

# New technologies in laboratory diagnosis

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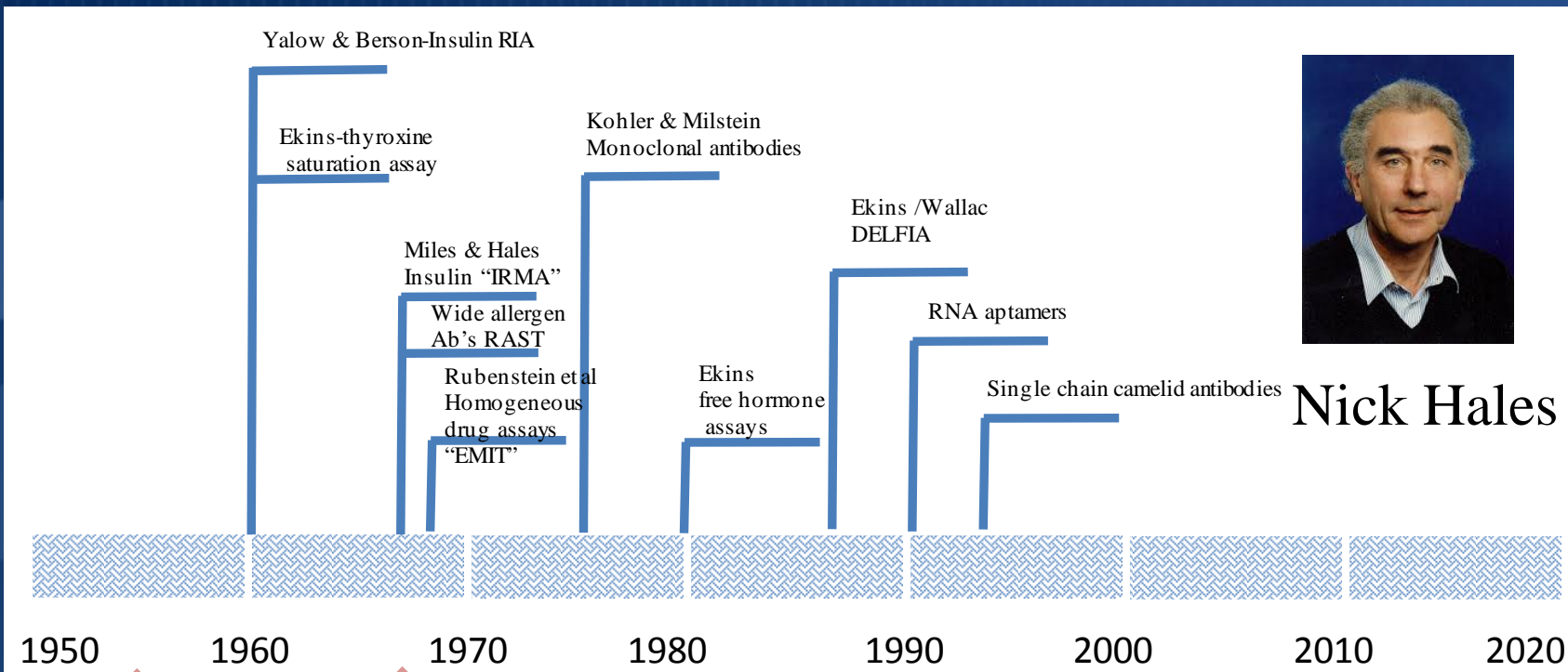
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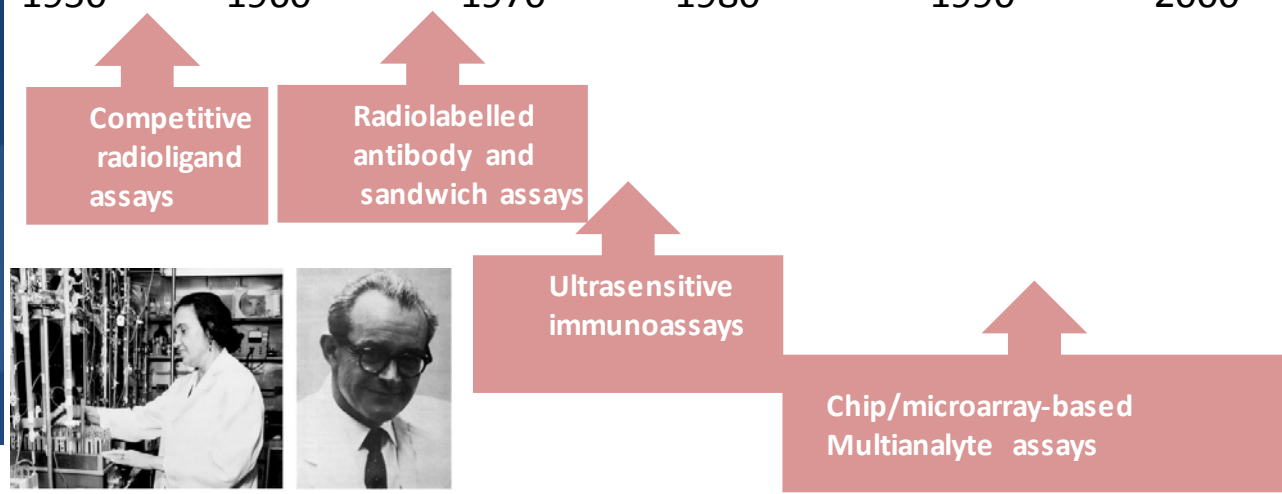
# Outline

- Structure of antibodies and characteristics of camelid heavy chain antibodies
- How to produce antigen-specific nanobodies
- Advantages of nanobodies
- Nanobodies as capture or detection agents





