Inducible strategies for controlling binding of intrabodies to their antigen targets

Technical Journal Club

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Francesca Catto

Paper #1

nature methods

BRIEF COMMUNICATION

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Optogenetic activation of intracellular antibodies for direct modulation of endogenous proteins

Daseuli Yu[1], Hansol Lee[1], Jongryul Hong[1], Hyunjin Jung[1], YoungJu Jo[2,5], Byung-Ha Oh[1,3], Byung Ouk Park[4*] and Won Do Heo[1,3,4*]

Paper #2

nature methods

BRIEF COMMUNICATION

https://doi.org/10.1038/s41592-020-0746-7

Chemogenetic Control of Nanobodies

Helen Farrants[1,2], Miroslaw Tarnawski[3], Thorsten G. Müller[4], Shotaro Otsuka[5,6], Julien Hiblot[1], Birgit Koch[1], Moritz Kueblbeck[5], Hans-Georg Kräusslich[4], Jan Ellenberg[5] and Kai Johnsson[1,2] ✉

What are intrabodies?

- Intrabodies are small format antibodies that function inside of cells

- Delivered as DNA packaged in a virus
- Intrabody DNA does not incorporate into cell DNA

Introduction

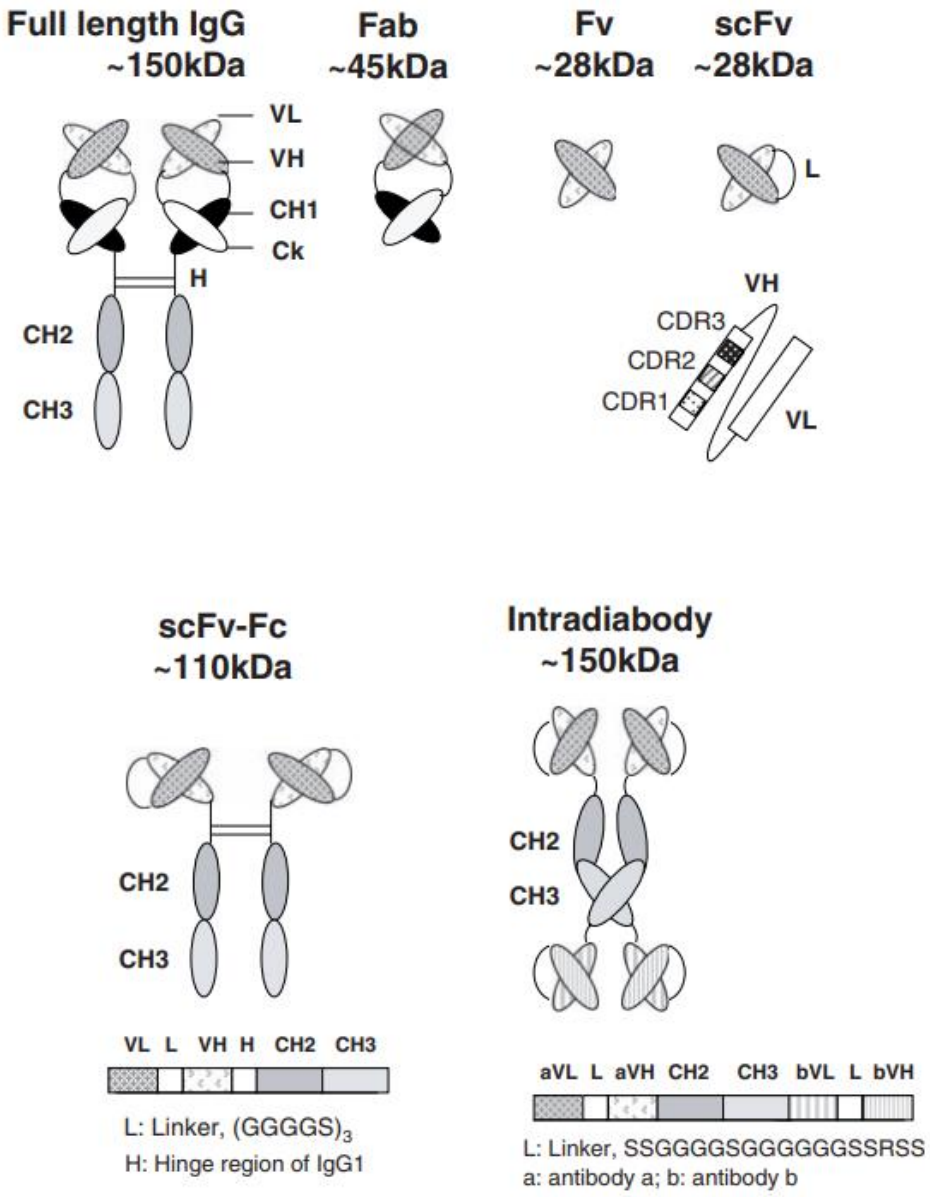


Fig1

Intrabodies

Produced in the cell, and bind an antigen within the same cell.

Antibodies are soluble proteins that are normally found circulating the body within the serum.

They are synthesized in the endoplasmic reticulum (ER) of B cells as separate heavy chain and light chains, which are then linked by disulfide bonds in the mature Ig.

However, the full-length antibody is not functional in the cytosol, prior to secretion, due to its reducing conditions, which affect protein folding and the intramolecular disulfide bonds that are required to maintain the antibody's conformation and stability.

Variable regions characterize an antibody with its exceptional target specificity.

--> **it is possible to use antibody fragments incorporating the specificity-providing regions within a single-chain variable fragment (scFv), which can be further engineered for cytosolic stability, to target intracellular antigens**

Introduction

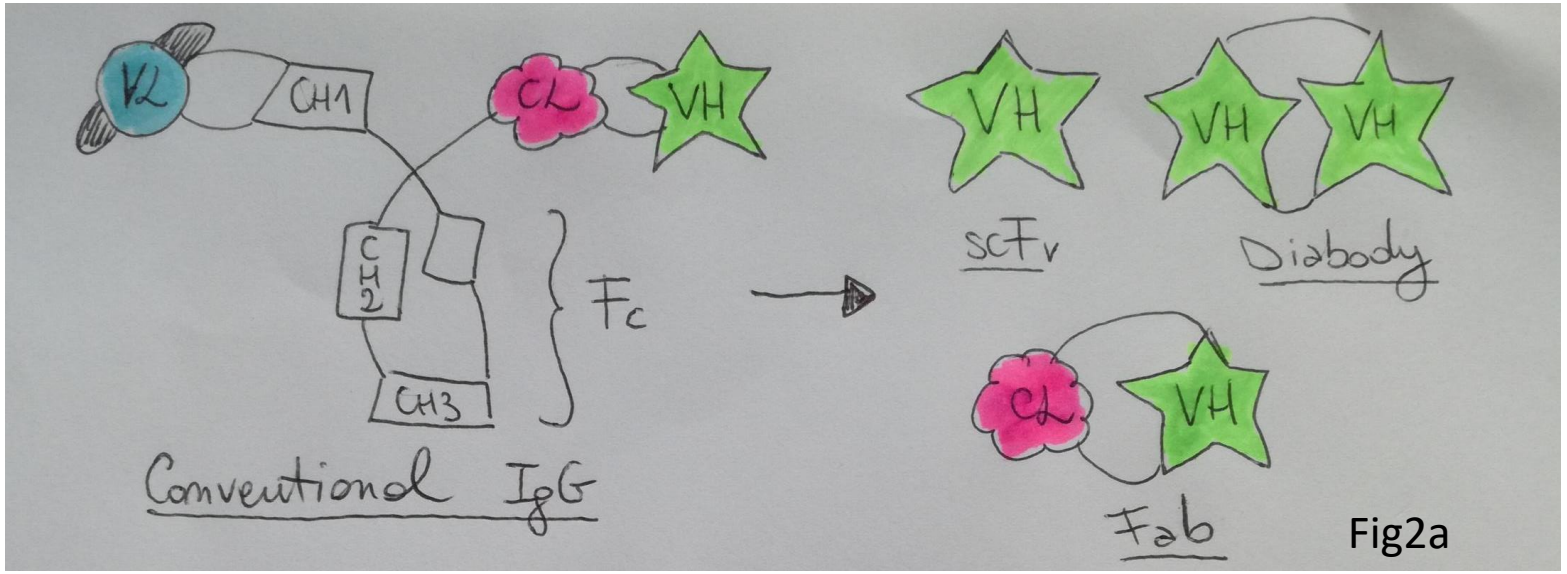
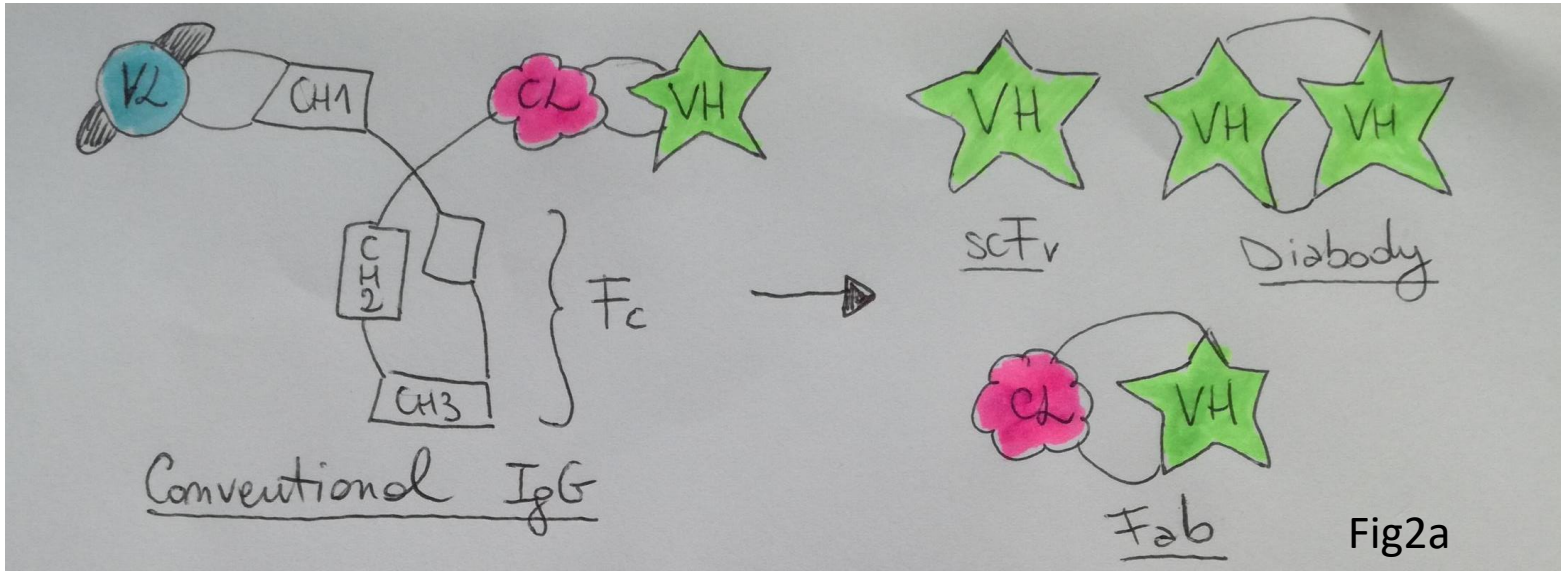


Fig2a

The variable (V) region domain can be used by itself to form a domain antibody or Dab. These can be engineered from conventional human Igs.

Introduction



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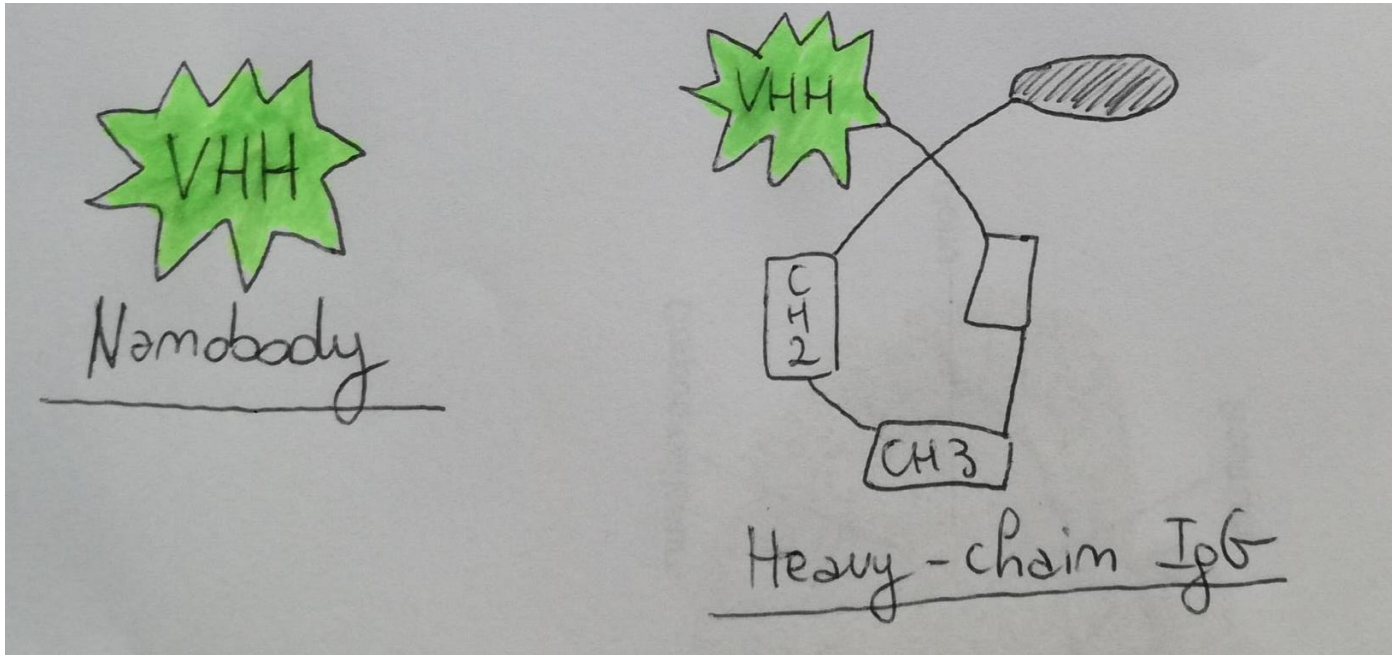


Fig2b

Single heavy chain V regions or light chain V regions can be expressed inside cells.

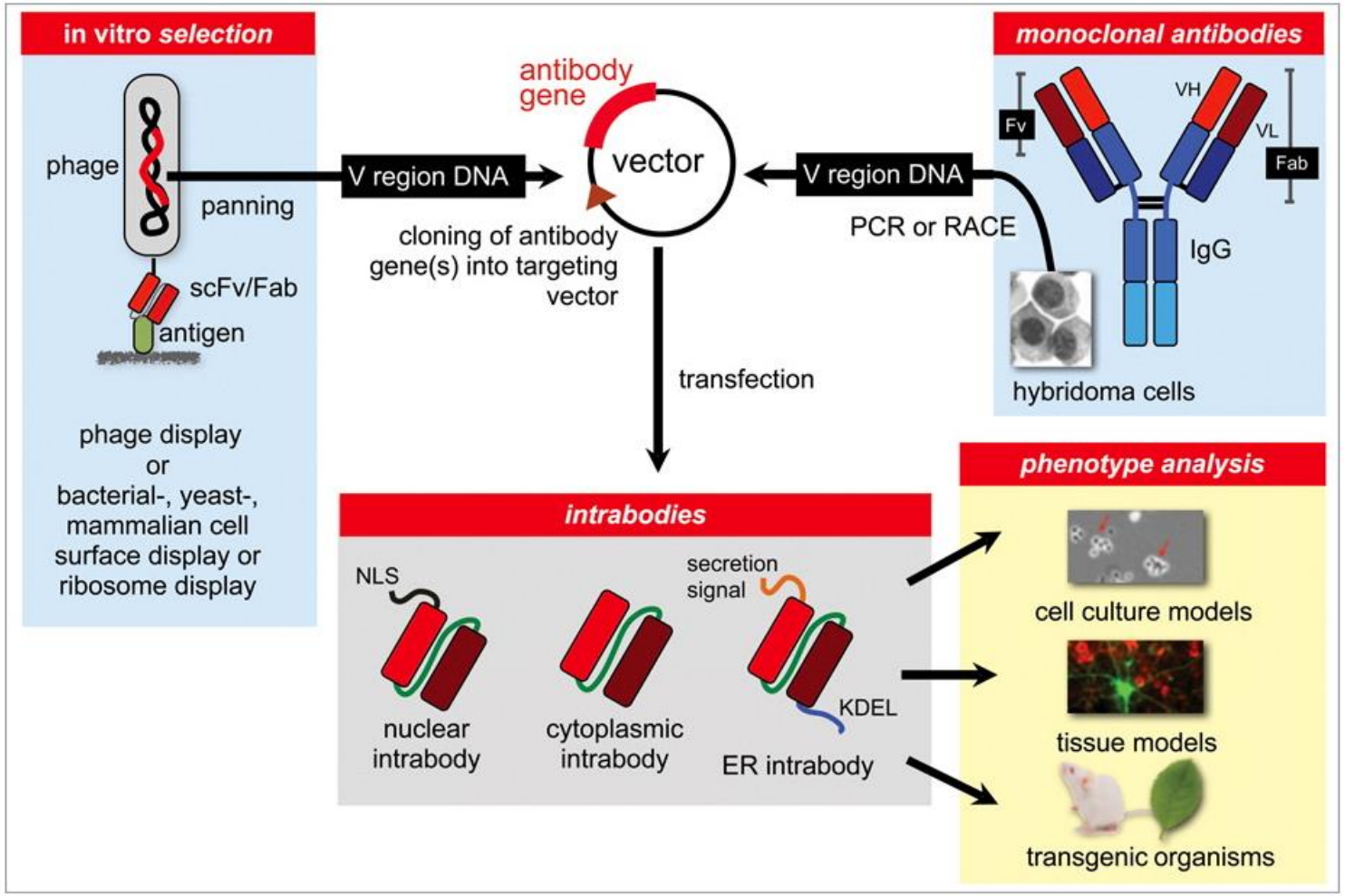
These are referred to as intracellular domain antibodies, which do not require intramolecular disulfide bonds for stability, hence representing **the smallest format of the antibody that retains target specificity while minimizing size.**

Introduction

What can they be used for?

