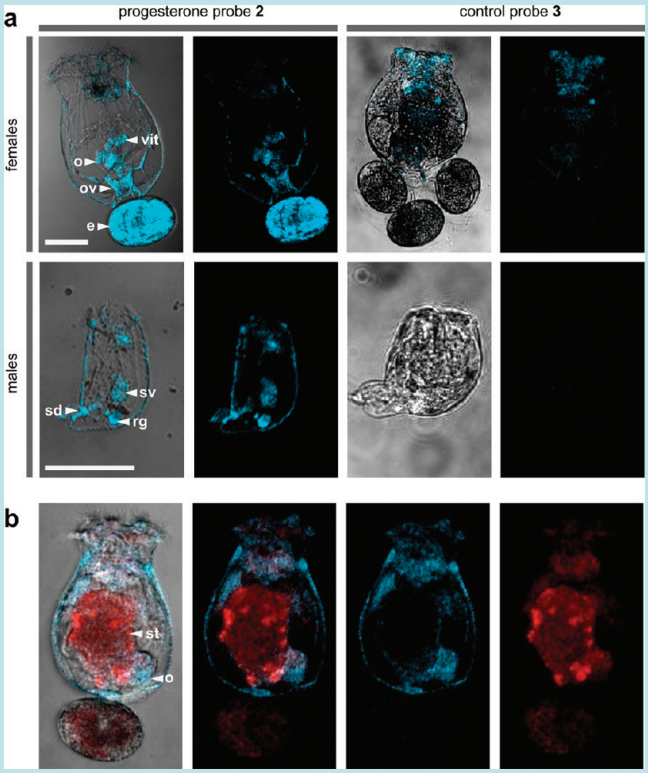
Spotlight

Sex and Progesterone in Invertebrates



Stout, E. P., *et al., Proc. Natl. Acad. Sci. U.S.A.*, DOI: 10.1073/pnas.1006074107. Copyright 2010 National Academy of Sciences, U.S.A.

Not much is known about the sex life of monogonont rotifers, which are submillimeter-sized, nonarthropod invertebrates that generally reside in freshwater environments. However, it is known that these tiny animals can reproduce both sexually and asexually, and recent evidence has implicated the steroid hormone system in the switch from asexual to sexual reproduction. Now, using an impressive suite of chemical and biological methods, Stout *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2010, published online June 14, 2010, DOI: 10.1073/pnas.1006074107) reveal the presence of progesterone and a progesterone receptor in the monogonont rotifer *Brachionus manjavacas*.

Examination of the *B. manjavacas* genome led to the identification of a gene sequence with homology to numerous membrane-associated progesterone receptors. To help find and characterize this receptor in the rotifer, two progesterone-based molecular probes were designed and synthesized. First, use of a fluorescent progesterone derivative demonstrated the presence of a progesterone binding molecule in the reproductive organs of both male and female rotifers. Next, along with an antibody against the fluorescent progesterone, the probe was used to help fish out progesterone-binding proteins from crude rotifer lysates. Immunoprecipitation experiments with the fluorescent progesterone probe, as well as use of a chemically reactive progesterone-derived probe designed to become covalently attached to the receptor active site, led to the identification of a protein that selectively bound to the progesterone probe. Mass spectrometry analysis confirmed that peptides derived from the protein indeed exhibited strong homology with other progesterone receptors. Finally, RNA interference experiments also confirmed the presence of the putative progesterone receptor, and demonstrated that inhibition of this gene led to a decrease in induction of sexuality in first-generation daughters of female rotifers. These findings strongly implicate progesterone in the regulation of the rotifer reproductive system and also offer clues into the evolutionary history of progesterone and the sex steroid system enjoyed by higher animals. **Eva J. Gordon, Ph.D.**

Next Generation Antibodies? Plastics

Antibodies exhibit remarkable affinity and specificity for their target molecules, making them extraordinarily good at their job of ridding the body of infectious or toxic agents. Much progress had been made in the effort to create synthetic antibodies that target a specific antigen, but few methods have yielded products that rival the efficacy of natural antibodies in a real biological setting. Now Hoshino *et al.* (*J. Am. Chem. Soc.* 2010, *132*, 6644−6645) use a molecular imprinting approach in the design of a "plastic antibody" capable of targeting and clearing an antigen from the bloodstream of live mice.

