BIOLABYRINTH: Navigating Academic Literature

Joseph Crognale & Akshaye Shah

We'll Cover:

- General cell biology
- Virology
- Evolution
- Oncology
- Immunology
- 3D & 4D Bioprinting
- Anything else you might be interested in!

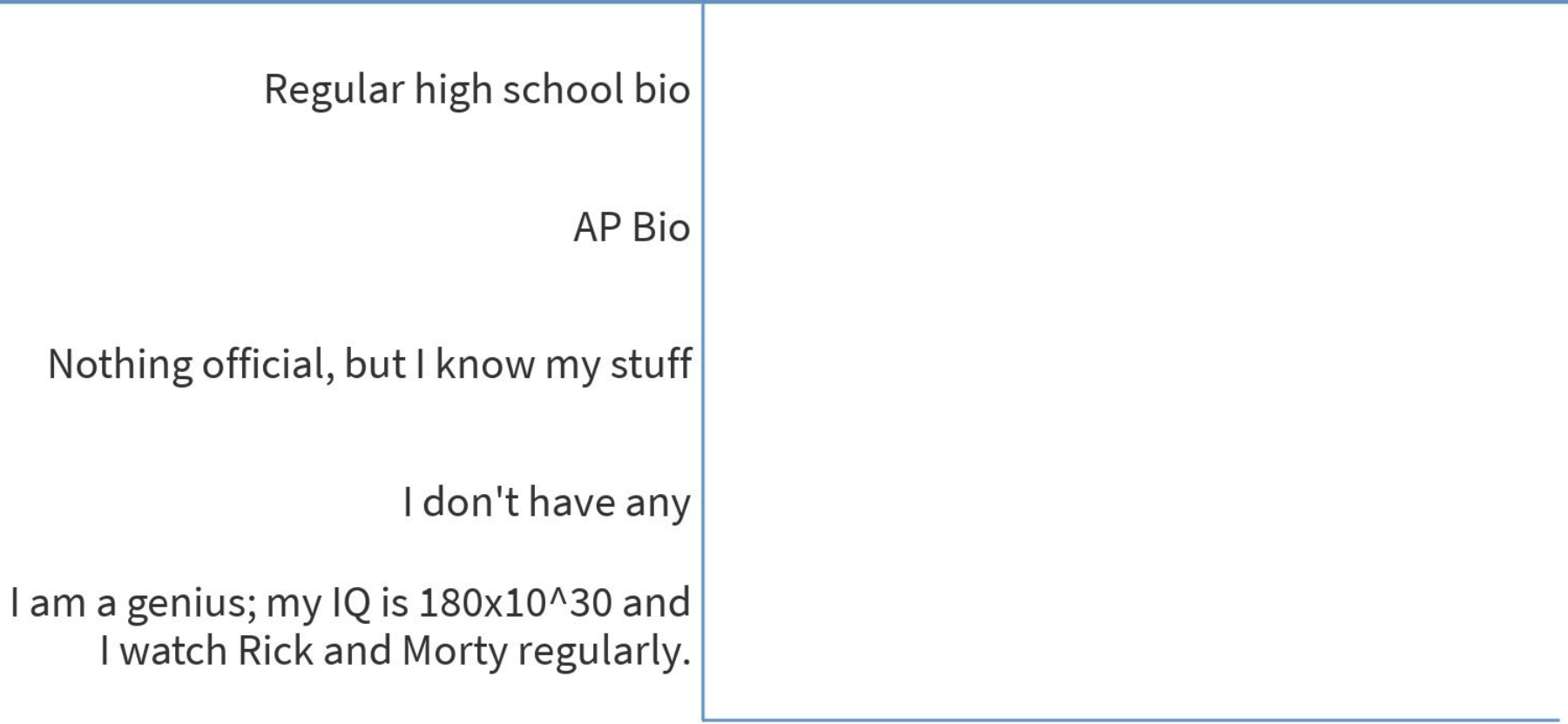
We hope you'll:

- Improve general biology knowledge
- Understand & apply laboratory techniques
- Be exposed to new & "hot" topics
- Improve reading and comprehension abilities
- Learn about the relationship between science, business, & ethics

Introduce Yourselves!

- Name and grade
- Why you’re interested in the class
- Your favorite cereal and why it’s Cinnamon Toast Crunch

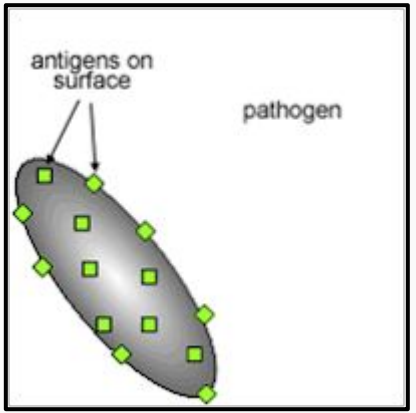
What is your biology background?



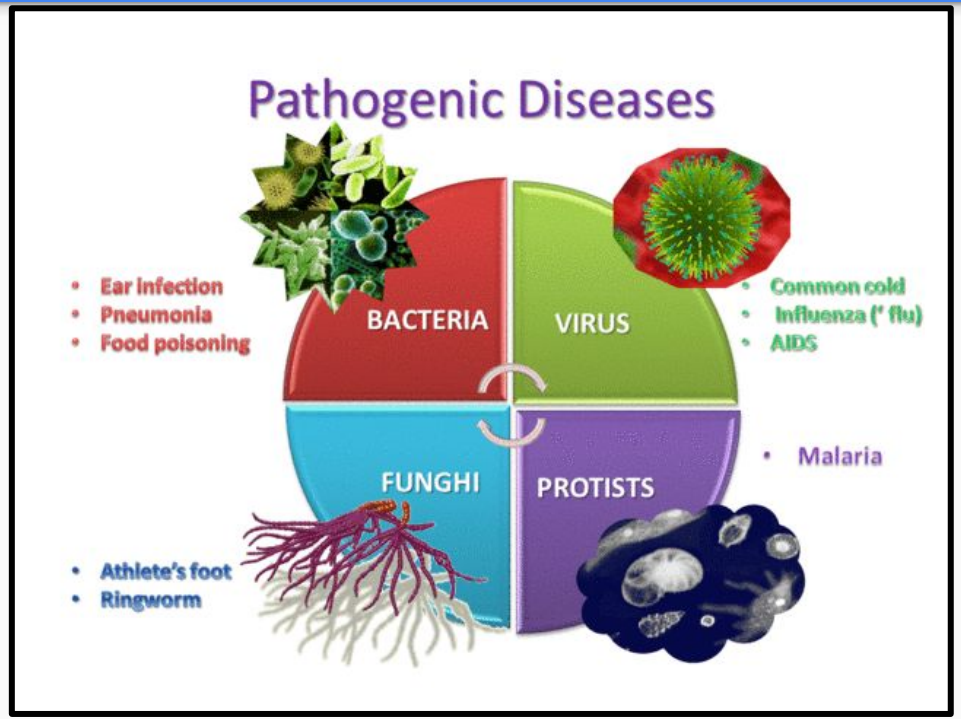
Is a hot dog a sandwich?

Immunology: The Basics

- **Immune system** = system of biological structures, molecules, and processes that protects an organism against disease and functions to:
  - Recognize a wide array of **pathogens**
    - **Pathogen** - Anything that can cause a disease (ie. **bacteria, viruses, algae, prions**)
    - Pathogens are what we used to call **germs**
    - Identified by cell surface proteins called **antigens**
  - Distinguishes invaders and their products from the organism's own products
    - Why is this important?



