

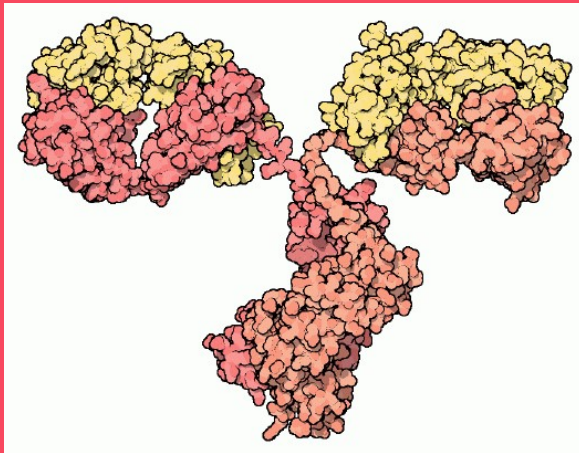
Antibody quality control in biomarker research

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COST CLINIMARK TRAINING SCHOOL, 25.9.2019 SPETSES



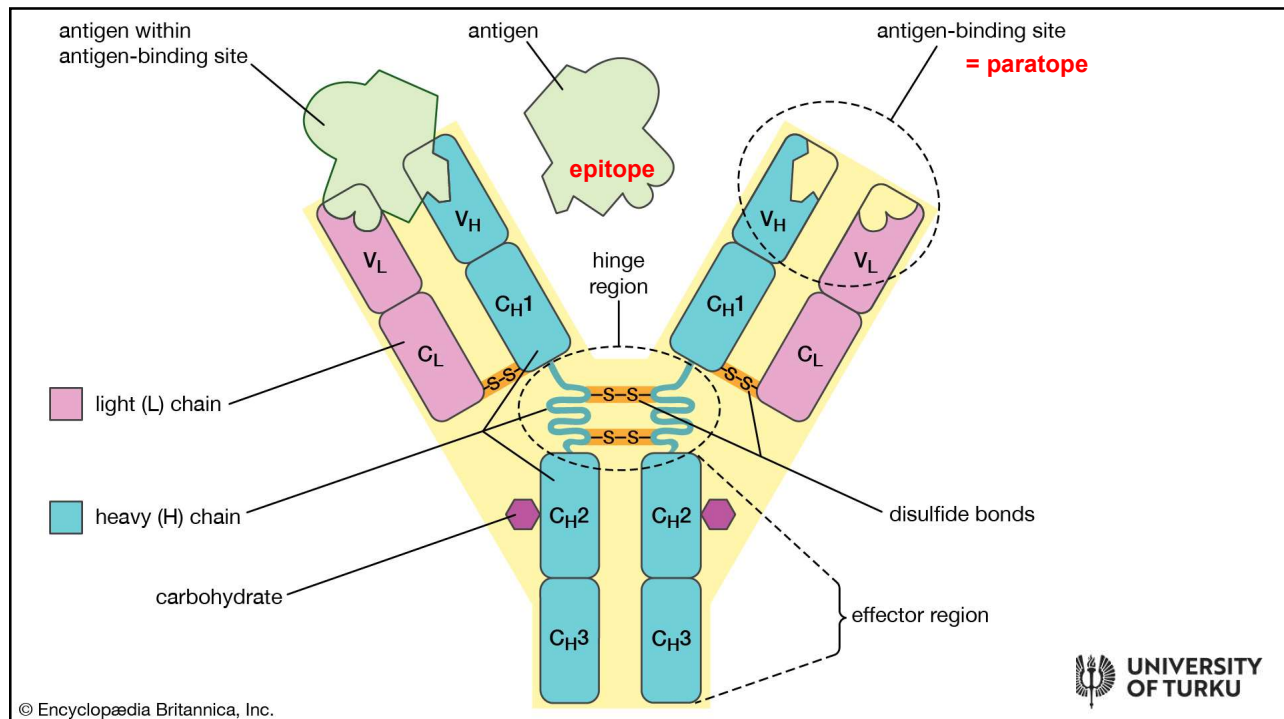


>\$2 billion spent on research tool
antibodies per year

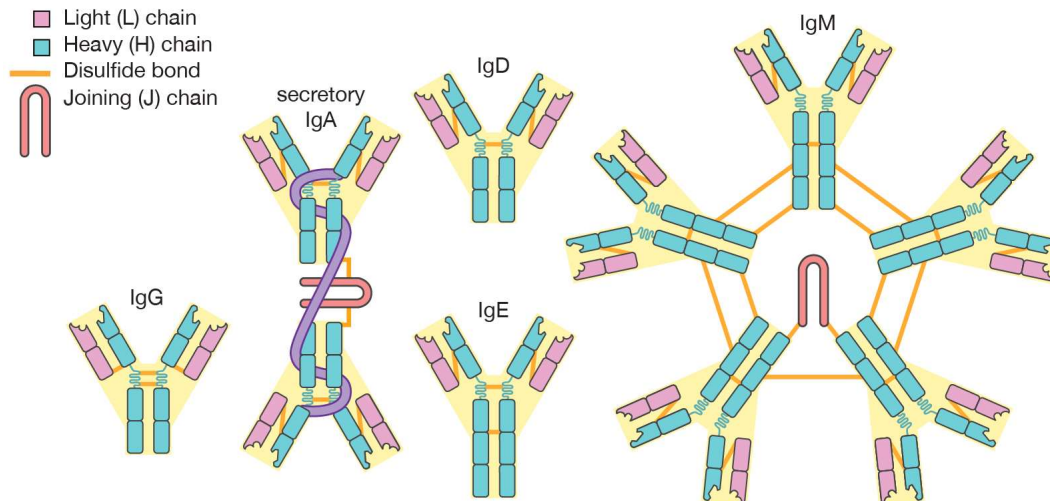
**BLAME IT
ON THE
ANTIBODIES**