Intrabodies can also be used to characterize the expression of their target proteins and study the in vivo knockdown of protein function, and can represent an alternative to generating gene knockout animal models.

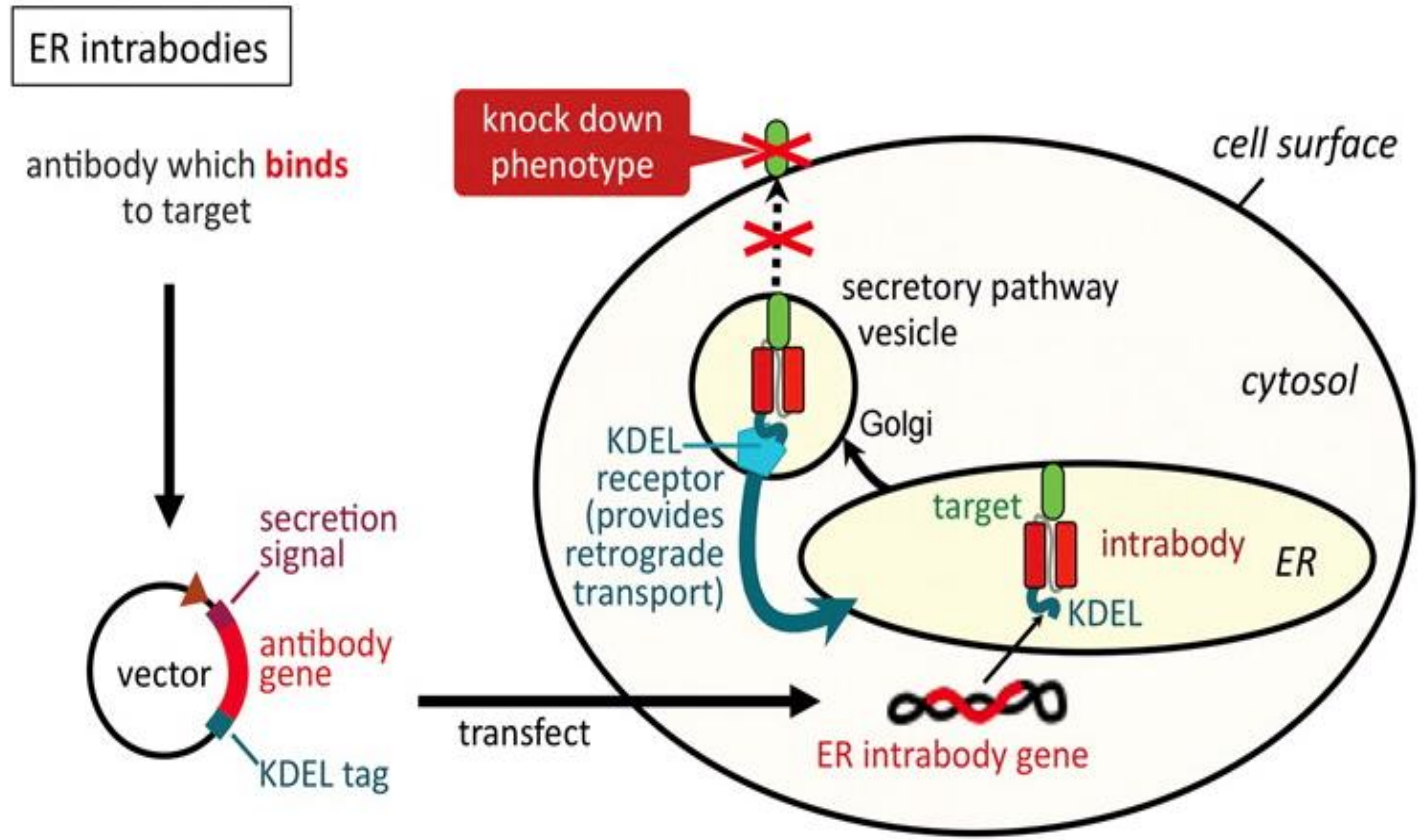
Protein knockdown strategies mediated by intrabodies



Andrea LJ Marschall et al., 2015

Introduction

What can they be used for?



Using intrabodies targeted to the ER (using a "KDEL" or "SEKDEL" sequence) allows the knockdown of proteins that are passing through the ER, thus abrogating their downstream function in a similar way to RNA interference and providing an **alternative strategy for silencing gene products**.

It has also been proposed that ER-targeting intrabodies may maintain silencing more effectively than short interfering RNA (siRNA) and their specificity may be easier to predict than the off-target effects of an siRNA.

Fig2: Via their retention signal KDEL, ER intrabodies retain antigens passing the ER by binding to them. As antibodies are naturally produced in the ER, no particular selection for special folding/stability properties is required.

Andrea LJ Marschall et al., 2015

Introduction

What can they be used for?

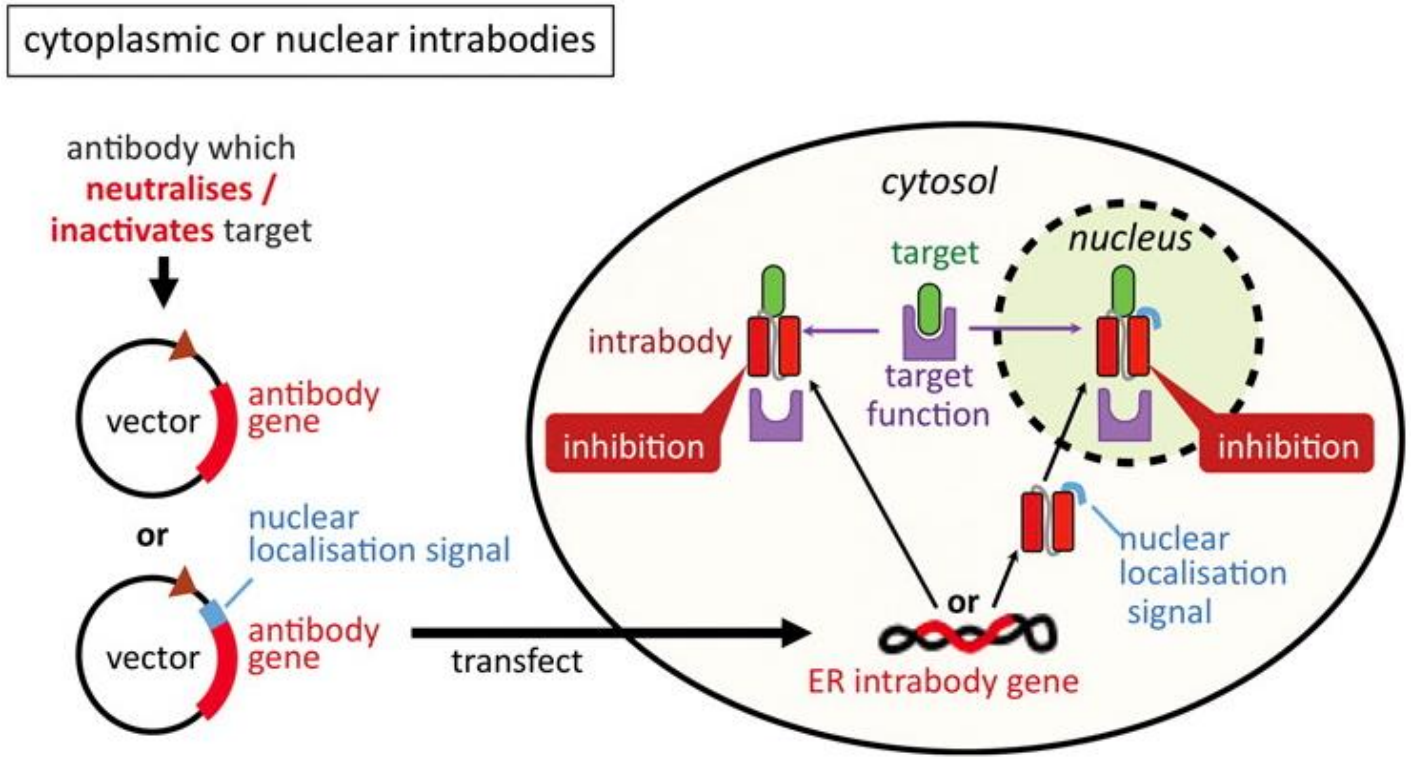


Fig 2:
Cytoplasmic/nuclear intrabodies need to fold correctly in the reducing milieu of the cytoplasm.

They need to be tested and screened to identify antibodies which are capable, in addition to binding, to neutralise or inactivate their target's activity in the cytoplasmic biochemical milieu.

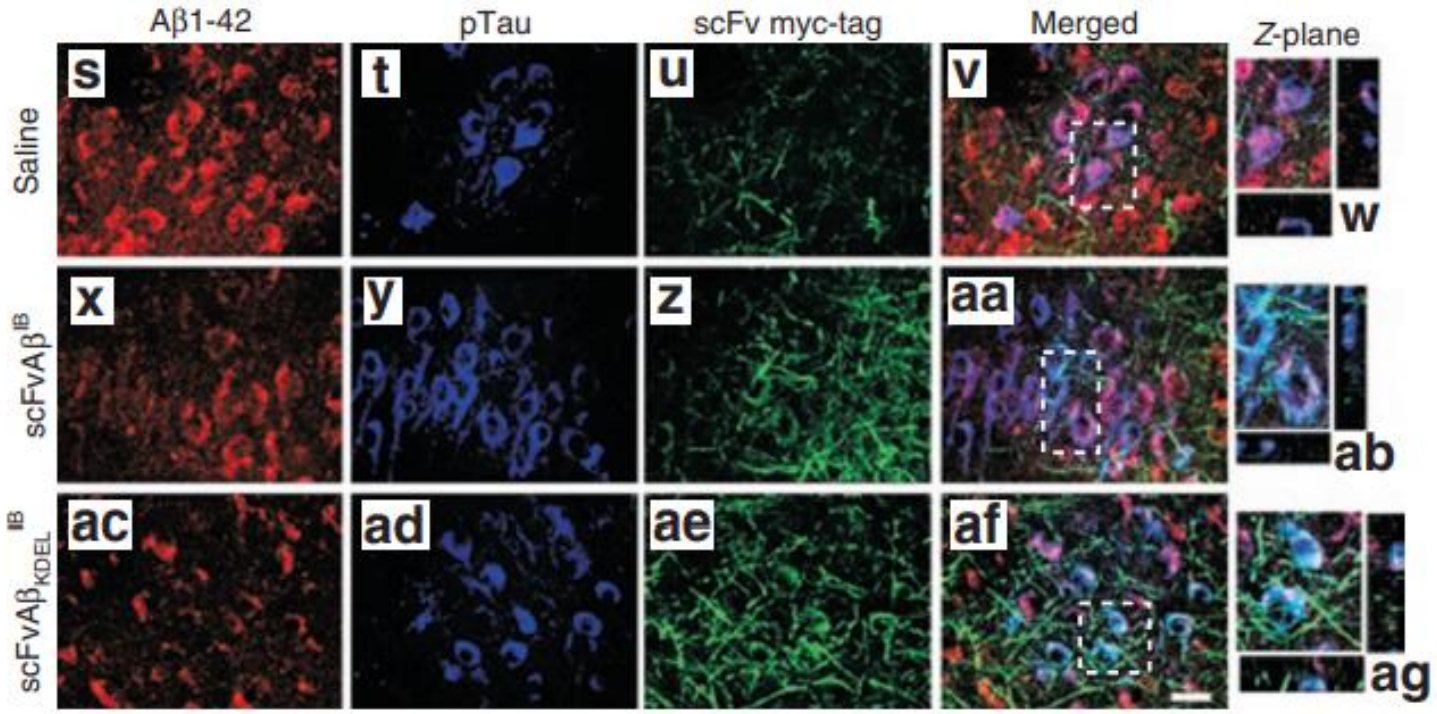
Andrea LJ Marschall et al., 2015

Specific Intrabodies as a Passive Vaccination Strategy for AD

AAV vector–mediated expression of the Aβ-specific intrabodies in 3xTg-AD mice reveals the intrabodies alter general patterns of cell-associated Aβ42

- To determine whether chronic Aβ42-specific intrabody (IB) expression in vivo could abrogate amyloid-related pathology
- To determine whether subcellular targeting of the IB would influence its effectiveness in abrogating amyloid-related pathology...

--> They subsequently packaged the rAAV-scFvAβIB and rAAVscFvAβKDEL IB plasmids into serotype 2 virions and delivered these constructs intrahippocampally to triple-transgenic AD mice (3xTgAD).



Sudol KL et al., 2009

Introduction

An intradiabody that simultaneously enabled the knockdown of VEGF-R2 and Tie-2 was able to reduce both tumor growth and angiogenesis in vivo.

Model of a bispecific, tetravalent intradiabody.

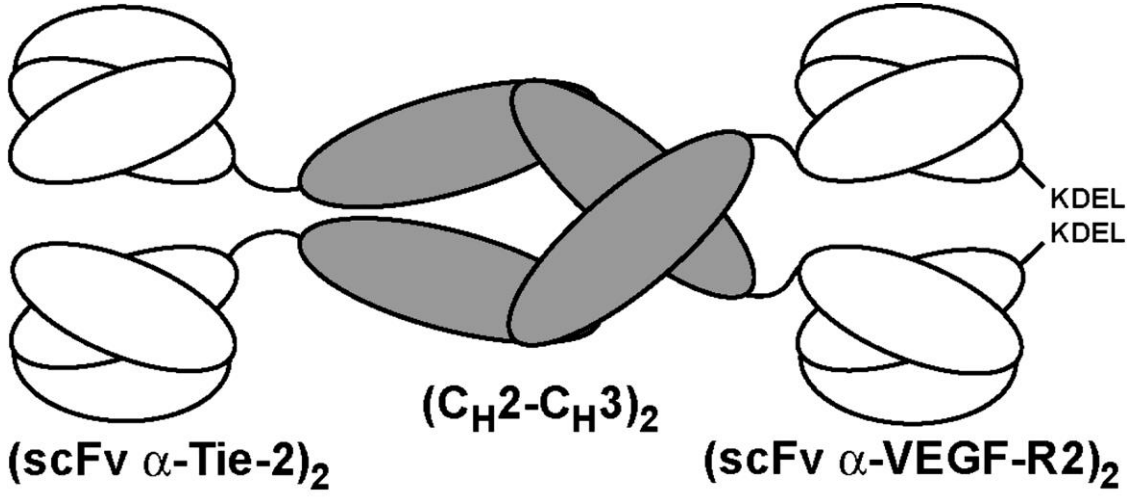
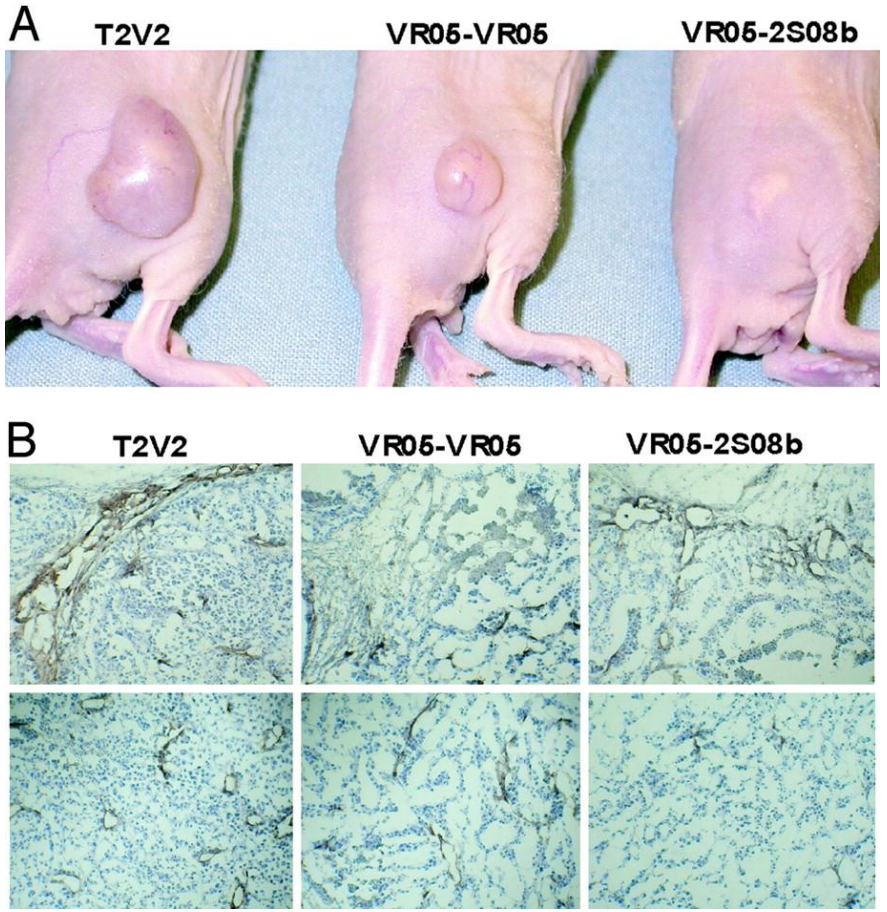


Fig 6

Vascularization of transplanted tumors.



