CHAPTER 1. OVERVIEW

Many interactions in chemistry can be described using a ***lock and key*** model system. In fact, the basic mechanism for learning about biosensors is based on the lock and key model. Here we will describe the working principle for these tools.

1.1 How does a lock and key work?

The most common lock is called a cylinder lock, but other lock and key systems work with a similar process (link here). The working mechanism of a cylinder lock is known as a "pin and tumbler". This system is comprised of upper and lower pins of varying length, along with small springs (**Fig 1**). The pins within the cylinder must be in the proper position to allow the lock to open. The proper alignment for this system to open is called the "shear line". The small springs provide constant downward force against the pins. When the upper region of the pin is within the shear line area, the lock will not open. When properly aligned, the upper pins (shown in orange, **Fig 1b**) rest just above the shear line, while the lower pins remain within the cylinder. The cylinder can now turn freely with no obstructions and the lock can be opened.

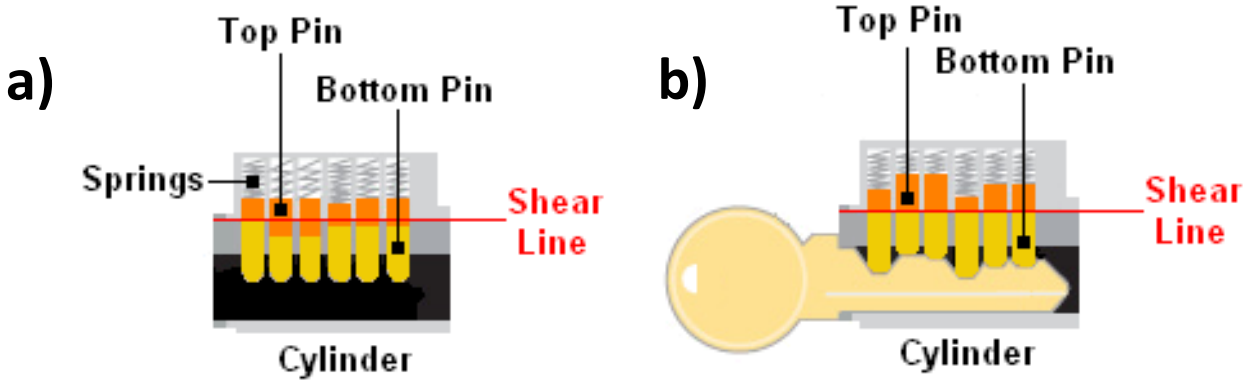

Figure 1. Simple schematic of pin and tumbler system in a cylinder lock. **a)** Locked state, where the pins overlap with the shear line. **b)** Open state, where the pins are arranged to leave a gap that overlaps with the shear line (link to reference for images is here).

1.2 The lock and key analogy in chemistry

The most common example of the lock and key analogy in chemistry is for describing general interactions of proteins and small molecules (called "ligands"); see Box #1. The analogy was used to derive mathematical equations that describe the rates of protein-ligand interactions (known as binding kinetics). In this analogy, the proteins are the "locks" and the ligands are the "keys". Protein-ligand interactions are very specific, and there are few mistakes made in biological systems. In the biological analogy of a lock and key model, the "pin and tumbler" (recall **Fig 1**) is replaced by a "binding pocket" (see **Fig 2**). As noted in the side box, some proteins can act as "reaction centers", driving reactions to take place after binding. In **Fig 2**, there are two zones of the binding pocket that are colored. The blue zone is the "binding site", where the ligand fits perfectly using a similar logic as the pin and tumbler system in a cylinder lock. Once the ligand binds, it is located next to a "reaction site", which results in the arrangement of molecules in such a way that a chemical reaction can take place.

Box #1: Protein discoveries

Proteins are organic compounds that are composed of one or more long chains of amino acids. See *Protein Assembly Activity*

Until the 1920's, the central role of proteins as "reaction centers" in living organisms was not fully appreciated, In 1926, J.B. Sumner showed that urease, a small protein known to perform chemical reactions, was in fact a protein. The next few decades saw an explosion in innovation related to protein research.

- *The first protein to be sequenced was insulin, by Frederick Sanger, who won the Nobel Prize for this achievement in 1958.*
- *The first protein structures to be solved were hemoglobin (Max Perutz, 1958) and myoglobin (Sir John Cowdery Kendrew, 1958).*
- *The three-dimensional structures of hemoglobin and myoglobin were first determined by X-ray diffraction analysis (Perutz and Kendrew shared the 1962 Nobel Prize in Chemistry for these discoveries).*

A link to the principles of biochemistry Open Source wiki book is here.