Antibodies are very good tools
if you know how to use them!



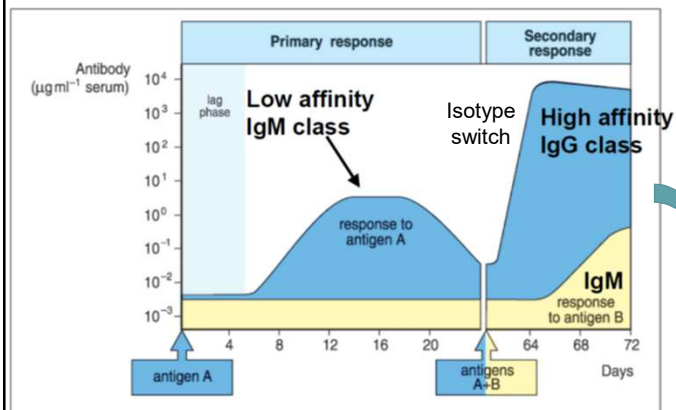
Antibody isotypes



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Antibody protein sequence and bioactivity changes during the immune response



Polyclonal antibody

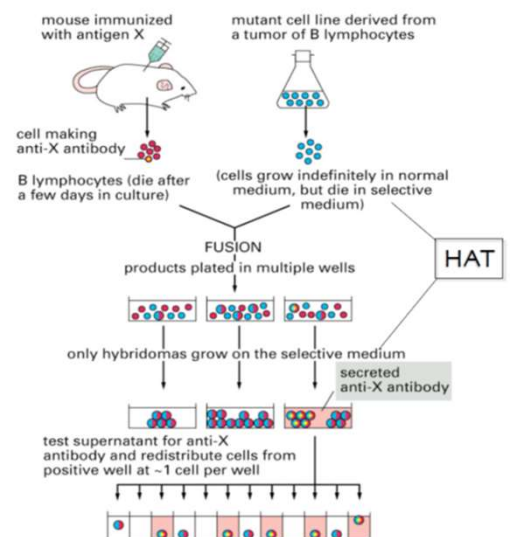
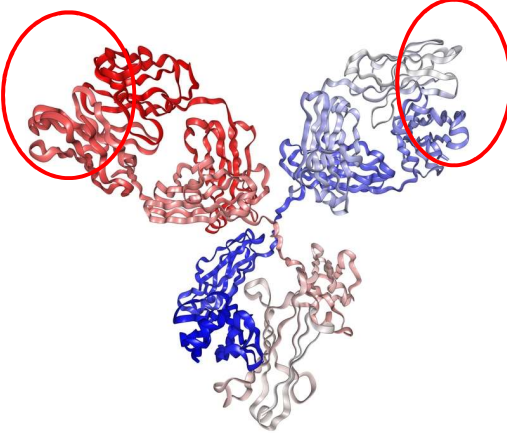


