Model an ELISA

1. Print these pieces on a color printer or color your own (p2)
2. Fit them together like a puzzle
3. Align the different pieces to the steps of an ELISA (see last slide in deck for explanations)
4. Discuss how this type of “puzzle” might work in something like a pregnancy test or rapid flu test (and why we don’t have one available yet for COVID19)

Assembled ELISA

(the answer key & explanation for this puzzle)

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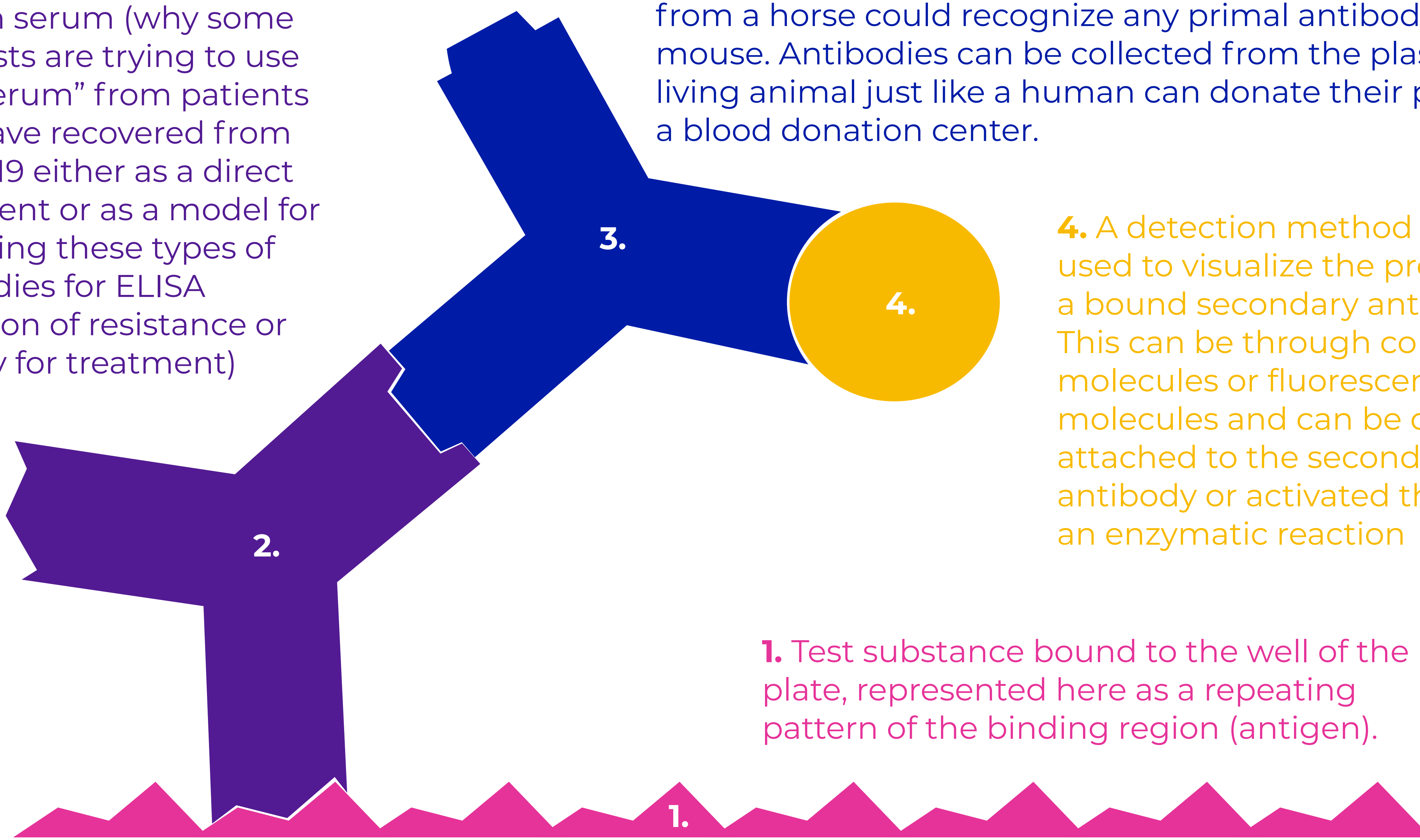
2. Primary antibody has recognition sequences for the antigen. Primary antibodies can come from human serum (why some scientists are trying to use “anteserum” from patients who have recovered from COVID19 either as a direct treatment or as a model for designing these types of antibodies for ELISA detection of resistance or directly for treatment)

3. Secondary antibody has recognition sequences for the stem of the primary antibody. Usually secondary antibodies are designed to generally recognize any antibodies made by a particular other species .e.g a secondary antibody from a horse could recognize any primal antibody from a mouse. Antibodies can be collected from the plasma of a living animal just like a human can donate their plasma at a blood donation center.