Where some pathogenic diseases come from



Physical barriers are the first line of defense

Primary Components of the Immune System

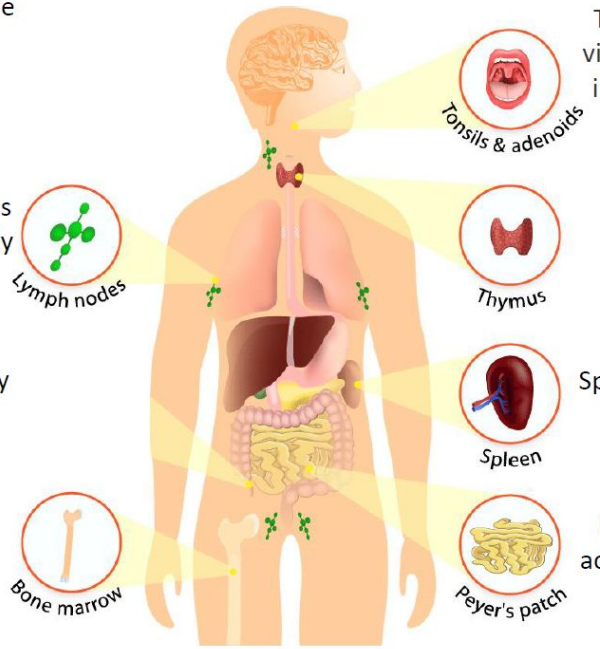
Skin – First line of defense

Bloodstream – contains circulating immune cells

Lymphatic system – carries immune cells through body to lymph nodes

Lymph nodes – samples information from the body to send out signals in response to pathogen

Stem cell source for immune cells



Trap bacteria and viruses and contain immune cells and antibodies

Site of T cell maturation

Specific areas enrich immune cells

Immune cells can access samples from the GI tract (mucosal tissue)

Two subsets of the immune system

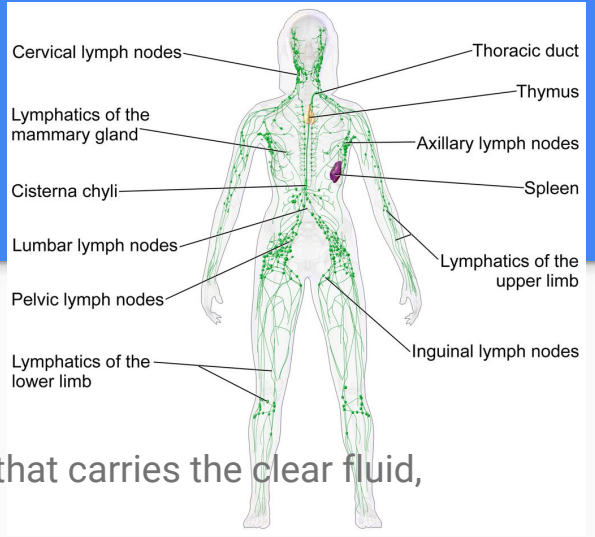
- **Innate immunity**: rapid response!
  - **Nonspecific** defense system activated soon after an antigen appears in the body
  - Includes both **physical barriers** and **immune cells**
- **Adaptive immunity**: slooooow response
  - **Antigen-specific** - goes after a specific pathogen, which is identified by its specific antigens
  - Causes immune system to produce **antigen-specific immune cells**
  - Creates **immunological memory** in case of future attacks
  - Only performed by immune cells
  - Can last for life or need to be “refreshed”

Examples of innate immune system?

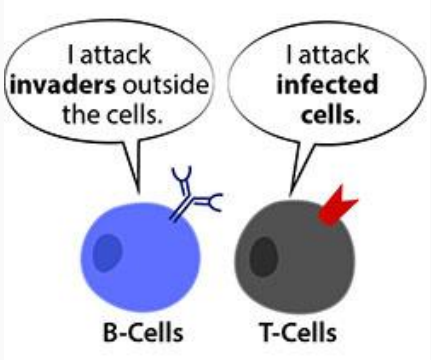
- **Skin**: sweat and organic acids
- **Eyes:** tears
- **Throat and mouth:** mucus, saliva
- **Gut:** gastric acid, “gut flora,” bile acids

Lysozyme is present in many of these bodily fluids — it catalyzes the degradation of bacterial cell walls (peptidoglycan)

Adaptive immune system

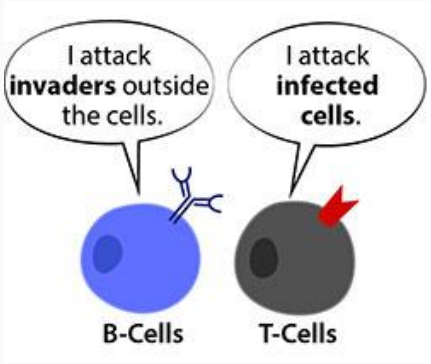


- Mainly **B & T lymphocytes** (aka **B cells & T cells**)
  - Constitutes up to 40% of the body's **white blood cells**
  - Located in tissue and **lymphatic system** (network of vessels that carries the clear fluid, **lymph**, around the body)
    - Like veins but carry immune cells
- Produced in the **bone marrow**
- **But there's more...**



Two parts of adaptive immune system

- **Humoral immunity** - **Macromolecules** found in extracellular fluids, recruited by **B cells**, **attack pathogens**.
- **Cell-mediated immunity** - **Immune cells** (not molecules) attack both **pathogens and infected cells,** and are recruited by **T cells.**
- **Both T and B cells bind the specific antigens of specific pathogens and call in the cavalry.**
- **Some T cells (cytotoxic) can also kill pathogens.**

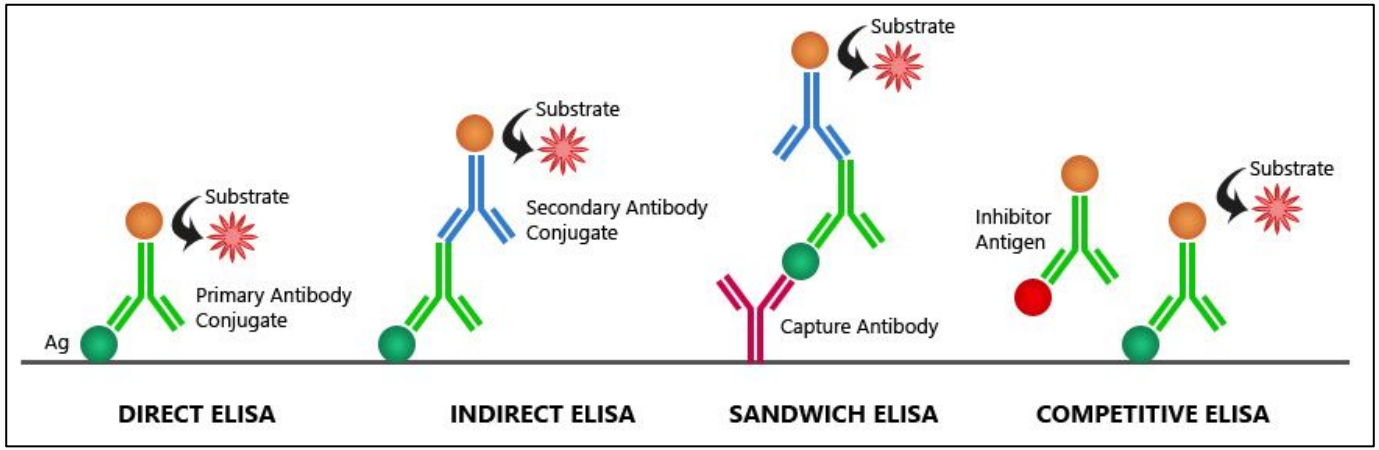


Humoral immunity: antibodies

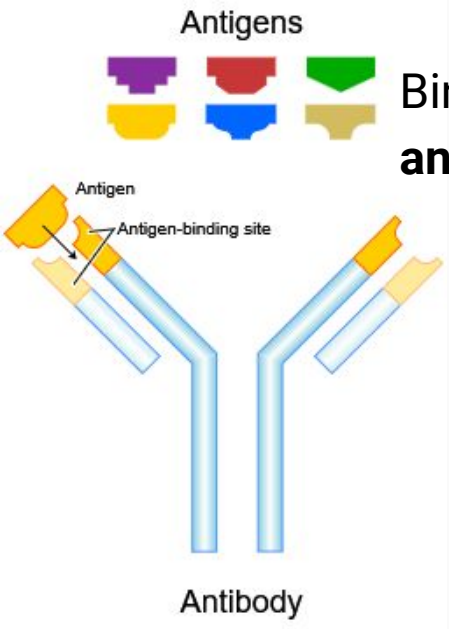
- **Antibodies** - part of *humoral immunity only*.
  - Also known as **immunoglobulins (IGs)**
  - Y-shaped protein produced by **plasma cells**
  - Are made to recognize specific antigens
  - **Bind and coat antigens** to prevent pathogens from entering or damaging healthy cells
  - **Stimulate other parts of immune system** to destroy pathogens

