

clues to understanding the ground rules governing allosteric control, giving insight into how nature designs allosteric proteins, and how these same principles can be applied to the rational design of signaling systems.

The important role played by the ensemble in determining allostery is demonstrated in the figure, which shows the allostery between an effector (I) and a functional (II) subunit. Depending on the energetic cost of interconverting from the active to the inactive state of each subunit, each state in the ensemble has a different stability (3, 16–18), which determines how much time the protein spends in that state. Addition of the effector ligand redistributes the ensemble and—provided that the ensemble equilibria are suitably poised—activates the functional domain.

Although conceptually simple, this ensemble view has important ramifications. Specifically, the ability of a protein to facilitate site-to-site allosteric coupling can be understood in terms of the stabilities of the states in the native ensemble (3, 16–18), without requiring detailed insights about the mechanical or structural pathways that connect the coupled sites (16). Of course, this does not mean that understanding allostery at the atomic level should not be an ultimate goal. To the contrary, the ensemble view suggests that the multitude of sequence and structural permutations that exist for a particular allosteric protein can be reconciled within a common framework.

The implications of an ensemble view of allostery are twofold. First, it provides mechanistic insight into, for example, how amino acid substitutions distal from the binding sites may affect allosteric coupling, or how different combinations of amino acid changes can combine to maintain coupling. Second, and perhaps more important, the potential robustness of coupling revealed in the ensemble model suggests that the evolution and design of allosteric systems may be much more straightforward than a detailed analysis of the structural interactions might suggest; this speculation has recent experimental support (19, 20).

Within the context of the ensemble model, evolving (or designing) allosteric coupling between subunits can be reduced to achieving two separate, albeit related goals. First, mutations must be introduced that position the equilibria between the active and inactive states of the different subunits in a regime that is suitable to facilitate coupling (3, 16). Second, mutations must be introduced that maintain a substantial interaction energy between one or the other (that is, active or inactive) states of each subunit (3, 16). In this respect, the ensemble model (see the figure) provides a framework for articulating the problem in energetic terms, providing the ground rules that govern coupling between two (or even many) sites. These ground rules may serve as the foundation for future design strategies targeting the development of signaling-competent systems.

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Iron and the Carbon Pump

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The concentration of carbon dioxide (CO₂) in Earth's atmosphere has risen by ~38% since the start of the industrial era as a result of fossil fuel burning and land use changes; if current trends continue, it is projected to increase further by at least a factor of 2 by 2100 (1). About a quarter of the CO₂ emitted through human activities has been absorbed by the ocean (1). On page 676 of this issue, Shi *et al.* show that the resulting acidification of ocean surface waters may decrease the biological availability of iron, which could in turn reduce the ability of the ocean to take up CO₂ (2).

When CO₂ dissolves in seawater, it reacts to release hydrogen ions and bicarbonate ions and consume carbonate ions (see the figure). The projected increase in surface ocean CO₂ by the end of the century should increase the hydrogen ion concentration by a factor of 3 over the preindustrial level, amounting to a decrease in pH from 8.2 to 7.7 (1, 3). The hydrogen ion concentration (and thus its negative log, pH) controls all acid/base reactions and influences most oxidation/reduction (redox) reactions (4); thus, the predicted changes in seawater acidity will fundamentally alter ocean chemistry (3) and the biology that depends on that chemistry.

Much attention has focused on projected decreases in carbonate ion concentrations, which should adversely affect many organ-

Ocean acidification may reduce the biological availability of iron, affecting the ocean's ability to absorb more carbon dioxide.

isms, such as corals and mollusks, that form their shells or skeletons from calcium carbonate (5), and on increases in CO₂ concentrations, which may increase rates of nitrogen fixation (conversion of dissolved N₂ to NH₃) by photosynthetic bacteria (6). Shi *et al.* demonstrate another potential effect: a decrease in the biological availability of iron to marine phytoplankton as a result of pH-linked changes in iron chemistry. Phytoplankton such as diatoms are responsible for almost all marine photosynthesis. They thus play a pivotal role in ocean carbon cycling.

These findings (2) provide an unexpected twist to two biological feedback processes in the ocean thought to regulate climate. The ocean's ability to absorb CO₂ is largely controlled by the biological carbon pump, a

