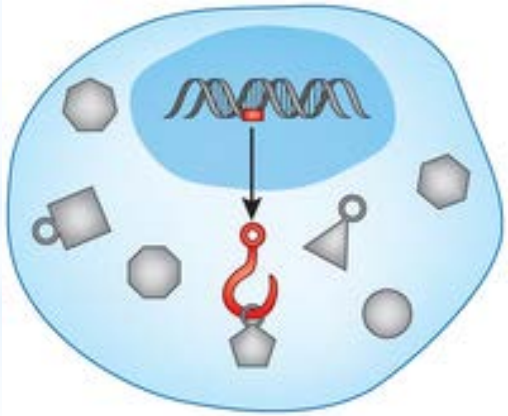
GFP+Nanobodies:

Genetically encoded probes for real-time detection and manipulation of cellular events



Vijay Chandrasekar

Journal club presentation

2.9.14

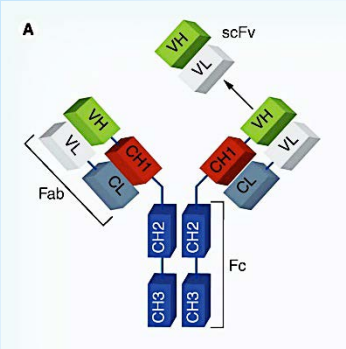
Nanobodies :

Small, recombinant, antibody-like proteins that bind to specific antigens.

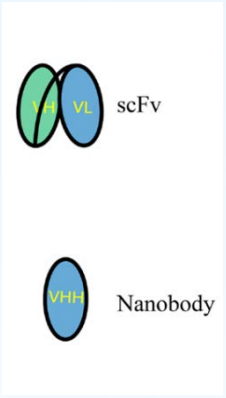
Engineered from animals such as camels or sharks that naturally produce very small single-domain antibodies or from larger mammalian antibodies

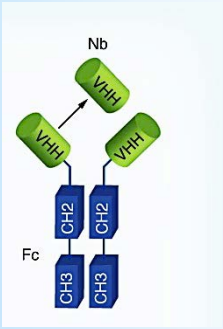
Serum contains also a considerable fraction of heavy-chain antibodies (HCAbs)

Nanobodies

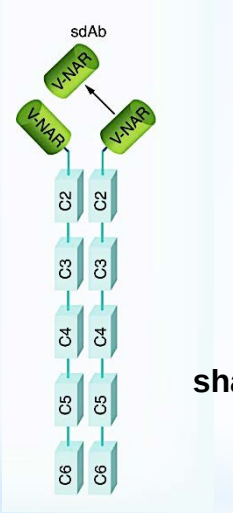


mammalian IgG antibodies





camelid heavy chain-only antibodies



shark Ig-NARs

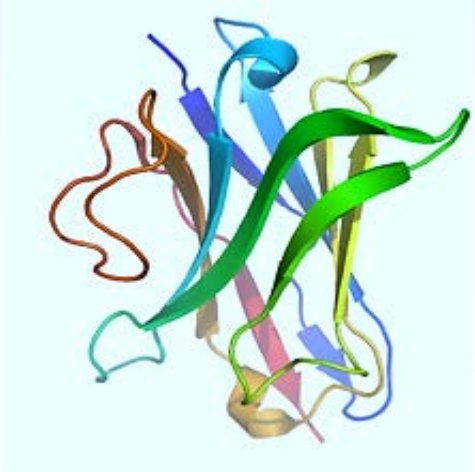
Nanobodies (VHH) are strictly monomeric, single domain antigen-binding entities, prolate shape in the nm range (diameter of 2.5 nm and height of 4 nm)

Nanobodies (VHH) have similar affinity to antigens as whole antibodies, but are more heat-resistant and stable towards detergents and high concentrations of urea.

Hassanzadeh-Ghassabeh et al., 2013

Nanobodies

Less lipophilic and more soluble in water, owing to their CDR3, which forms an extended loop covering the lipophilic site that normally binds to a light chain



Advantages:

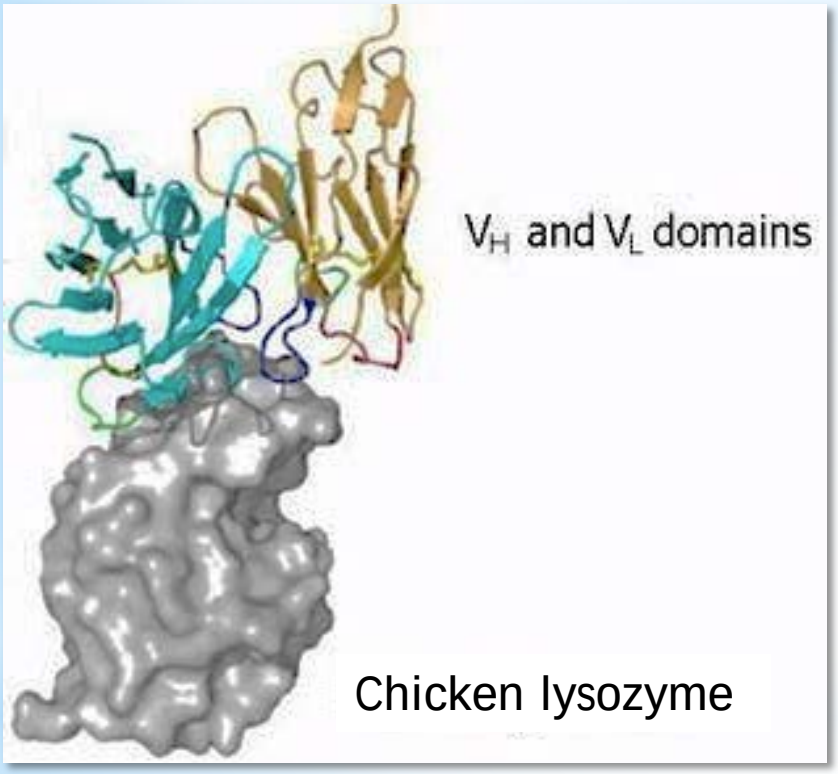
Comparatively low molecular mass leads to a better permeability in tissues, and to a short plasma half-life.

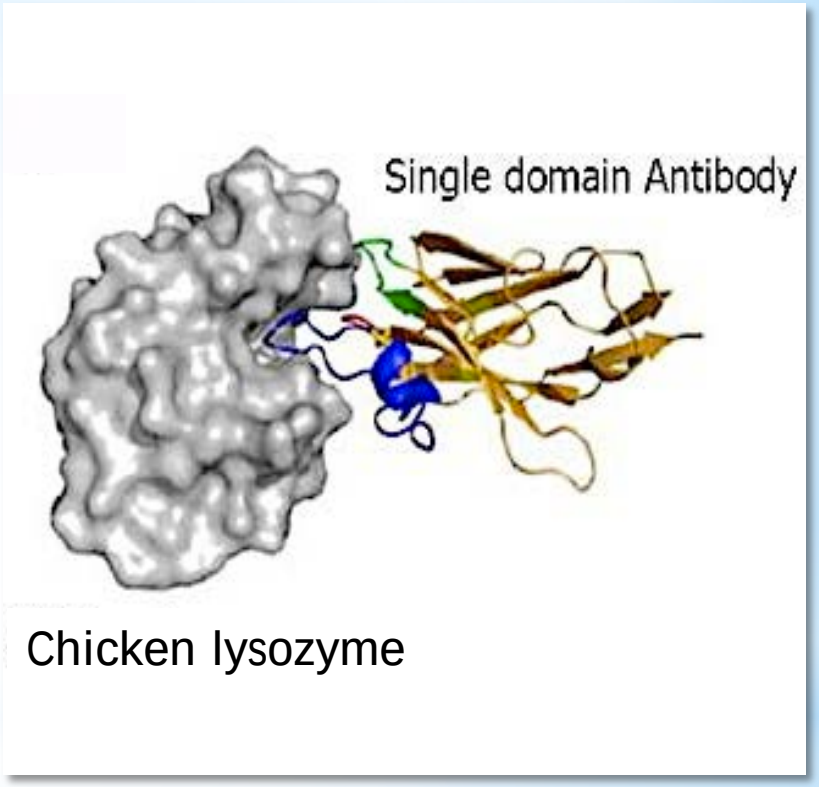
No complement system triggered cytotoxicity as they lack Fc region.

Able to bind to hidden antigens that are not accessible to whole antibodies,

Hassanzadeh-Ghassabeh et al., 2013

Potential uses for nanobodies:

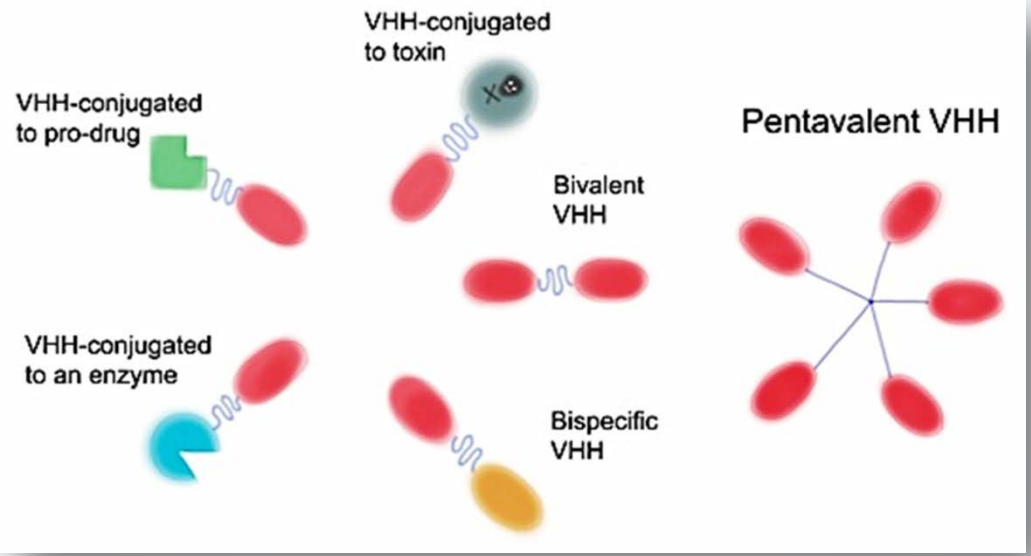




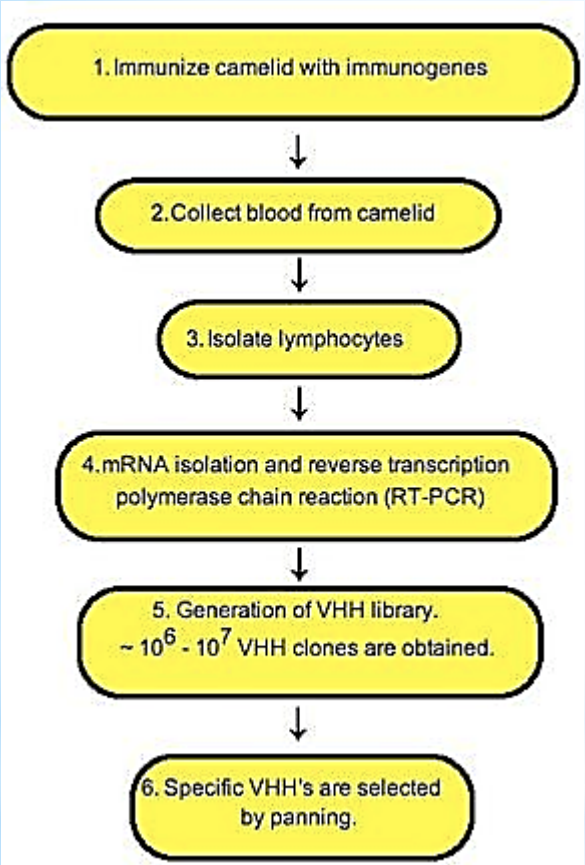
Nanobody binding to the cryptic epitopes recessed in antigen (lysozyme) cavities

