Llama antibodies and Nanobodies.

Prof. Dr. Serge Muyldermans

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Antibodies

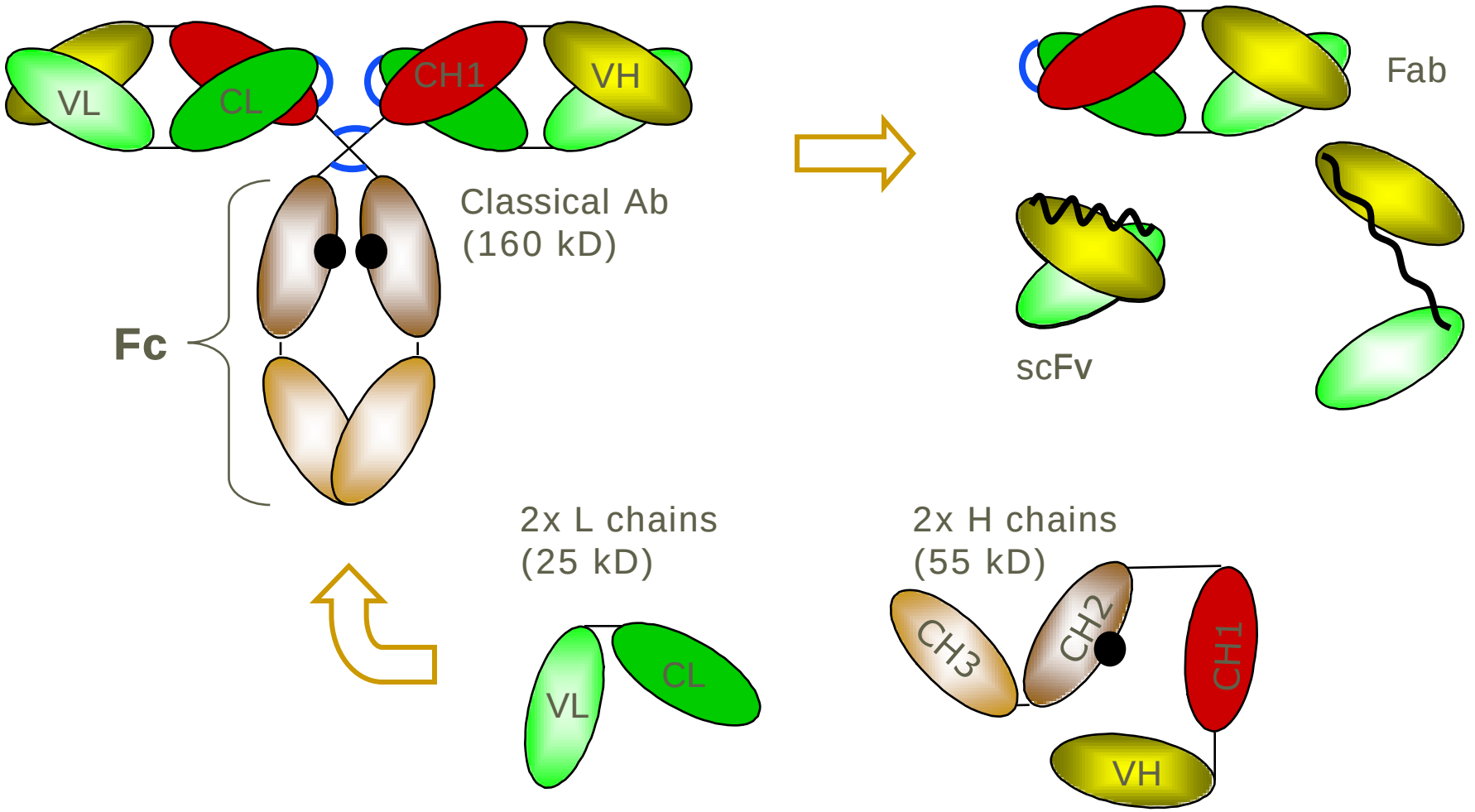
Antibodies are at the core of many diagnostic and therapeutic applications

- Can be raised against virtually any target (antigen)
- Highly specific for this antigen (epitope)
- Associate with high affinity
- Can be obtained in monoclonal form in nearly unlimited amounts.

In diagnostics, antibodies are used as capturing and/or as detection agents even in complex mixtures.

Antibodies are the natural therapeutics in vertebrates

Abs have conserved architecture



Outline of the today's presentation

A Nanobody is a generic tool. It can be used for research, for diagnostic applications and for therapy, to remediate environmental contaminations, to detect and treat veterinarian & human infections and diseases.

1. Basics of unique llama Heavy Chain Antibodies & recombinant single-domain antigen binding fragments (= Nanobodies)
2. How to obtain antigen-specific Nanobodies
3. Advantages of Nanobodies
4. Applications with Nbs as capturing or detection agents and in therapy.

Nanobodies: the next generation antibody products for research, diagnosis and therapy

1. Basics of unique llama HCAbs and Nanobodies
2. How to obtain antigen-specific Nanobodies
3. Advantages of Nanobodies
4. Applications with Nbs.

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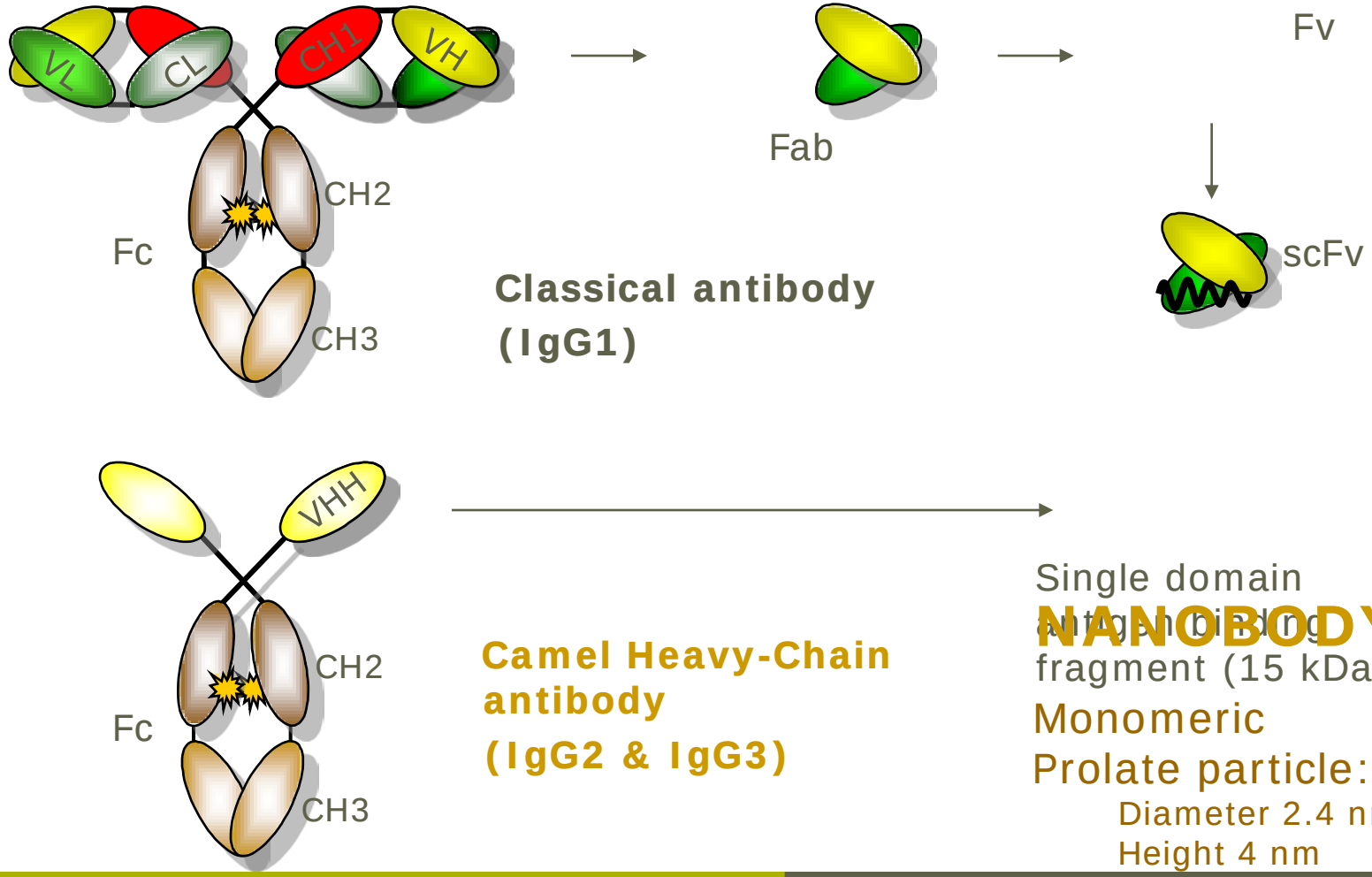
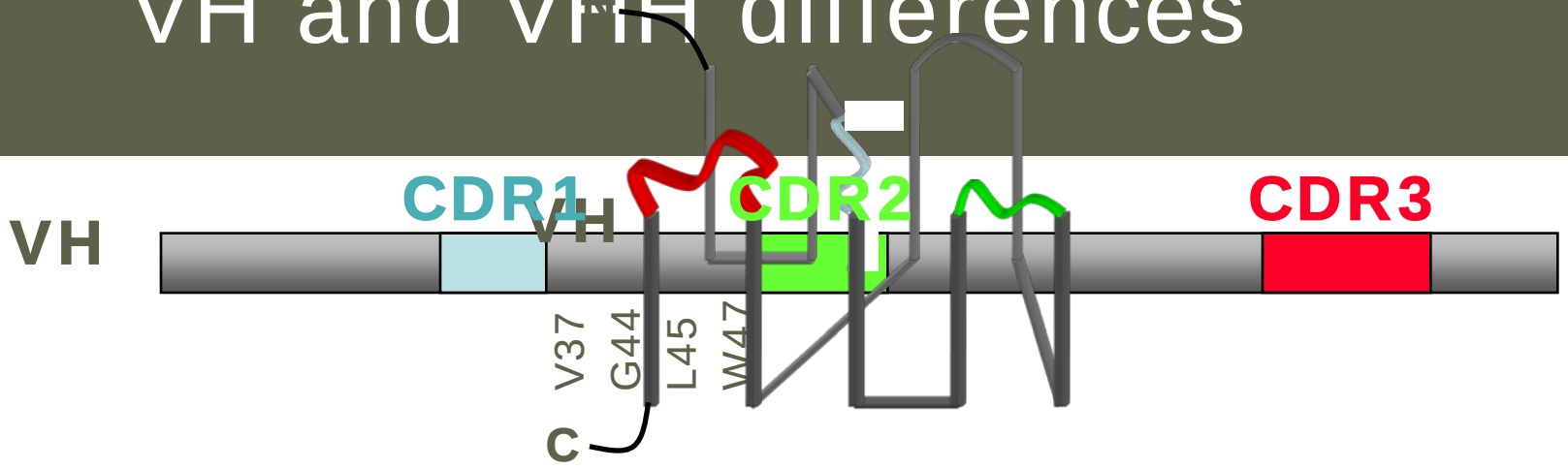
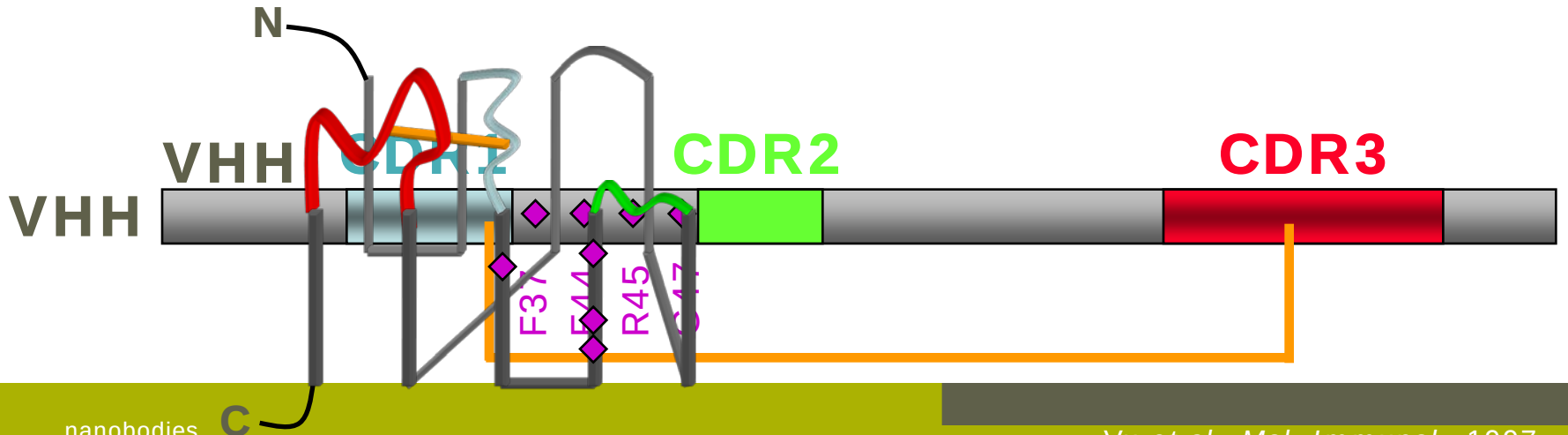
Camelid antibodies