Nick Hales



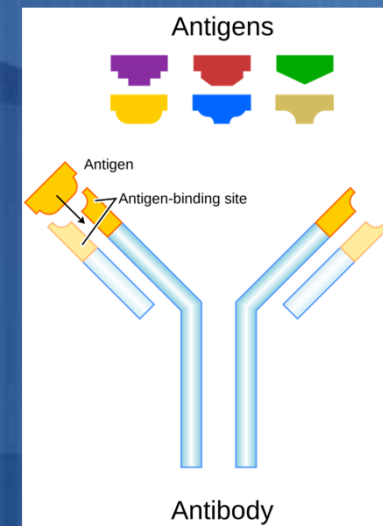
Rosalyn Yalow/Solomon Berson



Roger Ekins

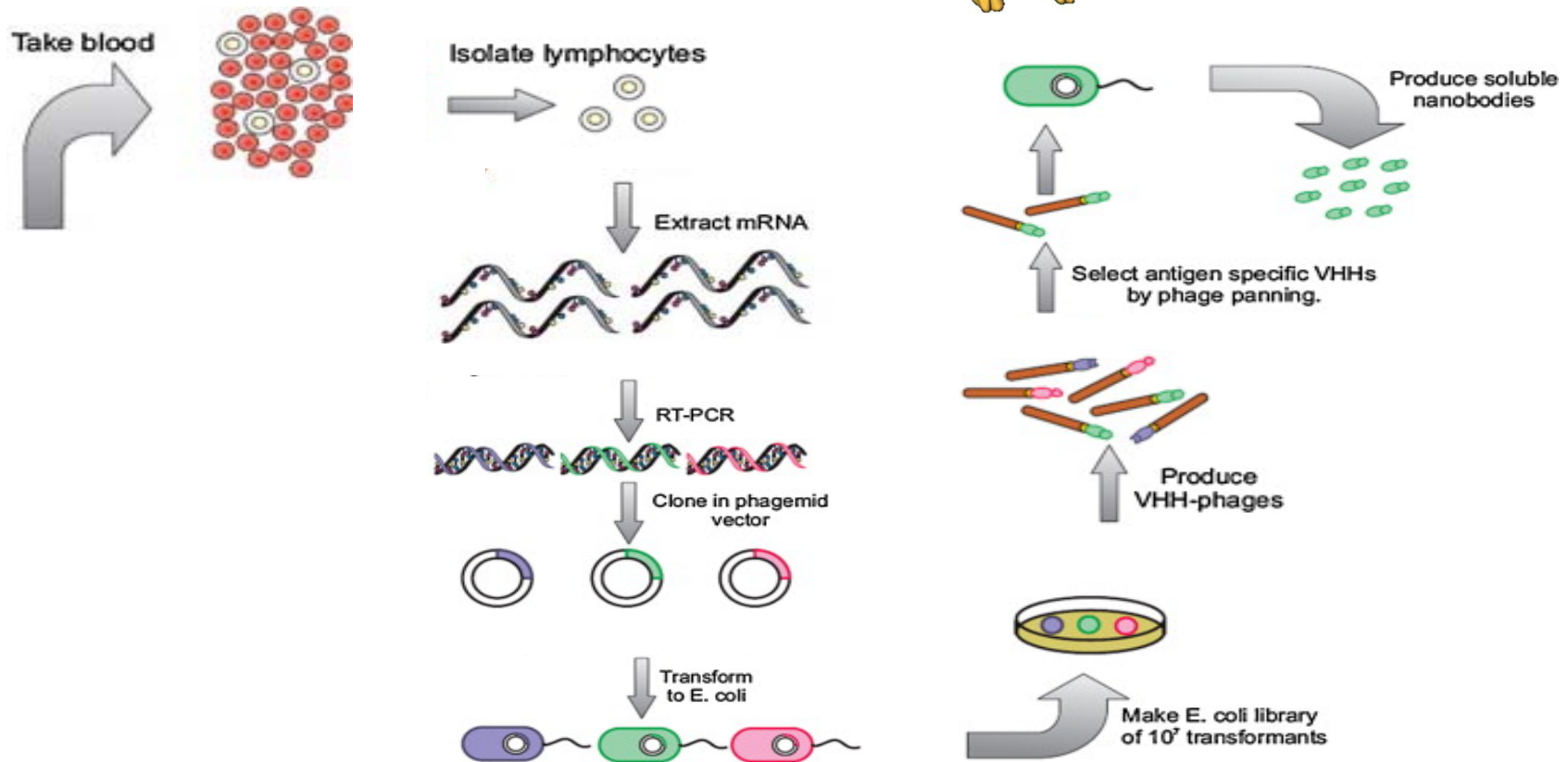
# Antibodies in medicine

- Mainstay of immunoassays-heart of diagnostic medicine and point-of-care testing devices
- Histopathology: immunohistochemistry
- Also important in therapeutic applications
- Antigenicity of proteins; Widely established for protein ligands especially; can be raised against virtually any epitope; highly specific for the epitope
- Bind with high affinity
- Polyclonal and monoclonal
- Monoclonals can be obtained in unlimited amounts
- New biomarker proteins or analytes
- More than diagnostic reagents: the emergence of biologics for therapy