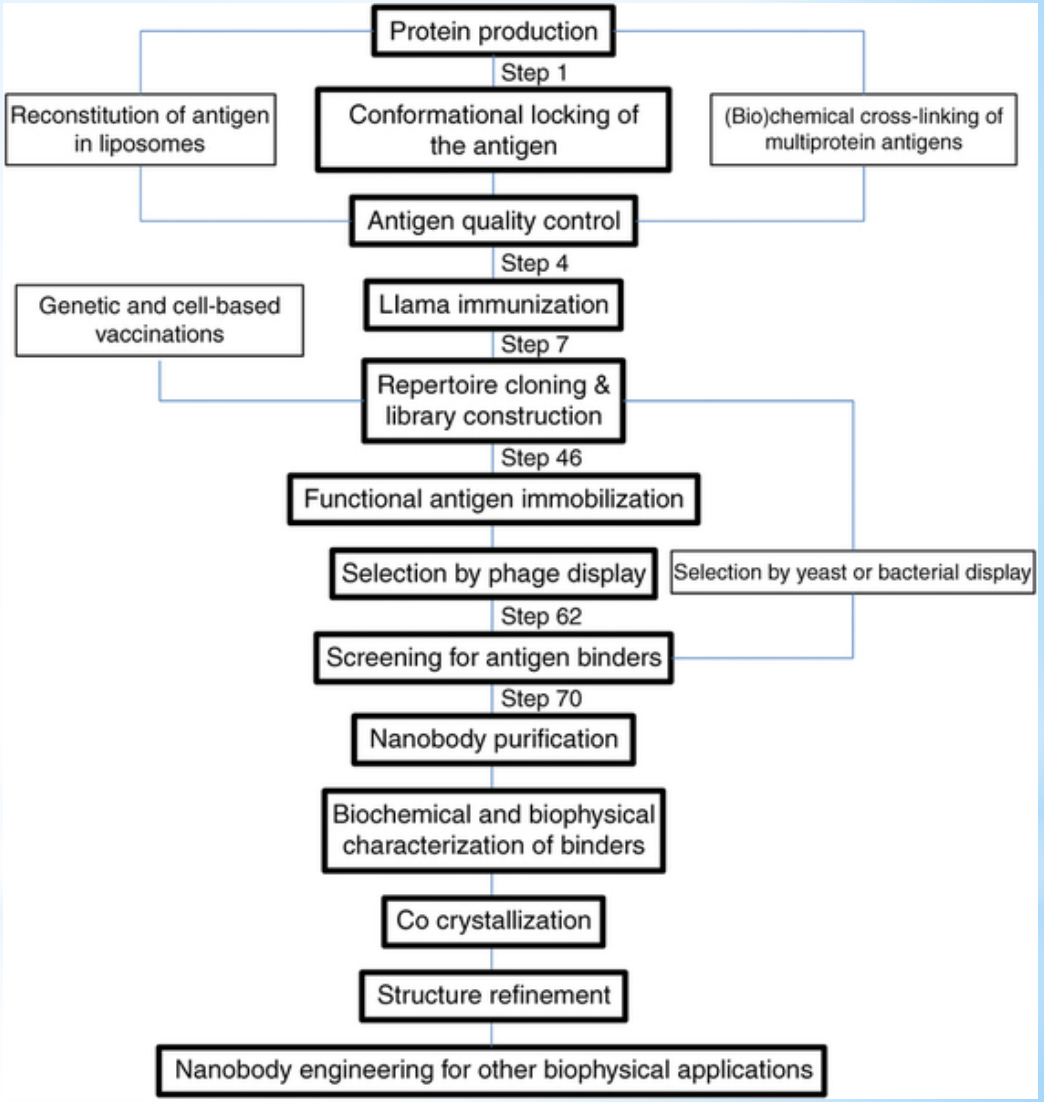
Weslolowski, 2001.

Potential uses for nanobodies:



Generation of Nanobodies





Pardon *et al. Nature protocols* 2014

LETTER

doi:10.1038/nature12000

Conformational biosensors reveal GPCR signalling from endosomes

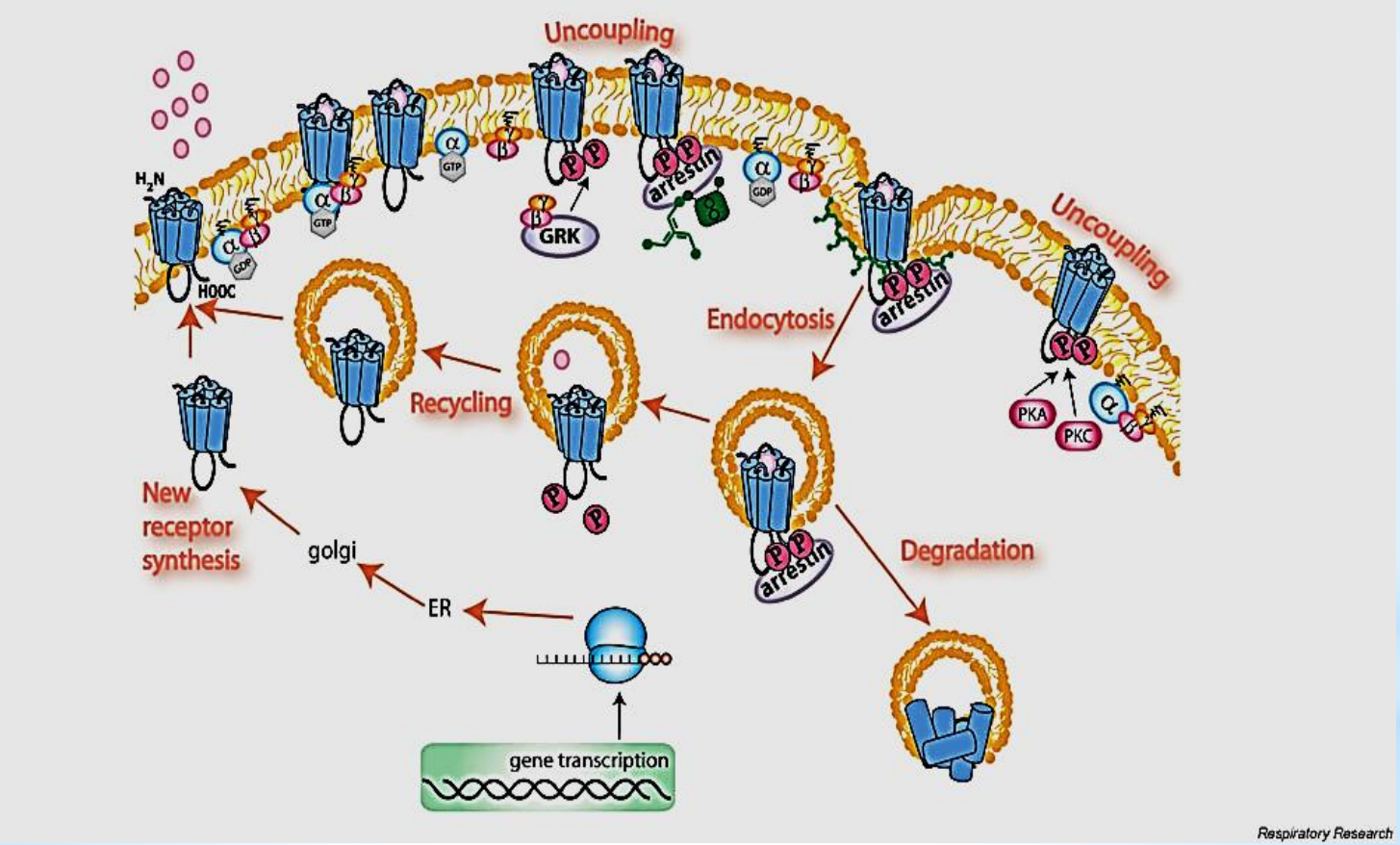
Roshanak Irannejad[1], Jin C. Tomshine[1], Jon R. Tomshine[1], Michael Chevalier[2], Jacob P. Mahoney[3], Jan Steyaert[4,5], Søren G. F. Rasmussen[6], Roger K. Sunahara[3], Hana El-Samad[2], Bo Huang[2,7] & Mark von Zastrow[1,8]

What is known so far

Canonical signal transduction mediated by G-protein-coupled receptor (GPCR) coupling to heterotrimeric G proteins is confined to the plasma membrane

Irannejad *et al. Nature* 2013

GPCR signalling pathway



Billington et al., 2003

LETTER

doi:10.1038/nature12000

Conformational biosensors reveal GPCR signalling from endosomes

Roshanak Irannejad[1], Jin C. Tomshine[1], Jon R. Tomshine[1], Michael Chevalier[2], Jacob P. Mahoney[3], Jan Steyaert[4,5], Søren G. F. Rasmussen[6], Roger K. Sunahara[3], Hana El-Samad[2], Bo Huang[2,7] & Mark von Zastrow[1,8]

Hypothesis

GPCR signalling occurs from endosomes as well as the plasma membrane

Irannejad *et al. Nature* 2013

Tool:

Conformation-specific single-domain camelid antibody (Nb80) against activated β_2-AR (agonist-occupied β_2-AR)