Hamers *et al.*, *Nature*, 1993

VH and VHH differences





Vu *et al.*, *Mol. Immunol.*, 1997

Desmyter *et al.*, *Nat.Struct.Biol.*, 1996

Llama antibodies and Nanobodies

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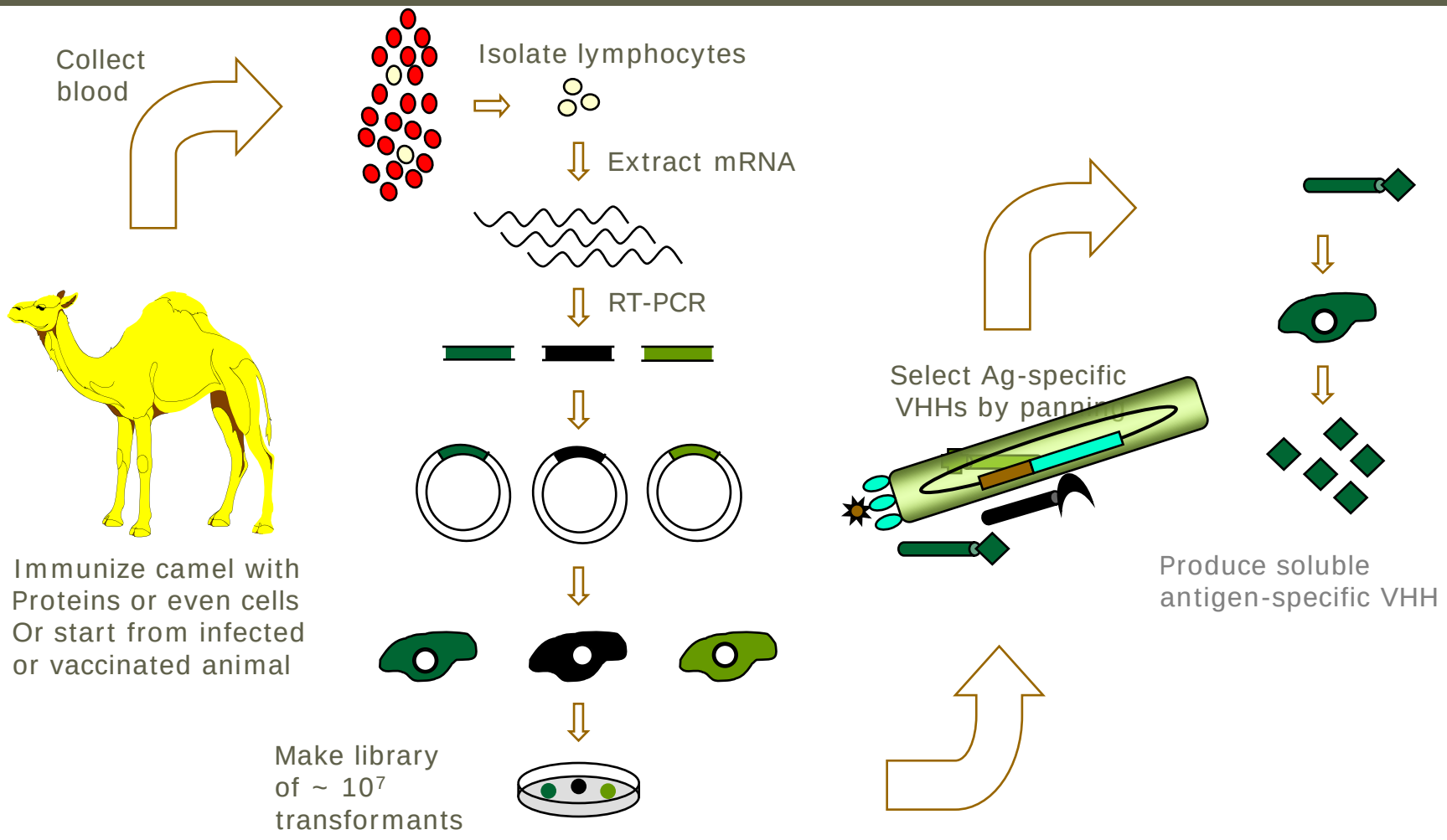
Animalarium: Dubaï, Tunisia, Quito

Selection of antigen-specific Nb



Ghahroudi *et al.*, *FEBS Letters*, 1997
Lauwereys *et al.*, *EMBO J.*, 1998

Llama antibodies and Nanobodies

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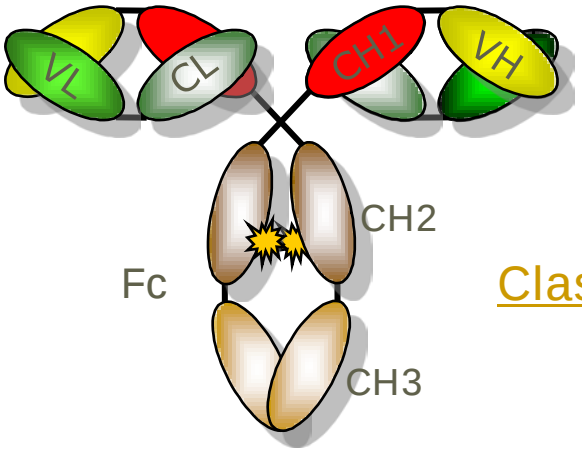
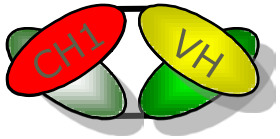
Antigen-binding fragments of Abs