Humoral immunity: antibodies

- Very useful for analytical tools in the lab!
  - **ELISA** - special plate coated with antibodies that bind specific antigens and then change color.
  - May also be coated with antigen instead, to recognize and bind antibodies.

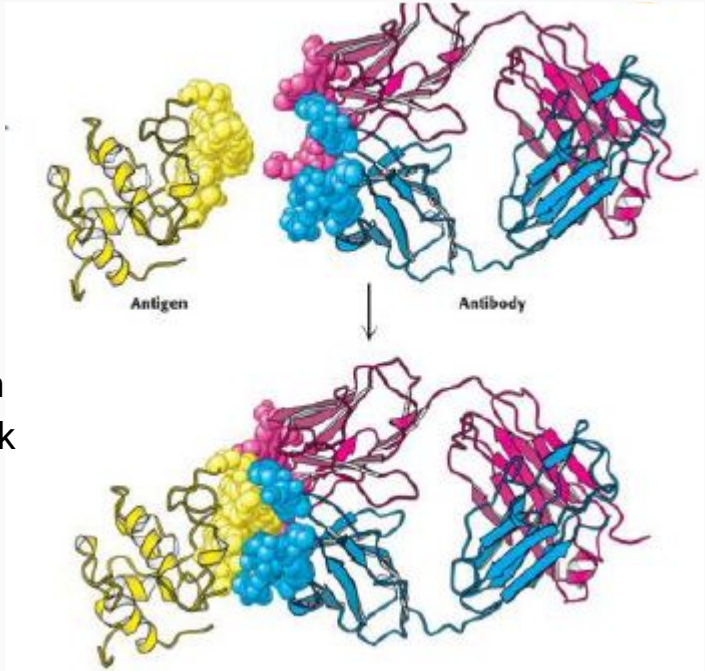


Humoral immunity: antibodies



Bind antigens via **epitopes** (aka **antigenic determinants**)

- A group of amino acids on antigen surface
- **Complementary shapes** between antibody and antigen — like a lock in a key



...And there are two types of antibodies

Monoclonal

- Copies of the **same antibody**
- Recognize **only a single epitope** of an antigen.

VS

Polyclonal

- **Different antibodies**
- Recognize **multiple epitopes** of the **same antigen.**

Questions?

Form groups!

Dissecting a Paper

- **SKIM the paper** - look at title (duh?), date, headings, and subheadings.
  - Get the general picture so you know it's both **relevant to your work** and **current**.
  - If there is an **abstract, *read it!***
- **Notes** are important but should be **minimal**
  - Knowing every detail isn't important
  - Highlighting can be faster than notes and is easy to read if you have to come back later
  - Have **Google** handy — look up anything that seems important
- **Graphs and tables are key**
  - These are often much easier to understand than the results section, and have helpful **captions! Read them!**

Now let's dive in...

molecular pharmaceutics

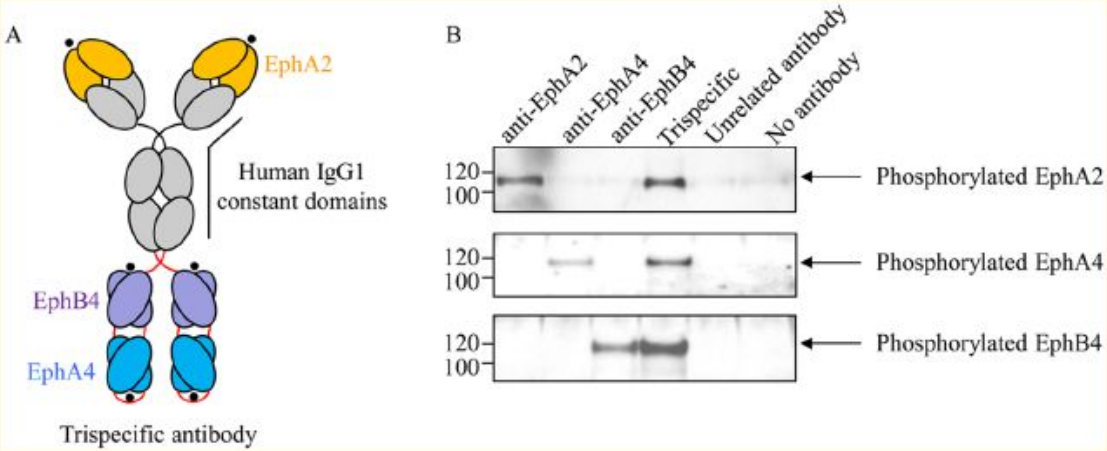
Brief Article

pubs.acs.org/molecularpharmaceutics

Development of a Trispecific Antibody Designed to Simultaneously and Efficiently Target Three Different Antigens on Tumor Cells

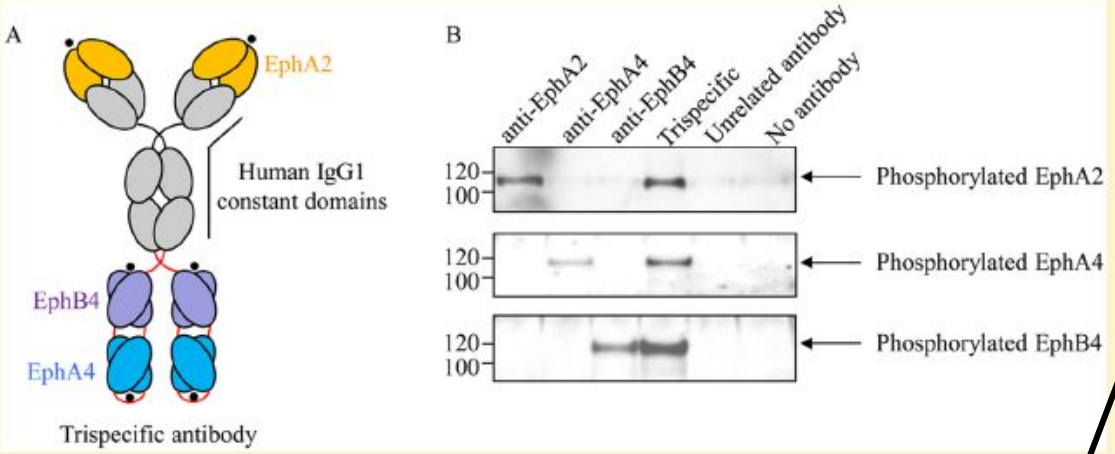
Nazzareno Dimasi,[*,†] Ryan Fleming,[†] Carl Hay,[‡] Rob Woods,[†] Linda Xu,[†] Herren Wu,[†] and Changshou Gao[*,†]

[†]Antibody Discovery and Protein Engineering and [‡]Oncology Research, MedImmune, Gaithersburg, Maryland 20878, United States



ABSTRACT: Targeting Eph (erythropoietin producing hepatoma) receptors with monoclonal antibodies is being explored as therapy for several types of cancer. To test whether simultaneous targeting of EphA2, EphA4, and EphB4 would be an effective approach to cancer therapy, we generated a recombinant trispecific antibody using the variable domain genes of anti-EphA2, anti-EphA4, and anti-EphB4 monoclonal antibodies. A multidisciplinary approach combining biochemical, biophysical, and cellular-based assays was used to characterize the trispecific antibody *in vitro* and *in vivo*. Here we demonstrate that the trispecific antibody is expressed at high levels by mammalian cells, monodispersed in solution, thermostable, capable of simultaneously binding the three receptors, and able to activate the three targets effectively as evidenced by receptor internalization and degradation both *in vitro* and *in vivo*. Furthermore, pharmacokinetic analysis using tumor-bearing nude mice showed that the trispecific antibody remains in the circulation similarly to its respective parental antibodies. These results indicate that simultaneous blockade of EphA2, EphA4, and EphB4 could be an attractive approach to cancer therapy.