Pathway: Prototypical GPCR, β_2-Adrenoceptor signalling pathway

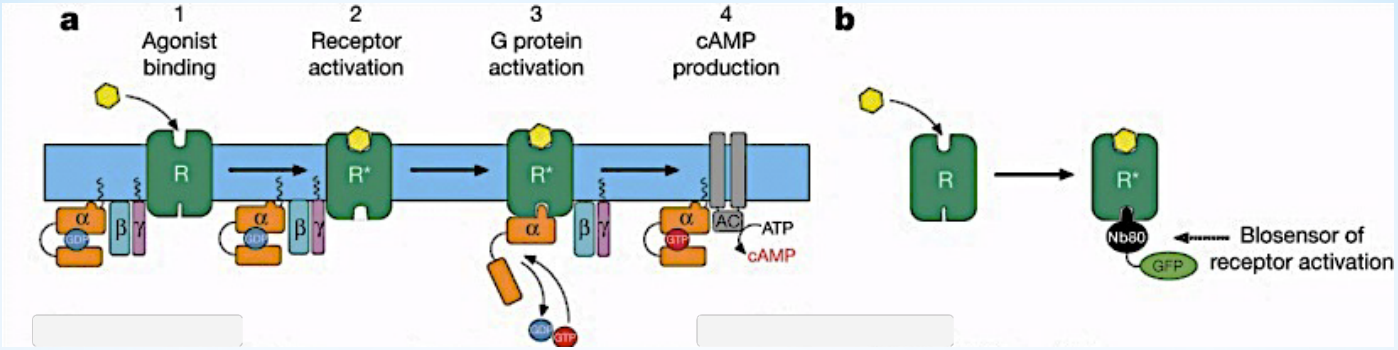
Cell model: HEK293 cells

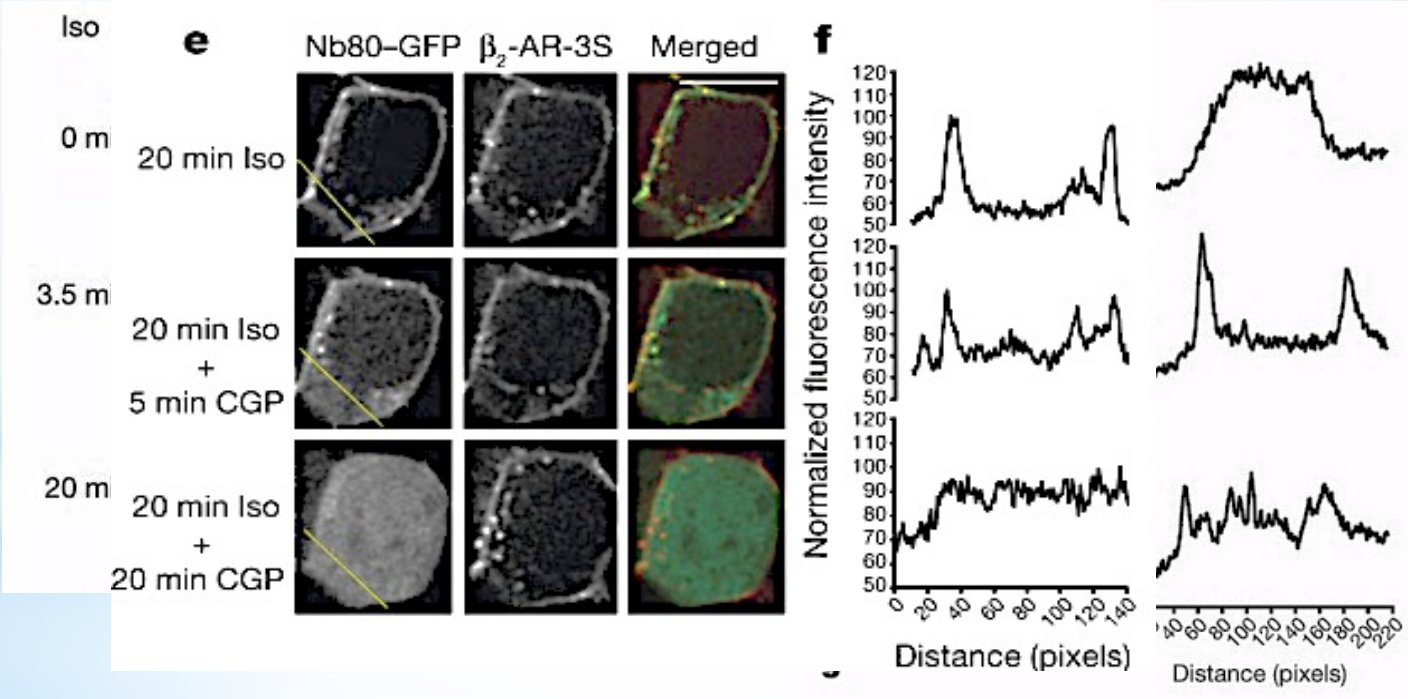
TIRF: Total internal reflection flourescence microscopy

Selectively detects events occuring in the plasma membrane and extending approx 100 nm into the cytoplasm

Irannejad *et al. Nature* 2013

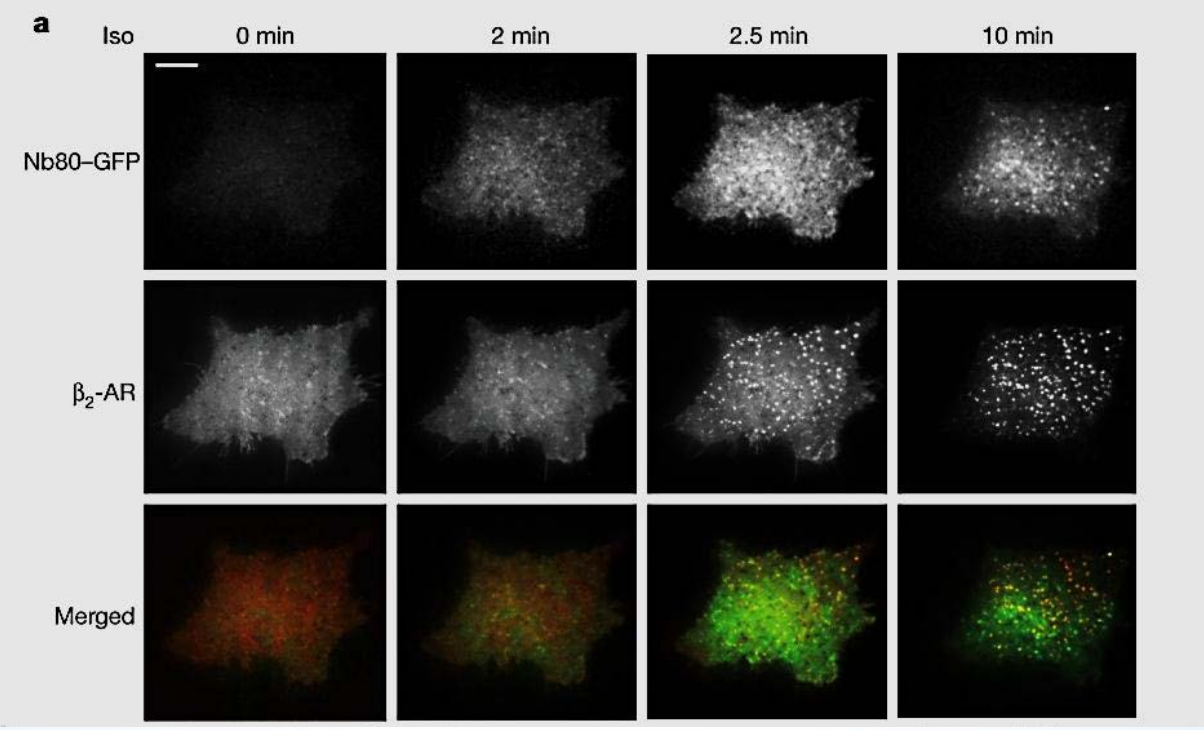
Nb80–GFP detects activated ß$_2$-ARs in the plasma membrane and endosomes

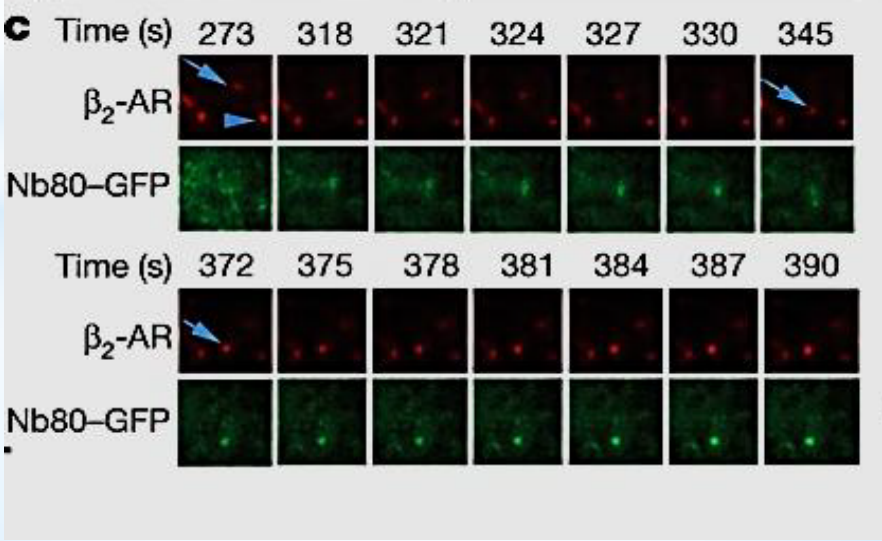




Irannejad *et al. Nature* 2013

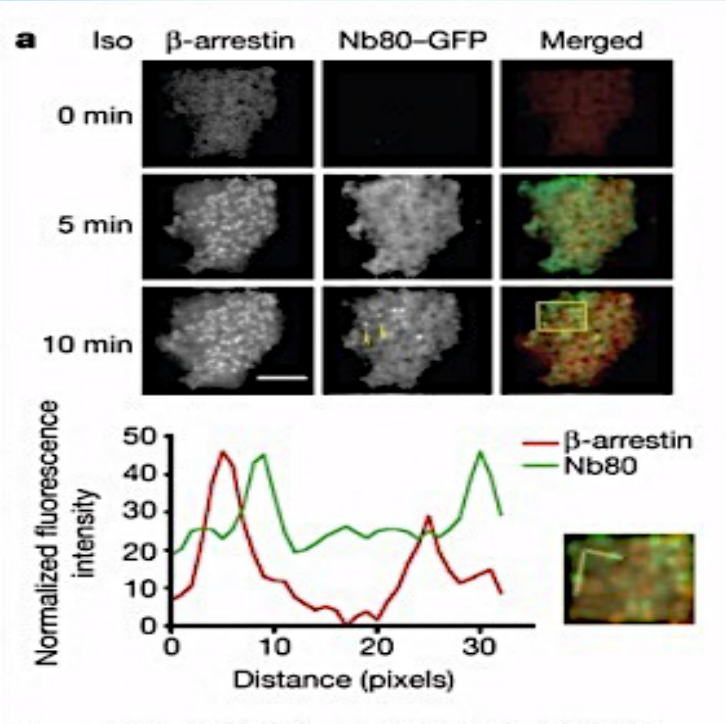
Nb80–GFP accumulates on ß$_2$-AR containing endosomes after their formation

