Open-Source Antibodies as a Path to Enhanced Research Reproducibility and Transparency

Adapted from mamaspark.blogspot.com

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thequiltshow.com

UC's public service mission includes making its research results available for public use and benefit.

The mission of the National Institutes of Health (NIH) is to seek fundamental knowledge about the nature and behavior of living systems and the application of that knowledge to enhance health, lengthen life, and reduce illness and disability.

Brain Disorders Have a Substantial Societal Impact

Estimated $1.7 trillion in direct global health-care spending on brain disorders in 2019.

With an aging global population, costs are expected to continue to rise.

Indirect costs (lost wages, caregiver expenses, impact on families, societal well-being, etc.) likely exceed this.

Mitchell et al., Lancet Public Health May 2025

Top ten neurological conditions worldwide

- stroke
- neonatal encephalopathy (brain injury)
- migraine
- dementia
- diabetic neuropathy (nerve damage)
- meningitis
- epilepsy
- neurological complications from preterm birth
- autism spectrum disorder
- nervous system cancers

Does not include non-diabetic chronic pain

Mental Illness

It is estimated that more than one in five U.S. adults live with a mental illness (59.3 million in 2022; 23.1% of the U.S. adult population).

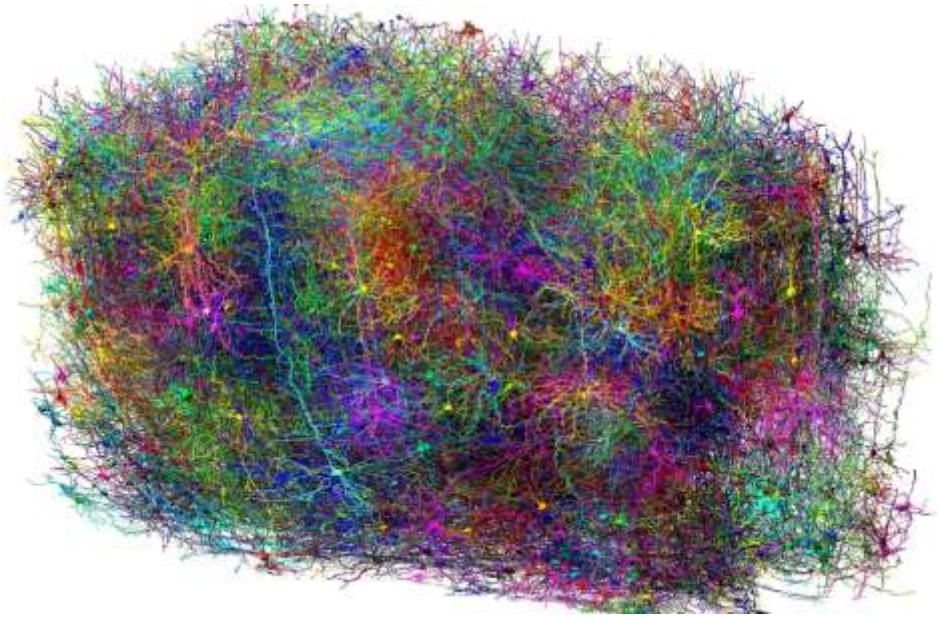
Source: National Institute of Mental Health

Brain Research is Advancing Rapidly

The New York Times April 9, 2025

An Advance in Brain Research That Was Once Considered Impossible

Scientists achieved "a milestone" by charting the activity and structure of 200,000 cells in a mouse brain and their 523 million connections.



A small subset of the neurons in a 1 mm^3 volume of mouse cortex

See the set of seven papers in the April 10, 2025 issue of Nature

To understand biological processes (and to be able to intervene in them for therapeutic benefit), it is necessary to reveal the molecular heterogeneity of cells and their subcellular compartments by gaining access to the location and interaction of all biomolecules.

"Spatial proteomics in neurons at single-protein resolution". Cell 187:1785, 2024

molecular heterogeneity of cells in the brain

Nature | Vol 624 | 14 December 2023 | 253

Cell types identified by gene-expression profiles and mapped to the brain

Mouse brain

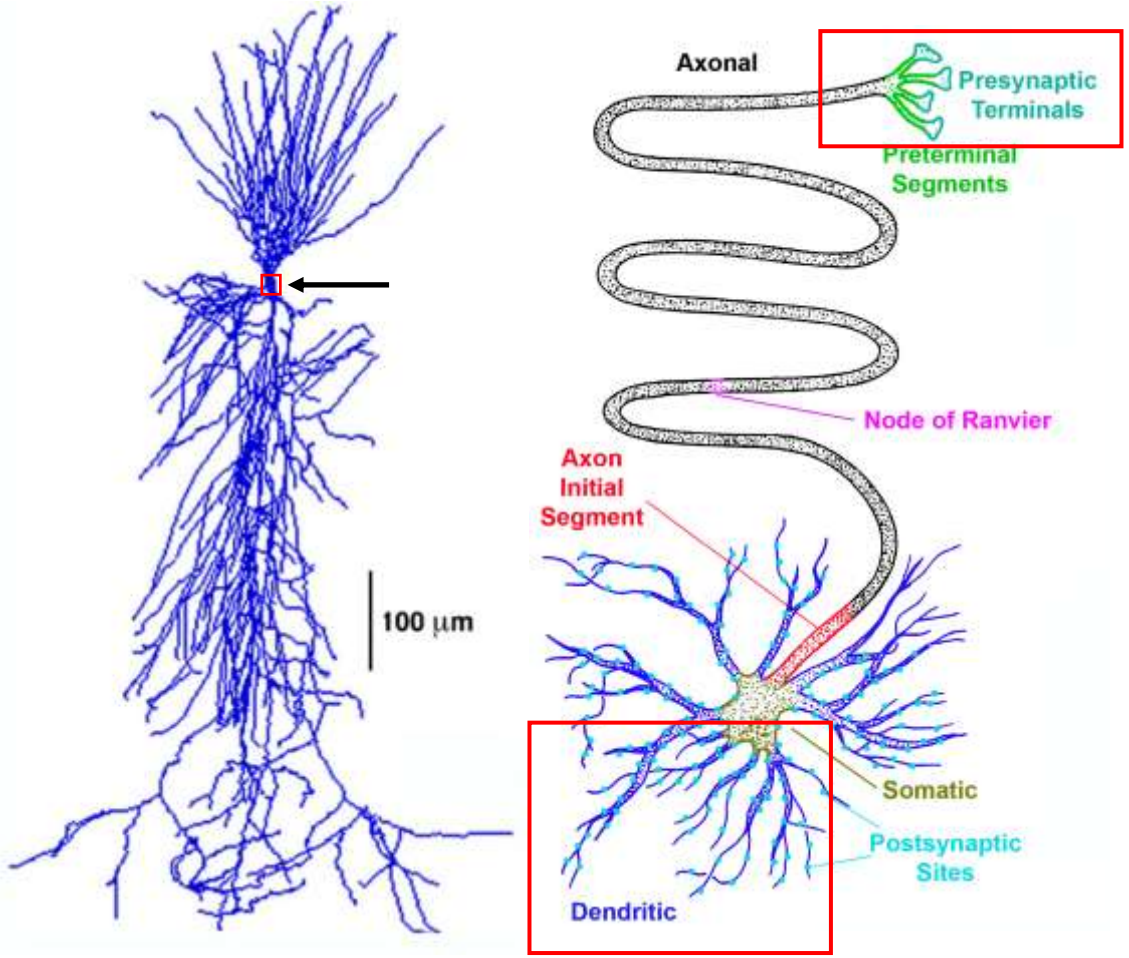
News & views

Cellular atlases of the entire mouse brain

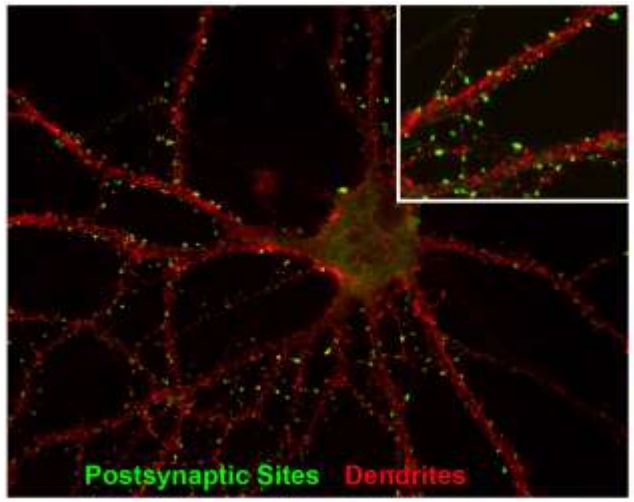
NIH BRAIN Initiative Cell Census Network: based on their RNA content, cells in adult mouse brain can be grouped into 5,000 cell types (almost all of which are different types of neurons)