KEYWORDS: *trispecific antibody, tumor targeting, EphA2, EphA4, EphB4, differential scanning calorimetry, analytical ultracentrifugation*



Eph genes are often mutated in melanoma patients.

ABSTRACT: Targeting Eph (erythropoietin producing hepatoma) receptors with monoclonal antibodies is being explored as therapy for several types of cancer. To test whether simultaneous targeting of EphA2, EphA4, and EphB4 would be an effective approach to cancer therapy, we generated a recombinant trispecific antibody using the variable domain genes of anti-EphA2, anti-EphA4, and anti-EphB4 monoclonal antibodies. A multidisciplinary approach combining biochemical, biophysical, and cellular-based assays was used to characterize the trispecific antibody *in vitro* and *in vivo*. Here we demonstrate that the trispecific antibody is expressed at high levels by mammalian cells, monodispersed in solution, thermostable, capable of simultaneously binding the three receptors, and able to activate the three targets effectively as evidenced by receptor internalization and degradation both *in vitro* and *in vivo*. Furthermore, pharmacokinetic analysis using tumor-bearing nude mice showed that the trispecific antibody remains in the circulation similarly to its respective parental antibodies. These results indicate that simultaneous blockade of EphA2, EphA4, and EphB4 could be an attractive approach to cancer therapy.

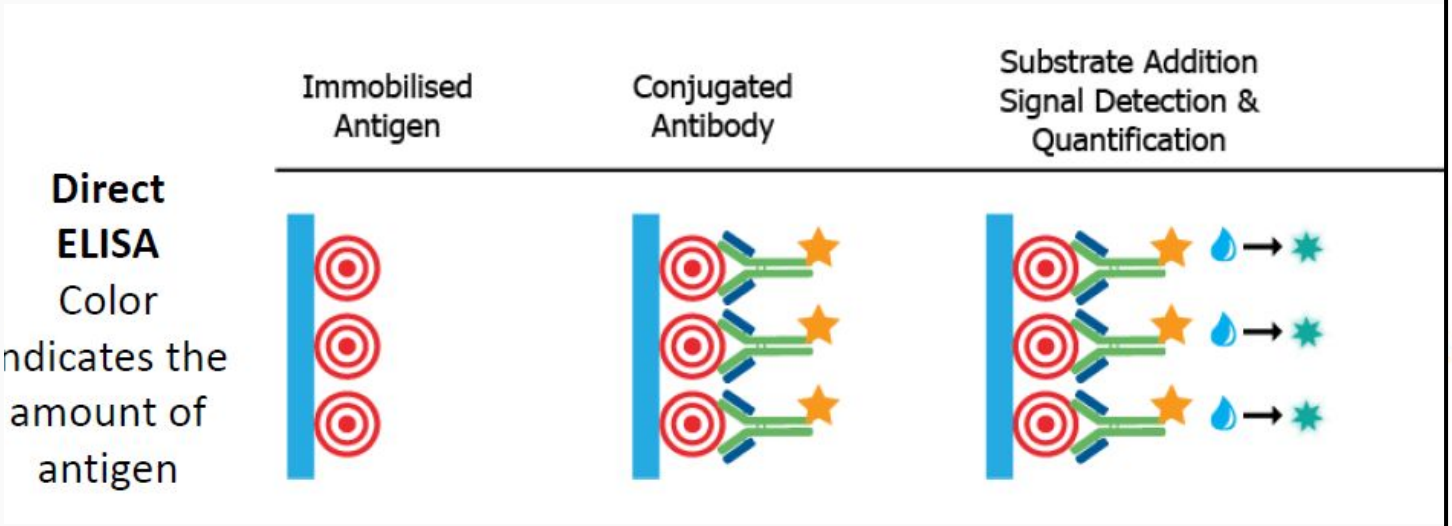
KEYWORDS: *trispecific antibody, tumor targeting, EphA2, EphA4, EphB4, differential scanning calorimetry, analytical ultracentrifugation*

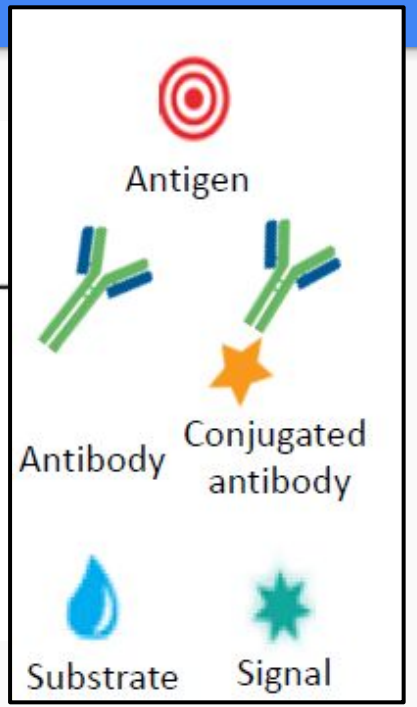
What is the goal of the study?

What is the goal of the study?

Respond at **PollEv.com/josephc611** Text **JOSEPHC611** to **22333** once to join, then text your message