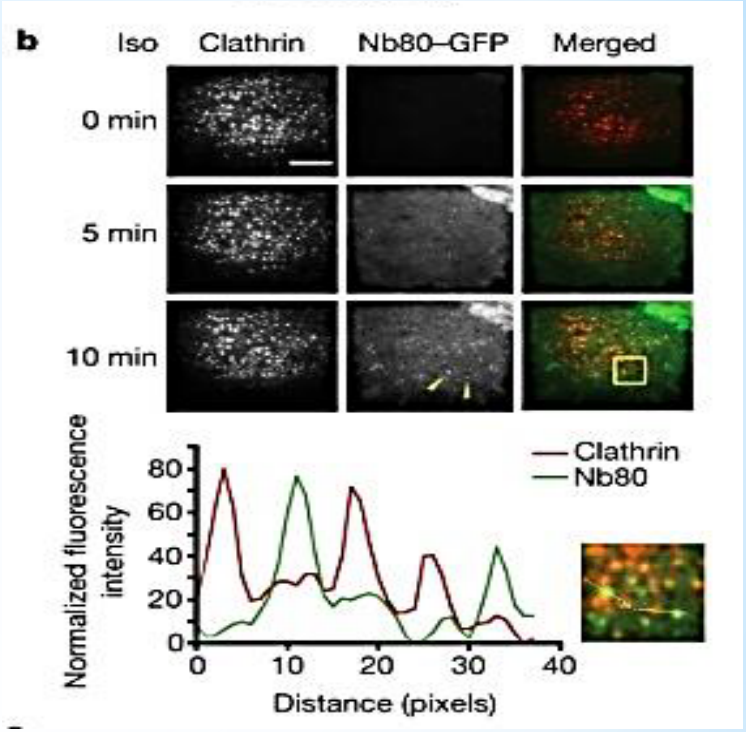




Irannejad *et al. Nature* 2013

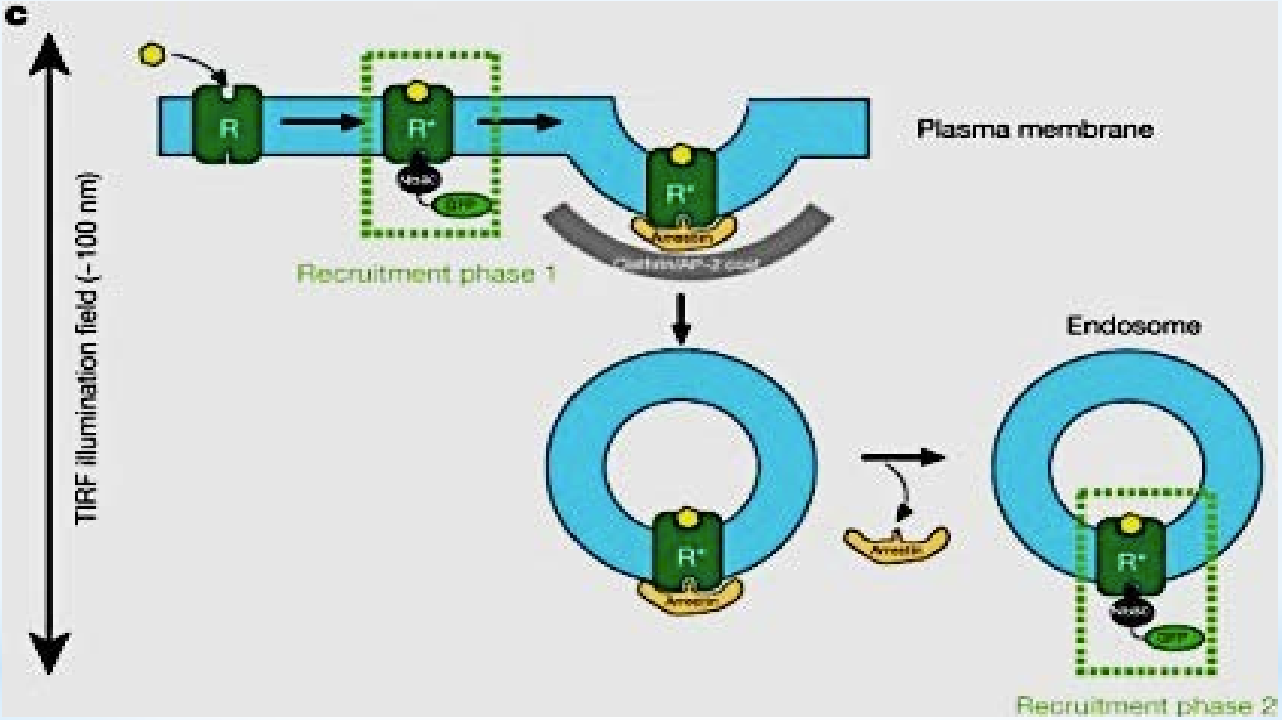
Nb80–GFP does not accumulate in clathrin-coated pits or vesicles





Irannejad *et al. Nature* 2013

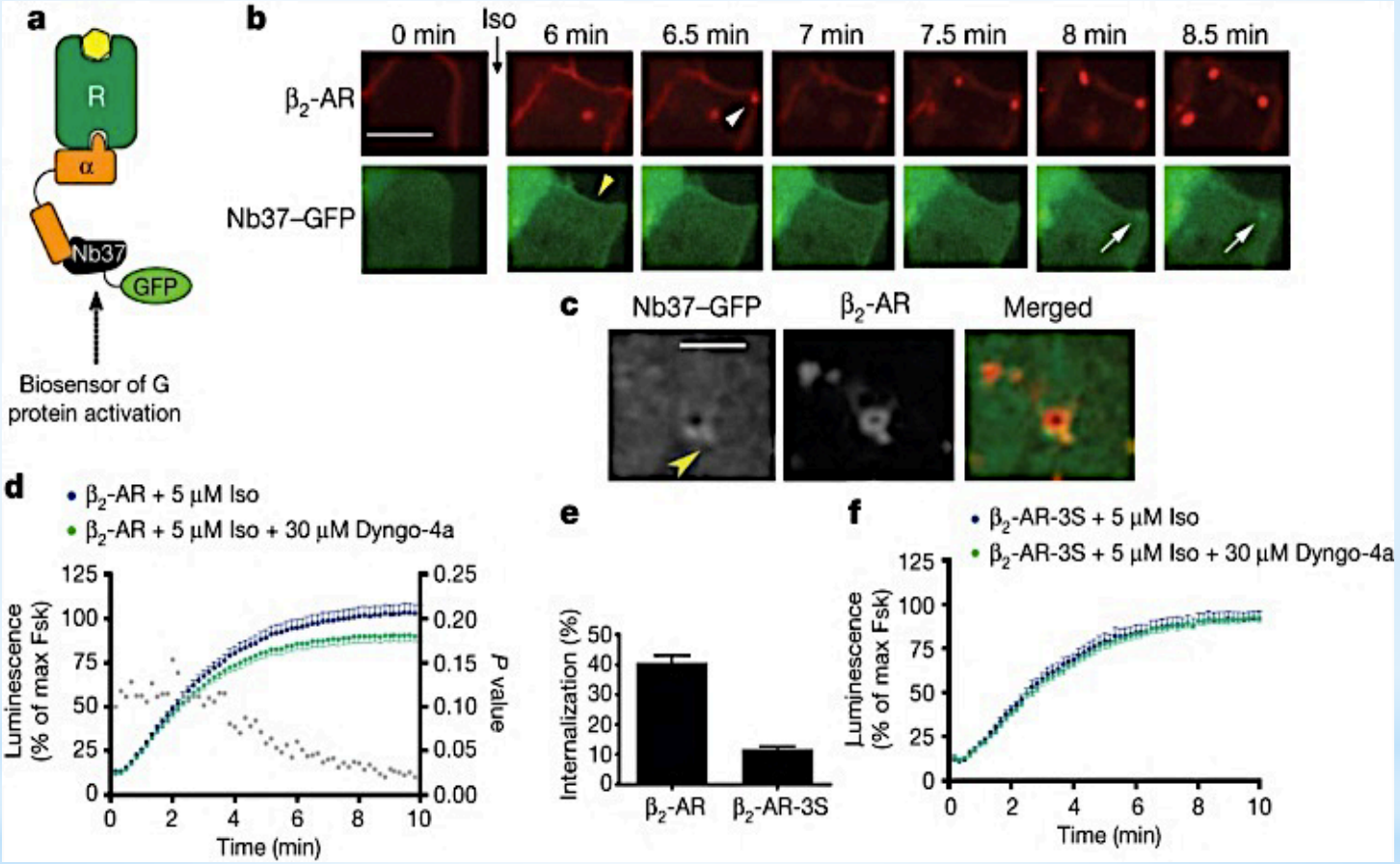
Nb80–GFP does not accumulate in clathrin-coated pits or vesicles



Nb80-GFP dissociation is accelerated by clustering process such as receptor phosphorylation and/or steric exclusion

Irannejad *et al. Nature* 2013

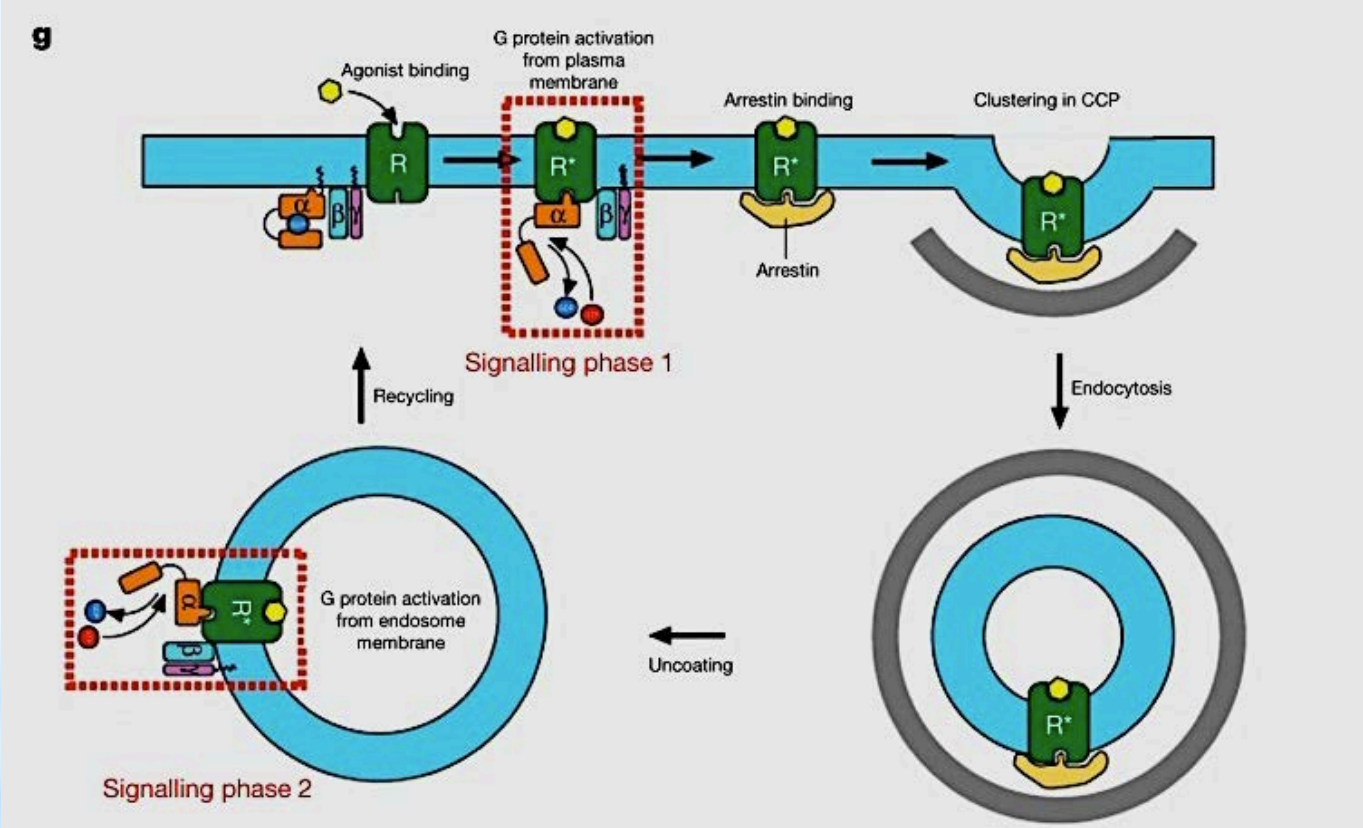
Internalized ß$_2$-ARs contribute to the acute cAMP response.