Nina Jendreyko et al. PNAS 2005

Introduction

Strategies for delivery of Antibodies to the Intracellular Compartment

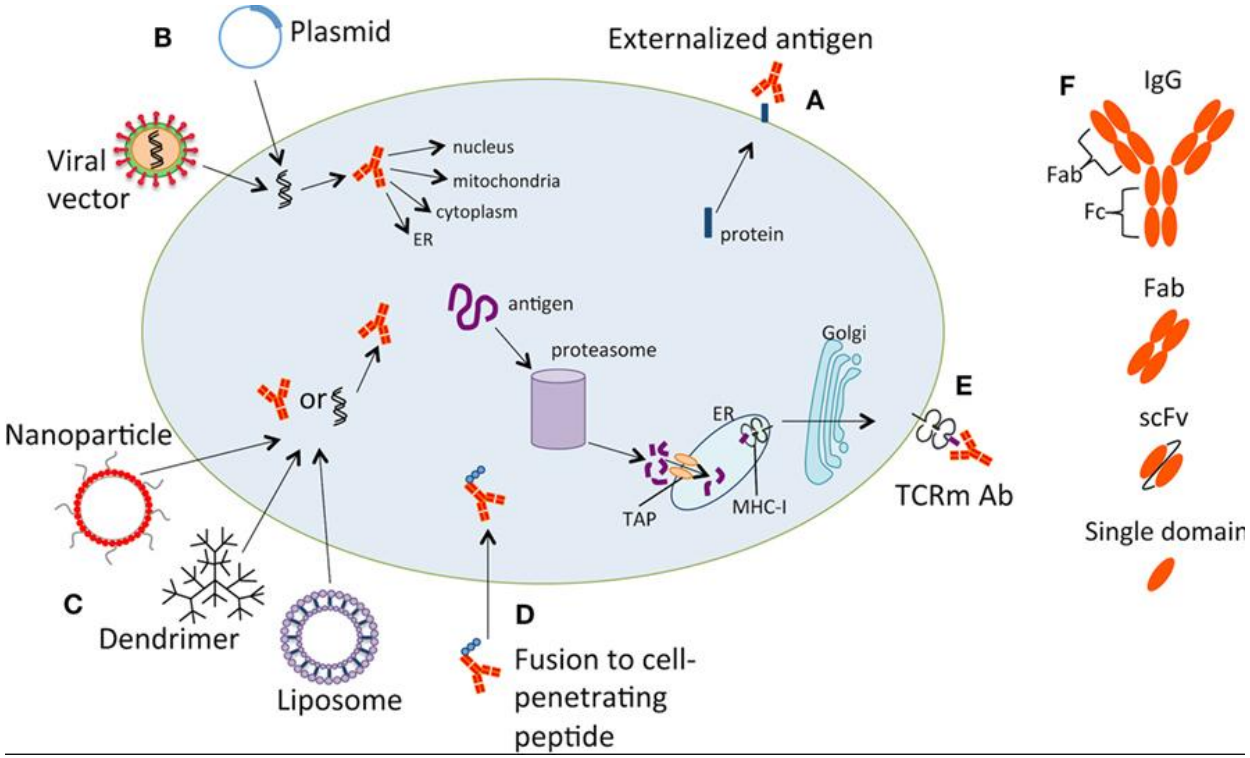


Fig 1: Trenevska I, Li D and Banham AH (2017) Therapeutic Antibodies against Intracellular Tumor Antigens. Front. Immunol.

(A) **Intracellular antigens can be externalized** on the cell surface or secreted, allowing targeting by antibodies.

(B) **Plasmids or viral vectors** can be used to deliver antibody-encoding genes into the cell. Once internalized, the DNA is transcribed into the targeting antibody, which can be designed to translocate to the nucleus, mitochondria, endoplasmic reticulum (ER), or cytoplasm.

(C) **Nanoparticles, dendrimers, or liposomes** can be used to deliver an antibody or an expression vector encoding the intracellular antibody into the target cell.

(D) **Antibodies can be fused to cell-penetrating peptides**, which allow internalization of the antibody.

(E) T**-cell receptor mimic (TCRm) antibodies can be used to target peptides bound to major histocompatibility complex class I (MHC-I) molecules on the cell surface.** The peptides are derived from intracellular proteins, which have been degraded by the proteasome into short peptides. Peptides are loaded onto MHC-I molecules in the ER, transported through the Golgi apparatus, and finally presented on the cell surface.

(F) The antibody depicted on the diagram could represent a full-length IgG, a Fab fragment, scFv or a single domain antibody.

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2019

Optogenetic activation of intracellular antibodies for direct modulation of endogenous proteins

Daseuli Yu[1], Hansol Lee[1], Jongryul Hong[1], Hyunjin Jung[1], YoungJu Jo[2,5], Byung-Ha Oh[1,3], Byung Ouk Park[4*] and Won Do Heo[1,3,4*]

They describe an optogenetically activated intracellular antibody (optobody) consisting of split antibody fragments and blue-light inducible heterodimerization domains.

They expanded this optobody platform by generating several optobodies from previously developed intracellular antibodies.

They demonstrated that photoactivation of gelsolin and β2-adrenergic receptor (β2AR) optobodies suppressed endogenous gelsolin activity and β2AR signaling, respectively.

Introduction

- Intrabodies: ability to manipulate endogenous protein function.

- Small antibody fragments, such as single-domain antibodies (V H H=nanobodies) and single-chain variable fragments (scFv), are used as efficient tools to define the function of endogenous proteins and determine their roles in signaling pathways.

Problem

- Still no techniques for directly controlling intrabody activity in living cells.

- The current methods for inducing intrabody activity in cells rely on chemically induced intrabody expression or degradation.

--> However, these methods do not allow for fine-tuned regulation of intrabody activity.

- Direct modulation of protein activity with an optogenetic tool adds further precision in terms of spatial and temporal regulation of proteins.

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--> However, these methods do not allow for fine-tuned regulation of intrabody activity.

- Direct modulation of protein activity with an optogenetic tool adds further precision in terms of spatial and temporal regulation of proteins.

Optogenetic platform for inducing activation of intrabodies that allows the function of endogenous target proteins to be precisely controlled in living cells.

They combined a split-protein system and light-responsive proteins to generate an optogenetically activatable intracellular antibody (optobody).

Curr Opin Chem Biol. 2011 Dec;15(6):789-97. doi: 10.1016/j.cbpa.2011.10.014. Epub 2011 Nov 7.

Split-protein systems: beyond binary protein-protein interactions.

Shekhawat SS[1], Ghosh I.

Author information

Abstract

It has been estimated that 650,000 protein-protein interactions exist in the human interactome (Stumpf et al., 2008), a subset of all possible macromolecular partnerships that dictate life. Thus there is a continued need for the development of sensitive and user-friendly methods for cataloguing biomacromolecules in complex environments and for detecting their interactions, modifications, and cellular location. Such methods also allow for establishing differences in the interactome between a normal and diseased cellular state and for quantifying the outcome of therapeutic intervention. A promising approach for deconvoluting the role of macromolecular partnerships is split-protein reassembly, also called protein fragment complementation. This approach relies on the appropriate fragmentation of protein reporters, such as the green fluorescent protein or firefly luciferase, which when attached to possible interacting partners can reassemble and regain function, thereby confirming the partnership. Split-protein methods have been effectively utilized for detecting protein-protein interactions in cell-free systems, Escherichia coli, yeast, mammalian cells, plants, and live animals. Herein, we present recent advances in engineering split-protein systems that allow for the rapid detection of ternary protein complexes, small molecule inhibitors, as well as a variety of macromolecules including nucleic acids, poly(ADP) ribose, and iron sulfur clusters. We also present advances that combine split-protein systems with chemical inducers of dimerization strategies that allow for regulating the activity of orthogonal split-proteases as well as aid in identifying enzyme inhibitors. Finally, we discuss autoinhibition strategies leading to turn-on sensors as well as future directions in split-protein methodology including possible therapeutic approaches.

Development of an optobody platform from nanobodies

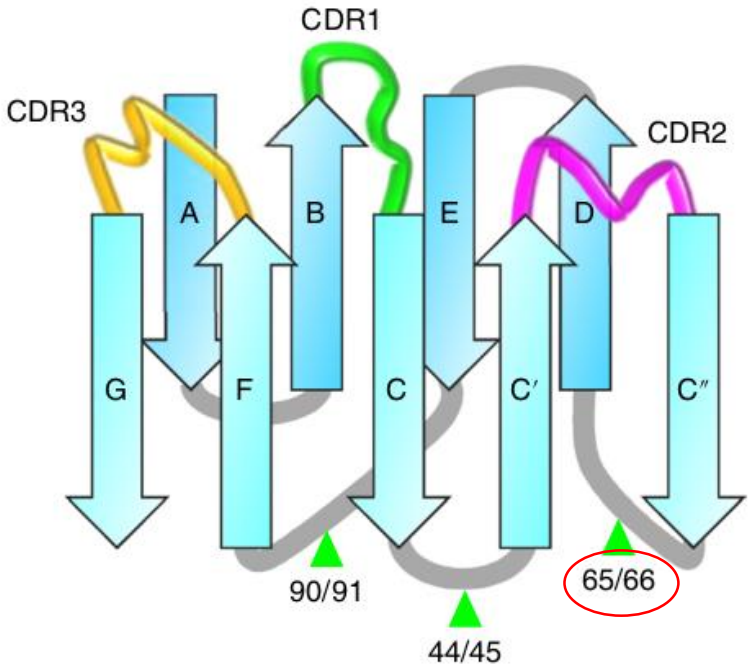
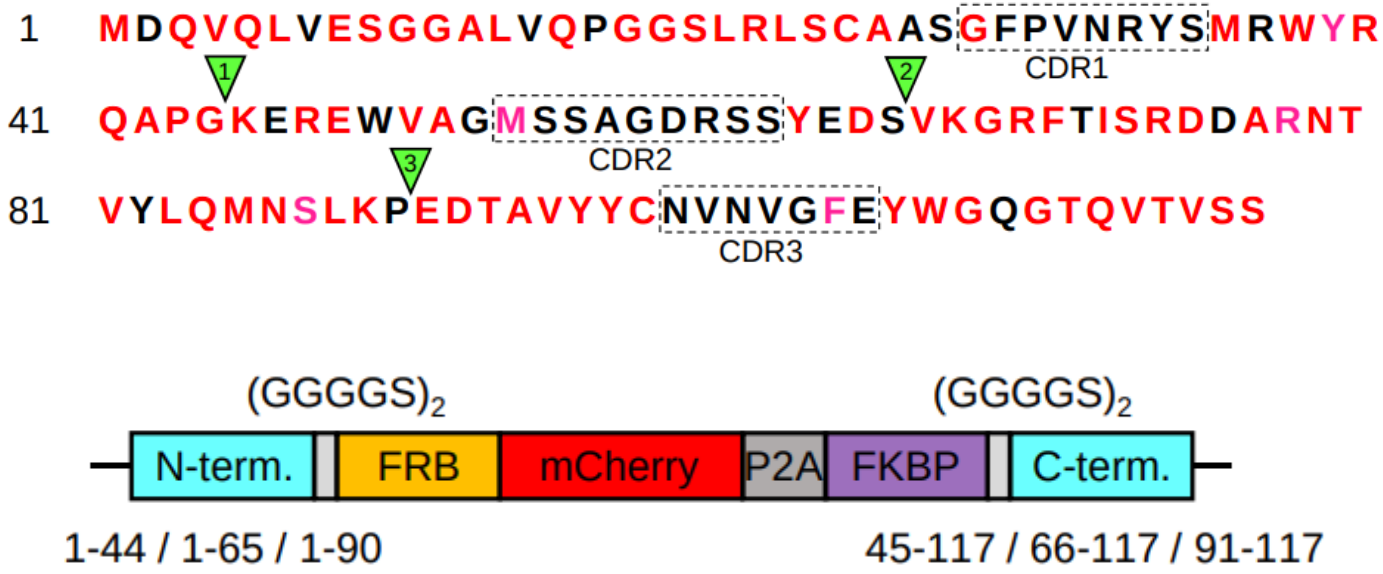


Fig 1a: green fluorescent protein (GFP) nanobody

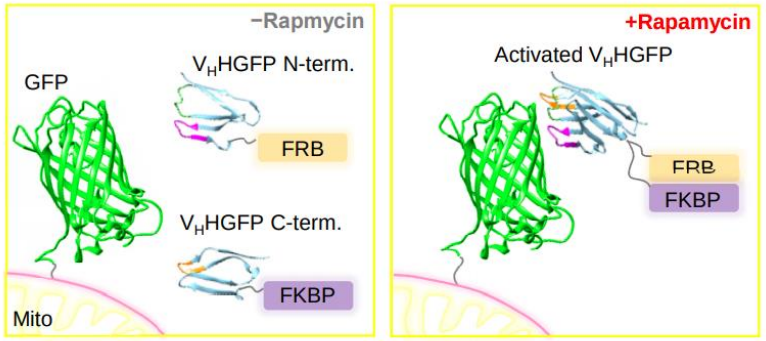


Supplementary fig 1

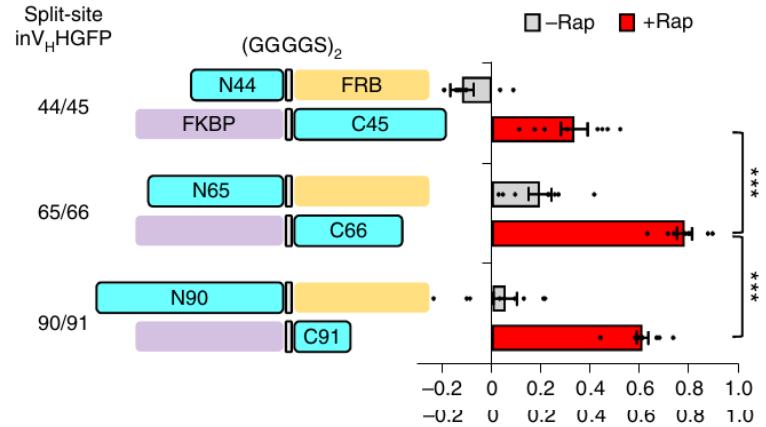
They first identified the best-suited split site on opposite sides of complementarity-determining regions (CDRs) in a green fluorescent protein (GFP) nanobody.

They conjugated each split GFP nanobody fragment to a FK506 binding protein fused to FKBP rapamycin binding protein (FKBP–FRB).

Development of an optobody platform from nanobodies



The efficiency of each split site was measured by assessing the association of the corresponding split GFP nanobody with Mito-GFP.



They found that the split sites, which divide CDR1/2 and CDR3, produced clear accumulation of GFP nanobody fragments with Mito-GFP after rapamycin treatment.