Fab

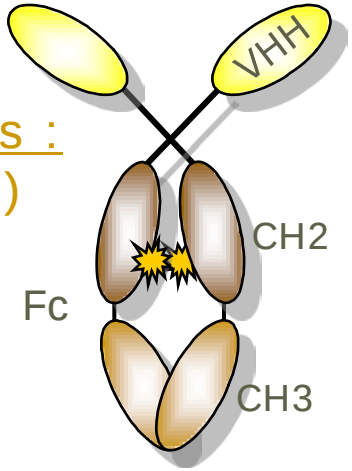
scFv

Scrambling of affinity matured VH-VL pairs

10^6 —> 10^{12}

Classical Ab & its fragments :

Heavy-chain Abs :
(camel or shark)



Nanobody

No scrambing of Ag-specific domain as only one gene fragment is amplified

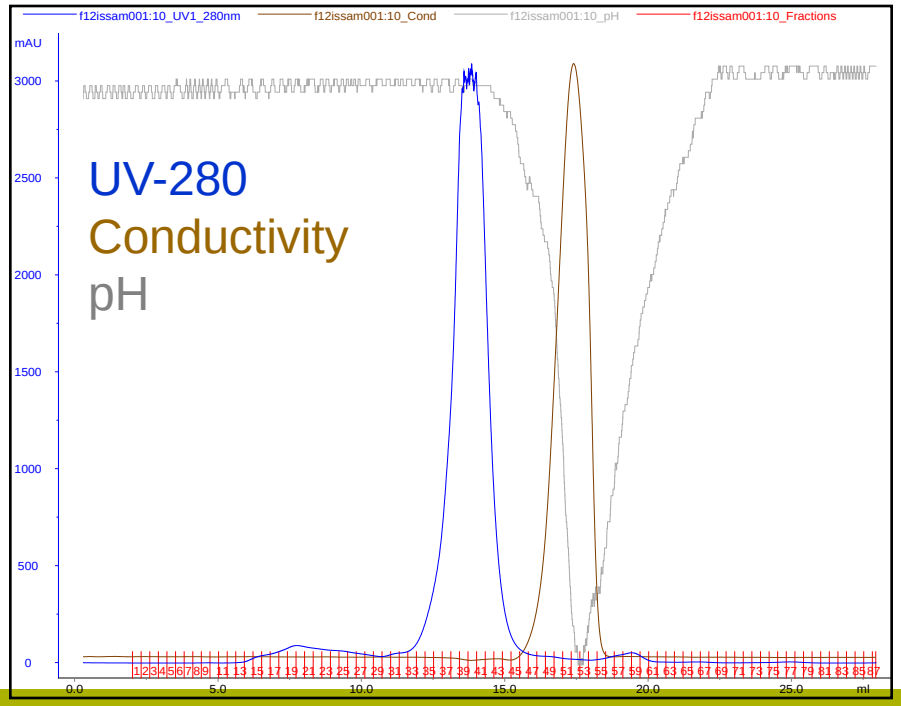
10^6 = 10^6

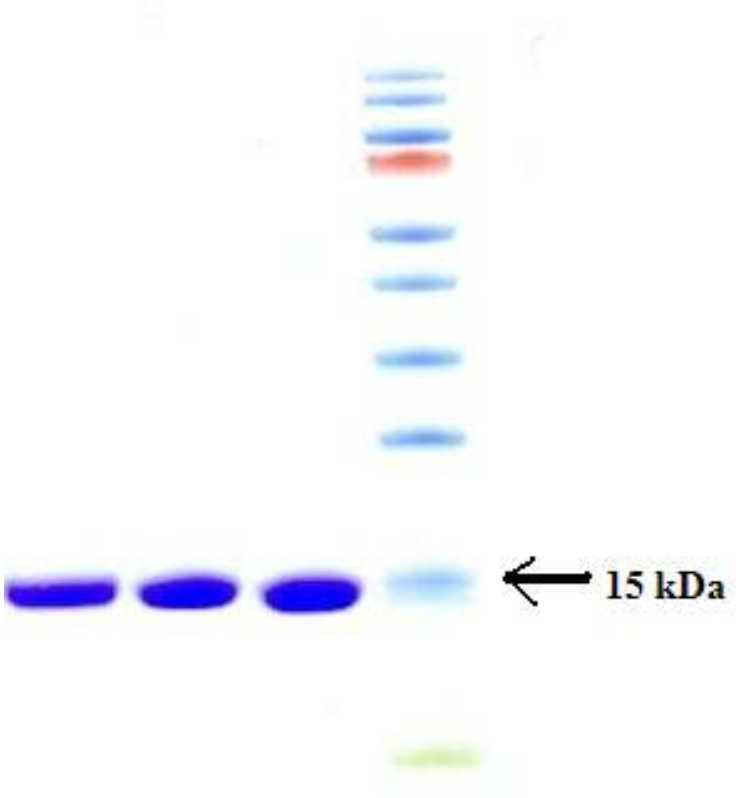
Nb properties versus scFv and Fab

❖ Efficient identification of Ag binders	Nb > scFv = Fab
❖ Good expression yields	Nb > scFv = Fab
❖ Good stability	Nb > Fab > scFv
❖ Good solubility	Nb > Fab > scFv
❖ Antigen specific	Nb = Fab = scFv
❖ High affinity for the Ag	Nb = Fab = scFv
❖ Nbs target unique epitopes	Nb ≠ scFv = Fab
❖ Easy tailoring	Nb > scFv = Fab

Purification of Nbs

Nb expressed in *E.coli*
Extracted from periplasm,
Immobilized Metal Affinity Chromatography,
Size Exclusion Chromatography





Hmila *et al.*, *Mol.Immunol.*, 2008
Ben Abderrazek *et al.*, *Biochem. J.*, 2009

The 4S HARE

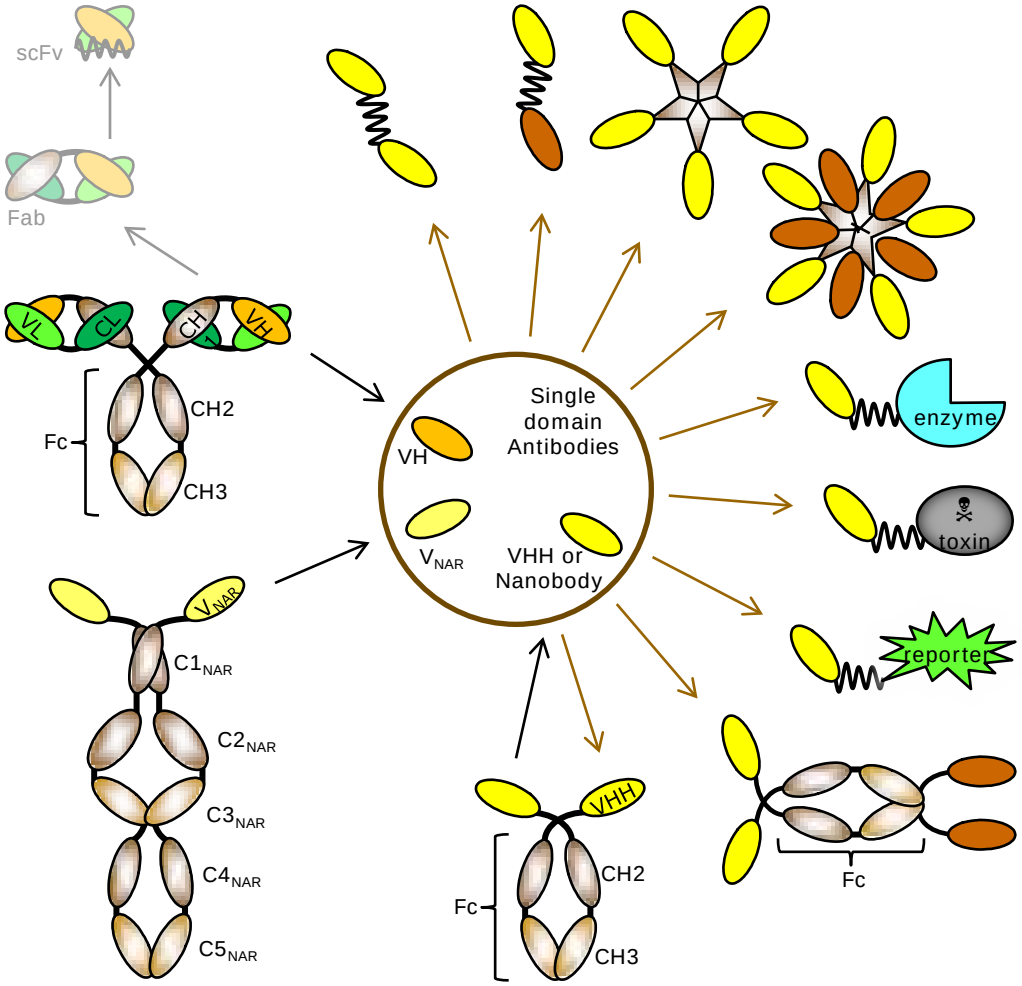
An optimal/practical binder fulfills the 4S HARE requirements

- S: **S**mall size
- S: **S**oluble in aqueous environment
- S: **S**table
- S: **S**pecific for antigen

- H: **H**uman sequence
- A: **A**ffinity for antigen
- R: **R**enewable and sustainable
- E: **E**conomic to produce (= good yield of **E**xpression)

Nanobodies are just perfect

Tailoring into pluripotent constructs



Bivalent:
Conrath et al., JBC 2001

Bispecific:
Conrath et al., JBC 2001

Pentavalent:
Zhang et al., JMB 2004

Decavalent/bispecific:
Stone et al., J Imm Meth 2007

Immuno-enzyme (ADEPT):
Cortez-Retamozo et al., Can Res 2004

Immuno-toxin:
Baral et al., Nat Med 2006

Chromobody:
Rothbauer et al., Nat Meth 2006

HCAb:
Hmila et al., Mol Immunol 2008

Scorpion (bispecific + Fc effector function)