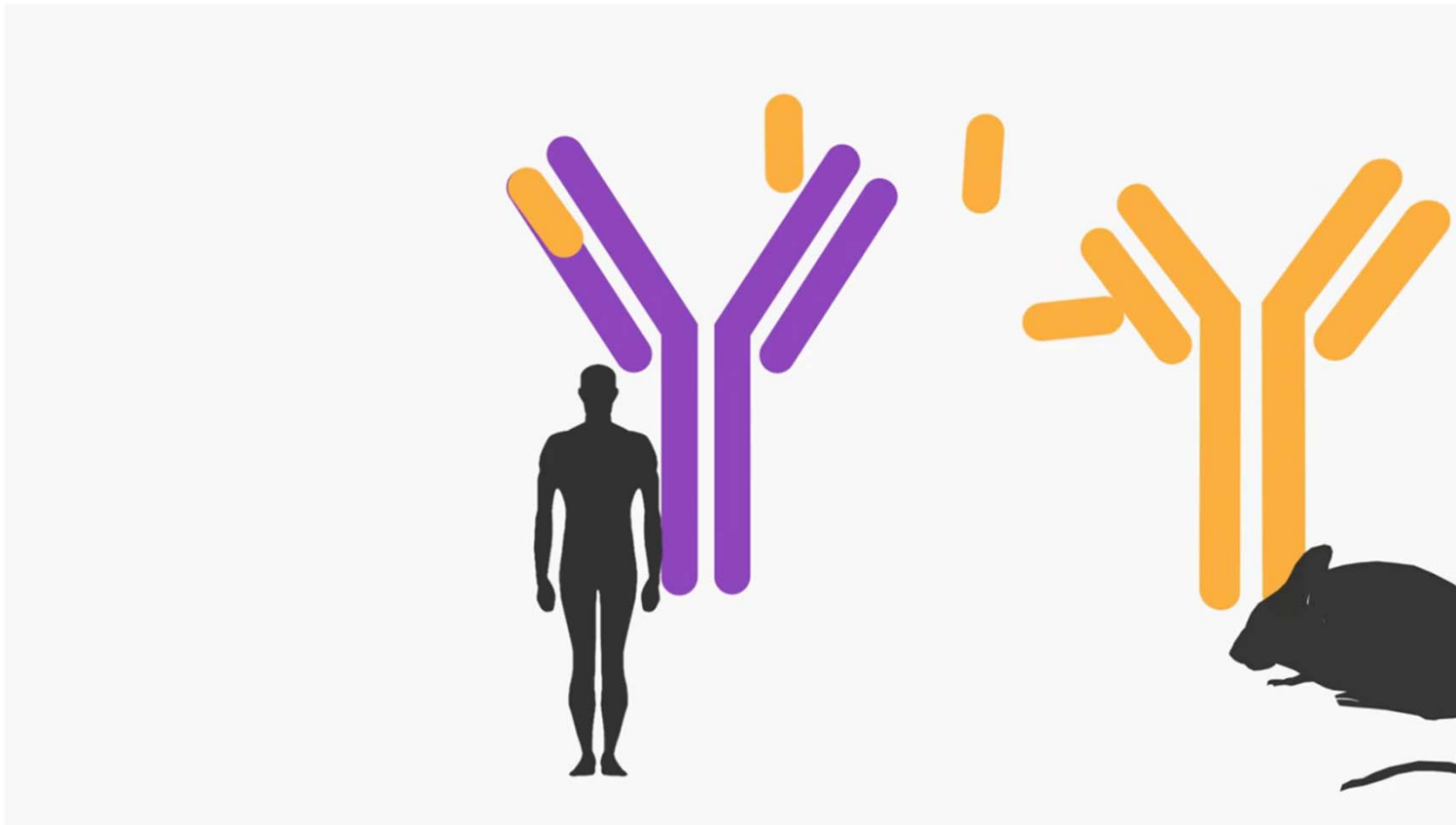




# Selection of antigen-specific VHH



## What are the challenges with traditional antibodies?





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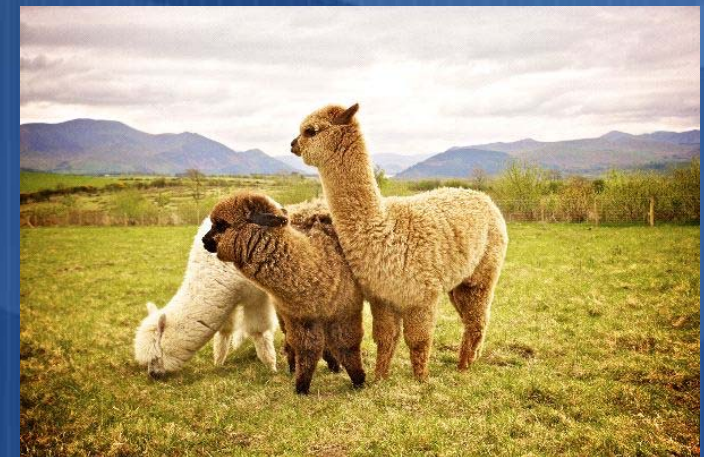
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# Taxonomy

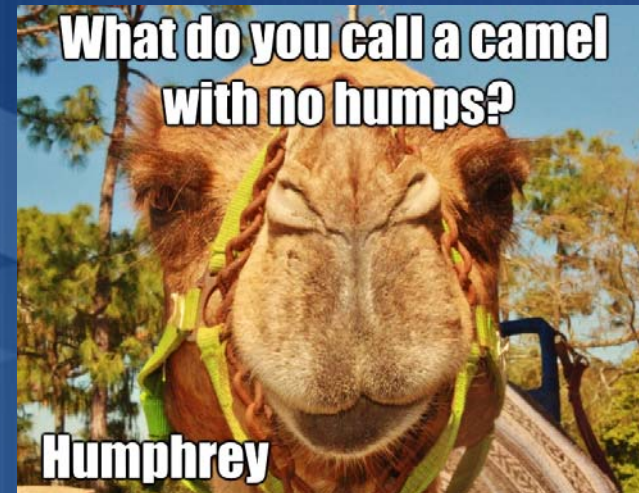
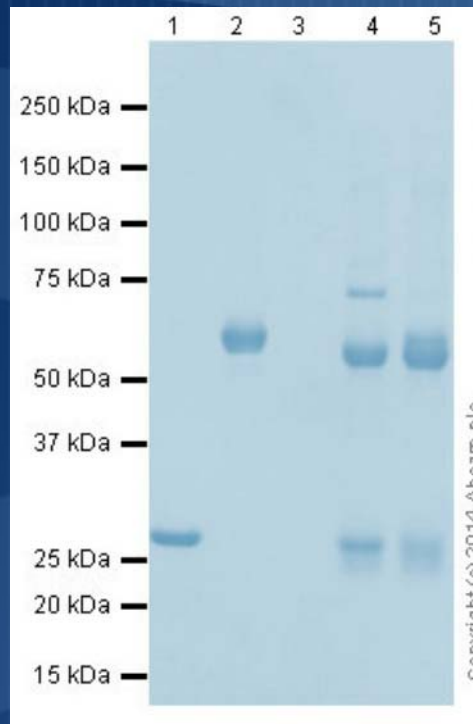


- **Camelidae**
  - Genus *Lama*
    - Llama, *Lama glama*
    - Guanaco, *Lama guanicoe*
  - Genus: *Vicugna*
    - Vicuña, *Vicugna vicugna*
    - Alpaca, *Vicugna pacos*, formerly *Lama pacos*
  - Genus *Camelus*
    - Dromedary, *Camelus dromedarius*
    - Bactrian Camel, *Camelus bactrianus*