Dyngo-4a: selective dynamin inhibitor blocks endocytosis and CCP function

Irannejad *et al. Nature* 2013

Internalized ß$_2$-ARs contribute to the acute cAMP response.



Irannejad *et al. Nature* 2013

Outlook:

Versatile tool for probing dynamic conformational change *in vivo* with high spatio-temporal resolution

GPCR signalling occurs from endosomes as well as the plasma membrane

TECHNICAL REPORTS

nature
structural &
molecular biology

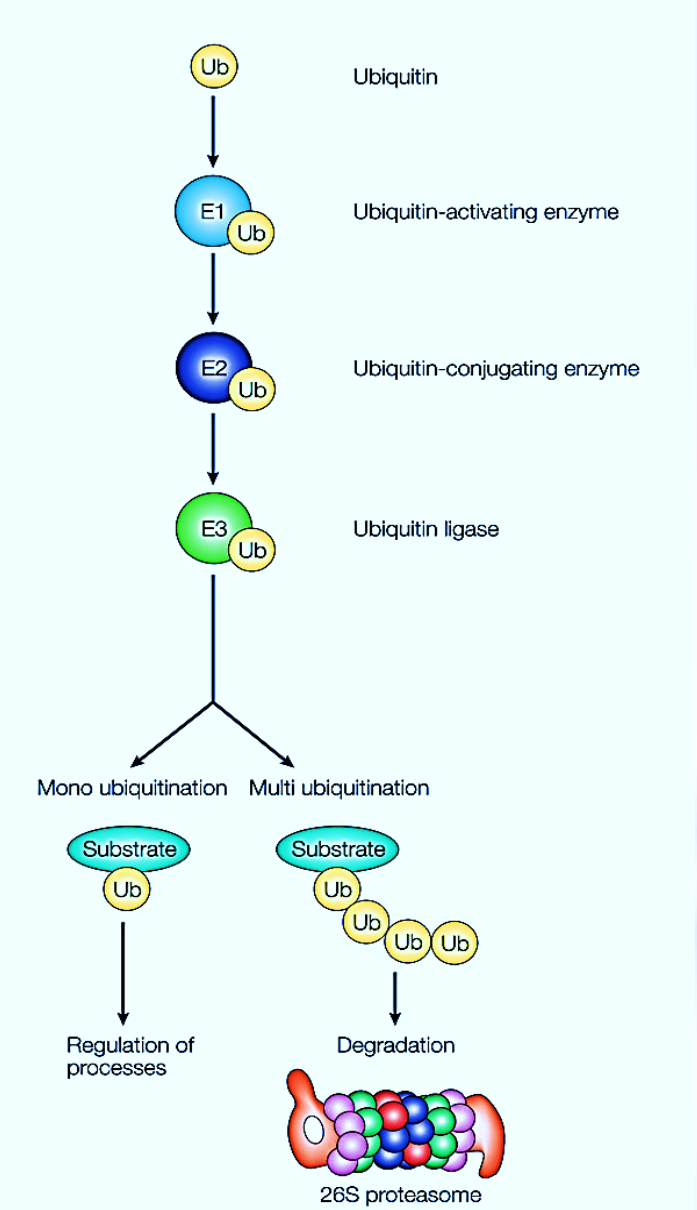
Fluorescent fusion protein knockout mediated by anti-GFP nanobody

Emmanuel Caussinus, Oguz Kanca & Markus Affolter

To knock out GFP fusions in any eukaryotic model system

Caussinus et al, 2012

deGradFP: Utilising the ubiquitin pathway



Jesenberger et al., 2002

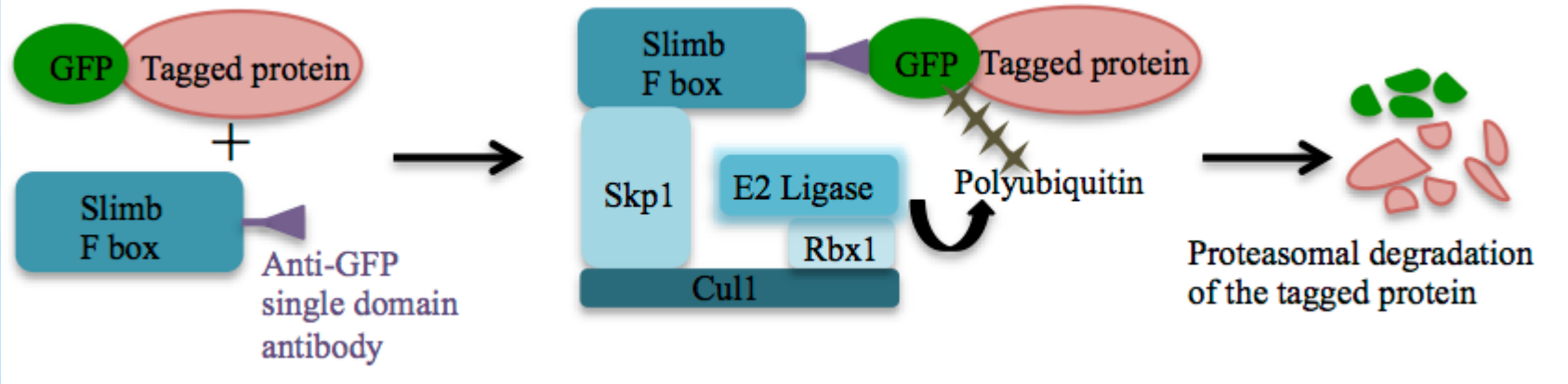
deGradFP: Utilising the ubiquitin pathway

SKP1–CUL1–F-box protein ligase complexes (SCFs): E3 enzymes with S-phase kinase-associated protein 1, cullin 1 and RING proteins and Slimb as subunits

VhhGFP4: nanobody against GFP

Engineered NSlmb-vhhGFP4 obtained by fusing the F-box domain contained in the N-terminal part of Slmb to a single-domain antibody fragment

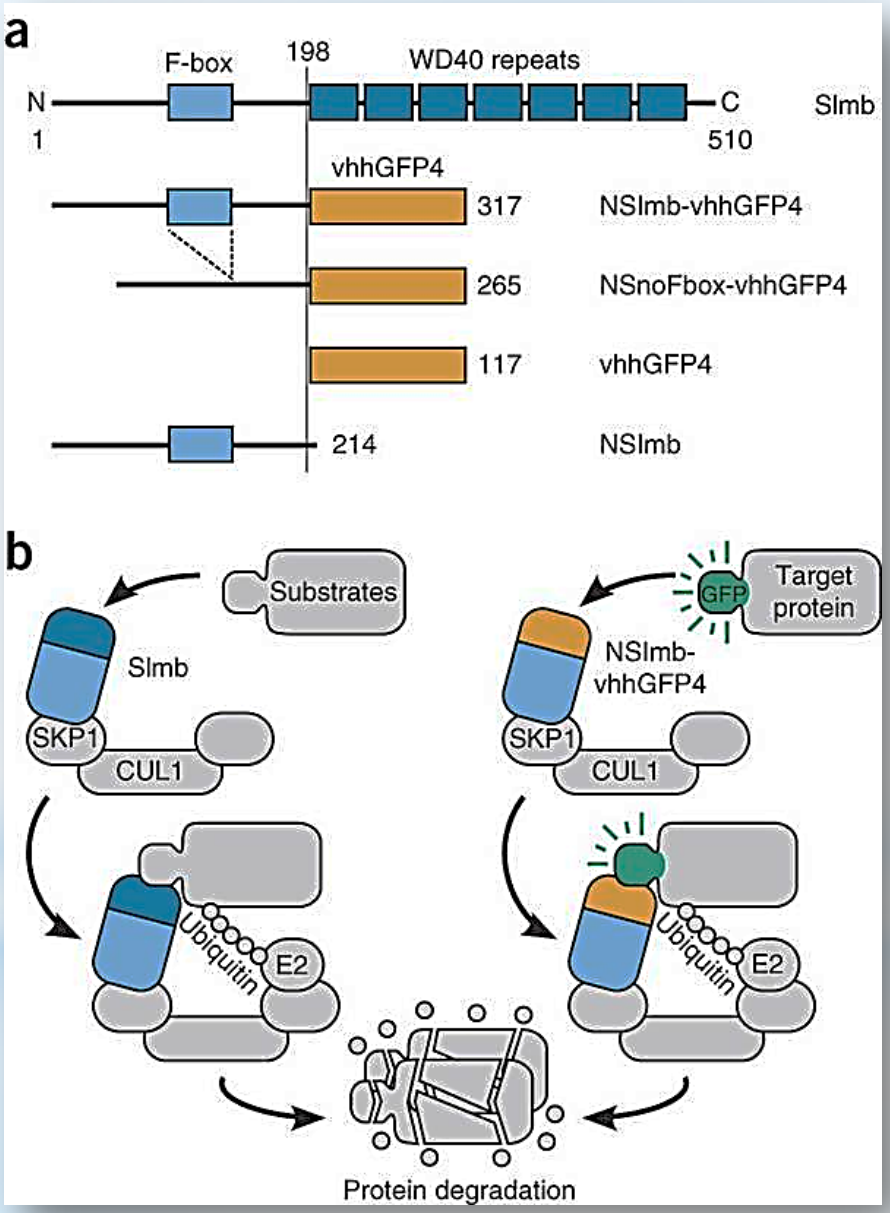
VhhGFP4 is remains active in fusion proteins and is directed against GFP, Venus, YFP.