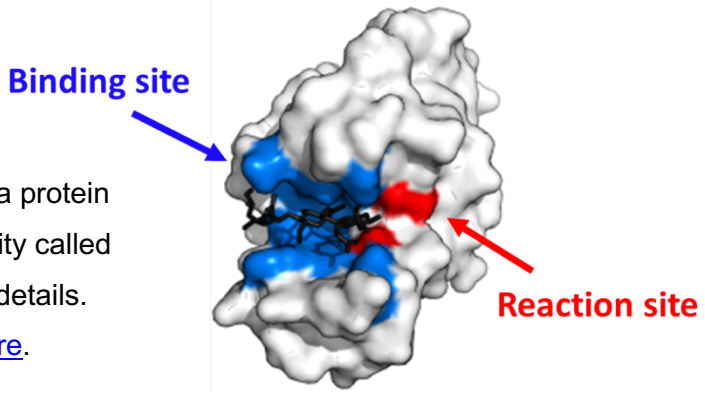

Figure 2. Ligand binding site in a protein called a lysozyme. See the activity called *Substrate Specificity* for more details. Reference for image is here.

What is a Biosensor? How does it work?

A biosensor is a tool that uses biological materials to measure a specific target such as a small molecule (sugars, gases, salts), virus, or whole cell (bacteria, algae). In a biosensor, targets (keys) bind to receptors (locks) based on the lock and key model described above. There are two things that can happen after these molecules stick together: Class #1) binding of the target by the receptor does not produce an action, or Class #2) binding of the target by the receptor produces an action that can be measured; see Box #2. In both cases, a biosensor may be developed if a change in energy state (called transduction) occurs.

Box #2: Transduction in biosensors
Transduction is defined as a change in energy state. In biosensors, transduction is the step that is used to collect a useful signal that results from *lock and key* interactions. There are two major classes of transduction in biosensors:

Class #1) Engineered transduction-In this type of device, binding of the target by the receptor does not produce an action. Thus, at least one extra engineering process must be developed to extract useful data from the sensor.

Class #2) Inherent transduction-In this type of device, binding of the target by the receptor produces an action that can be measured. Little or no additional engineering is needed to obtain data.

The process for all biosensors follows a scheme called RTA: Recognition-Transduction-Acquisition. In the lock and key RTA system (**Fig 3**, top), interaction of the lock and the *correct* key results in recognition of the key, with the pins in the lock confirming the key is correct by aligning along the shear line. The analogous process in a biosensor is when a target molecule enters the binding pocket and aligns with the binding site (recall **Fig 2**, blue highlighted region). There are multiple chemical bonds with the target that must coincide with the target, ensuring the target is the correct match (see *Substrate Specificity Kit* activity for details). The second step in RTA is transduction, when the tumbler turns in the cylinder. The analogous biosensor step is when the reaction site drives a chemical reaction involving the target (recall **Fig 2**, red highlighted region located adjacent to blue binding site region). Note that this example is for *inherent transduction*, as no additional processes are required to produce a measurable signal (see **Fig 3b** and Box #2). Also of importance is that if any of the pins in the shear line change, or if there are any changes to the binding pocket or target, transduction will not occur. In the last step, the lock has been opened and the latch is released. The analogous biosensor step is the release of the reacted chemical (called a reaction byproduct). In both cases, the lock remains intact and can be used again,

which is a very important aspect of biosensing. For an example of engineered transduction, see **Fig 3c**.

