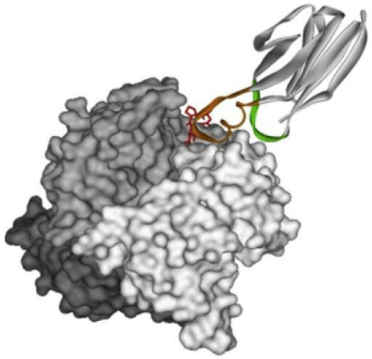
Single domain antibodies for the knockdown of cytosolic and nuclear proteins



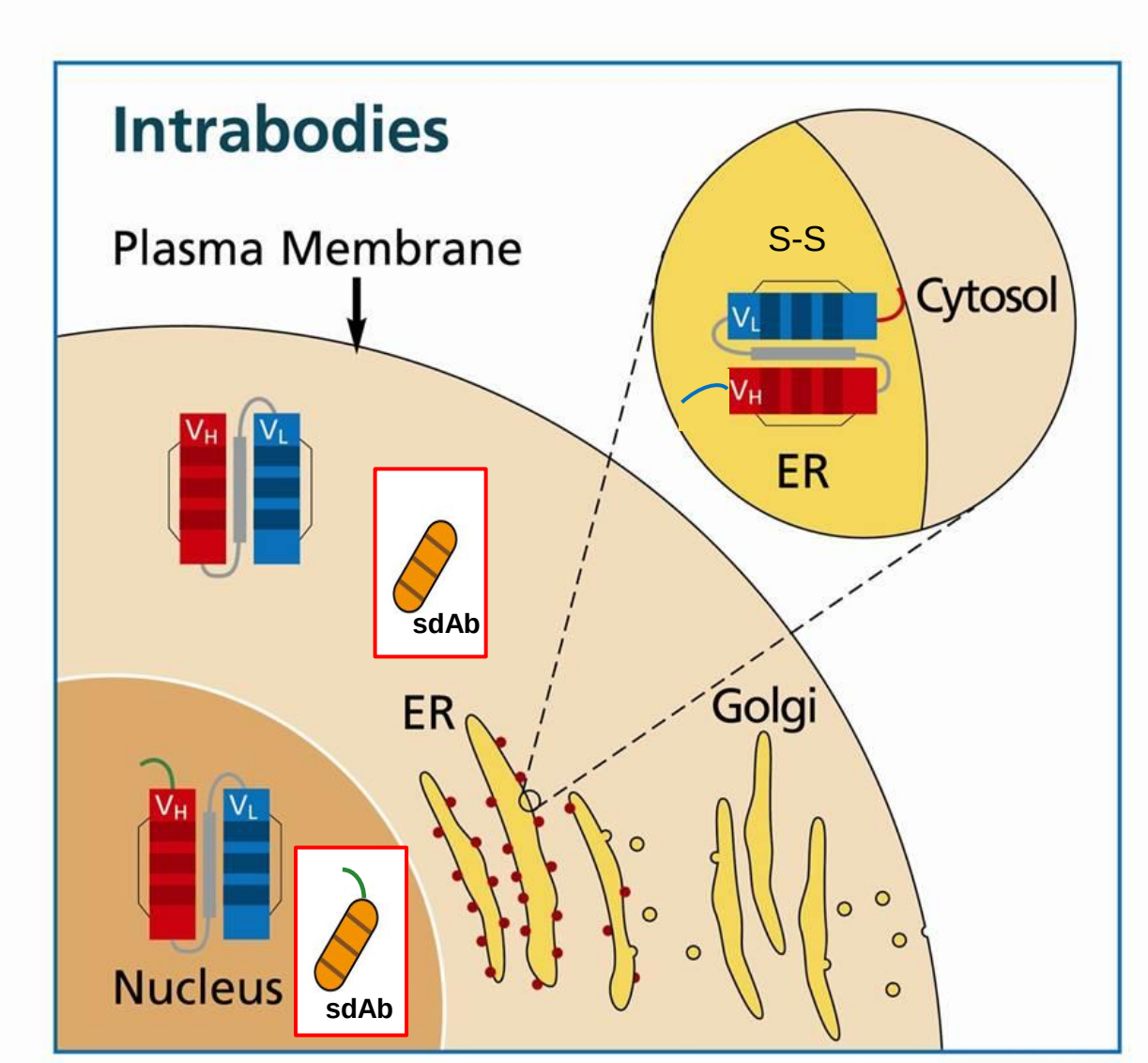
8th European Immunology Conference
Madrid, Spain

