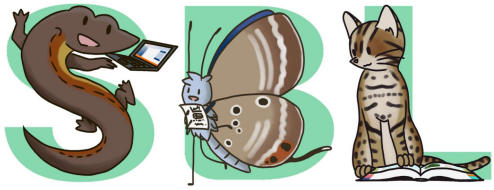

Main Problems

SAMPLE

"Pull me DOWN NOW"

Difficulty: Hard

This sample problem will give you a glimpse of how Main Problems in the contest will be structured.

All the best for SBL 2024!

Last modified: 21 April 2024

Pull Me DOWN NOW

(200 points)

"Pull me down if you want to and I hope that you want to." ~ Pull Me Down by Mikky Ekko

Co-Immunoprecipitation (Co-IP) is an assay to investigate the interactions between proteins in the *in vivo* state. An antibody specific to the antigen of the protein of interest is immobilised on a solid-state substrate. Proteins that do not interact with the antibody are washed out. Proteins of interest that interact will bind to the antibodies, be precipitated and subsequently eluted out.

Huntington's disease is a rare, inherited disease that causes the progressive degeneration of nerve cells in the brain. The mutant protein involved, huntingtin (Htt), is a 350kDa protein. Dr Sudduth cloned Htt into an expression vector with a FLAG tag (DYKDDDDK) and transformed the plasmids into competent BL21(DE3) cells. He then homogenised the cells to produce a lysate, which was subjected to Co-IP with anti-FLAG antibodies. SDS-PAGE was then performed on the eluted proteins and the gel was stained with Coomassie Blue. The results are seen in Figure 1.

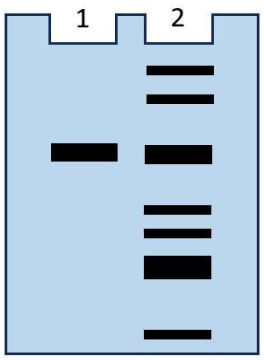

Figure 1: Results of Co-IP on bacterial lysate.
Lane 1: Pure Htt protein. Lane 2: Co-IP eluate.
SDS-PAGE gel was stained with Coomassie Blue.

Q1. [Multiple Choice] What do the bands in Lane 2 of Figure 1 most likely represent? **(20 points)**
(Select the correct option.)

A. SDS
B. The different epitopes of FLAG
C. Non-specific bands of proteins present in the lysate
D. Proteins that interact with Htt
E. Alternative splices of Htt
F. The anti-FLAG antibodies.

The insulin-like growth factor 1 receptor (IGF-1R)/Akt pathway is known to block huntingtin-induced toxicity. Dr Sudduth knows that protein X interacts with IGF-1R in human cells and is interested in investigating whether protein Y interacts as well. He performs Co-IP on lysates from wild-type cells (Lanes 1-4) and cells with a 100-base deletion in the gene coding for protein X (Lanes 5-8) using anti-FLAG antibodies immobilised on Dynabeads. Co-IP eluate (Bound, Lanes 1, 2, 5, 6) and wash (Unbound, Lanes 3, 4, 7, 8) are subjected to SDS-PAGE and Western Blot using either anti-X (Lanes 1, 3, 5, 7) or anti-Y antibodies (Lanes 2, 4, 6, 8). The results are seen in Figure 2. Some lanes are hidden.

