ularly complex because they are bounded by two lipid bilayers, and their proteins must pass through both of these hydrophobic barriers to reach host cells. Gram-positive bacteria are generally simpler in structure in that they lack the second lipid bilayer, yet surprisingly, our understanding of virulence factor secretion in these organisms is rudimentary. Although they have been known to lack the canonical Type I to Type VI systems of Gram-negative bacteria, it was only recently that the ESX-1 system was discovered.

A large number of low molecular weight proteins are secreted by *M. tuberculosis* when the bacteria are cultured in vitro. Two of these are potent immune response elicitors—early secreted antigenic target–6 (ESAT-6) and culture filtrate antigen 10 kD (CFP-10). These proteins form a heterodimer and secretion of ESAT-6 depends on the presence of CFP-10 (*4*, *8*, *9*). The genes encoding both antigens are localized on the region of difference 1 (RD1). RD1 encompasses most of a 15-gene cluster called ESX-1, which is duplicated a number of times in the *M. tuberculosis* genome (*10*). It is also a part of the *M. tuberculosis* genome that is absent from the attenuated *M. bovis* bacillus Calmette-Guérin (BCG) or the avirulent *M. microtii* strains (*11*, *12*).

At least five genes encoded within the ESX-1 cluster, *Rv3868* through *Rv3871* and *Rv3877*, are required for secretion of ESAT-6 and CFP-10 (*3*–*5*, *13*). *Rv3877* encodes a transmembrane protein that may form all or part of a channel. *Rv3869* also encodes a membrane-associated protein (see the figure). *Rv3868*, *Rv3870*, and *Rv3871* encode ATPases (adenosine triphosphatases) that may transduce the energy of ATP hydrolysis into mechanical work. CFP-10, but not ESAT-6, interacts with the ATPase Rv3871 (*4*).

The study by DiGiuseppe Champion *et al*. takes a large step toward elucidating the means by which CFP-10 and ESAT-6 are targeted for secretion. The authors constructed truncations and single–amino acid variants in the CFP-10 protein to define the regions important for associating with ESAT-6 and Rv3871. Interaction with Rv3871 is mediated by the carboxyl-terminal seven amino acids of CFP-10. Specific substitution of residues in this region abolished this association, but did not affect complex formation with ESAT-6, showing that these interactions are separable. The same substitutions also abolished secretion of CFP-10, suggesting that its interaction with Rv3871 is a prerequisite for export. Remarkably, mutations in CFP-10 that abolished its secretion also completely blocked export of ESAT-6, implying that both proteins are targeted to the secretion machinery as a complex. Moreover, the nuclear magnetic resonance solution structure of the ESAT-6–CFP-10 complex reveals extensive contacts between the two proteins, but that the carboxyl-terminal 15 amino acids of CFP-10 extend from the complex and adopt an α-helical structure (*14*). An open question that arises from the DiGiuseppe Champion *et al.* study is whether ESAT-6 and CFP-10 are secreted as a folded protein complex or whether one or more of the essential ATPases is required to tear the complex apart before export, allowing each protein to be secreted separately.

DiGiuseppe Champion *et al*. further demonstrate that when the key seven amino acids of CFP-10 are amended to another protein, the protein is exported from bacteria. Thus, the signal is portable and can target the secretion of heterologous proteins. This is of particular interest because it may provide a means to engineer more effective live attenuated vaccines. It has long been known that vaccination with BCG is effective only when administered live, suggesting that active secretion is necessary to generate an effective immune response. Enhanced secretion of antigens by engineering of the ESX-1 secretion system may therefore ultimately provide a mechanism for boosting protection against tuberculosis.

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CHEMISTRY

The Organic Approach to Asymmetric Catalysis

Benjamin List and Jung Woon Yang

Small organic molecules are increasingly used as asymmetric catalysts, complementing the enzymes and metal complexes traditionally used to make chiral products.

When chemists make chiral compounds—molecules that behave like object and mirror image, such as amino acids, sugars, drugs, or nucleic acids—they like to use asymmetric catalysis, in which a chiral catalyst selectively accelerates the reaction that leads to one mirror-image isomer, also called enantiomer. For example, the "Monsanto process" uses a chiral rhodium catalyst to synthesize the drug L-dopa, used to treat Parkinson's disease (*1*).