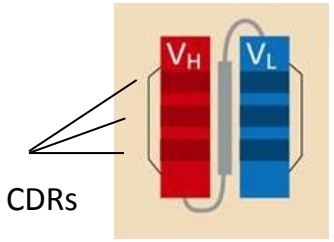
Dr. Thomas Böldicke
Helmholtz-Centre for Infection Research, Germany
Structure and Function of Proteins

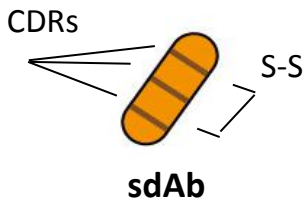
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Intrabodies

- Recombinant antibody fragments, format: scFv or single domain antibody
- Expressed in target cells expressing the corresponding antigen
- Protein Knockdown of the antigen
- Main advantage: Specificity
- Targeting of proteins passing the ER, nuclear and cytosolic proteins.







Camelid VHH
vNAR (shark antibody)
Human VH/VL

ScFvs and sdAbs targeted to subcellular compartments

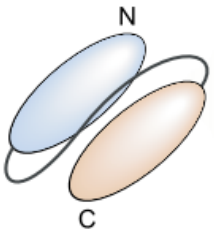
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Intrabodies

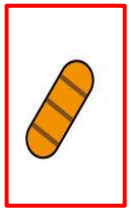
ER-intrabodies: knockdown of about 35 transitory targets

Single domain antibodies
knockdown of about 40 cytosolic and nuclear targets

Usage for tracking of intracellular proteins



scFv



Single domain antibody

Main advantage of intrabodies is its specificity and inhibition of unique targets

