**What is Immunohistochemistry?**

IHC is a popular technique used to determine the existence and abundance of target molecules in tissue. A protein or other antigen of interest can be visualized via microscopy by the binding of a series of antibodies. In some cases, multiple proteins can be probed simultaneously to assess their colocalization.

**Antigen Retrieval**

Fixing brain slices with paraformaldehyde can cause cross-linking that obscures binding sites for your protein of interest. Heating slices in a high pH citrate buffer releases these bonds.

**Blocking**

No antibody binds perfectly. Blocking with serum helps to prevent non-specific binding and lowers background signal. *Make sure to match your serum host to the secondary host*

**Primary Antibody**

Specifically binds to your protein of interest. Often there can be multiple antibodies for the same protein, so make sure to pick the one that can be used for IHC.

**Secondary Antibody**

A more general antibody that binds to any primary sharing the same host. It is attached to a reporter molecule that aids in visualization of the antigen. We have two main types:

- **Fluorescent tagged**: light sensitive and will fluoresce at a specific wavelength during imaging.
- **Biotinylated**: conjugated to biotin, which has many binding sites and is used to amplify signal when imaging. Further steps are needed to visualize, often a fluorescent tertiary or DAB staining.

**DAB Staining**

After application of a biotinylated secondary, the slices undergo a further incubation to form a complex between biotin and an avidin detection enzyme. When the chemical substrate DAB binds to our detection enzyme, it causes a color change, darkening where the antigen is detected. This non-light sensitive method allows for images to be taken via light microscopy.