For decades, the generally accepted view has been that there are two classes of efficient asymmetric catalysts: enzymes and synthetic metal complexes (*2*). However, this view is currently being challenged, with purely organic catalysts emerging as a third class of powerful asymmetric catalysts.

Most biological molecules are chiral and are synthesized in living cells by enzymes using asymmetric catalysis. Chemists also use enzymes or even whole cells to synthesize chiral compounds. Such biological catalysis is increasingly used on an industrial scale and is particularly preferred in hydrolytic reactions. The other class of accepted and efficient chiral catalysts, metal complexes, are reagents based on inorganic chemistry. Transition metal catalysts are particularly useful for asymmetric hydrogenations, but may leave possibly toxic traces of heavy metals in the product.

In contrast, in organocatalysis, a purely organic and metal-free small molecule is used to catalyze a chemical reaction. In

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addition to enriching chemistry with another useful strategy for catalysis, this approach has some important advantages. Small organic molecule catalysts are generally stable and fairly easy to design and synthesize. They are often based on nontoxic compounds, such as sugars, peptides, or even amino acids, and can easily be linked to a solid support, making them useful for industrial applications. However, the property of organocatalysts most attractive to organic chemists may be the simple fact that they are organic molecules.

Organocatalysts have been used sporadically throughout the last century; indeed, an organic catalyst was used in one of the very first examples of a nonenzymatic asymmetric catalytic reaction (*3*). But recently, this area has grown at a breathtaking pace. Within a few years, powerful organocatalysts for a wide range of reactions have been designed and developed (*4*–*6*).

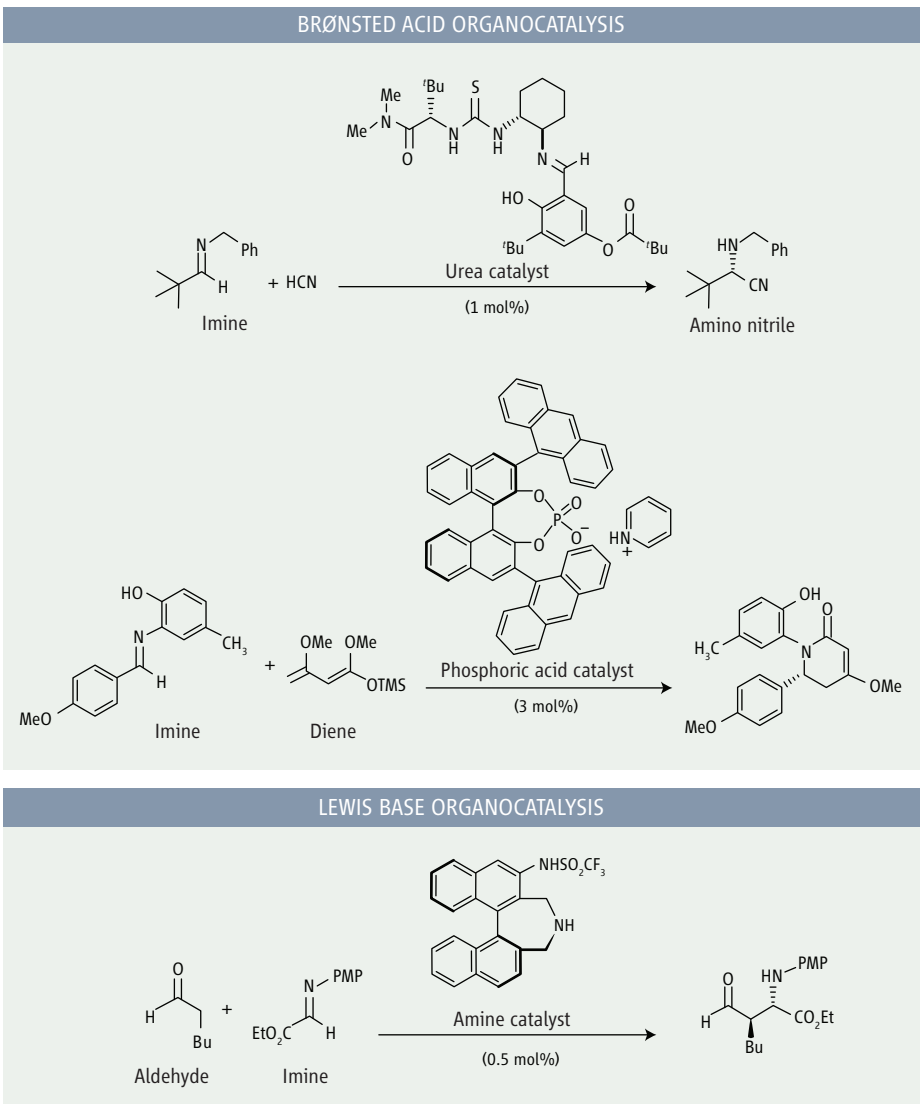
A particularly appealing discovery of great potential is the use of chiral Brønsted acids as organocatalysts. Organic Brønsted acids function by donating a proton to the substrate. They have been used as catalysts for a variety of reactions since the beginnings of modern chemistry, but applications in asymmetric catalysis have been extremely rare. A breakthrough in this area came when Jacobsen *et al.* developed highly active Brønsted acid organocatalysts that incorporate a urea motif as the active principle (*7*).