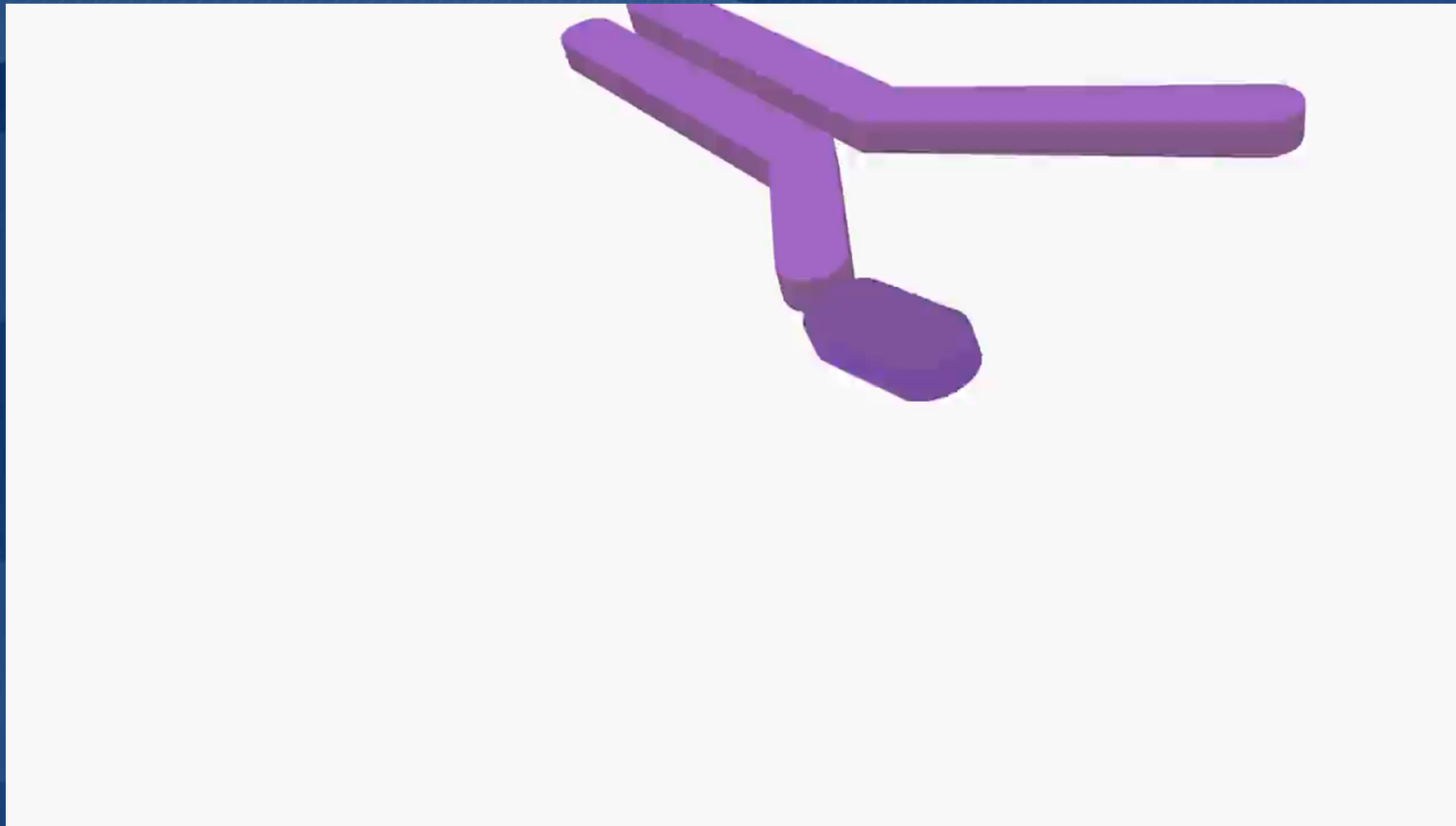


# Discovery of camelid single chain antibodies

- Hammers-Castemann, a graduate student



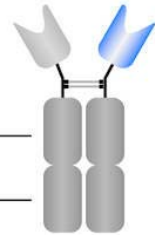
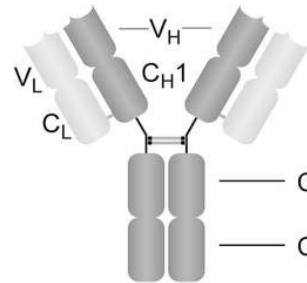
# Single domain antibodies



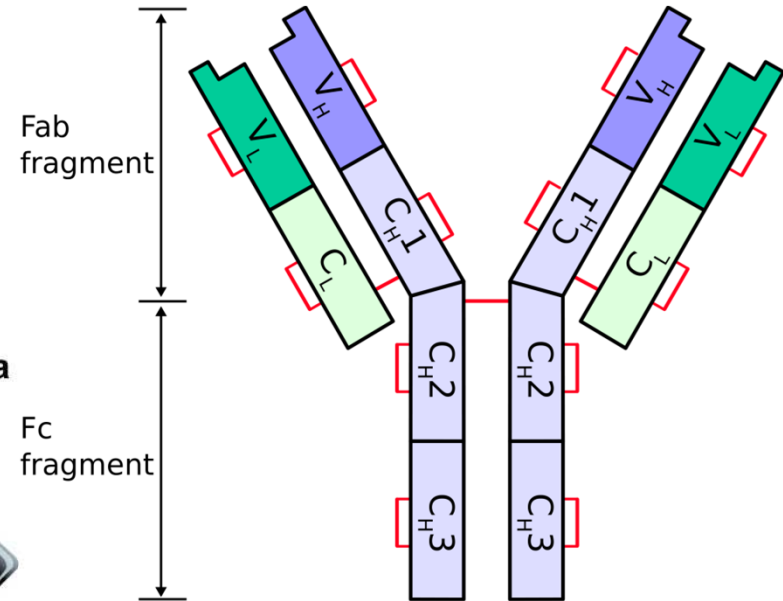
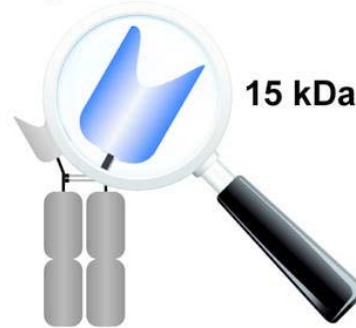


Conventional Antibody - 150 kDa

Camelidae Antibody



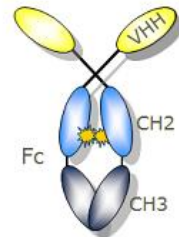
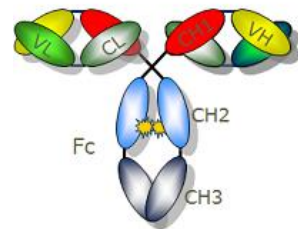
V<sub>H</sub>H fragment