4. A detection method must be used to visualize the presence of a bound secondary antibody. This can be through colored molecules or fluorescent molecules and can be directly attached to the secondary antibody or activated through an enzymatic reaction

1. Test substance bound to the well of the plate, represented here as a repeating pattern of the binding region (antigen).

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ELISA explained

Step-by-step

Enzyme-Linked ImmunoSorbent Assay: This is a test (assay) that detects an adsorbed (sorbent) substance using detection similar to one's immune system (immuno) and is detected by these immune system molecules—antibodies—being bound to an enzyme that makes a substance change color and thus can be seen with our eyes.

How an ELISA actually works:

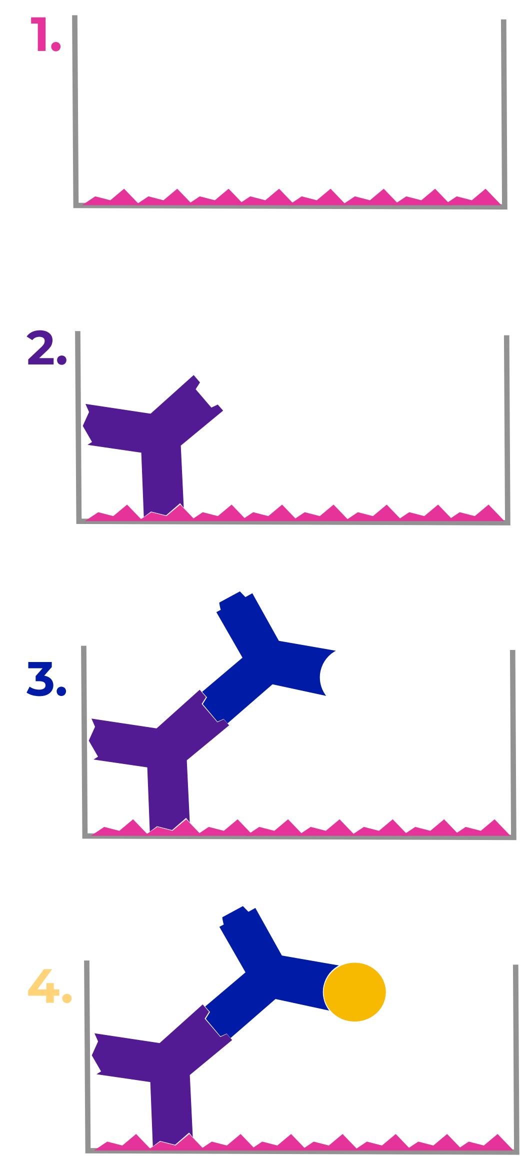
Step 1: A plastic well (usually the size of your pinky nail or smaller) is coated with the molecule you want to study (e.g.). Proteins often stick to plastic, it turns out.

Step 1b: Use a boring protein that we have a lot of to stick to all of the rest of the plastic.

Step 2: Add an antibody that will specifically recognize and bind to the molecule you're interested in. If it's there, they bind and the antibody stays stuck in the well. If they don't bind, it will get washed away in the next wash steps.

Step 3: Add another antibody that is bound to an enzyme that can specifically make a molecule change color. This antibody should recognize the general class of antibodies you used in Step 2 (often this is based off of the species of animal used to make the antibody—they're not just in people!).

Step 4: Add the color-changing substance. If the right molecules are present, the enzyme will cause a color change and give you a positive result. No color change means the molecule was not present.



What is an antibody?

