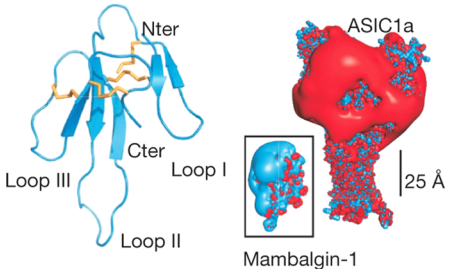
ACS chemical biology

Spotlight

pubs.acs.org/acschemicalbiology

## TAKING A BITE OUT OF PAIN

A bite from a poisonous animal would usually be associated with extreme pain, but a recent screen to identify inhibitors of one pain receptor turned up a fascinating contrast. The black mamba, the legendary venomous snake from Africa actually injects painkilling peptides with its deadly bite.

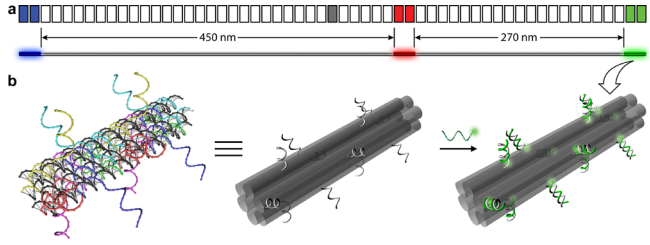



Reprinted by permission from Macmillan Publishers Ltd: *Nature*, Diochot, S., *et al.*, *490*, 552−555, copyright 2012.

Diochot *et al.* (*Nature* 2012, *490*, 552−555) initially went on the hunt for blockers of acid-sensing ion channels (ASICs), key pain receptors in the central and peripheral nervous system. When they uncovered mamba venom as a potent blocker, they fractionated the snake's venom to identify two active peptides and eventually clone the two genes. Both peptides had a potent inhibitory effect on ASICs channel gating both in a heterologous COS cell system or in mouse spinal or sensory neurons. They named the 57 amino acid peptides, mambalgins, and determined that they fold into a triple stranded structure stabilized by 4 disulfide bonds. This type of fold places mambalgins in a family with other three-finger toxins alongside a cohort of neurotoxic peptides. Instead of killing neurons, mambalgins showed potent analgesic effects in mice, at times displaying similar strength to the opiate, morphine. The peptides could quell both acute and inflammation-induced pain. A drug to block opiate analgesia, naloxone did not inhibit mambalgin's effects, indicative of a separate pathway. In addition, the knockout mouse for ASIC1a confirmed that the mambalgin works through binding that receptor in a reversible manner to alter acute pain reception. The mambalgins also invoked a central analgesia, indicating that ASIC2 protein might be involved as well. The knockdown experiment directed toward this latter gene indicated that its protein is also blocked by mambalgin binding. Many important tools for neurobiology have been uncovered through isolating the active agents in natural toxins and venoms. This study represents another step forward in that arena with a new drug for studying the biology of ASICs and pain. This study also indicates a possible direction for medicine, designing the next generation of pain management drugs with the help of a poisonous snake from Africa. **Jason G. Underwood, Ph.D.**

## BUILDING BETTER BARCODES

Although fluorescence imaging gives biologists a powerful way to observe chemical interactions, one limitation has been the number of fluorophores that researchers can track simultaneously. Now Lin *et al.* (*Nat. Chem.* 2012, *4*, 832−839) present a new technique based on DNA nanorods that can produce up to 216 distinct barcodes that researchers can observe simultaneously.




Reprinted by permission from Macmillan Publishers Ltd: *Nat. Chem.*, Lin, C., *et al.*, *4*, 832−839, copyright 2012.

These new barcodes self-assemble by "folding" a long single-stranded DNA molecule using more than 200 short oligonucleotides. To make distinguishable combinations, the researchers placed three attachment points for red, green and blue fluorescent dyes separated by 450 and 270 nm on the DNA strands. The spacing produces three distinct spots: one set apart, and two grouped more closely together. That initial step produces 27 different barcodes. Lin et al initially tested 5 individual barcodes to validate their structure and geometry. They then examined mixtures, first of 2 barcodes and then all of the initial 27. To make 216 combinations, Lin et al also used combinations of 2 fluorophores at each of the three spots to produce cyan, pink, and yellow and validated those structures.

