Tools for understanding brain computations

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MIT BE Biological Engineering

brain+cognitive sciences

CNBE MIT Center for Neurobiological Engineering

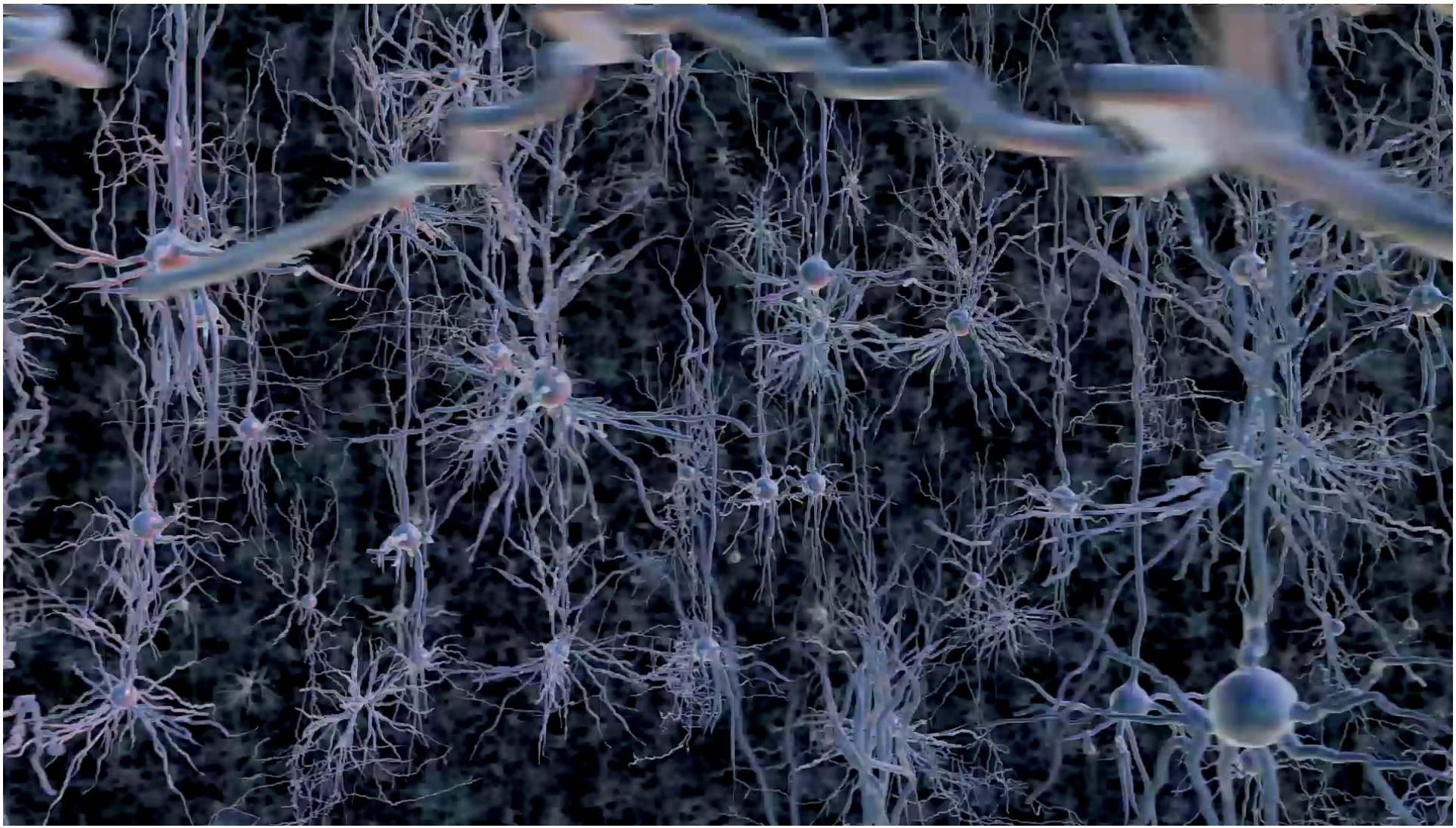
NYSCF The New York Stem Cell Foundation

Understanding complex biological systems, like the brain, is difficult because of wide-ranging spatial and temporal scales

Space: Nanoscale building blocks, spanning centimeters

Understanding complex biological systems, like the brain, is difficult because of wide-ranging spatial and temporal scales

Time: Millisecond events, spanning seconds to years

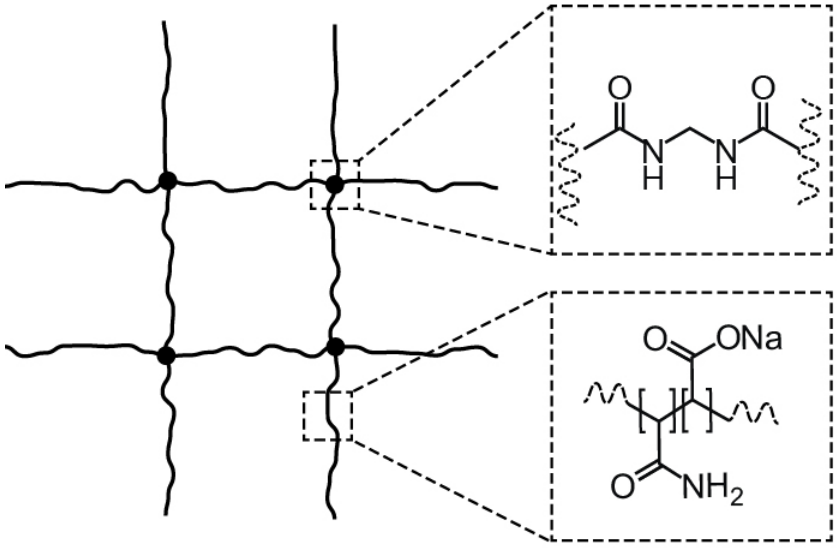


Map: molecules

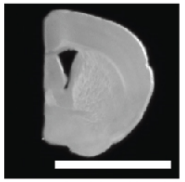
How can we map complex biological systems, like brain circuits, with molecular precision?