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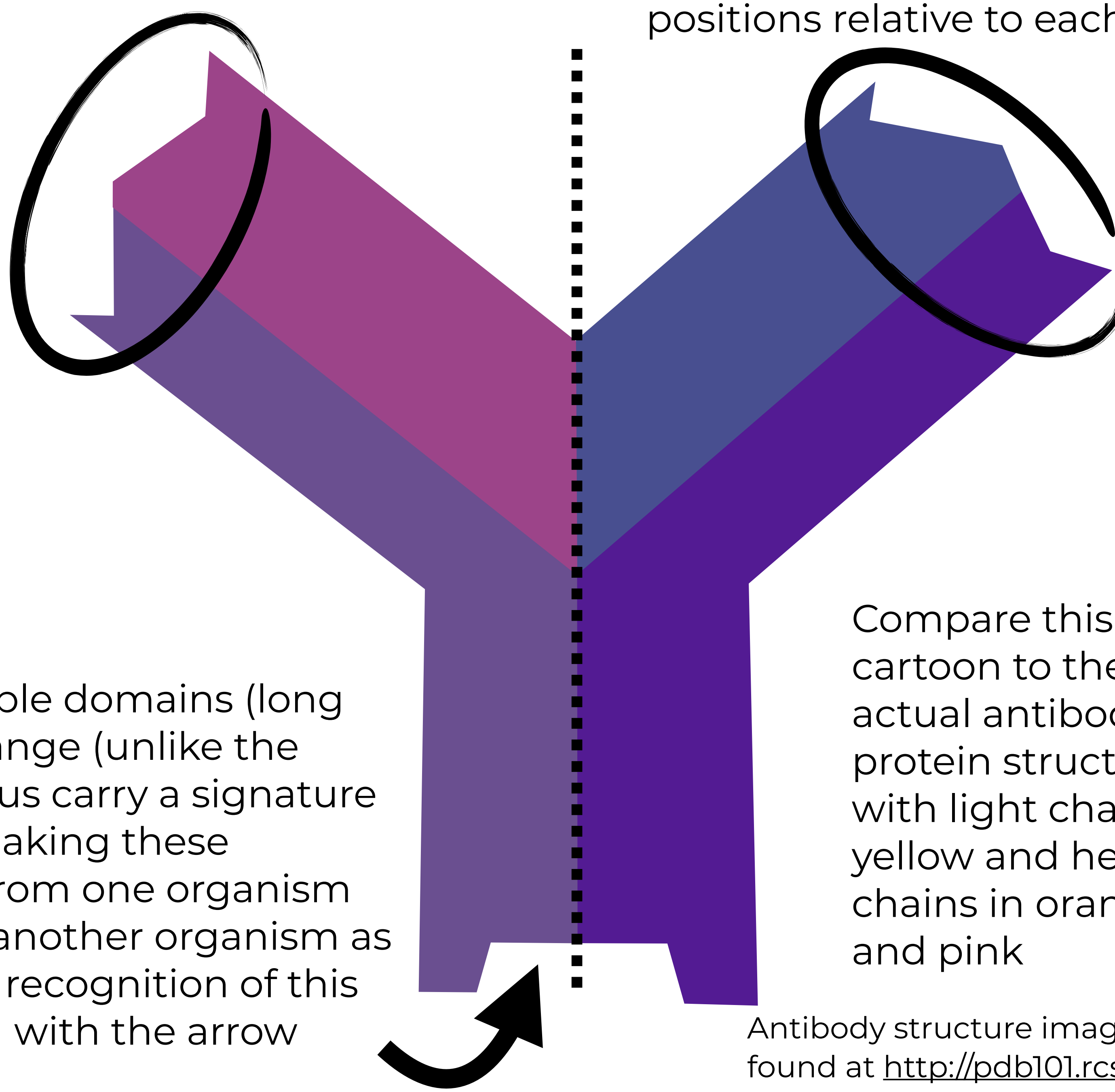
An **antibody** is a molecule made by mammals (including humans) immune systems as a way to recognize and initiate a defense mechanism within our bodies

Antibody symmetry: an antibody has mirror-image symmetry (as if this dotted line was a mirror); while the domains actually twist around each other more than this model shows, the diagram gives you a sense of the different components and their positions relative to each other

Binding domain: changes easily and our bodies make many combination (like carrying a ring of keys that might potentially open doors). Each antibody contains two identical binding domains, each one circled in our model antibody



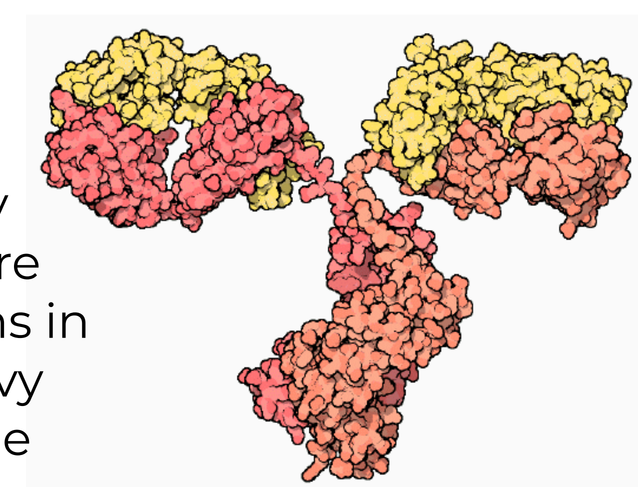
The components

●● (purples) Two heavy chains extend the whole length of the antibody

●● (pink/blue) Two light chains connect to the binding-end of each heavy chain

Antibody stem: the purple domains (long chains) do not easily change (unlike the binding domain) and thus carry a signature for the host organism making these antibodies. Antibodies from one organism detect antibodies from another organism as foreign, usually through recognition of this stem region highlighted with the arrow

Compare this cartoon to the actual antibody protein structure with light chains in yellow and heavy chains in orange and pink



Antibody structure image & more antibody resources can be found at http://pdb101.rcsb.org/learn/paper-models/antibody