The plastic antibody is composed of acrylamide-derived nanoparticle polymers that have been designed to bind melittin, the toxin in bee venom. To create polymeric nanoparticles specific for the toxin, libraries of nanoparticle monomers were screened for melittin binding, and the composition of the nanoparticle was repeatedly adjusted to enhance the specificity of the interaction. Polymerization of the nanoparticles in the presence of melittin, fol-

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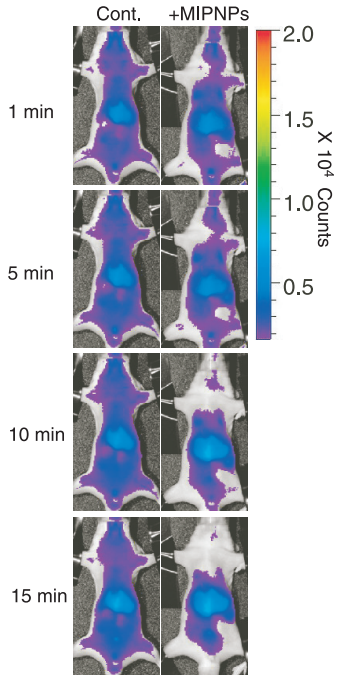

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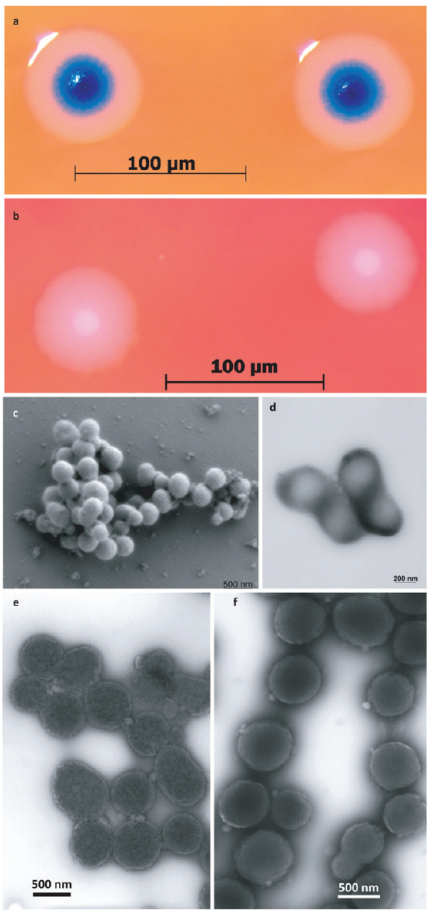
lowed by its removal by dialysis, yielded an antibody-sized polymeric nanoparticle with high affinity and specificity for melittin. To explore the effects of the plastic antibodies in a biological setting, it was first demonstrated that the polymeric nanoparticles themselves were not toxic in cultured cell lines or in live mice. Next, it was shown that approximately 60% of mice exposed to both melittin and the plastic antibodies survived, whereas in the absence of polymeric nanoparticles, all mice injected with melittin died. Fluorescent or radiolabeled nanoparticles were also created, enabling the visualization of the biodistribution of the compounds in the mice as well as their accumulation in the liver. Thus, the plastic antibodies appear to target and remove melittin from the bloodstream before being transported to the liver for destruction. This clever technology holds promise for the design of plastic antibodies targeting other molecules as well. **Eva J. Gordon, Ph.D.**

Life from a Digital File

The invention in the early 1980s of machines that could chemically synthesize DNA oligonucleotides in an automated fashion, coupled with revolutionary advances in DNA sequencing that have enabled the rapid and affordable decoding of the entire genomes of numerous organisms, has paved the way for an explosion of applications in fields such as molecular biology and genetic engineering. Surrounding these tremendous advances in our understanding of genomics over the last few decades, one question at the forefront is can we create a living, functioning organism using entirely synthetic DNA? Now, Gibson *et al.* (*Science*, 2010, published online May 20, 2010, DOI: 10.1126/science.1190719) report the creation of a viable bacterial cell under the control of a genome made up exclusively of synthetic DNA.



