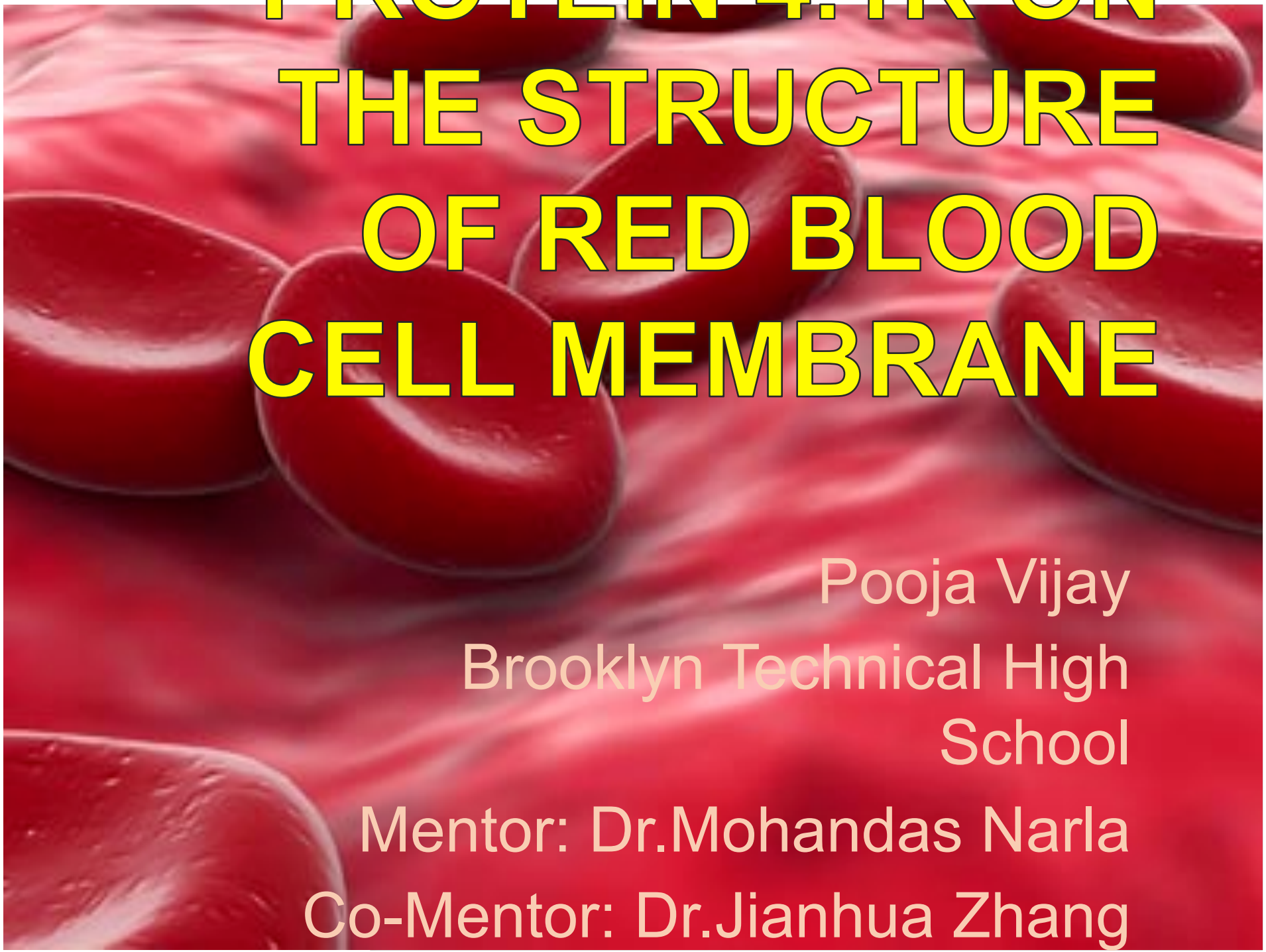
PROTEIN 4.1R ON THE STRUCTURE OF RED BLOOD CELL MEMBRANE



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WHAT WE'RE CURIOUS ABOUT

- Red blood cells perform the most important blood duty. A single drop of blood contains millions of red blood cells which are constantly traveling through your body delivering oxygen and removing waste. If they weren't, your body would slowly die. Because of this, An essential attribute of the red cell is its

WHY WE'RE DOING THIS RESEARCH…

- It has become clear that the simple model of red cell membrane organization, which endured for so long based on an irreversibly assembled membrane skeleton and a population of

THE HYPOTHESIS…

- We suggest that 4.1R protein organizes a macromolecular complex of skeletal and transmembrane proteins at the junctional node and that perturbation of this macromolecular complex

4.1R PROTEIN..

