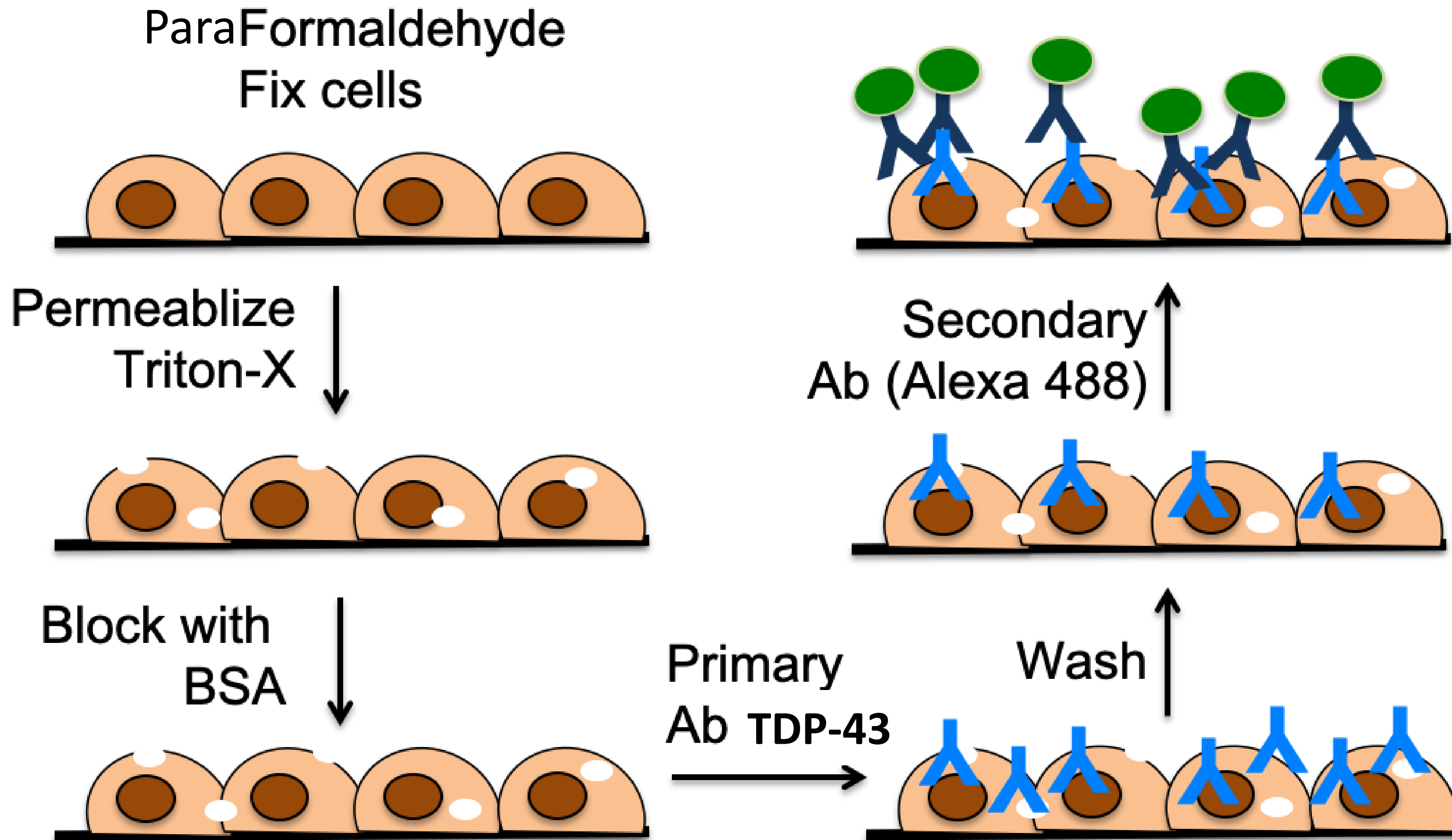
Using immunofluorescence (IF): steps in protocol



Fixation with paraformaldehyde (PFA) covalently crosslinks proteins

Healthy Cell	Fixed Cell
Proteins are in flux	
Cell is alive	
Cellular structures are mobile	
Cellular processes are active	
Cell membranes are pliable	

Fixation with paraformaldehyde (PFA) covalently crosslinks proteins

Healthy Cell	Fixed Cell
Proteins are in flux	Proteins are crosslinked
Cell is alive	Cell is dead
Cellular structures are mobile	Cellular structures are immobile
Cellular processes are active	Cellular processes are halted
Cell membranes are pliable	Cell membranes are rigid

