

CHEMISTRY

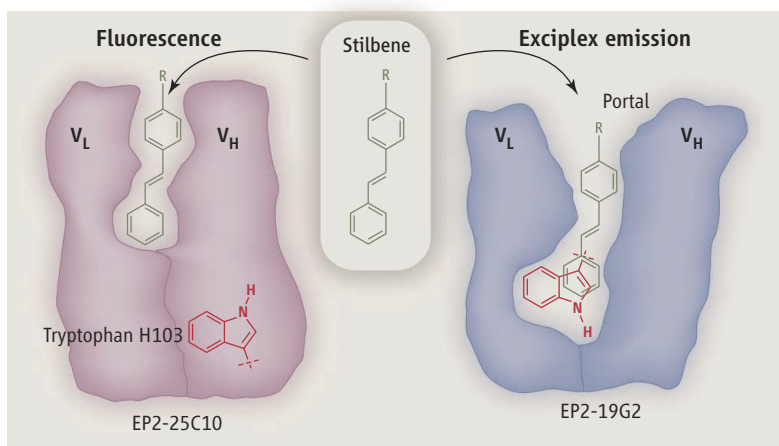
An Enlightening Structure-Function Relationship

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Small-molecule cofactors are widely used in biological systems to augment or alter the function of a biomolecular partner. For example, cofactors participate in electron-transfer reactions catalyzed by redox enzymes. In 2000, Simeonov *et al.* inverted this paradigm, reporting a system in which proteins (specifically, monoclonal antibodies) acted as cofactors for activating the fluorescence of a small molecule (a *trans*-stilbene derivative) (*1*). The resulting complexes have since been used to make fluorescent biosensors for various analytes. On page 1232 of this issue, Debler *et al.* (*2*) reveal a fascinating relation between the structure of the antibody-stilbene complex and the mechanism by which emission is enhanced.

The original paper (*1*) and a subsequent report from the same group (*3*) yielded a catalog of antibody-stilbene complexes emitting blue, purple, or green wavelengths of light. In most cases, the monoclonal antibody simply provided a constrained environment to inhibit photoisomerization of the stilbene (*4*), leading to strong fluorescence. The exception was the blue-emitting complex. Here, steady-state and time-resolved experiments indicated that enhanced emission resulted not only from constraining the dye, but also from the formation of an excited-state complex. A crystal structure suggested that this “exciplex” forms between the stilbene and a π -stacked tryptophan residue (Trp^{H103}) located at the deepest point in the binding pocket.

Debler *et al.* have now mutated residues in the stilbene-binding pocket and analyzed the impact on the emission properties. As expected, mutation of Trp^{H103} to phenylalanine (Trp^{H103}



Structure and function. The same fluorogenic dye molecule is bound by two antibodies in different ways, leading to different emission mechanisms and colors. (**Left**) The EP2-25C10 antibody binds the dye in a shallow pocket, and purple fluorescence is enhanced due to the conformational constraints. (**Right**) The EP2-19G2 antibody binds the dye in a much deeper pocket that allows π stacking with a tryptophan residue. An exciplex is formed after excitation, and charge recombination leads to blue luminescence.

→ Phe) eliminated exciplex emission without reducing the affinity of the protein for stilbene. In contrast, a Tyr^{L34} → Phe mutant retained exciplex emission, albeit with lower intensity. In the crystal structure of the complex, Tyr^{L34} lies perpendicular to the stilbene and evidently helps to hold it in place stacked against Trp^{H103}. Presumably, replacing the tyrosine OH with an H in phenylalanine sufficiently alters the structure to weaken exciplex formation.

In an exciplex, some charge transfer takes place between the two components; in the extreme case, complete transfer of an electron yields a contact radical ion pair (*5*). This property of exciplexes introduces a subtle but important point: Because the ion pair is an excited-state species, emission results from charge recombination, as opposed to simple relaxation of a locally excited state.