- Protein 4.1R (4.1R) is a multifunctional component of the red cell membrane. It forms a ternary complex with actin and spectrin, which defines the nodal junctions of the membrane-skeletal network, and its

WHAT WE ALREADY KNOW…

- By performing various western blots,We have previously shown that the membranes of mouse red cells lacking 4.1R have greatly impaired shear resistance.The same is true of human 4.1R-deficient cells. This is because we now know that deletion of 4.1R in mouse red cells leads to a large diminution of actin accompanied by extensive

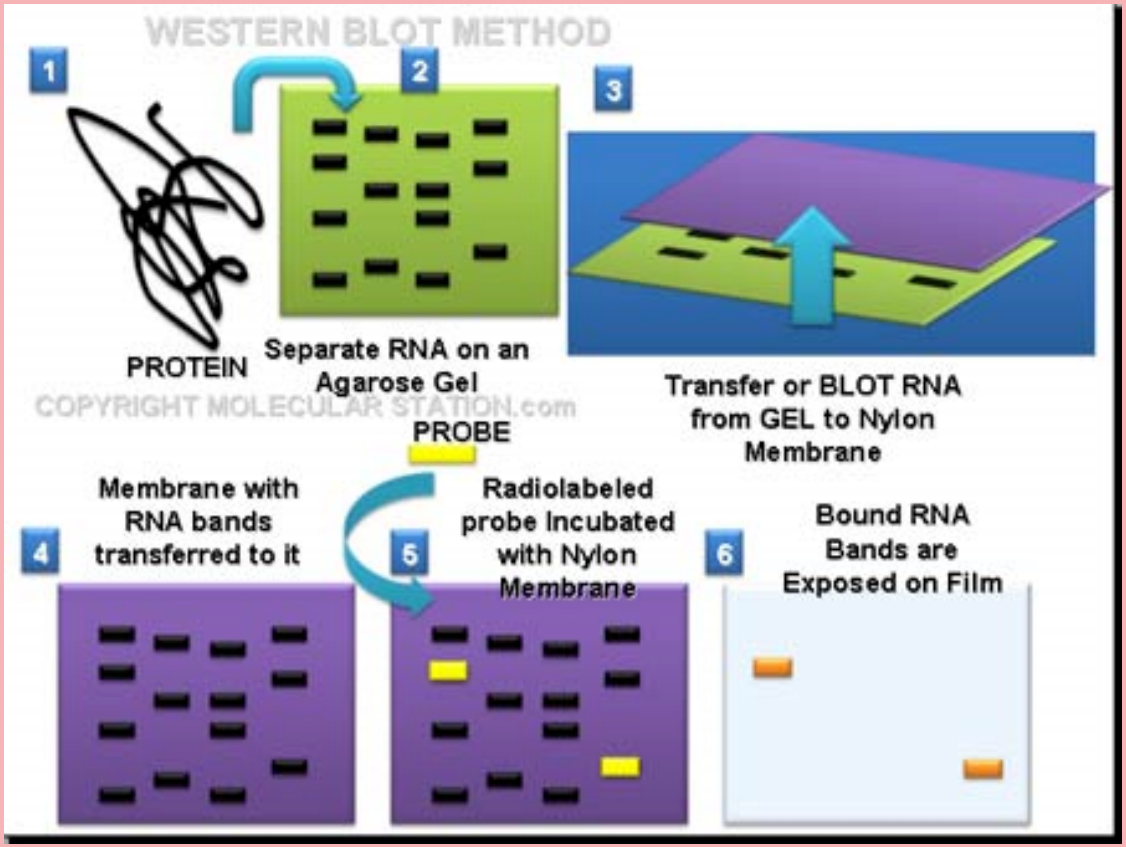
MATERIALS

- A SulfoLink kit
- A Cholesterol Quantification Kit
- pMAL vector
- MBP resin
- monoclonal anti-MBP antibody
- a DC Protein Assay Kit
- SDS/PAGE
- Electrophoresis reagents
- SuperSignal West Pico chemiluminescence detection kit
- HRP-conjugated anti-mouse IgG and HRP-conjugated anti-rabbit IgG