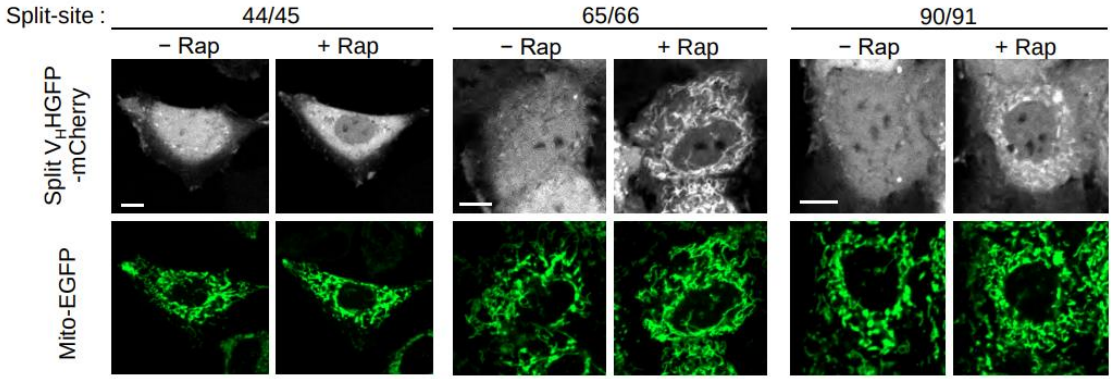
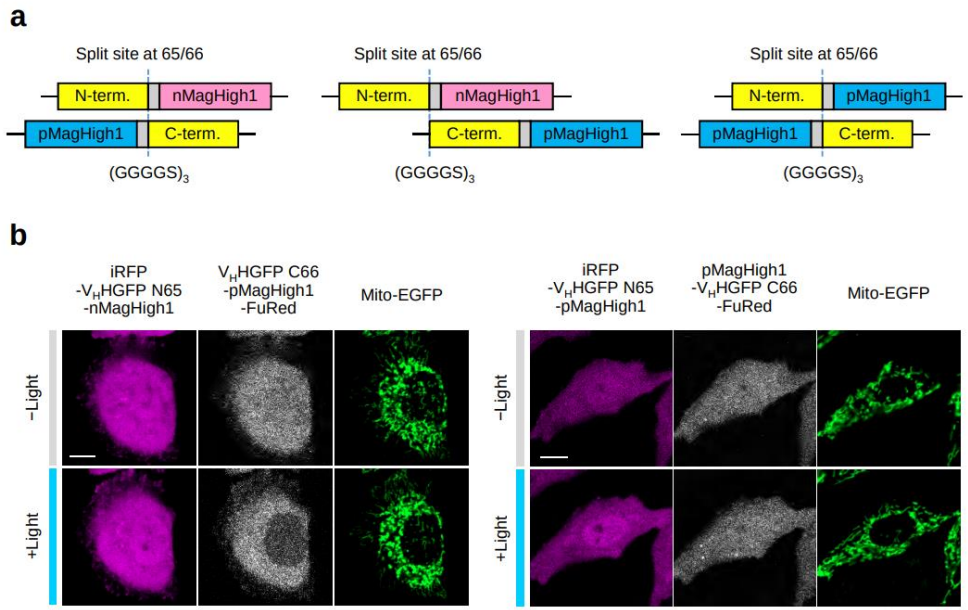
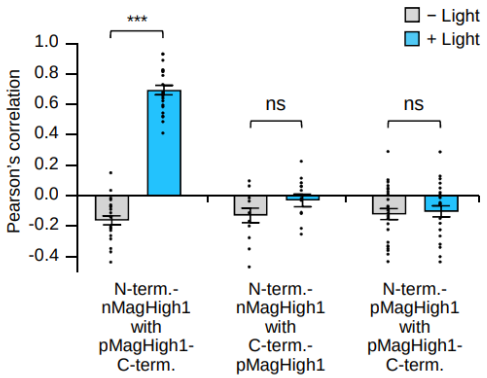


Fig 1b and suppl. fig 1

The N65/C66 split site was more efficient in rapamycin-mediated targeting of GFP.

Creation of an optobody platform from nanobodies

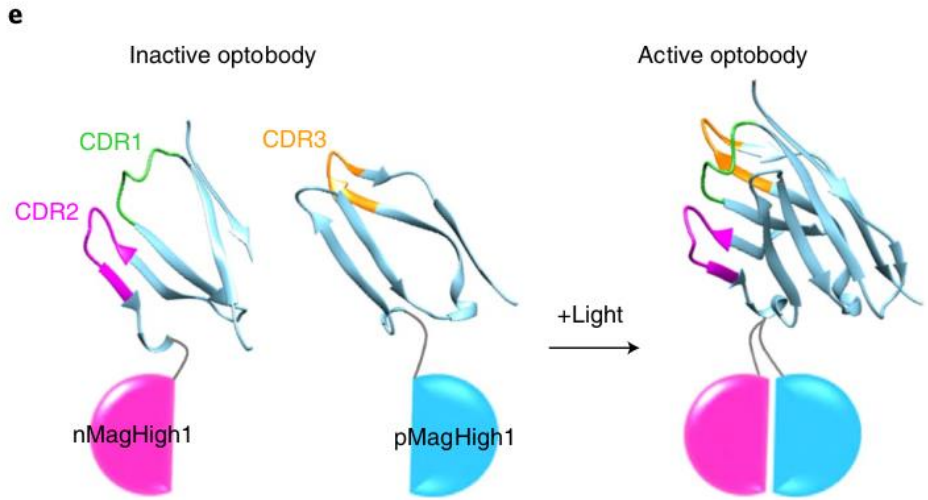




They linked the GFP nanobody fragments N65 and C66 to the Magnet optical dimerization tools nMagHigh1 and pMagHigh1, respectively.

The GFP nanobody N65-nMagHigh1 was paired with two different orientations of pMagHigh1 and GFP nanobody C66: pMagHigh1-C66 and C66-pMagHigh1.

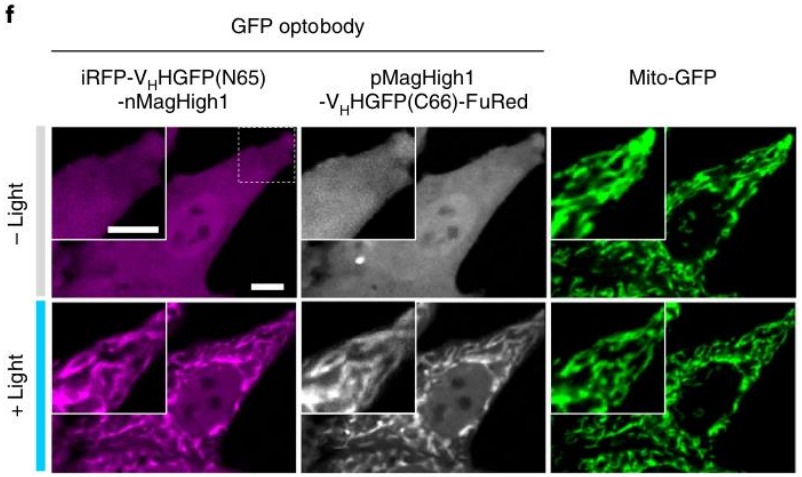
N65-nMagHigh1 paired with pMagHigh1-C66 showed efficient targeting to Mito-GFP in response to light stimulation.

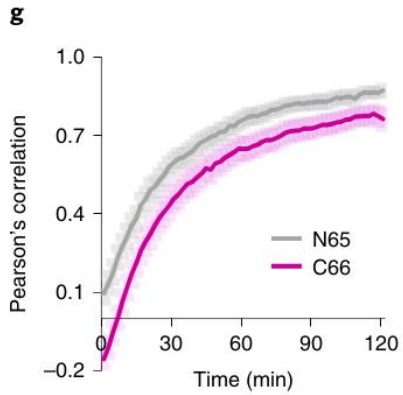


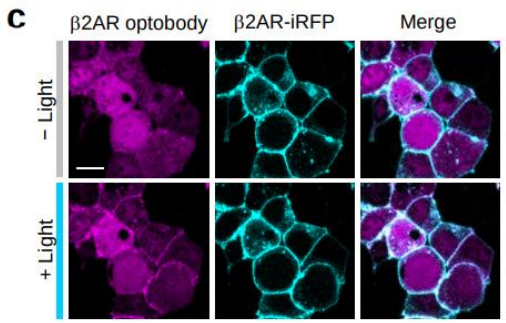
These results establish the N65/C66 split site and the indicated configuration of the Magnet fragments as the optimal optobody platform.

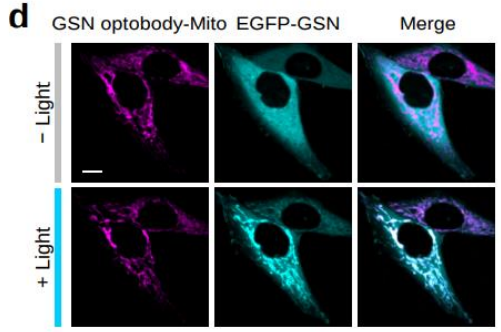
Suppl. fig 3 and Fig 1e

Creation of an optobody platform from nanobodies









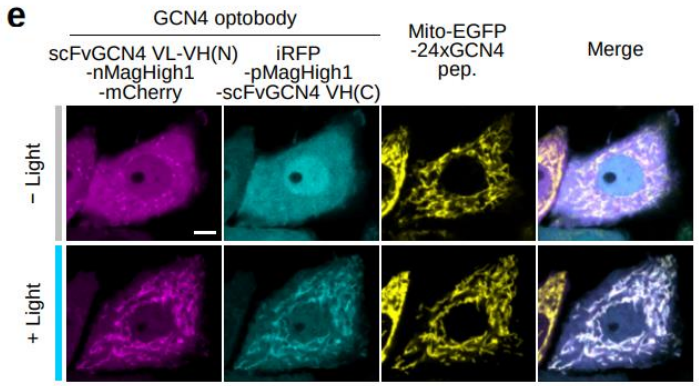
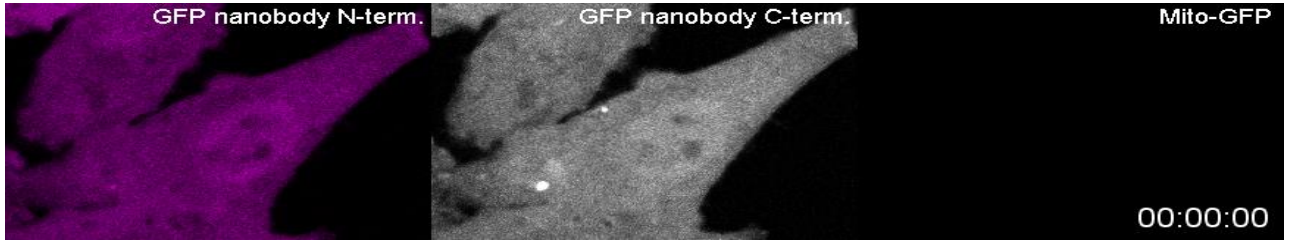


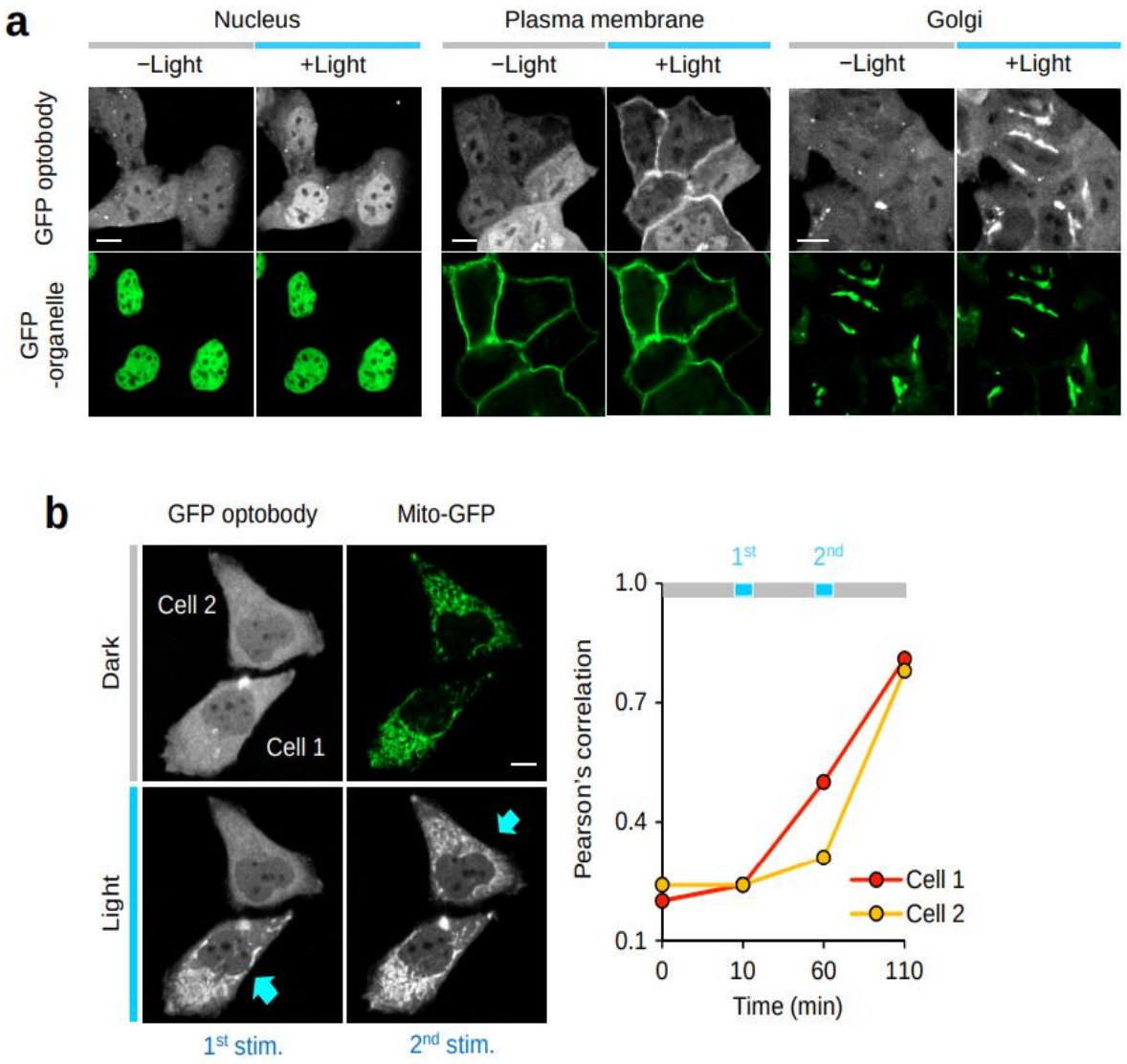
Fig 1 and Suppl. fig 12

Separate GFP optobody fragments were accumulated at Mito-GFP immediately after light stimulation, reaching saturation after about 1 h.



GFP Optobody activation on light illumination HeLa cells co-expressing GFP optobody and Mito-GFP were imaged for 20 min without blue-light stimulation and then illuminated with 488 nm light every 2 min for 2 h. Numbers indicate hours:minutes:seconds.

Creation of an optobody platform from nanobodies



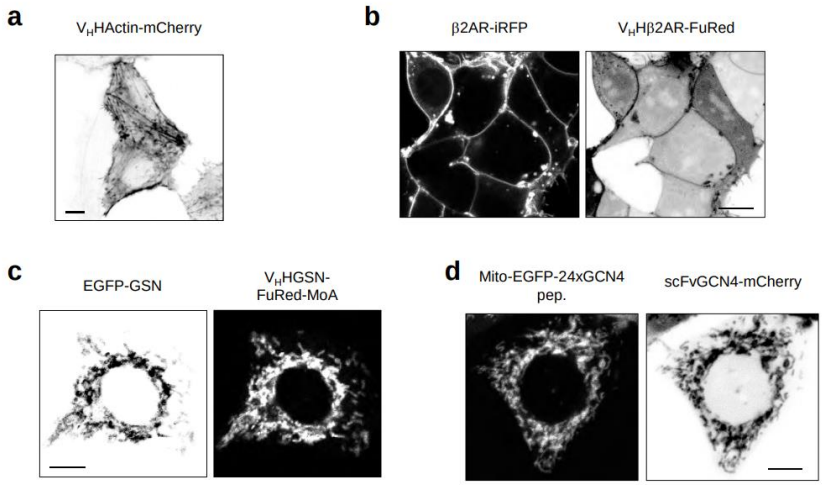
The GFP optobody can specifically target GFP within the nucleus, at the plasma membrane, and on the cytoplasmic
face of the Golgi apparatus.

The local stimulation in individual cells effectively activated the GFP opto-
body with high spatial resolution.

Single pulse of light power efficiently turned on the GFP optobody.
Increase of duration of light input at modest power density induced higher-amplitude activation of the GFP optobody.

Supplementary Fig. 5a, Fig 5b

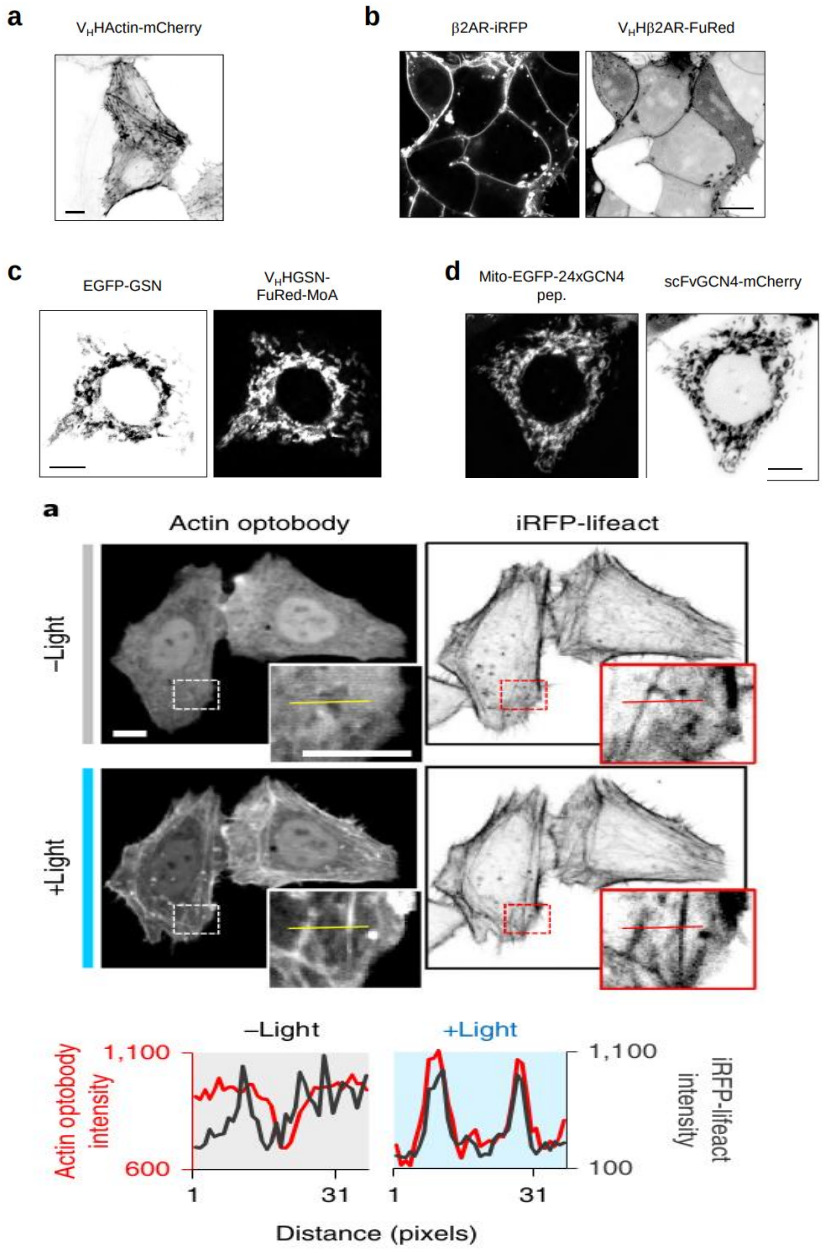
Expansion of the versatility of optobody to targeting of endogenous proteins



They generated a:
- actin optobody
- β2AR optobody
- gelsolin (GSN) optobody (gelsolin=actin-binding protein)
generated from nanobodies
and a:
- GCN4 optobody
generated from a scFv.

