receptors belonging to the rhodopsin-like class A group that transmit signals to heterotrimeric GTP-binding proteins (G proteins) and other effectors like β-arrestins (*7*). Many 5-HT receptors display a close evolutionary relationship with receptors for other biogenic amines (such as the neurotransmitters dopamine and norepinephrine). This, along with structural similarities among the biogenic amines themselves, explains why drugs that target specific 5-HT receptors are especially prone to produce untoward side effects through "off target" receptors. One example is the severe vasoconstriction that can result from supratherapeutic doses of ergot alkaloids, which are thought to exert their beneficial antimigraine effects through 5-HT_{1B}, and 5-HT_{1D} receptors but also can act on α-adrenergic receptors with dangerous consequences (*8*). Another prominent example is the fibrotic cardiac valvulopathy (thickening of heart valves) associated with a metabolite of fenfluramine that can act on the 5-HT_{2B} receptor. Fenfluramine is a component of the notorious and now defunct anorexogenic drug combination Fen-Phen (*9*), whose recall was the largest in U.S. history (*10*).

Rational drug design, in which the known structure of an endogenous agonist of interest is used as a basis to generate derivatives with potential selectivity for a subset of receptors, has played an important role in developing more selective 5-HT receptor–targeting drugs with improved side-effect profiles (*11*). A prime example is the 5-HT_1–selective "triptans," which are the drug class of choice for treating migraine headache. The 5-HT receptor structures will guide the tailoring of candidate drug molecules to bind selectively to particular receptor subtypes. Differences in the ligand-binding pockets of the 5-HT_{1B} and 5-HT_{2B} receptors could, for example, be exploited to eliminate agonist effects at the 5-HT_{2B} site that are associated with cardiotoxicity.

At first glance, structures of these receptors appear highly similar to that of rhodopsin, the first G protein–coupled receptor (GPCR) whose structure was solved by x-ray crystallography (*12*). Indeed, pairwise root mean square deviations (RMSDs) between the 5-HT receptors and rhodopsin are ~2.3 to 2.7 Å among the core of ~260 Cα positions (or ~80% of the receptor sequence), demonstrating a structural triumph whereby the same overall GPCR topology is maintained despite markedly different amino acid sequences (*13*). However, the differential ligand- and effector-binding specificity of the structures provides an important pharmacological story. Wacker *et al*. and Wang *et al*. could determine the 5-HT_{1B} and 5-HT_{2B} receptors, respectively, in the presence of the same ligand. By comparing the two structures, they noted differences in the intracellular region that interacts with G proteins or arrestins. Both receptors show biased signaling, in which agonists preferentially activate one pathway over the other. The comparison revealed that ergotamine biases the signaling of 5-HT_{2B} through β-arrestin by inducing conformational changes within the cytoplasmic portion of the receptor that are distinct from changes observed in the 5-HT_{1B} structure that enable coupling to G proteins. Such structural insights can open a whole new avenue of investigation of ligand-induced differential signaling.

The serotonin receptor family is like the mythical Roman god Janus, often depicted with two faces pointed in opposite directions. These receptors can be dangerous, like 5-HT_{2B}, which is often referred to as a death receptor because of its cardiotoxic effects. On the other hand, chocolate, which contains high concentrations of the serotonin precursor tryptophan and serotonin-like monoamines, elicits cravings through these same receptors and brings much pleasure to our lives (*14*). Structural information about GPCRs continues to have important implications for developing new pharmaceuticals (*15*). We can look forward to the design of noncardiotoxic 5-HT receptor ligands that will make the outcome of stimulating these receptors as good as chocolate.

References and Notes

1. M. M. Rapport, A. A. Green, I. H. Page, *Science* **108**, 329 (1948).
2. V. Erspamer, B. Asero, *Nature* **169**, 800 (1952).
3. G. Reid, M. Rand, *Nature* **169**, 801 (1952).
4. M. Berger *et al.*, *Annu. Rev. Med.* **60**, 355 (2009).
5. D. Wacker *et al.*, *Science* **340**, 615 (2013); 10.1126/science.1232808.
6. C. Wang *et al.*, *Science* **340**, 610 (2013); 10.1126/science.1232807.
7. B. L. Roth, *Neuropharmacology* **61**, 348 (2011).
8. W. J. Meyler, *Cephalalgia* **16**, 5 (1996).
9. D. R. Boughner, *Can. Med. Assoc. J.* **157**, 705 (1997).
10. G. Cowley, K. Springen, *Newsweek* **130**, 46 (1997).
11. P. P. Humphrey, *Headache* **47** (suppl. 1), S10 (2007).
12. K. Palczewski *et al.*, *Science* **289**, 739 (2000).
13. T. Mirzadegan *et al.*, *Biochemistry* **42**, 2759 (2003).
14. K. Bruinsma, D. L. Taren, *J. Am. Diet. Assoc.* **99**, 1249 (1999).
15. J. A. Salon, D. T. Lodowski, K. Palczewski, *Pharmacol. Rev.* **63**, 901 (2011).

Acknowledgments: This work was supported by NIH grant EY008061.

10.1126/science.1238521

CREDIT: C. BICKEL/*SCIENCE*

Downloaded from www.sciencemag.org on May 2, 2013