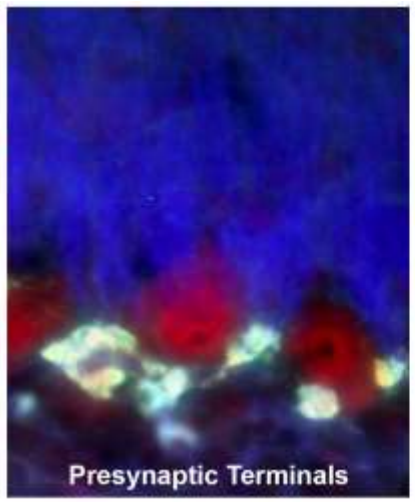
BRAIN INITIATIVE BRAIN RESEARCH THROUGH ADVANCING INNOVATIVE NEUROTECHNOLOGIES

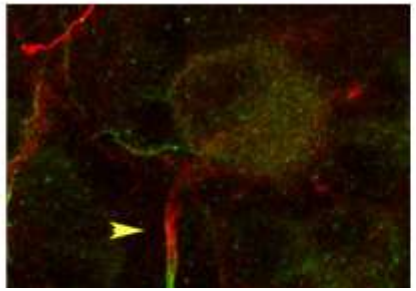
Neurons Exhibit Extreme Morphological Complexity and Subcellular Compartmentalization

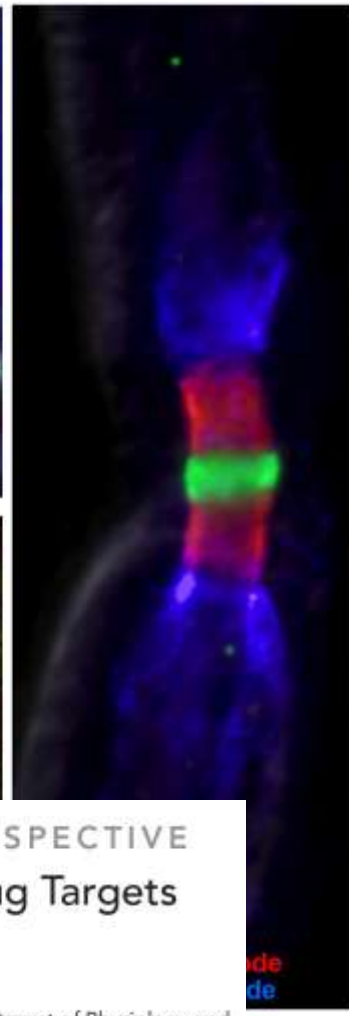


A mouse CA1 hippocampal neuron
(axon not shown)









J. Gen. Physiol. Vol. 131 No. 5 407–413

PERSPECTIVE

Antibody-based Validation of CNS Ion Channel Drug Targets

Kenneth J. Rhodes[1] and James S. Trimmer[2,3]

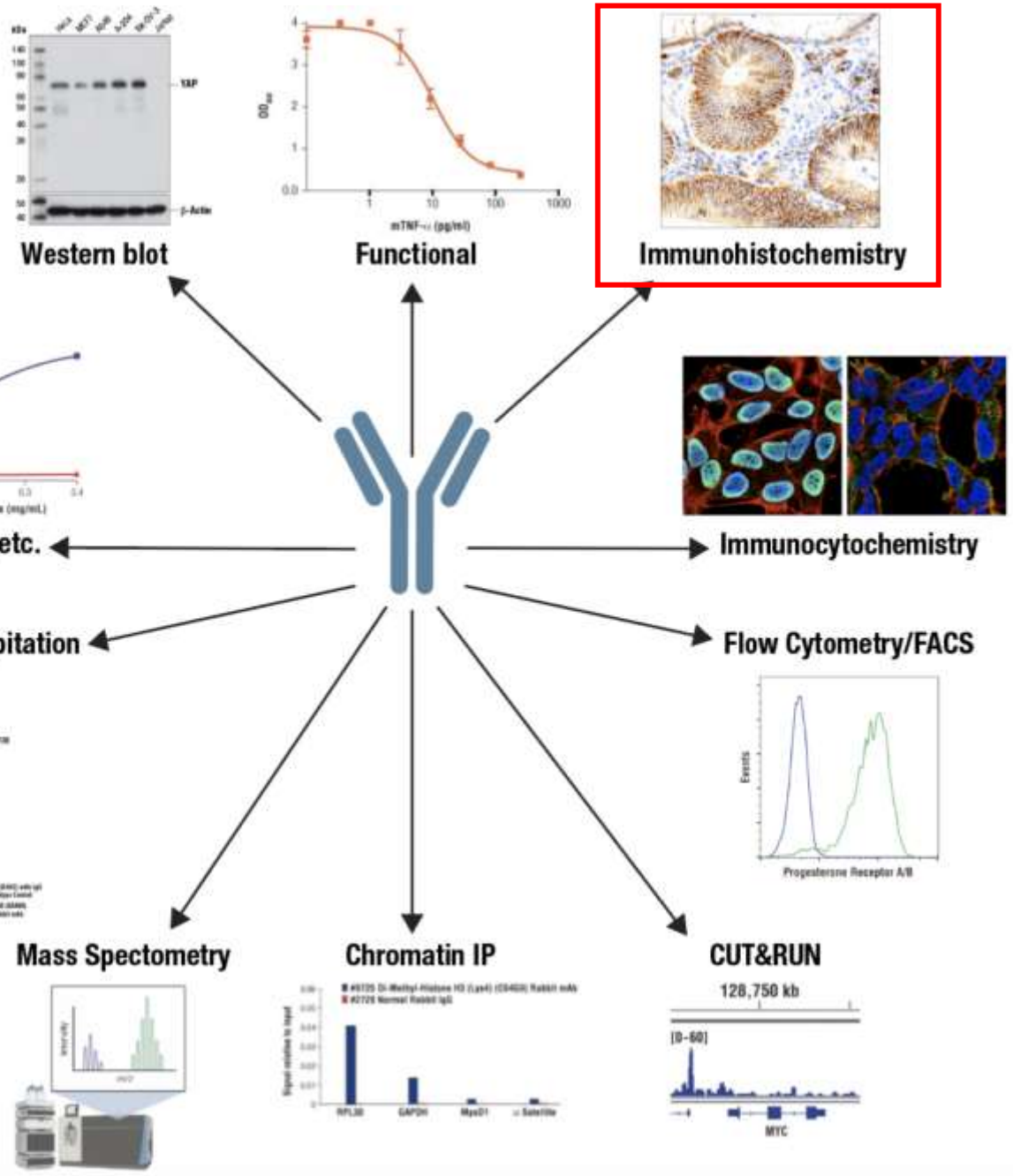
[1]Discovery Neurobiology, Biogen Idec, Cambridge, MA 02142
[2]Department of Neurobiology, Physiology and Behavior, College of Biological Sciences, and [3]Department of Physiology and Membrane Biology, School of Medicine, University of California, Davis, CA 95616

, 2015

Target ID and Validation → **Hit Identification** → **Lead Optimization** → **Efficacy and Safety** → **Development Feasibility**

Preclinical drug discovery

Antibodies Are Crucial Reagents for Proteomics Level Research Including Spatial Proteomics

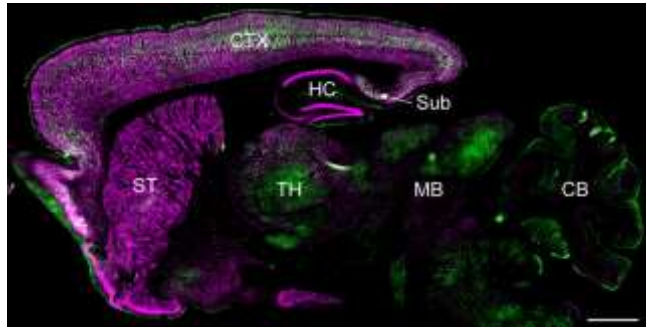


Cell Signaling Technology Website

Spatial Proteomics: a field that studies the distribution and organization of proteins within cells and tissues.