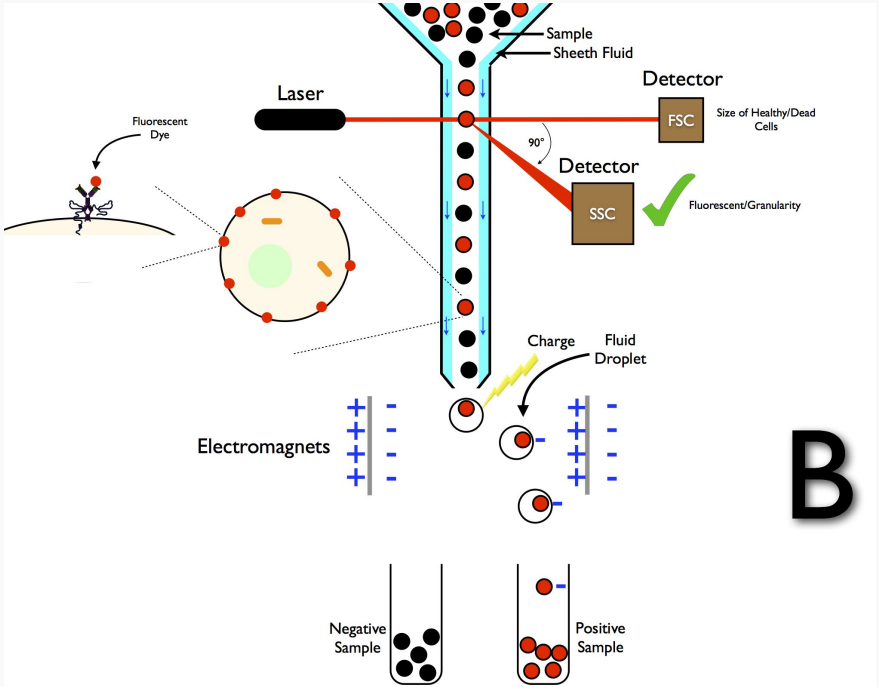
Methods: ELISA





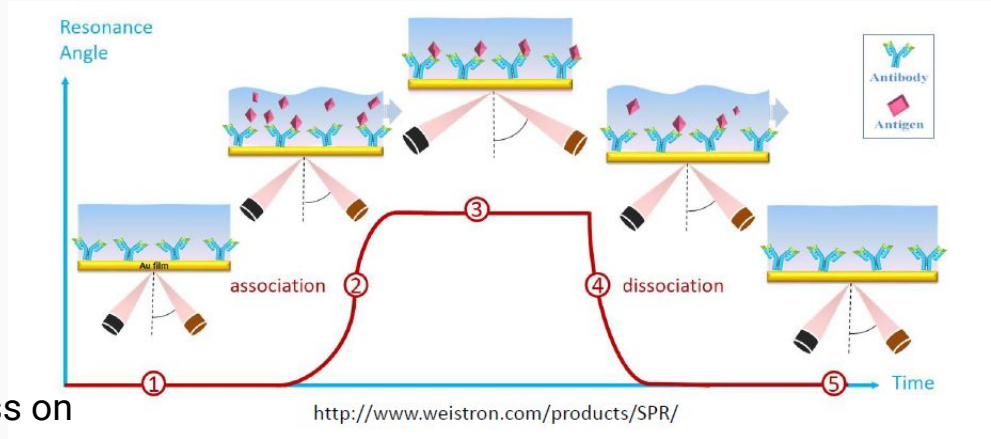
Methods: Flow cytometry

- Sorts cells at **very high rate** (1000s of cells per second)
- Passes cells through electric detection apparatus that applies charge to cells based on their **physical and chemical characteristics**
- Cells then **separated** into separate containers **based on charge**



Methods: Surface plasmon resonance

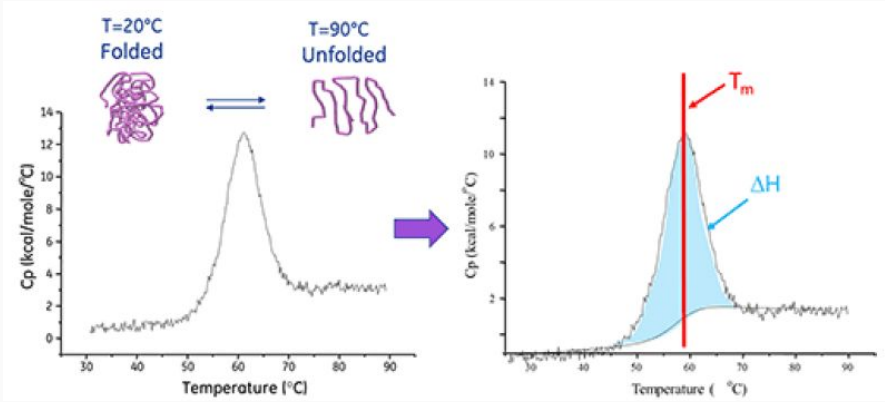
- **Real-time**, label-free detection of **biomolecular interactions**
- Polarized light strikes electrically conducting surface at interface between the two media
    - The intensity of reflected light at various resonance angles is proportional to mass on surface.
- **For this study: shows if antibody and antigens are bound together!**



http://www.weistron.com/products/SPR/

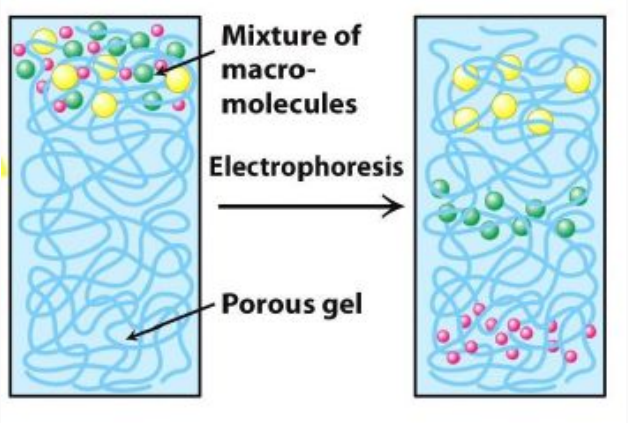
Methods: Differential scanning calorimetry

- Evaluates how a substance's **heat capacity changes** with temperature.
- Heats or cools a known substance, and compares to unknown.
- **Peak maxima** = **temp of denaturation (unfolding)**
- Ultimately, this allows us to **compare the stability** of substances.

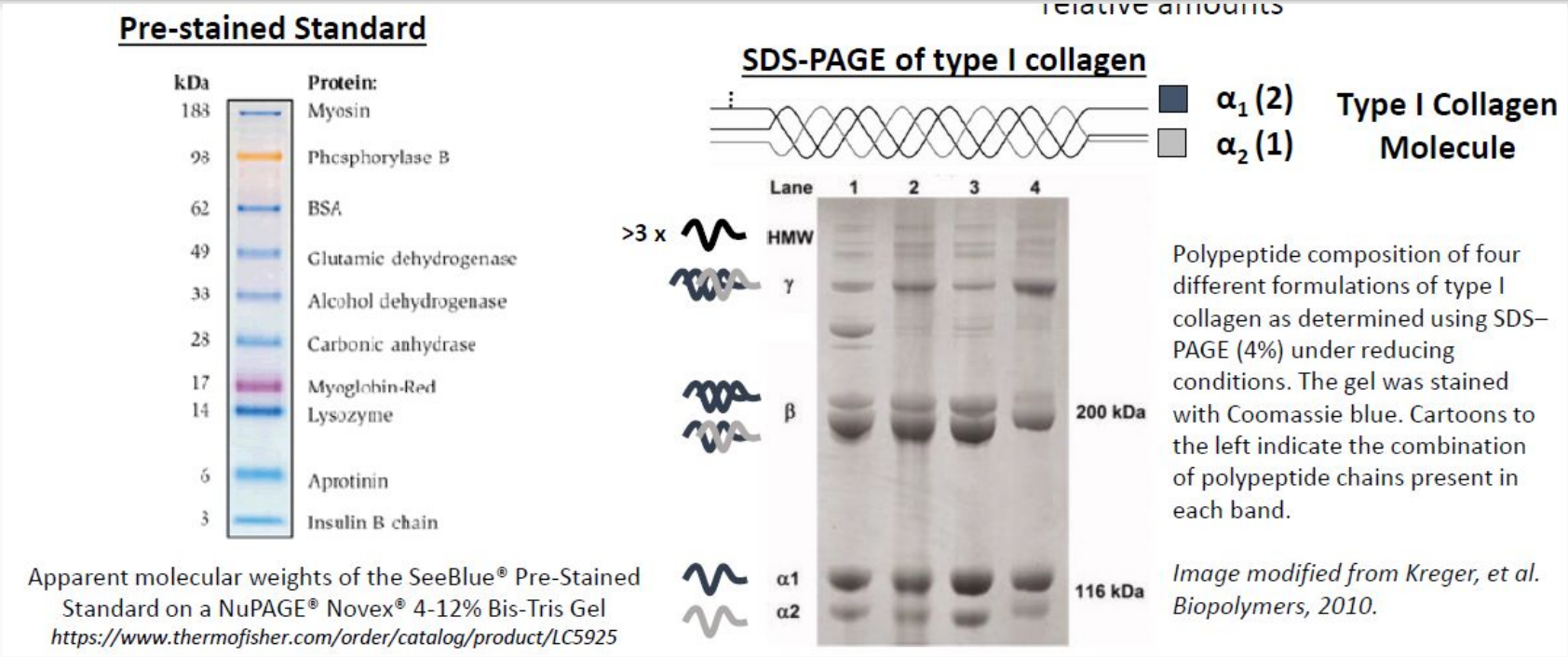


Methods: Gel electrophoresis (SDS-PAGE)

- **SDS-PAGE** separates macromolecules **by size.**
- **Smaller molecules run faster**, so when the gel is ended, they have **traveled farther down the gel.**
- Can show both **composition** and **relative amounts of substance.**
- **“Ladder”** of known substance for comparison.