Conventional antibody

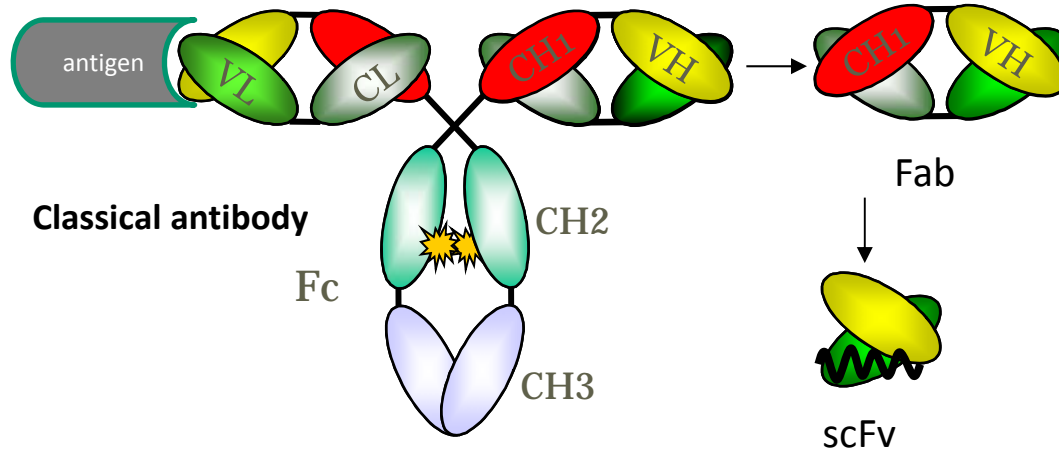
Camel Heavy-Chain antibody

V<sub>H</sub>H or Nanobody





# Camelid antibodies - nanobodies



Classical antibody

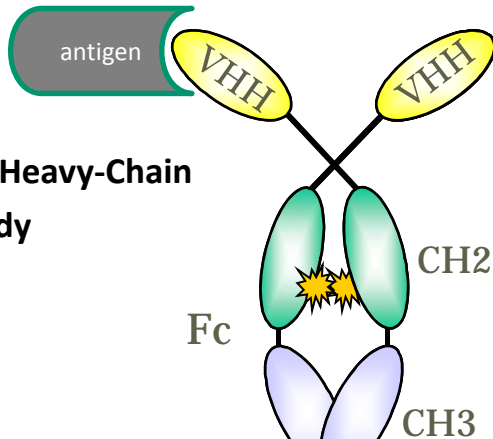
Fc

CH2

CH3

Fab

scFv



Camel Heavy-Chain antibody

Fc

CH2

CH3

VHH

Monomeric : 15 kDa  
Diameter 2.4 nm  
Height 4 nm

Smallest intact antigen-binding fragment derived from a functional immunoglobulin

➤ Nbs target unique epitopes

(poorly immunogenic by classical antibodies)

**Nb ≠ scFv = Fab**

➤ Antigen specific

**Nb = Fab = scFv**

➤ High affinity for the Ag

**Nb = Fab = scFv**

➤ Efficient identification of Ag binders

**Nb > scFv = Fab**

➤ Good expression yields

**Nb > scFv = Fab**

➤ Good stability

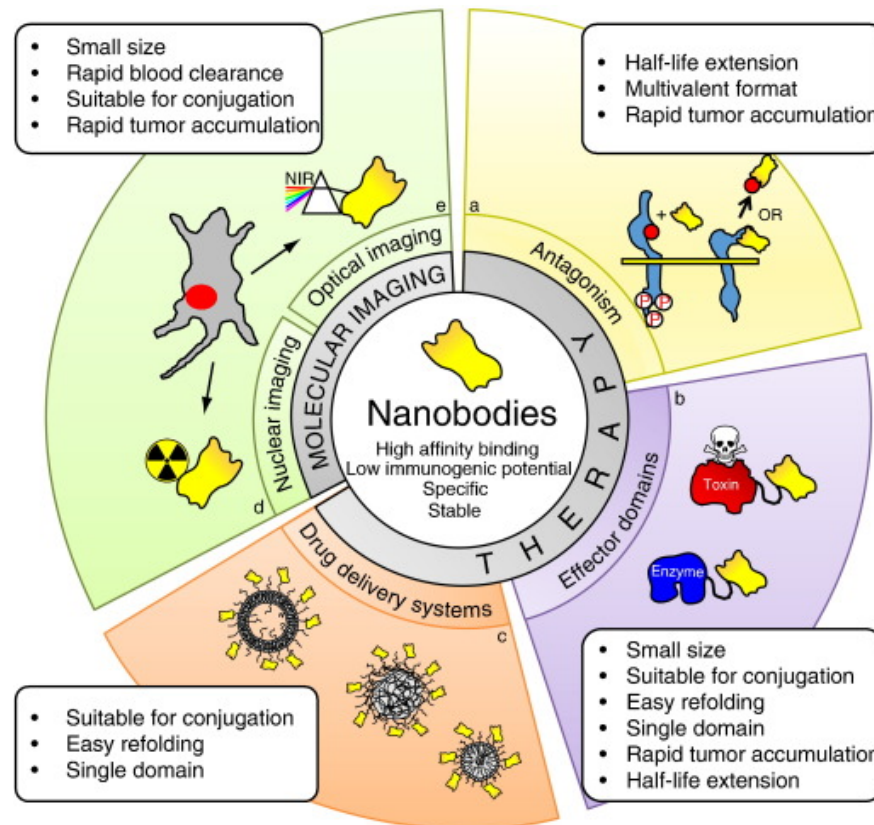
**Nb > Fab > scFv**

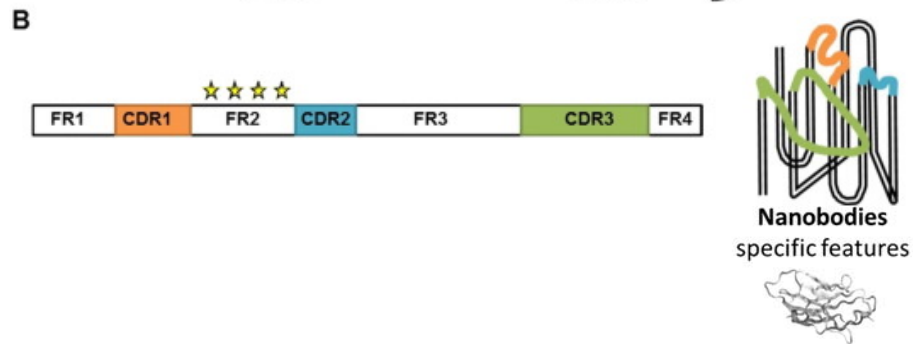
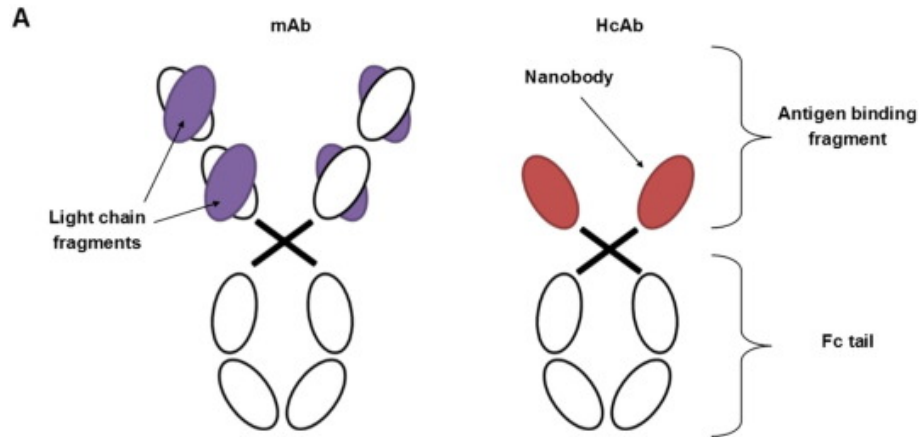
➤ Good solubility