Provides insights into how proteins interact and function within their native environment.

Provides a three-dimensional view of protein localization and interactions.



In (too) Many Cases, the Antibodies That Are Available May Not Be Suitable

2006

The Journal of Neuroscience, August 2, 2006 • 26(31):8017–8020 • 8017

Antibodies as Valuable Neuroscience Research Tools versus Reagents of Mass Distraction

Kenneth J. Rhodes[1] **and James S. Trimmer**[2]

[1]CNS Research, Johnson and Johnson Pharmaceutical Research and Development, Spring House, Pennsylvania 19477, and [2]Department of Pharmacology, School of Medicine, University of California, Davis, California 95616

2012

29 MARCH 2012 | VOL 483 | NATURE | 531

Raise standards for preclinical cancer research

C. Glenn Begley and **Lee M. Ellis** propose how methods, publications and incentives must change if patients are to benefit.

2015

BLAME IT ON THE ANTIBODIES

Antibodies are the workhorses of biological experiments, but they are littering the field with false findings. A few evangelists are pushing for change.

BY MONYA BAKER

Baker, Nature 521: 274-276, 2015

2024

Feature

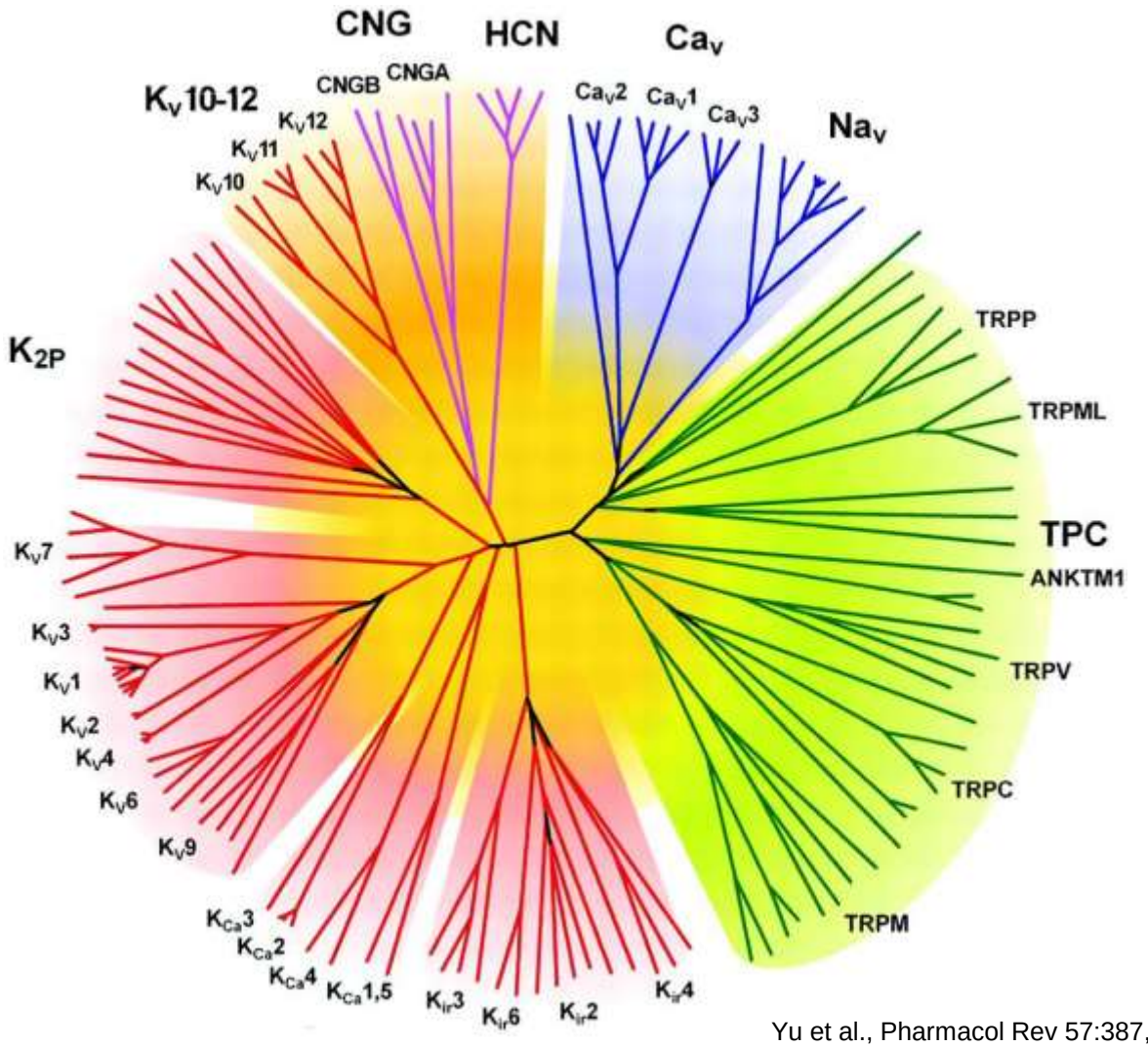
Nature | Vol 635 | 7 November 2024

THE QUEST TO RID LABS OF THE REAGENTS THAT RUIN EXPERIMENTS

Poorly performing antibodies have plagued biomedical sciences for decades. Several fresh initiatives hope to change this. **By Diana Kwon**

"Poorly characterized antibodies probably contribute more to the problem than any other laboratory tool". Glenn Begley.

Defining Where Ion Channels Are Localized Within Neurons is Critical to Understanding Their Function in Brain and Their Suitability as Targets for Therapeutics

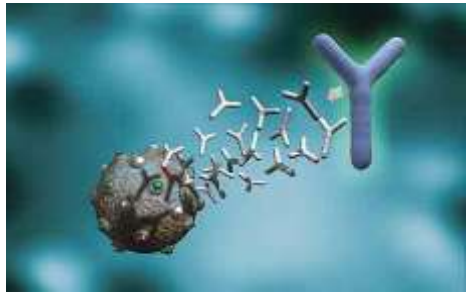


143 members in humans

Yu et al., Pharmacol Rev 57:387, 2005

Monoclonal Antibodies (mAbs) as Renewable Research Reagents

Fuse B cells to myeloma cells to create immortal hybridoma cell lines secreting a single monoclonal antibody (mAb).



Hybridoma cells in culture secrete mAbs into the culture medium. Can use “as is” or as source of purified mAbs.

Simple and scalable in any lab that performs mammalian cell culture.

Standard lab tissue culture
yields 20-50 µg mAb/ml of medium

Genentech Vacaville:
8 x 25,000 liter bioreactors
6,500 kgs of mAbs/year

Hybridoma cells can be archived indefinitely by cryopreserving in liquid nitrogen.