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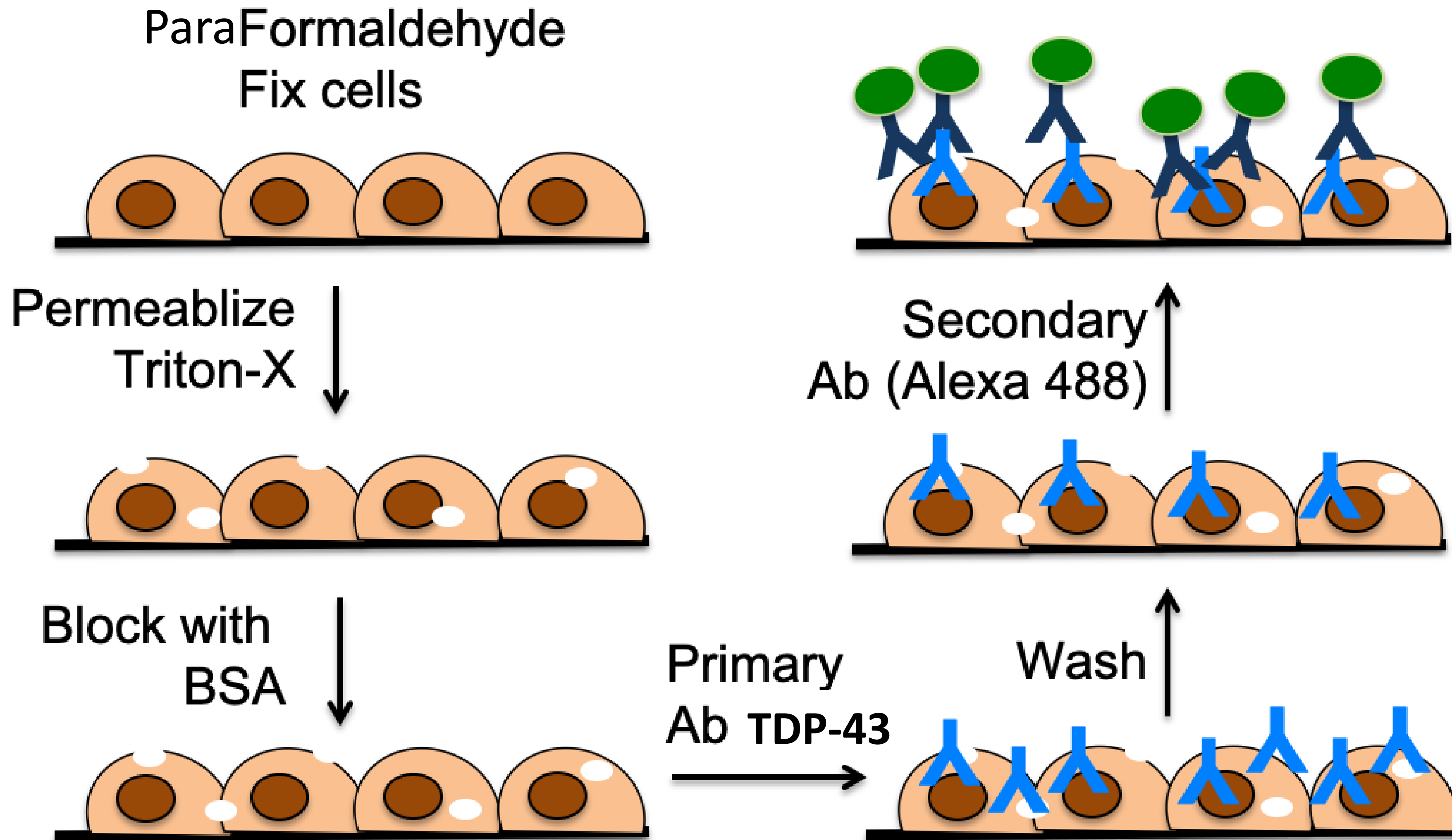
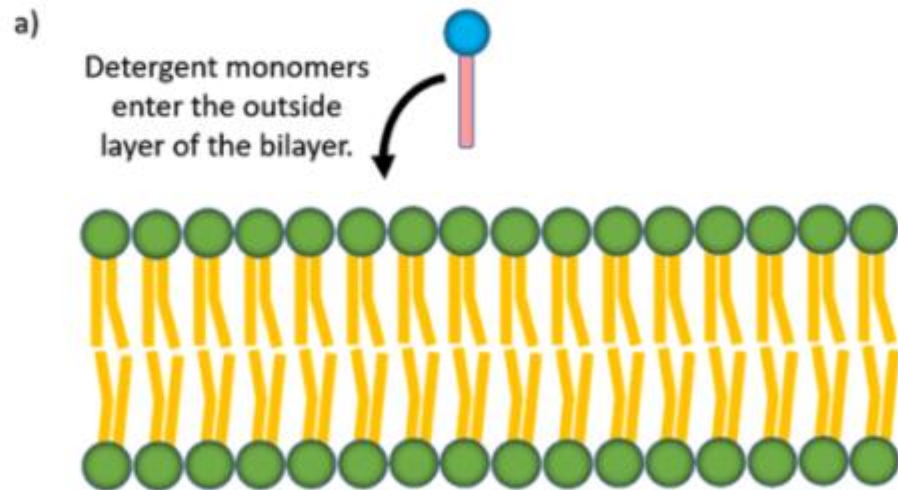
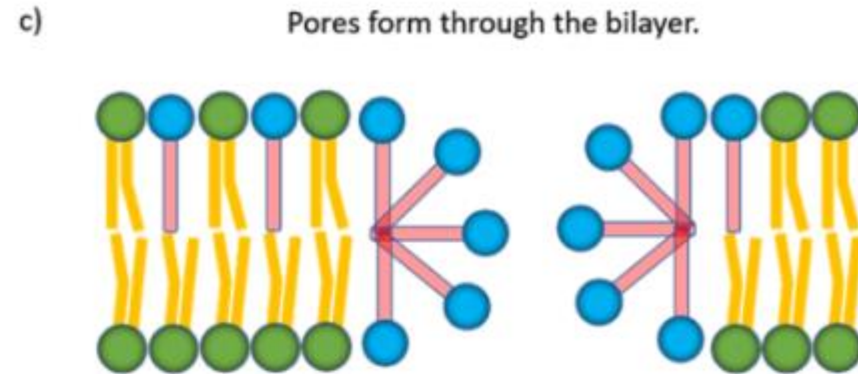