The researchers also demonstrated variations of these barcodes for super-resolution microscopy and more complex barcodes that arrange the fluorescent dots in a three-pointed star pattern. Finally, they demonstrated how these barcodes could be used to label cells in situ. Lin et al labeled barcodes with biotin molecules and those structures specifically bound to yeast cells coated with an antibody linked to streptavidin.

In addition to presenting a greater number of tagging combinations, these molecules are smaller than previous barcodes: 400−800 nm in diameter. Rather than using enzymatic reactions or other interventions, these barcodes self-assemble. The stiffness of the nanorods and the ability to generate super-resolution probes could offer the potential for thousands of distinct labels. However, particularly when imaging cells, researchers will need to overcome additional technical challenges, such as the possibility that barcodes could aggregate on cell surfaces. **Sarah A. Webb, Ph.D.**

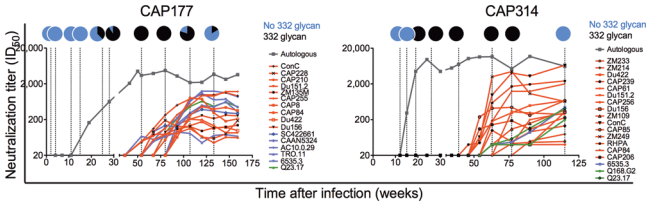
## THE SWEET PATH OF ESCAPE

Since its discovery, HIV has infected over 60 million people, and more than 20 million have died from AIDS. The disturbing ability of the virus to evade the immune response has made development of vaccines targeting HIV particularly challenging. Intriguingly, however, after many years of infection a small number of individuals naturally develop powerful types of antibodies called broadly cross-neutralizing (BCN) antibodies, which can inactivate viral activity. Now, Moore *et al.* (*Nat. Med.* advance online publication October 21, 2012; DOI: 10.1038/nm.2985) uncover a method by which HIV-1 subtype C evades

**Published:** November 16, 2012

   dx.doi.org/10.1021/cb300591c | *ACS Chem. Biol.* 2012, 7, 1770−1772

the body's initial immune response and inadvertently promotes development of BCN antibodies as a result.

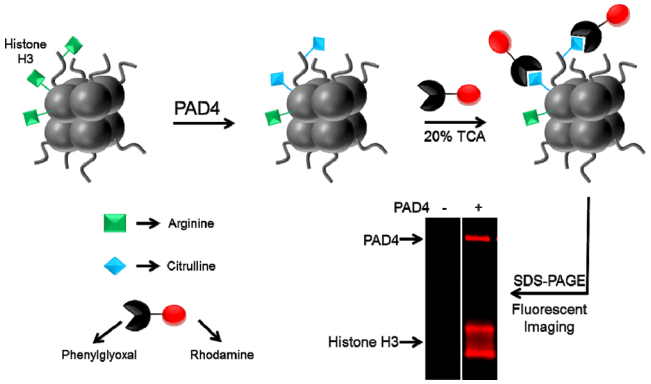



Reprinted by permission from Macmillan Publishers Ltd: *Nat. Med.*, advance online publication 21 Oct 2012, DOI: 10.1038/nm.2985.

The C3 region of HIV-1 subtype C is highly immunogenic and contains several glycosylation sites, including the BCN epitope at position 332. The authors examined viruses from two infected individuals who developed BCN antibodies. They found that though the virus responsible for the initial infection lacked the glycan at position 332, it was present 6 months later. The appearance of glycan 332 coincided with the development of BCN antibodies as well as the loss of a neighboring glycan at position 334. Ironically, after 2 years of infection, glycan 332 again disappeared, replaced by a glycan at position 334. This shift was likely an attempt to circumvent the BCN antibodies. The authors hypothesize, using large sequence and neutralization data sets, that the initial absence of the glycan at position 332 may have conferred an advantage to the virus in the early stages of infection, though pressure from the immune system then nudged the glycan over to the highly conserved position 332, which is susceptible to targeting by BCN antibodies. Delineation of this unusual mechanism of immune evasion by HIV is a valuable contribution toward the development of vaccines targeting this elusive virus. **Eva J. Gordon, Ph.D.**

## CHARACTERIZING CITRULLINATION

Arginine residues in proteins can be posttranslationally modified to the amino acid citrulline, in which the side chain guanidinium group is converted to a urea moiety. Enzymes called protein arginine deiminases are responsible for this transformation, which is increased in numerous human diseases including rheumatoid arthritis, ulcerative colitis, and cancer. However, the role of protein citrullination in normal and pathological processes is not well characterized, in part due to the lack of molecular tools tailored toward its investigation. Now, Bicker *et al.* (*J. Am. Chem. Soc.* 2012, *134*, 17015−17018) report the development of a small molecule probe uniquely designed to detect citrulline.