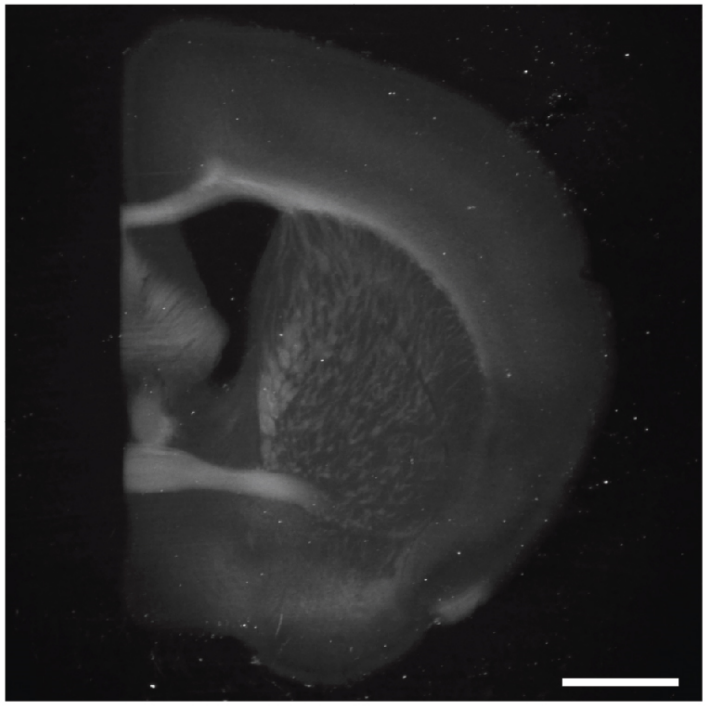
Physical magnification: a new principle of microscopy

Sodium polyacrylate:
polymer that swells in water
(found in baby diapers)



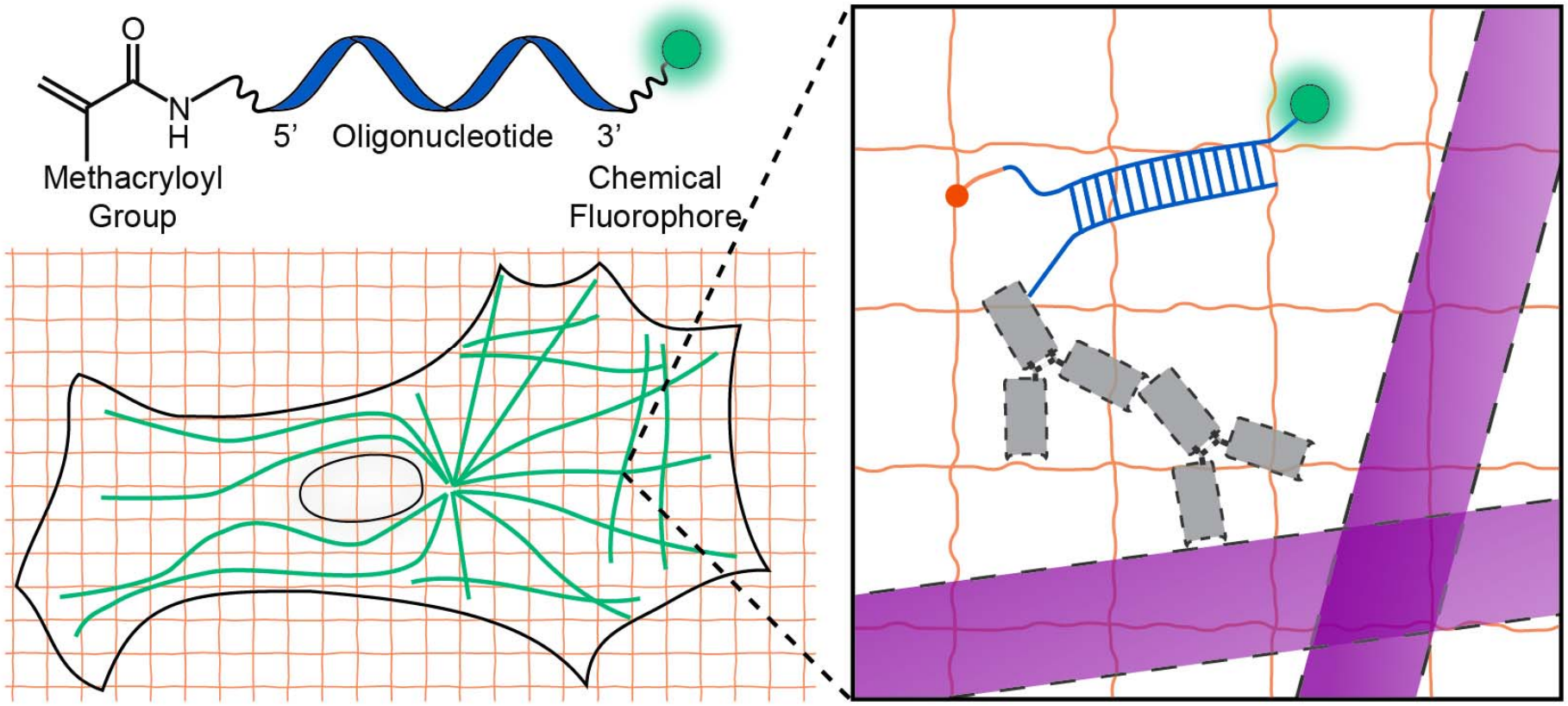
Embed brain
in permeating polyacrylate
network, then add water





Chen*, Tillberg*, Boyden (2015) *Science* 347(6221):543-548. http://expansionmicroscopy.org
Commercialized by Expansion Technologies, Inc. (ESB is co-founder)

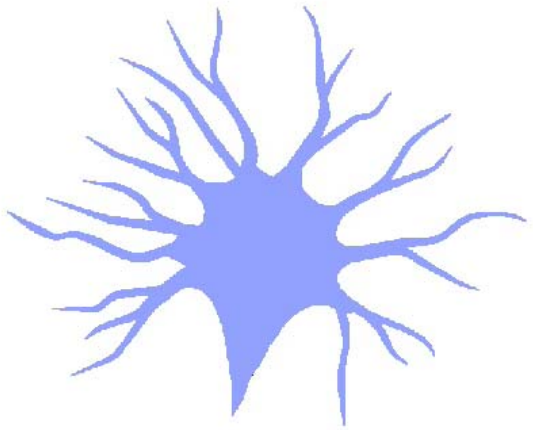
Expansion Microscopy (ExM)



1. Preserve tissue by fixation and permeabilization.
2. Immunostain with a fluorescent label bearing a polymer-linkable group.
3. Diffuse in acrylate monomer, and polymerize, linking fluorescent label to polymer.
4. Use protease to digest everything but the polymer and polymer-anchored labels.
5. Add water to swell the polymer.

Chen*, Tillberg*, Boyden (2015) *Science* 347(6221):543-548.

ExM Process

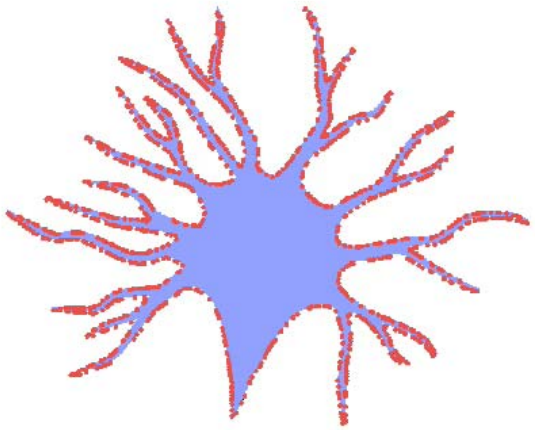


10 microns

- Fix specimen
- Tri-functional label
- Form swellable gel
- Digest specimen
- Expand in water

Chen*, Tillberg*, Boyden (2015) *Science* 347(6221):543-548.