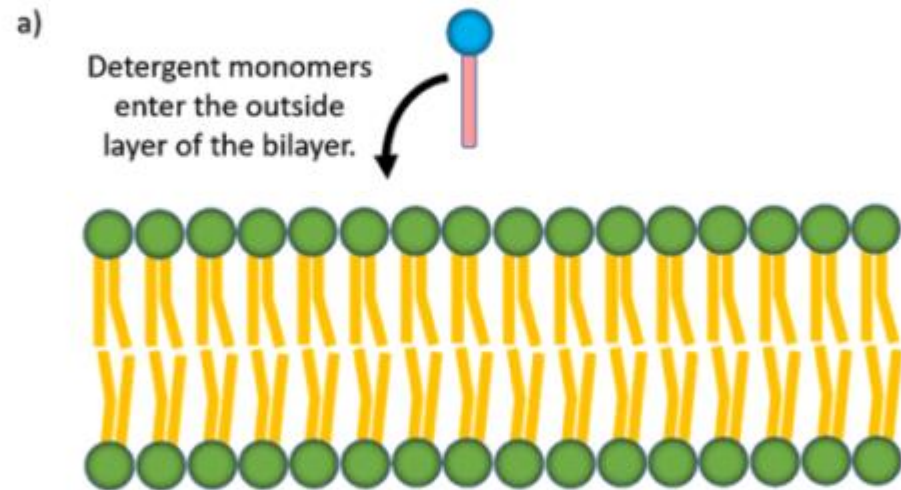
Triton-X blows holes through the membrane

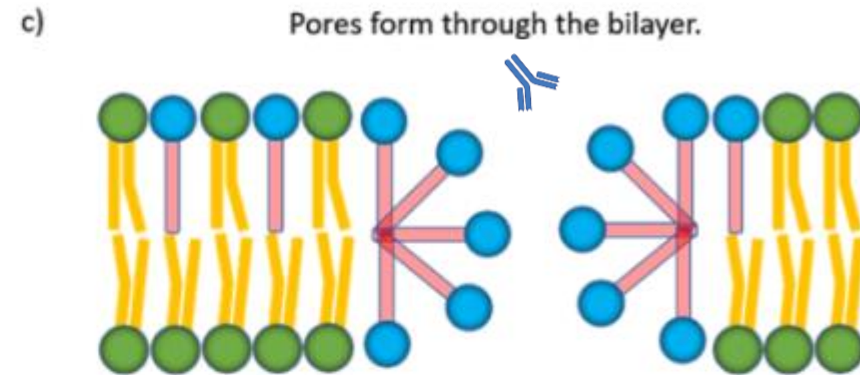




(Life Canvas Technologies)

Permeabilization allows our antibodies in





(Life Canvas Technologies)