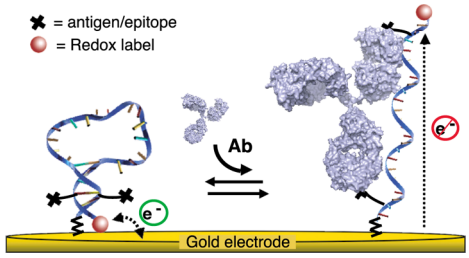



Reprinted with permission from Bicker, K. L., *et al.*, *J. Am. Chem. Soc.*, *134*, 17015−17018. Copyright 2012 American Chemical Society.

The citrulline-specific chemical probe exploits the pH-dependent reaction of glyoxals with either arginine, under basic conditions, or citrulline, under acidic conditions. By conjugating a glyoxal-derivative to a rhodamine molecule, citrulline could be readily detected using standard biochemical techniques. Using the autodeimination of protein deiminase 4 as a test case, the authors demonstrate that the probe is comparable to other commercially available citrullation detection agents in terms of sensitivity, but is quicker and simpler to execute. They also show that they can use the probe to investigate the kinetics of citrullination, visualize changes in citrullination, and detect citrullinated proteins for potential diagnostic applications. Using a mouse model of ulcerative colitis, they were able to detect a decrease in the citrullination of specific proteins in the presence of a protein arginine deiminase inhibitor. The ability of this novel probe to interrogate protein citrullination in complex biological environments points to its potential for delineating the role of citrullination in various pathological processes. **Eva J. Gordon, Ph.D.**

## SWITCHING UP DIAGNOSTICS

The ability to detect the presence of antibodies directly in whole blood could revolutionize the field of diagnostics. While current methods for antibody detection can be highly sensitive and selective, they often require multiple steps, sophisticated instrumentation, and trained technicians. These issues delay diagnosis and limit application of these methods in developing regions. Now, Vallée-Bélisle *et al.* (*J. Am. Chem. Soc.* 2012, *134*, 15197−15200) report the development of a DNA-based electrochemical "switch-based" sensor for the rapid, simple, and quantitative detection of antibodies in whole blood samples.




Reprinted with permission from Vallée-Bélisle, A., *et al.*, *J. Am. Chem. Soc.*, *134*, 15197−15200. Copyright 2012 American Chemical Society.

The sensor is composed of either a single- or double-stranded DNA displaying two strategically positioned identical antigens and also containing methylene blue at one end, which serves as a redox label, and a thiol group at the other end, which enables attachment to a gold electrode surface. In the absence of antibody, the DNA adopts a folded conformation that promotes rapid electron transfer. However, antibody binding causes a significant conformational change, forcing the DNA to extend in order to accommodate the binding of both antigens to a single antibody. This elongation, in turn, causes the electron transfer rate to decrease, producing an easily measured change in current when interrogated using square wave voltammetry. Having the detection signal reliant on this conformational change, rather than the actual binding event, renders the system much less sensitive to nonspecific binding than other single-step antibody detection methods. To demonstrate the utility of this approach, several sensors were generated to respond to antibodies to various well-characterized antigens including 2,4-dinitrophenol, digoxigenin, and an epitope from the HIV-1 protein gp41. Indeed, even in the presence of low nanomolar amounts of antibodies, change in


dx.doi.org/10.1021/cb300591c | *ACS Chem. Biol.* 2012, 7, 1770−1772

current indicating the presence of antibody was readily observed in buffer as well as in whole blood. This highly innovative approach has potential to be adapted to point-of-care diagnostics in a global setting. **Eva J. Gordon, Ph.D.**


dx.doi.org/10.1021/cb300591c | *ACS Chem. Biol.* 2012, 7, 1770−1772