To elucidate the mechanisms by which stilbene luminescence is activated by the two types of antibodies, the authors turned to high-resolution structural analysis of antibody-stilbene cocrystals. In the exciplex-forming antibody, EP2-19G2, the hydrophobic stilbene-binding pocket is deep within the antibody, between the V_H and V_L domains (see the figure, right panel). The Trp^{H103} that forms the exciplex is highly conserved and normally participates in contacts between two domains that stabilize the

Mutation and structural data elucidate distinct mechanisms by which different antibodies bind and induce luminescence of dye molecules.

antibody fold. For this amino acid to form an exciplex with the stilbene, the interface between the two domains must rearrange. This interdomain distortion generates a surface depression that creates a portal to the deep binding pocket [see figure 2B in (*2*)].

The crystal structures of the green and purple antibody-stilbene complexes, which do not show exciplex emission, reveal a binding pocket that is also quite hydrophobic but at least 0.6 nm shallower. There is no distortion of the V_H/V_L interface and no surface depression or portal to the stilbene-binding pocket [see the figure, left panel, and figure 2A

in (*2*)]. With no long portal in the green and purple antibodies, stilbene is unable to approach the Trp^{H103}, and no exciplex emission occurs.

EP2-19G2 has been used in several biosensor applications, including fluorescent labeling of DNA that was modified with stilbene dyes (*6*), high-throughput screening of chiral catalysts (*7*), discrimination of small-molecule stereoisomers (*8*), and detection of mercury (*9*). These sensors exploit the unique structural features of the antibody, i.e. the depth and shape of the portal leading to the stilbene-binding pocket.

Other biomolecules such as nucleic acid aptamers (*10*) and single-chain variable-fragment antibodies (scFvs) (*11*) can selectively bind fluorogenic dyes and enhance their fluorescence by a factor of more than 1000. Because there is no evidence for exciplex formation, these cases are analogous to the green and purple antibody-stilbene complexes. In the case of the scFvs that bind thiazole orange and malachite green, considerable spectral tuning and improved binding affinity was generated through directed evolution (*11*). This approach could be used to generate stilbene-binding scFvs with improved affinity, higher fluorescence quantum yields, or more diverse colors. Such modified biomolecules could find applications in fluorescence imaging and sensing.

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The discovery of the exciplex-forming antibody was fortuitous in that immunization and in vivo selection were based solely on binding to the stilbene antigen, not on activation of emission. Methods that allow direct selection of luminescent scFvs—for example, by flow cytometry (11)—would be more efficient and might even allow discrimination between normal fluorogenic and exciplex-forming scFvs as

a result of the different emission properties expected for such protein-dye complexes.

References

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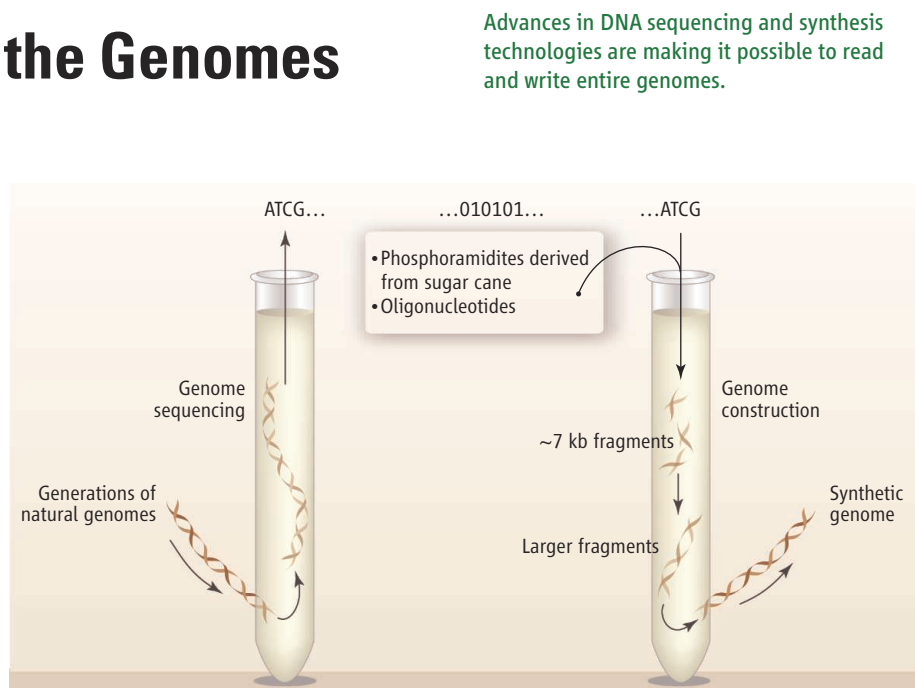
GENOMICS