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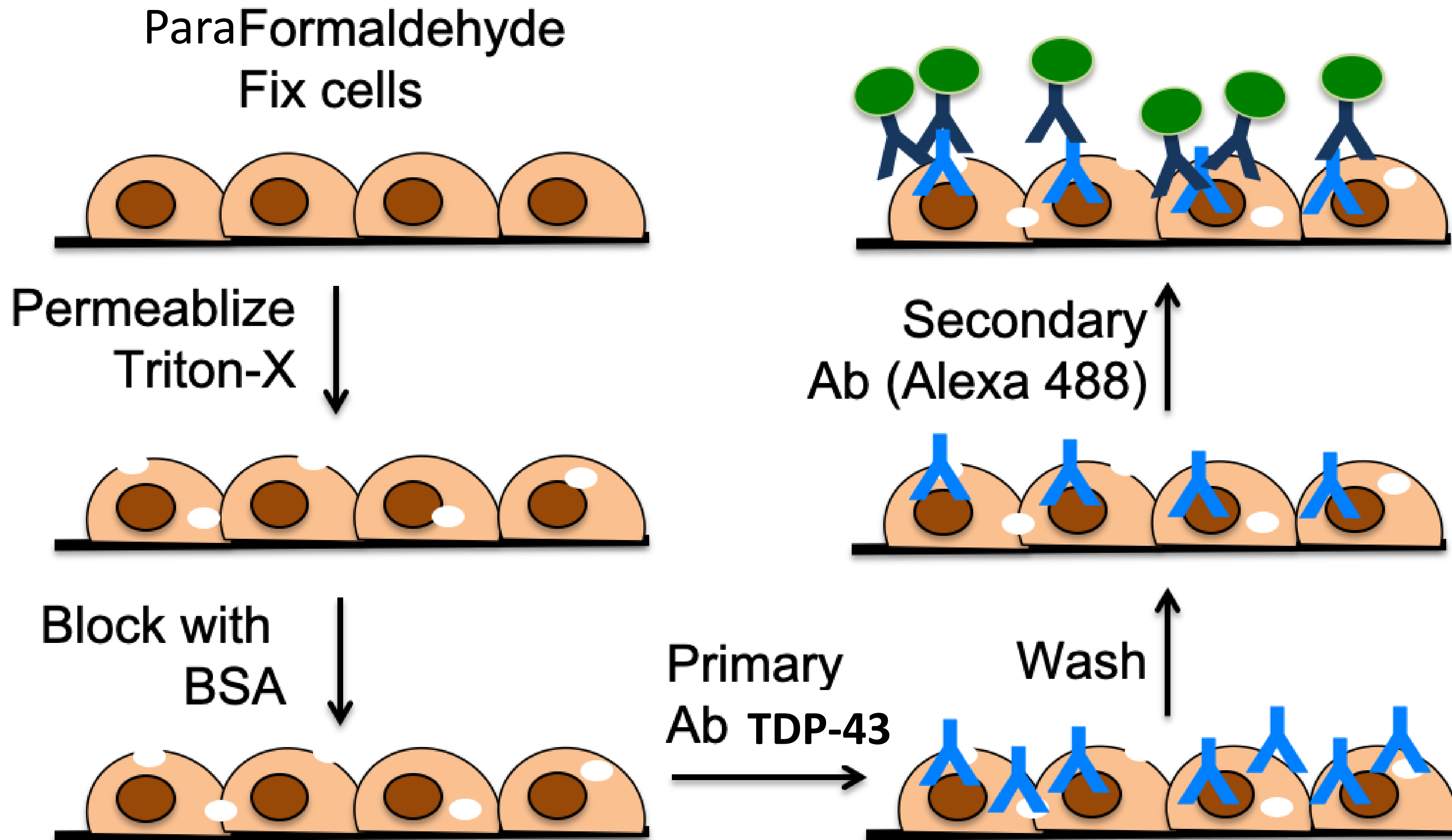
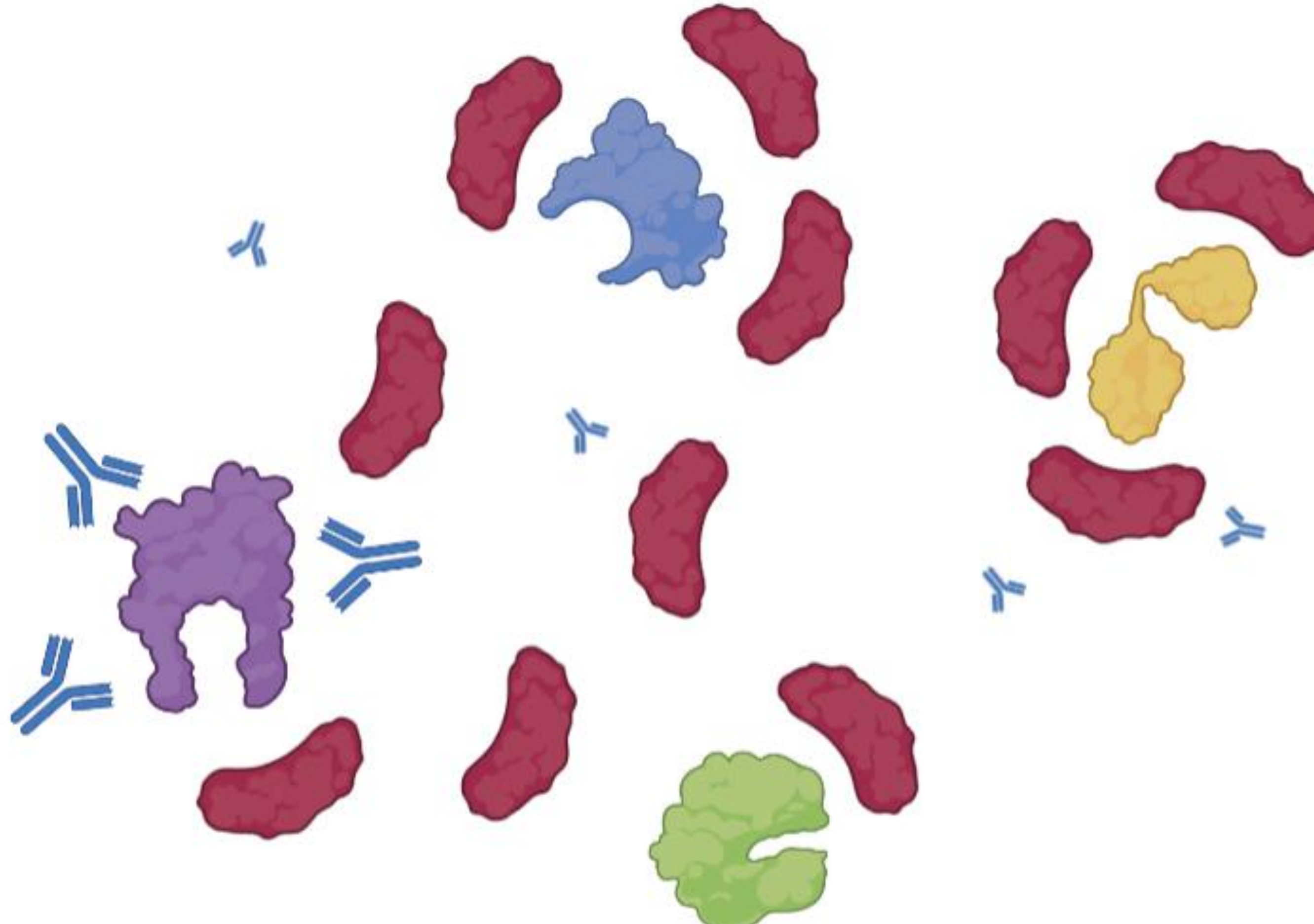