HeLa S3 cells, Transgenic *Drosophila* lines

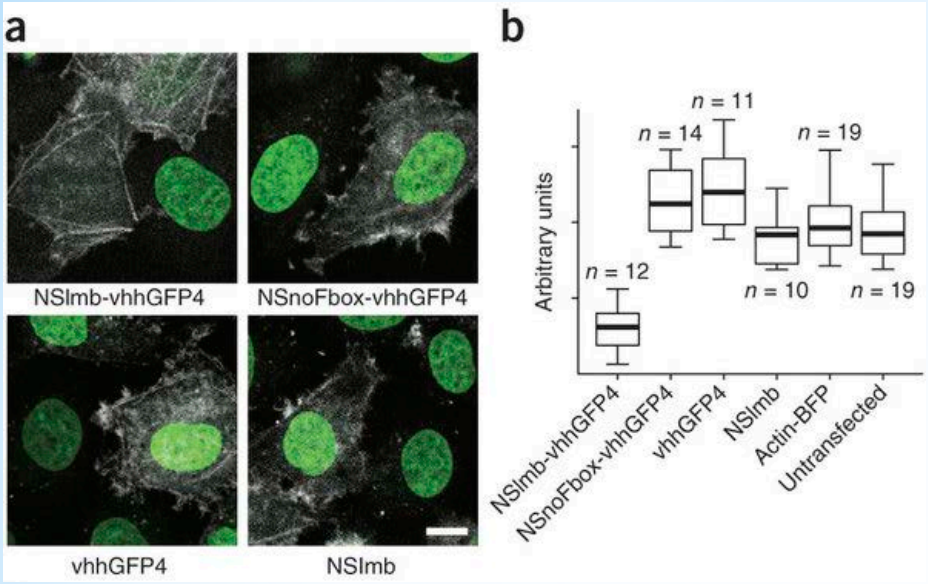
Caussinus et al, 2012

Schematic illustration of deGradFP

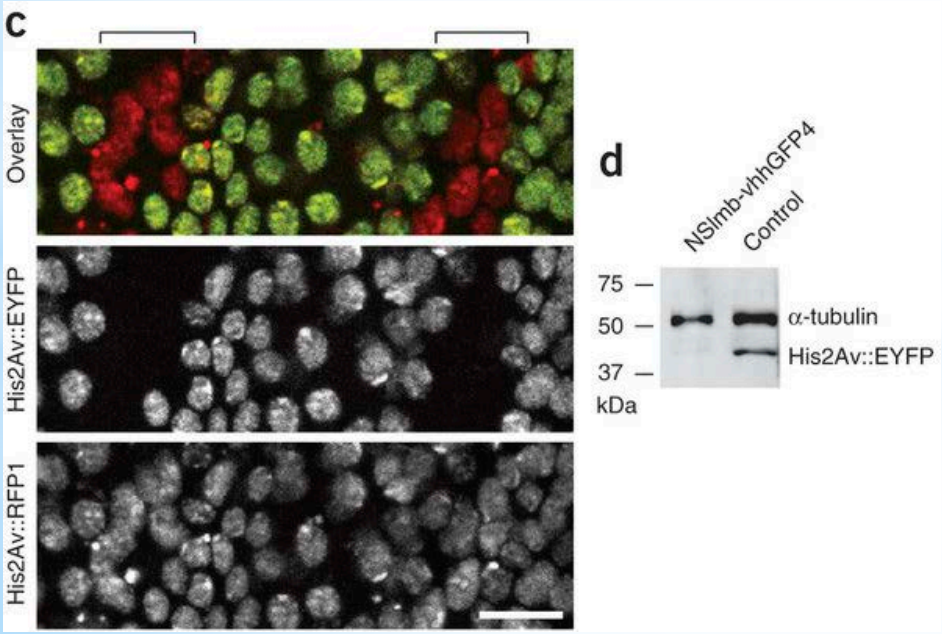


Caussinus et al, 2012

NSlmb-vhhGFP4 mediates degradation of H2B-GFP in mammalian cells and His2Av::EYFP in Drosophila.



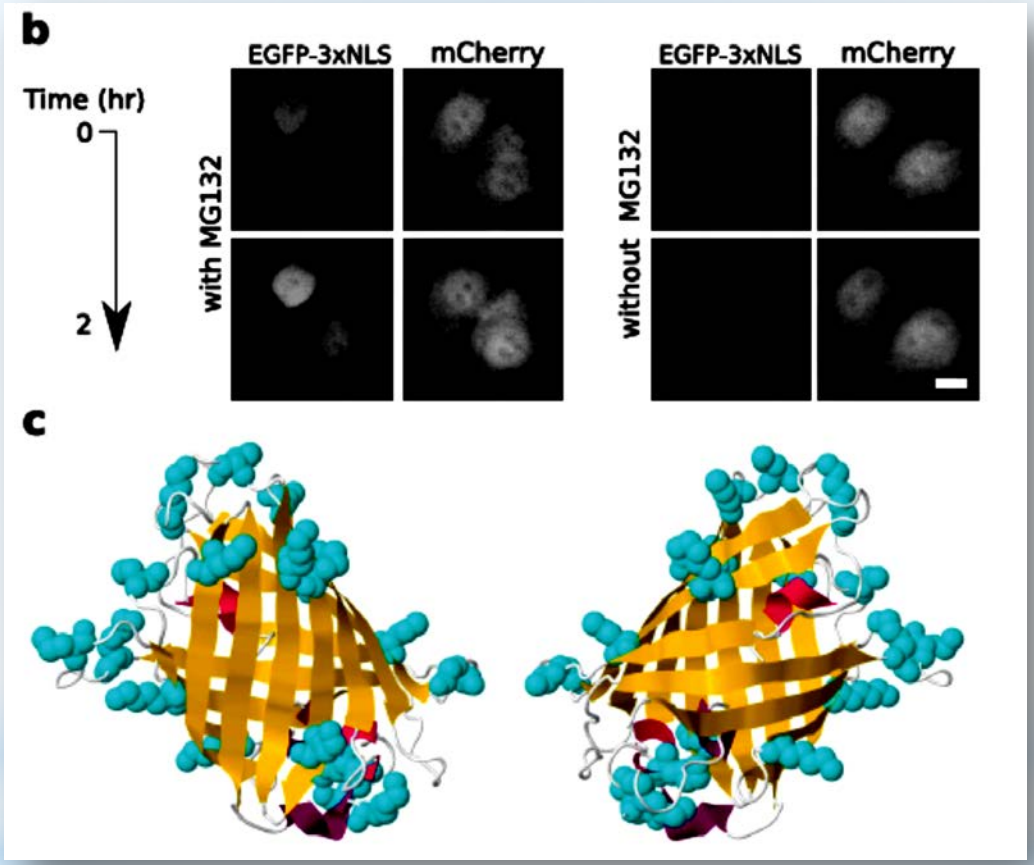
Target protein H2B-GFP (HeLa S3 cells)



Target protein His2AV-GFP (Epiderm of a stage 12 *Drosophila* embryo)

Caussinus et al, 2012

The ubiquitin-proteasome pathway is involved in the mechanism of action of deGradFP



deGradFP inhibited by proteosomal inhibitors

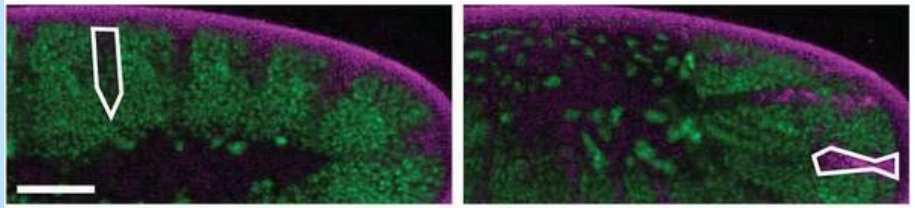
Caussinus et al, 2012

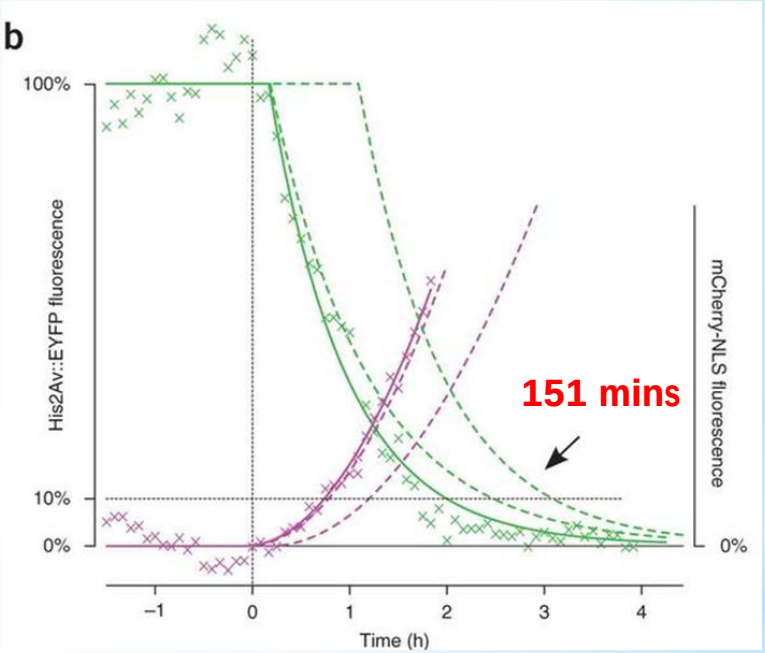
Kinetics of target protein degradation by deGradFP in Drosophila

Confocal life imaging and image processing of embryos

z-stack sum projections of an embryo at

stage 10 stage 12



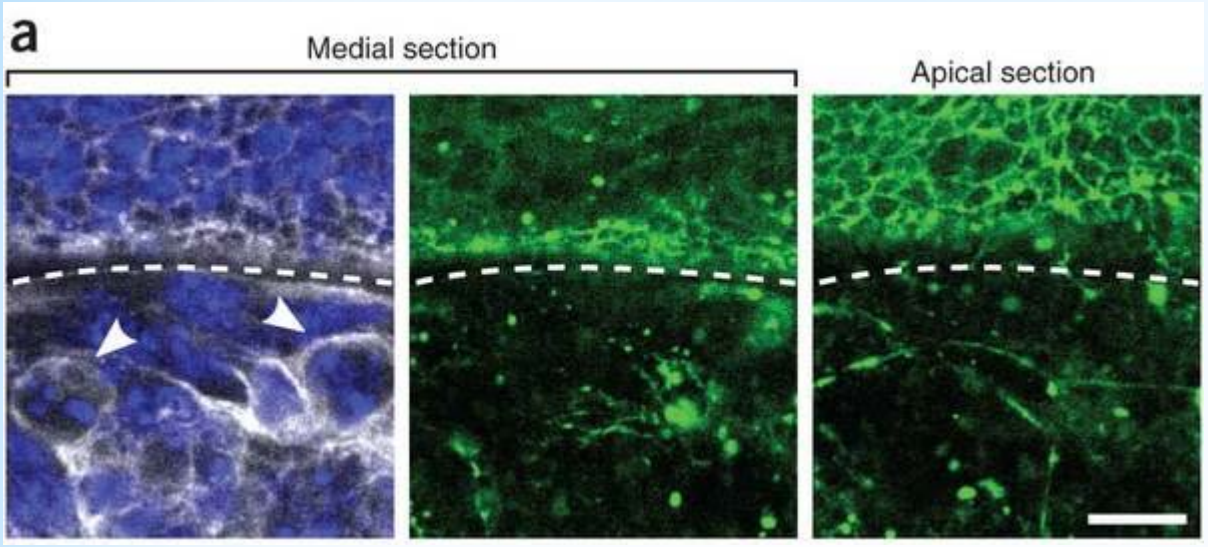


Plot of His2Av::EYFP (green symbols) and mCherry-NLS (purple symbols) signals expressed in arbitrary units (amount of fluorescence per volume) in en expression areas manually tracked