ExM Process



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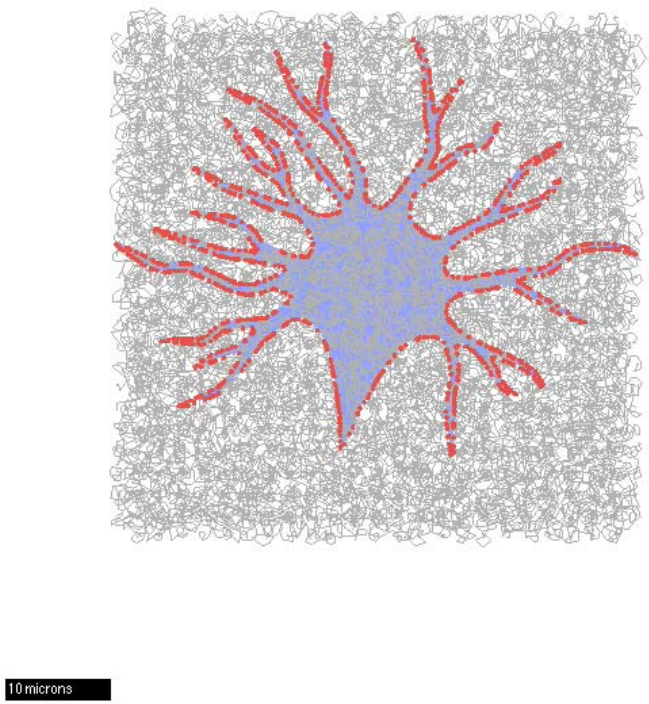
ExM Process



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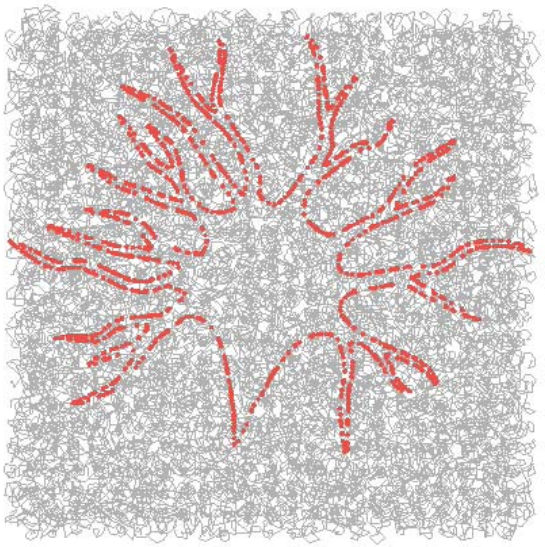
ExM Process



- Fix specimen
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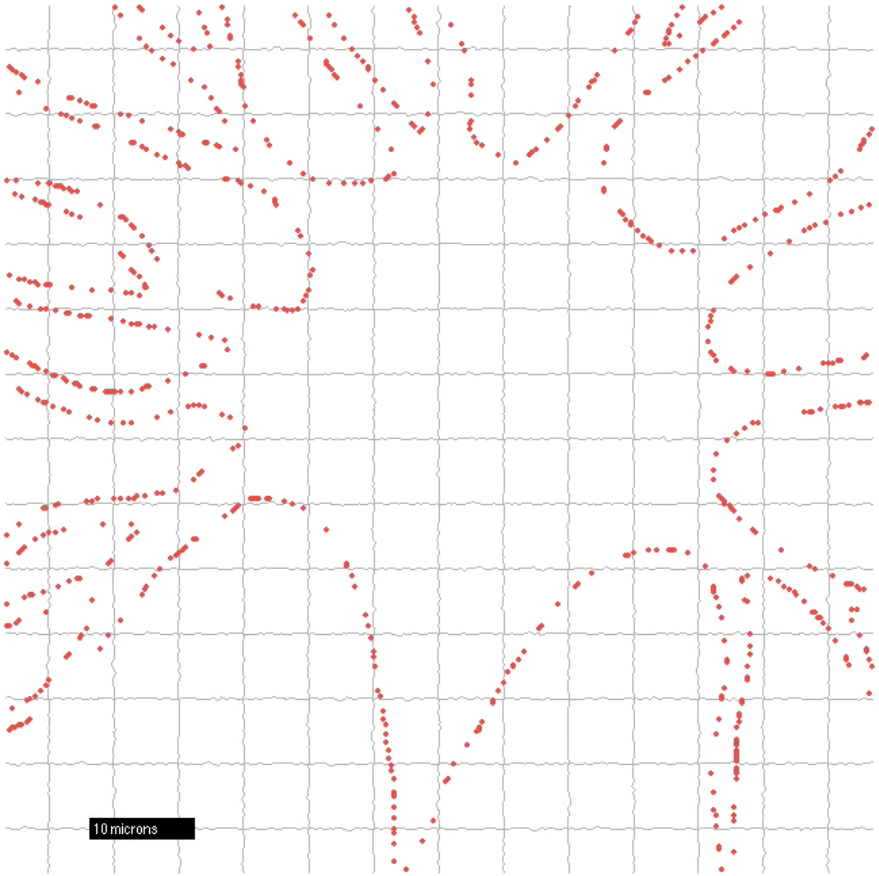
Chen*, Tillberg*, Boyden (2015) *Science* 347(6221):543-548.

ExM Process

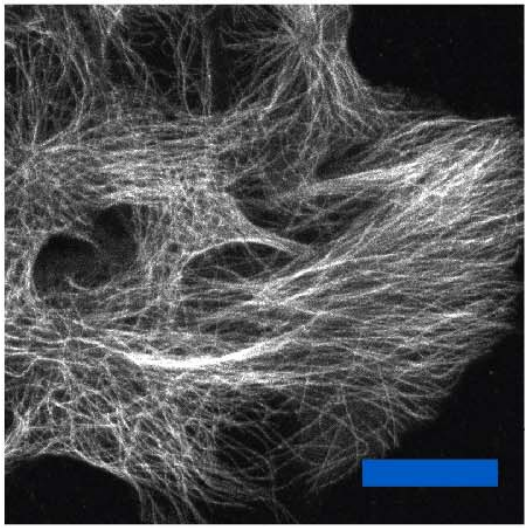


- Fix specimen
- Tri-functional label
- Form swellable gel
- Digest specimen
- **Expand in water**

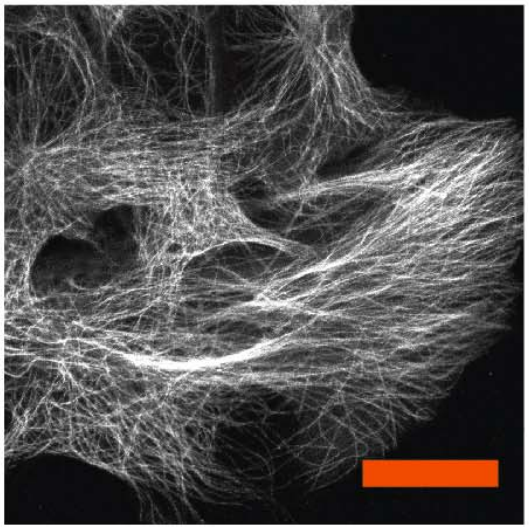
Chen*, Tillberg*, Boyden (2015) *Science* 347(6221):543-548.