Supplementary Fig. 11

Expansion of the versatility of optobody to targeting of endogenous proteins



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generated from nanobodies
and a:
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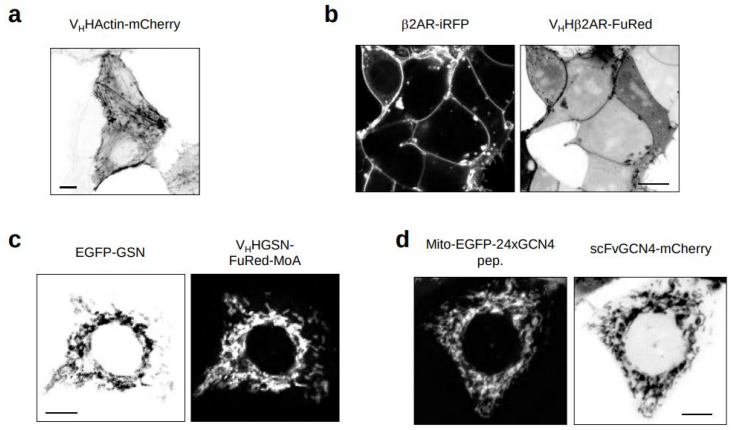
generated from a scFv.

They first converted the actin nanobody to an actin optobody.

--> In darkness, the inactive actin optobody was dispersed in the cytosol of cells, but light stimulation induced activation of the actin optobody, resulting in its targeting to actin filament.

Supplementary Fig. 11

Expansion of the versatility of optobody to targeting of endogenous proteins



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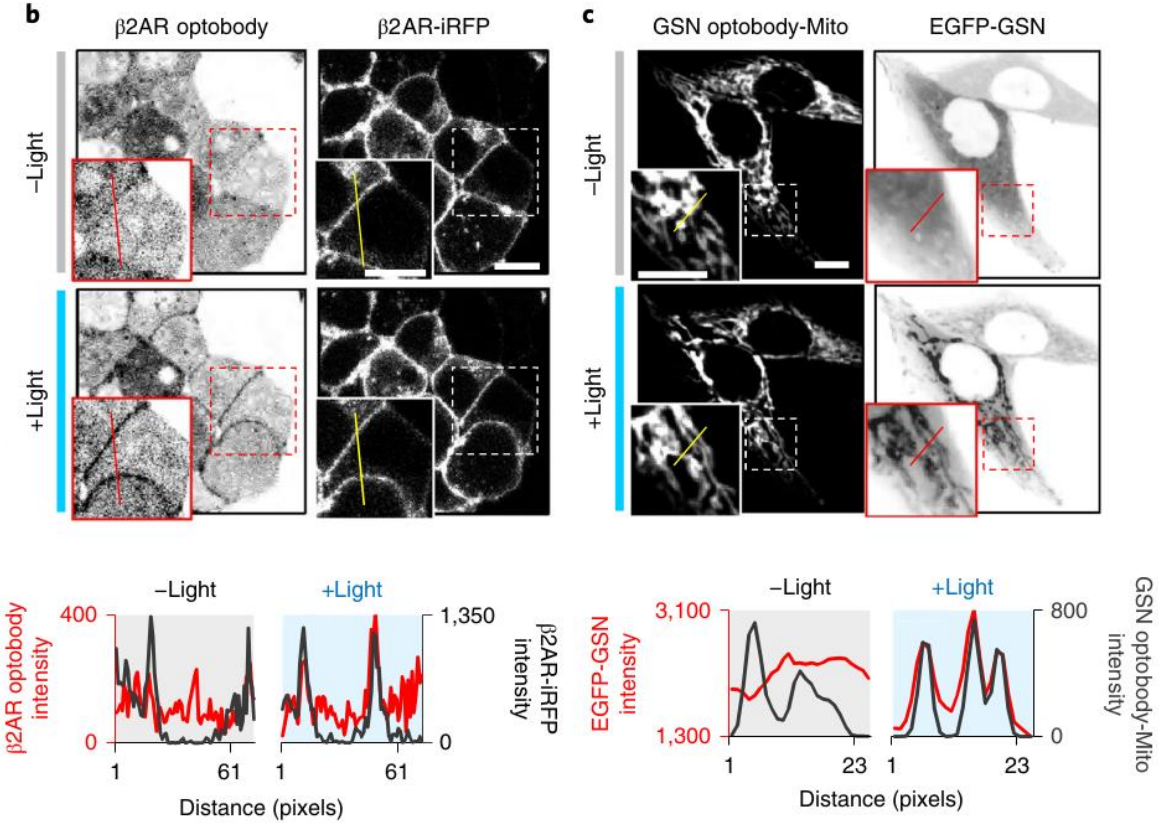


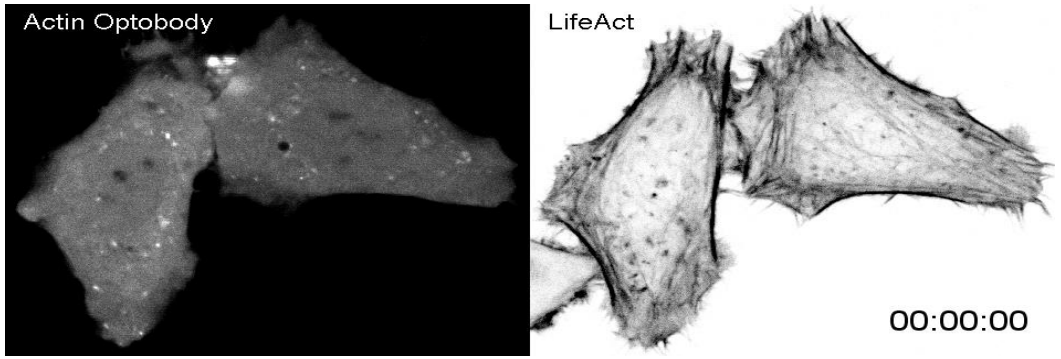
β2AR optobody:
they engineered the nanobody, Nb60, which detects an inactive form of β2AR. Light illumination triggered translocation of the activated β2AR optobody to the membrane, where it targeted inactive β2AR.

GSN nanobody:
they used GSN Nb11 as a template for a GSN optobody, which specifically recognizes the GSN G2 domain.

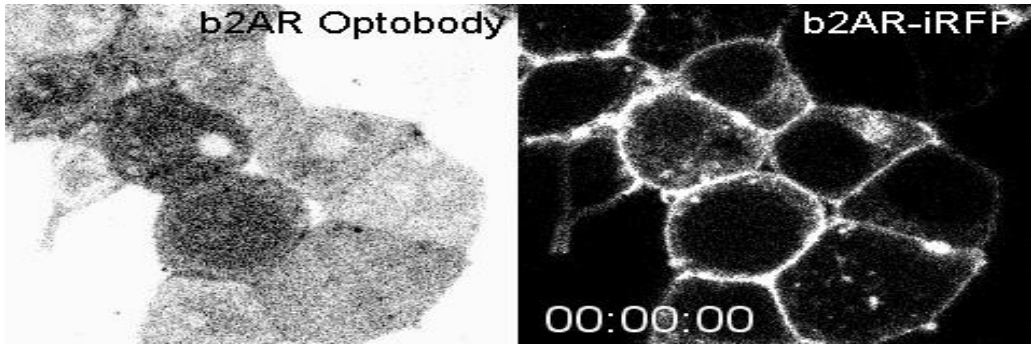
On light stimulation, the mito-conjugated GSN optobody was activated and EGFP-GSN clearly moved toward the mitochondria.

Supplementary Fig. 11

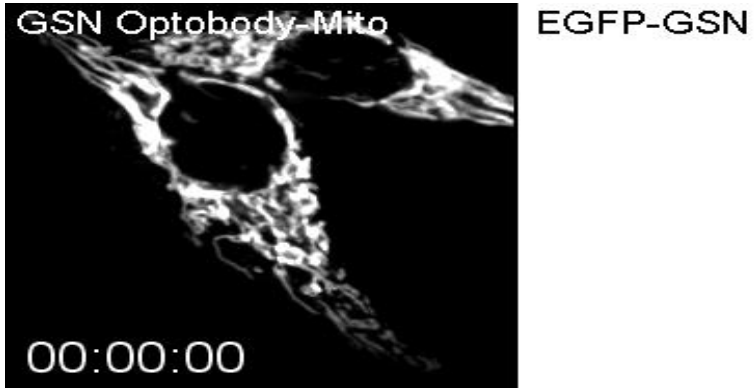
Expansion of the versatility of optobody to targeting of endogenous proteins



Light stimulation induced activation of the actin optobody, resulting in its targeting to actin filaments.



Light illumination triggered translocation of the activated β2AR optobody to the membrane, where it targeted inactive β2AR.

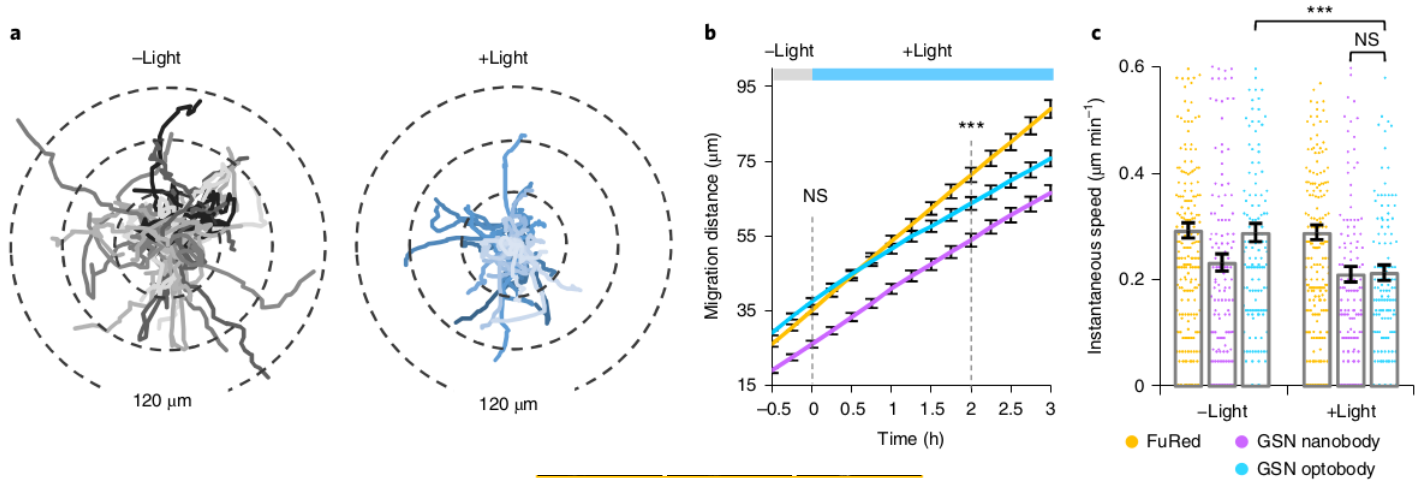


On light stimulation, the mito-conjugated GSN optobody was activated and EGFP-GSN clearly moved toward the mitochondria.

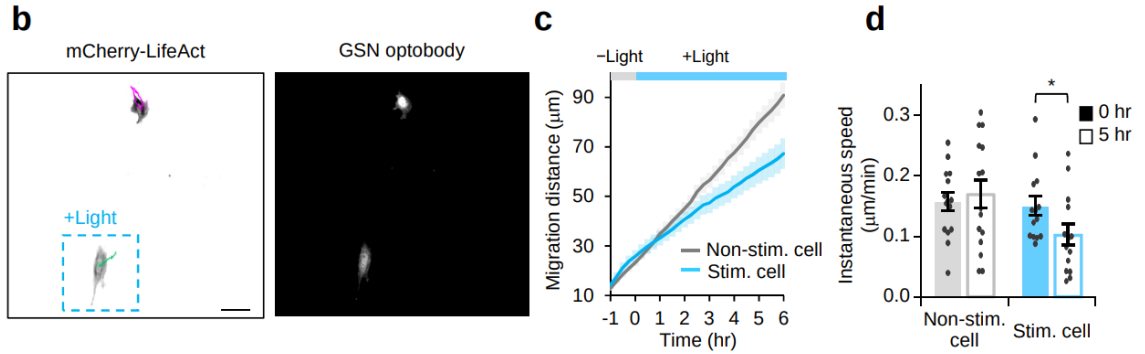
Suitability of GSN optobody as new tools for optogenetic inhibition on their endogenous target

Investigation of light-induced perturbation of GSN function by GSN optobody:

--> we monitored migration of NIH3T3 cells expressing a GSN optobody, with or without light stimulation.



Cells illuminated with blue light explored a more restricted territory than cells in darkness, exhibiting a considerable decrease in migration distance and instantaneous speed.



Spatiotemporal activation of GSN optobody recruited GSN protein at the subcellular level, showing that local stimulation of the GSN optobody in a specifically targeted cell induced a spatially controlled defect in cell migration.

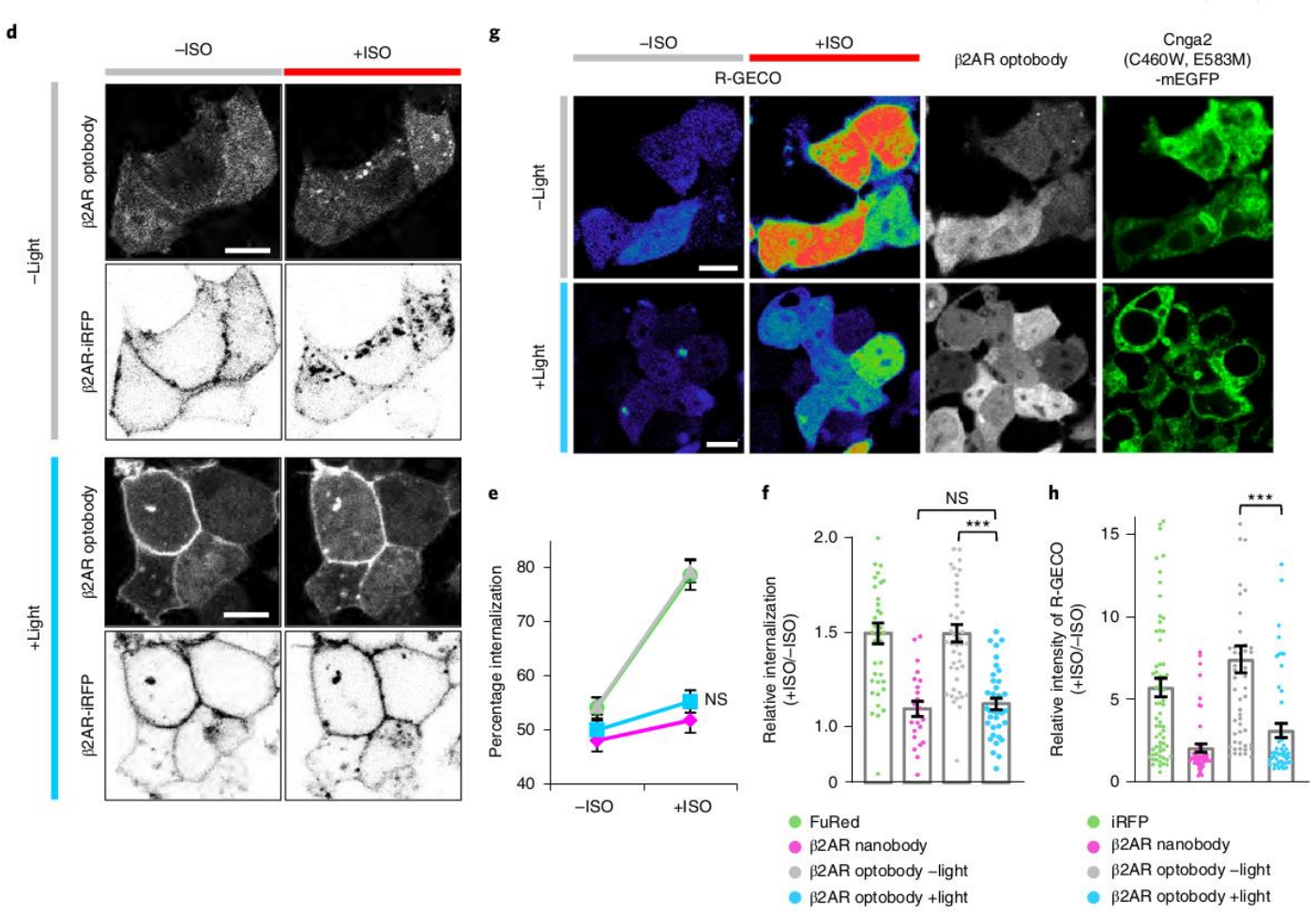
--> This capability provides an opportunity for optically modulating endogenous GSN in a spatio-temporal manner.