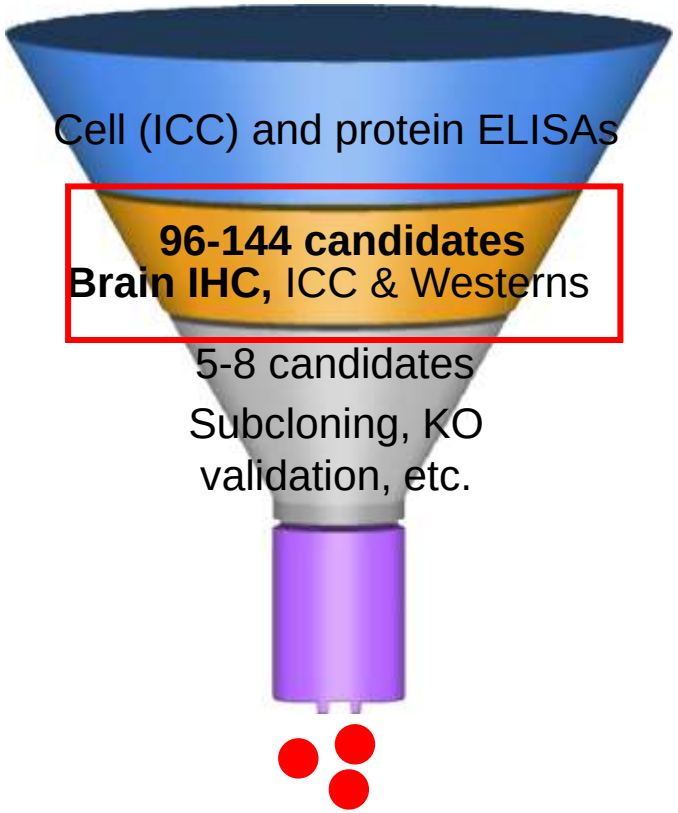
mAbs represent a renewable research resource, as hybridomas can be recovered from cryopreservation and cultured to produce the same mAb time and again.

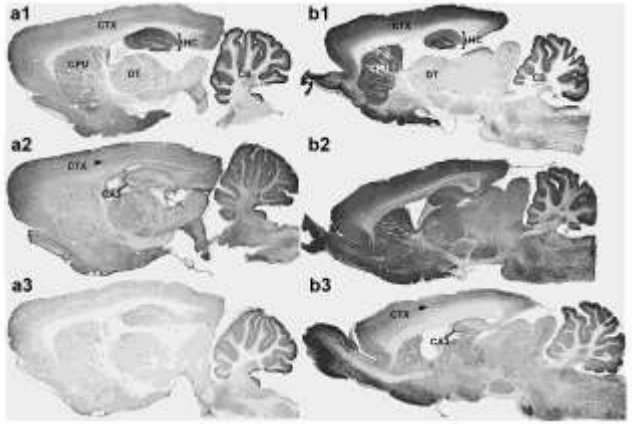
The use of renewable research reagents enhances research reproducibility.

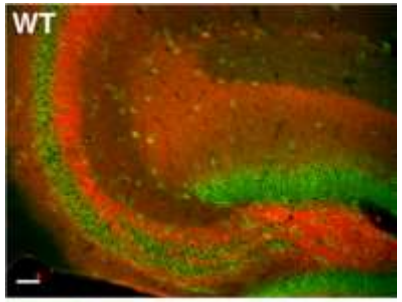
A Monoclonal Antibody Development Platform Aimed at Developing Tools for Spatial Proteomics in Brain

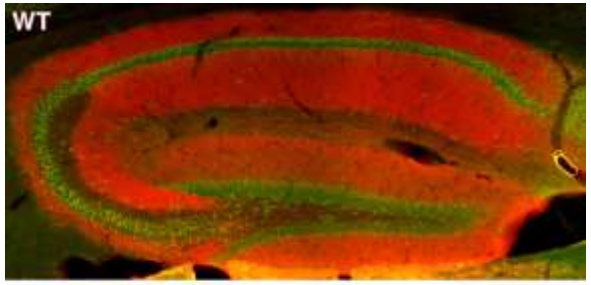
mAb screening platform

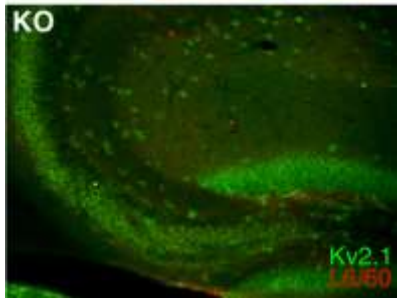
2,944 candidate samples

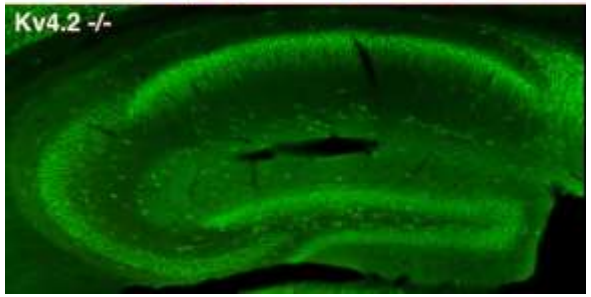


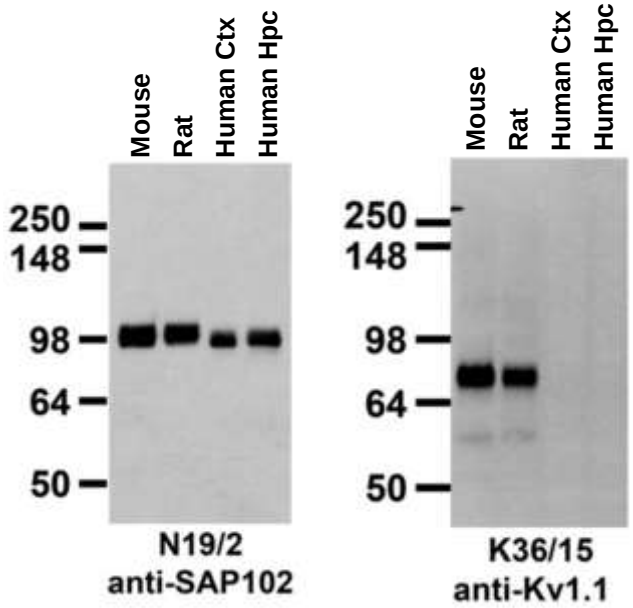












Trimmer Lab/NeuroMab Monoclonal Antibody Collection

Generated over a 30-year period in the Trimmer lab including since 2005 at the UC Davis/NIH NeuroMab Facility.

>800 projects against a wide variety of targets

Ion channels	Cell adhesion molecules	Epigenetics
Receptors	Cell type markers	Rare disease
Transporters	Activity markers	+Many Others
Scaffolding proteins		

We have made the top mAbs selected from these projects available as “NeuroMabs”. https://neuromab.ucdavis.edu/

Since 2005 we have been disseminating NeuroMabs through a low-cost distribution system to which UC Davis has contributed by not charging licensing fees or royalties.

≈75,000 vials of NeuroMabs have been distributed through this system, resulting in savings of tens of millions of research dollars in the direct cost of antibodies alone.

≈5,000 research publications citing NeuroMab as the source of antibodies used in the paper. Publications listed on NeuroMab website along with detailed experimental protocols, info on antibody characterization, etc.

Open Access to the Hybridoma Cells That Produce Our Monoclonal Antibodies Allows for Very Low-Cost Antibody Production

NeuroMab Hybridomas and ready to use mAbs

DSHB ANTIBODIES FOR RESEARCH

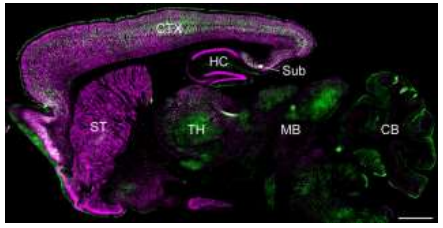
OPEN ACCESS

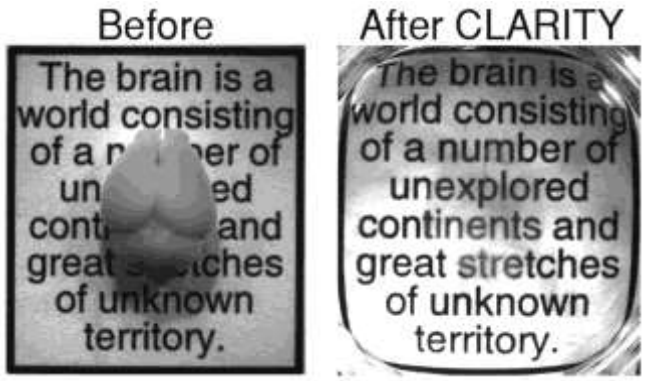
Costs of Ready To Use Antibodies

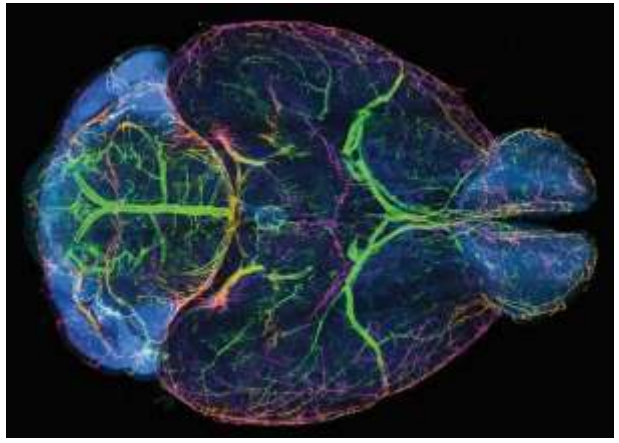
Source	Amount	Cost	Cost/100 µg
Commercial mAb	100 µg	$400	$400*
DSHB	50 µg	$75	$150
Outsource	50 mgs	$800	$1.60
Grow your own	Scalable	$50/ 1 L culture	$0.1-$0.2