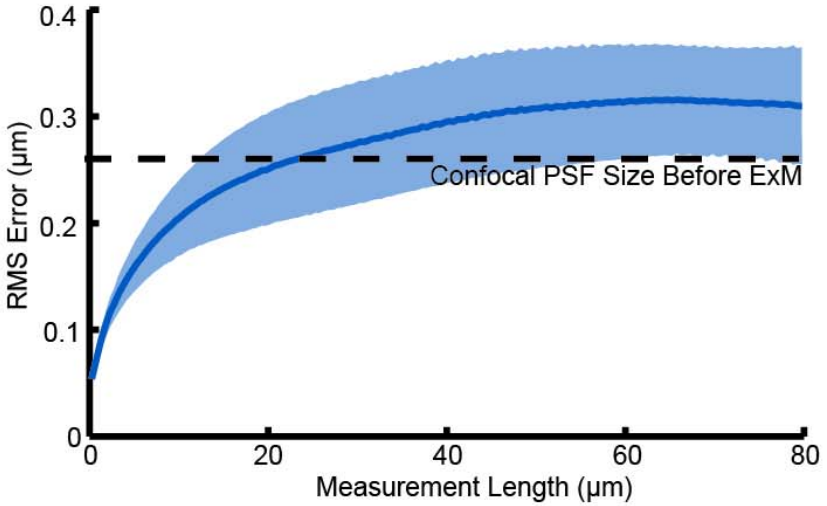
ExM preserves global isotropy: cultured cells, microtubules

Before expansion: confocal microscopy



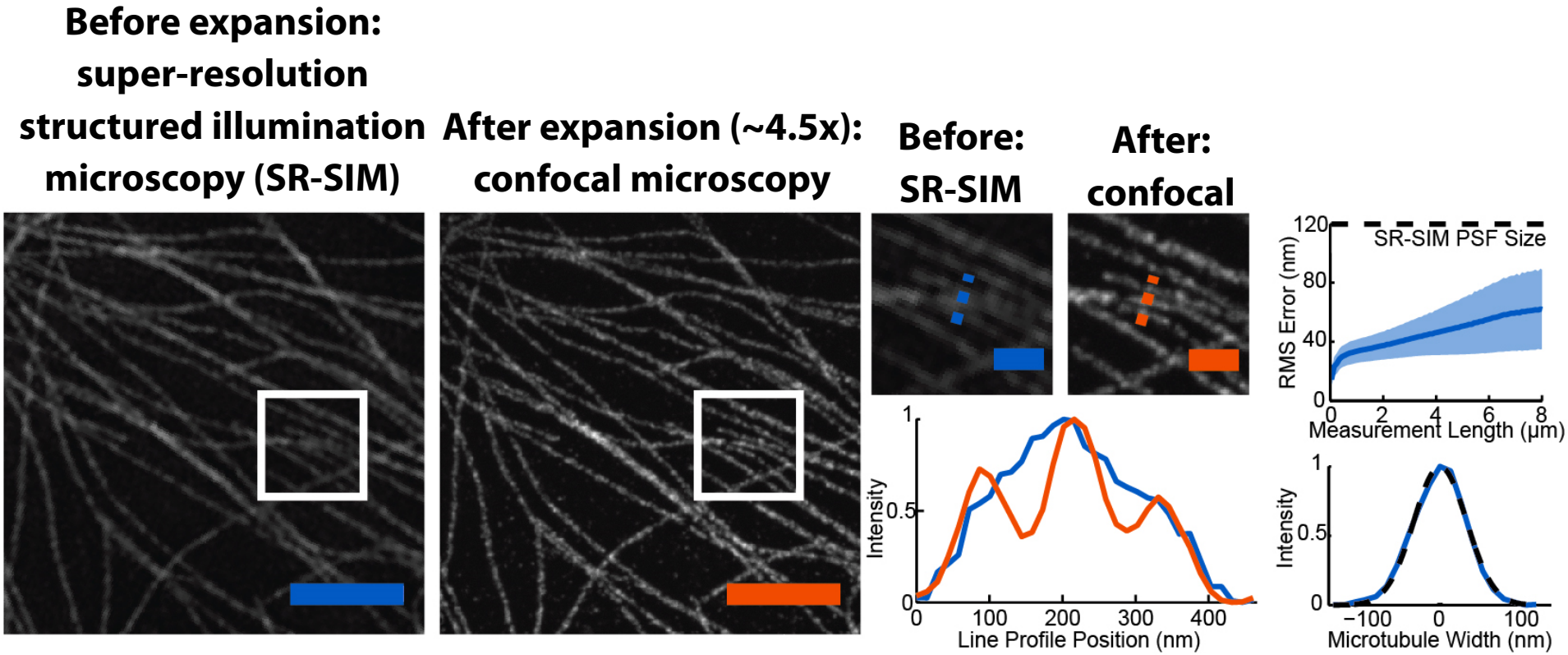
After expansion (~4.5x): confocal microscopy





Chen*, Tillberg*, Boyden (2015) *Science* 347(6221):543-548.