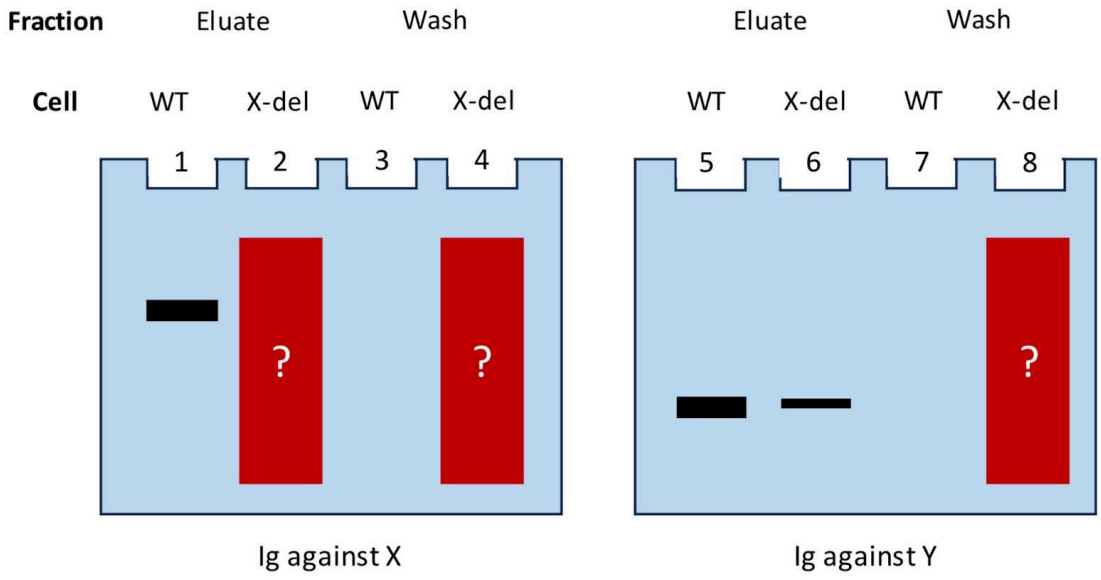

Figure 2: Western Blot results. Ig against X and Y were used. Lanes 2, 4 and 8 are hidden.

Q2. [Multiple Response] In which hidden lanes should no bands be seen? **(20 points)**
(Select all correct options.)

A. Lane 2
B. Lane 4
C. Lane 8
D. None of the lanes

Q3. [True-False Set] Indicate whether the following statements are true or false. **(40 points)**
(Mark each statement as true or false.)

A. Protein X has a lower molecular weight than Protein Y.
B. Protein X increases the affinity of binding of Protein Y to IGF-1R.
C. Protein Y can bind directly to IGF-1R.
D. If the entire experiment was repeated but Co-IP was performed using anti-Y instead of anti-FLAG antibodies, compared to Figure 2, Lane 1 will be similar while Lane 6 will have a thicker band.

Huntington's disease can cause involuntary movement problems and impaired voluntary movements as they affect the muscles. Dr Sudduth hopes to understand how different conditions can affect muscle performance. He extracts skeletal muscle tissue from an unaffected male and performs the following experiments on it.

He purifies the sample of skeletal muscle tissue and performs SDS-PAGE with Western Blot using anti-actin, anti-myosin and anti-GAPDH antibodies on the tissue lysate. Then, he performed Co-IP on the tissue lysate using anti-actin, anti-myosin, or mutated non-specific IgG antibodies (which do not bind to any protein) and repeated the SDS-PAGE and Western Blot on the eluate. These were repeated with extra ADP or ATP added to the tissue lysate before Co-IP. Figure 3 shows the Western Blots from all experiments. Results from Co-IP are hidden.

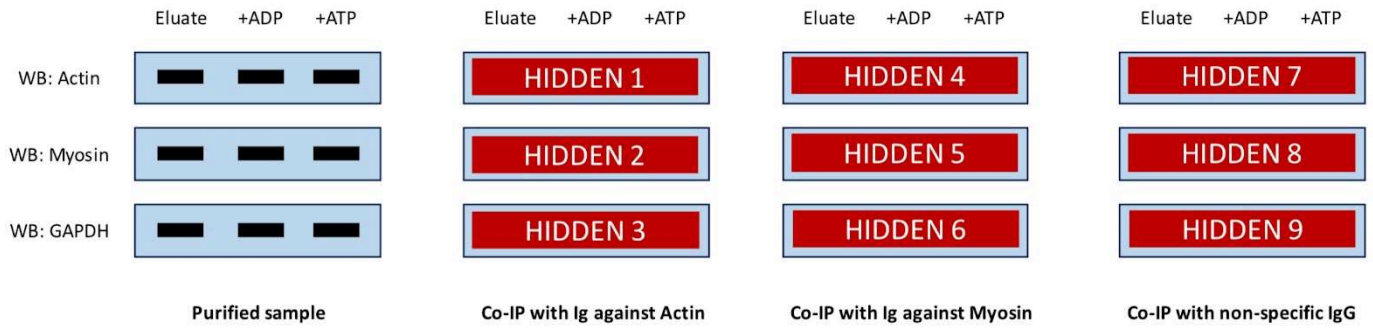

Figure 3: Western Blot (WB) on skeletal muscle tissue lysate and Co-IP eluate. Co-IP results are hidden under red panels.

Figure 4 shows possible results for the Western Blots performed.