*Can approach $1,000

Advances in Sample Preparation, Microscopy and Image Processing Allow for Spatial Proteomics on Intact Brains

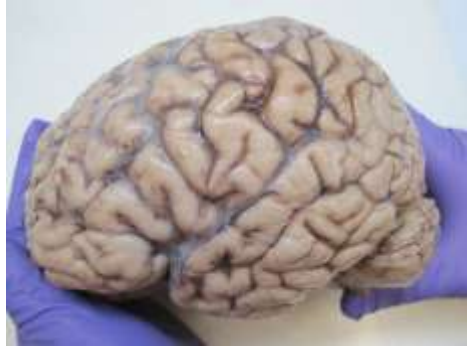






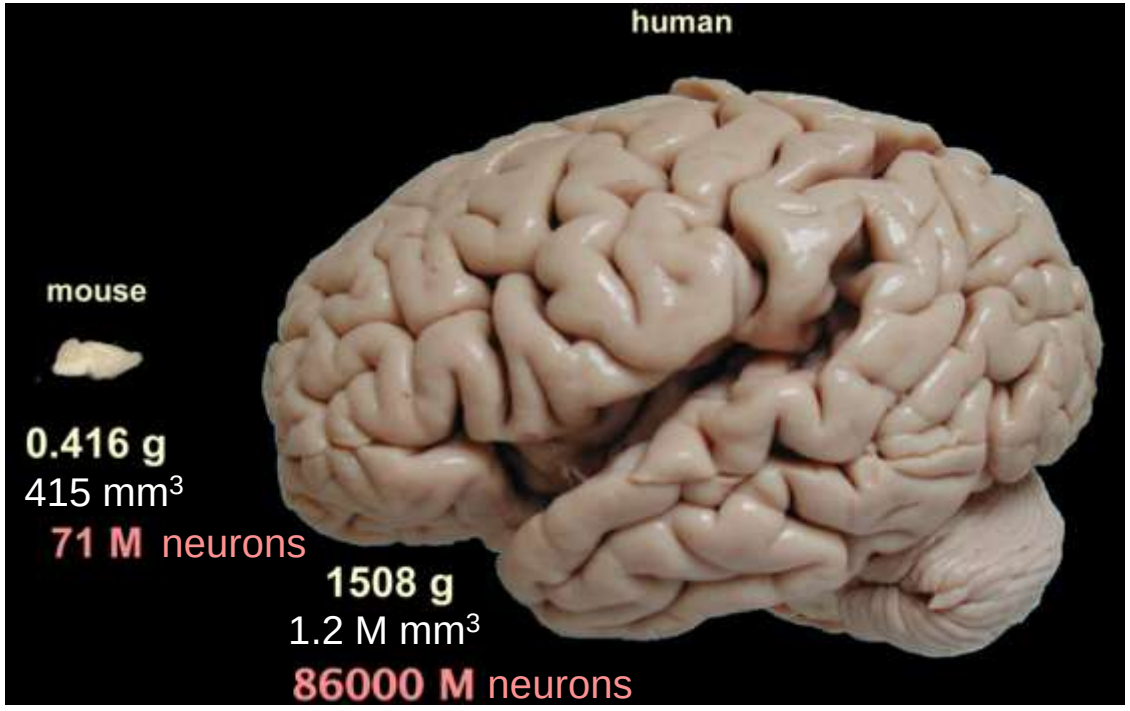
Blood vessels in an intact mouse brain

40 mgs of antibody are needed to label a single intact cleared human brain (1.5 L volume)



NIH BRAIN Initiative Cell Atlas Network (BICAN): Comprehensive Center on Human and Non-human Primate Brain Cell Atlases

Aim is to label ≈100 intact cleared human brains with 50-100 antibodies



$800M-$1.6B would be need to spent on commercial antibodies (at $400/100 μg)

Total funding for BICAN program: $90M

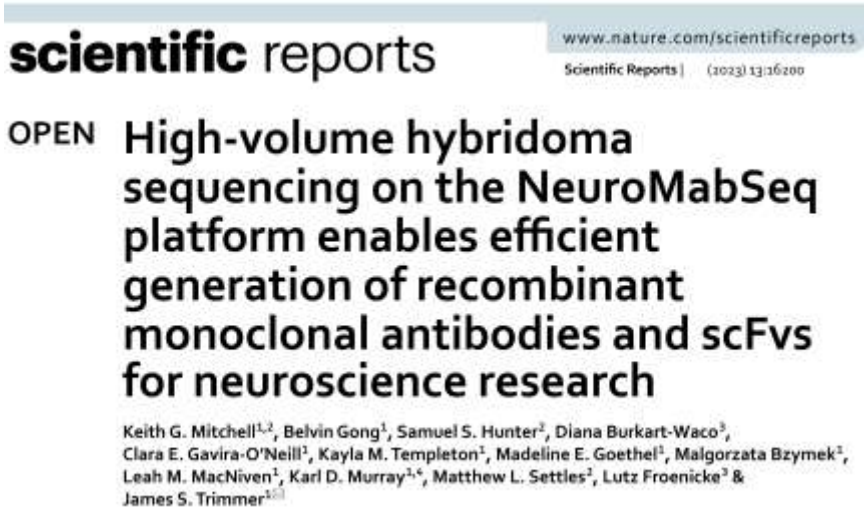
Requires the 4000X cost savings open access antibodies provide

Recombinant Monoclonal Antibodies (R-mAbs) Further Enhance Research Transparency and Reproducibility

- Once you have an R-mAb and its sequence, you no longer need to maintain collections of cryopreserved hybridoma cells, can permanently archive R-mAbs *in silico* as DNA sequence and/or as plasmid DNA
- Easier to disseminate R-mAbs as DNA sequence and/or as plasmids than as hybridoma cells
- R-mAbs are molecularly-defined reagents, can sequence the plasmid before each use and verify that it still encodes the same exact antibody sequence
- Can often obtain more reliable and higher expression from transfected cells than from hybridomas
- Can engineer R-mAbs to enhance their properties

High Throughput Sequencing of NeuroMabs from Cryopreserved Hybridoma Samples

https://neuromabseq.ucdavis.edu/


scientific reports
www.nature.com/scientificreports
Scientific Reports | (2023) 13:16200
OPEN

High-volume hybridoma sequencing on the NeuroMabSeq platform enables efficient generation of recombinant monoclonal antibodies and scFvs for neuroscience research

Keith G. Mitchell[1,2], Belvin Gong[1], Samuel S. Hunter[1], Diana Burkart-Waco[3], Clara E. Gavira-O'Neill[1], Kayla M. Templeton[1], Madeline E. Goethel[1], Malgorzata Bzymek[1], Leah M. MacNiven[1], Karl D. Murray[1,4], Matthew L. Settles[2], Lutz Froenicke[3] & James S. Trimmer[1,2]

