

Therapeutic antibodies (Tab-IP) and recombinant antibodies**Year of publication 2018**

Sandrine Moutel, Clément Nizak, Franck Perez (2018 Sep 10)

Selection and Use of Intracellular Antibodies.

Methods in molecular biology (Clifton, N.J.) : 491-503 : [DOI : 10.1007/978-1-4939-8648-4_25](https://doi.org/10.1007/978-1-4939-8648-4_25)

Summary

Intracellularly expressed recombinant antibodies, or intrabodies, are powerful tools for cell biology studies as well as therapeutic applications. Cell biologists use them to either block the intracellular antibody target or to image endogenous target dynamics. We describe here methods to select recombinant antibodies from antibody phage display libraries and to subsequently express them as fluorescent intrabodies.

Year of publication 2017

Valentina Galli, Rafael Sebastian, Sandrine Moutel, Jason Ecard, Franck Perez, Aurélien Roux (2017 Oct 13)

Uncoupling of dynamin polymerization and GTPase activity revealed by the conformation-specific nanobody dynab.

eLife : [DOI : 10.7554/eLife.25197](https://doi.org/10.7554/eLife.25197)

Summary

Dynamin is a large GTPase that forms a helical collar at the neck of endocytic pits, and catalyzes membrane fission (Schmid and Frolov, 2011; Ferguson and De Camilli, 2012). Dynamin fission reaction is strictly dependent on GTP hydrolysis, but how fission is mediated is still debated (Antonny et al., 2016): GTP energy could be spent in membrane constriction required for fission, or in disassembly of the dynamin polymer to trigger fission. To follow dynamin GTP hydrolysis at endocytic pits, we generated a conformation-specific nanobody called dynab, that binds preferentially to the GTP hydrolytic state of dynamin-1. Dynab allowed us to follow the GTPase activity of dynamin-1 in real-time. We show that in fibroblasts, dynamin GTP hydrolysis occurs as stochastic bursts, which are randomly distributed relatively to the peak of dynamin assembly. Thus, dynamin disassembly is not coupled to GTPase activity, supporting that the GTP energy is primarily spent in constriction.

Ronan Crepin, Gianluca Veggiani, Selma Djender, Anne Beugnet, François Planeix, Christophe Pichon, Sandrine Moutel, Sebastian Amigorena, Franck Perez, Nicolae Ghinea, Ario de Marco (2017 Oct 12)

Whole-cell biopanning with a synthetic phage display library of nanobodies enabled the recovery of follicle-stimulating hormone receptor inhibitors.

Biochemical and biophysical research communications : 1567-1572 : [DOI :](https://doi.org/10.1016/j.bbrc.2017.10.011)

[S0006-291X\(17\)32006-5](https://doi.org/10.1016/j.bbrc.2017.10.011)

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Summary

Antibodies are essential reagents that are increasingly used in diagnostics and therapy. Their specificity and capacity to recognize their native antigen are critical characteristics for their in vivo application. Follicle-stimulating hormone receptor is a GPCR protein regulating ovarian follicular maturation and spermatogenesis. Recently, its potentiality as a cancer biomarker has been demonstrated but no antibody suitable for in vivo tumor targeting and treatment has been characterized so far. In this paper we describe the first successful attempt to recover recombinant antibodies against the FSHR and that: i) are directly panned from a pre-immune library using whole cells expressing the target receptor at their surface; ii) show inhibitory activity towards the FSH-induced cAMP accumulation; iii) do not share the same epitope with the natural binder FSH; iv) can be produced inexpensively as mono- or bivalent functional molecules in the bacterial cytoplasm. We expect that the proposed biopanning strategy will be profitable to identify useful functional antibodies for further members of the GPCR class.

Ronan Crépin, David Gentien, Angeline Duché, Audrey Rapinat, Cecile Reyes, Fariba Némati, Gérald Massonnet, Didier Decaudin, Selma Djender, Sandrine Moutel, Klervi Desrumeaux, Nathalie Cassoux, Sophie Piperno-Neumann, Sebastian Amigorena, Franck Perez, Sergio Roman Roman, Ario de Marco (2017 Feb 1)

Nanobodies against surface biomarkers enable the analysis of tumor genetic heterogeneity in uveal melanoma Patient Derived Xenografts.