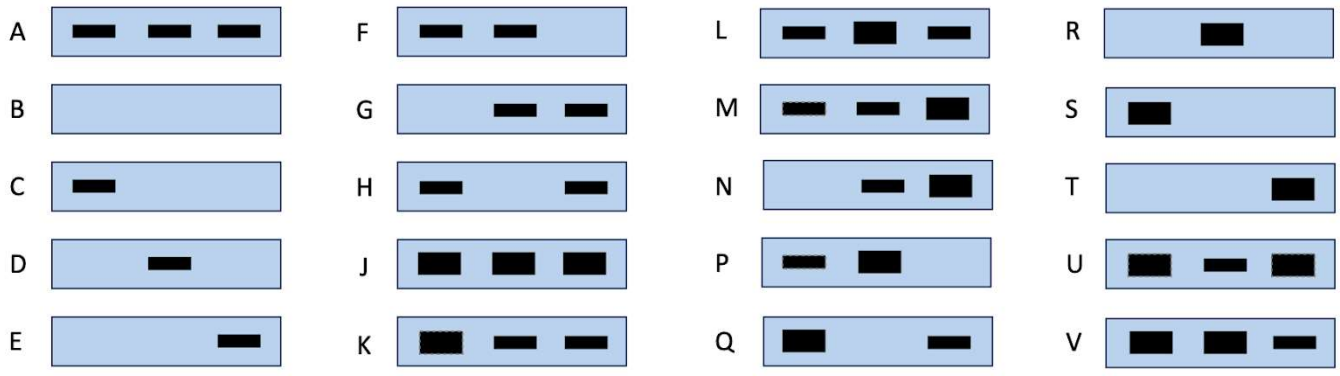

Figure 4: Possible Western Blot results. For clarity, options I and O are omitted.

Q4. [Matching] By considering the roles of ADP and ATP in the sliding filament theory of muscle contraction, indicate what are the most likely results under each of the hidden panels in Figure 3. **(60 points)**
(Match the correct letters to the correct numbers.)

Hidden Panel	**Likely WB Result**
1	
2	
3	
4	
5	
6	

Q5. [Short Answer] If the cell completely lacks Ca^{2+}, what will be the letter in Figure 4 that represents the WB: Myosin result behind Hidden Panel 2? **(10 points)**
(Enter a single capital letter.)

Q6. [Numeric Response] Each of the Hidden Panels 7, 8 and 9 can be represented by one of the possible Western Blot results in Figure 4. How many individual bands should be seen in total behind the three hidden panels 7, 8 and 9? **(10 points)**
(Enter a whole number.)

Q7. [Short Answer Set] Different ions are involved in the transmission of the nervous impulses to the contraction of the muscle. Indicate the **symbol** of the element of the ion involved in the following processes. Enter *-1* if there is no such ion. **(40 points)**
(Give the symbol of the element of the ion. For example, if your answer is H^+, leave your answer as H.)

Process	**Symbol of element of ion**
Ion directly involved in the release of acetylcholine vesicles into the synaptic cleft	
Ion that is actively transported into the sarcoplasmic reticulum	
Ion that counters the effect of calcium ions in muscle cells by binding competitively to sites	
Ion whose influx into the axon initiates the action potential	

Answers and Explanations

Q1.

Answer: **D**

Explanation: We see that there are extra bands, implying that the contents of these bands were eluted out. For them to be found in the eluate, they must either bind to the column by having a FLAG tag or bind to something that has a FLAG tag. Since FLAG is artificial and not found in natural proteins, the other bands must be proteins that bind to Htt and avoid being washed out like non-specific proteins. SDS denatures the proteins and separates them from Htt, so they appear as separate bands on the gel when stained by Coomassie Blue.

Anti-FLAG antibodies should not be found in the Co-IP eluate because they are bound to the media used in Co-IP, typically beads. Even if they were eluted, they should form a single band with consistent molecular weight instead of the multiple bands seen.

Coomassie Blue does interact with SDS, but this stabilises the neutral form of the dye. SDS-PAGE gels are washed before staining with Coomassie Blue and even if this step is omitted, the entire gel would simply be stained.

Q2.

Answer: **A, B**

Explanation: The bound solution contains proteins that bind to IGFR-1 while the unbound solution contains proteins that do not interact with IGF-1R. In the Western Blot, a visible band shows that the antibody is bound to the corresponding protein, indicating its presence. In Lanes 1 and 5, we see that X and Y are present, indicating that both X and Y bind to IGF-1R. As expected, Lanes 3 and 7 are empty as all the X and Y was bound to IGF-1R and avoided being washed out.

The 100-base deletion in gene X causes a frame-shift mutation and alters the conformation of protein X. Thus, mutant protein X does not bind to IGF-1R in Co-IP and is not found in the eluate, leaving Lane 2 empty. Lane 4 is also empty even though mutant protein X is present in the unbound solution because anti-X antibodies would be unable to bind to the malformed protein.