Fig. 3 and Supplementary Fig. 16

Ability of β2AR optobody to inhibit endogenous β2AR

Activation of β2AR with isoprenaline (ISO), resulting in β2AR internalization and cAMP-mediated Ca 2+ influx, was blocked by the intact β2AR nanobody

They photo-stimulated HEK293 cells expressing β2AR optobody and monitored down-stream β2AR signaling.



In β2AR optobody-expressing cells, light illumination inhibited the internalization of β2AR-iRFP, significantly decreasing the relative internalization ratio to a degree comparable to that of the β2AR nanobody.

The blue-light illumination of β2AR optobody-transfected cells also induced a decrease in Ca 2+ influx into the cells, even with ISO treatment.

Fig. 3

Summary

- They have developed an optogenetic platform, termed Optobody, for light-inducible regulation of intrabody activity and manipulation of endogenous target protein function.

- They used blue-light responsive photoreceptors for generating optobodies.

- The concept underlying the optobody is a split intrabody fragment and reassembly via an inducer, and is thus not limited to blue-light stimulation.

- **Advantage: The representative intrabodies that they have developed showed a clear expression pattern in cells.**

- **Disadvantage: Intrabodies sometimes aggregate.**

<u>To keep in mind:</u> To choose an intrabody as a backbone for optobody, it is important to consider the expression pattern of original intrabody inside cells.
(When some fragments of optobody such as C-terminal fragment of GFP optobody are highly expressed in cells, they have a self-aggregated tendency before stimulation)

The optobody might thus be applied to engineering of conventional antibody techniques to convert these tools to inducible antibody-activated systems, suggesting a broader drug-activating concept.

nature methods

BRIEF COMMUNICATION

https://doi.org/10.1038/s41592-020-0746-7

Check for updates

Chemogenetic Control of Nanobodies

Helen Farrants[1,2], Miroslaw Tarnawski[3], Thorsten G. Müller[4], Shotaro Otsuka[5,6], Julien Hiblot[1], Birgit Koch[1], Moritz Kueblbeck[5], Hans-Georg Kräusslich[4], Jan Ellenberg[5] and Kai Johnsson[1,2] ✉

Engineered nanobody whose affinity to green fluorescent protein (GFP) can be switched on and off with small molecules.

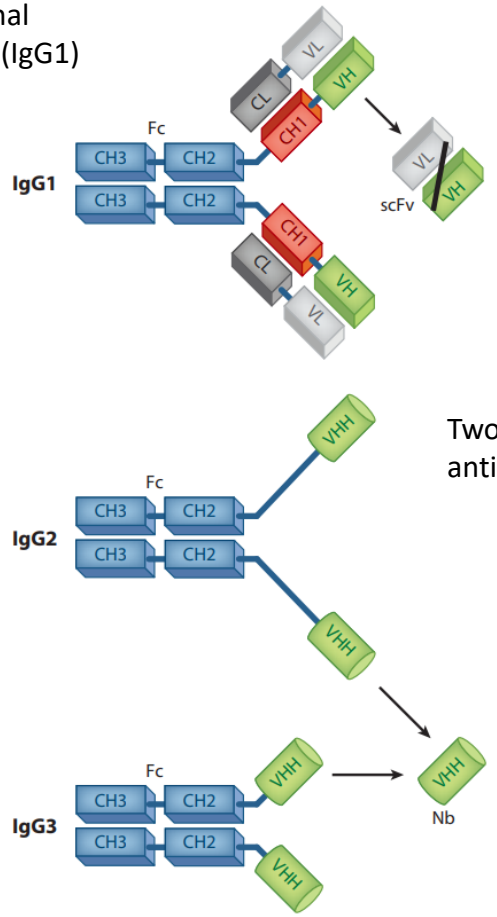
It allows interrogation of the role of fusion proteins in basic biological processes.

The binding affinities of other nanobodies can be controlled by small molecules.

Introduction

Schematic representation of naturally occurring antibodies in sera of camelids

Conventional antibodies (IgG1)



The smallest intact functional antigen-binding fragment that can be generated from conventional antibodies, consisting of a VH-VL pair and linked by an oligopeptide.

Two types of homodimeric heavy-chain antibodies (HCAbs), IgG2 and IgG3

Nanobodies

The range of applications of nanobodies would be greatly expanded if their binding affinity toward their target could be rapidly switched on and off with a cell-permeable and nontoxic molecule.

Introduction **Applications of nanobodies**

Need to control their binding affinity toward their target through rapidly switching on and off system with a cell-permeable and nontoxic molecule.

Recent discoveries:

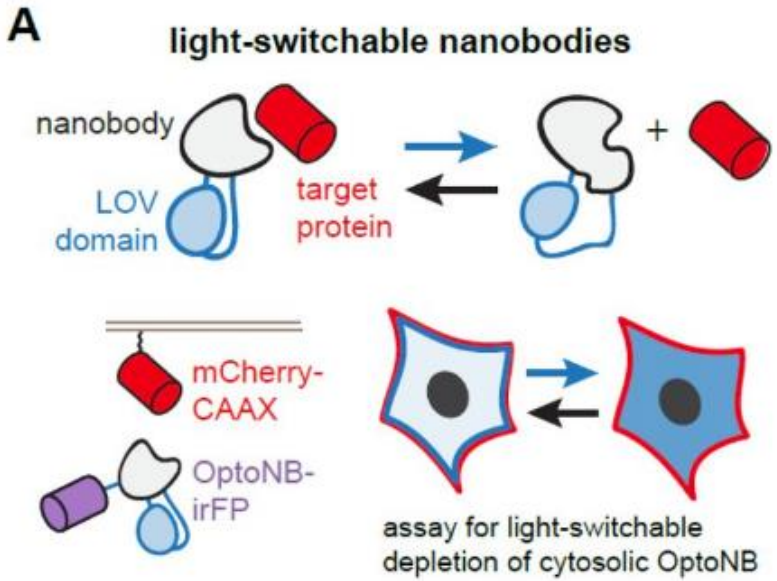
insertion of a light-oxygen-voltage (LOV) domain:

- Opto-Nanobodies (OptoNBs) enable light-regulated binding to a wide range of protein targets.

- They identify an optimized LOV domain and two loop insertion sites for light-regulated binding.

- OptoNBs function in vivo: when expressed in cells and fused to signaling domains, OptoNBs enable light-activated and light-inhibited Ras/Erk signaling.

- OptoNBs function in vitro: Target proteins can be reversibly bound to OptoNB-coated beads and separated through size-exclusion chromatography.



Introduction **Applications of nanobodies**

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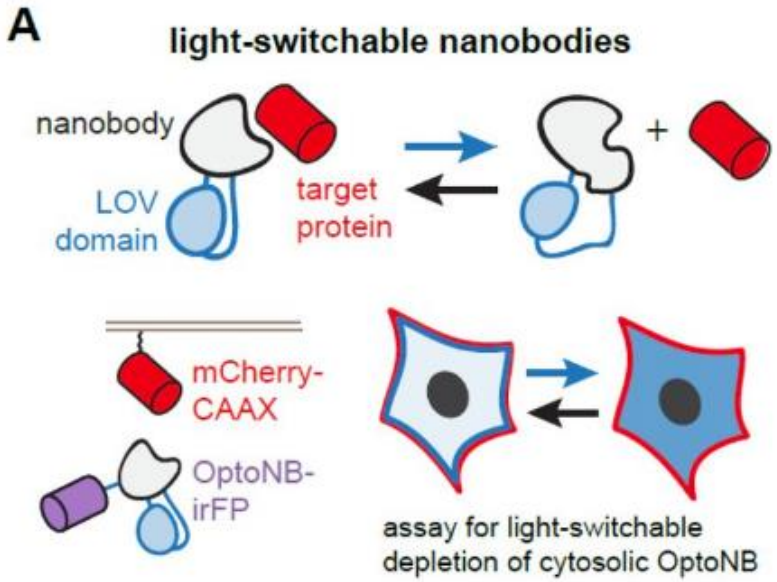
fusion of split antibody fragments to light-inducible heterodimerization domains (seen before).

--> However, insertion of the light-oxygen-voltage domain results in only a modest 5.5-fold change in affinity on irradiation, whereas the activation of the split antibody fragments is irreversible.



nature methods BRIEF COMMUNICATION
https://doi.org/10.1038/s41592-020-0746-7

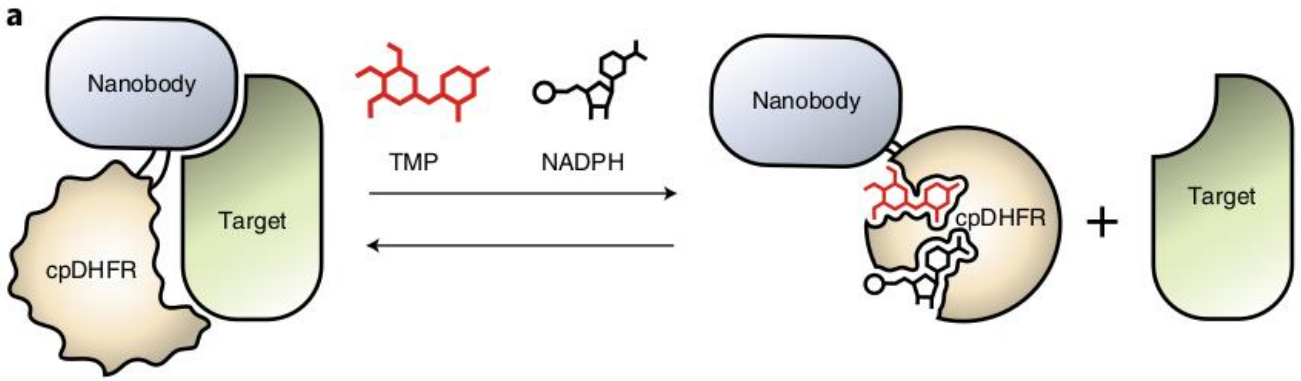
What is new in this paper?

Chemogenetic Control of Nanobodies

Helen Farrants[1,2], Miroslaw Tarnawski[2], Thorsten G. Müller[4], Shotaro Otsuka[5,6], Julien Hiblot[1], Birgit Koch[1], Moritz Kueblbeck[5], Hans-Georg Kräusslich[4], Jan Ellenberg[5] and Kai Johnsson[1,2]

They introduce ligand-modulated antibody fragments (LAMAs), which combine:
the high selectivity and specificity of nanobodies
with
the fast temporal control offered through the use of small molecules

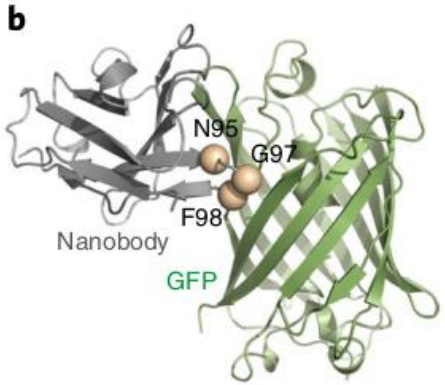
LAMAs generation



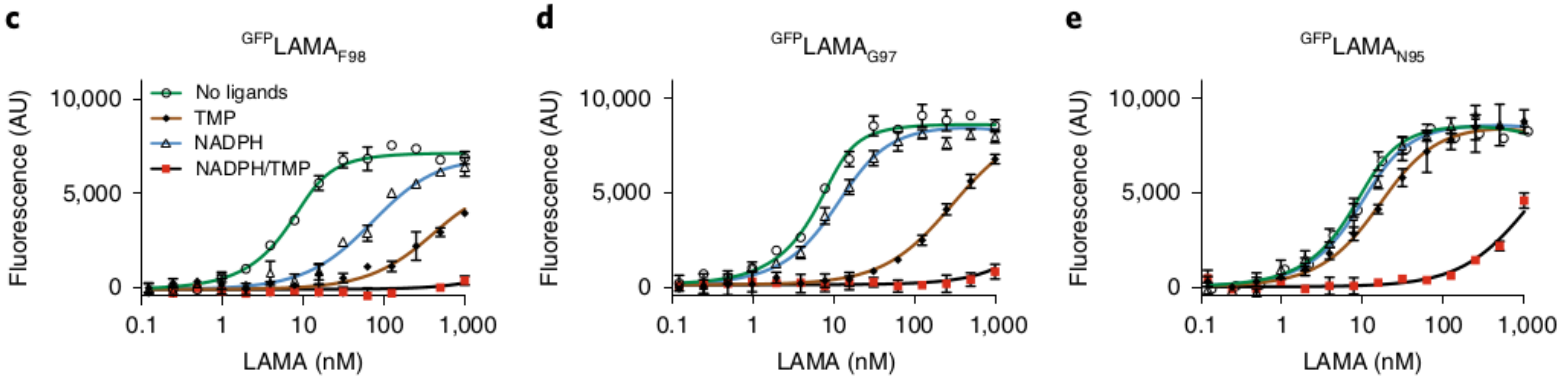
- Insertion of a circularly permutated bacterial dihydrofolate reductase (cpDHFR) into nanobodies.
- cpDHFR is partially unfolded in the absence of its cofactor nicotinamide adenine dinucleotide phosphate (NADPH) and DHFR inhibitors such as trimethoprim (TMP).
- TMP is a clinically approved antibacterial drug that has excellent cell and tissue permeability and is not toxic to mammalian cells.
- LAMAs disrupt the binding of the nanobody to its target by exploiting the change in conformation of cpDHFR on binding of NADPH and DHFR inhibitors.

Fig. 1

Enhancer antibody for GFP



The target here is GFP



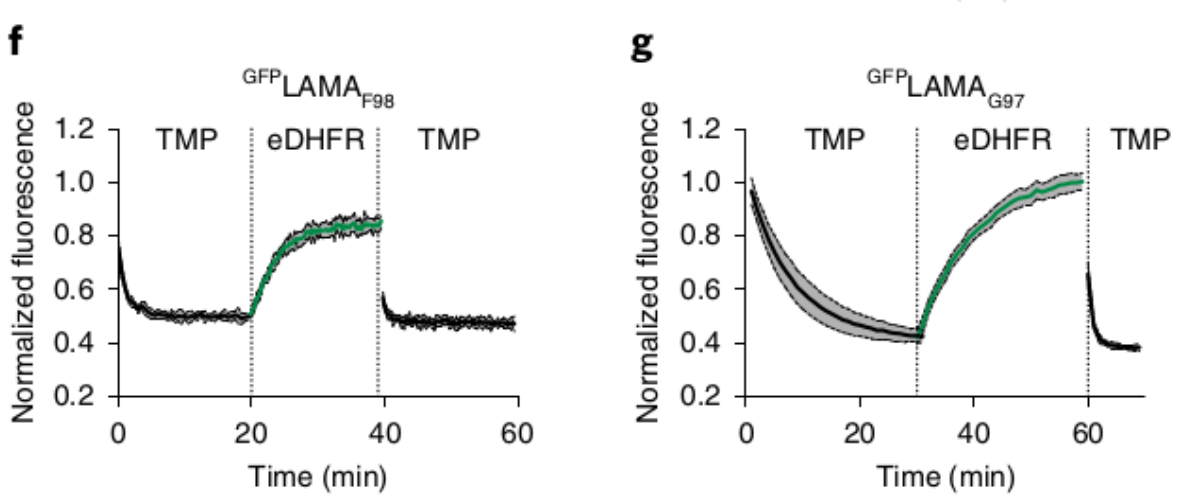
- ❑ They inserted cpDHFR into various sites of the enhancer nanobody.
- ❑ They measured the binding affinities of the protein chimeras to wild-type GFP (wtGFP) in the presence and absence of the ligands NADPH and TMP.

- The most promising insertion hits were in the complementary-determining region 3 (CDR3).
- CDR3 is often essential for making high affinity contacts between nanobodies and their targets.
- All three GFP LAMAs retained a single-digit nanomolar affinity to GFP in the absence of ligands.
- For all three nanobodies, the affinity toward GFP was dramatically decreased in the presence of NADPH and TMP such that no binding to GFP could be detected for GFP LAMA F98 and GFP LAMA G97.
- For GFP LAMA F98 the presence of NADPH alone also affected the binding affinity to GFP, whereas the affinity of GFP LAMA G97 and GFP LAMA N95 was not affected by NADPH.