→ **Alternative to RNAi technology and knockout mice**

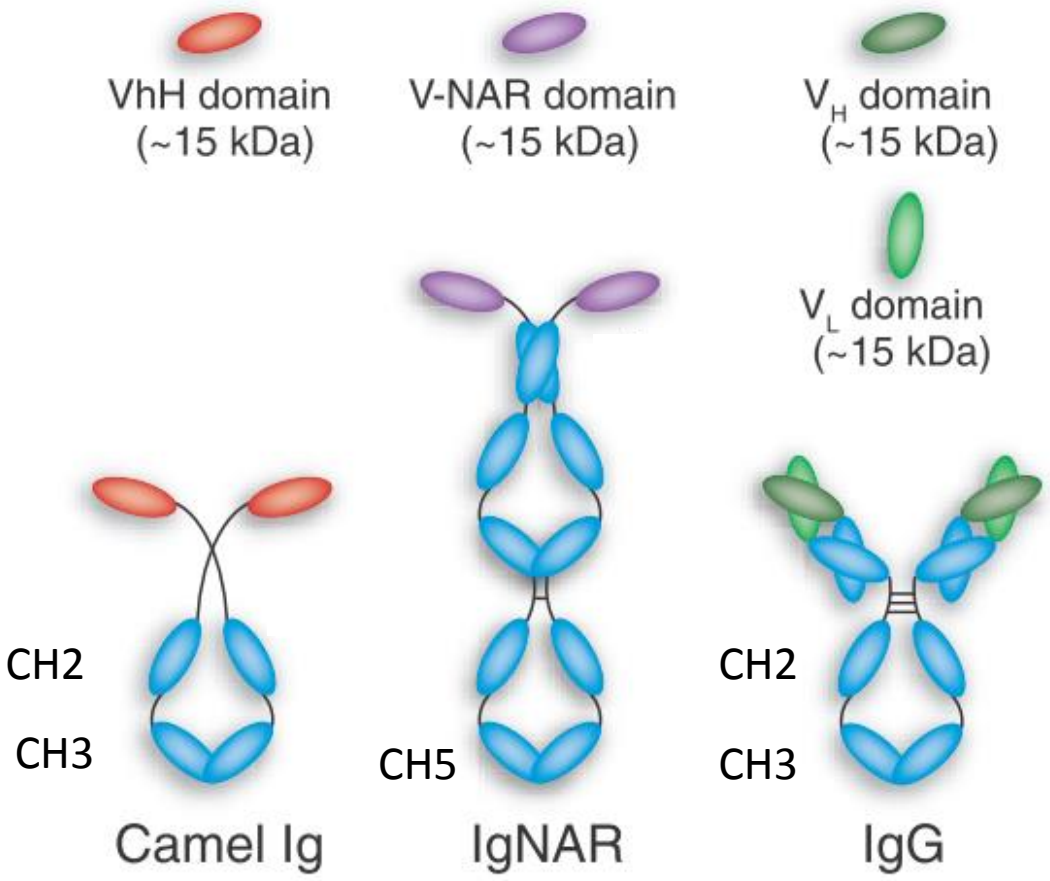
→ protein interaction regions

→ conformers

→ splice variants

→ protein isoforms

→ posttranslational modifications



Schematic representation of single domain antibodies from camels, shark and human IgG

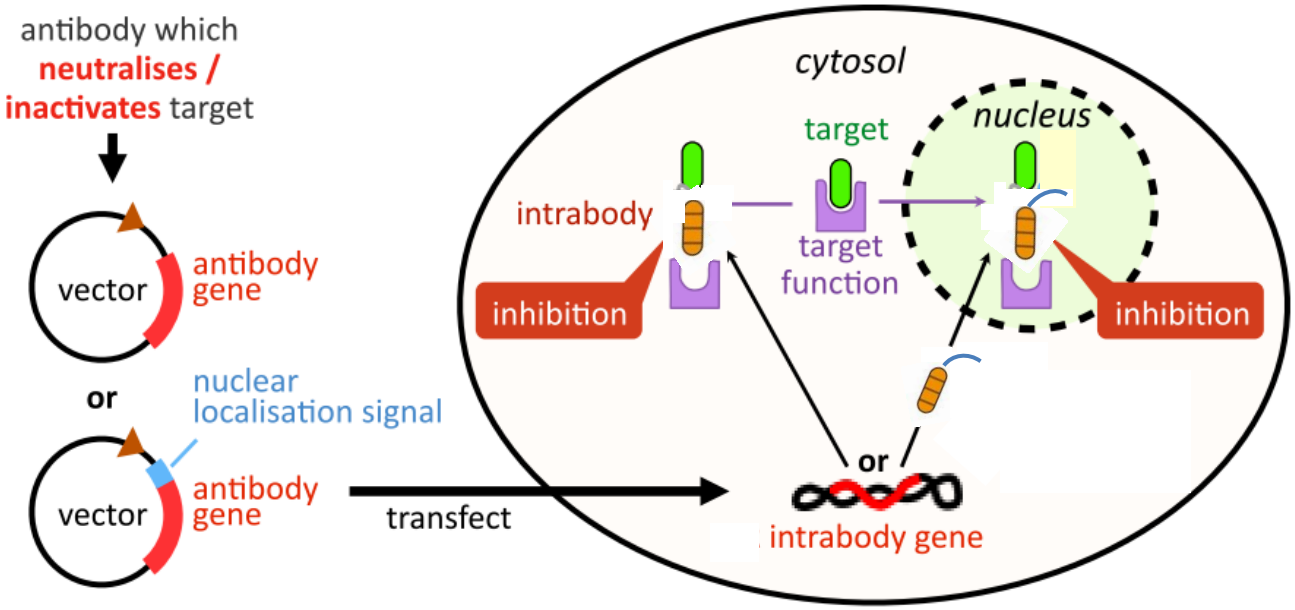
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Inhibition of cytosolic/nuclear proteins by sdAbs

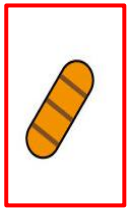
- intracellular enzymes including kinases
- oncogenic proteins
- toxins
- proteins of the nervous system
- virus proteins
- proteins from plants and drosophila