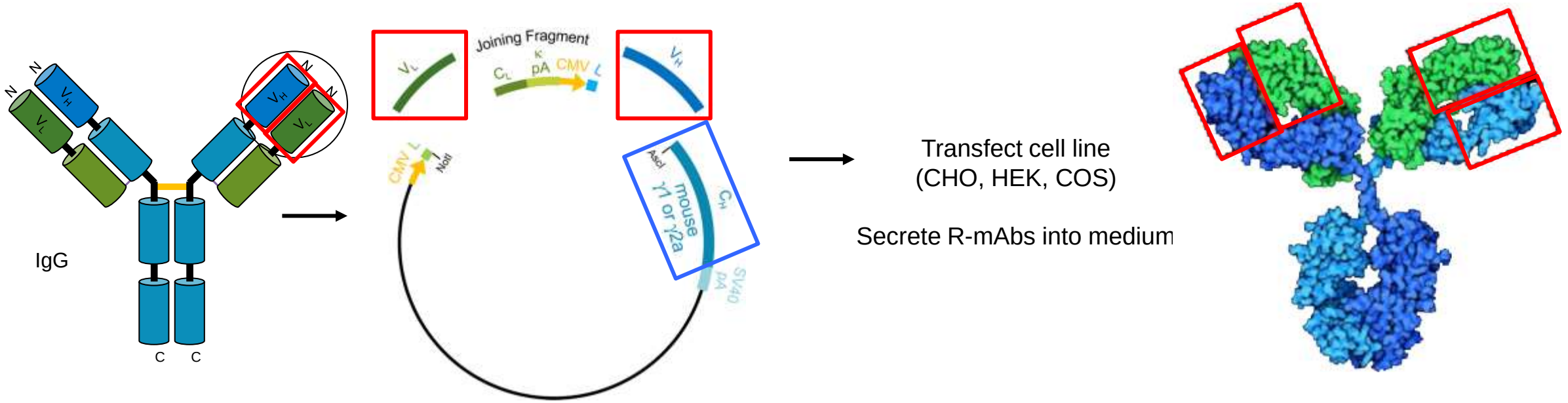
Publicly available dataset from high throughput Illumina sequencing of >8,000 hybridoma samples

The searchable database includes detailed sequencing information for each entry, as well as analyses of the crucial domains of each antibody that define their structure, efficacy and specificity.

OPEN ACCESS

Mitchell et al., Sci. Rep. 13:16200, 2023

Converting Monoclonal Antibodies Into Recombinant Form



Synthesize gene fragments encoding antibody variable domains

Gibson Assembly ligation into an expression plasmid

Express recombinant mAb (R-mAb) from transfected mammalian cell lines

Validate R-mAb in side-by-side comparisons to conventional mAb by IHC, ICC, IB

Mitchell et al., Sci. Rep. 13:16200, 2023

Open Access Availability of Our Recombinant Monoclonal Antibodies

Have now deposited ≈700 validated R-mAb plasmids at Addgene

Addgene has distributed ≈>2,200 plasmid samples on our behalf

addgene
The nonprofit plasmid repository

Have also partnered with Addgene to produce ready-to-use R-mAbs from these plasmids

*For the first time, researchers can obtain the ready-to-use antibody, the plasmid that encodes it and the entire plasmid sequence from a single source

BRAIN Initiative award NIH U24 NS119916: NABOR: A Sustainable, High-quality “Neuroscience AntiBody Open Resource”

OPEN ACCESS

Open-Source Software and Antibodies

Computers are machines that execute programs written in software code.

Open-source software makes this code widely available to the user community.

This greatly expands the opportunity for informed use and subsequent improvement.

The result is higher quality software, increased security through vulnerability detection, and greater customization for end users, all while often being cost-effective due to its free access.

Cells are biological machines built by executing programs written in the genetic code of four nucleotides.

The code for open-source antibodies is widely available to the user community.

This greatly expands the opportunity for informed use and subsequent improvement.

The result is higher quality antibodies, increased confidence by using a molecularly defined reagent, and greater customization for end users, all while often being cost-effective due to its free access.

Asay CD. Software's legal future. Front Res Metr Anal 7:980744, 2022

Open-Source Antibodies

New BIOTECHNOLOGY 87 (2025) 121–129

Contents lists available at ScienceDirect

New BIOTECHNOLOGY

journal homepage: www.elsevier.com/locate/nbt

Open-source antibodies as a path to enhanced research reproducibility and transparency

Meghan Rego [a], Douglas W. Houston [b], Melina Fan [a], Karl D. Murray [c], James S. Trimmer [c,*]

[a] Addgene, Watertown, MA, United States
[b] Developmental Studies Hybridoma Bank, Department of Biology, University of Iowa, Iowa City, IA, United States
[c] UC Davis/NIH NeuroMab Facility, Department of Physiology and Membrane Biology, University of California School of Medicine, Davis, CA, United States

We encourage researchers to take advantage of existing open-source antibodies to enhance the transparency and reproducibility and cost-effectiveness of their own research.

We encourage researchers who have developed antibodies to make them open source, with a goal of enabling more reproducible, transparent and cost-effective research in the community at large.

We encourage funding agencies to support the further development of open-source antibody resources.

Acknowledgements

NeuroMab

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UNIVERSITY OF CALIFORNIA

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Nick Andrews
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Karl Murray
Kayla Templeton

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Randy Stewart
Ned Talley
Natalie Trzcinski

BRAIN INITIATIVE
BRAIN RESEARCH THROUGH ADVANCING INNOVATIVE NEUROTECHNOLOGIES

THE UNIVERSITY OF CALIFORNIA · LET THERE BE LIGHT · DAVIS

UC Davis Genome Center Cores

DNA TECH
DNA Technologies & Expression Analysis Core Laboratory

Lutz Froenicke, Director
Diana Burkart-Waco

UC Davis Bioinformatics Core

Matt Settles, Director
Sam Hunter

addgene
The nonprofit plasmid repository

Melina Fan, CSO
Meghan Rego

DSHB
Developmental Studies Hybridoma Bank
Antibodies at the University of Iowa for use in research

Doug Houston, Director
Karla Daniels

Publicly available sources for products of large-scale antibody initiatives

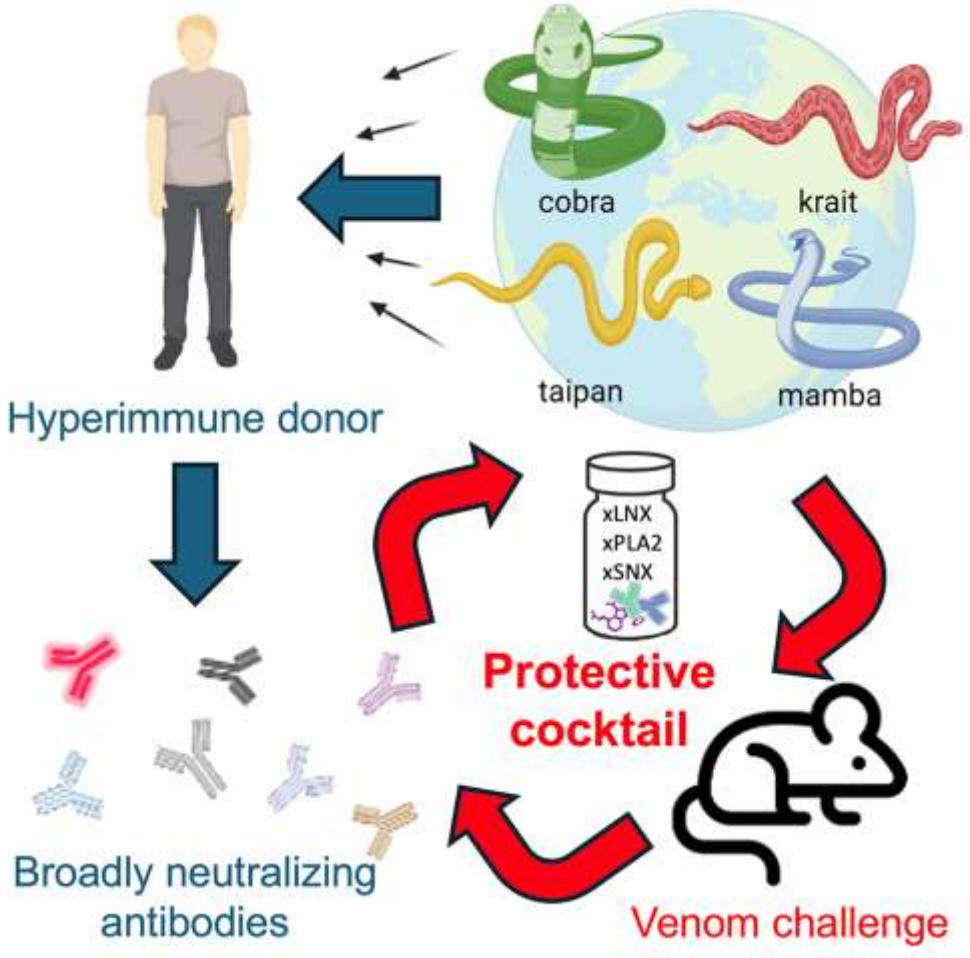
Initiative	Funding Source	Primary target areas#	Form	Number of Abs made available
Human Protein Atlas	Wallenberg Foundation	Entire proteome	pAbs	20,000
Affinomics	EU	Protein kinases and related signaling proteins	mAbs	250
Trimmer Lab/NeuroMab	NIH	Receptors, ion channels, scaffolds, epigenetics, cell markers	mAbs	500*
Trimmer Lab/NeuroMab			R-mAbs	800*
PCRP	NIH	Transcription factors	mAbs	1,200
PCRP			R-mAbs	850
CPTAC	NIH	Diverse cancer targets	mAbs	850
CPTAC			R-mAbs	100
BICCN	NIH	Diverse brain targets	mAbs	25 (out of 600 made)
BICCN			R-mAbs	0