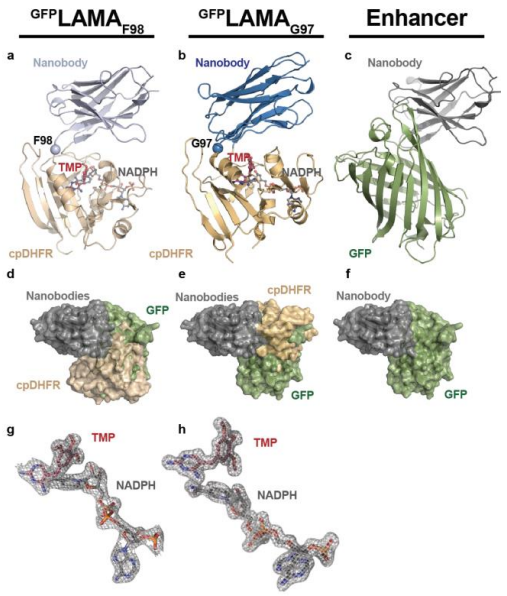
Fig. 1

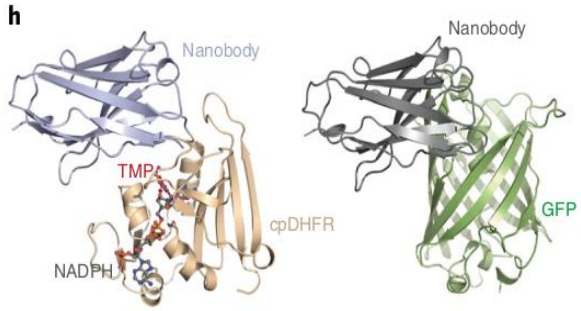
Enhancer antibody for GFP

- The kinetics of dissociation of the complexes between GFP and GFP LAMA F98 or GFP LAMA G97 on addition of TMP were on the timescale of minutes.
- Subsequent removal of TMP by addition of wild-type DHFR resulted in reformation of the complexes within minutes.
- The complex could then be dissociated again by addition of an excess amount of TMP.



Comparing:
The crystal structures of GFP LAMA F98 and GFP LAMA G97 in complex with NADPH and TMP
with
The structure of enhancer nanobody bound to GFP





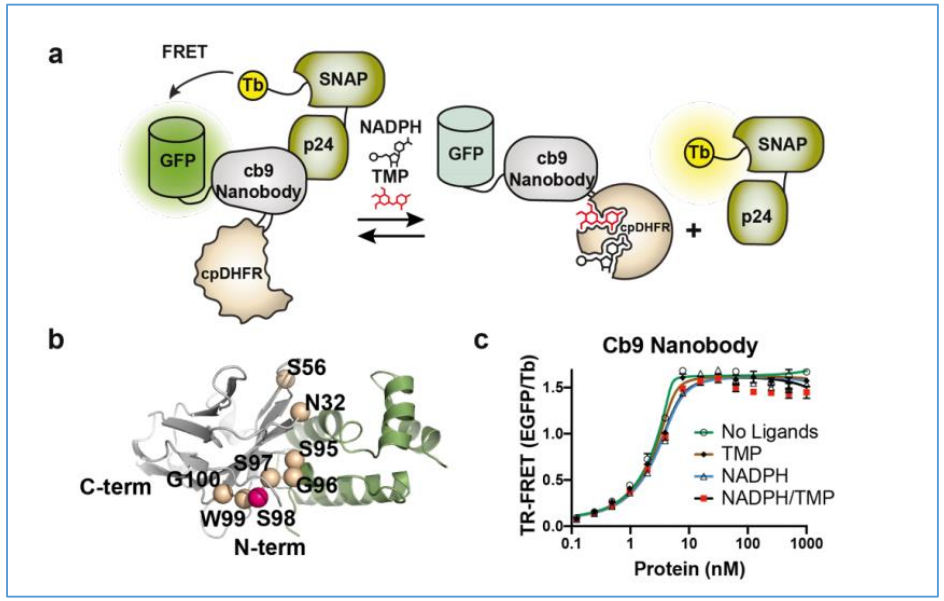
suggests that folded cpDHFR sterically hampers binding to GFP

Fig. 1 and Supplementary Fig. 5

Expansion of the LAMA concept to other targets

Given the large number of nanobodies that have been selected and characterized they attempted to expand the LAMA concept to other targets

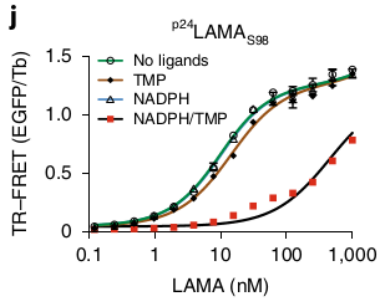
A nanobody for the C-terminal region of the p24 HIV capsid protein (PDB ID 2XV6) could be readily converted into a LAMA on insertion of cpDHFR into the CDR3 loop.



Insertion of cpDHFR into other nanobodies

Schematic illustration of the TR-FRET assay to monitor binding between the nanobodies (Nb) and target protein.

The Nb is expressed as an EGFP fusion (FRET acceptor) and the target expressed as a SNAPtag fusion, labelled with Tb-cryptate (FRET donor).

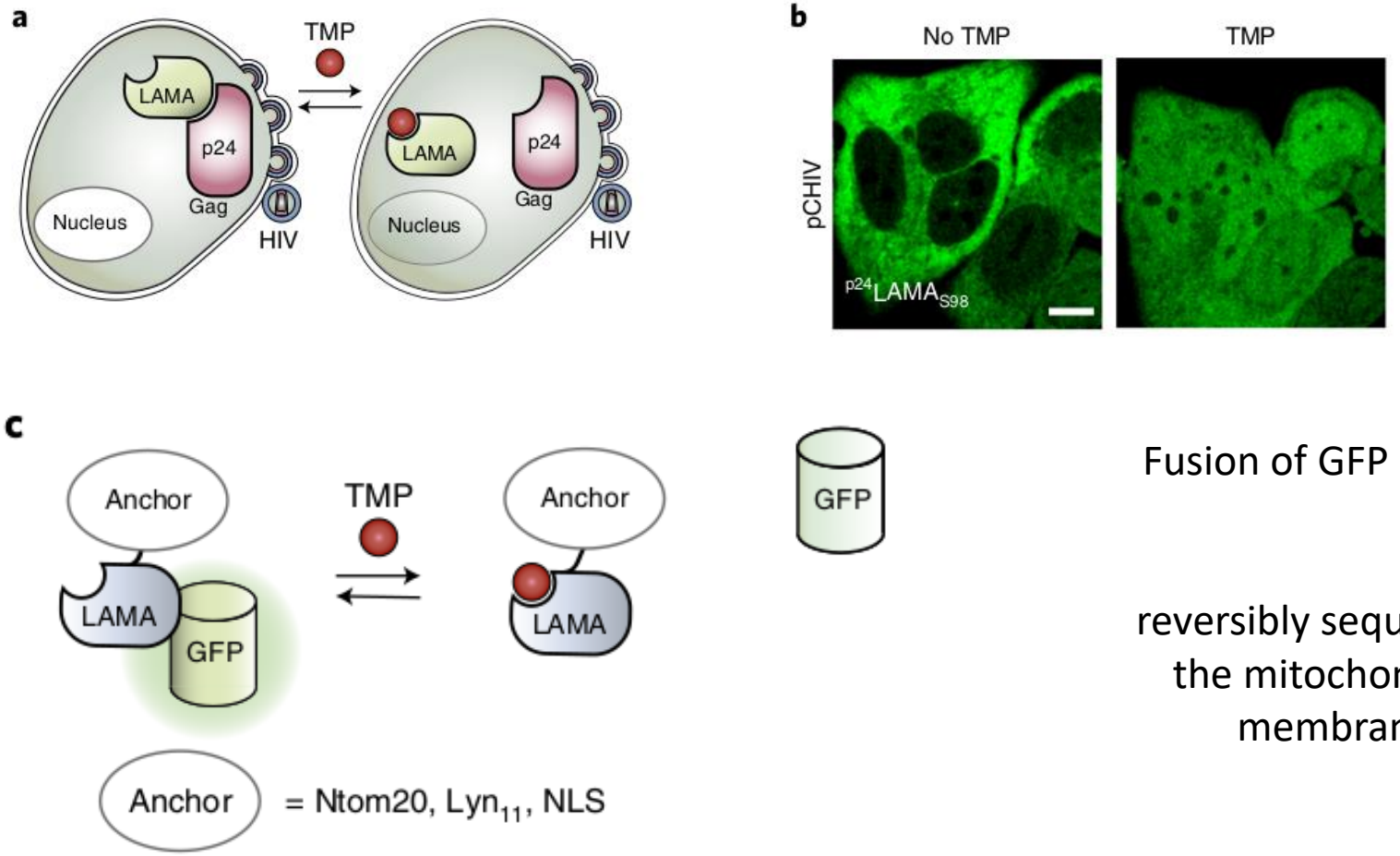


The p24 LAMA S98 showed low nanomolar affinity for p24 HIV capsid protein when no ligands were present. Neither TMP nor NADPH alone could decrease the affinity of p24 LAMA S98 for its target, but addition of both ligands reduced the affinity 70-fold (Fig. 1j).

Fig 1 and Suppl. Fig. 7 and 8

ON and OFF switching in live cells

The binding of both the p24 LAMA and GFP LAMAs to their targets could be switched on and off through the addition of TMP in live cells.



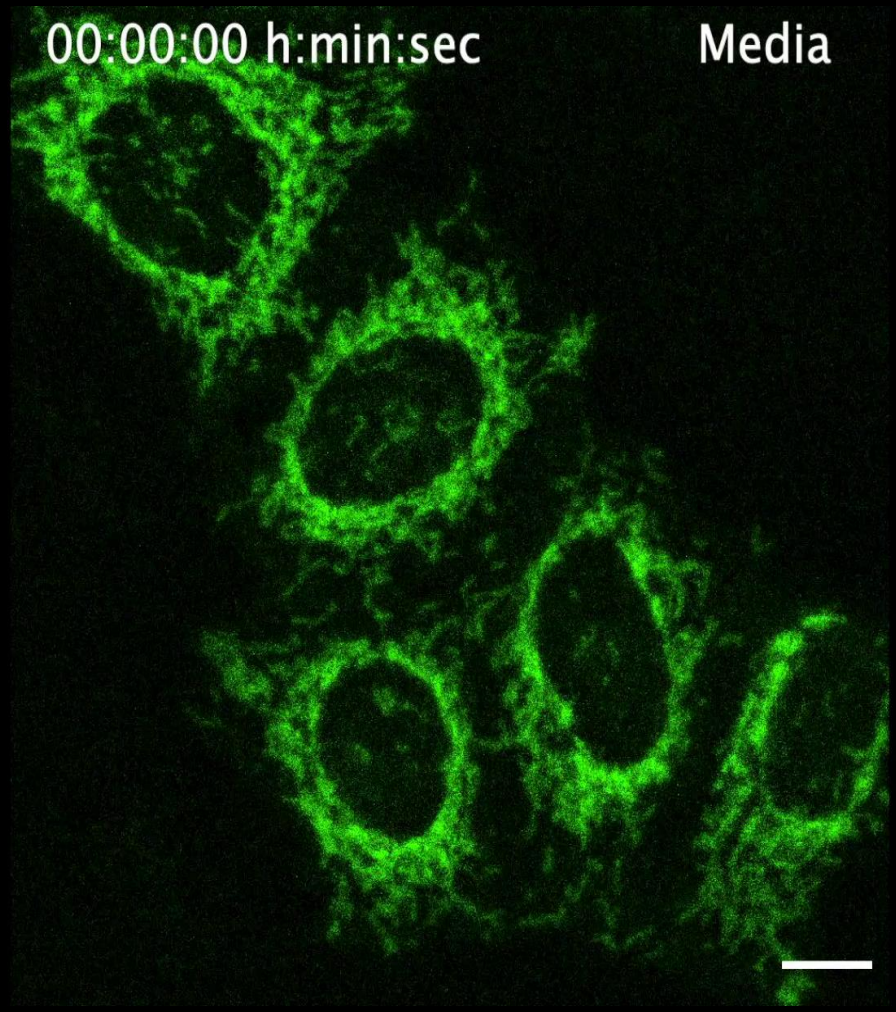
The expression of the cytosolic p24 precursor polyprotein Gag in HIV transfected cells stably expressing an enhanced GFP-(EGFP-) p24 LAMA S98 fusion resulted in sequestering of the LAMA in the cytosol.

Fusion of GFP LAMAs to Ntom20, Lyn 11, and a nuclear localization sequence:

reversibly sequestered EGFP to the outer membrane of the mitochondria, to the inner leaflet of the plasma membrane, and to the nucleus, respectively.

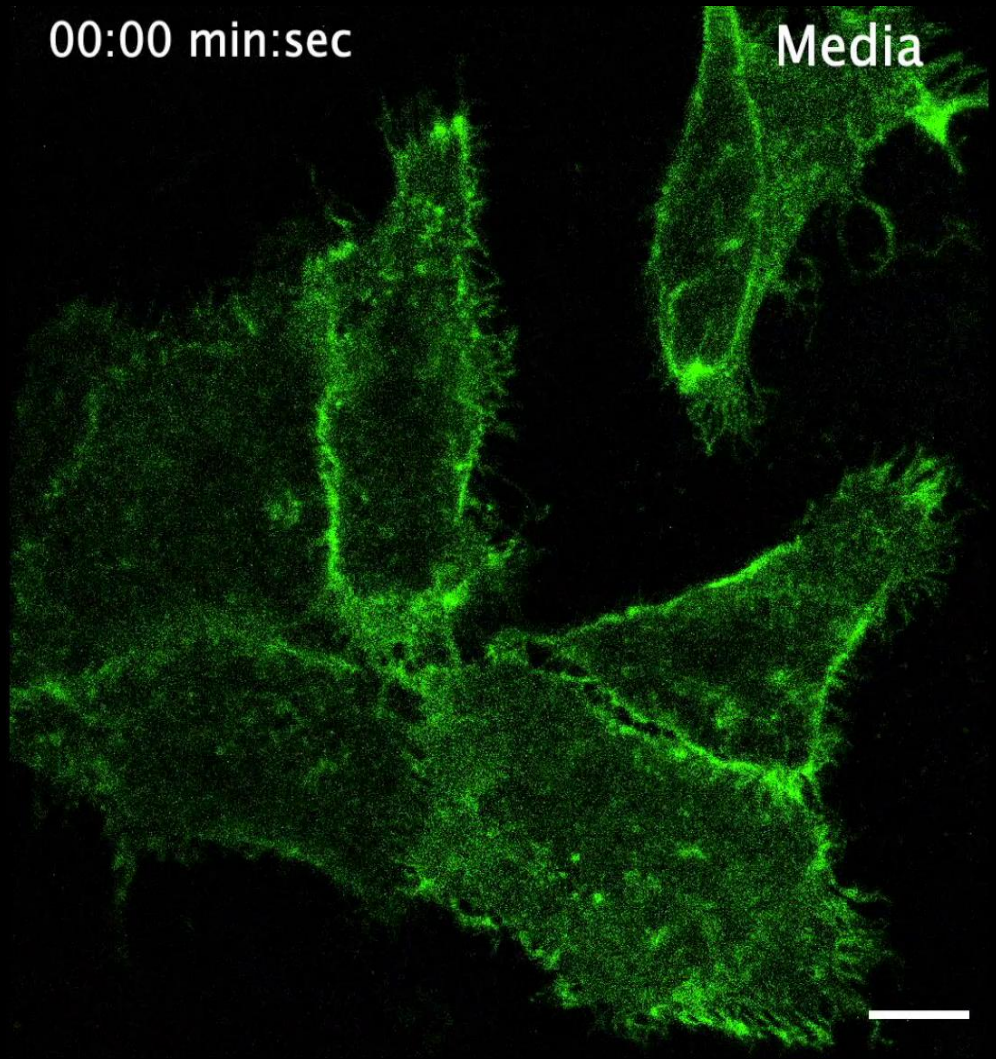
Fig. 2

Reversibility of mito-GFPLAMAF98 with EGFP in live cells



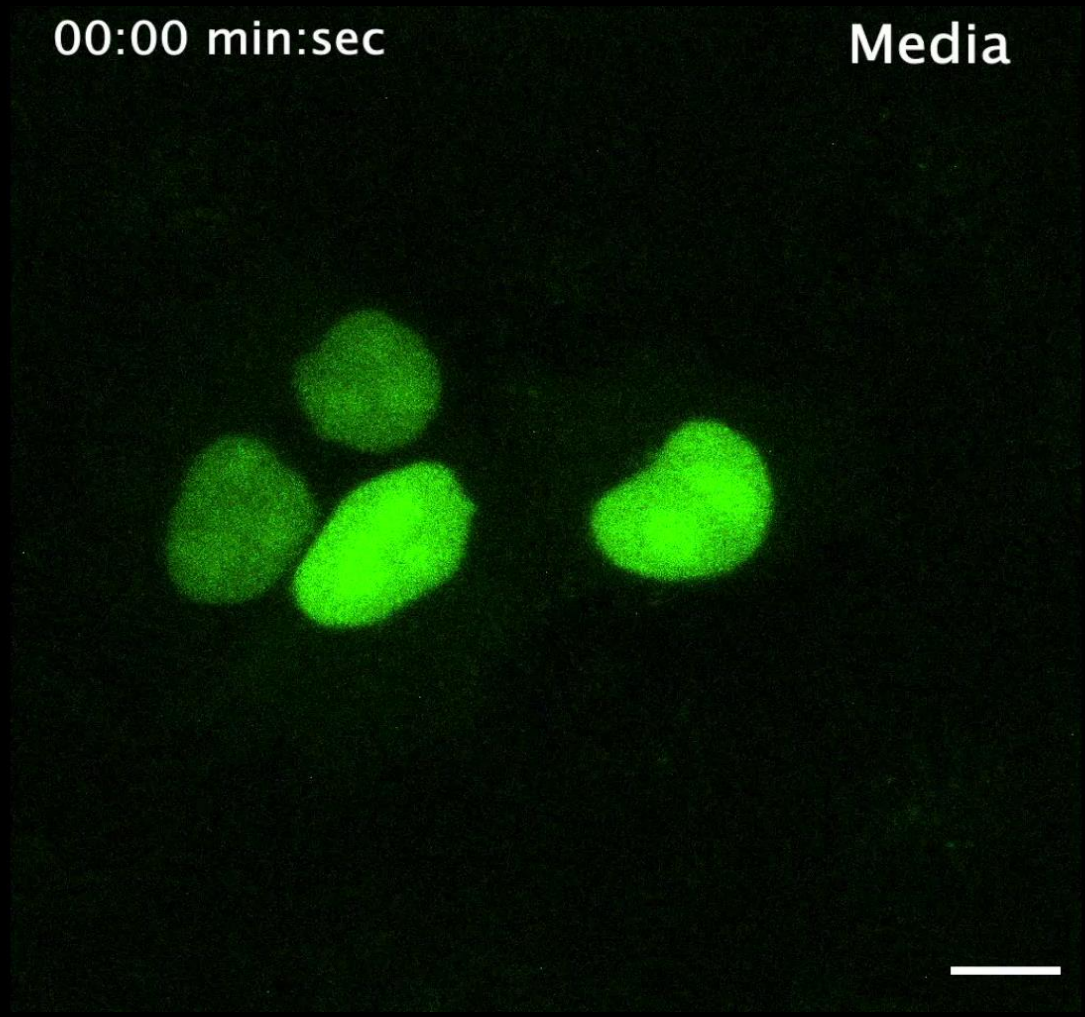
HeLa Kyoto cells expressing mito-GFPLAMAF98 IRES EGFP.