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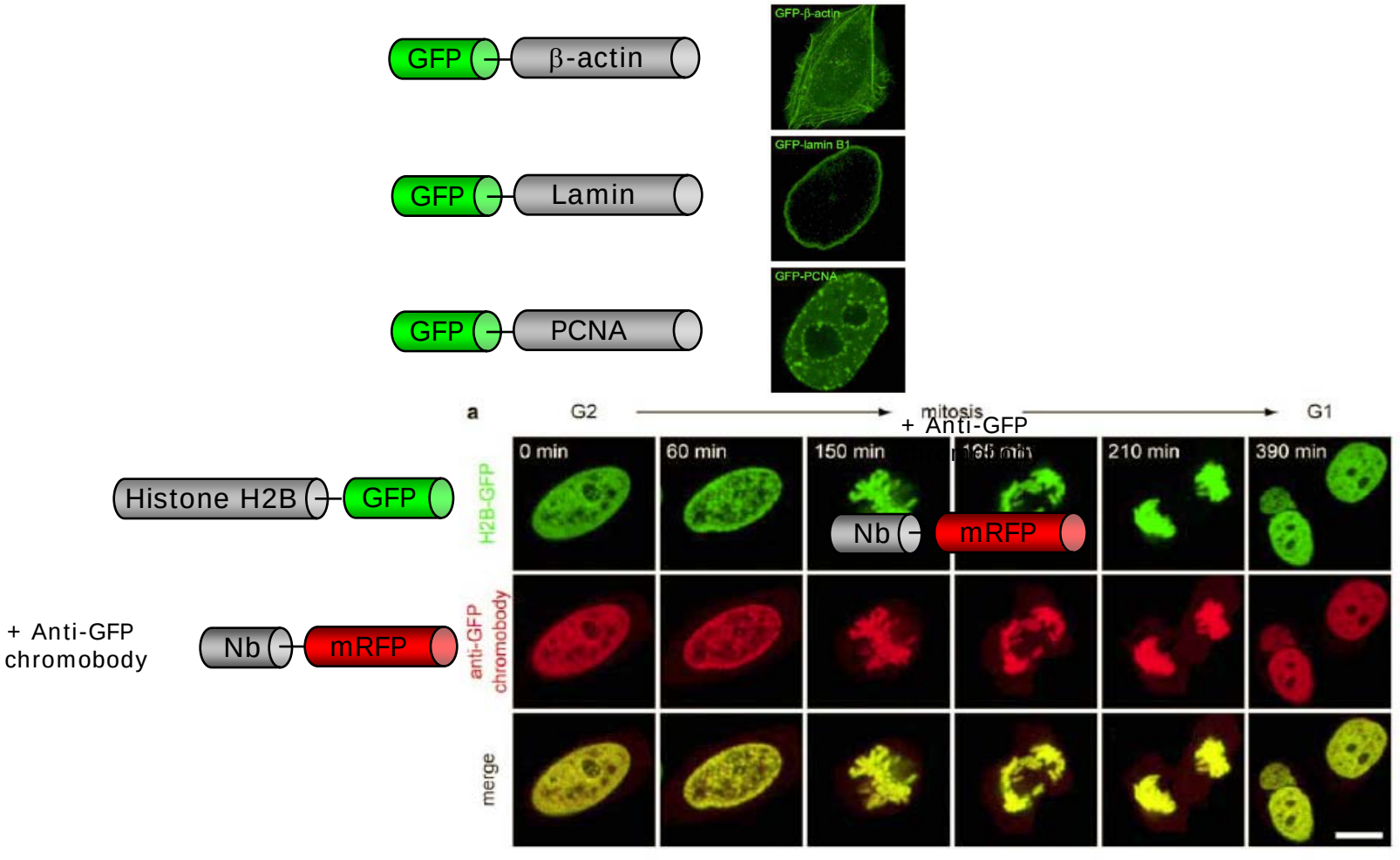
Molecular imaging: *In vivo* cell staining



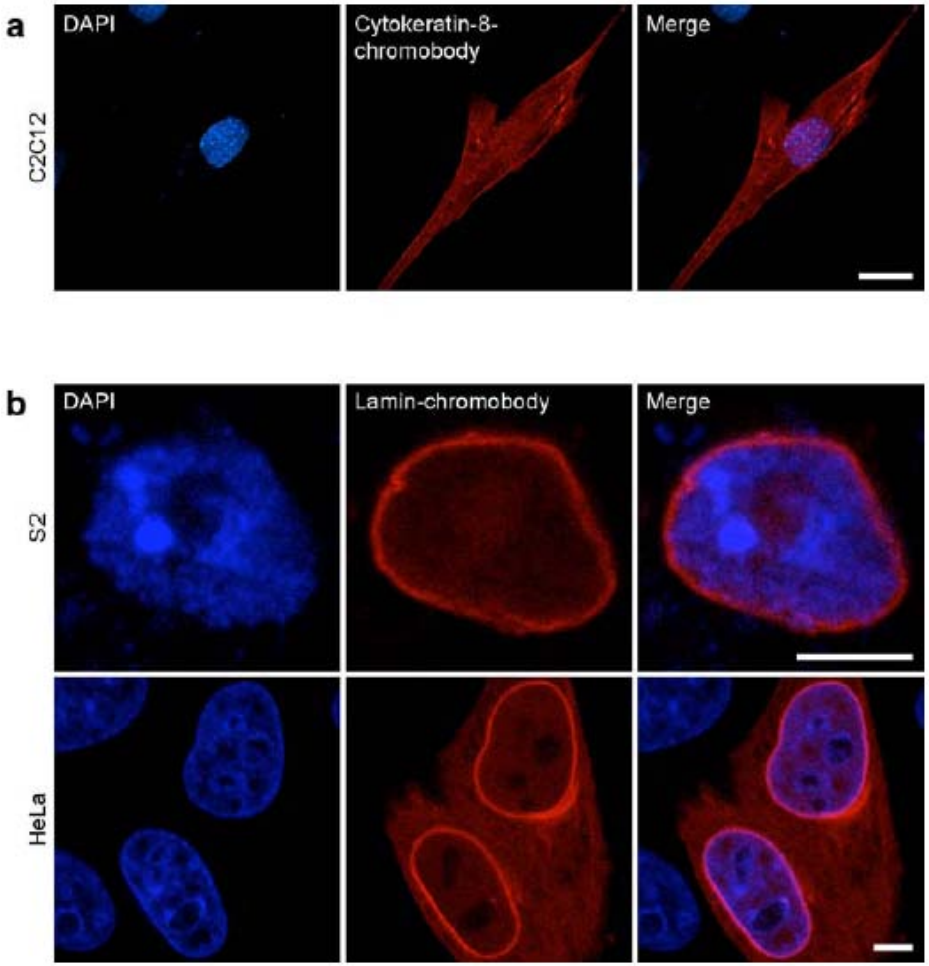
Rothbauer *et al.*, *Nat.Meth.*, 2006

Molecular imaging: *In vivo* cell staining

+ chromobody:

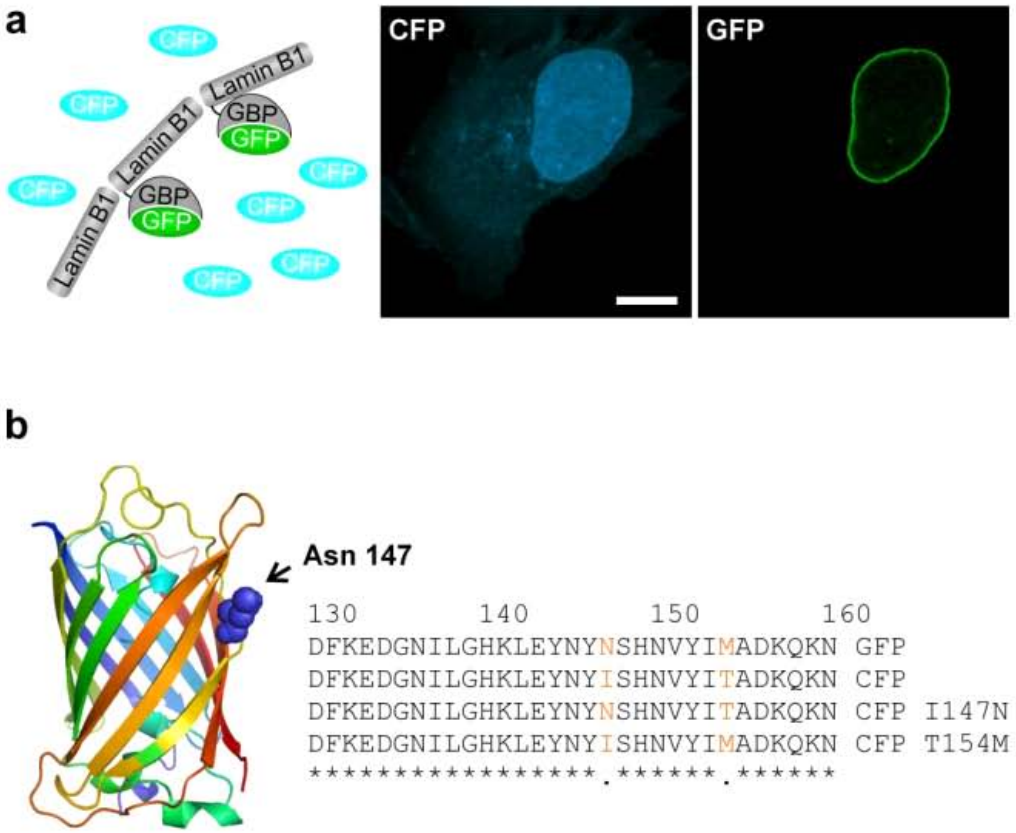
anti-cytokeratin 8

anti-Lamin



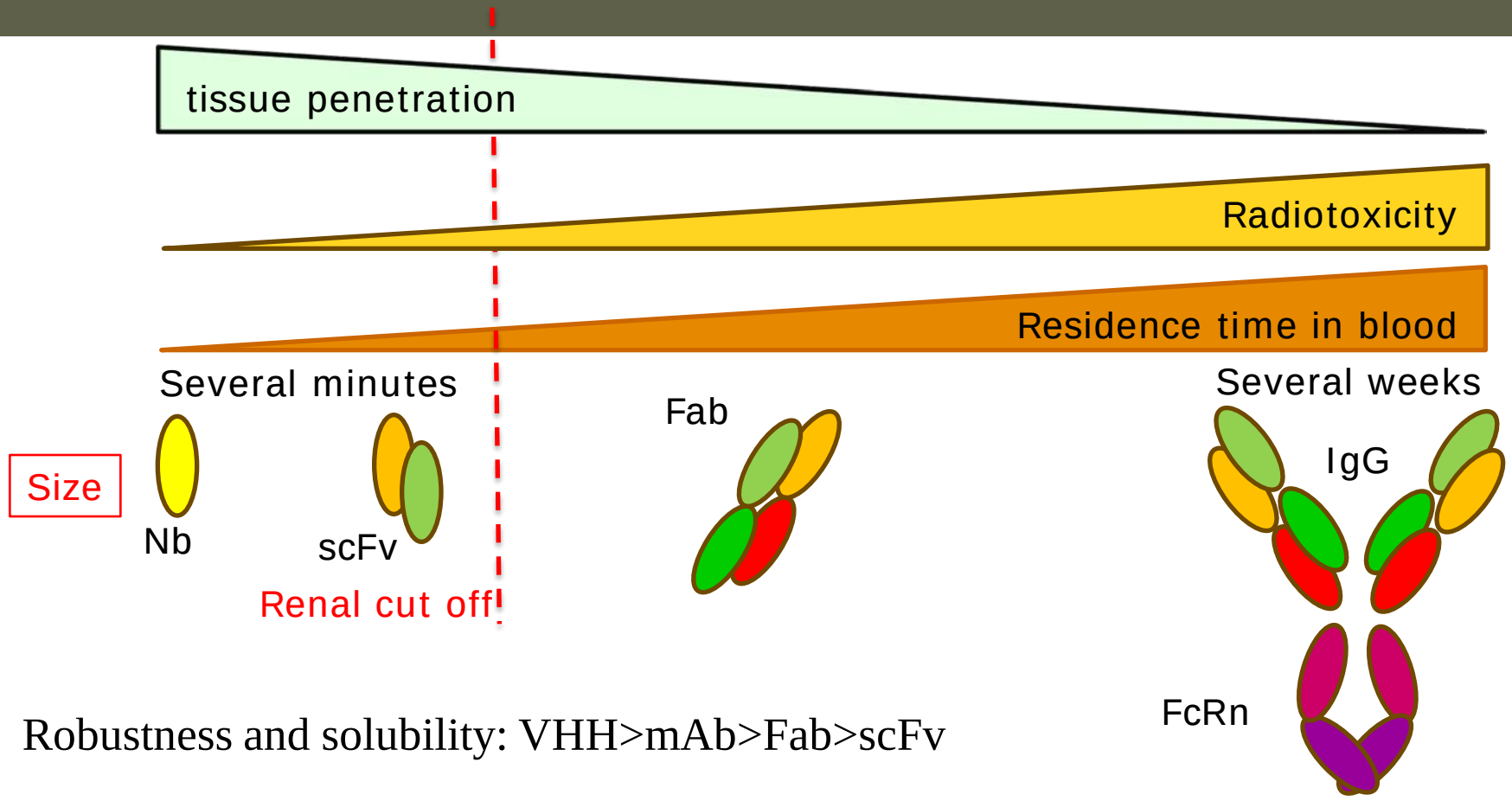
Rothbauer *et al.*, *Nat.Meth.*, 2006

Nb specificity + use as intrabody



Rothbauer *et al.*, *Mol Cell Prot*, 2008

Blood retention versus Ab size

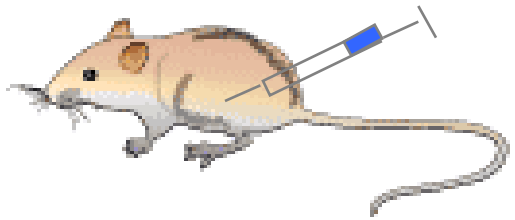


Robustness and solubility: VHH>mAb>Fab>scFv

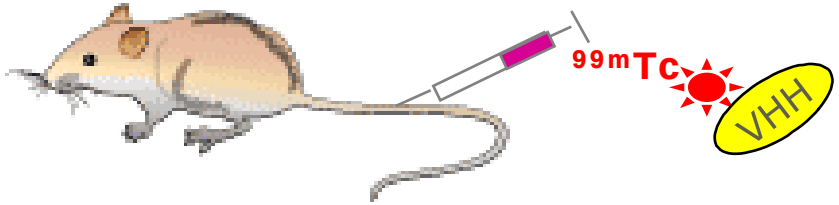
Most important factor for imaging: **Contrast** (tumor load/blood ratio)

Experimental setup

subcutaneous injection of
$2x10^6$ HER2 positive tumor cells
in hind limb of athymic nu/nu mice

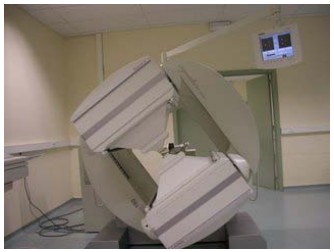


10-12d (tumor size ≈ 250-300 mm^3)