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cytoplasmic or nuclear intrabodies



IB format sdAb



Mechanism of knockdown of cytosolic or nuclear intrabodies

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sdAb → **Cytosolic, nuclear intrabody**

Camelid VHHs
Immune, naive, synthetic
Phage display sdAb library

Shark IgNARs
Immune, naive, synthetic
Phage display sdAb library

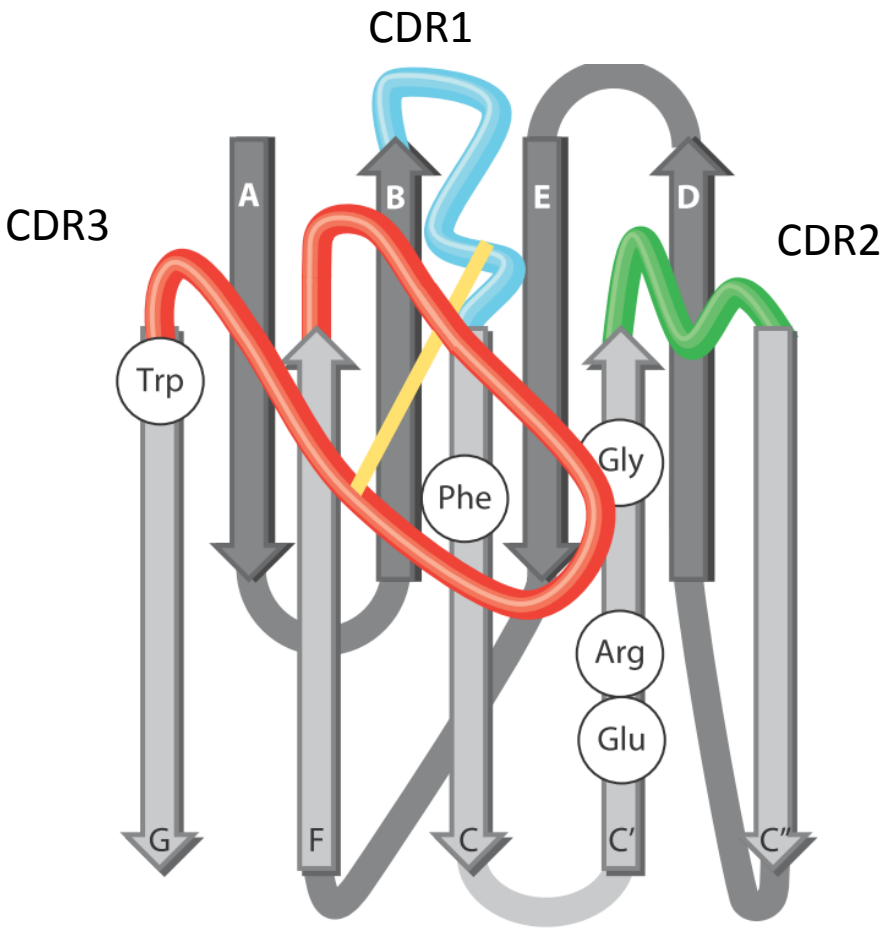
Human VH, VL
Synthetic VH or VL
Phage display sdAb library