Blocking with BSA reduces non-specific antibody binding

No Blocking

Blocking with BSA reduces non-specific antibody binding

With Blocking



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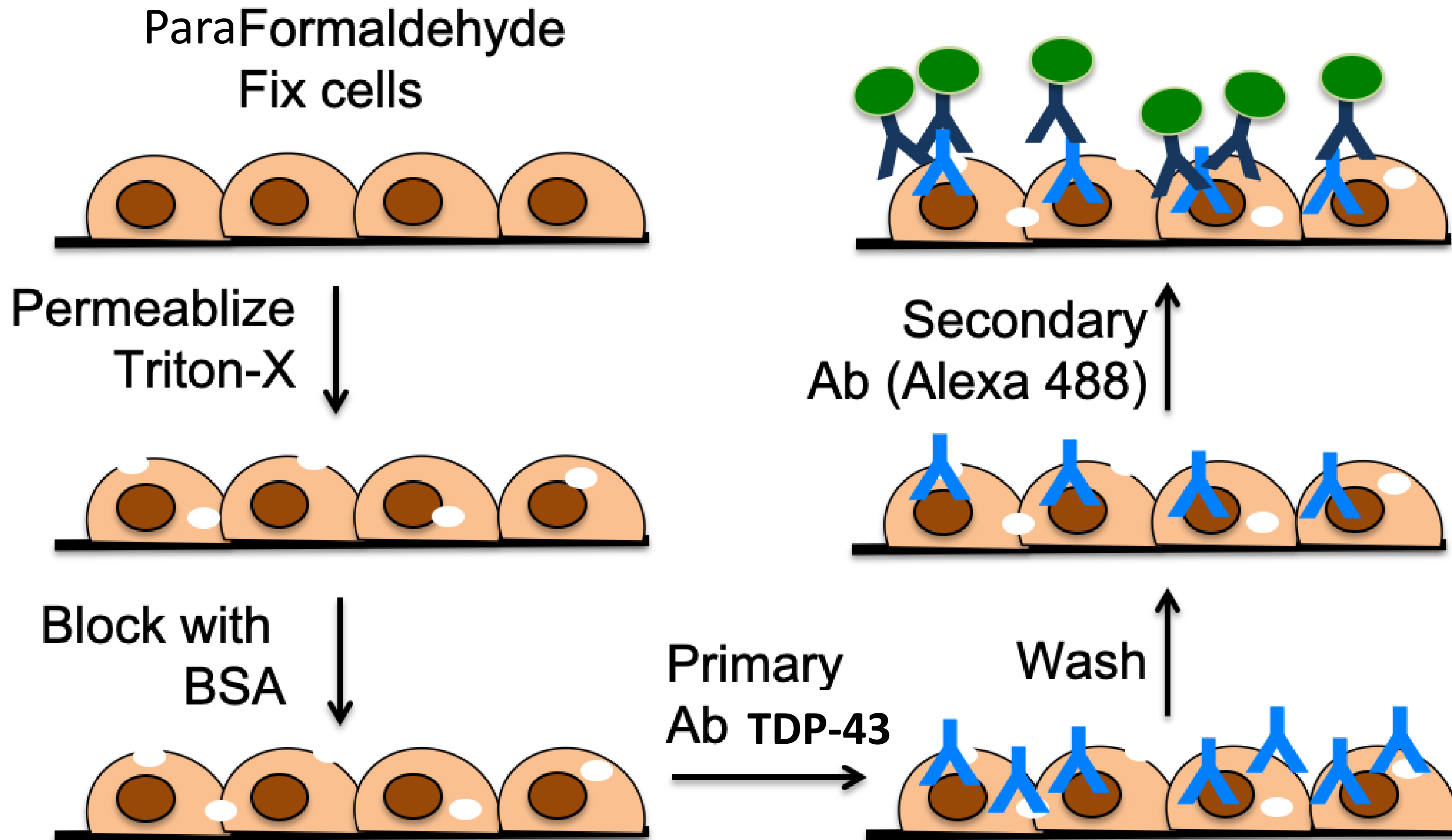
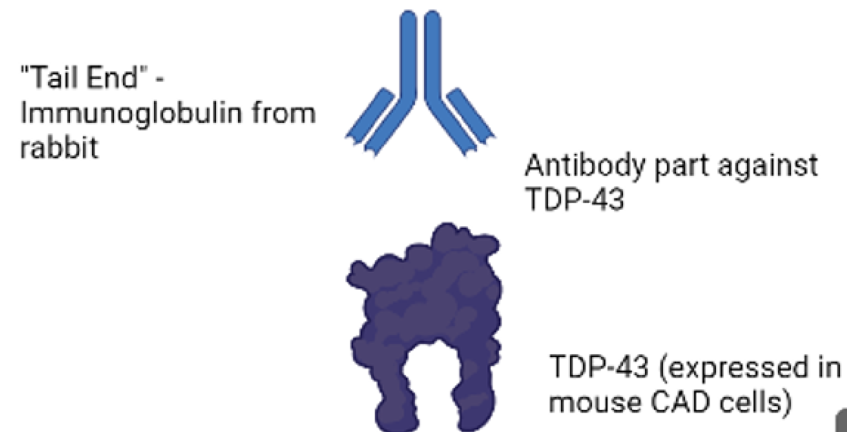
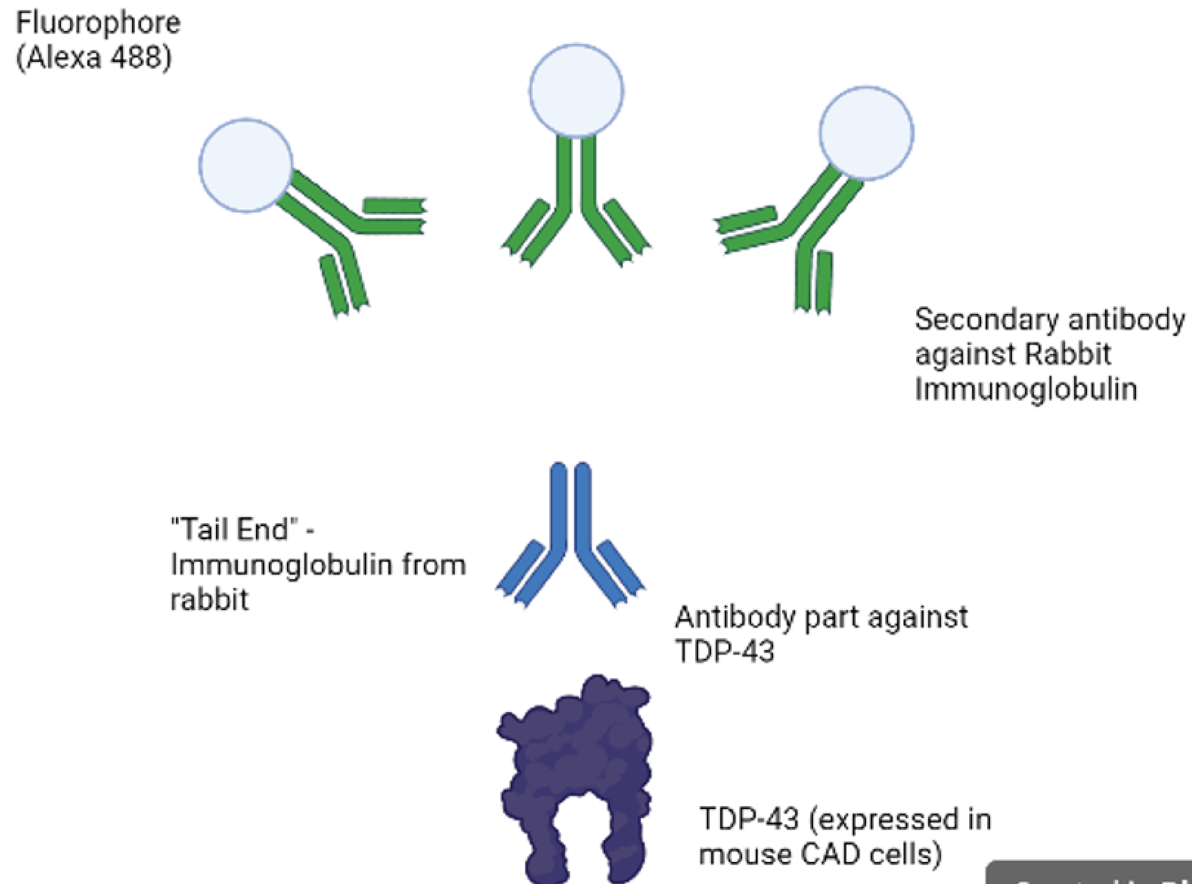