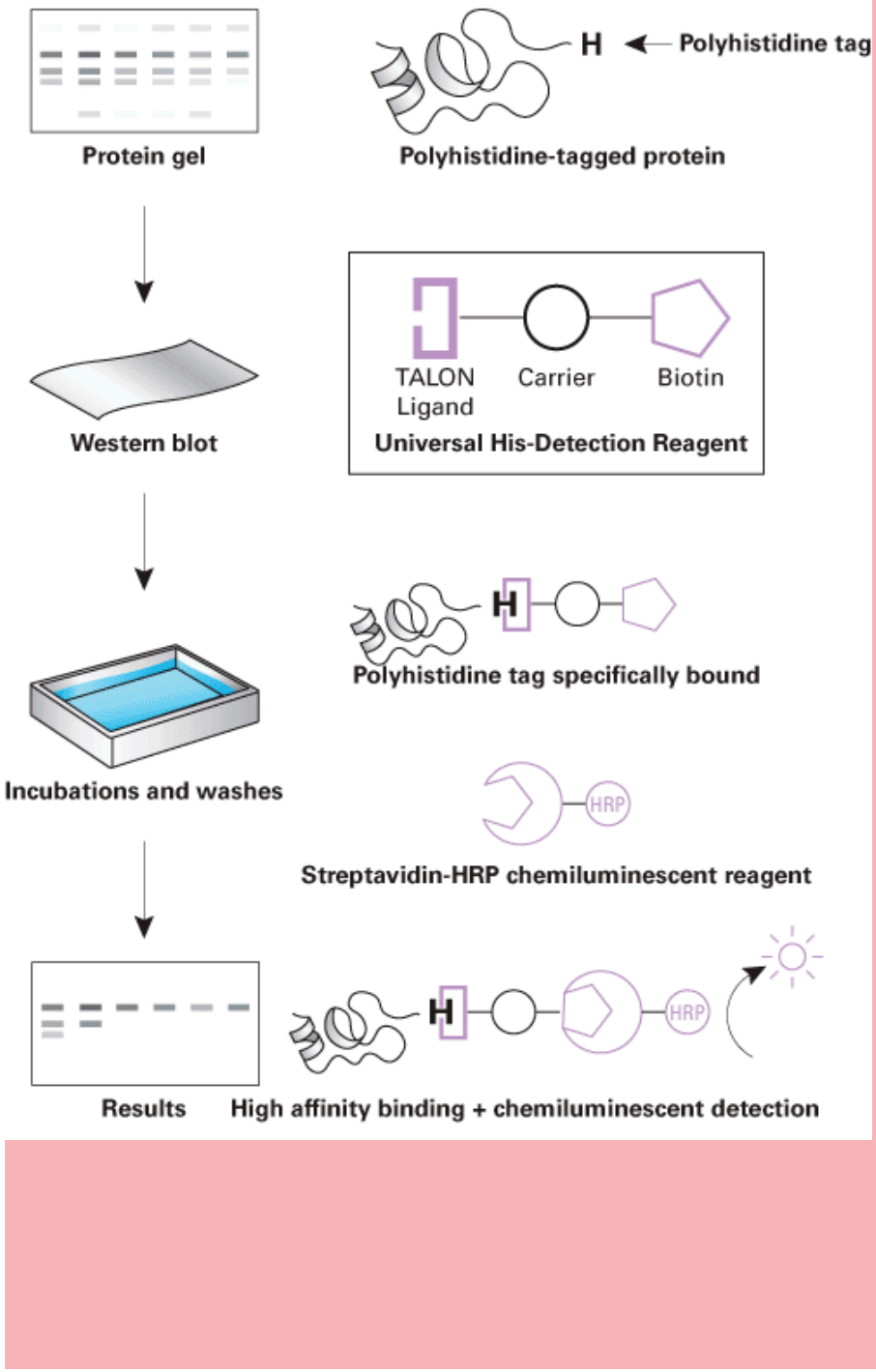
WHAT WE'VE DONE SO FAR

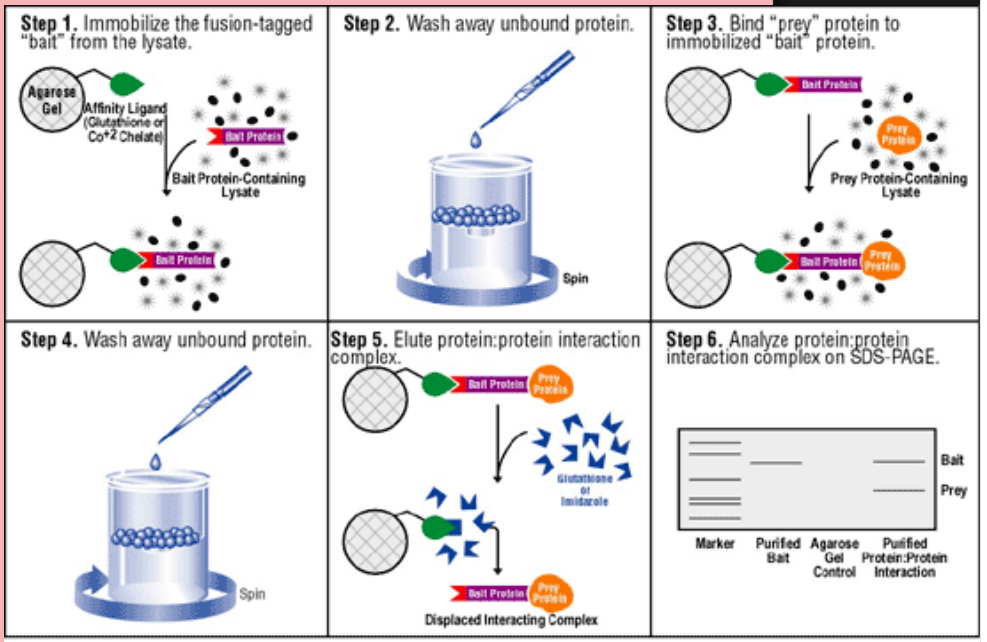
- **Mice -**4.1R knockout mice had to be generated
- **Generation of Antibodies-**Antibodies against mouse transmembrane proteins GPC, band 3, Rh, RhAG, XK, Kell, Duffy, LW, and CD47 were raised in rabbit by using synthetic peptides as antigens.
- **Phalloidin Staining of Red Cells.-**Freshly drawn blood was washed three times in PBG buffer
- **Electron Microscopy-** Mouse erythrocytes were attached to polyl-lysine-coated coverslips by centrifugation at 200 × *g* for 5 min at room temperature
- **Preparation of Red Cell Membranes-** RBCs

WHAT IS A WESTERN BLOT?



Western blotting is a technique used to identify and locate proteins based on their ability to bind to specific antibodies. So, the Western Blot