Caussinus et al, 2012

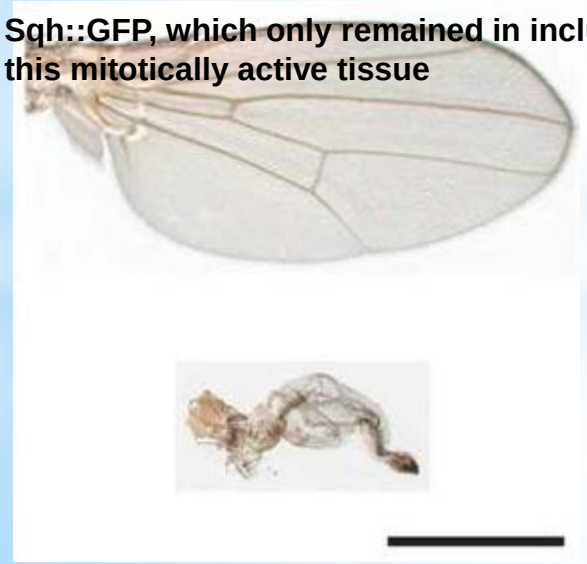
deGradFP phenocopies loss-of-function mutations

sqhSqh::GFP third instar larval wing discs



Sqh (*spaghetti squash*): Myosin II regulatory light chain

Sqh::GFP, which only remained in inclusion bodies, and produced many big multinucleated cells in this mitotically active tissue

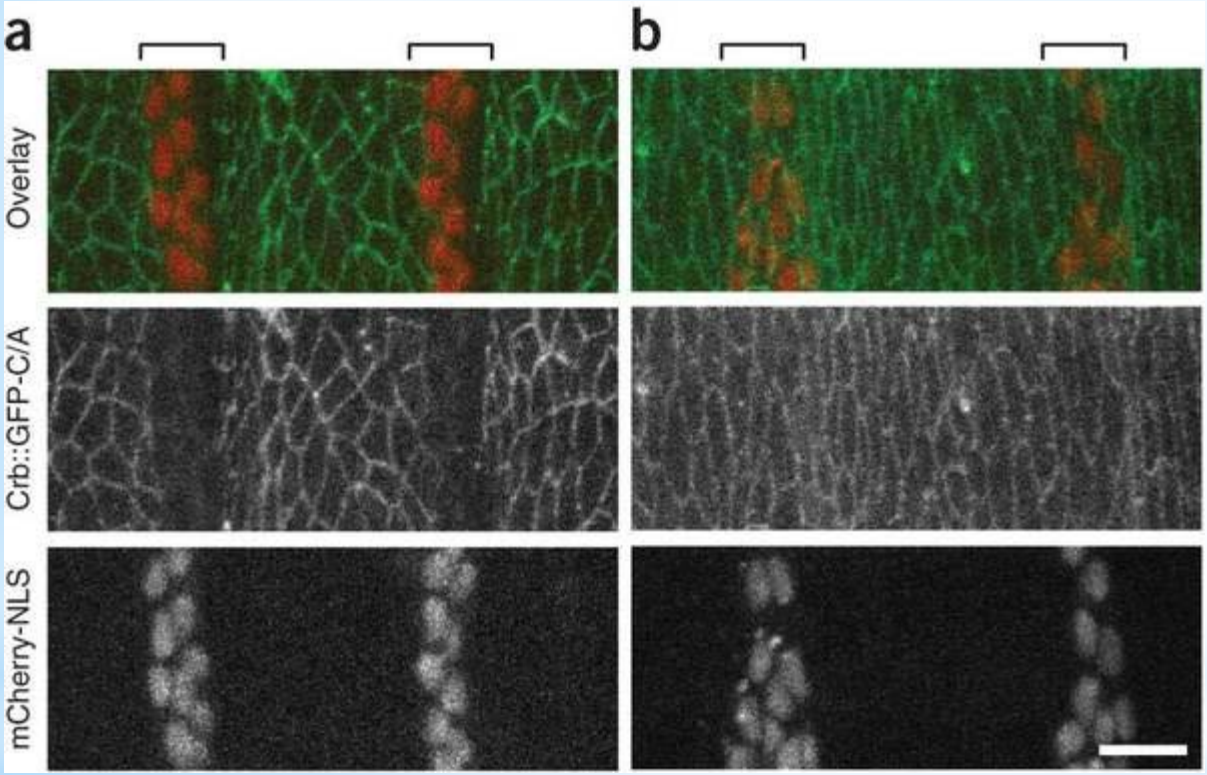


ap (apterous)::GFP- TF required for wing formation

Caussinus et al, 2012

deGradFP is able to target the intracytoplasmic, but not the extracytoplasmic, part of transmembrane proteins.

Epiderm of stage 12 embryos by live imaging.



enGal4 to express both NSlmb-vhhGFP4 and mCherry-NLS in Drosophila embryos carrying either crb::GFP-C or crb::GFP-A, two GFP fusion knock-in alleles of crumbs.

deGradFP is not only active on cytoplasmic (Sqh) and nuclear (Ap) proteins, it is also active on transmembrane proteins (Crb)

Caussinus et al, 2012

Outlook:

deGradFP allows the target protein knockout to be easily monitored by fluorescence microscopy during live imaging, while the phenotype is being documented.

Limitations:

Needs fusion proteins tagged with GFP/YFP/Venus

Target proteins embedded in big protein complexes might be inaccessible for NSImb-vhhGFP4

deGradFP did not deplete GFP on its own, possibly because the small size of GFP, which might not be properly exposed to the SCF-recruited E2 enzyme

Caussinus et al, 2012

Cell

Resource

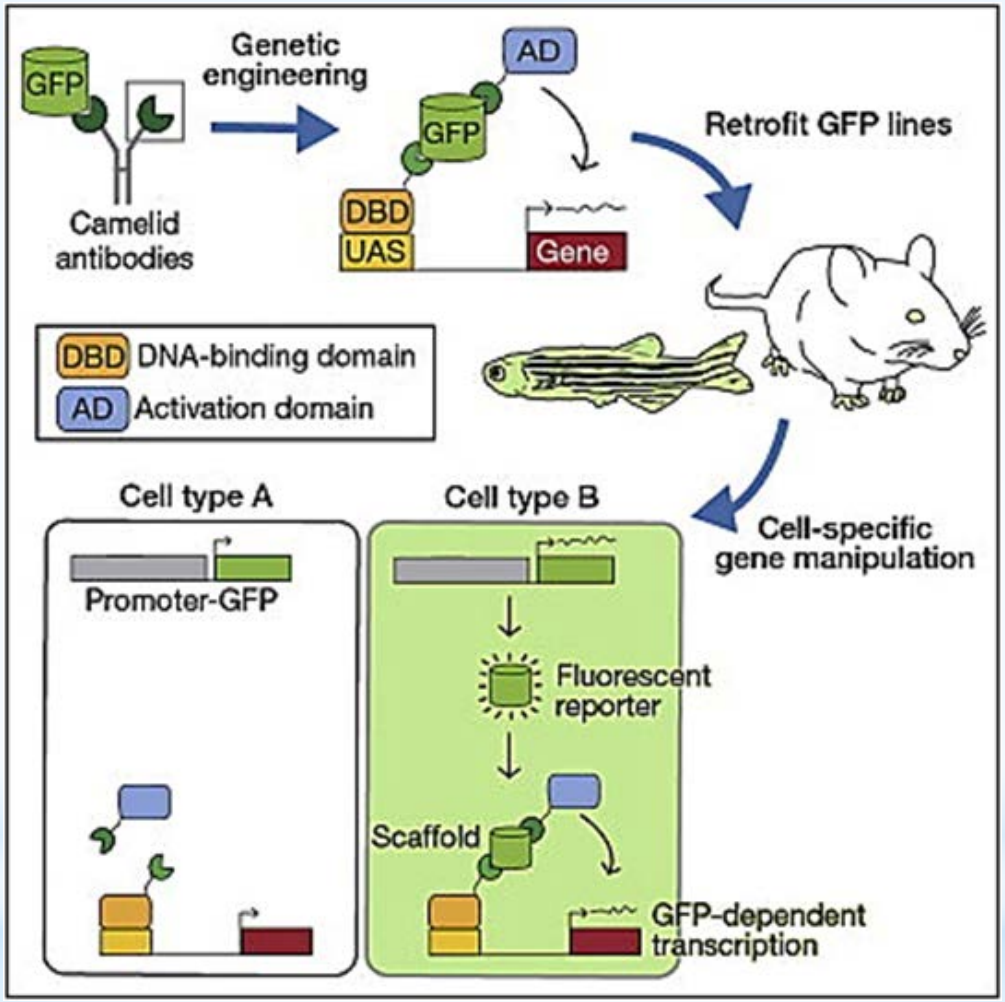
A Nanobody-Based System Using Fluorescent Proteins as Scaffolds for Cell-Specific Gene Manipulation

Jonathan C.Y. Tang,[1,2] Tamas Szikra,[4] Yevgenia Kozorovitskiy,[1,3] Miguel Teixiera,[4,5] Bernardo L. Sabatini,[1,3] Botond Roska,[4] and Constance L. Cepko[1,2,*]
[1]Howard Hughes Medical Institute
[2]Departments of Genetics and Ophthalmology
[3]Department of Neurobiology
Harvard Medical School, Boston, MA 02115, USA
[4]Neural Circuit Laboratories, Friedrich Miescher Institute for Biomedical Research, 4058 Basel, Switzerland
[5]University of Basel, 4003 Basel, Switzerland
*Correspondence: cepko@genetics.med.harvard.edu
http://dx.doi.org/10.1016/j.cell.2013.07.021

GFP-dependent transcription system enabling control of any target gene for functional studies across tissues and organisms

GFP would act like a small-molecule "dimerizer," bridging the association of distinct modular domains or protein fragments to reconstitute useful activities such as transcription and recombination