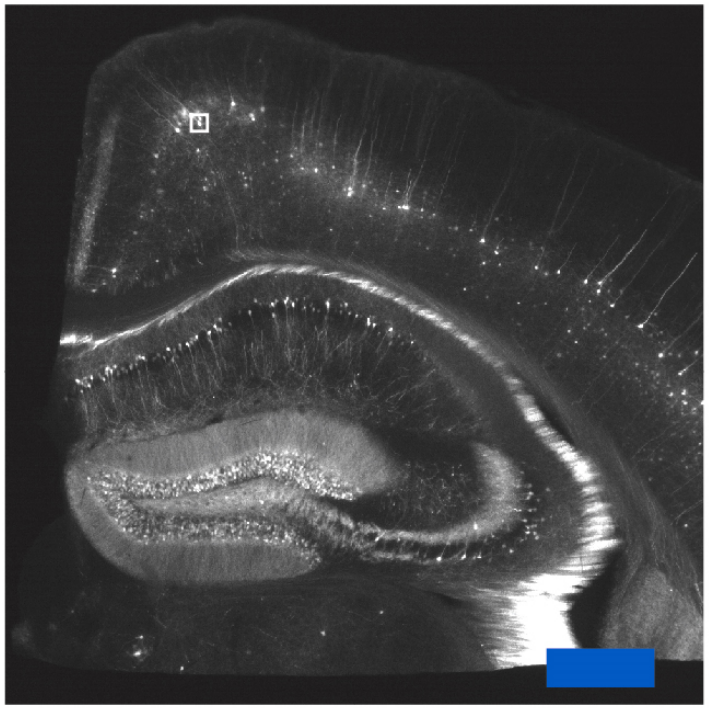
ExM preserves nanoscale isotropy: cultured cells, microtubules



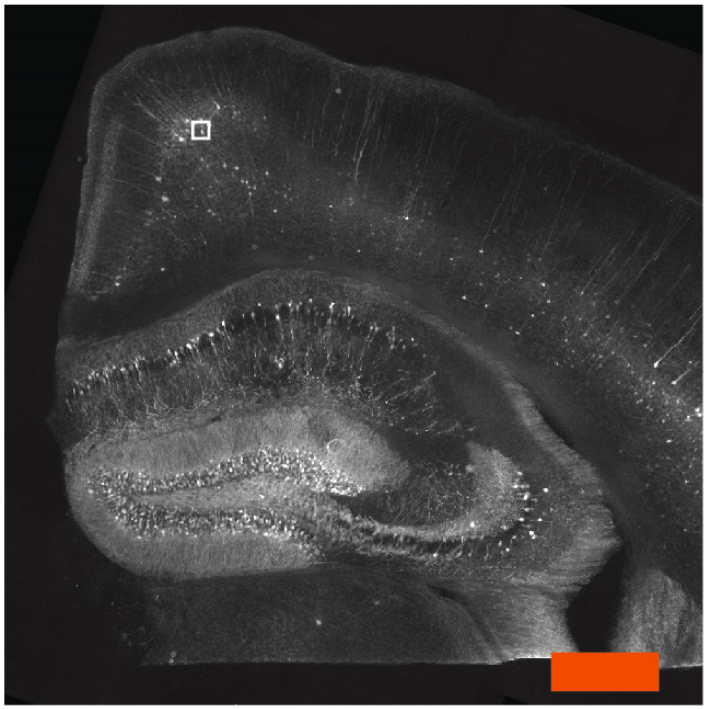
Chen*, Tillberg*, Boyden (2015) *Science* 347(6221):543-548.

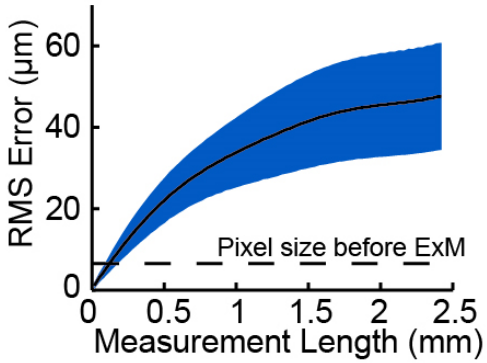
ExM preserves global isotropy: brain, YFP-expressing neurons

Before expansion: confocal microscopy



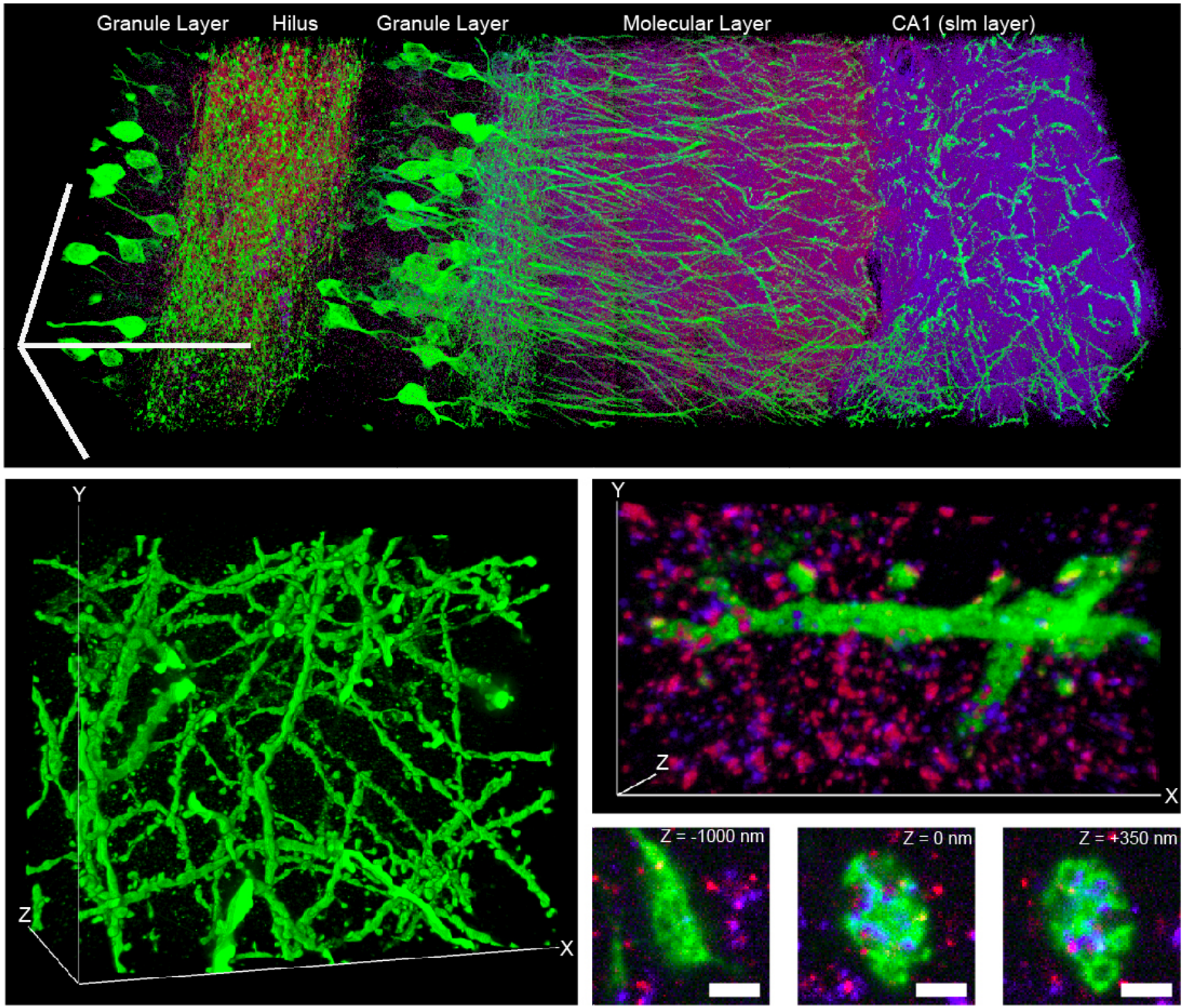
After expansion (~4.5x): confocal microscopy