Pigment cell & melanoma research : [DOI : 10.1111/pcmr.12577](https://doi.org/10.1111/pcmr.12577)

Summary

Monoclonal antibodies specific for biomarkers expressed on the surface of uveal melanoma (UM) cells would simplify the immune-capture and genomic characterization of heterogeneous tumor cells originated from patient derived xenografts (PDXs). Antibodies against four independent tumor antigens were isolated by panning a nanobody synthetic library. Such antibodies enabled flow-cytometry-based sorting of distinct cell sub-populations from UM PDXs and to analyze their genomic features. The complexity and specificity of the biochemical and genomic biomarker combinations mirrored the UM tumor polyclonality. The data showed that MUC18 is highly and universally displayed at the surface of UM cells with different genetic background and consequently represents a reliable pan-biomarker for their identification and purification. In contrast, the other three biomarkers were detected in very variable combinations in UM PDX cells. The availability of the identified nanobodies will be instrumental in developing clone-specific drug evaluation and rational clinical strategies based on accurate genomic profiling. This article is protected by copyright. All rights reserved.

Year of publication 2016

Sandrine Moutel, Nicolas Bery, Virginie Bernard, Laura Keller, Emilie Lemesre, Ario de Marco,

Therapeutic antibodies (Tab-IP) and recombinant antibodies

Laetitia Ligat, Jean-Christophe Rain, Gilles Favre, Aurélien Olichon, Franck Perez (2016 Jul 20)

NaLi-H1: A universal synthetic library of humanized nanobodies providing highly functional antibodies and intrabodies.

eLife : [DOI : 10.7554/eLife.16228](https://doi.org/10.7554/eLife.16228)

Summary

In vitro selection of antibodies allows to obtain highly functional binders, rapidly and at lower cost. Here, we describe the first fully synthetic phage display library of humanized llama single domain antibody (NaLi-H1: Nanobody Library Humanized 1). Based on a humanized synthetic single domain antibody (hs2dAb) scaffold optimized for intracellular stability, the highly diverse library provides high affinity binders without animal immunization. NaLi-H1 was screened following several selection schemes against various targets (Fluorescent proteins, actin, tubulin, p53, HP1). Conformation antibodies against active RHO GTPase were also obtained. Selected hs2dAb were used in various immunoassays and were often found to be functional intrabodies, enabling tracking or inhibition of endogenous targets. Functionalization of intrabodies allowed specific protein knockdown in living cells. Finally, direct selection against the surface of tumor cells produced hs2dAb directed against tumor-specific antigens further highlighting the potential use of this library for therapeutic applications.

Year of publication 2015

Marina Mikhaylova, Bas M C Cloin, Kieran Finan, Robert van den Berg, Jalmar Teeuw, Marta M Kijanka, Mikolaj Sokolowski, Eugene A Katrukha, Manuel Maidorn, Felipe Opazo, Sandrine Moutel, Marylin Vantard, Frank Perez, Paul M P van Bergen en Henegouwen, Casper C Hoogenraad, Helge Ewers, Lukas C Kapitein (2015 Aug 12)

Resolving bundled microtubules using anti-tubulin nanobodies.

Nature communications : 7933 : [DOI : 10.1038/ncomms8933](https://doi.org/10.1038/ncomms8933)

Summary

Microtubules are hollow biopolymers of 25-nm diameter and are key constituents of the cytoskeleton. In neurons, microtubules are organized differently between axons and dendrites, but their precise organization in different compartments is not completely understood. Super-resolution microscopy techniques can detect specific structures at an increased resolution, but the narrow spacing between neuronal microtubules poses challenges because most existing labelling strategies increase the effective microtubule diameter by 20-40 nm and will thereby blend neighbouring microtubules into one structure. Here we develop single-chain antibody fragments (nanobodies) against tubulin to achieve super-resolution imaging of microtubules with a decreased apparent diameter. To test the resolving power of these novel probes, we generate microtubule bundles with a known spacing of 50-70 nm and successfully resolve individual microtubules. Individual bundled microtubules can also be resolved in different mammalian cells, including hippocampal neurons, allowing novel insights into fundamental mechanisms of microtubule organization

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in cell- and neurobiology.