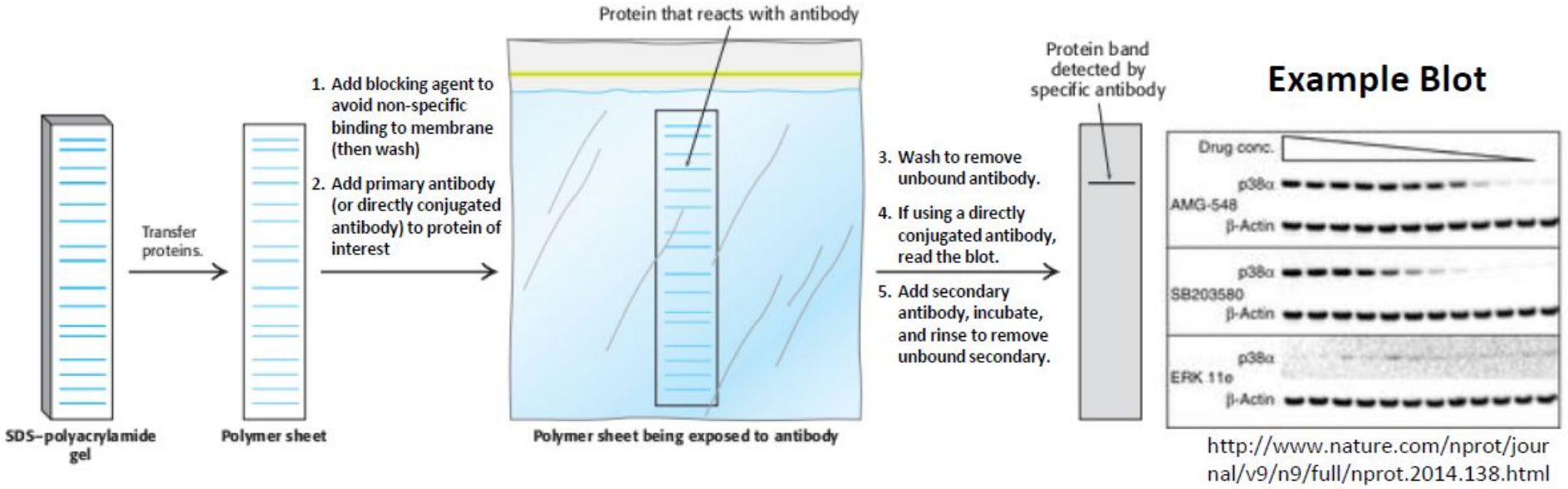
Methods: Gel electrophoresis (SDS-PAGE)



Methods: Western blots

- **Identify proteins** in a sample, and/or **compare proteins across samples** after electrophoresis (separation).

Methods: Western blots



http://www.nature.com/nprot/journal/v9/n9/full/nprot.2014.138.html

Methods: Western blots

- **Identify proteins** in a sample, and/or **compare proteins across samples** after electrophoresis (separation).
  - **Positive controls -** samples known to express protein. Show's it's working.
  - **Negative controls** - samples known to not express protein. Shows false positives and non-specific binding.
  - **Loading controls** - ensures even transfer between gel and membrane, and checks lanes contain same amount of sample.

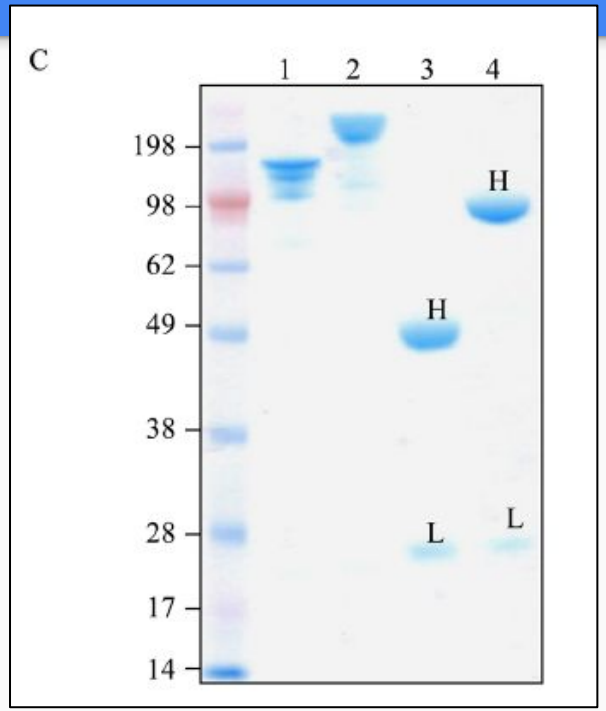
All of these methods are trying to show something. What is it?

Understanding Figures

Each group will be a assigned a figure to study and then explain to the class. Questions are welcome and I can go back to previous slides. You may need to read around in other parts of the paper as well.

- Figure 1C - Gel electrophoresis (SDS-PAGE)
- Figure 3 - ELISA & Flow Cytometry (special type called FACS)
- Figure 4 - Surface plasmon resonance
- Figure 6 - Western Blots
- Figure 7 - Western Blots
- Figures 8A, 8C - Western Blots

Figure 1C



“SDS-PAGE analysis of protein A purified trispecific antibody under nonreducing (line 2) and reducing conditions (line 4). The parental antibody anti-EphA2 is also shown under nonreducing (line 1) and reducing conditions (line 3). The light and heavy chains under reducing conditions (line 3 and 4) are denoted with L and H, respectively. The molecular mass standards are shown.”