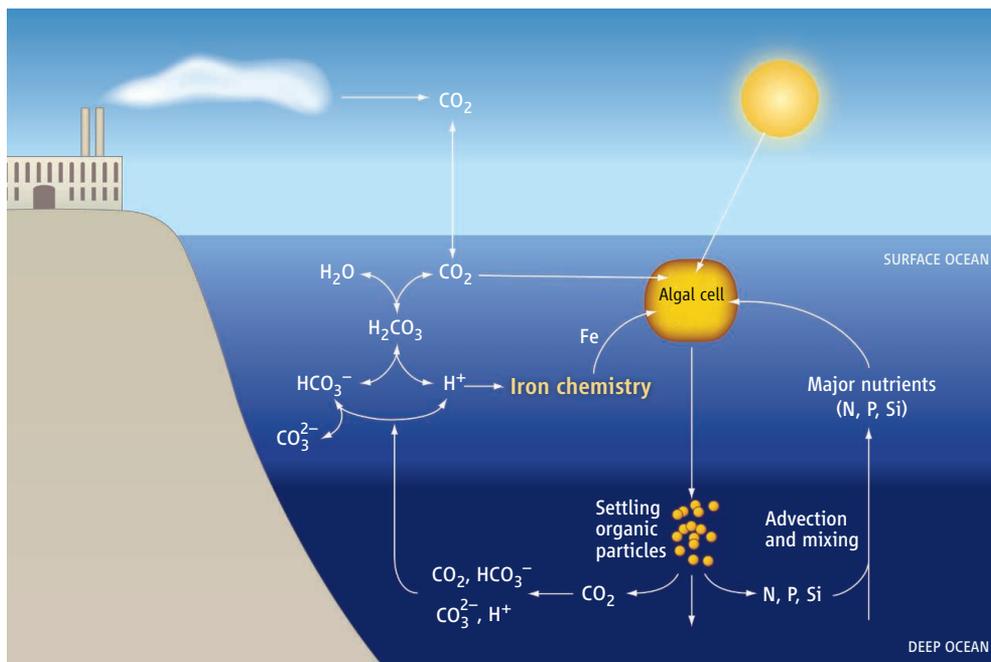
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mechanism consisting of photosynthetic fixation of CO_2 in sunlit surface waters and subsequent transfer of fixed carbon to the ocean's depths through the settling of plankton and other particulate organic material (see the figure). The settling particles are eventually respired back to CO_2 by bacteria, increasing the CO_2 concentration in the ocean's interior by a factor of 2 to 3 over that in surface waters. In large high-latitude regions, especially the Southern Ocean, phytoplankton fixation of carbon, and hence the carbon pump, is restricted by inadequate supplies of iron, an hypothesis first proposed by the late John Martin (7–9). Iron concentrations in seawater are extremely low because of the low solubility of Fe(III), the thermodynamically stable redox form of iron. Iron concentrations in the Southern Ocean are especially low (10) because of low inputs of iron-rich wind-borne dust from arid land regions, the major pathway of iron input to the ocean (11).

In a related process, iron can further regulate the ocean's carbon pump by limiting nitrogen fixation by marine photosynthetic bacteria, a process that controls the ocean's biologically available chemical forms (primarily nitrate) of nitrogen (12). Photosynthesis in much of the ocean (such as the mid-ocean gyres) is not limited directly by iron but by the availability of nitrogen (13). By limiting nitrogen fixation, iron indirectly influences the ocean's biological pump by influencing the supply of nitrogen (12).

Thus, any process that reduces the availability of iron to phytoplankton should restrict the ocean's biological carbon pump, thereby increasing atmospheric CO_2 concentrations and global warming. Shi *et al.* found that an increase in CO_2 and concomitant decrease in pH within the range expected to occur in surface seawater by 2100 decreased iron uptake by a diatom species by 10 to 20% but had no effect on the relation between specific growth rate and cellular iron concentration. By itself, this finding suggests that a lowering of the ocean water pH from increasing CO_2 may decrease iron availability to phytoplankton, thereby restricting the biological carbon pump.

But when we consider other complicating factors, the implications are less clear. The decrease in biological iron uptake with decreasing pH is believed to be largely linked to a pH-driven increase in the binding of iron by dissolved organic molecules (called



The carbon pump. Anthropogenic CO_2 emissions increase surface ocean concentrations of CO_2 and hydrogen ions, which in turn affects iron chemistry and iron-limited photosynthetic fixation of CO_2 by algal cells. This CO_2 fixation regulates the ocean's ability to absorb CO_2 by the settling of algal-derived particulate organic matter to the deep ocean. CO_2 is released into the deep ocean by bacterial respiration of the settling particles; here, it undergoes the same chemical reactions as shown for surface ocean waters. In deep ocean waters (below ~800 m depth), CO_2 has a residence time of ~1000 years.

ligands), which reduces the concentration of highly biologically available dissolved inorganic Fe(III) species. Independent measurements indeed indicate that lower pH increases iron binding to organic ligands in seawater (14). But increased organic binding should also decrease the tendency of iron to precipitate as Fe(III) oxides and to adsorb on particle surfaces—two key mechanisms by which iron is removed from seawater—and could thus increase iron retention rates and dissolved iron concentrations.

Moreover, rates of Fe(III) reduction to soluble Fe(II) also generally increase [and rates of reoxidation to Fe(III) decrease] with decreasing seawater pH (3). These changes may increase concentrations of biologically available Fe(II). In a recent mesocosm experiment with coastal seawater, a tripling of the CO_2 partial pressure over current levels increased concentrations of ferrous iron by a factor of 2, but effects on biological uptake were not assessed (15).

Finally, inputs of iron from wind-borne desert dust (11) will likely change with changing regional climate patterns, and inputs of highly soluble, iron-rich soot particles from the burning of oil and other fossil fuels may increase (16).

Thus, the availability of iron to phytoplankton in a future CO_2 -enriched Earth will be controlled by a number of complex and

poorly resolved factors, whose combined effect is uncertain. By providing new information on iron availability at low pH, Shi *et al.* help to reduce that uncertainty and provide new insights needed to refine ocean carbon cycling models. Their findings also highlight the important issue of potential future changes in iron regulation of ocean productivity and climate with increasing anthropogenic CO_2 emissions.

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