Although we could not see or feel inside the box, several groups came up with the same hypothesis about the connections in the box. This is drawn below.

In summary, we decided that the box contained a direct connection between $a$ to $d$, $b$ to $f$ and $c$ to $e$, but that $a$ to $d$ had a loop. In some boxes, there was something blocking the flow of $f$ to $b$, more so than $b$ to $f$. That is, whereas 100% of the time, 100% of the balls could move $b$ to $f$ in the TOP-UP state, the reverse was not true for the BOTTOM-UP state.

If this system was very important to a biologist, they may wish to model it, even though they have no real data or detail on the connections within the box. What they want to model is their understanding of the connections between the letters shown above. The objectives would be to test whether the hypotheses of what is in the box are supported when implemented into a model and the model output compared to the “biological” data. A mechanistic model is therefore required.

What should such a model consist of and how do we do it?

We need to start asking some questions about the property of the network. **What are the objects we need to represent in the network?** Are they simply $a$, $b$, $c$, $d$, $e$, and $f$? How would we deal with the “valve” between $b$ and $f$ and the loop between $a$ and $d$?

The answer is that we should consider the valve and the loop to be objects in the system even though we have no data for them. They are important features of the system and affect its properties.

How should we represent the loop? To answer this question, we need to consider **how we are going to describe the relationships between the objects** in the system?.

Are the relationships between the objects always the same? They are always connected to each other in the same order. But the flow between objects is determined by the orientation of the box, or on the **state of the system** (which side of the box is facing up?). Furthermore, the flow of contents among different areas of the loop depends on the state of the system. The complexity arises from the properties of the objects changing for each state of the system (orientation of the box).

Although the loop is continuous, the model objectives are to achieve a largely qualitative rather than strictly quantitative output and therefore the different parts of the loop can be considered as discrete compartments rather than as a continuous connection. In order to represent the flow in the loop, the loop therefore needs to be divided into parts that represent arbitrary compartments in the loop. As shown in the figure below, 5 compartments (objects) $h$, $i_1$, $i_2$, $i_3$ and $j$ are sufficient to describe all
parts of the loop within the box. Parts \( h \) and \( j \) are included because the balls often rested at that point rather than going directly from \( i1 \) to \( d \) or \( i3 \) to \( a \).

\[
\text{Top} \\
\text{Left} \quad \text{h} \quad \text{j} \\
\text{a} \quad \text{d} \\
\text{b} \quad \text{g} \quad \text{e} \\
\text{c} \quad \text{f} \\
\text{Bottom}
\]

How are we going to implement these concepts into a model? One possibility is to use a Boolean network to describe the state of the objects as containing or not containing balls. That is, on or off (1 or 0). Will that allow us to account for intermediate values, such as when only half the balls move as in from \( f \) to \( b \)? Not easily. This may be important for the case of the valve or if the box is flipped from top to bottom up and the contents are split between objects (compartments). This would be the cases of if the box was flipped from top to bottom.

As the hypothesis considers that the tubes are continuous, we could represent flows in and out of the compartments using differential equations. This is not necessary as the system will quickly reach a steady state that can be represented with simple addition and subtraction and this also corresponds to the timeframe in which the data was collected (after contents had stopped moving).

We could consider the loop as a series of compartments that can only transfer balls to adjacent compartments. That is, when the box is turned from the topside up to the left side up, we could capture the mechanism that the contents move from \( a \) to \( h \) to \( i3 \) rather than simply stating that \( i3 \) becomes equal to \( a \), by explicitly representing the structure of the compartments in the model. Time permitting, an L-system model will be presented to you in class that describes this approach.

An alternative approach to the approach above is to consider the mathematical relationships among the components in each state of the system rather than relying on representing the physical structure of the system.

For example, if Top is up then \( c \) will be it’s value at the last step, plus the value of \( e \); and \( e \) will become 0. As flow through \( g \) is uninhibited in this orientation, then \( f \) becomes it’s value at the last step + \( g \) + \( b \); and \( b \) and \( g \) become 0. In contrast, if Bottom is up, then we need to consider the valve. Lets say that only half the contents that reach the valve at a given rotation step can move through \( g \). On this basis \( b \) becomes 0.5 x (\( g \)+\( f \)); \( g \) becomes 0.5 x \( f \) and \( f \) becomes 0.

Assessment Requirements
1. Write a list of hypotheses that describe the system and can be used to achieve point 2 below.
2. Write a rule-based mechanistic model that describes the simple relationships between the components for each state of the system.
3. Prescribe an initial state of the box with the tubes facing down with all the balls showing at the bottom and print results for the contents of each compartment at each step of turning the box 6 times to the left and 6 times to the right.