Imaging

Intravenous injection of
^{99m}Tc-labeled Nanobody®


SPECT

Micro CT

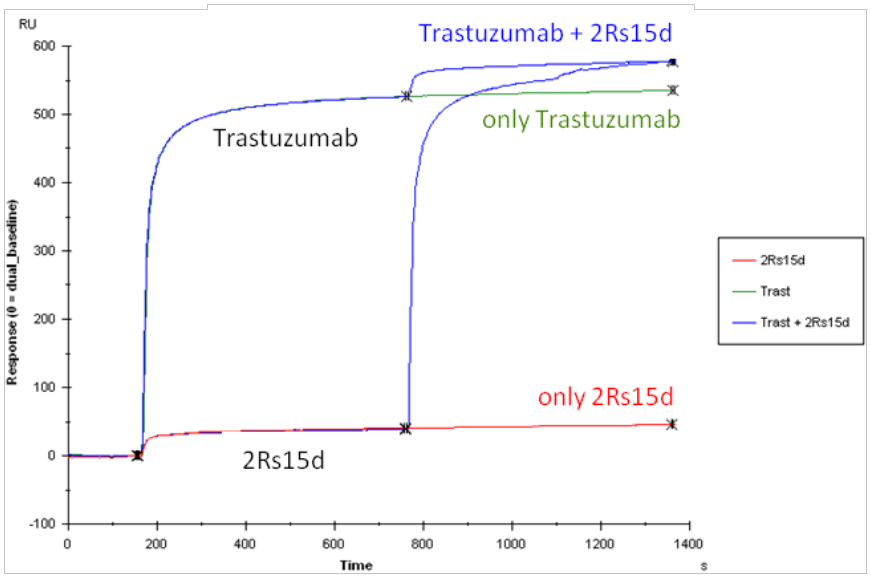
In-vivo non invasive imaging

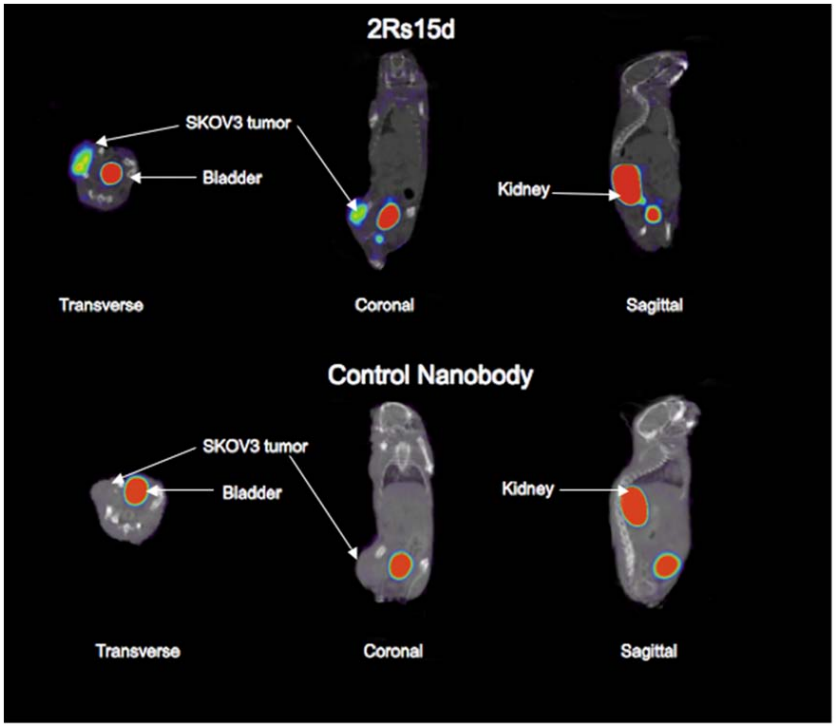
~40 Nbs against Her-2

Select best binder for non-invasive imaging without overlap with Trastuzumab

Produce under GMP and evaluate in breast cancer patients

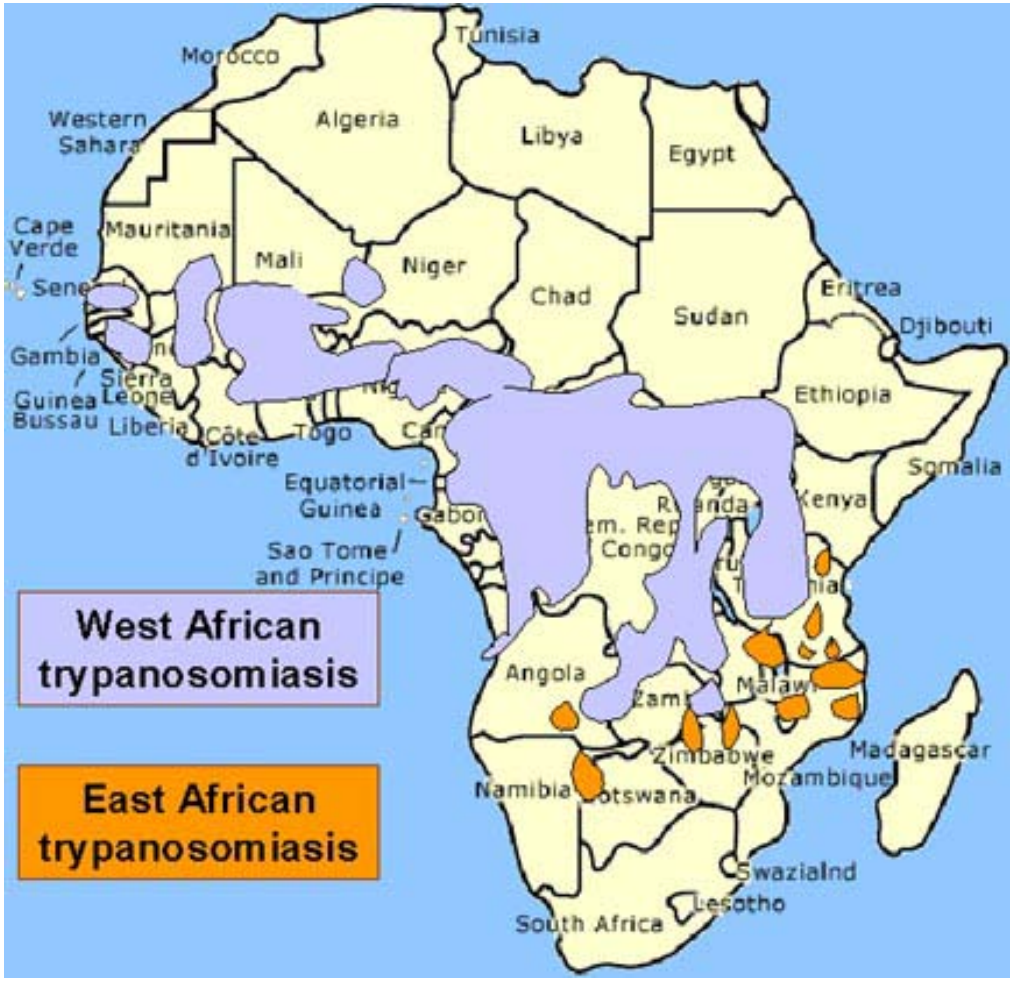
~1M € translational medicine grant (UZBrussel)

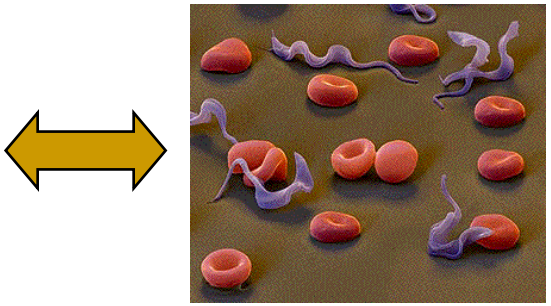




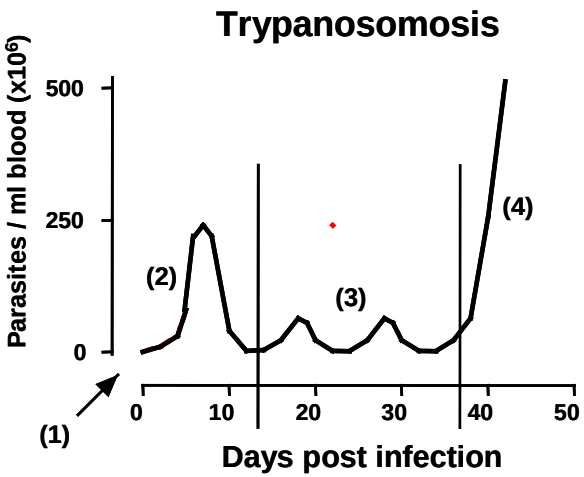
Vaneycken *et al.*, *FASEB J.*, 2011

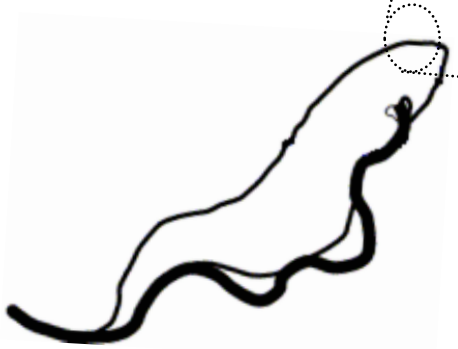
Nbs against African trypanosomes



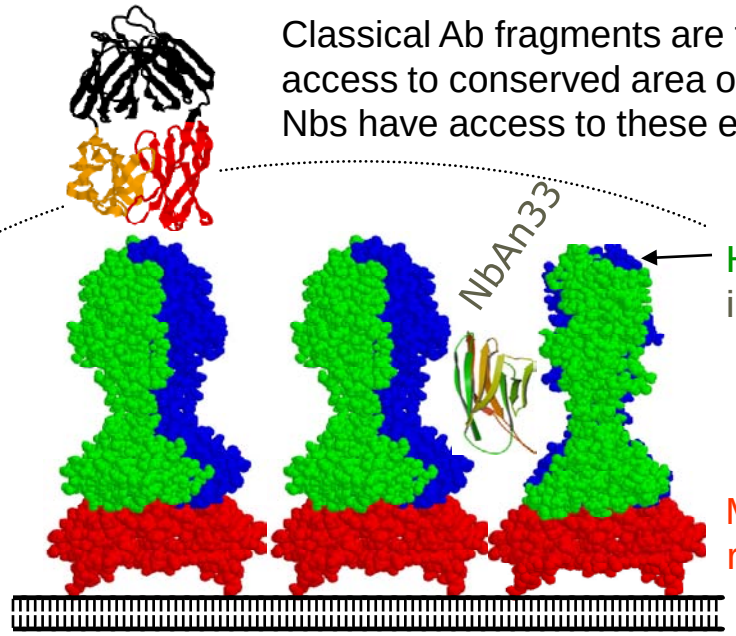


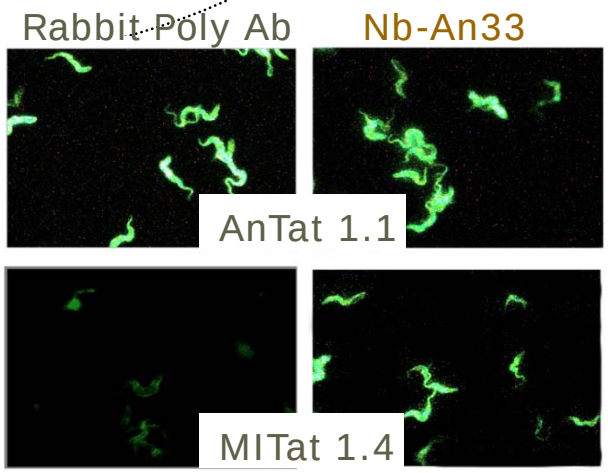
Antigenic variation



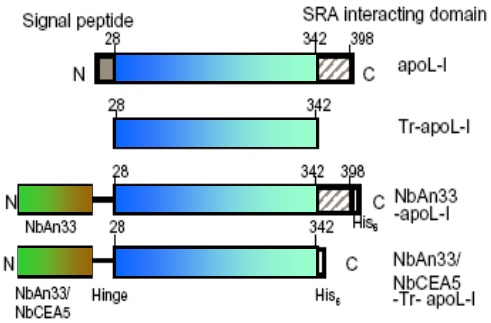


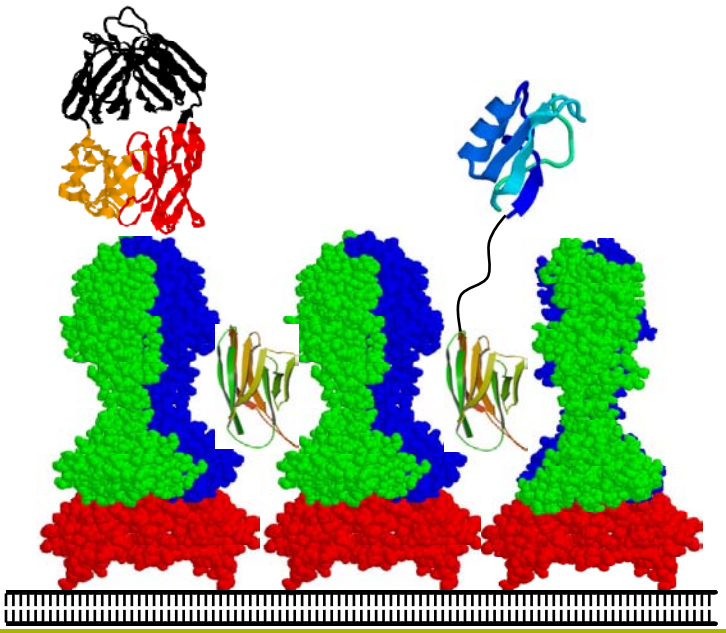
Classical Ab fragments are too large to have access to conserved area of VSG. Small-sized Nbs have access to these epitopes