Figure 3

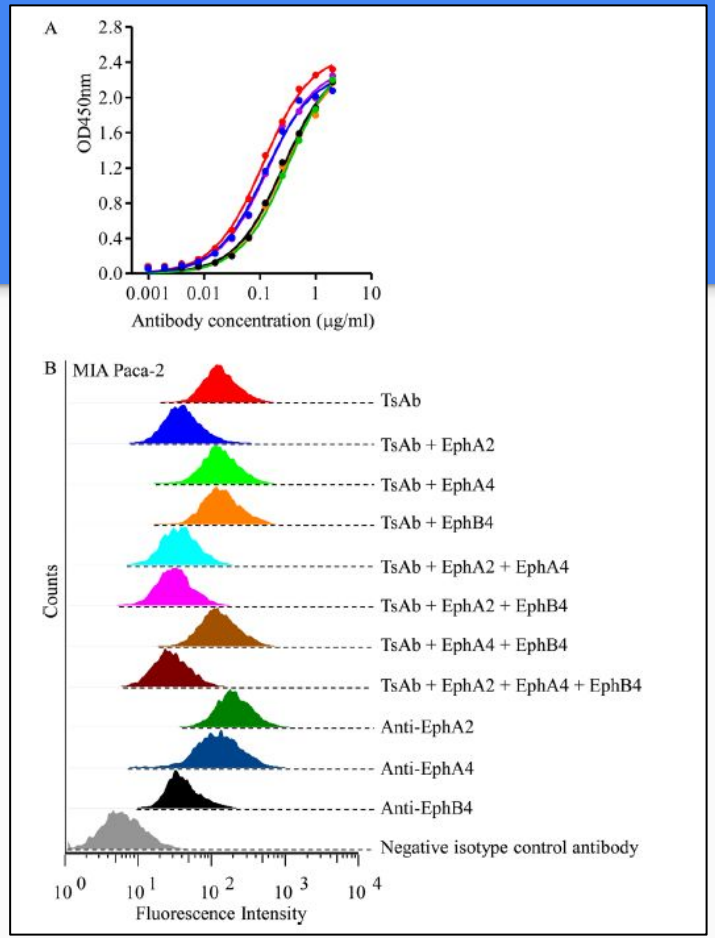


Figure 3. Binding analysis of the trispecific antibody (TsAb) determined using ELISA and FACS. (A) The binding of the trispecific antibody to EphA2, EphA4, and EphB4 antigens is shown in blue, red, and green, respectively. The simultaneous binding to EphA2 and EphA4 is shown in orange, the simultaneous binding to EphA2 and EphB4 is shown in black, and the concurrent binding to EphA4 and EphB4 is shown in violet. ELISA analysis revealed that the trispecific antibody is able to bind to its individual antigens and simultaneously engage pairs of antigens. (B) Flow cytometry was used to assess the binding capacity of the trispecific antibody to the cell surface antigens (EphA2, EphA4, and EphB4) expressed on MIA PaCa-2 pancreatic carcinoma cells. The cells were incubated with their respective antibodies as shown and specific binding was visualized. The FACS binding assay showed that the trispecific antibody binds to its cognate antigens expressed on the cell surface. The binding of the trispecific antibody is decreased upon preincubation of the trispecific antibody with recombinant antigens.

Figure 4

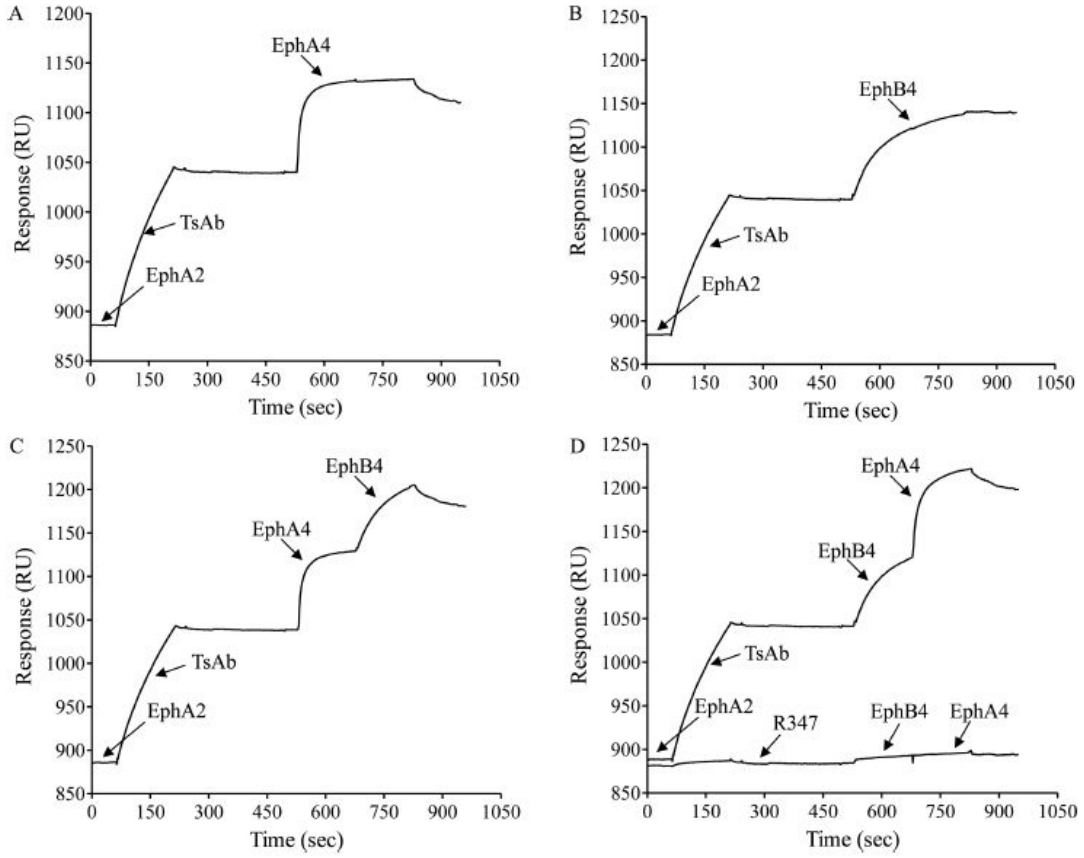


Figure 4. Cuncurrent binding of the trispecific to antigens using BIAcore. In these experiments EphA2 was immobilized on the BIAcore censor chip. Simultaneous binding of the trispecific (TsAb) to EphA2 and EphA4 (A) and to EphA2 and EphB4 (B). Concurrent binding to the three antigens (C,D). The trispecific antibody binds to its three antigens regardless of the sequence of antigen used, and the binding to antigens is specific since there is no detectable binding when the isotype control antibody (R347) is used (D).

Figure 6

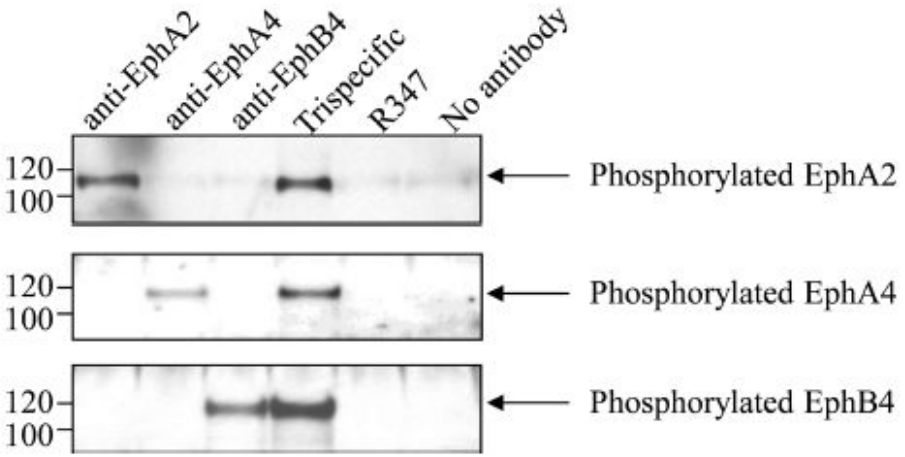


Figure 6. Induction of receptor phosphorylation by the trispecific antibody. MIA PaCa-2 cells were incubated with the trispecific and parental antibodies as schematically shown. An isotype negative antibody (R347) and no antibody treatments were included as negative controls. After incubation the cells were lysed, and phosphorylated EphA2, EphA4, and EphB4 were immunoprecipitated using receptor specific antibodies. Western blots were probed with antiphosphotyrosine HRP antibody and developed using enhanced chemiluminescence (ECL). The respective phosphorylated EphA2, EphA4, and EphB4 are schematically labeled. The molecular weights are reported in KDa.

