have now been well characterized (i.e., [23–26]). Another potential drawback is the lower chemical stability of RNA relative to DNA.

One often wonders what structures a single-stranded DNA could adopt. We have hundreds of examples of structures of double-stranded DNAs in the crystal and in solution, but very little is known regarding the conformational variations in a single strand. The work described here provides probably just a glimpse at the potential complexity of DNA structure when noncanonical pairing modes are considered. Although DNA cannot possibly match RNA in terms of structural complexity and numbers of folding motifs, its chemistry offers an ideal basis for the formation of predictable 2D and 3D arrays. The work by Paukstelis and coworkers is an excellent demonstration that DNA "wears many hats" and, used correctly, can serve as an ideal building material for supramolecular assemblies and nanoscale structures.

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Pheromone Unwrapping by pH Flip-Flopping

The Asian elephant utilizes the same sex pheromone as a number of moth species, (*Z*)-7-dodecen-1-yl acetate encapsulated in a serum-derived albumin. The chemical signal is emitted in the urine and received in the mucus of the trunk. The unwrapping of the package is pH mediated.

The Asian elephant [1], the cabbage loop moth, and many other moth species [2] share a common sex pheromone, (*Z*)-7-dodecen-1-yl acetate (Z7-12Ac), but the packing and processing of this chemical signal is remarkably different in elephants and moths. Female moths advertise their readiness to mate and reproduce by releasing sex pheromones, which are utilized by male moths in long-range odorant-oriented navigation toward females. Sustainable flight and orientation requires a dynamic, sensitive, and selective olfactory system [3–6] to detect specifically pockets of chemical signals that are separated by small clean air spaces in a pheromone plume [7]. To be able to follow the trail, males have only a few milliseconds to reset the olfactory system while navigating through clean air [4, 8]. Three major groups of proteins play pivotal roles in the dynamics, selectivity, and sensitivity of pheromone reception in insects. They are the pheromone receptors (PRs), pheromone binding proteins (PBPs), and pheromone-degrading enzymes (PDEs) [4, 5, 8]. While PBPs serve as liaison between the external environment (air) and the PR, PDEs are essential for inactivation of chemical signal and consequently resetting the receptors [9]. Upon binding pheromones, PBPs transport the chemical signals to their receptors while avoiding premature inactivation by PDEs [4, 5, 8]. Interaction with negatively charged membrane surfaces in the proximity of the pheromone receptors leads to a pH-dependent conformational change in PBPs [10, 11] and delivery of the pheromones to the receptors [4, 5, 8]. Elephants have a much less stringent requirement for the dynamics of pheromone reception. It seems that they do not have a pheromone carrier/protector in the mucus of the trunk. As opposed to the unique helix-rich structures of insect PBPs [12–14], the major odorant binding protein (OBP) in the mucus of the Asian

elephant is a lipocalin, which apparently functions as a scavenger of the pheromone [15]. It has been suggested that the elephant pheromone binds to the OBP in the mucus rather slowly and with moderate affinity. This implies that the pheromone alone may be sufficiently soluble to reach the pheromone receptors [15], provided that PDEs are absent in the mucus. In insects, chemical signals as soluble as ethanol need to be carried and protected by an odorant binding protein [4]. The reception of pheromones in elephants and moths highlights differences in the modus operandi of the same molecule (Z7-12Ac) with identical type of signal (pheromone) in two different animals. Although it is tempting to conclude that the reception of an identical pheromone would occur with identical molecular partners (receptors and OBPs) in two animals from different orders, it is generally unwarranted. *Drosophila melanogaster* and *Caenorhabditis elegans*, for example, smell 2,3-butanedione (diacetyl), yet there is no odorant receptor in the fruit fly with significant amino acid similarity to the nematode diacetyl receptor, odr10 [16].

In this issue of *Chemistry & Biology*, Lazar and colleagues [17] provide enlightening evidence on pheromone signaling in the Asian elephant. In marked contrast to moth and other insects that produce pheromones in specialized glands and let them evaporate to permeate the environment and form a plume, the Asian elephant pheromone is "wrapped" in a protein and delivered in the urine, with its titer increasing toward the periovulatory period. Although mammalian pheromones are normally "fixed" by proteins of the lipocalin family, the fixative in the Asian elephant pheromone is an albumin. The authors did not detect any lipocalins in the Asian elephant urine either by MALDI-MS or with a polyclonal antibody against the mouse urinary protein. The elephant urinary protein seems to "leak" from the serum (thus the name ESA, elephant serum albumin) and carries along the trapped pheromone. Bioassays clearly indicate that ESA functions as a fixative of the pheromone in the environment. While a long-lasting pheromone signal may disrupt communication in moths [5], it may enhance the chances of male-female encounters in the Asian elephant. The principle of protein molecules serving as packages for odorants (nanocapsules) has been implemented at least twice in evolution, with proteins belonging to different folding families [18].