One “Novel” Approach to Targeted Antibody Development

“He injected himself with snake venom hundreds of times (2000-2018). His blood could ‘revolutionize’ snakebite treatment”

https://www.cnn.com/2025/05/02/science/antivenom-snakebite-treatment-tim-friede

Is now employed by biotechnology company Centivax



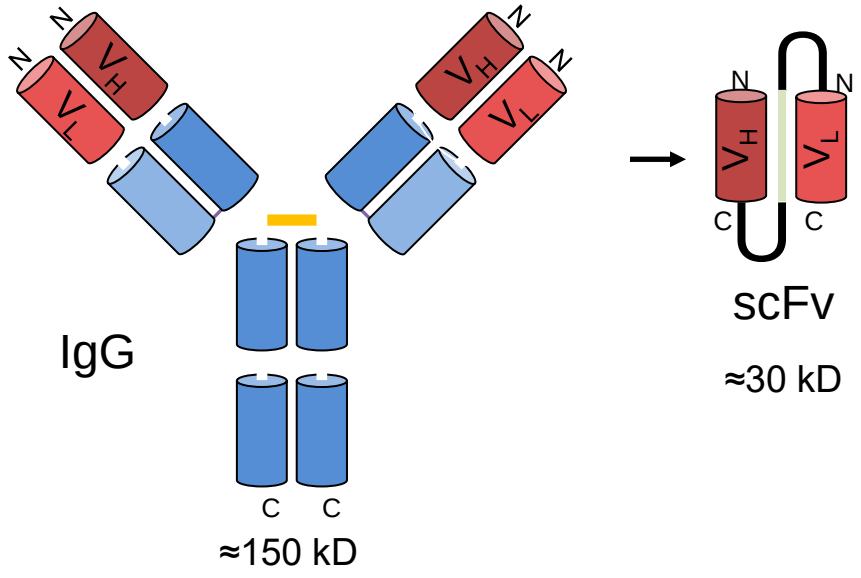
Glanville et al., Cell Apr 30, 2025. Online ahead of print.

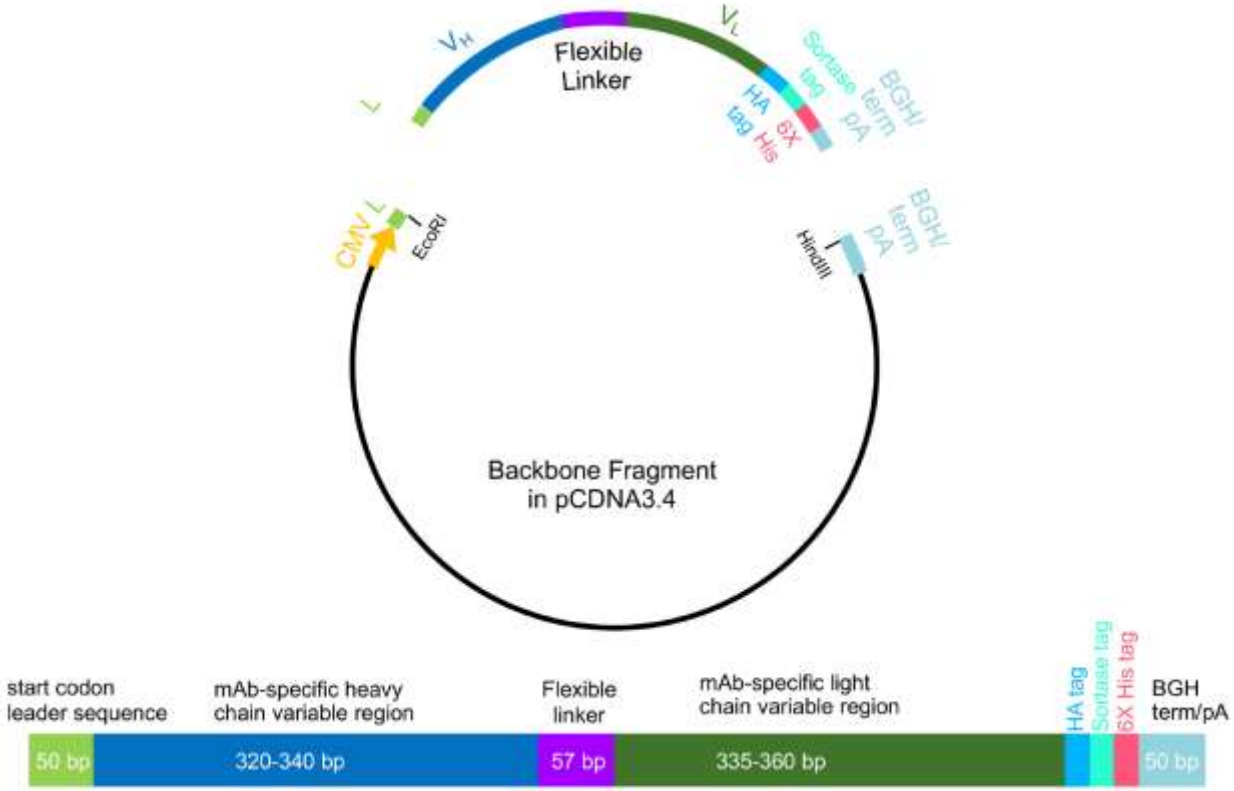
Generating Single-Chain Variable Fragments (scFvs) from Hybridoma-derived Sequences

Small size of scFvs offers advantages for higher imaging resolution and enhanced tissue penetration.

Can also be used as genetically-encoded intracellular antibodies or intrabodies.