Reversibility of Lyn-GFPLAMAF98 with EGFP in live cells



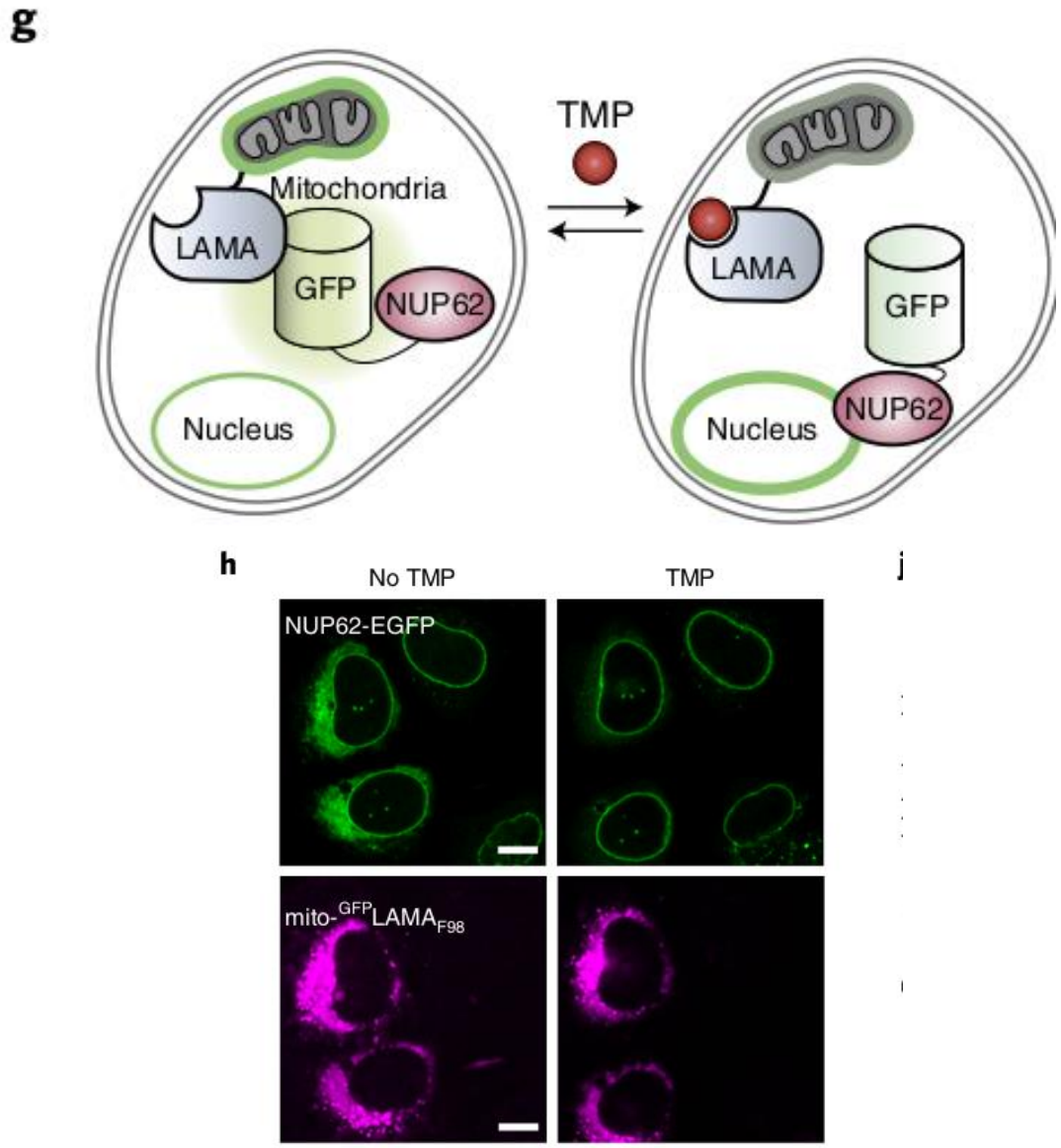
HeLa Kyoto cells expressing Lyn-GFPLAMAF98 IRES EGFP

Reversibility of nuc-GFPLAMAF98 with EGFP in live cells



HeLa Kyoto cells expressing nuc-GFPLAMAF98 IRES EGFP

The high affinity of GFP LAMAs for GFP also allows to mislocalize GFP fusion proteins that are part of larger protein complexes

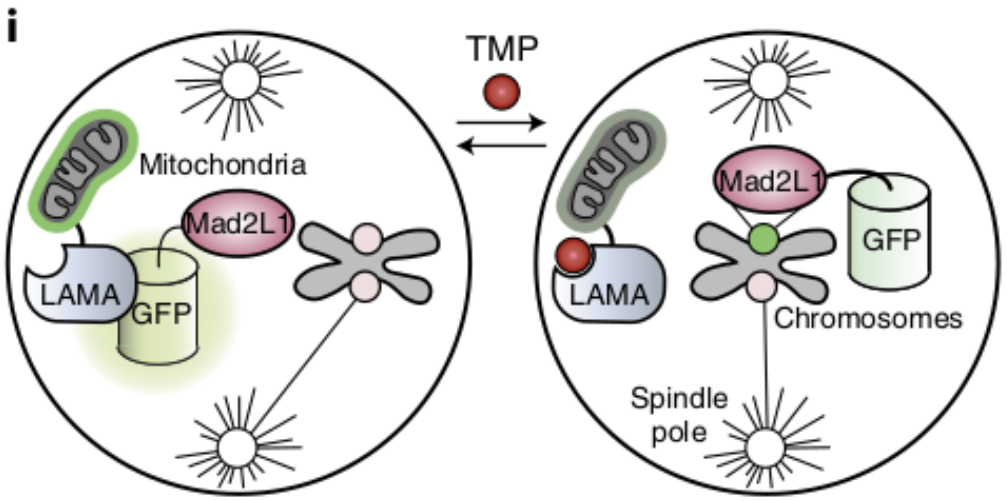


Example:
transient transfection of mito- GFP LAMA F98 into a genome-edited cell line expressing NUP62 monomeric EGFP (mEGFP), a component of the nuclear pore complex

resulted in sequestering of NUP62-mEGFP from the nuclear envelope to the mitochondria in the absence of TMP.

GFP-LAMAs offer a new way to probe the function of GFP fusion proteins

Demonstrate the potential of GFP LAMAs for mechanistic studies:

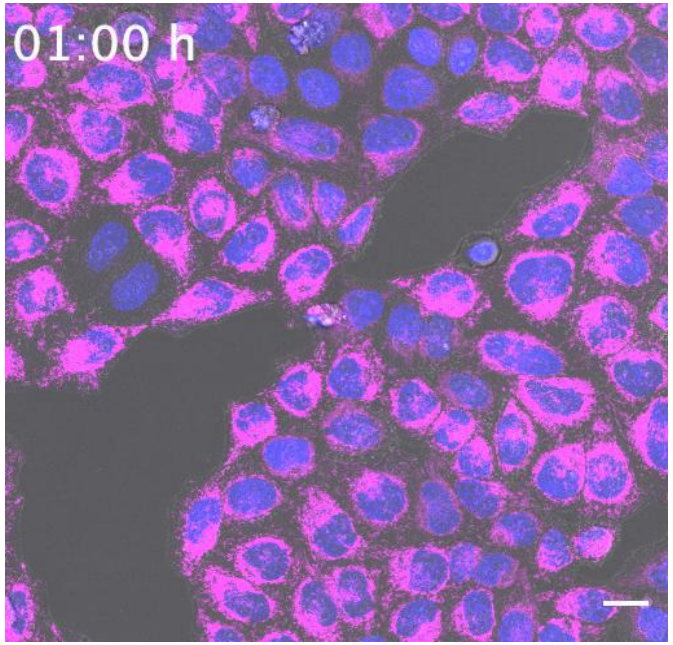


--> they used mito-GFP LAMA F98 to control the function of a GFP fusion of Mad2L1, an important component of the mitotic checkpoint complex.

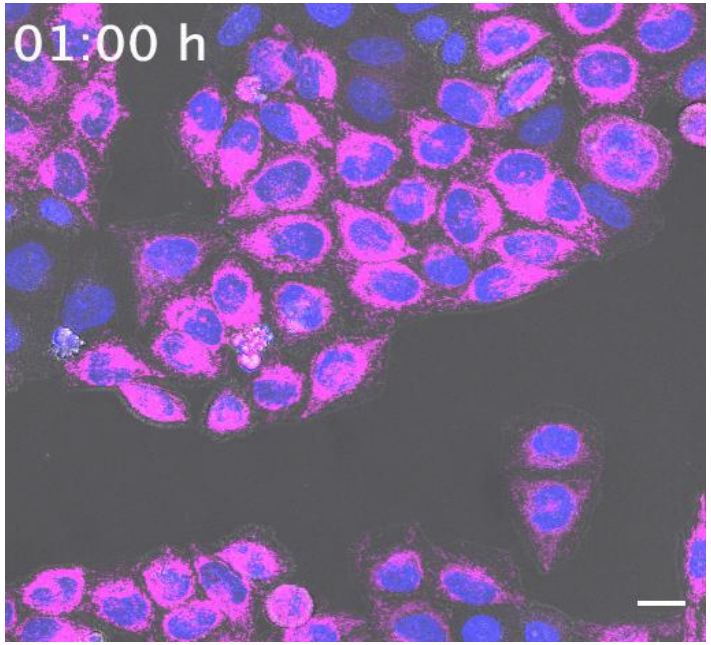
Knock-down of Mad2L1 reduces mitotic duration and increases the percentage of polylobed nuclei.

- They stably expressed mito-GFP LAMA F98 in the Mad2L1-EGFP cell line.
- They observed how sequestering Mad2L1-EGFP to the mitochondria affected the outcome of cell division

GFP-LAMAs offer a new way to probe the function of GFP fusion proteins



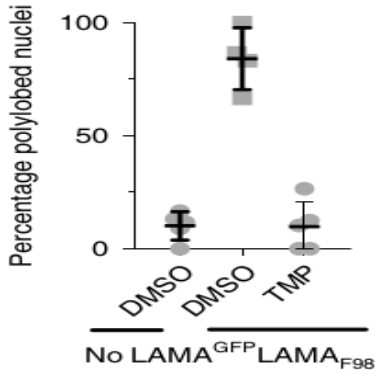
in the absence of TMP



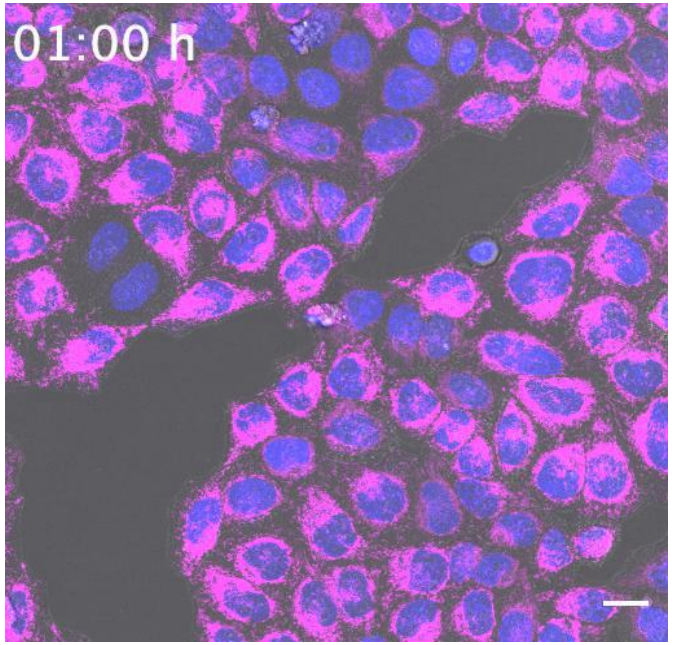
in the presence of TMP

In the absence of TMP:
Increase in the percentage of polylobed nuclei following mitotic events in cells not expressing mito-GFP LAMA F98.

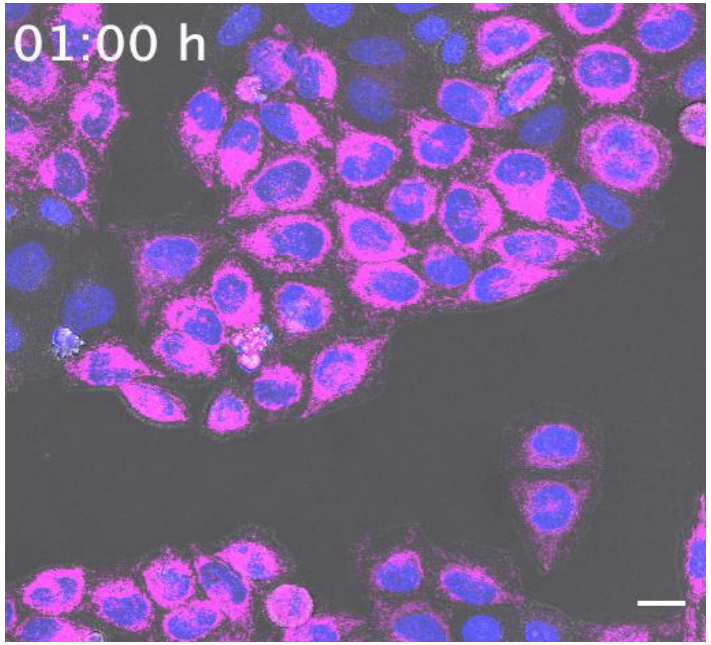
In the presence of TMP:
Reduction of the levels of polylobed nuclei following mitotic events in cells expressing mito- GFP LAMA F98.



GFP-LAMAs offer a new way to probe the function of GFP fusion proteins



in the absence of TMP



in the presence of TMP

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Increase in the percentage of polylobed nuclei following mitotic events in cells not expressing mito- GFP LAMA F98.

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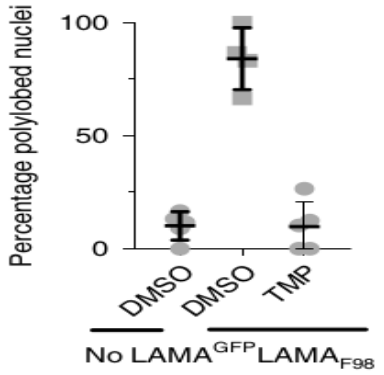

Treatment with nocodazole

- A small molecule that prevents attachment of microtubules to kinetochores
- Added to activate the mitotic checkpoint complex
- After treatment with nocodazole, cells in which Mad2L1-EGFP had been sequestered at the mitochondria were able to override mitotic arrest
- Treatment with nocodazole and TMP lead to mitotic arrest

These data show that the function of Mad2L1-EGFP in the mitotic checkpoint complex can be controlled through its TMP-dependent interaction with mito- GFP LAMA F98 .

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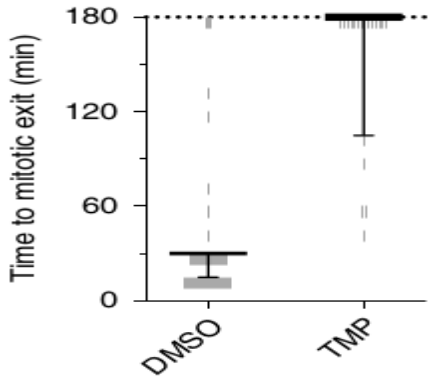

Summary

- LAMAs are a generally applicable chemogenetic tool to reversibly control the location and function of proteins, including the most commonly used class of fusion proteins (GFP).

- Nanobodies have been selected for a large variety of targets, all of which could serve as starting point for the generation of new LAMAs.

- As TMP is a clinically approved drug, the approach might also be applicable in vivo.

- The design principle introduced here should be applicable for the generation of other switchable proteins.

Thank you!