phage display sdAb repertoire



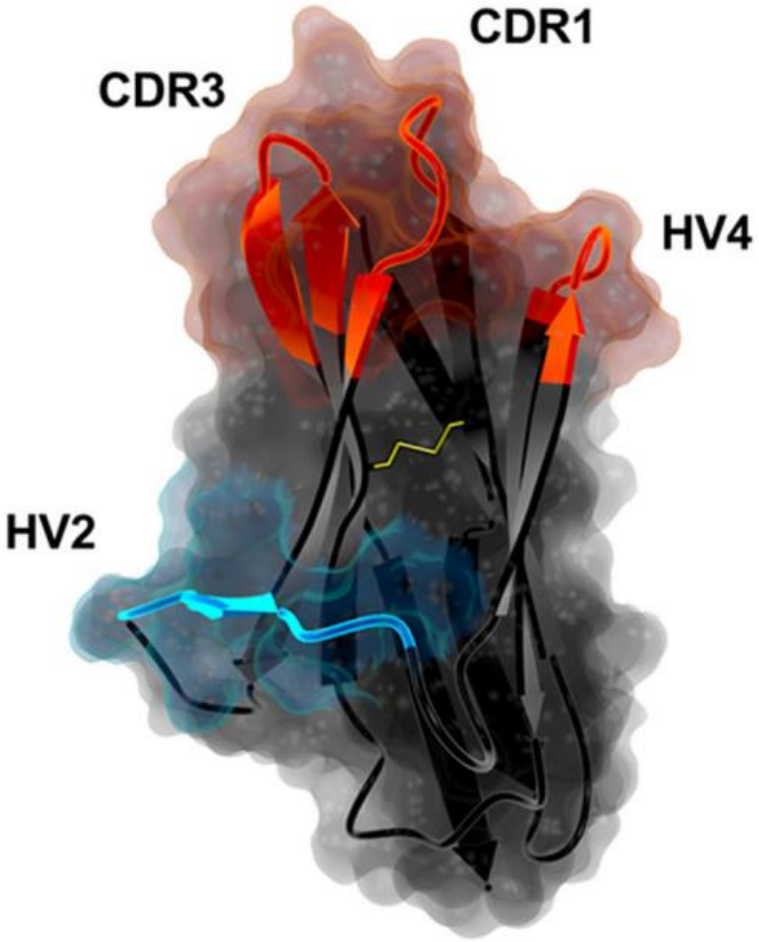
Nanobody, shark sdAb, human VH, VL

Source of sdAbs for construction of cytosolic, nuclear intrabodies



Structure of a VHH single domain antibody of camelid

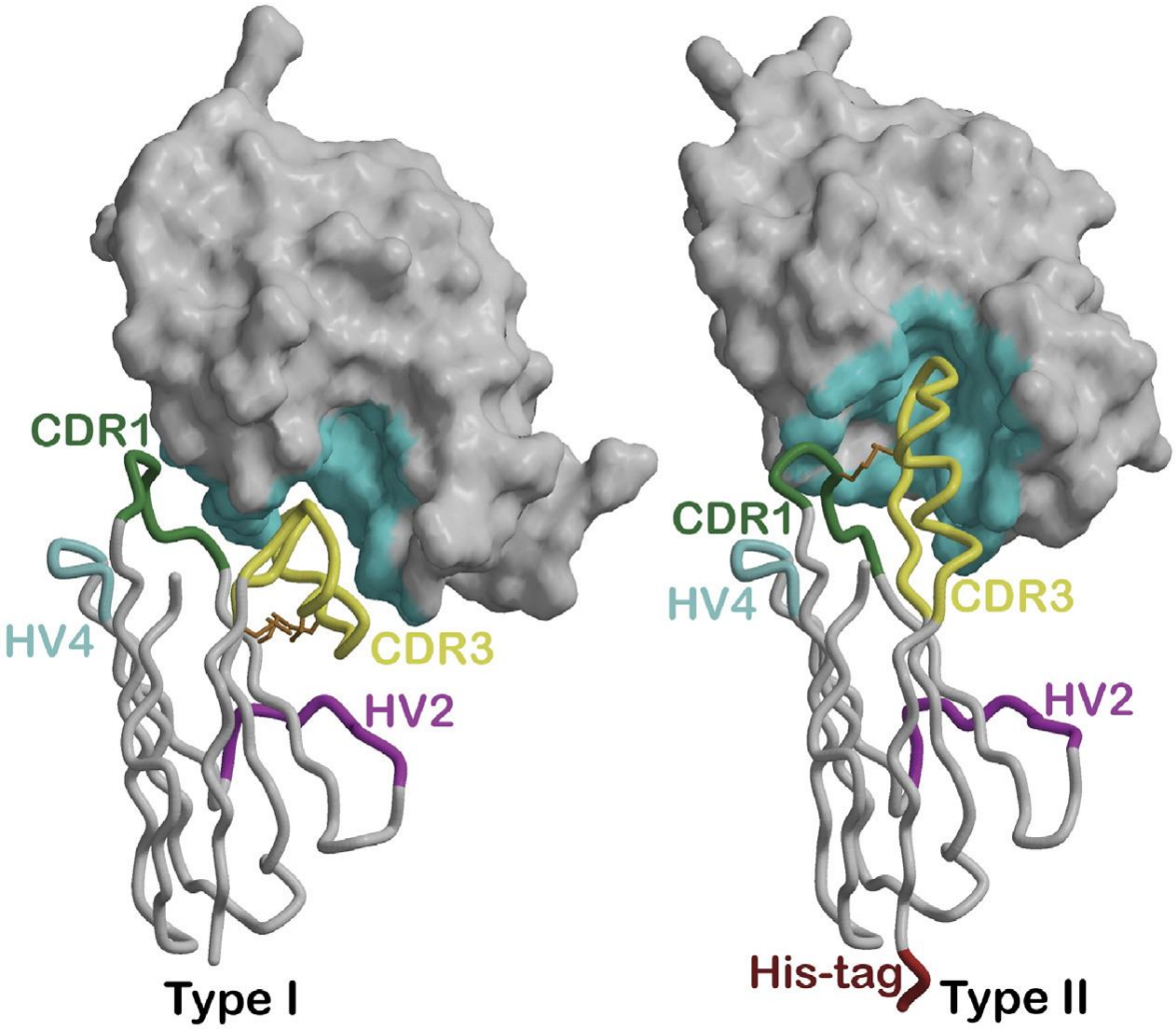
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Protein subdomain of a vNAR scaffold