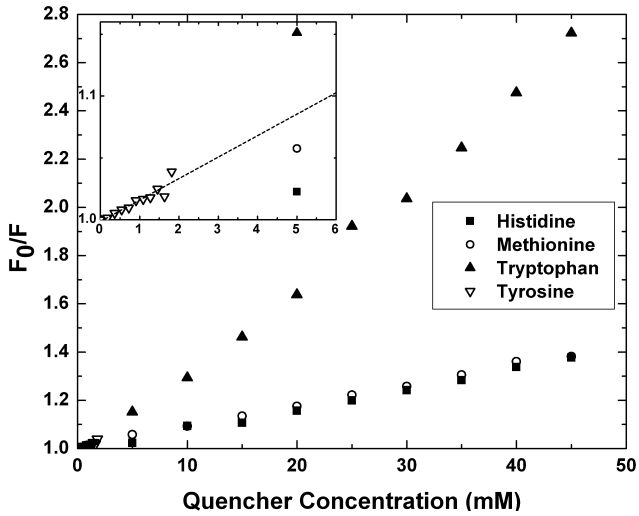
From Gibson, D. G., *et al.*, *Science*, May 20, 2010, DOI: 10.1126/science.1190719. Reprinted with permission from AAAS.

The relatively small complement of genes required for independent growth in cell culture made certain species of mycoplasma bacteria an appealing choice for the transplantation and expression of a synthetic genome. The genome from *Mycoplasma mycoides* was selected as the donor genome, and *Mycoplasma capricolum* was chosen as the recipient host cell. Beginning just with the approximately 1 million base-pair *M. mycoides* genome sequence in digitized form, its reconstruction using synthetic DNA was undertaken. First, 1078 1kb DNA cassettes were synthesized. These cassettes were then assembled into 111 10-kb synthetic intermediates, which were subsequently assembled into 11 100-kb synthetic intermediates, which were finally assembled into the complete genome. Importantly, the synthetic DNA contained watermark sequences in four

distinct locations so that it could be clearly distinguished from the natural genome. Once the assembly process was complete, the genome was transplanted into a *M. capricolum* host cell whose own DNA had been removed. Remarkably, cells with the transplanted DNA formed colonies with the expected phenotypic properties. This achievement is an exciting proof of principle that designed genomes can be synthesized and inserted into cells to create viable organisms. **Eva J. Gordon, Ph.D.**

The Quest for Quenching Mechanisms

Labeling of proteins with small fluorescent molecules has become a central tool for investigating protein function. While the activity of labeled proteins is often examined to ensure that the presence of the label does not perturb protein activity, rarely is the consequence of protein attachment on the behavior of the fluorophore investigated. Now, Chen *et al.* (*J. Am. Chem. Soc.* 2010, *132*, 7244−7245) probe the effect that protein labeling may have on the fluorescence properties of the Alexa Fluor dyes, which are commonly used to label proteins.



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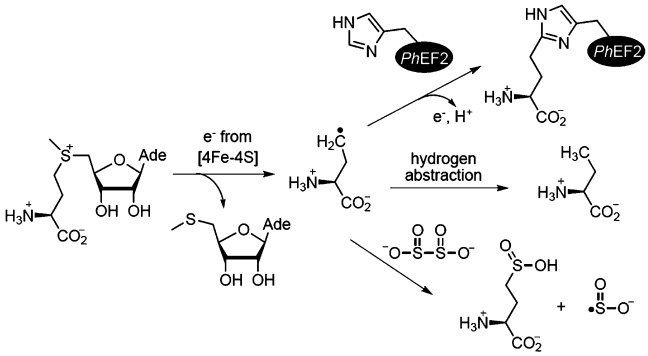
The fluorescence emission properties and excitation lifetimes were examined for three dyes, Alexa 488, 555, and 594, in the presence and absence of each of the 20 naturally occurring L-amino acids. All three dyes were quenched by tryptophan, and Alexa 488 was also quenched by tyrosine, histidine, and methionine. Fluorescence quenching generally occurs through either dynamic or static mechanisms. Dynamic quenching results from molecular collisions between the fluorophore and the quencher during the excited state of the fluorophore; static quenching occurs when a nonfluorescent complex forms between the two molecules. By analyzing the fluorescence intensities and lifetimes in the absence and presence of the quencher, it was possible to determine which of these mechanisms was responsible for the quenching of Alexa 488 by the four amino acids. While quenching by tryptophan and histidine appeared to have both static and dynamic properties, quenching by tyrosine and methionine occurred predominantly through a dynamic mechanism. This study reveals the potential effects of proteins on the fluorescence properties of molecular dyes and exposes the key amino acids capable of quenching the dyes. These results facilitate more effective design of labeling sites and offer a warning for careful interpretation of quantitative fluorescence intensity data when such amino acids are in the vicinity of the fluorophore. **Eva J. Gordon, Ph.D.**

A Pathogen'S Radical Tagging Strategy

Corynebacterium diphtheriae takes a two-pronged approach in causing infectious disease. The bacterium secretes a protein complex that tags a critical modified histidine in the eukaryotic translation elongation factor 2 (EF2). That unique post-translational modification marks a bulls-eye on EF2, a critical protein for ribosomal protein synthesis, and the diphtheria toxin then zeroes in on that target.