Figure 8-6 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

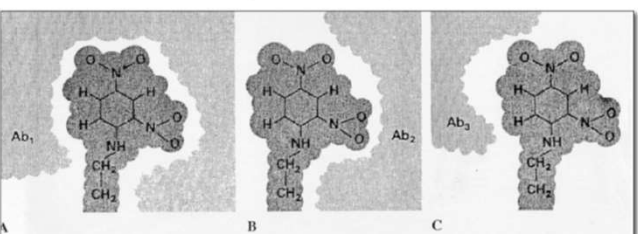
Monoclonal antibody





High affinity

Low affinity



The Law of Mass Action

$$[Ag] + [Ab] \xrightleftharpoons[k_{off}]{k_{on}} [AgAb]$$

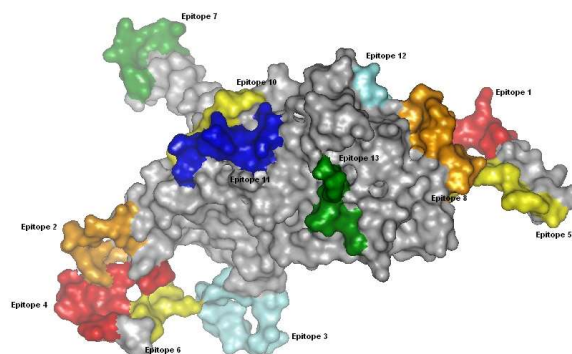
<https://www.immunology.org/structure-antibody-1972>

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What we want from an antibody in biomarker research?

⇒ Efficient and specific binding to the target molecule!

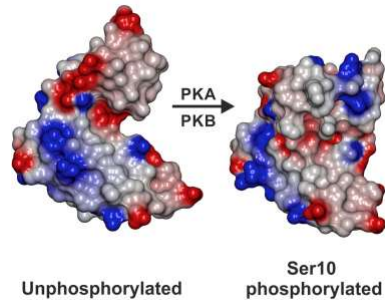
Antibody only specific for a certain epitope = a certain molecular surface structure



<https://www.proimmune.com/proarray-ultra-b-cell-linear-epitope-mapping/>

**If the interacting surface structure of the target molecule (epitope)
or of the antibody (paratope) is changed, binding is affected!**

Conformation change of target (denaturation, phosphorylation, mutation)
= decrease(/increase) in affinity

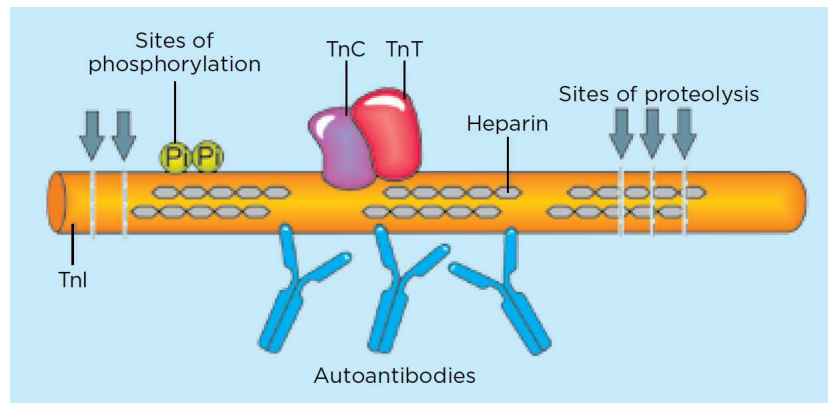


[https://www.cell.com/structure/abstract/S0969-2126\(16\)30134-4](https://www.cell.com/structure/abstract/S0969-2126(16)30134-4) – cysteine string protein



**If the interacting surface structure of the target molecule (epitope)
or of the antibody (paratope) is changed, binding is affected!**

Epitope unavailable on target
(degradation, complexation, splicing variant, anticoagulants, autoantibodies, other species...)
= no binding



<https://www.hytest.fi>

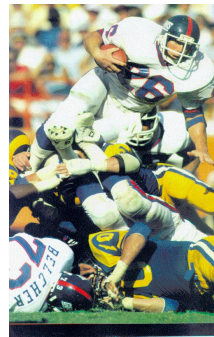


If the interacting surface structure of the target molecule (epitope) or of the antibody (paratope) is changed, binding is affected!