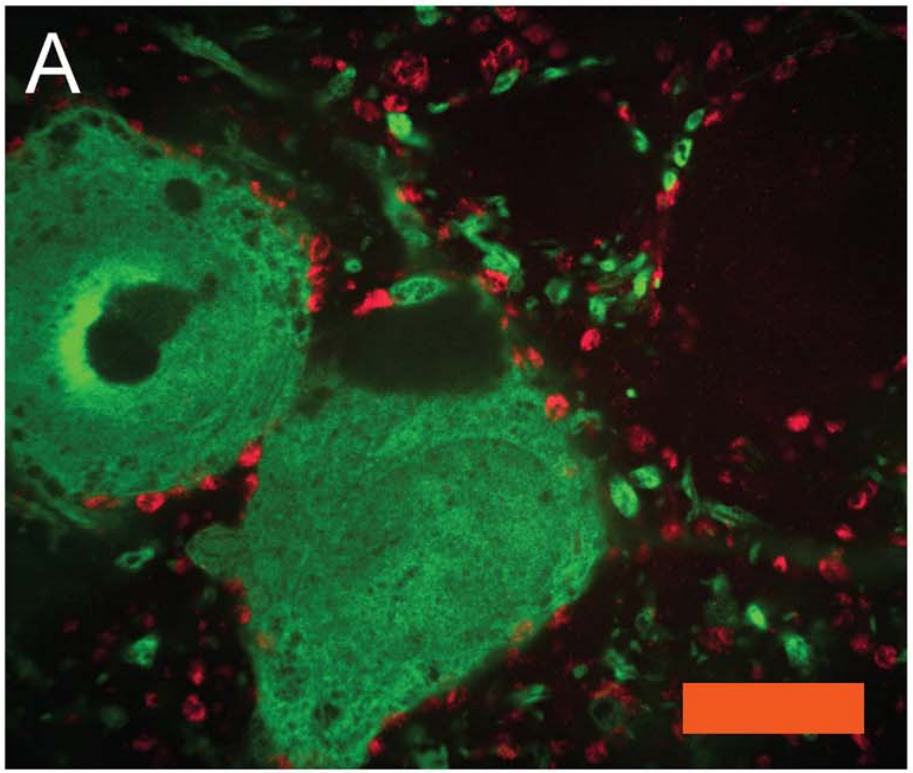
Chen*, Tillberg*, Boyden (2015) *Science* 347(6221):543-548.

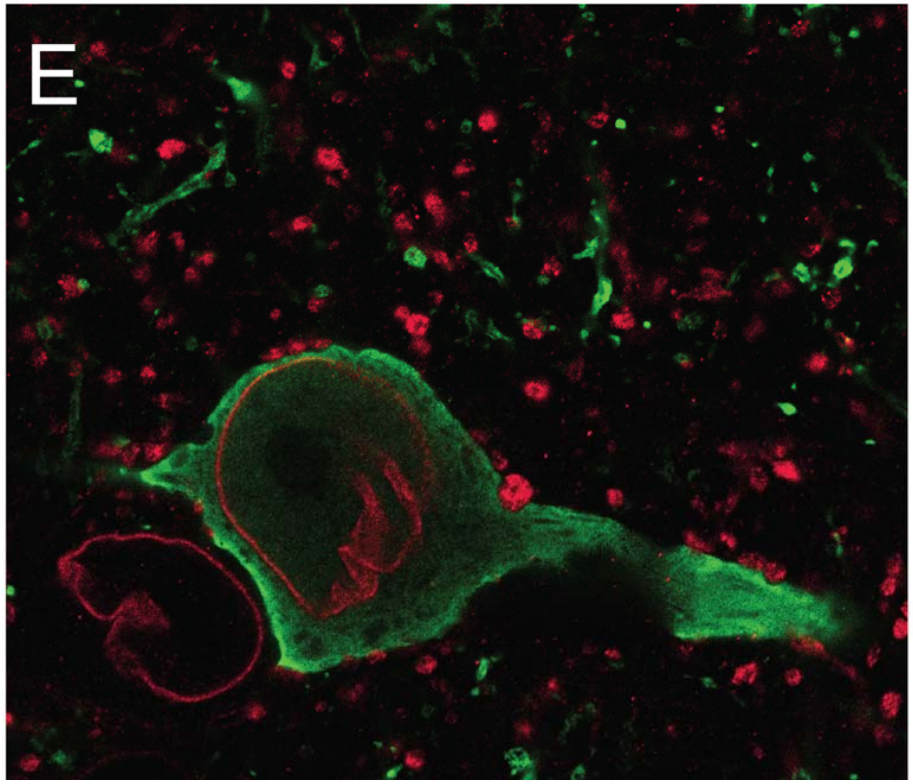
ExM: voxel sizes of a super-resolution microscope, voxel rates of a diffraction-limited microscope



Chen*, Tillberg*, Boyden (2015) *Science* 347(6221):543-548.