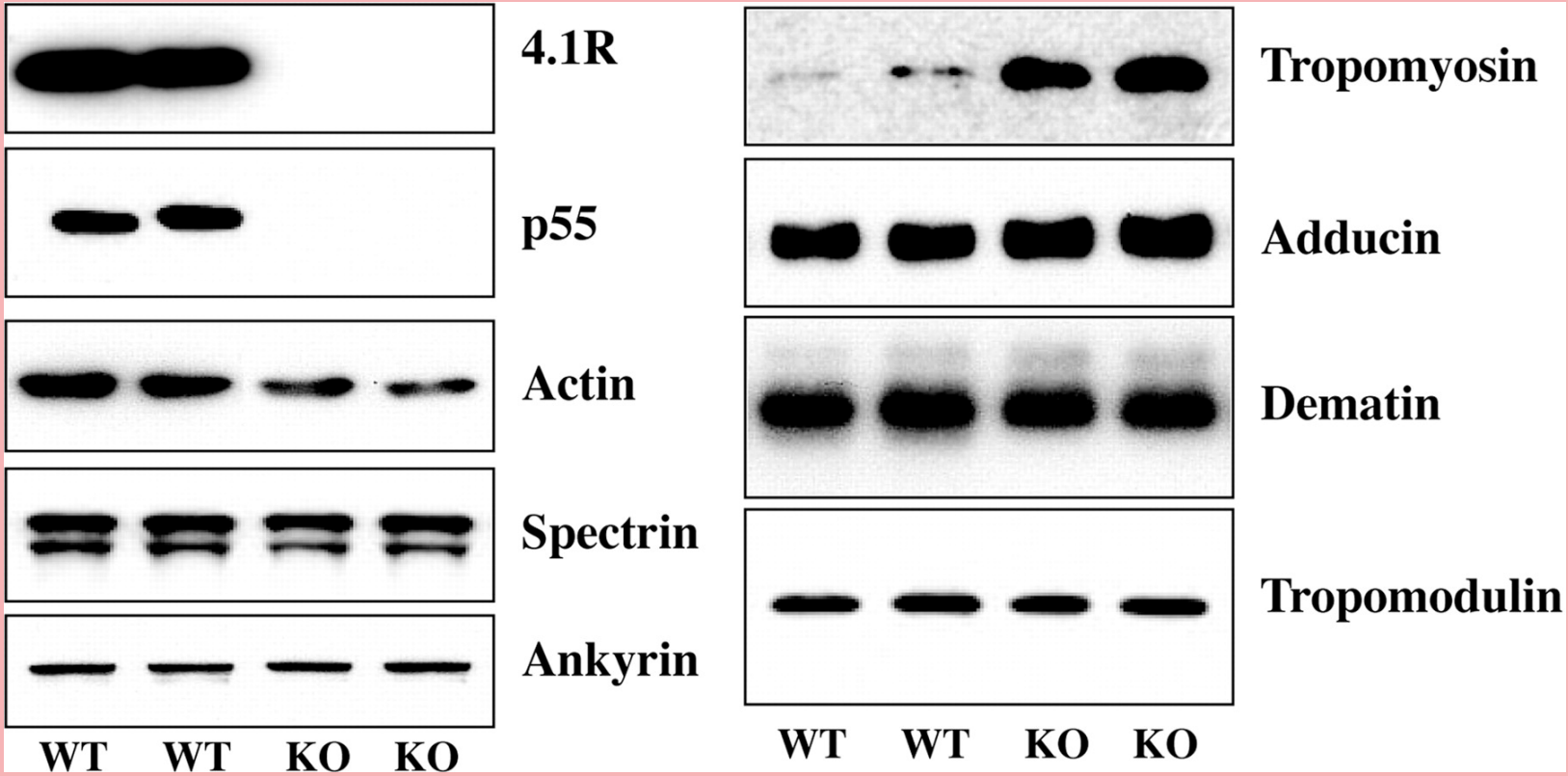

Protein gel
H
Polyhistidine tag
Polyhistidine-tagged protein
Western blot
TALON Ligand
Carrier
Biotin
Universal His-Detection Reagent
Incubations and washes
Polyhistidine tag specifically bound
HRP
Streptavidin-HRP chemiluminescent reagent
Results
High affinity binding + chemiluminescent detection


Step 1. Immobilize the fusion-tagged "bait" from the lysate.
Agarose Gel
Affinity Ligand (Glutathione or Co+2 Chelate)
Bait Protein
Bait Protein-Containing Lysate
Step 2. Wash away unbound protein.
Spin
Step 3. Bind "prey" protein to immobilized "bait" protein.
Prey Protein
Prey Protein-Containing Lysate
Step 4. Wash away unbound protein.
Step 5. Elute protein:protein interaction complex.
Glutathione or Imidazole
Displaced Interacting Complex
Step 6. Analyze protein:protein interaction complex on SDS-PAGE.
Bait
Prey
Marker
Purified Bait
Agarose Gel Control
Purified Protein:Protein Interaction
= Fusion Tag (GST or polyHis)