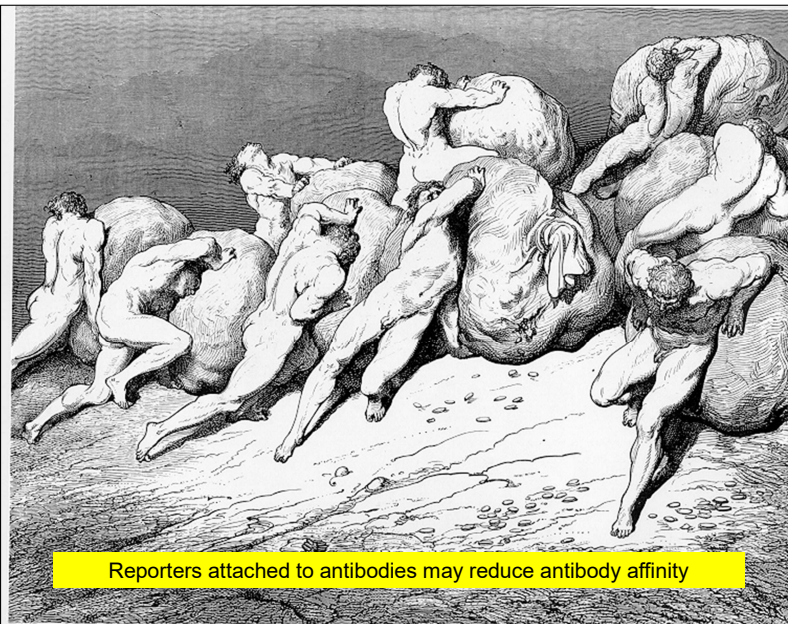
Immobilisation of antibodies may effect the affinity of an antibody dramatically



Y Y Y Y
EXPECTATION



Y Y Y Y
REALITY

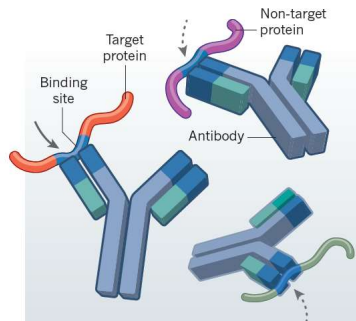


Reporters attached to antibodies may reduce antibody affinity



If similar surface structure found on other molecules

=> off-target binding!



Binding specificity of an antibody: not an absolute measure but a continuous variable directly dependent on binding affinity

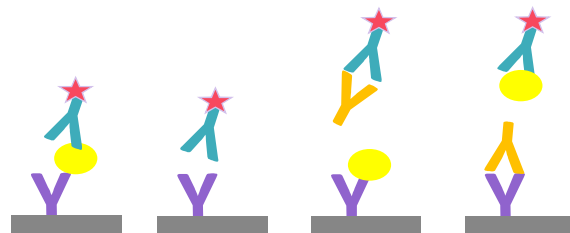
Minor cross-reactivity (affinity towards off-target << target) will cause major problems if concentration of off-target >> target



Baker M. 276 | NATURE | VOL 521 | 21 MAY 2015

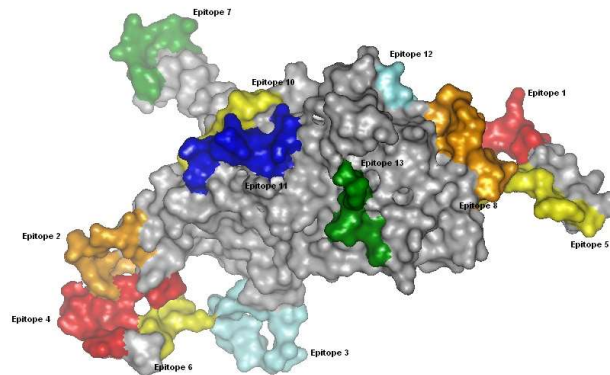
Also binding conditions matter!