Lots of interesting things to look at

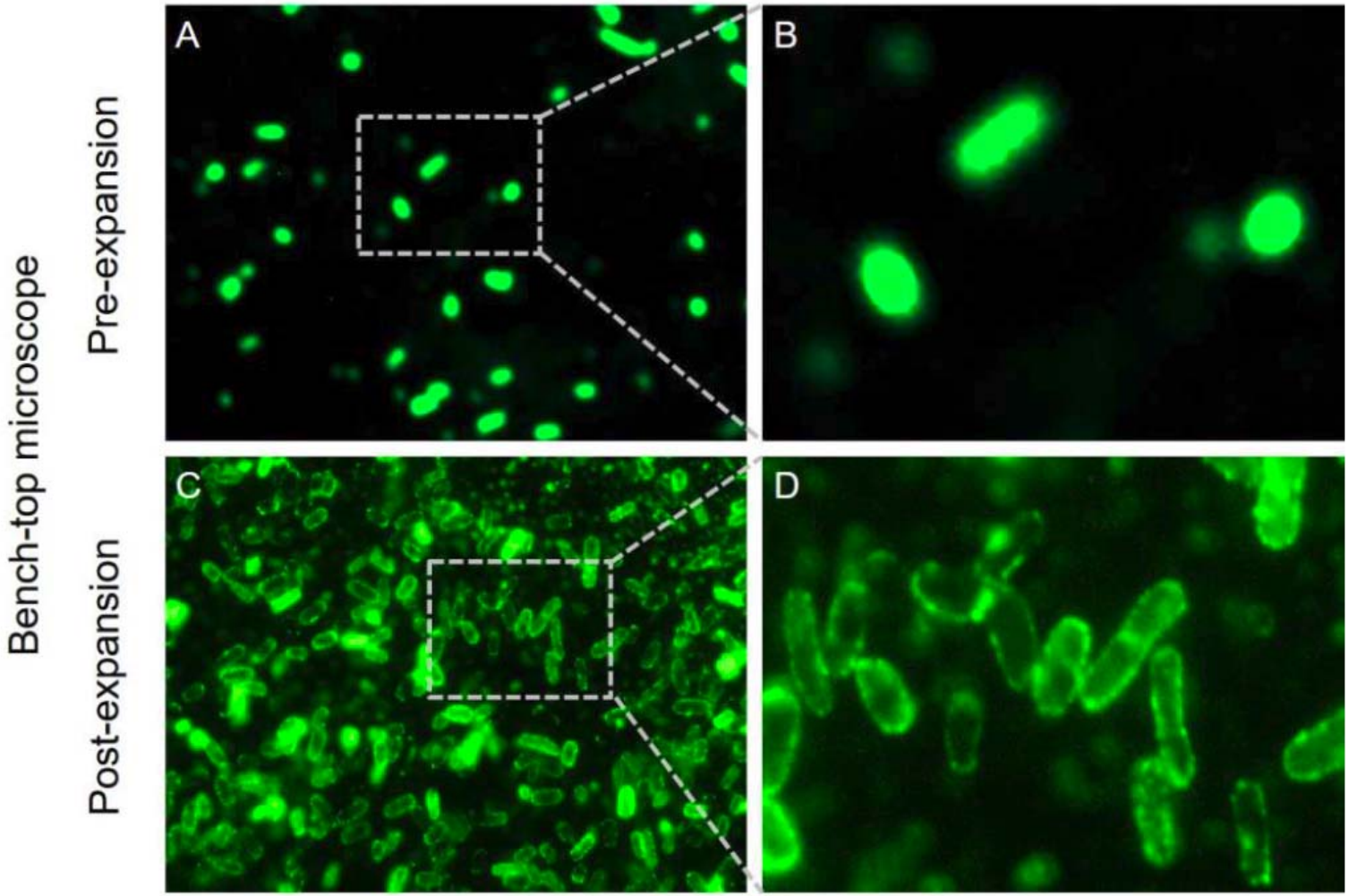




ExM imaging of antibodies of interest in neuroscience and biology. Confocal images of expanded Thy1-YFP mouse brain cerebral cortex sections stained with anti-GFP (green) and antibodies against other proteins (red) as follows: (A) GAD65/67 (*22*), (B) ChAT (*23*), (C) CaMKII (*24*), (D) GABA (*25*), (E) Lamin A/C (*26*), (F) NMDAR2a/b (*27*). Scale bars: (A) 10 μm in pre-expansion units (physical size post-expansion, 45 μm); (B) 10 μm (47 μm); (C) 10 μm (40 μm); (D) 10 μm (44 μm); (E) 10 μm (43 μm); (F) 10 μm (43 μm).

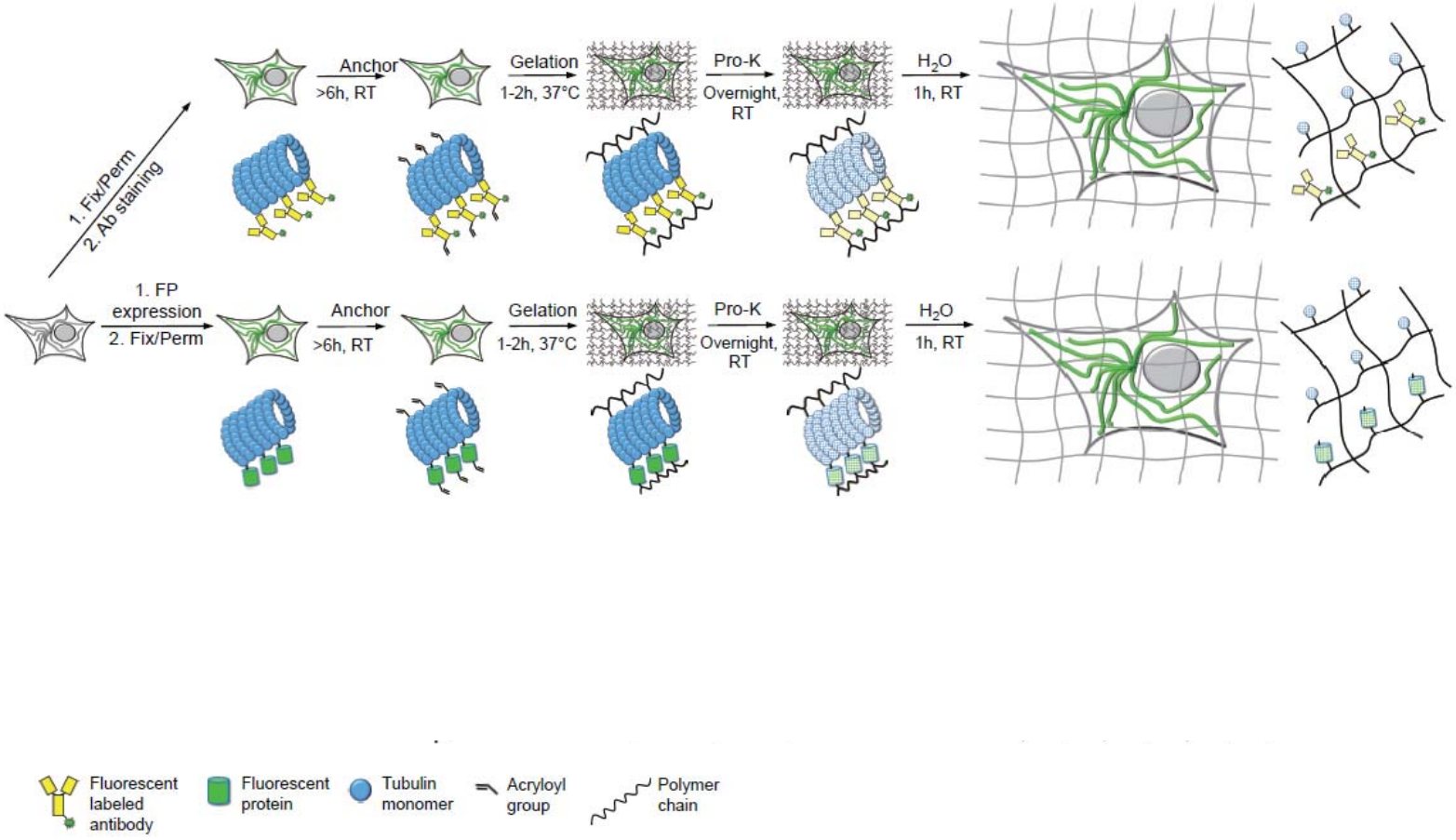
Chen*, Tillberg*, Boyden (2015) *Science* 347(6221):543-548.