Year of publication 2014

Selma Djender, Aurelie Schneider, Anne Beugnet, Ronan Crepin, Klervi Even Desrumeaux, Chiara Romani, Sandrine Moutel, Franck Perez, Ario de Marco (2014 Sep 17)

Bacterial cytoplasm as an effective cell compartment for producing functional VHH-based affinity reagents and Camelidae IgG-like recombinant antibodies.

Microbial cell factories : 140 : [DOI : 10.1186/s12934-014-0140-1](https://doi.org/10.1186/s12934-014-0140-1)

Summary

The isolation of recombinant antibody fragments from displayed libraries represents a powerful alternative to the generation of IgGs using hybridoma technology. The selected antibody fragments can then be easily engineered into (multi)-tagged constructs of variable mass and complexity as well as reconstituted into Camelidae IgG-like molecules when expressed fused to Fc domains. Nevertheless, all antibody constructs depend on an oxidizing environment for correct folding and consequently still belong to the proteins difficult to express in bacteria. In such organisms they are mostly produced at low yields in the periplasmic space.

Year of publication 2012

Sandrine Moutel, Clément Nizak, Franck Perez (2012 Aug 22)

Selection and use of intracellular antibodies (intrabodies).

Methods in molecular biology (Clifton, N.J.) : 667-79 : [DOI : 10.1007/978-1-61779-974-7_37](https://doi.org/10.1007/978-1-61779-974-7_37)

Summary

Intracellularly expressed recombinant antibodies, or intrabodies, are powerful tools for cell biology studies as well as therapeutic applications. Cell biologists use them to either block the intracellular antibody target or to image endogenous target dynamics. We describe here methods to select recombinant antibodies from antibody phage display libraries and to subsequently express them as fluorescent intrabodies.

Year of publication 2009

Sandrine Moutel, Franck Perez (2009 Dec 29)

[Intrabodies, potent tools to unravel the function and dynamics of intracellular proteins].

Médecine sciences : M/S : 1173-6 : [DOI : 10.1051/medsci/200925121173](https://doi.org/10.1051/medsci/200925121173)

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Summary

In the 1980s, progress in molecular biology enabled the manipulation and cloning of antibody fragments as functional scFv (single chain Fv). Because of their small size and relative ease of expression, scFv opened the road for new medical and biotechnological applications. scFvs can be easily expressed and targeted to different cellular compartments (cytosol, nucleus, endoplasmic reticulum, mitochondria, inner surface of the plasma membrane, etc.), using specific signals to target or retain them in a given compartment. Recombinant antibodies can thus be used as intracellular antibodies (intrabody) to neutralize, disrupt or track endogenous antigen. Intrabodies not only represent new tools for fundamental research to study the dynamics of endogenous proteins, but may also bring interesting options for applied research in terms of intracellular immunization for therapeutic use.

Ole Vielemeyer, Hebao Yuan, Sandrine Moutel, Rénette Saint-Fort, Danming Tang, Clément Nizak, Bruno Goud, Yanzhuang Wang, Franck Perez (2009 May 29)

Direct selection of monoclonal phosphospecific antibodies without prior phosphoamino acid mapping.

The Journal of biological chemistry : 20791-5 : [DOI : 10.1074/jbc.M109.008730](https://doi.org/10.1074/jbc.M109.008730)

Summary

In the current post-genomic era, large scale efforts are underway to functionally explore the proteome by assembling large antibody libraries. However, because many proteins are modified post-translationally to regulate their function, collections of modification-specific sensors are also needed. Here we applied a novel approach to select monoclonal phosphospecific antibodies directly from the full-length protein and without up-front phosphoamino acid identification. We chose as antigen GRASP65, a well studied Golgi phosphoprotein. Bacterially produced full-length protein was first incubated with mitotic cytosol, thus allowing modification by naturally occurring kinases, and then used directly for affinity-based antibody selection using a single chain variable fragment phagemid library. In less than 1 week, three distinct and highly functional monoclonal phosphospecific antibodies against two GRASP65 epitopes were obtained and subsequently characterized. The presented approach is carried out fully in vitro, requires no prior knowledge of the phosphoamino acid identity, and is fast and inexpensive. It therefore has great potential to be an attractive alternative to classic animal-based protocols for the selection of post-translation modification sensors and thus to become an invaluable tool in our quest to understand the proteome in all its complexity.

