# How-To-Do-It

## Crackase and Flippase

*A Demonstration of What Happens in the Enzyme Active Site*

James E. Gaw

Students of introductory biology, especially if they have not studied much chemistry, often have a difficult time visualizing phenomena at the molecular level. Laboratory exercises with enzymes tend to relate substrate, concentration, temperature and inhibitors to enzyme activity by means of changes in pH, color or other macroscopic properties. Such experiments do not give any indication of what occurs within the active site. This article describes a simple demonstration, by analogy, of the dynamic nature of the active site which shows the specificity of every enzyme for its substrate, with regard to both binding and catalytic activity. The demonstration has been found to be effective in the author's general biology class (grades 10-11). It takes about 25-30 minutes to perform.

### Materials

The following materials can be found in most kitchens: three eggs (one is a spare); a pair of very thick mittens (not gloves); a round-bottomed bowl or dish (about 1000 ml); a food strainer or colander; a frying pan (electric is most convenient); a little cooking oil; one chopstick; and a spatula. Three differently colored shirts or jackets are also desired. You should obtain a beaker (200 ml) and about 50 ml of 6 M hydrochloric acid from the laboratory. The demonstration should be done in front of a blackboard.

### The Demonstration and Its Accompanying Commentary

The teacher tells the class that a sequence of enzymatic reactions will be modeled, using an egg as the initial substrate and a fried egg, over easy, as the final product. The teacher will play the roles of the several enzymes and writes this sequence on the blackboard:

$$\text{egg} \xrightarrow{1} \text{cracked egg} \xrightarrow{2} \text{egg in pan} \xrightarrow{3} \text{fried egg, over easy}$$

It should be stated that this sequence also represents a biochemical pathway which might be compared to, for example, glycolysis. In addition, it should be emphasized that enzymes are essential components of all biochemical pathways.

With all materials assembled and the pan starting to heat up, the teacher (wearing one of the three shirts) explains that he or she is the enzyme and that arms represent the folding of the polypeptide chain. Step one in the sequence requires that the enzyme bind the egg and crack it neatly into a bowl. The hands, thus, become the active site. Donning a pair of mittens the teacher shows how difficult it is to bind the egg and crack it neatly. So it is apparent that the mittens represent an active site that is inappropriate for binding the substrate (the egg) and catalyzing the reaction (cracking it neatly). Why? Wrong amino acids are in the active site. After taking off the mittens, it becomes apparent that ten naked fingers bind the substrate and catalyze the reaction quite well, and the egg may now be cracked into the bowl. Why do the naked fingers work so well? A different set or sequence of amino acids construct the proper active site. It is also to be noted at this point that proper folding of the polypeptide chain is necessary for the two components of the active site (the two hands) to come together. Hence, elbows very far apart represent a different or possibly a denatured enzyme. (The importance of environmental conditions, eg, pH and temperature will be considered below). A suggested name for the enzyme catalyzing step one is "crackase".

For step two, an enzyme ("transferase") puts the egg into the frying pan. For starters, the teacher alters his or her appearance by putting on a different shirt. It should be stated that crackase does not change into transferase and that transferase is a completely different enzyme, coexisting with crackase in the cell. It is clear that transferase must have a different active site, composed of different amino acids. For transferase the bowl itself appears to be an ideal active site. The bowl represents a sequence or collection of amino acids which is different from that found in the active site of crackase. Before the egg is actually transferred to the pan, teachers might ask their students whether the food strainer would represent a better active site. After the obvious response (related to binding of substrate and catalytic effectiveness), transferase pours the egg into the frying pan. Again, the hands and arms of the teacher represent the rest of the protein that is necessary for the proper functioning of the active site.

For step three, "flippase" must turn the egg over. The teacher puts on the third shirt. As before, it should be emphasized that transferase is not converted to flippase and that flippase coexists with crackase and transferase in the cell. When the egg is nearly

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ready to be flipped, the enzyme has a chopstick for the active site. It clearly fails to catalyze the reaction. Why? Inappropriate amino acids are in the active site with the wrong enzyme. The proper enzyme (the real "flippase") has a spatula in the active site composed of amino acids different (in sequence, at least) from those represented by the chopstick. This results in good binding of substrate and rapid catalysis.

At this point is should be asked whether crackase, for instance, could catalyze reactions 2 and 3. This question and its obvious answers point out the high degree of specificity of enzymes for their substrates. It should also be pointed out that because each step of a biochemical pathway involves substrates and products that are different from every other step of the pathway, the enzyme that catalyzes a given step must be different from the enzymes of all other steps. For this reason the amino acids of each enzyme's active site must be unique, as represented by the hand of crackase, the bowl of transferase and the spatula of flippase. Also, it should be stated that a given enzyme in a biochemical pathway does not function in isolation. For instance, crackase accomplishes nothing if the bowl of transferase is not present to receive the products of the first catalyzed step. In case a student asks why doesn't crackase just crack the egg into the frying pan, the teacher may respond by saying that if we suppose steps 1 and 3 occur in different places of the cell, perhaps on different sides of a membrane, then steps 1 and 3 require an intermediate step.

Teachers can conclude the demonstration by addressing the importance of environmental conditions, for instance, pH and temperature. The teacher reassumes the role of crackase and, with bare hands, attempts to bind a new egg, asking the class to imagine that the enzyme is attempting to function in a more acid or basic environment. The change in pH changes the ionic nature of the amino acids that make up the entire protein and, because of positive-positive or negative-negative repulsions, the side chains of the enzyme (the elbows) are pushed far enough apart that the amino acids of the active site (the bare hands) cannot bind the substrate. The result, a denatured enzyme, can be made more dramatic by putting a bit of uncooked egg white into a beaker containing dilute hydrochloric acid. The observable change that results from putting the egg into acid is the result of changes in the folding of the protein. As a check of the students' understanding of enzyme structure, the teacher might then ask the class whether enzymes are susceptible to the same kinds of conditions that affect egg whites.

Similarly the importance of temperature can be covered by asking the class to imagine trying to bind and neatly crack the egg with very cold hands. The fingers don't work well; at best the process slows down considerably. What about the effect of elevated temperatures? Particles move faster at higher temperatures and all components of a given enzyme, for instance, crackase, will move about more with respect to each other at higher temperatures. By moving the arms a little, it becomes apparent that at elevated temperatures the amino acids of the active site (the hands) cannot maintain the orientation and distance needed for proper catalysis. At very high temperatures, the enzyme will become denatured, which can be represented by the arms locked into such a grotesque position that the active site is totally destroyed. To make the point even clearer, the teacher may now hold up the fried egg and ask why the egg is white, in contrast to its uncooked appearance. The answer is that, as with the acid, the observable change that resulted from heating the egg is the result of changes in the folding of the protein. It should be stated that every enzyme has its pH and temperature optimum and that these conditions are the conditions that prevail in the natural environment of the enzyme.

Thus, with a few props, much adlibbing and class participation, a lot can be conveyed to students about protein structure and enzymatic activity. (It should be considered, however, that very hungry students may not be able to concentrate on the purpose of the demonstration). High school teachers of introductory biology or biochemistry may find this demonstration particularly helpful before undertaking some of the more classic enzyme/substrate experiments. The students will then have some notion of what is taking place in the active site when they observe a color or pH change. At the least, the demonstration should give students a greater appreciation for the commonly encountered textbook drawings of how the substrate fits into the enzyme.