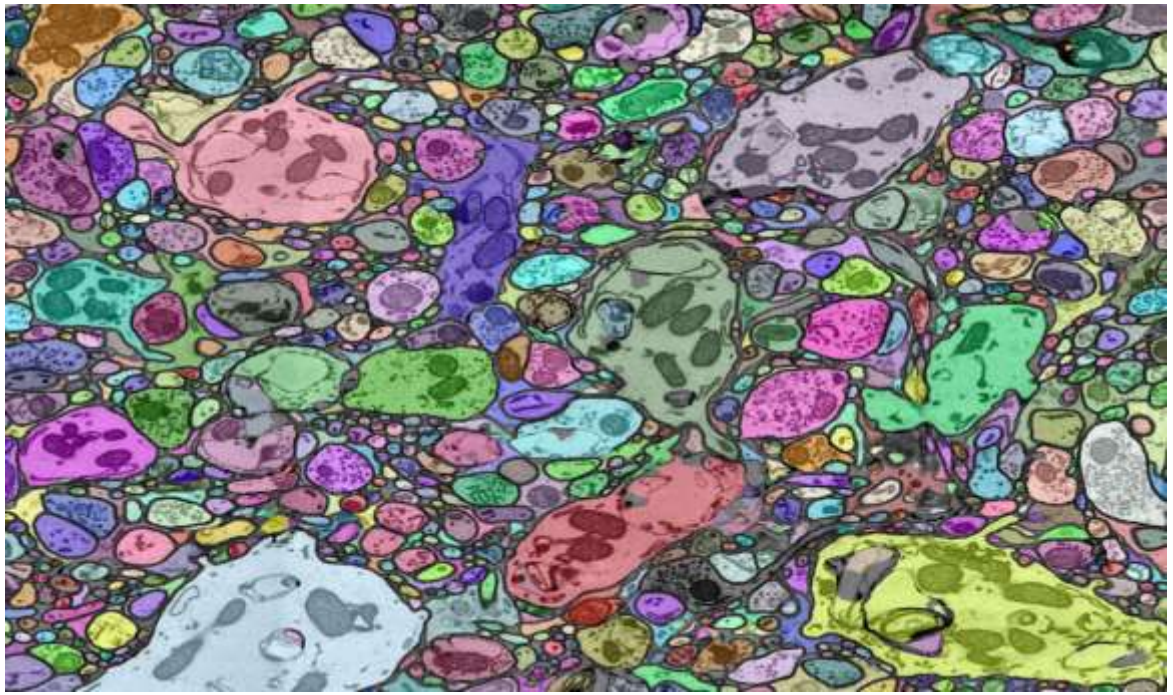
All plasmids for validated scFvs have been deposited at Addgene.





The Enhanced Tissue Penetration of scFvs Enables Higher Resolution Imaging of Brain Tissue at the Ultrastructural Level

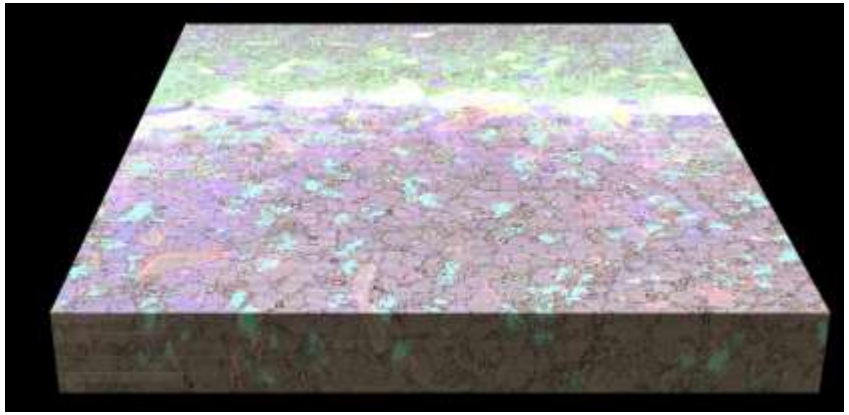
30 nm thick section

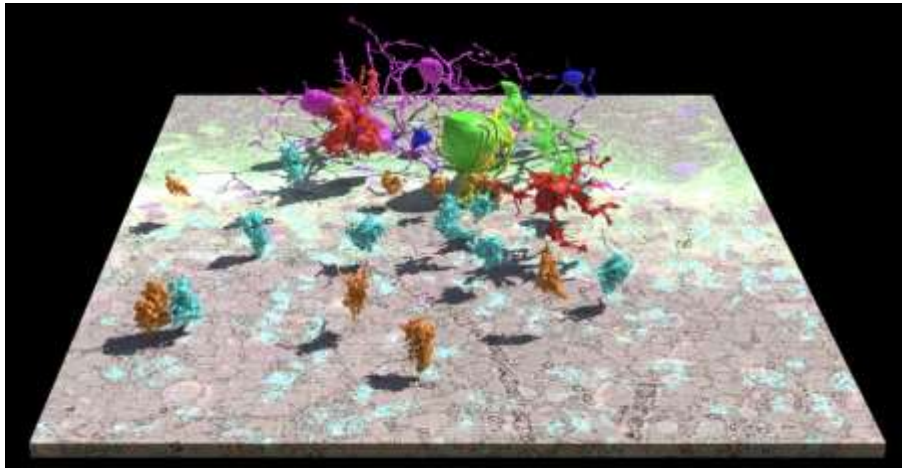


Directly labeled scFvs allow for efficient multiplex labeling of brain samples prepared without detergent permeabilization

Allows for excellent ultrastructure in correlative light and electron microscopy

≈30 µm thick block





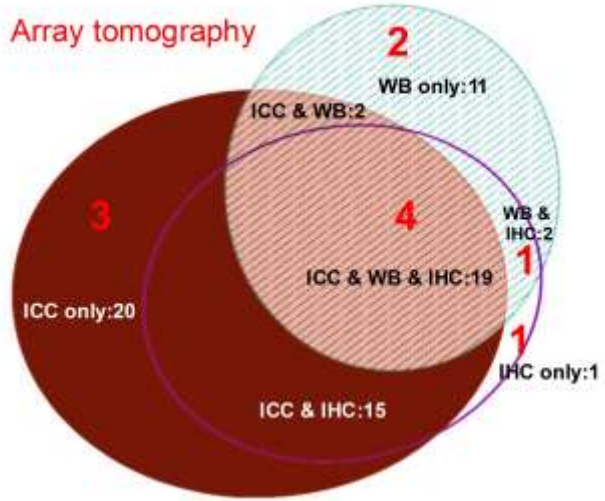
Also see “Probing Molecular Diversity and Ultrastructure of Brain Cells with Fluorescent Aptamers.” bioRxiv doi: 10.1101/2023.09.18.558240.

Han et al., 2024. Nat Commun 15:6648.

Designer Antibodies

Improving existing antibodies

Solubility
Stability
Off-target “stickiness”
Humanization
Many other characteristics related to efficacy, specificity and developability



De novo design

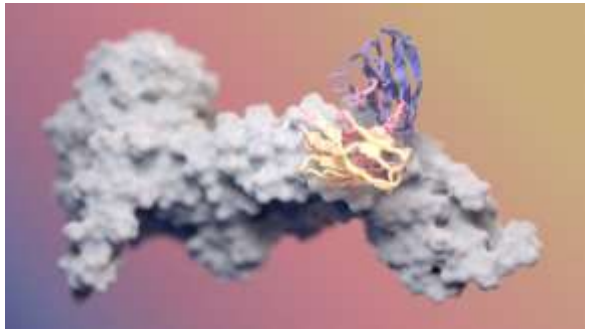
Nobel Prize in Chemistry 2024
David Baker: for computational protein design
Demis Hassabis: for protein structure prediction
John Jumper: for protein structure prediction

Atomically accurate de novo design of antibodies with RFdiffusion

bioRxiv [Preprint]. Posted March 18, 2024.
PMID 38562682

Today we are sharing a significant update in the use of AI to generate antibodies. In a new preprint, we introduce a version of RFdiffusion fine-tuned to design human-like antibodies. We are also making this software free to use for both non-profit and for-profit research, including drug development.

Baker lab website



How much money is wasted on “bad” antibodies?

Research antibody market ≈$1.6B in 2023 (clinical diagnostic ≈$110B, therapeutic ≈$250B)

World life science research spending ≈$300B
NIH budget ≈$48B, or ≈16% of total life science research spending

Published antibody functionality 5-49%, depending upon assay

Suggests worldwide ≈$800M-$1.5B (51-95%) is wasted **each** year on research antibodies that don’t work

If everything is proportional, NIH spends $130M-$240M annually on antibodies that don’t work.

This is direct costs (antibody purchase price) only, does not factor in staff salary and benefit costs for time spent doing failed/worthless experiments, cost of associated reagents, wasted patient samples, etc., as well as the huge cost (the ripple effect) of misinformation in the scientific literature.

Info courtesy of Andrew Bradbury, LANL. 2014. Updated 2023.
Also see Bradbury & Plückthun, Nature 518, 27–29 (05 February 2015)