Sandrine Moutel, Ahmed El Marjou, Ole Vielemeyer, Clément Nizak, Philippe Benaroch, Stefan Dübel, Franck Perez (2009 Feb 28)

A multi-Fc-species system for recombinant antibody production.

BMC biotechnology : 14 : [DOI : 10.1186/1472-6750-9-14](https://doi.org/10.1186/1472-6750-9-14)

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Summary

Genomic, transcriptomic and proteomic projects often suffer from a lack of functional validation creating a strong demand for specific and versatile antibodies. Antibody phage display represents an attractive approach to select rapidly in vitro the equivalent of monoclonal antibodies, like single chain Fv antibodies, in an inexpensive and animal free way. However, so far, recombinant antibodies have not managed to impose themselves as efficient alternatives to natural antibodies.

Sandrine Moutel, Ole Vielemeyer, Hulin Jin, Séverine Divoux, Philippe Benaroch, Franck Perez (2009 Jan 22)

Fully in vitro selection of recombinant antibodies.

Biotechnology journal : 38-43 : [DOI : 10.1002/biot.200800246](https://doi.org/10.1002/biot.200800246)

Summary

Antibodies are essential for the identification and characterization of proteins. In the current postgenomic era the need for highly specific antibodies has further increased not only for research applications but also because they represent one of the most promising therapeutic options, especially in the field of cancer treatment. One appealing approach for rapid and inexpensive antibody generation is the use of phage display. This technique allows for a fast and animal-free selection of highly functional alternatives to classical antibodies. However, one strong limitation of this recombinant approach has been the difficulty in producing and purifying antigens. These steps have to be adjusted for each new target, are time consuming and sometimes present an insurmountable obstacle. Here we report the development of new antibody selection approach where antigens are produced through in vitro translation and are used directly and without the need for purification. With this approach we were able to rapidly select recombinant antibodies directed against GFP and the mammalian protein tsg101, respectively. We believe that our method greatly facilitates antigen preparation and thus may broaden the use of the recombinant approach for antibody generation, especially since the technique could in the future be adapted to a high-throughput technology, thus further accelerating antibody selection.

Year of publication 2008

Ariane Dimitrov, Mélanie Quesnoit, Sandrine Moutel, Isabelle Cantaloube, Christian Poüs, Franck Perez (2008 Oct 16)

Detection of GTP-tubulin conformation in vivo reveals a role for GTP remnants in microtubule rescues.

Science (New York, N.Y.) : 1353-6 : [DOI : 10.1126/science.1165401](https://doi.org/10.1126/science.1165401)

Summary

Microtubules display dynamic instability, with alternating phases of growth and shrinkage

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separated by catastrophe and rescue events. The guanosine triphosphate (GTP) cap at the growing end of microtubules, whose presence is essential to prevent microtubule catastrophes in vitro, has been difficult to observe in vivo. We selected a recombinant antibody that specifically recognizes GTP-bound tubulin in microtubules and found that GTP-tubulin was indeed present at the plus end of growing microtubules. Unexpectedly, GTP-tubulin remnants were also present in older parts of microtubules, which suggests that GTP hydrolysis is sometimes incomplete during polymerization. Observations in living cells suggested that these GTP remnants may be responsible for the rescue events in which microtubules recover from catastrophe.

Sandrine Moutel, Franck Perez (2008 Mar 19)

“Antibodies-Europe. Engineering the next generation of antibodies”.

Biotechnology journal : 298-300 : [DOI : 10.1002/biot.200800011](https://doi.org/10.1002/biot.200800011)

Summary