**Nb > Fab > scFv**



# Role of nanobodies in medicine





- Small size (aprox. 15 KDa)
- High affinities (nano- to picomolar)
- Easy to engineer
- Possibility of multimerization /formatting
- Chemical/Thermo stability
- Better solubility
- Deep tissue penetration
- Fast blood clearance
- Cavity binding (hidden epitopes)
- Alternative routes of administration
- Low immunogenicity
- Manufacturing in microbial cells (Low production costs)

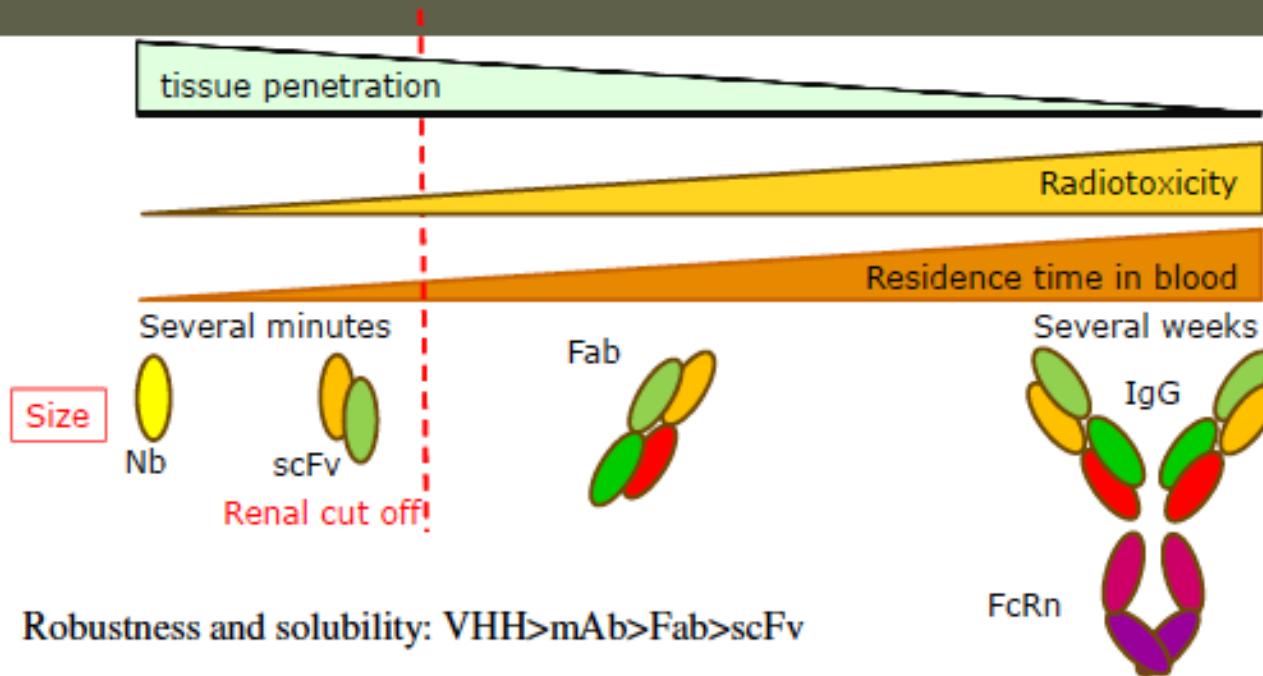
**DIAGNOSTICS**

- Recombinant fluorescent probes [52]
- Imaging [12, 13]
- Immunochemical assay for small molecules and protein detection [2, 3]

**THERAPEUTICS**

- Oncology [57]
- Neurodegenerative diseases [26]
- Infectious diseases [6]
- Inflammation processes [6]
- Envenoming
- Toxicity [12]

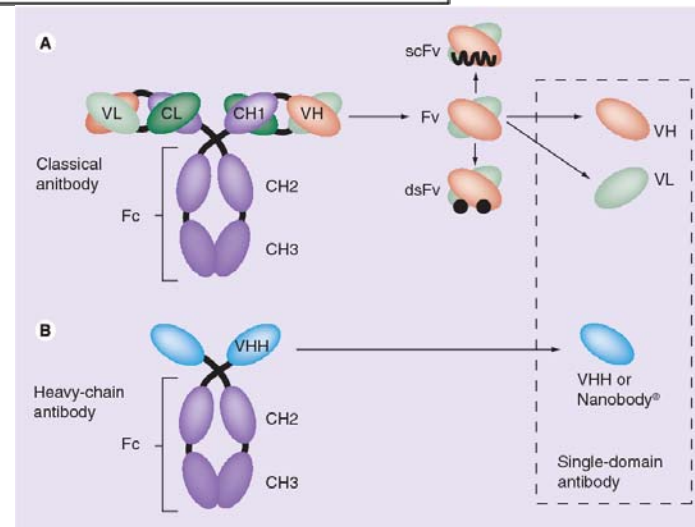
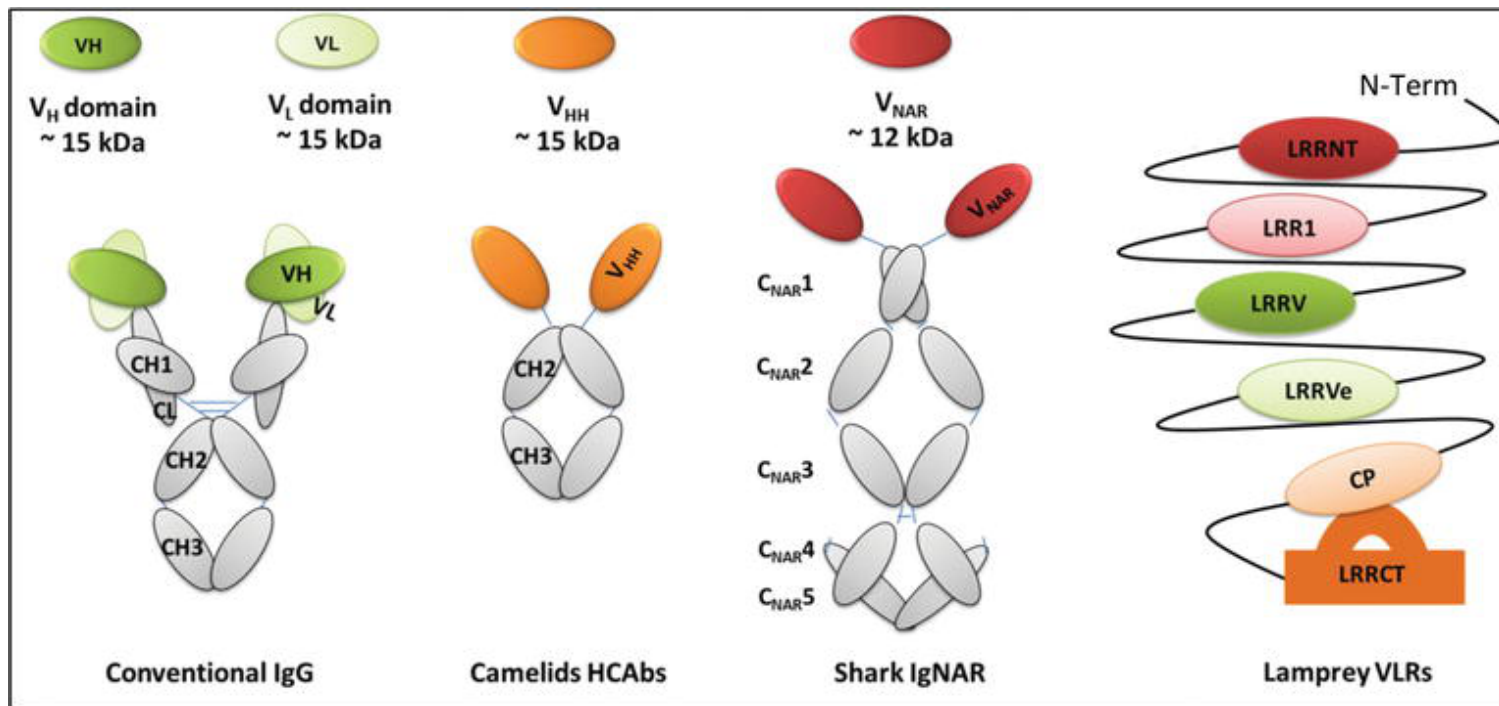
# Blood retention versus Ab size