We observe that the Y band in Lane 6 is thinner than the band in Lane 5, implying that without protein X, less protein Y binds to IGF-1R. This also implies that the remaining protein Y has been washed out in the unbound solution, so lane 8 will not be empty.

Q3.

Answer: **FTTT**

Explanation:

A. Since SDS is denaturing, any protein complexes are broken up and the bands represent pure proteins. Since the band for Protein X has travelled a shorter distance from the well, it is more retarded by the gel and has a higher molecular weight than Protein Y.
B. The band for protein Y is thicker in Lane 5 than Lane 6, so more protein Y binded to IGF-1R in the presence of functional protein X.
C. As seen in Lane 6, protein Y is still eluted in the absence of functional protein X, indicating that it can bind directly to IGF-1R.
D. Both Proteins X and Y bind to IGF-1R. Protein X helps Protein Y bind to IGF-1R, so both proteins will be eluted out together with antibodies against Protein Y. Hence lane 1 will have a band indicating the presence of Protein X. Lane 6 will have a thicker band similar to that in lanes 1 and 5 as all Protein Y will be eluted out.

Q4.

Answer: **A, P, B, P, A, B**

Explanation: First, we need to understand the sliding filament model of muscle contraction. Myosin is first bound to ATP and is not bound to actin. The myosin head then hydrolyses ATP into ADP, leaving it in its high-energy configuration. This allows the myosin head to bind to the actin filament forming a cross-bridge. The release of ADP and inorganic phosphate is coupled to a power stroke by the myosin head returning it to its low-energy configuration. This shifts the actin filament closer to the centre of the sarcomere, forming a contraction. A new molecule of ATP then binds to the myosin head and the cycle repeats.

GAPDH does not bind to actin or myosin and will not be eluted out so Hidden Panels 3 and 6 will be empty and will appear as Result B. If the antibody of a protein is used to elute the protein out, it will appear on the Western Blot using the same antibodies as the protein is present. Thus Hidden Panels 1 and 5 will appear as Result A.

For Hidden Panel 2, we look at whether myosin is bound to actin. In the lysate, it is expected that normal amounts of myosin will be bound to actin, and thus a normal-sized band will be seen. The addition of ADP means more myosin heads are in the high-energy configuration and ready for contraction, so more cross-bridges are formed, and more myosin is bound to actin, so a thicker band should be seen. With ATP, most myosin heads are left unbound to actin, so myosin is not eluted out and no band should be seen. Hence the answer is Result P.

Similarly, for Hidden Panel 4, we should see a normal-sized band for the lysate. In the presence of ADP, more myosin is bound to actin, so more actin will be eluted out and a thicker band should be seen. In the presence of excess ATP, myosin is unbound from actin, so actin is not eluted out and no band is seen. Hence the answer is also Result P.

Q5.

Answer: **B**

Explanation: The lack of Ca^{2+} means no Ca^{2+} will bind to troponin in the troponin complex, so the myosin binding sites are not exposed. This prevents contraction and the myosin head cannot bind to actin. Thus, regardless of the presence of ADP or ATP, myosin cannot bind to actin. For a Co-IP with anti-actin antibodies, no myosin will be eluted out in all three conditions, so no bands will be observed.

Q6.

Answer: **0**

Explanation: With mutated non-specific antibodies, nothing will be eluted out as no protein is bound to the antibodies in Co-IP. Thus, no actin, myosin, or GADPH will be detected and zero bands will be observed.

Q7.

Answer: **Ca, Ca, Mg, Na**

Explanation:

When the action potential reaches the axon terminal, the calcium channels open allowing calcium ions to enter which induces the vesicles containing acetylcholine to fuse with the membrane releasing acetylcholine into the synaptic cleft. As this action potential travels down the T tubule, calcium ions are released from the sarcoplasmic reticulum. These calcium ions bind to troponin. As acetylcholinerase removes acetylcholine from the synaptic cleft, the calcium ions are actively transported back into the sarcoplasmic reticulum via pumps, causing the cross-bridge to detach.

Magnesium ions reduce the rate of calcium ions binding to sites that bind both ions competitively like Ca^{2+}-Mg^{2+}-type sites in troponin in relaxed muscles, as bound magnesium ions take a long time to dissociate and be unbound.

During depolarisation, when the threshold potential is exceeded, the voltage-gated sodium channels open allowing sodium ions to enter the axon, initiating the action potential.

All the best for SBL 2024!

Credits

All figures are original work.