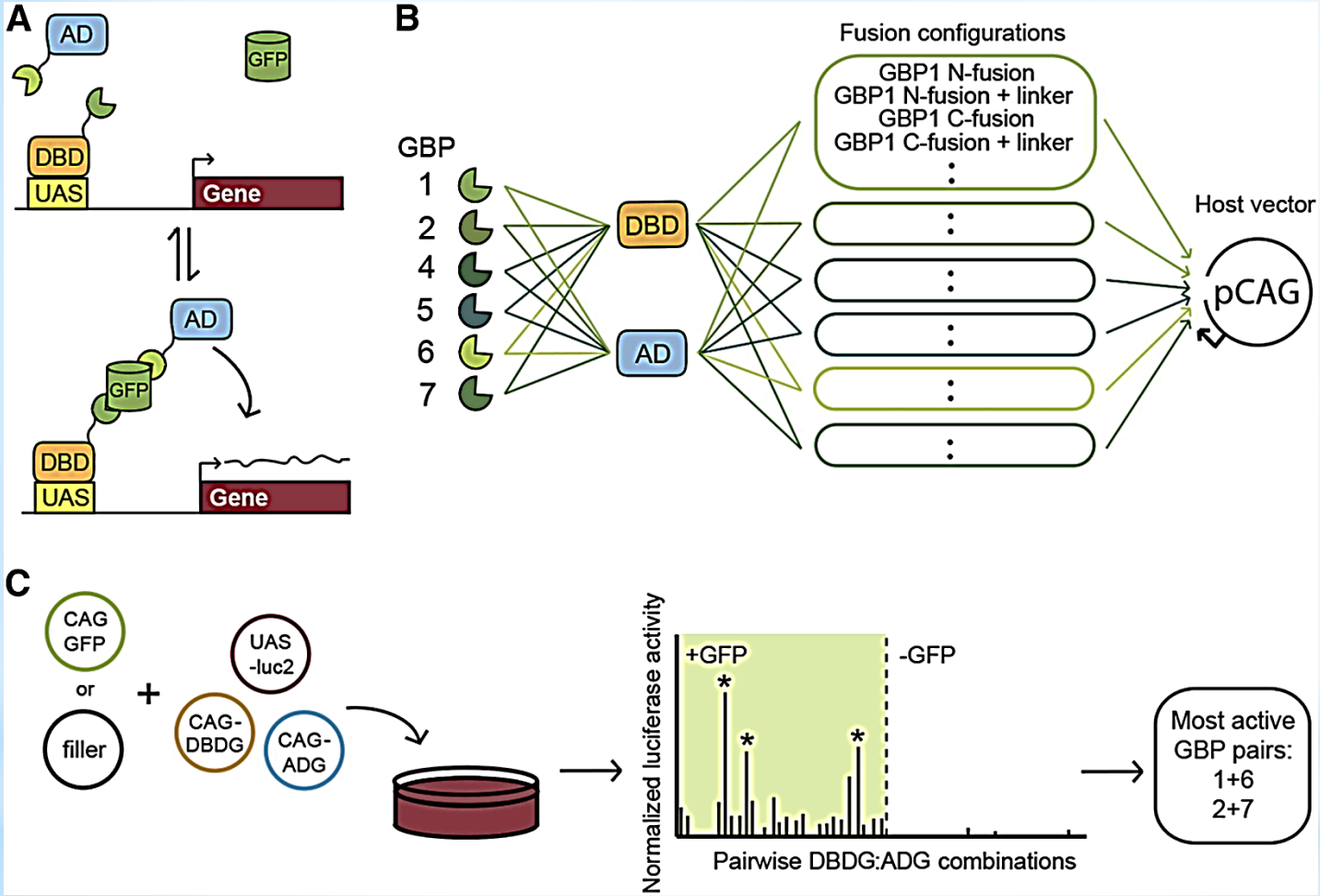
Tang et al., 2013



293T cells or the mouse retina for screens

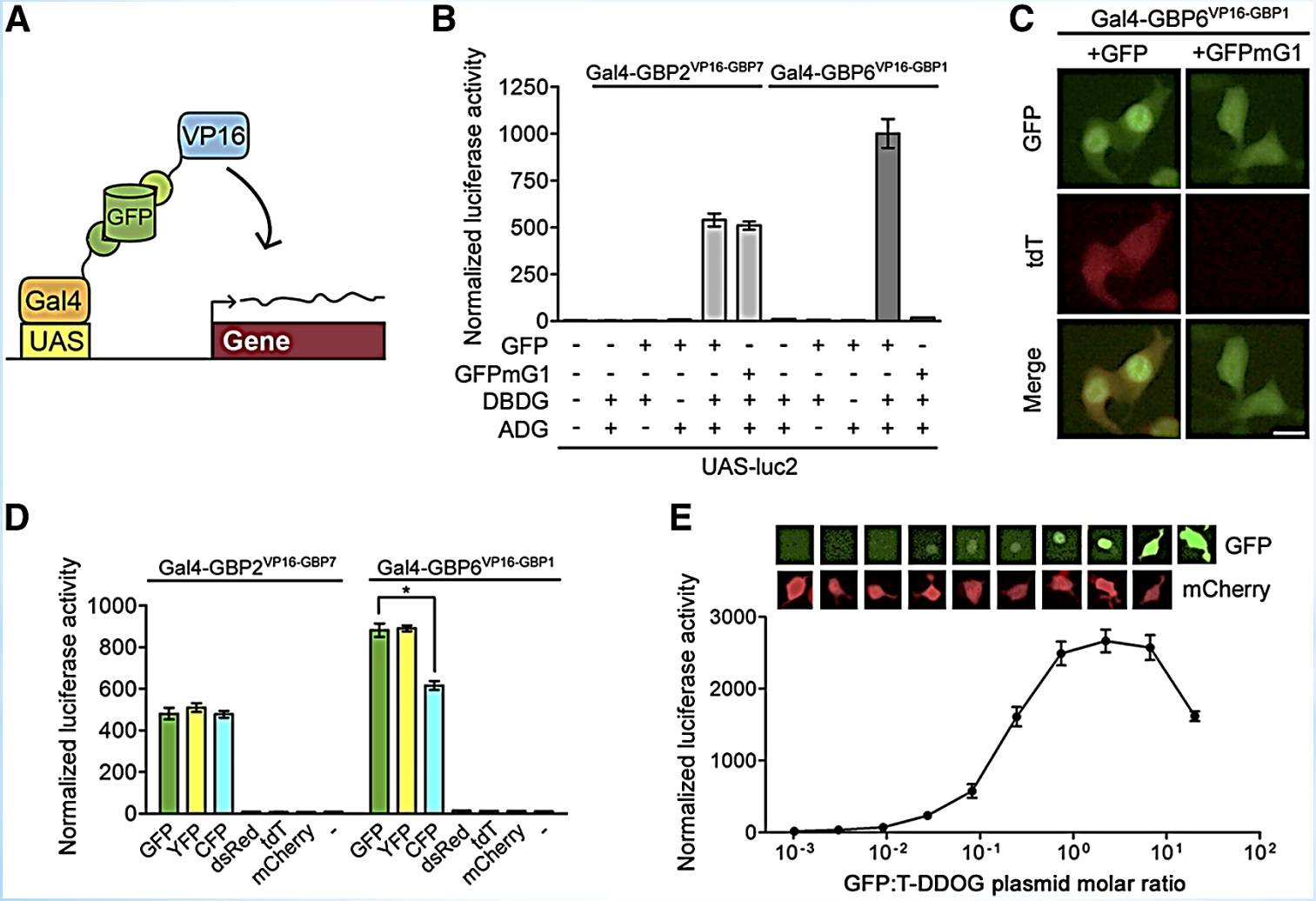
Tang et al., 2013

In Vitro Screen Used to Identify Functional GBP Pairs for the GFP-Dependent Transcription System



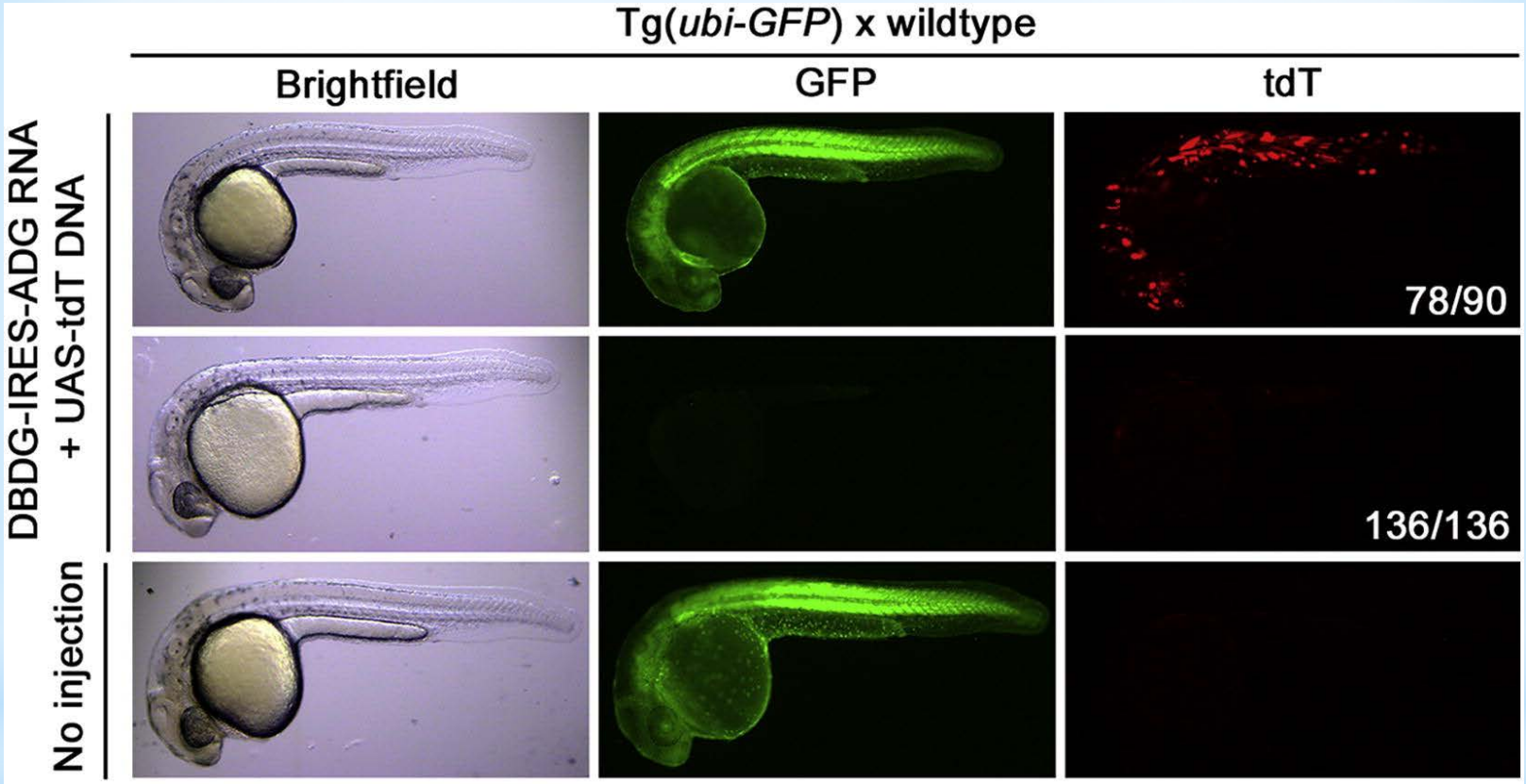
Tang et al., 2013

Characterization of the GFP-Dependent Transcription System



Tang et al., 2013

GFP-Dependent Transcription in Transgenic Zebrafish



Tang et al., 2013

Outlook:

Selective manipulation of GFP-labeled cells across transgenic GFP lines and establishing components for the design of synthetic circuits using nanobodies

Exogenous molecules like GFP and nanobodies showing little connection to host protein networks can be used to study and manipulate cellular process across a wide spectrum of model systems.

Tang et al., 2013

Thank you for your attention