Module Overview

Day	Lecture	Lab
1	Introduction	DNA library synthesis (PCR)
2	SELEX I: Building a Library	DNA library purification (agarose gel electrophoresis)
3	SELEX II: Selecting RNA with target functionality	RNA library synthesis (*In vitro* transcription = IVT)
4	SELEX III: Technical advances & problem-solving	RNA purification and heme affinity selection
5	Characterizing aptamers	RNA to DNA by RT-PCR
6	Introduction to porphyrins: chemistry & biology	Post-selection IVT Journal Club 1
7	Aptamer applications in biology & technology	Aptamer binding assay
8	Aptamers as therapeutics	Journal Club 2

Aptamer Applications

20.109 Lecture 7

6 March, 2012

Today's Objectives

- Consider, through discussion, an increasingly important application area for aptamer technology

- Overview of antibodies as affinity reagents to provide a context for appreciating when to consider aptamers versus antibodies

Objective

- As Team Leader at AptUs™ Biotechnologies (77 Mass. Ave. in Cambridge), you would like to develop a rapid and sensitive method for uniquely and simultaneously detecting 50 proteins. These proteins have homologies ranging from 30-60% (i.e. they are highly similar to each other).

- The relative abundance of these proteins in human serum samples correlates with diabetes risk.

- You are highly motivated to use your team's primary expertise to generate aptamers that can unambiguously distinguish these proteins.

- The newest team member, ***Hot Shot***©, asks about using antibodies to tackle this problem. After the massive group laughter subsides, you promise to discuss later.

Challenges

- *What are some key SELEX-related issues you'll need to address?*
- *What are your strategies for addressing these issues?*
- *How will you integrate your aptamers into a detection device?*
  - What will be your readout?
  - How will you calibrate your system?
  - Keep in mind that you will likely want to analyze hundreds of samples simultaneously!

Discuss

Challenges

- *What are some key SELEX-related issues to address?*
  - Large number of proteins and selection optimization space to consider
  - High degree of similarity between the various targets => potential for cross-reactivity is high
  - Obtaining target proteins (all 50?!)
    - Pure?
    - Mixture?

Challenges

- *What is your strategy for addressing these issues?*

  - Large number of proteins and selection optimization space to consider
    - Automation (options?)

  - High degree of similarity between the various targets => potential for cross-reactivity is high

    - Counter-selection (how might you efficiently do this?)
    - Perform SELEX on the isolated, non-homologous protein regions only
      - Advantages and disadvantages?

  - Obtaining pure target proteins (all 50?!)
    - Recombinantly express each individually
    - Complex mixture SELEX (problems?)

Challenges

- *You've successfully obtained aptamers for 45/50 targets!*
  - How do you verify their respective specificities?
  - What cross-reactivities are you concerned about?
    - Between highly related classes of proteins
    - "Non-specific" interactions with other unrelated proteins
      - Especially high abundance proteins (e.g. albumin)

Challenges

- You now start thinking about how you'll use these aptamers to test the levels of these various proteins in blood samples.
  - *What test format do you choose?*
    - Solution phase
    - Immobilized (which component do you immobilize?)
    - How do you detect/visualize a binding reaction?
  - You anticipate doing high throughput testing. Which formats are most compatible with having to run tests for 100 samples/day?
    - Microarray
    - Microfluidics

Aptamers versus antibodies

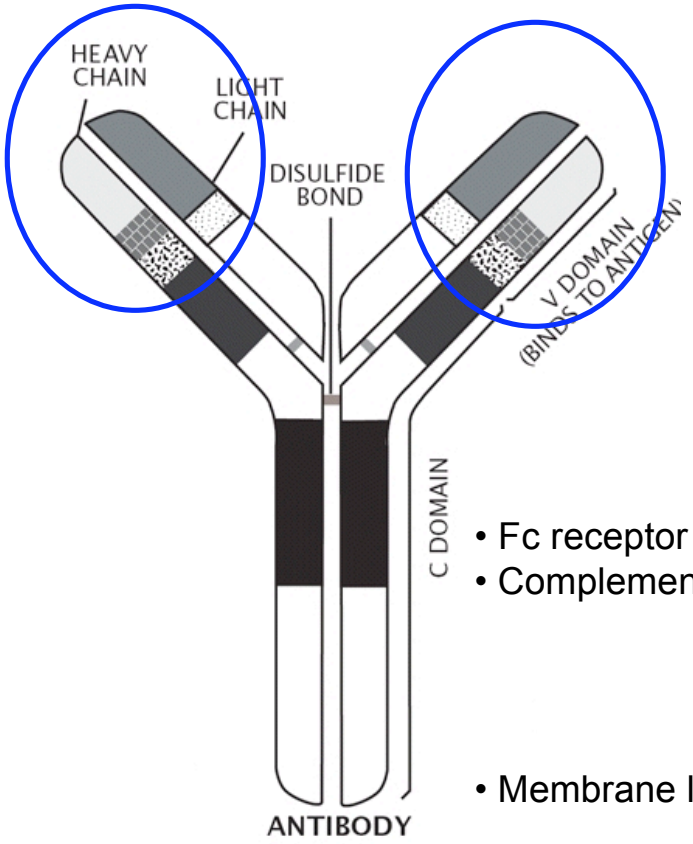
Antibodies: General

- Antibodies are proteins
  - Produced by B cells
  - Each B cell produces one type of antibody