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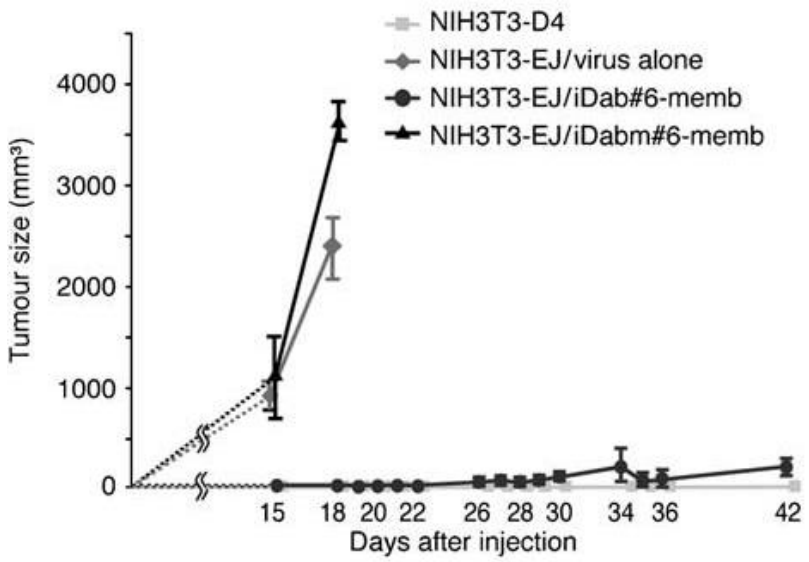


Comparison of modes of binding of the type I IgNAR and Type II IgNAR variable domains to lysozyme