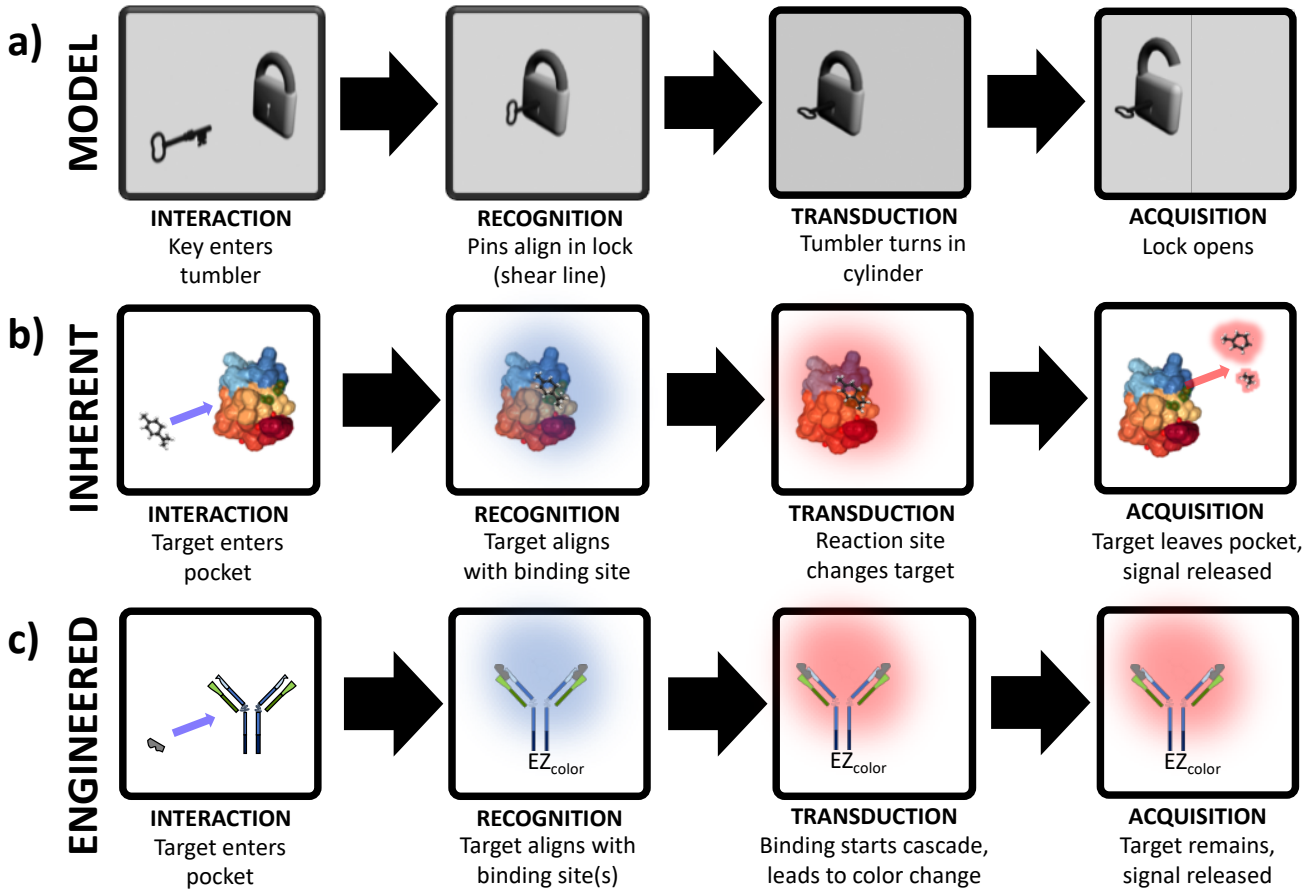

Figure 3. Lock and key analogies for biosensors. In the model system, the lock is reusable, and the key does not change physical form. The two classes of biosensor systems are more complex. For inherent transduction (middle pane), the target leaves the pocket as a byproduct, and signal is produced without addition of an extra engineering step. For engineered transduction (bottom pane), an additional engineering process (such as inclusion of a coloring enzyme, EZ_{color}) is necessary for obtaining signal.

What are the important features of a biosensor?

There are five important features of sensor performance. These include sensitivity, selectivity, response time, range, limit of detection, and hysteresis. Each of these key terms is summarized below; see the glossary for expanded definitions.

- Sensitivity: The relationship between output signal and target. The most common parameter for comparing standards, but of limited value to users.
- Selectivity: The accuracy and precision of the sensor for detecting the target. One of the most important parameters to users, but a challenging parameter to engineer.
- Response time: Defined by IUPAC as the time required for the sensor to reach 95% of the total equilibrium response to a step change in target concentration. This parameter can be highly important for users, depending on the context and type of sensor.
- Range: The range of input signals which can be converted to output signal. For moderate to advanced users, this parameter is typically of high importance. As a component of range, limit of detection is commonly calculated. This is the minimum target concentration that can be differed from background noise. Depending on the target, this can either be critically important or not meaningful
- Hysteresis: The reusability of the sensor after exposure to target. This can be one of the most important parameters for a user, particularly for sensors that require continuous use.

Box #3: Characterization of sensors
The other parameters are xx and y

According to the International Union of Pure and Applied Chemistry (IUPAC) gold book (link here), there are at least 10 key parameters for defining biosensor performance. In addition to the five described here, these include reproducibility, bandwidth, signal-to-noise ratio, drift, and stability.

What are examples of biosensors?