Primary antibody binds to protein of interest



Secondary antibody binds primary antibody

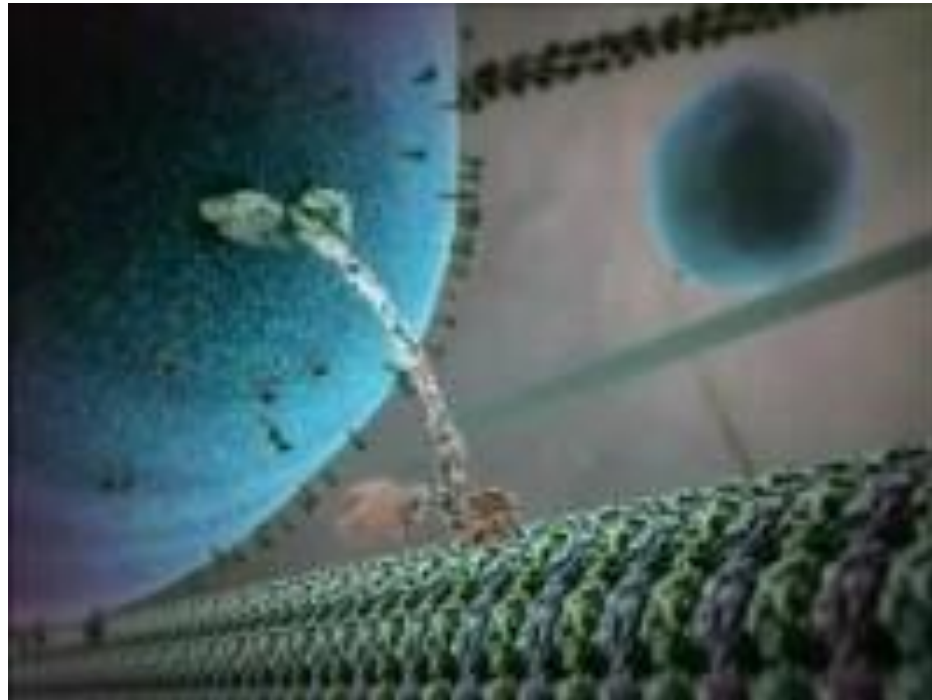


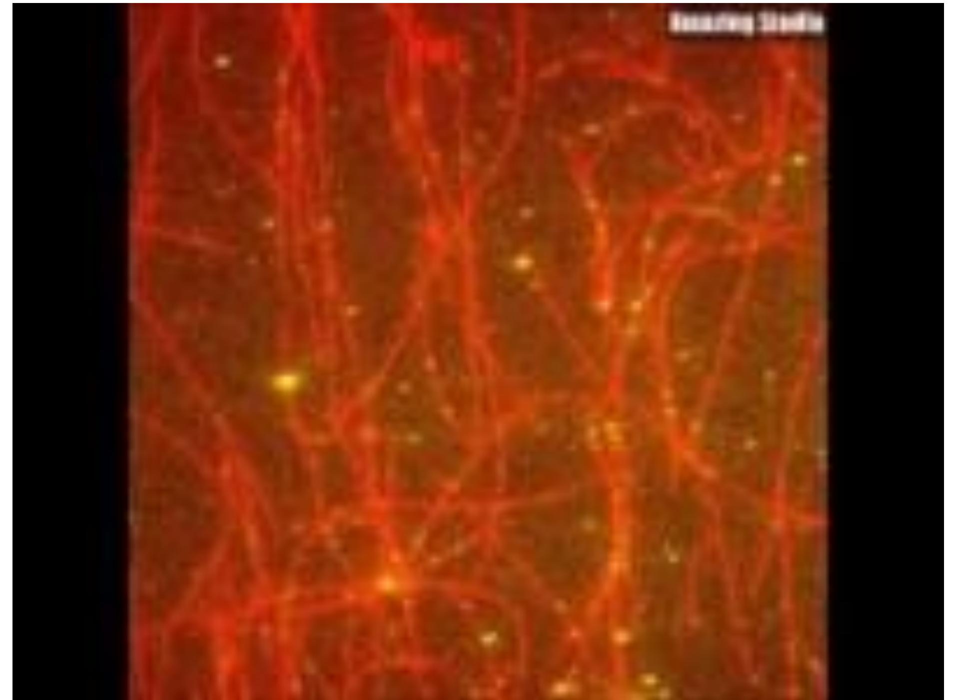
A couple of very good questions came up…

1) Why are we not imaging live cells?