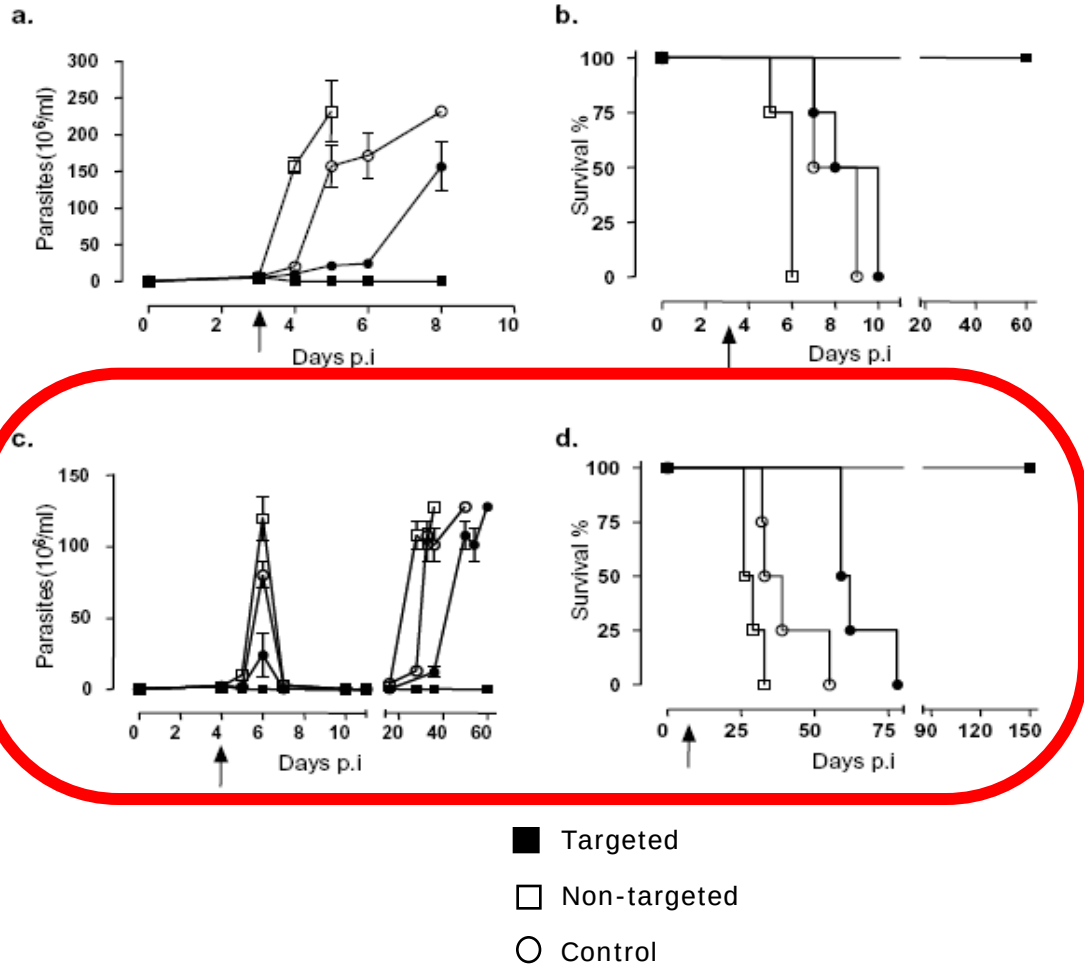




Trypanolytic Nbs







Stijlemans *et al.*, *J.Biol.Chem.*, 2004
Baral *et al.*, Nat Med., 2006

Nbs against scorpion toxin

Scorpion in Tunisia:

Androctonus australis hector

Extract venom

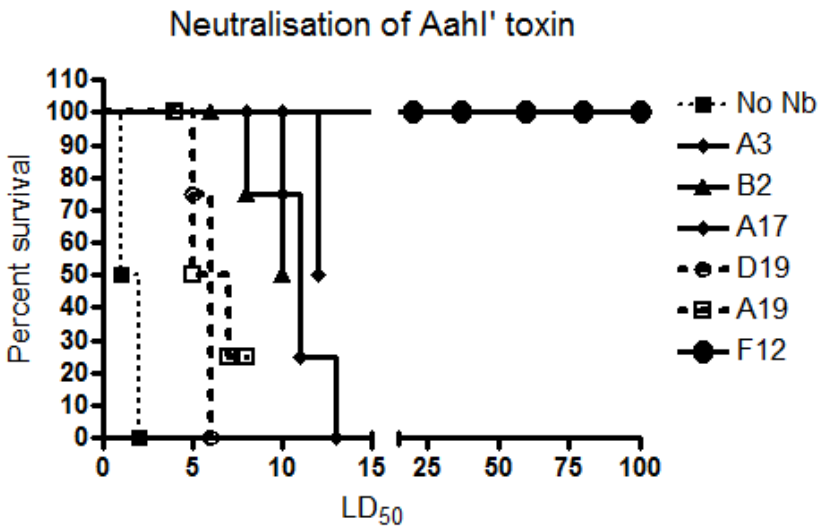
(SEC over Sephadex-G50, followed Mono-S FPLC and C-8 reverse phase HPLC to purify AahI' and AahII (LD50 in Swiss mouse ≈ 3 ng for i.c.v. and 250 ng for s.c.)

Immunise dromedary with AahI' or AahII enriched fractions and identify Nbs against AahI' or against AahII

AahI' neutralisation with Nbs (i.c.v.)

1. Inject (icv) variable amounts of purified AahI' toxin in mice) to determine LD_{50} = 3 ng AahI' per mouse

2. Mix variable amounts of toxin with Nb, inject ivc and monitor survival



NbAahI'F12 has an exceptionally high neutralisation capacity reaching 100% neutralisation of 100 LD50.

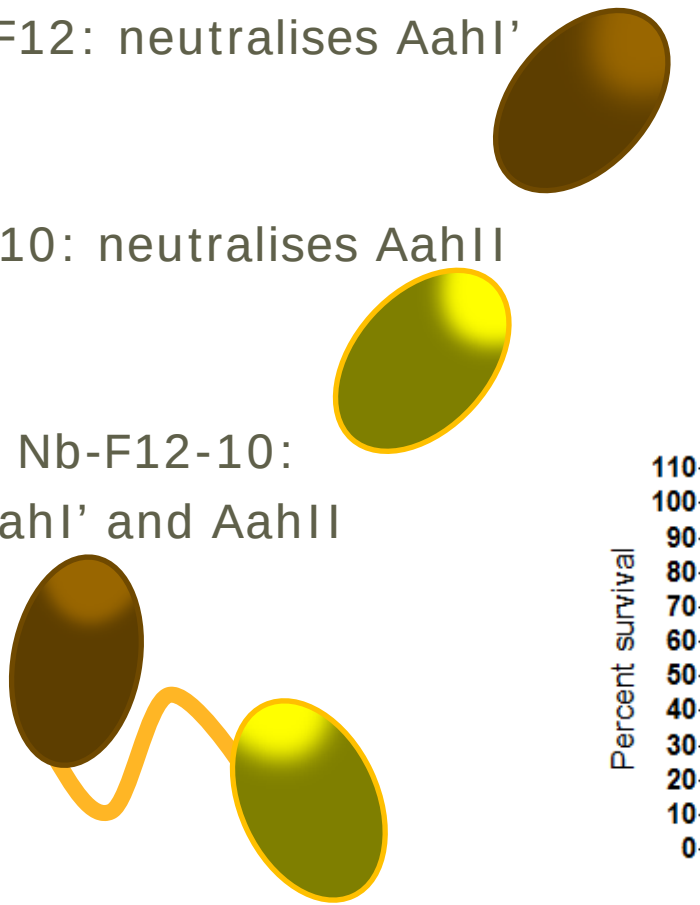
Such neutralisation capacity was never observed before for any other antibody preparation.