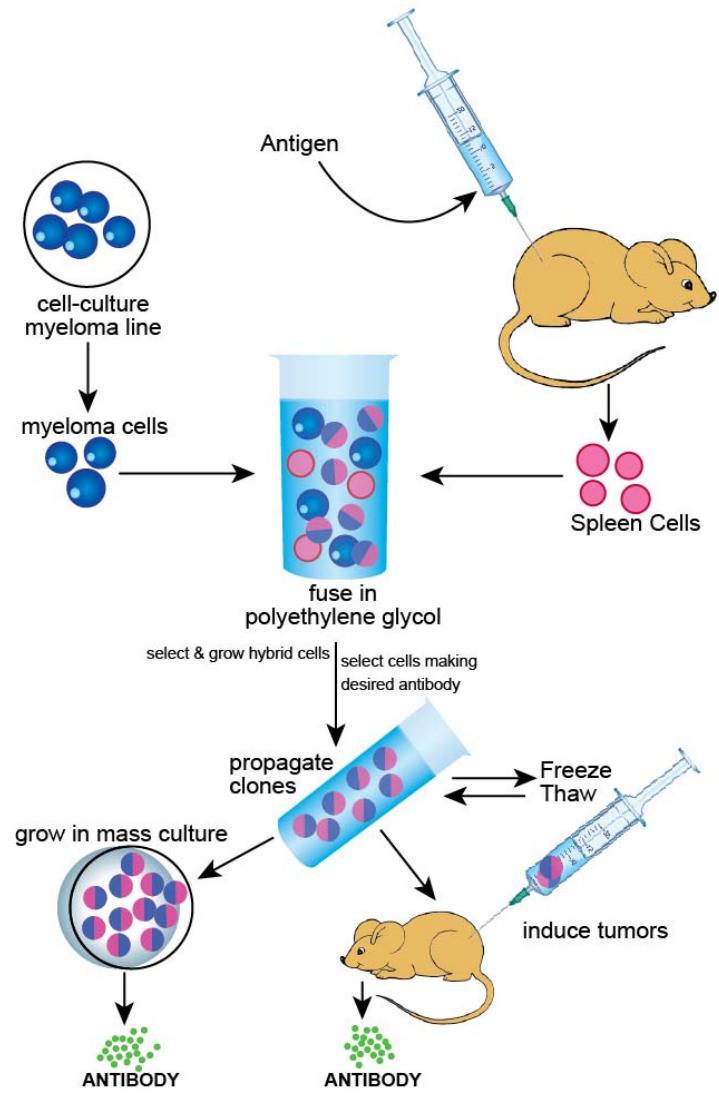
Robustness and solubility: VHH>mAb>Fab>scFv

Most important factor for imaging: **Contrast** (tumor load/blood ratio)