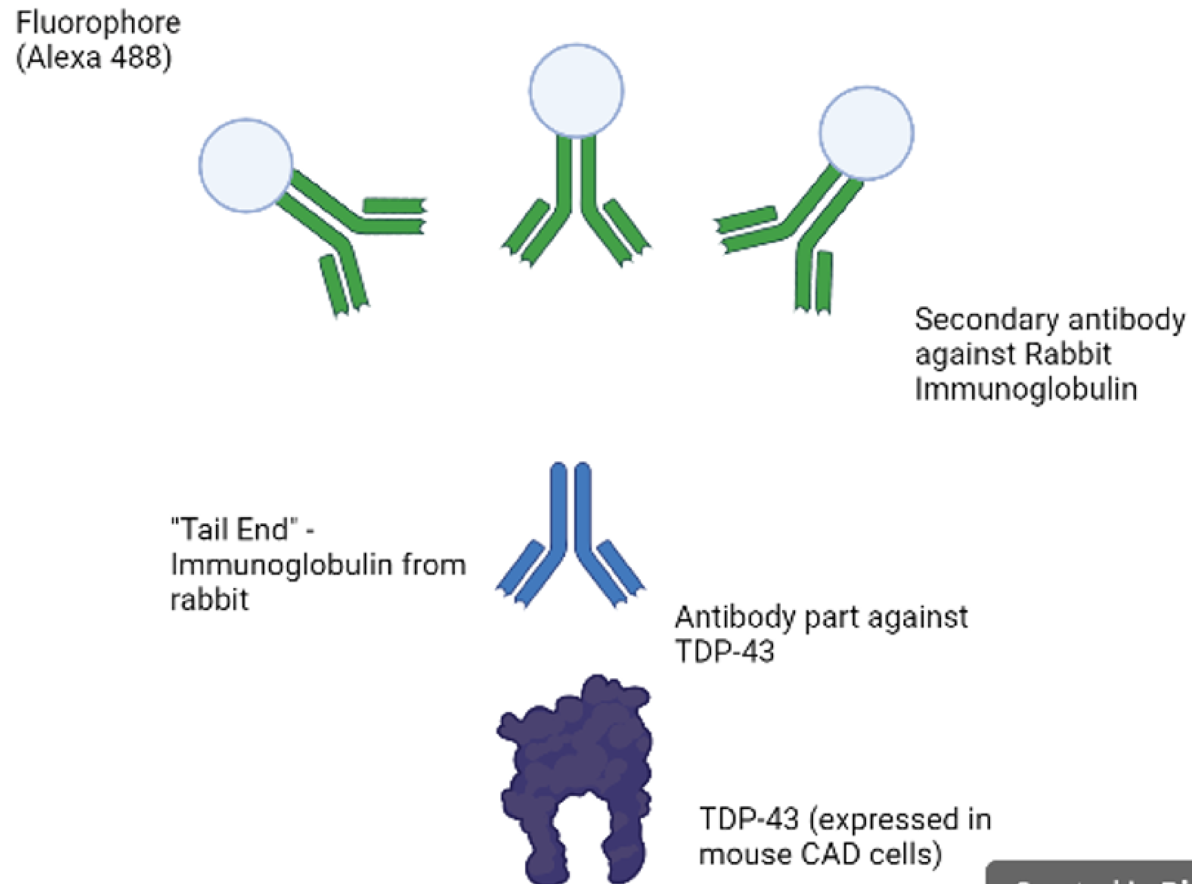
2) Why not use a primary conjugated to a fluorophore? Why use secondary at all?

Live imaging is fantastic – but how would you label what you need to label?

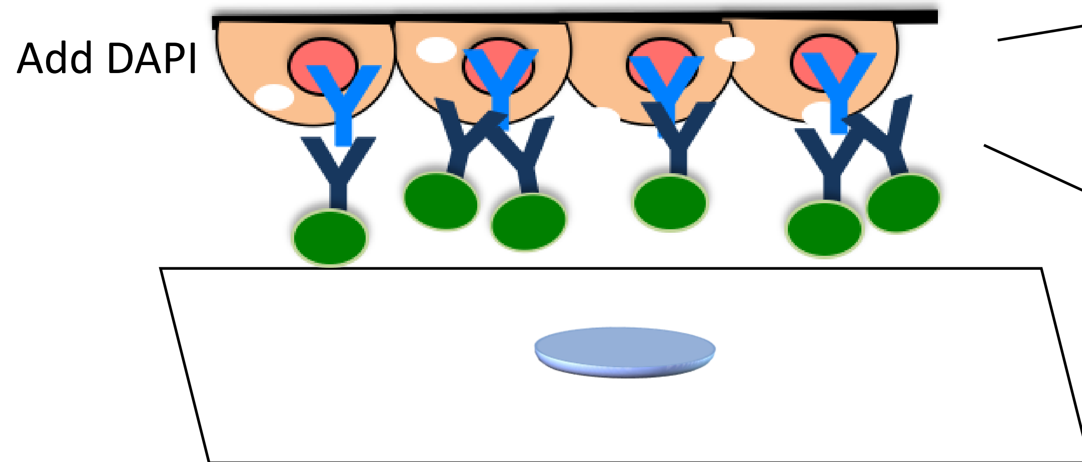




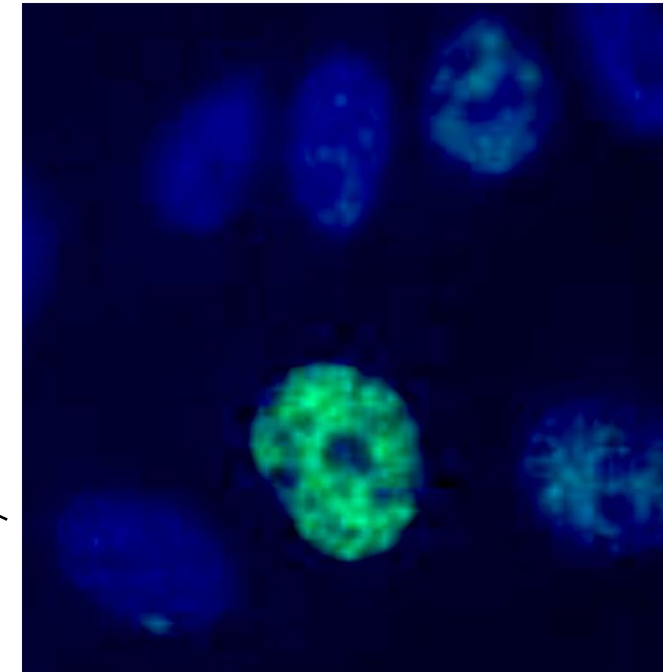
Secondary antibodies can *amplify* signal



Finish IF by adding DAPI, then mount slides for imaging



Mount coverslip on glass slide
with mounting media



Blue= DAPI
Green= antibody staining

Later on, I have a fun data analysis story regarding teaching my cat how to sit