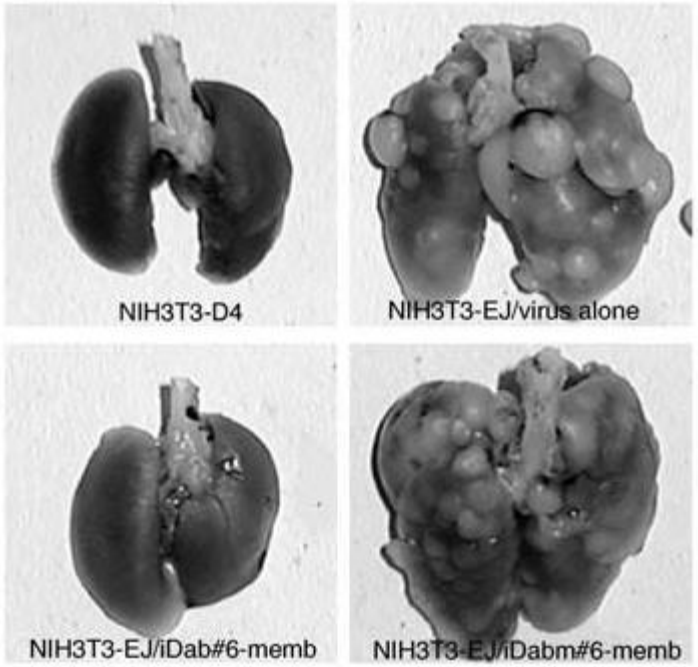
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Human anti-RAS VH sdAb inhibits tumorigenesis

A



B

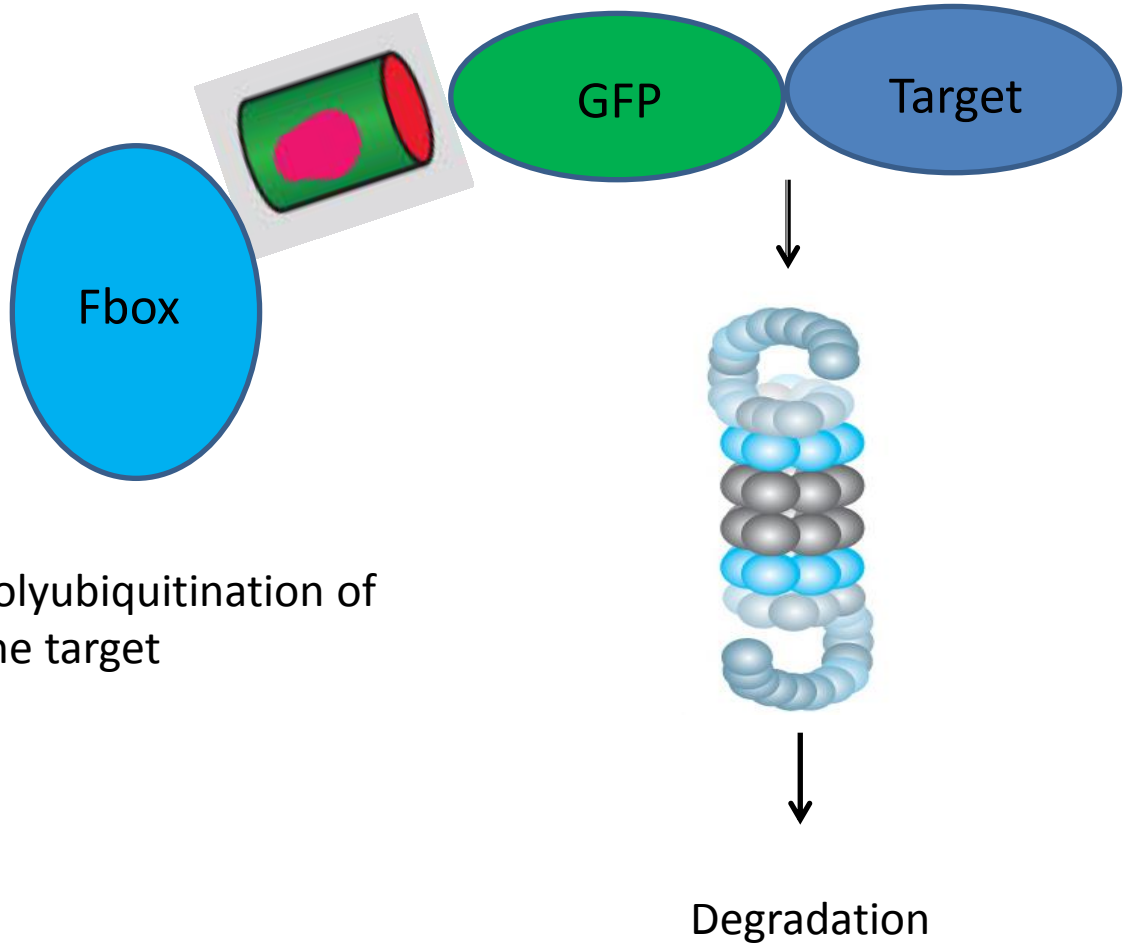


The anti-RAS single domain inhibits tumorigenesis and metastasis in a mouse model