One powerful application of these catalysts is in the Strecker reaction (*7*, *8*), one of the most general and useful ways to make enantiomerically pure amino acids (see the figure, top). In this transformation, hydrogen cyanide reacts with imines (which contain a carbon-nitrogen double bond). In the presence of the Jacobsen urea catalyst, amino nitriles are obtained in high enantiomeric ratios (er; this is the ratio of the two mirror image isomers); hydrolysis of these compounds yields valuable amino acids. This type of catalysis, in which the catalyst donates a hydrogen bond to a reactive intermediate, is very common in enzymes, but only became a powerful tool for asymmetric organocatalysis through the work of Jacobsen and co-workers (*7*, *8*).

Akiyama *et al.* recently developed even stronger Brønsted acids based on the phosphoric acid motif. These acids catalyze reactions such as the hetero-Diels-Alder reaction between imines and dienes (the latter contain two carbon-carbon double bonds) (see the figure, middle) (*9*). The resulting cyclic molecules are of interest in drug development. Independently, Terada *et al.* have recently shown that such phosphoric acid catalysts are highly active and have the potential of becoming as efficient as even the most active metal- or biocatalysts (*10*).

Yet, there is more to organocatalysis than Brønsted acids. Lewis base organocatalysis, in which the catalyst donates not protons but electrons to the substrate, is another very actively researched area. Catalysts include amines, phosphines, and even carbenes. The amino acid proline is an exceptionally simple yet versatile amine catalyst and has attracted a lot of attention (*11*, *12*). Another useful example of amine-catalyzed reactions was recently published by Maruoka and co-workers. (*13*). This group has designed a highly active and selective catalyst for the Mannich reaction, which also involves imines (see the figure, bottom). The catalyst activates aldehydes (widely used organic substrates that contain a carbon-oxygen double bond) to react with imines to give useful intermediates for the synthesis of biologically active compounds.

The reactions discussed here are just a small subset of the increasing number of highly selective and efficient organocatalytic transformations. Finally, an organic approach to asymmetric catalysis that allows organic chemists to design and to understand their catalysts themselves is at hand. Organocatalysis complements the traditional and highly developed inorganic and biological approaches to asymmetric catalysis. Because of its many attractive features, applications in the pharma-



Powerful organocatalysts. In the emerging field of organocatalysis, Brønsted acid organocatalysts (**top**, **middle**) and Lewis base organocatalysts (**bottom**) have received particular attention. The reactions shown have enantiomeric ratios of at least 98.5:1.5.

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ceutical industry can be expected. There is little doubt that organocatalysis is here to stay.

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STRUCTURAL BIOLOGY

Dynamic Visions of Enzymatic Reactions

Experimental evidence is provoking further discussion of a stochastic view of protein behavior.

