

that the fastest atomic motions during this change occur along one particular axis of the crystal, and are related to pairs of vanadium atoms moving apart from one another (see the figure, left). Only at later times do the other crystallographic planes expand (see the figure, right). The transformation pathway between stable monoclinic and tetragonal phases is then shown to pass through an unstable, tetragonal unit cell, which is compressed along one of the axes. The authors thus establish a direct connection between the femtosecond dilation of the V-V bond and the equally fast changes in conductivity that can be measured with other techniques (7).

It is impressive that Baum *et al.* achieve this femtosecond time resolution in the reflection mode of diffraction, because the inherent velocity mismatch between electrons and photons can, in principle, smear out the time response and hinder the observation of the femtosecond movements of the atoms. To this end, the authors ingeniously tilted the front of the optical pump pulse, thus matching the speeds with which the surface of the sample is

excited optically and swept by the diffraction probe. Reflection geometry opens the way to femtosecond electron diffraction in most bulk solids, whereas previous experiments performed in transmission were limited to very thin films (8, 9).

In thinking of new advances in the studies of ultrafast structural dynamics, a few key considerations come to mind. Electron pulses can be incorporated in a microscopy apparatus, as shown previously by Zewail and co-workers (10). This microscopy advance is important for the study of strongly correlated transition-metal oxides discussed here, which are known to exhibit important phase separation phenomena that are quite difficult to investigate (11).

The next frontier will be a time resolution of 10 fs or below, which will allow the movements of the lightest atoms that compose many important organic compounds and liquids to be resolved. Femtosecond electron diffraction is evolving hand in hand with x-ray techniques (12), which have developed in the recent past with both tabletop and accelerator-based techniques, offering

similar time resolutions but also important spectroscopic capabilities (13). The capability of interrogating matter with ultrafast electron and x-ray pulses is opening new horizons that could only be dreamed of as recently as a decade ago.

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10.1126/science.1150672

CHEMISTRY

No Protection Required

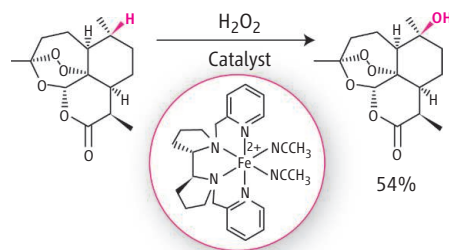
Robert H. Crabtree

Organic synthesis has traditionally relied heavily on activating groups and protecting groups to steer synthetic reactions to the desired products. Activating groups such as halides enhance the reactivity of reactants, whereas protecting groups such as amides or esters block reactivity at undesired sites. On page 783 of this issue, Chen and White (1) show that such activating and protection groups are not always required even in reactions involving complex molecules.

With the rise of green chemistry (2), more attention is being paid to eliminating activating and protecting groups, wherever possible, for two reasons. First, they generate waste. Second, both activating and protecting groups require extra synthesis steps to be introduced into reactants; protecting groups also need extra steps to be removed after reaction. Catalysis can give reactivity and selectivity without the need for activating or protecting groups. The ideal catalyst reacts with an un-

activated reactant with a selectivity that can be tuned by choice of catalyst. In practice, however, we are still far from the goal, particularly when the substrate is a complex organic molecule with multiple functional groups.

Perhaps the greatest challenge is finding catalysts that selectively attack C–H bonds,



Selective conversion. The antimalarial compound artemisinin (left) is extracted from a shrub used in herbal form in Chinese traditional medicine. Although artemisinin has numerous C–H bonds and a delicate peroxide functional group, it gives a single product (right) when the Chen-White catalyst is used in conjunction with hydrogen peroxide. This implies that the catalyst has high selectivity even for a complex molecule, but predictability for other cases will require more detailed study.

An iron catalyst converts C–H bonds to C–OH groups with predictable selectivity even in very large molecules.

which are ubiquitous in organic compounds but are often very unreactive. A number of catalysts are known for this “C–H activation” reaction (3), but they act only on simple molecules such as hydrocarbons. In more complex organic molecules, such as those commonly encountered in pharmaceuticals, numerous oxygen or nitrogen-containing functional groups are distributed over a core held together by carbon-carbon bonds. In such a polyfunctional molecule, unselective attack at any of a number of C–H bonds can result in a cocktail of final products.

Chen and White now report a striking counterexample that shows how C–H bonds can be activated selectively even in complex polyfunctional molecules (see the figure for an example). The authors used an iron catalyst to convert specific C–H bonds in a wide variety of molecules to C–OH groups; the benign and inexpensive hydrogen peroxide serves as the ultimate source of the oxygen atom.

Depending on the specific case, the authors ascribe the remarkably high selectivity to a combination of a number of causes. These include the reactive C–H bond being either inherently more reactive than any other, or

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more physically accessible to the catalyst. The catalyst can also be attracted to a specific location by binding to a pre-existing functional group within the reactant, thus attacking only a nearby C–H bond. A goal in the area is to understand the relevant selectivity trends from the previous results, in order to predict the outcome in any subsequent case. Predictability is essential for the design of a multistep synthetic route relying on a selective, late-stage C–H

activation, because failure at a later step would vitiate the entire scheme.