Construction of bispecific Nbs

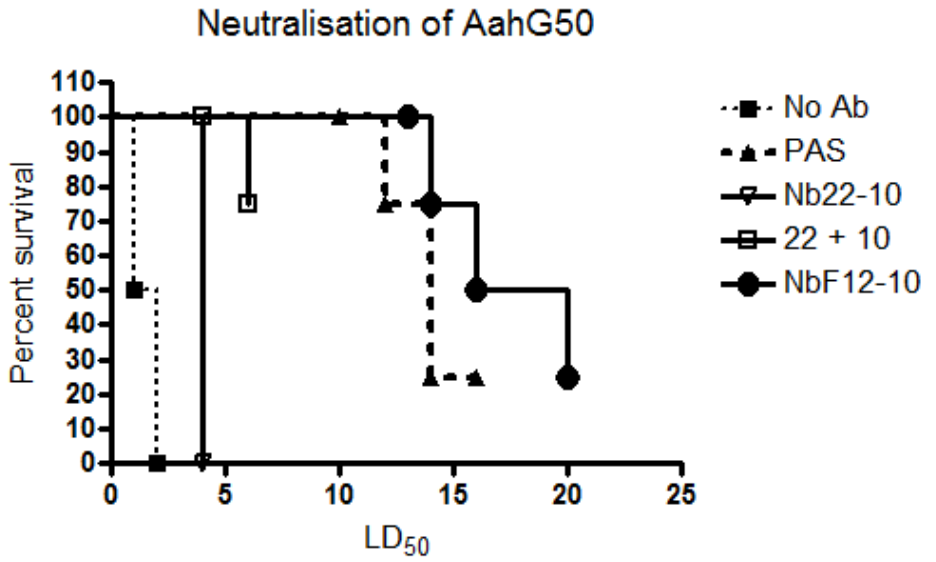
NbAahI'-F12: neutralises AahI'

NbAahII-10: neutralises AahII

Bispecific Nb-F12-10:
targets AahI' and AahII

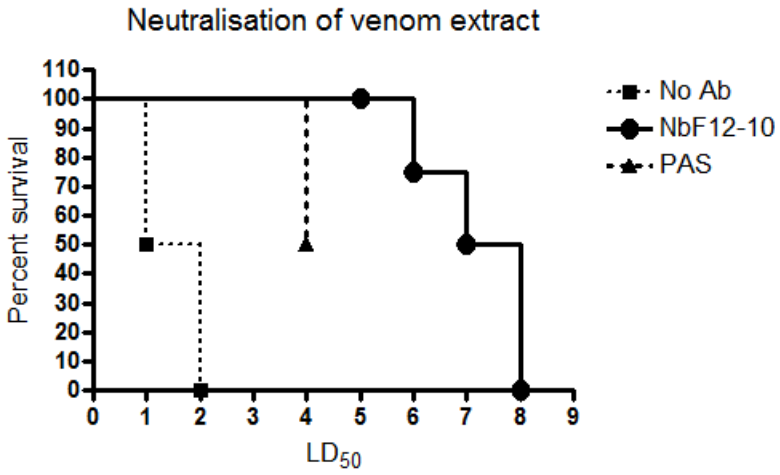


Mix AahG50 venom (contains AahI' and AahII) with bispecific and inject icv to monitor protection



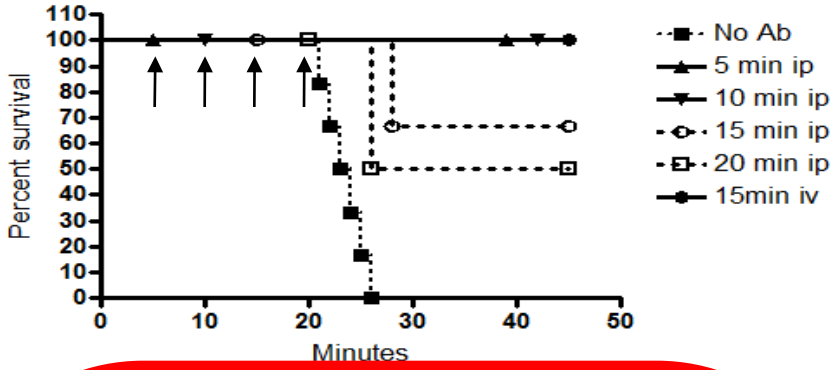
Protection by bispecific Nb

1. Inject variable amounts of venom (sc) and then inject (iv) Nb or horse polyclonal serotherapeutic

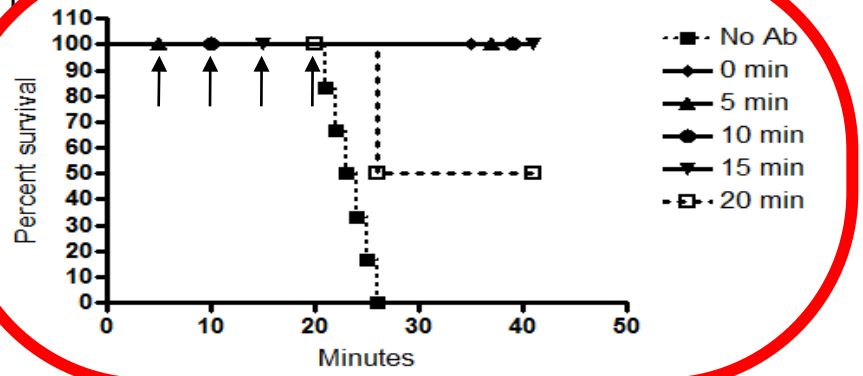


2. Inject (sc) 1.5 LD_{50} of AahG50 (A) or total venom (B) in mouse and at variable times inject (iv) 85 µg of bispecific Nb and monitor survivals

A



B



Acknowledgments

Postdocs in our group

Gh. Hassanzadeh (NSF),
N. Devoogdt
C.Vincke
B. Stijlemans
S. Magez
P. De Baetselier
T. Lahoutte,
V. Caveliers
(D. Saerens,
K. Conrath,
NT Baral)

Non-VIB collaborations

CVRL (Dubai, UAE)

U. Wernery
R. Wernery
J. Kinne
K. Khazanehdari

Tunisia (PTI)

I. Hmila
R. Ben Abderrazek
Z. Benlasfar
H. Dabbek
B. Bouhaouala
M. El Ayeb

Tech transfer & spin off

Foundation of Ablynx NV (December 2001)

70 M € from venture capitalists (3 rounds)

November 2007: Introduction at EURONEXT (85 M€) + 55 M € at SPO

Research collaborations:

- Proctor & Gamble (July 2004 & April 2006)
- Genencor (2004), Centocor (2006)
- Novartis (2006), Kirin (2006)
- WYETH pharmaceuticals (212 M$, anti-TNF)
- BOEHRINGER Ingelheim (265 M$, alzheimer)
- Merck Serono Boehringer (XXXXX M€)

Achievements

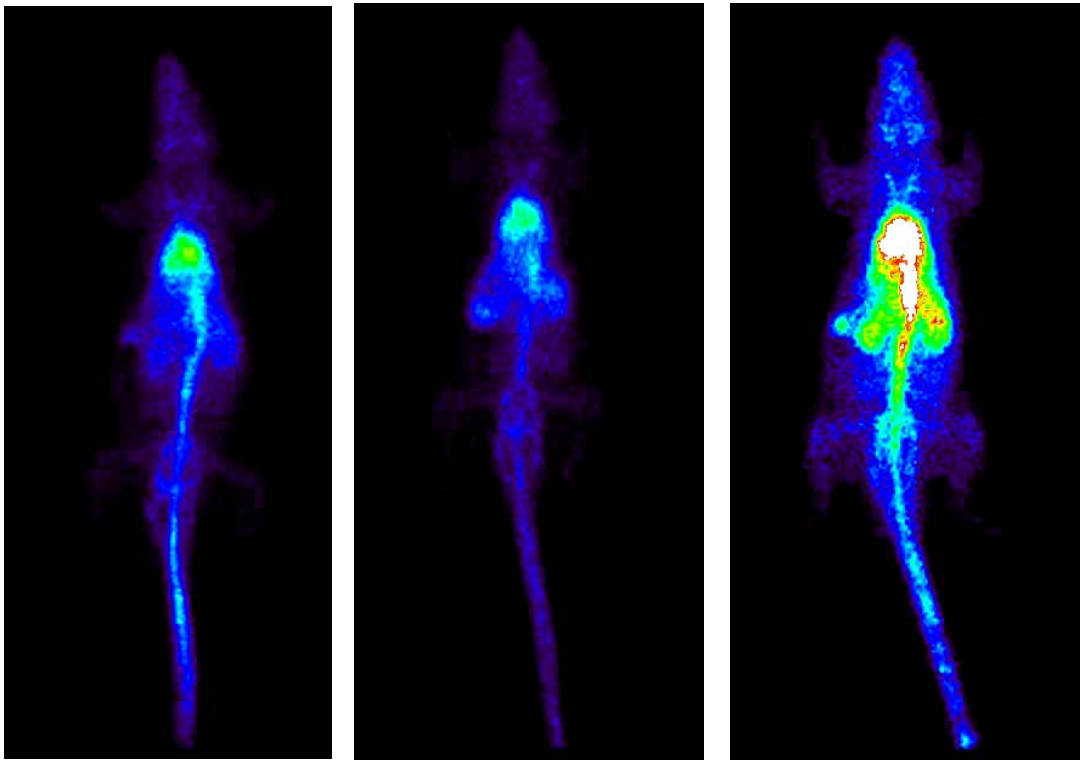
- Phase II for anti-trombotic (ALX081 & ALX0681)
- Phase II for anti-TNF (ATN103)
- Phase II for IL6R (ALX061)
- Phase I for anti-RANKL (ALX0141)
- Phase I for anti TNF (ATN_192
- Phase I for CXCR4 (ALX0651)
- Phase I for antiRSV (ALX0171)

Ablynx

2002 = 5 man
2003 = 10 man
2004 = 20 man
2005 = 40 man
2006 = 70 man
2007 = 90 man
2008 = 190 man
2009 = 230 man
2010 = 250 man
2011 = 290 man

Nanobodies are cleared fast by renal excretion

time-lapse SPECT studies



Fab Nb F12 Nb 10