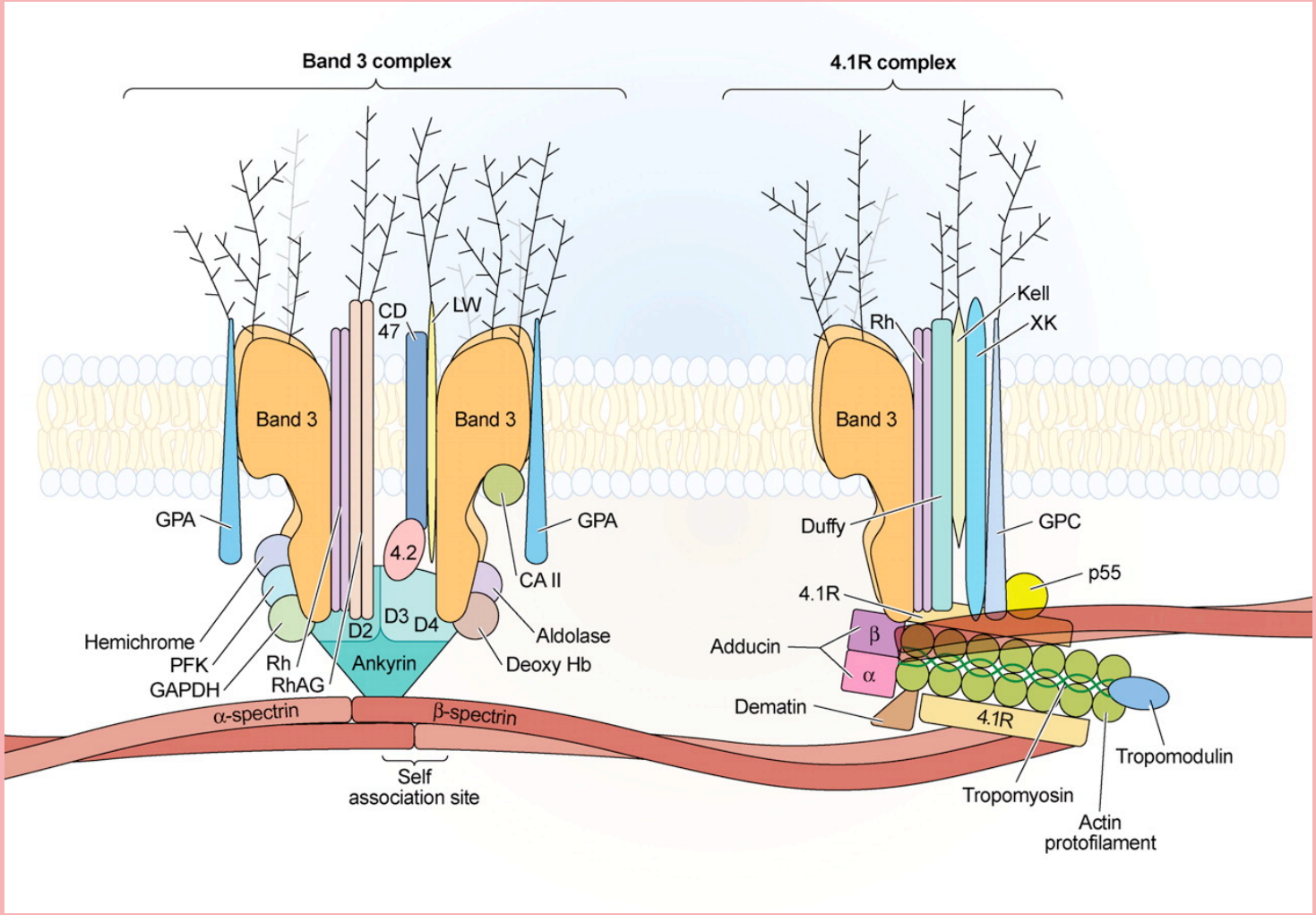
WHAT WE'RE PLANNING TO DO IN THE FUTURE

- **SDS/PAGE and Western Blot Analysis-** Aliquots of RBC ghosts, matched for cholesterol content, have to be separated by 10% SDS/PAGE. The proteins have to be transferred to a nitrocellulose membrane.
- **Flow Cytometry Analysis -**RBCs from wild-type and 4.1R knockout mice have to be washed three times in PBS supplemented with 0.1% BSA (PBS-BSA). Cells have to bewashed four times with PBS-BSA before flow cytometry analysis.
- **Preparation of Recombinant Proteins -** The full-length 4.1R 80-kDa, GST-tagged 30-kDa, 16-kDa, 10-kDa, and 22/24-kDa domains of 4.1R have to be constructed and purified as described previously
- **Pull-Down Assay -** To measure the binding of

Immunoblots of membrane skeletal proteins in red cells of 4.1R+/+ and 4.1R−/− mice



Schematic representation of two types of multiprotein complexes in the red cell membrane



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And….
MY AUDIENCE =). THANK YOU FOR LISTENING

ANY

QUESTIONS?