This remarkable work is part of an emerging trend, in which different types of selective, catalytic C–H activation reactions are being successfully applied to more complex molecules than previously envisaged (4–6). With the conceptual barrier breached for hydroxylation, further striking applications to complex molecules are likely to emerge in the near future.

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10.1126/science.1150982

SYSTEMS BIOLOGY

A Clock with a Flip Switch

Andy C. Poon and James E. Ferrell Jr.

Two years ago, Takao Kondo's group showed that when a phosphate source (adenosine 5'-triphosphate) and three purified proteins were mixed in a test tube, they spontaneously generated sustained oscillations in the phosphorylation state of one of the proteins (1). The three proteins—KaiA, KaiB, and KaiC—were previously identified as important for the daily patterns of activity and behavior (circadian rhythms) in the cyanobacterium *Synechococcus elongates*. Astonishingly, oscillations of phosphorylation in the reconstituted system were similar to the bacterium's natural circadian rhythm of about 24 hours. Moreover, mutations in one of the proteins, KaiC, that change the circadian period in vivo had nearly identical effects in vitro. Thus, a relatively simple and highly robust timekeeper seemed to set the pace for this complicated organism. The big remaining challenge was to determine how this clock works. Two studies, reported by the Kondo group (2) and by Rust *et al.* on page 809 of this issue (3), now provide a satisfying answer to this question. The oscillations arise from the slow, orderly addition and then subtraction of two phosphates from the KaiC protein. This provides a fascinating example of reductionistic systems biology, where the ability to pick apart a complex system has yielded an understanding of how the whole system works.

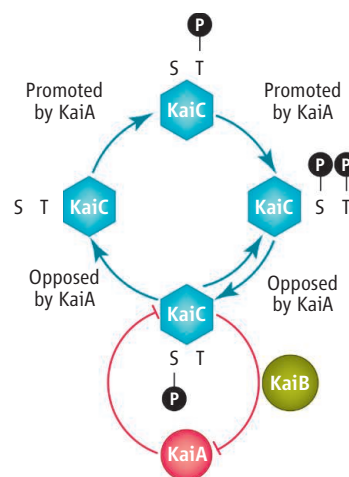
The addition and removal of phosphate can alter a protein's function, and if the protein is part of a network of interacting factors, its phosphorylation status may relay information that impinges on some cell behavior. The dynamics of reversible phosphate addition

and removal in cells are usually rapid—occurring on time scales of seconds or minutes—and so seem poorly suited for slow circadian rhythms. Both studies examine details of the timing of KaiC phosphorylation during oscillations. KaiC is phosphorylated at two sites and in a particular order: first on a threonine residue, then on a serine. Subsequently, the threonine and then serine are dephosphorylated and the KaiC returns to an unphosphorylated state (see the figure). The KaiA protein regulates these transitions by promoting autophosphorylation and inhibiting autodephosphorylation by KaiC.

But a cycle of phosphorylations and dephosphorylations would not necessarily be expected to generate oscillations. Consider, for example, activation and inactivation of the enzyme Erk2 (extracellular signal-regulated protein kinase 2). Like KaiC, Erk2 cycles among four chemical states, modified by enzymes that phosphorylate a tyrosine residue and then a threonine (4), and then by enzymes that dephosphorylate tyrosine first and then the threonine (5). But there is no hint that this system oscillates. What, then, keeps the cyclic phosphorylation and dephosphorylation of KaiC from settling into a static steady-state and allows it to oscillate?

The key insight was the discovery by Rust *et al.* that the serine-phosphorylated form of

The heart of circadian timekeeping in cyanobacteria is a toggle switch that controls the periodic phosphorylation of a key circuit protein.



The cyanobacterial circadian clock. Cyclic phosphorylation and dephosphorylation of the clock protein KaiC on serine (S) and threonine (T) becomes oscillatory through a double-negative-feedback loop (red) that toggles between two states with high or low concentration of free KaiA protein. KaiB is the third protein of the oscillator.

KaiC (S-KaiC) binds stoichiometrically to both KaiA and KaiB. The formation of the KaiA-KaiB-KaiC complex prevents KaiA from activating KaiC phosphorylation. Thus, when S-KaiC concentration is high, KaiA is sequestered by S-KaiC and KaiB, and KaiC dephosphorylation predominates; when S-KaiC concentration is low, KaiA is released and KaiC phosphorylation is activated.

Through modeling studies, Rust *et al.* show that the stoichiometric inhibition of KaiA by S-KaiC allows the cyclic phosphorylation-dephosphorylation system to become an oscillator. This inhibition closes a feedback loop and makes KaiA and S-KaiC mutually antagonistic. KaiA is a negative regulator of S-KaiC, because it pushes the balance between S-KaiC and ST-KaiC (phosphorylated on serine and threonine) toward the latter. Conversely, S-KaiC is a negative regulator of KaiA, because it sequesters KaiA (with the help of KaiB). This mutual antagonism, or double-negative-feedback loop (see the figure), allows S-KaiC and KaiA to function as a bistable toggle switch with two alternative stable steady-states; oscillations could then arise from the successive flipping of the KaiA/S-KaiC switch between these two states. Starting with unphosphorylated KaiC and the KaiA/S-KaiC switch in its low-S-KaiC concentration state, phosphorylation

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