- **interferences of the sample matrix**
 - serum: anti-species antibodies, rheumatoid factor, complement...
 - urine: pH
- **assay buffer**
 - BSA! and other additives
 - pH
 - salt concentration...



When buying an antibody against a protein...
 ...you are buying an antibody **recognising a certain epitope** that was present on
 the **antigen product** used for immunization/binder panning

↓ does it represent your target in the sample?



"cTnI antibody ≠ cTnI antibody ≠ cTnI antibody"

"dairy product ≠ dairy product ≠ dairy product"

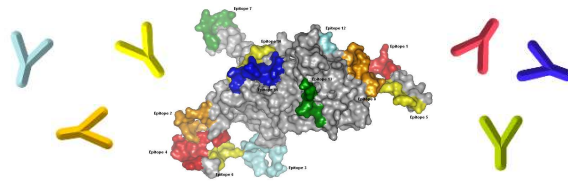


To get equal results, you have to use exactly the same antibody!

Antibody products

• Polyclonals

- By definition a mixture of antibodies only part of which bind to the aimed target
- Batch variation inevitable due to the nature of production
 - *Antisera different in each animal and even in the same animal after repeated immunisations*
- Cheap but should not be used unless limitations well understood!



Antibody products

• Monoclonals

- All molecules the same detecting the same epitope similarly
- No batch variation
- Some sustainability concerns if not produced with care

• Recombinant antibodies

- Confirmed structure to the level of genetic code
- No batch variation
- Expensive to produce and limited selection (*for now*)

Quality issues ("antibody does not work")

"Reproducibility crisis" – published results cannot be reproduced

Blame the antibodies?

- To be able to replicate the results, **exactly the same antibody must be used** in exactly the same experimental setting!
 - *Do not use polyclonals - batch-to-batch variation!*
- **Antibodies not identified well enough to replicate work**
- **Solutions**
 - *Detailed information in publications (duty of researchers, referees, journals)*
 - *Universal identifiers for antibodies*
 - *Forced use of recombinant antibodies?*



Quality issues ("antibody does not work")

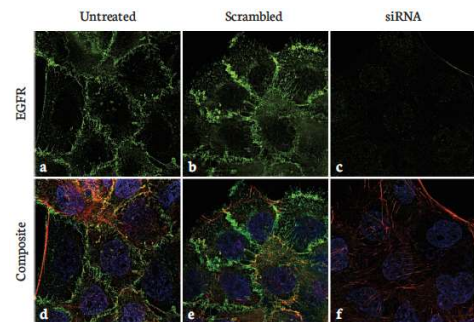
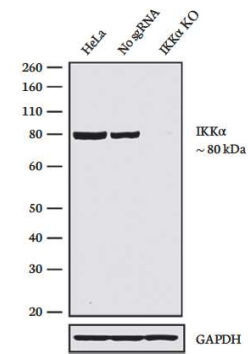
Antibody not fit for purpose

- **Performance of antibody different in different settings**
 - *The target may be in different form in different settings*
 - e.g. good Western blotting antibodies not good for immunoassays
 - target present in different forms in the sample (e.g. degradation, complexation, other species)
 - *Modifying the antibody can affect its function dramatically*
 - *Interfering substances in the experimental setting*
- **Solution**
 - Sufficient batch-specific validation data on fitness for purpose
 - *By suppliers*
 - *By users*



Antibody validation for purpose

- Performance with positive and negative controls
- ELISA etc.
 - Buffer with/without standard material
 - Target sample matrix with/without standard material
 - Target sample matrix with/without endogenous material
 - (Target sample matrix with likely off-target)
- Assay validation!
 - Dynamic range
 - Accuracy
 - Imprecision



It is not it hard work to make antibodies,
it is hard work to find the ones that work well!

How to find antibodies of "good quality"?

- Look for specific antibodies (monoclonals) which have been successfully used for the same purpose before
- Look for suppliers who provide thorough validation data
- Do not opt for polyclonals (if possible)
- Check the online tools
 - Antibodypedia.com, antibodies-online.com (validation data comparison)
 - Pabmabs.com/wordpress ("tripadvisor" for antibodies)
- **Start with multiple alternative antibodies!**



It is always the users responsibility to do the final validation of the antibody in a specific experimental setting!



Antibodies are excellent tools if used correctly!