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The histidine modification, known as diphthamide, requires the addition of a 3-amino-3-carboxypropyl (ACP) group from *S*-adenosyl-L-methionine (SAM) to a carbon of the imidazole ring in its first step. The chemistry is unusual: post-translational modifications rarely involve carbon−carbon bond formation, and SAM typically donates methyl groups in enzymatic reactions. The process also requires four different proteins. Researchers have struggled to tease out the underlying chemical mechanism.

But now Zhang *et al.* (*Nature*, 2010, *465*, 891−896) present compelling evidence for a radical reaction facilitated by an iron−sulfur enzyme. Their first clue to an oxygen-sensitive reaction center came from their inability to reconstitute enzymatic activity under aerobic conditions, even with all four required proteins. An X-ray crystal

structure of *Pyrococcus horikoshii* Dph2(PhDph2) showed a cluster of three conserved cysteine residues suggesting that the protein may bind an iron−sulfur cluster. Absorbance, EPR and Mössbauer spectra confirmed a $[4Fe\text{-}4S]^{+}$ cluster.

When purified anaerobically, this single protein severs the critical carbon−sulfur bond within SAM, to form methylthioadenosine *in vitro*. If the *P. horikoshii* EF2 substrate is not present, the ACP portion is trapped as 2-aminobutyric acid or homocysteine sulphinic acid, two products that would be formed via a radical intermediate.

With these findings, the researchers have described a new type of iron−sulfur cluster enzyme. The enzyme with the closest structural similarities, quinolinate synthase, uses its iron−sulfur cluster to carry dehydration chemistry without the SAM cofactor. The unusual SAM chemistry suggests a different orientation of the cofactor within the active site. But further studies will be needed to understand the involvement of the other critical proteins and other details of this novel biochemistry. **Sarah A. Webb, Ph.D.**

The Tarantula Toxin with Two Fangs

Natural toxins from venomous creatures act on a diverse set of cellular targets ranging from the essential molecular machines that make RNA or protein to the array of receptors and channels that keep cells alive and responsive to their environment. As such, some of nature's nastiest toxins have played critical roles in our understanding of basic cell biology and physiology. One family of agents, the inhibitory cysteine knot (ICK) peptides, share a common disulfide architecture but rather divergent sequences and mechanisms of action. Now, scientists investigating one tarantula's painful venom have uncovered how gene duplication during evolution can result in an ICK peptide of nearly irreversible potency.

Bohlen *et al.* (*Cell* 2010, *141*, 834−845) named the new peptide double-knot toxin, or DkTx, because of its remarkable architecture of two separate ICK domains connected by a tethering linker sequence. In contrast to other ICK toxins from scorpions or tarantulas that target potassium channels, DkTx binds with high affinity to the capsaicin and heat-sensitive receptor, TRPV1, the same receptor that communicates the "heat" of spicy chili peppers. But unlike the reversible nature of a spicy stimulus, the authors showed that treatment of cultured mouse neurons with DkTx resulted in stimulation that could not be washed out with fresh media. Taking apart this essentially irreversible toxin into two ICK domains still retained TRPV1 activation but lacked the extreme potency inherent to the double-knot structure.

In an impressive display of comparative genomics, the authors went on to find key residues in TRPV1 that mediate sensitivity to DkTx. The frog TRPV ortholog responded to capsaicin and extracellular protons but not to DkTx, so a series of chimeric receptors with a rodent TRPV1 receptor helped narrow down the search. Honing in on fine differences uncovered one extracellular amino acid, alanine 657, that is key to DkTx efficacy. In fact, changing just the frog proline residue at that position to an alanine conferred the frog receptor DkTx-sensitive. Further mutagenesis experiments showed the footprint of DkTx on mammalian TRPV1 receptors. The peptide binds one side of the extracellular pore domain of the TRPV1, but because of its bivalent structure, it probably binds to two adjacent sites at the same time, thus explaining its irreversible action. Binding keeps the pore open, leading to continued painful stimulus since the cell cannot reset to its proper potential. Because of its irreversible nature, DkTx should be an excellent gift from an 8-legged tarantula to the two-legged scientific community seeking a tool to study TRPV receptors and the downstream signal transduction. **Jason G. Underwood, Ph.D.**