- Overall structure
  - 2 heavy chains
  - 2 light chains
  - Both intra- and inter- chain disulfide bonds important for maintaining structure and function

- Functional Regions
  - Variable
    - Antigen binding
      - Both heavy & light chains contribute to binding site
  - Constant regions

Diversity: ≥ 10^{11}

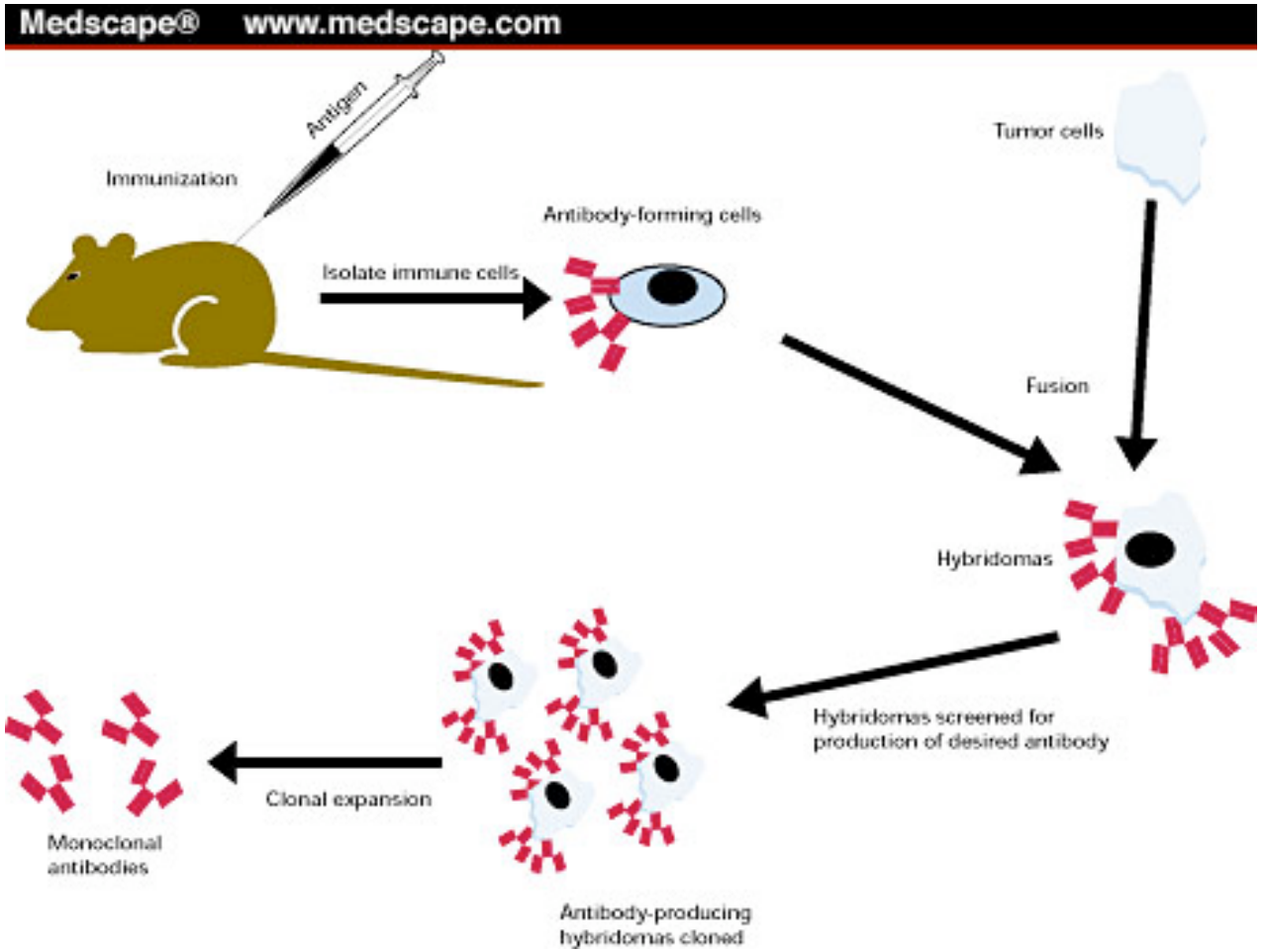


- Fc receptor
- Complement binding

- Membrane localization

Antibodies

- How are antibodies produced?
  - Immunize an animal (e.g. mouse, goat, rabbit)
  - Several months later, antibodies can be isolated (if immunization successful)