The two most common examples of a biosensor are: 1) a glucose meter and 2) a pregnancy test. The glucose meter is a biosensor that uses a protein (called GOx, or glucose oxidase) extracted from a mold (called *Asperillus niger*). The protein has a lock and key interaction with glucose in blood, and is highly specific for glucose only (i.e., not fructose, sucrose, maltose, or other sugars). The interaction produces an electrical signal that is recorded and used to calculate the total amount of glucose in the sample (1 glucose molecule=2 electrons) (**Fig 4**). The glucose meter uses inherent transduction based on the activity of the oxidase protein, and provides a small potential to drive the reaction faster than would normally occur.

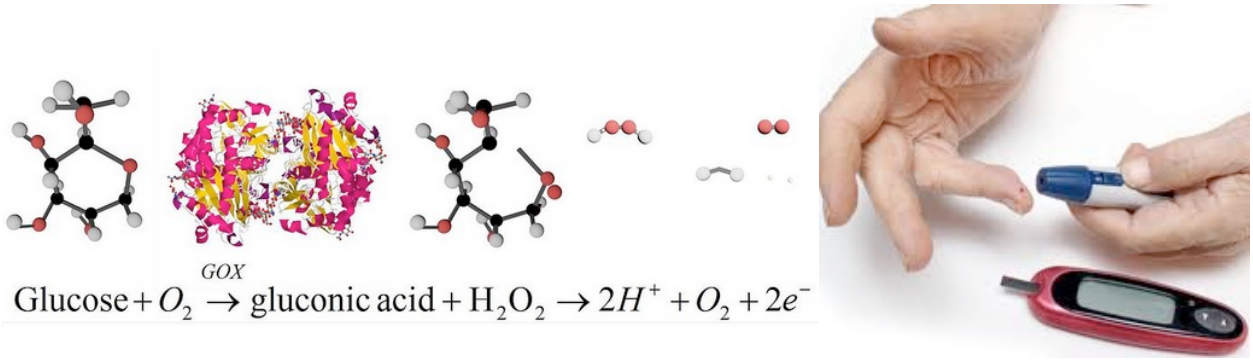

Figure 4. The glucometer is an electrical device that serves as an example of a biosensor with inherent transduction.

The pregnancy test uses a lock and key mechanism between a protein found in mammals (called IgG, or immunoglobulin) and a marker in urine found in pregnant women (called hCG, or human chorionic gonadotropin). The image below shows the working mechanism for a modern pregnancy test (**Fig 5**). The pregnancy test uses engineered transduction and depends on a coloring enzyme to produce the device output after the hcG and IgG bind.

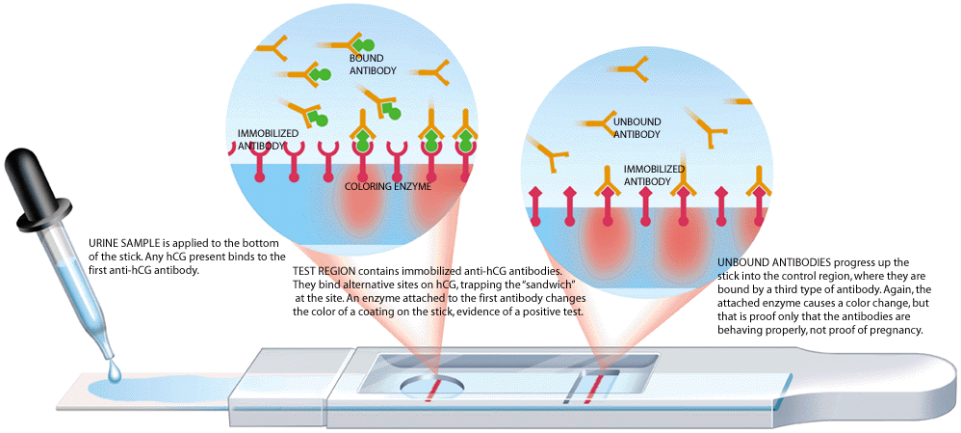

Figure 5. A pregnancy test is an optical device that serves as an example of a biosensor with engineered transduction. A secondary coloring enzyme is used to provide the color change that indicates binding.

In this activity book, we provide individual exercises (each approximately one half day) for youth ages 6th grade through 12th grade. Many of the activities are also applicable to higher education, but the more detailed scientific principles are not covered herein. For more details

regarding application of the activities in advanced high school, or higher education contact Eric McLamore at emclamore@ufl.edu.

Key terms from Chapter 1 (see glossary for definitions):

ligand, protein, kinetics, binding pocket, binding site, reaction site, biosensor, receptor, transduction, engineered transduction, inherent transduction, acquisition, sensitivity, selectivity, response time, range, limit of detection, hysteresis, glucose oxidase, immunoglobulin.