Figure 7

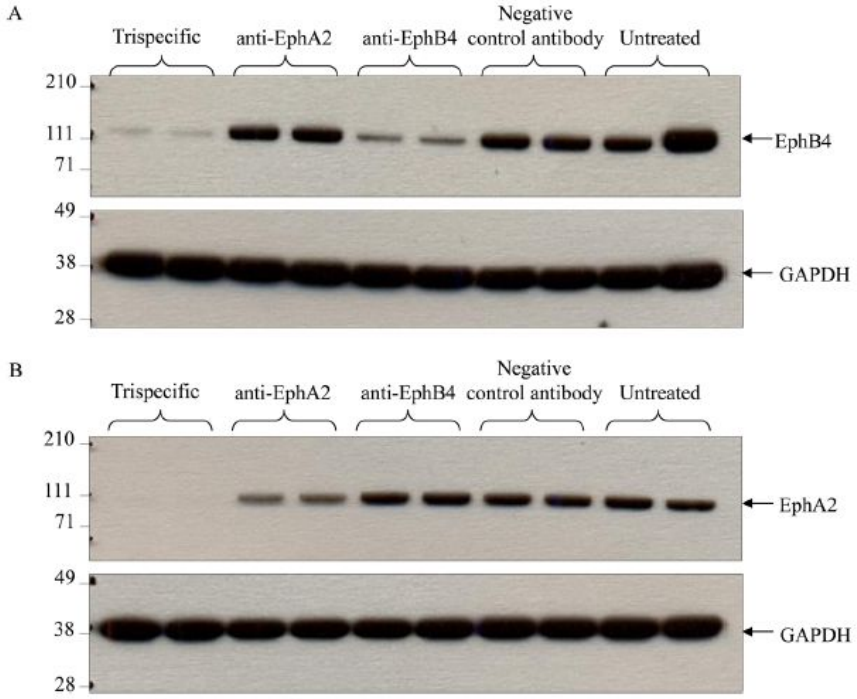


Figure 7. *In vitro* degradation of EphB4 and EphA2 in PC-3 cells by the trispecific antibody. The cells were treated with the trispecific or control antibodies (anti-EphA2, anti-EphB4, negative control R347 antibody or untreated) at 67 nM, as schematically shown. Trispecific and control antibodies were incubated with PC-3 cells for 4 h for EphB4 degradation (A) and 24 h for EphA2 degradation (B). Western blots were probed with anti-EphA2, anti-EphB4, and anti-GAPDH antibodies. The positions of EphB4, EphA2, and GAPDH (glyceraldehyde 3-phosphate dehydrogenase) are schematically shown. The molecular weights are reported in KDa.

Figure 8A

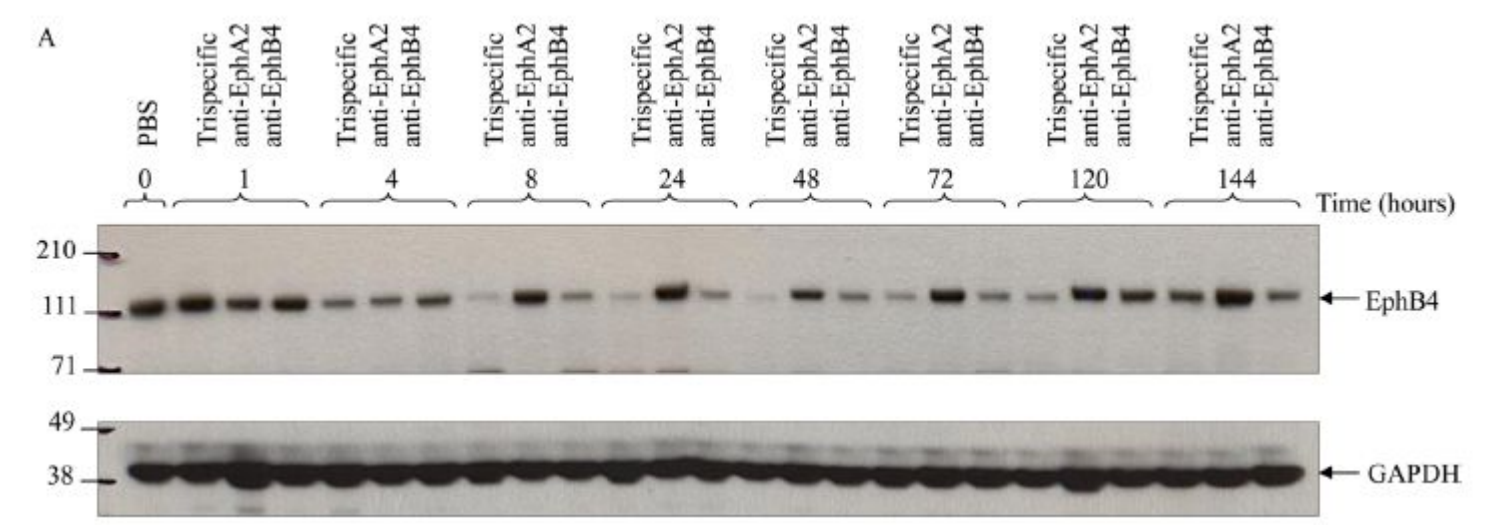
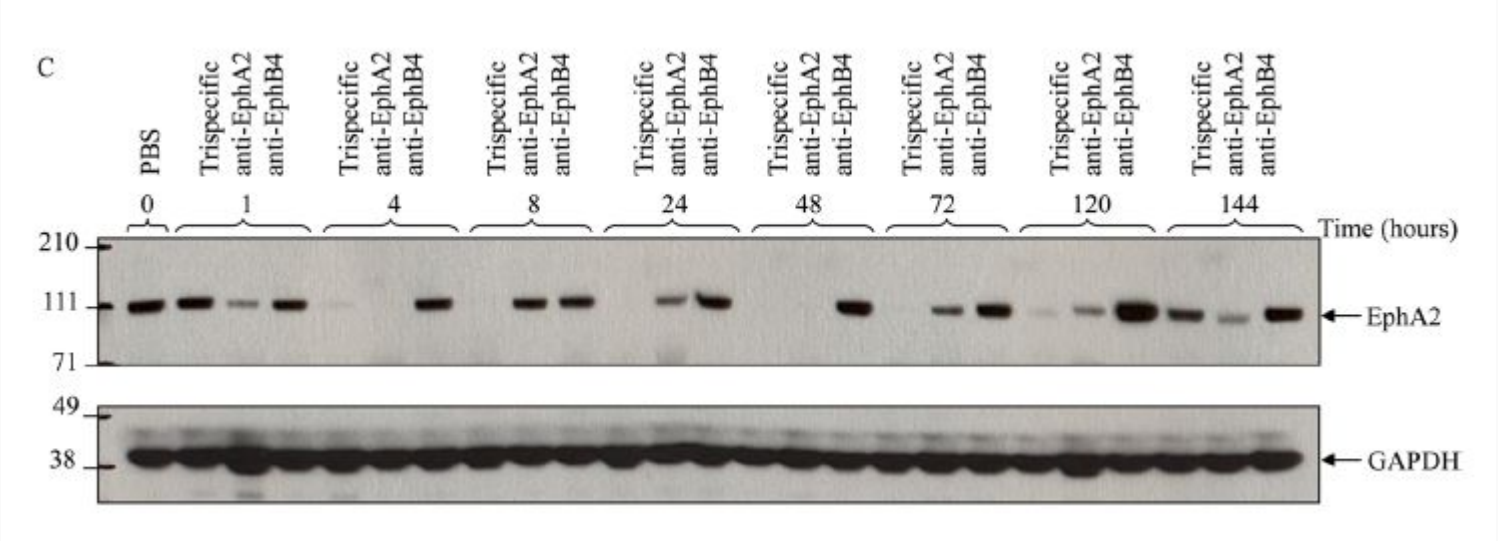

A
PBS
Trispecific anti-EphA2 anti-EphB4
0
1
4
8
24
48
72
120
144
Time (hours)
210
111
71
EphB4
49
38
GAPDH

Figure 8C


C
PBS
Trispecific
anti-EphA2
anti-EphB4
0
1
4
8
24
48
72
120
144
Time (hours)
210
111
71
EphA2
49
38
GAPDH

What did the authors learn by the end of the study?

Questions?

Questions, comments, and concerns?

For more on the immune system...

Overview

Antigen presentation and recruitment of cytotoxic T cells

The paper