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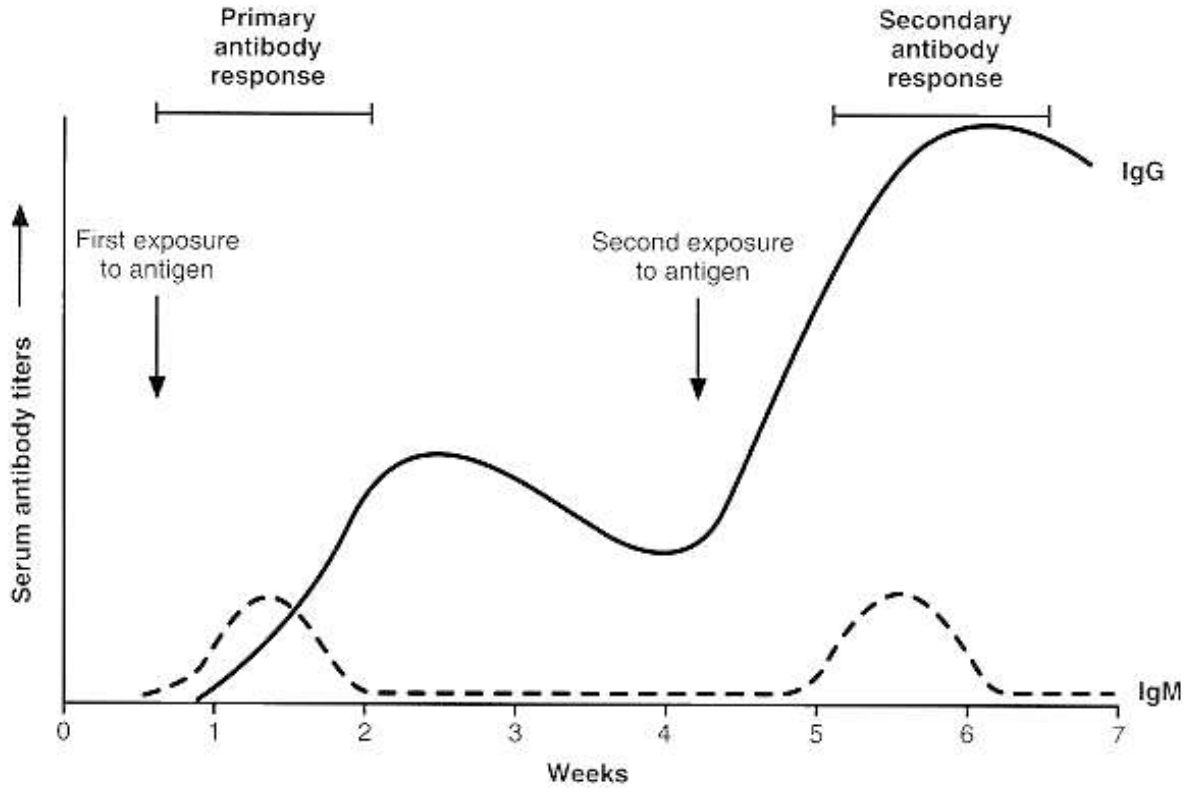

Typical time course for antibody production after immunization

Antibodies

- Are there any similarities between antibody and aptamer selections?
  - An animal makes large, randomized antibody pools (B cell clones)
  - Antibodies to the target antigen are **selected** from this pool
- Do you expect an antibody to every possible target will be present in an animal's antibody repertoire?
  - Consider representation within space considerations
  - Any other factors?
    - Yes--Antibodies that interact strongly with self-proteins are stringently selected against
    - B-cell clones capable of making these antibodies are eliminated from an animal's antibody repertoire

Antibodies

- What are some **advantages** to using or working with antibodies for binding reactions?
  - Lots of existing expertise!
  - Stability
  - Nature has optimized the selection process
    - Robustness of antibody production process
    - Potentially higher success rate of identifying an antibody
  - Selection stringency high ==> skewed towards identifying high affinity antibodies
  - Selection occurs (unattended) in immunized animal

Antibodies

- What are some **disadvantages** to using or working with antibodies for binding reactions?
  - Limited to targets that are not toxic to the animal!
  - Cannot easily tune the selection stringency
  - No guarantee that antibody will function in non-physiological conditions
  - Limited antibody reuse
  - Requires using animals (costly to house)
  - Batch-to-batch variability can be high

Aptamers versus antibodies

- Limited to targets that are not toxic to the animal!
- Aptamers to toxic substances can be developed

- Cannot easily tune selection stringency
- Stringency easily tuned

- No guarantee that antibody will function in non-physiological conditions
- Aptamers can be selected under conditions in which they will be used

- Limited antibody reuse
- Aptamers can be refolded and reused

- Requires using animals (costly to house)
- No live animals required

- Batch-to-batch variability can be high
- Chemically well-defined aptamers can be reproducibly synthesized

Summary

- Aptamers are a viable strategy for binding and distinguishing closely related protein family members

- For multi-target, high throughput protein identification studies, there is a need to:
  - Increase the throughput for producing aptamers to distinct targets
  - Use aptamers in a format compatible with processing many samples in parallel

- Antibodies are the gold standard affinity reagents in biology/biotechnology
  - Many desirable characteristics
  - Aptamers can rival antibodies
  - Must carefully consider your applications to decide which affinity agent is more suitable