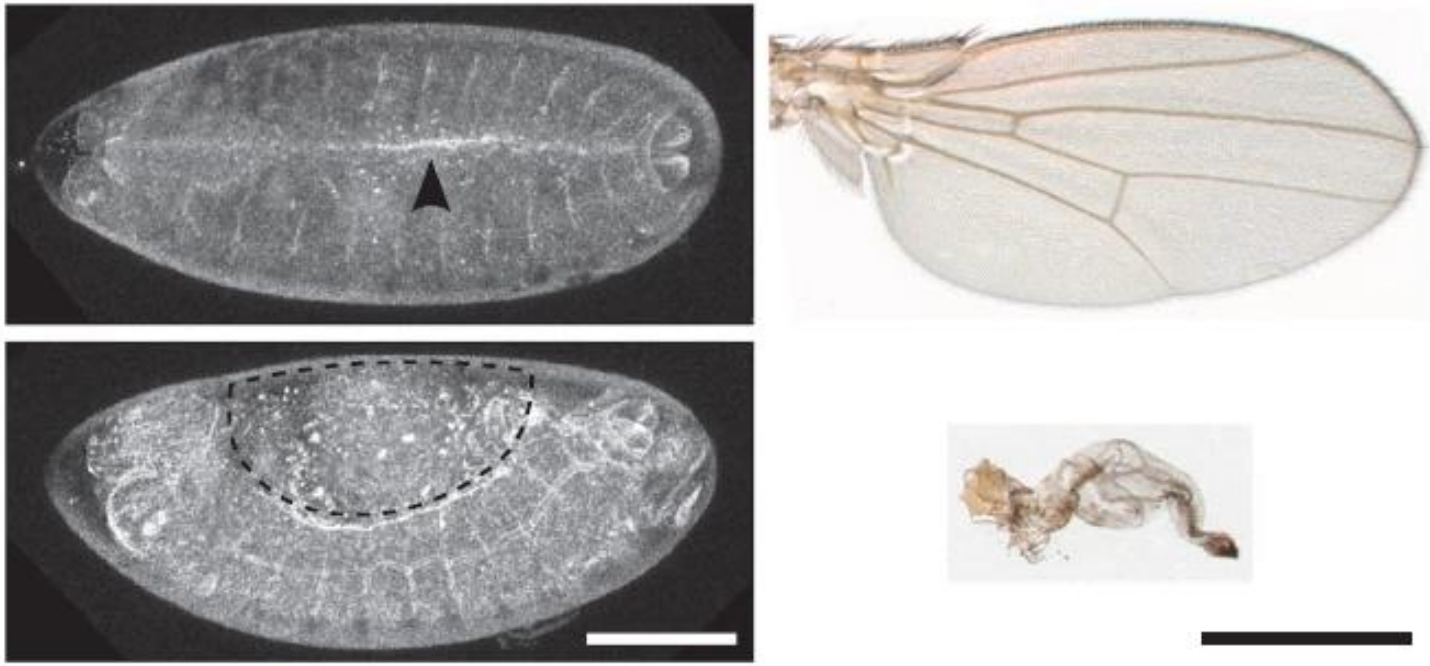
Tanaka T, Williams RL, Rabbits TH

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Fluorescent fusion protein knockout mediated by anti-GFP nanobody



Schematic illustration of direct depletion of target GPF fusions using nanobody



Sqh = myosin II regulatory light chain

Nanobody mediated knockdown of a) Sqh-GFP and Apterous-GFP in transgenic flies

Caussinus E, Kanca O, Affolter M

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Conclusion

Single domain antibodies from camels, shark and humans can be stable expressed inside the cytosol or nucleus.

They are very useful to efficiently and specifically knock down cytosolic and nuclear proteins *in vivo*.