Lazar and colleagues provide an elegant demonstration that the pheromone is delivered from the urine to the olfactory system by a pH flip-flopping. They carried out two different types of binding assays, using the intact pheromone and a photoaffinity labeling compound; the latter type of compound has been demonstrated to mimic semiochemicals in insects [19, 20]. Lazar and colleagues tested binding activity at various pH values from the alkaline pH (8.4 at preovulation period) of the urine to the acidic pH (5.5) in the mucus of the trunk. Demonstration of high binding affinity of the pheromone by ESA at alkaline pH and low affinity at acidic pH were supplemented by behavioral assays, indicating that ESA is essential for retention of pheromonal activity and that this retention is higher at alkaline pH. Since males sample the urine directly with the tip of the trunk, and the pH of the mucus is low, it is convincingly argued that the pH-dependent binding affinity is physiologically relevant. Such pH-mediated unwrapping of a pheromone is a hitherto unknown mechanism to deliver a chemical signal from the external environment to an olfactory system. It is somewhat similar to the release of pheromones from the pheromone-PBP complexes inside the insect olfactory system. In the latter case, however, the unwrapping is triggered by a localized pH (at the negatively charged surface of dendrites), whereas the bulk low pH of the mucus in the Asian elephant is significant for the reception of the signal.

The study suggests some interesting questions for future research. Is the fixation of pheromone the only role played by ESA, or is the pheromone being protected from degrading enzyme(s) in the urine? Is the pH-triggered release a unique feature of elephant pheromones, or is it a common delivery system in mammalian pheromones? Are there pheromone binding proteins in the mucus, which could not be detected in the previous studies? I eagerly await the answer to these and other questions.

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Aptamers Meet Allostery

Engineered RNAs have demonstrated remarkable properties of molecular recognition and allosteric function. Liu and colleagues now report the isolation and in vivo function of a ligand-dependent RNA-based transcription factor that opens wide the door for allosterically controllable aptamers.

RNA is a highly versatile biopolymer capable of exhibiting fundamental biochemical properties once believed to be unique to the realm of protein factors and enzymes. The numerous and varied activities that cellular RNAs fulfill as catalysts or regulators of biological processes have shattered the view of RNA as a simple biological intermediary. Moreover, engineered RNAs have served to further expand the repertoire of biochemical capabilities ascribable to RNA and have offered unique insights to RNA's inherent potential for catalysis [1], molecular recognition and discrimination [2], and allosteric function [3]. Such engineering efforts are made possible by RNA's unique tractability to both rational design and combinatorial selection techniques [1], the latter of which is facilitated by the dualistic character of RNA as an informational and functional molecule. RNA is thus regarded as an attractive biopolymer for tailoring novel molecular therapeutic agents and biotechnological tools.

In this issue of *Chemistry & Biology*, Liu and colleagues report the successful exploitation of RNA's molecular recognition and allosteric capabilities in the creation of an RNA-based transcriptional activator that is facilely modulated by an effector compound in yeast [4]. The transcriptional activator functionality is derivative of a previously isolated RNA aptamer that binds an unidentified host factor and activates reporter gene expression when localized to the promoter region of DNA [5]. By integrating a second RNA aptamer domain that binds tetramethylrosamine (TMR) [6], Liu and coworkers sought to modulate the function of the adjacent transcriptional activator through conformational changes in aptamer structure arising from TMR interaction, and have succeeded in generating the first biologically active allosteric aptamer (Figure 1A).

Such integration of functional RNA domains has previously been achieved in the generation of allosteric RNA catalysts by joining aptamer and ribozyme domains [7]. The union of ligand binding and catalytic functions through rational design strategies has proven to be moderately successful. Such judicious integration of functional domains typically relies on a phenomenon of RNA-ligand interaction termed adaptive binding [2], in which ligand binding stabilizes local RNA structure. By replacing a critical element of a catalyst's secondary structure with an aptamer domain, ligand-induced structural stabilization and ribozyme activation has been demonstrated [8]. However, this design strategy can be significantly augmented with combinatorial strategies, in which nucleotide positions in the region conjoining functional domains are randomized, and individuals are selected on the basis of optimal allosteric performance [9]. In this manner, allosteric nucleic acid catalysts that are either activated or inhibited by ligand binding have been isolated.

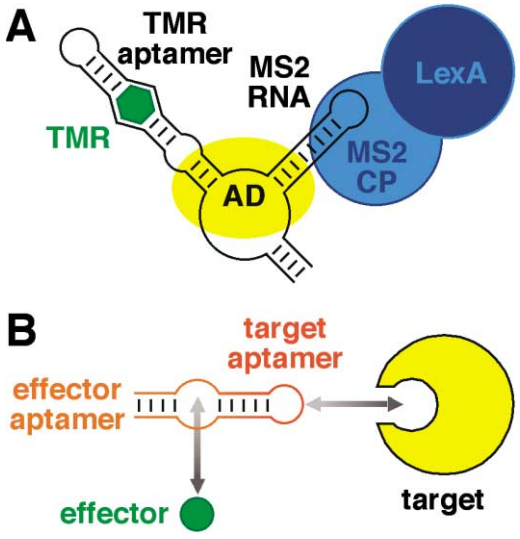

Figure 1. Allosteric Aptamers

(A) TMR-dependent transcriptional activator isolated by Liu and coworkers [4]. An RNA aptamer that functions as a transcriptional activation domain (AD) in yeast by binding an unidentified host factor is integrated with the TMR aptamer in such a manner that TMR binding promotes formation and function of the activation domain. The RNA is localized to the promoter of a reporter gene through the respective RNA and DNA binding activities of an MS2 coat protein (MS2 CP)-LexA fusion protein.

(B) General scheme for allosteric aptamer function. Integration of effector and target aptamer domains as interdependent or mutually exclusive functional domains might achieve effector activation or inhibition of target aptamer function, respectively.