Michele Vendruscolo and Christopher M. Dobson

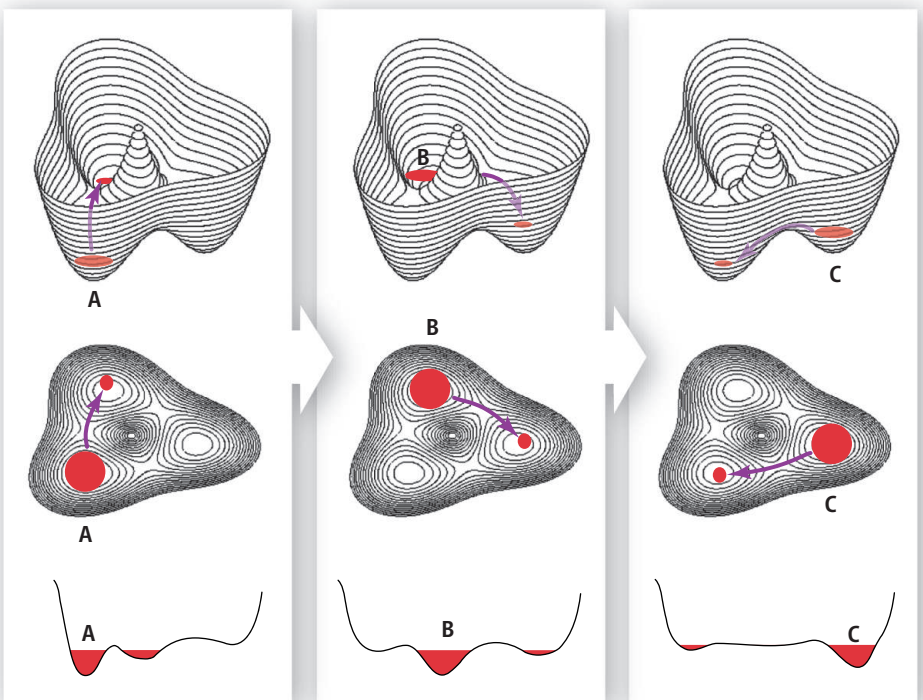
The action of many proteins involves large-scale conformational changes that typically take place on the millisecond time scale. Examples include the cooperative transitions that enable efficient oxygen transport by hemoglobin in the blood, and the series of motions involved in muscle contraction (*1*). But even proteins that do not undergo such dramatic conformational excursions are not the rigid objects that structural models often imply. The conformations of all proteins constantly fluctuate, with some motions taking place on time scales of a picosecond or less and involving displacements in atomic positions of ~0.1 nm (*1–6*) .

Are these motions simply inherent properties of molecules held together by relatively weak interactions, or have they evolved to enhance their functional efficiency? There is increasing evidence that both views may in fact have validity and that biology has channeled the inherent motions of proteins into directions that enhance their effectiveness. For example, structural fluctuations of enzymes appear to increase the probability of binding certain ligands, although more studies are needed to establish their effects on the catalytic rates themselves. On page 1638 of this issue, Boehr *et al*. (*7*) report experimental evidence that is interpreted in terms of this view of enzymology. They also indicate that many fluctuations can be linked together into whole reaction cycles that carry out a complex chemical process with great efficacy.

In recent years, the idea that random conformational fluctuations of proteins are channeled into productive events has gained popularity. This concept is rooted in a statistical view that has revolutionized, for example, our understanding of protein folding (*1*, *2*, *8–10*), a process now perceived not as a deterministic sequence of well-defined conformations, but rather in terms of stochastic events along free-energy landscapes that funnel the molecular fluctuations toward their native structures. The "jigglings and wigglings" of protein molecules anticipated by Feynman (*11*) appear therefore to have been harnessed for specific purposes during molecular evolution.

Application of this statistical view to enzyme behavior suggests that conformational fluctuations resulting from the concerted motions of many atoms can push the unbound states of enzymes into conformations closely resembling the bound states, thereby priming them to form complexes with specific ligands (*5*, *12*, *13*). Thus, although the unbound state of a protein is inherently flexible, fluctuations are not random. Rather, they take place preferentially in a way that prepares the protein to bind to its cofactors and substrates. The free-energy landscapes of the free and the bound states differ just enough to cause changes in the relative populations of their principal states. After binding, the free-energy landscape (*7*) is plastically deformed just enough to make a slightly different state of the protein become the most populated (see the figure).

Boehr *et al*. suggest that certain enzymes can combine flexibility with plasticity to



A free-energy channel model for enzymatic behavior. The binding of a ligand shifts the predominant population of enzyme molecules from the free state (**A**) to a bound state (**B**) that was previously sampled transiently through stochastic fluctuations. Further conformational fluctuations from **B** enable the catalytic state (**C**) to be accessed. When the enzyme returns to state **A**, the cycle can begin again.

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The Organic Approach to Asymmetric Catalysis

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