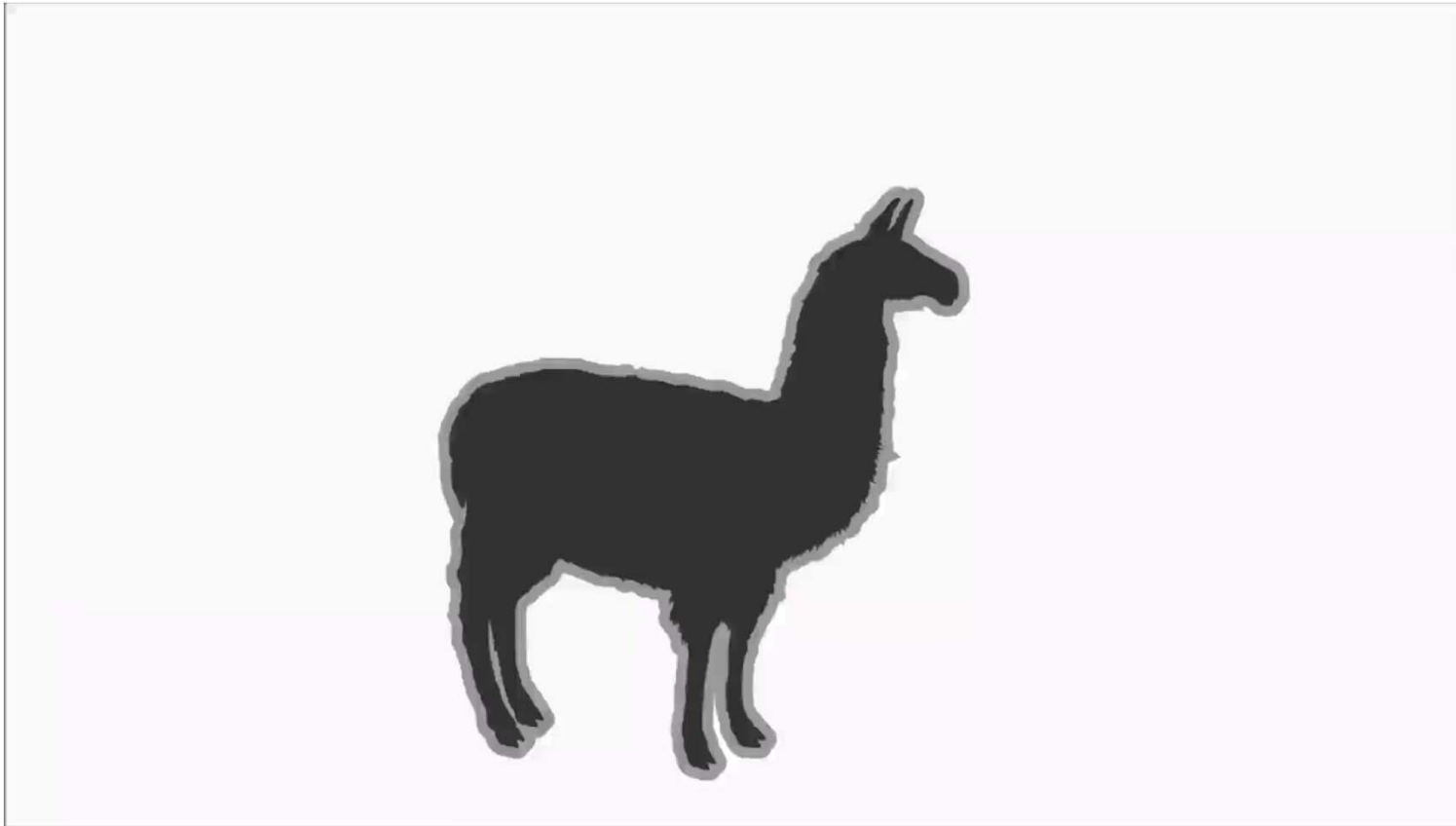




# Generation of conventional monoclonal antibodies



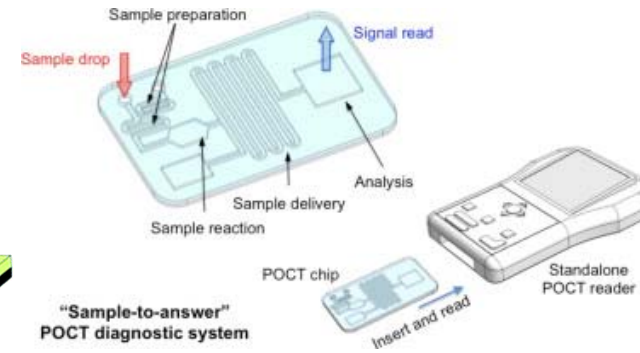
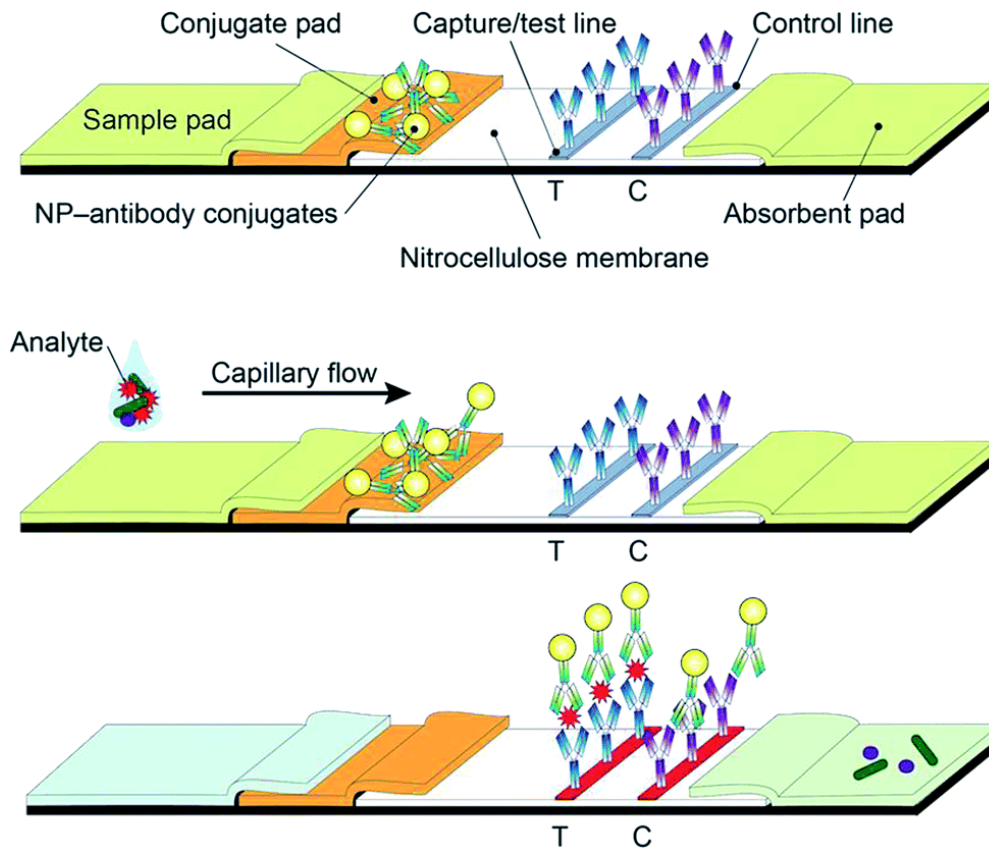
## Production of antibodies in camelids, eg. Llama or alpaca











# Outline

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- Structure of antibodies and characteristics of camelid heavy chain antibodies
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## In conclusion

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# Nanobody research in Iran

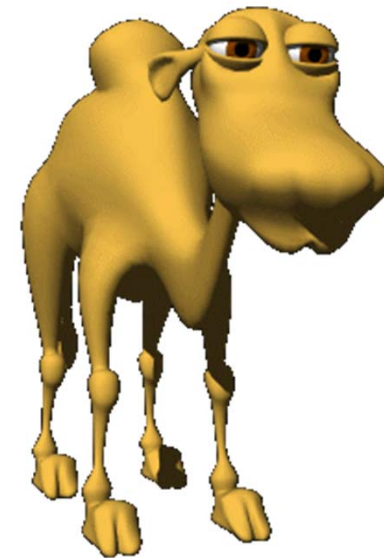
- Shiraz University
- Tabriz University







**Thank you for your attention**



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