Reconstruction of the Genomes

Drew Endy

“I am the family face; flesh perishes, I live on, projecting trait and trace through time to times anon, and leaping from place to place over oblivion.” So starts the poem *Heredity* by Thomas Hardy, whose protagonist personifies the observation that all life exists through a process of direct descent from one generation to the next. Scientifically, the replication and propagation of genetic material, as DNA or RNA, is the primary mechanism by which each generation transmits the instructions underlying the traits and traces of their offspring. On page 1215 of this issue, Gibson *et al.* (1) bypass nature’s constraint of direct descent by combining information and raw chemicals to construct the entire set of genetic material, or genome, encoding a bacterium (see the figure). This first construction of a genome encoding a self-reproducing organism heralds important opportunities in both genetics and biotechnology, highlights the need for improved DNA construction technology, and reinforces the value of ongoing public discussion of the impacts of making organisms easier to engineer.

Gibson *et al.* used a multistage process to construct the genome of *Mycoplasma genitalium*. First, information defining the 582,970–base pair (bp) DNA sequence of the genome to be synthesized was obtained from a computer database and divided into shorter sections, or cassettes of DNA up to ~7000 bp long. Commercial DNA suppliers then constructed these cassettes. Raw chemicals derived from sugar cane were combined to synthesize specific oligonucleotides, short fragments of DNA up to several hundred base pairs long (2). The suppliers then combined



Genome construction. DNA sequencing technology decodes the genome of an organism. DNA synthesis and genome construction technologies enable the opposite process. Bacterial genomes can be built from DNA sequence information and raw chemicals.

subsets of oligonucleotides to produce the requested cassettes (3). Gibson *et al.* used a hierarchical scheme to assemble, check, and, as needed, repair ever-longer DNA fragments, eventually producing the full-length genome.

Given that all life is encoded by genetic material, ongoing and future advances in DNA synthesis and genome construction technology will be important. For example, the U.S. National Institutes of Health is estimated to spend ~\$1.5 billion annually supporting the manual manipulation of DNA (4). Such work consumes most of the experimental effort for many biologists and biological engineers, a hidden opportunity cost that is harder to quantify. Moreover, the required slavish mastery of ad hoc methods and tedious tools for DNA manipulation discourages most students and researchers in fields such as physics, electrical engineering, and computer science from exploring biomedical and bio-

Advances in DNA sequencing and synthesis technologies are making it possible to read and write entire genomes.

technology research. Thus, an improved ability to provide any DNA molecule quickly, reliably, and economically would enhance and expand life sciences and engineering research (5), and might well become the goal of well-coordinated public research programs. Unfortunately, no such programs exist today.

Meanwhile, consider that most early discoveries of genetically encoded functions depended on analysis of the linkage between natural or randomly generated mutations and phenotypes (6), a powerful approach akin to blindly smashing many cars with a hammer and then determining which broken parts matter by attempting to drive each machine. Over the past 30 years, the invention (7) and development (8) of DNA sequencing technology have provided a complementary approach for discovering genetic functions. By comparing DNA sequence information from different organisms, researchers can

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