Bacteria



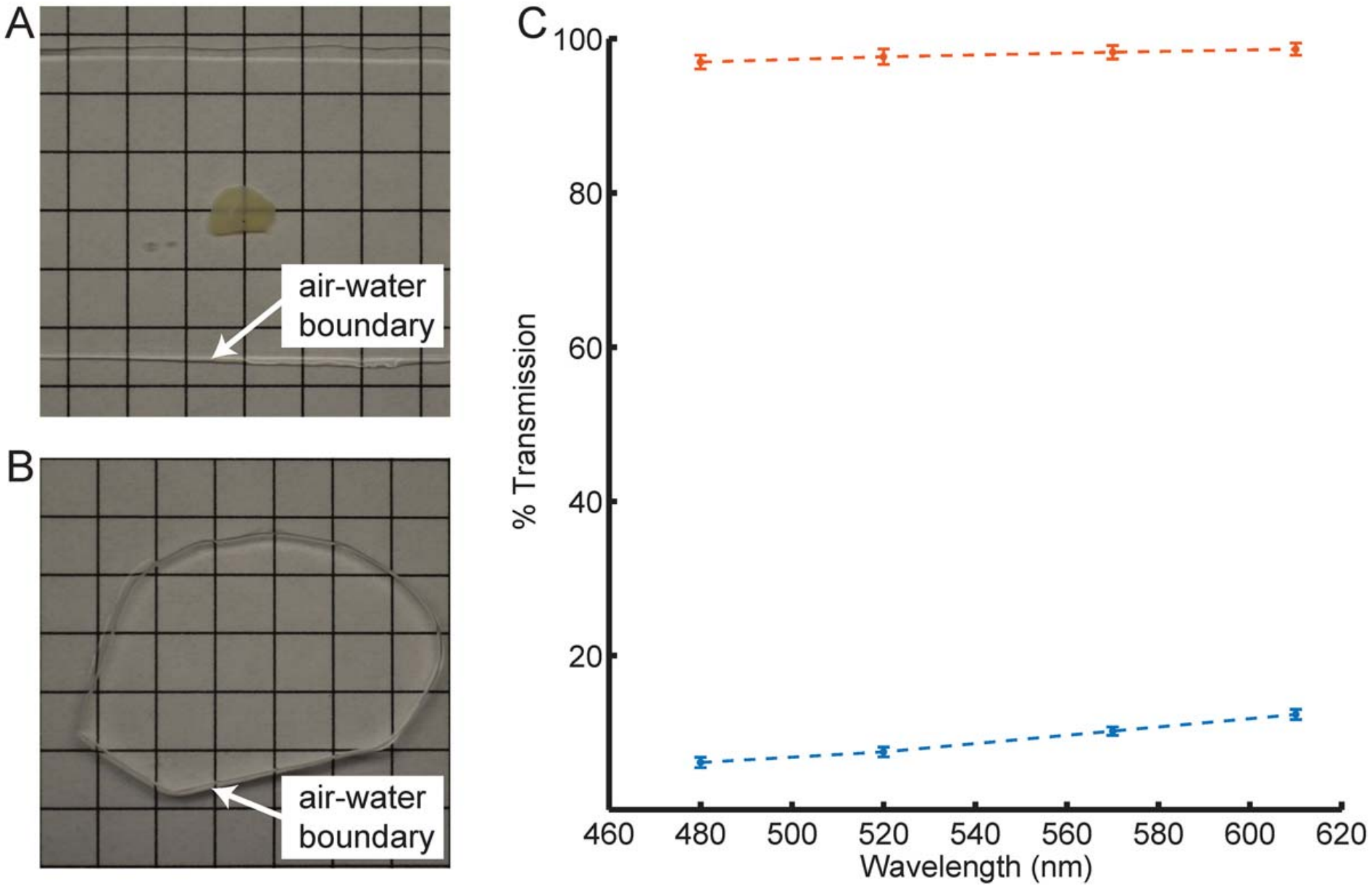
Zhang YS*, Chang JB*, et al. (2016) *Scientific Reports* 6:22691.

proExM: ExM with commercially available chemicals, like a normal immunohistochemistry workflow



Paul W. Tillberg, Fei Chen, Kiryl D. Piatkevich, Yongxin Zhao, Chih-Chieh (Jay) Yu, Brian P. English, Linyi Gao, Anthony Martorell, Ho-Jun Suk, Fumiaki Yoshida, Ellen DeGennaro, Douglas H. Roossien, Robert Desimone, Dawen Cai

Expanded samples are almost completely transparent



Chen*, Tillberg*, Boyden (2015) *Science* 347(6221):543-548.

Record: dynamics

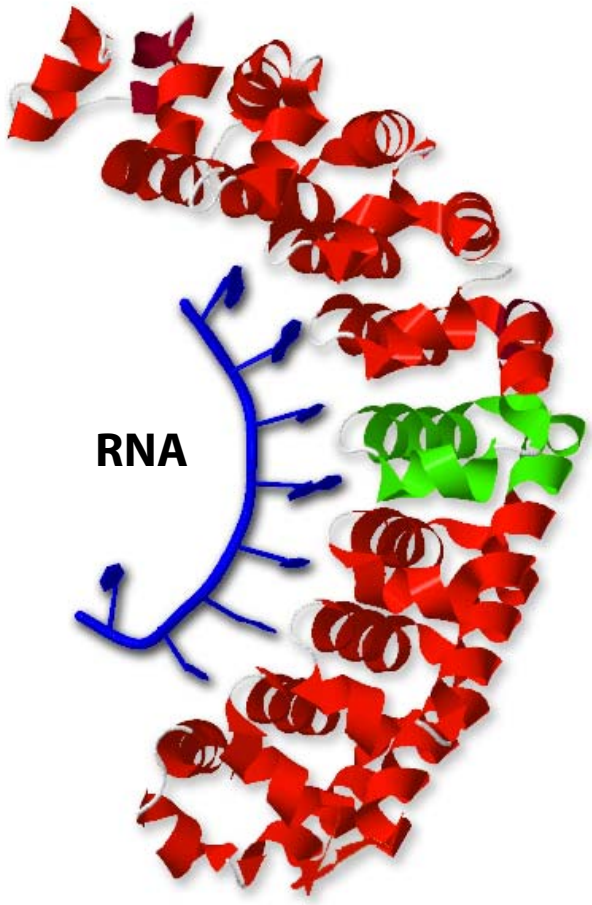
How can we record biological dynamics with precise temporal, and broad spatial, scale?

A scaffold for designing RNA-binding proteins

Pumilio homolog 1 (PumHD): sequence-specific RNA-binding protein that regulates translation and mRNA stability by binding the 3'-UTR of mRNA targets.

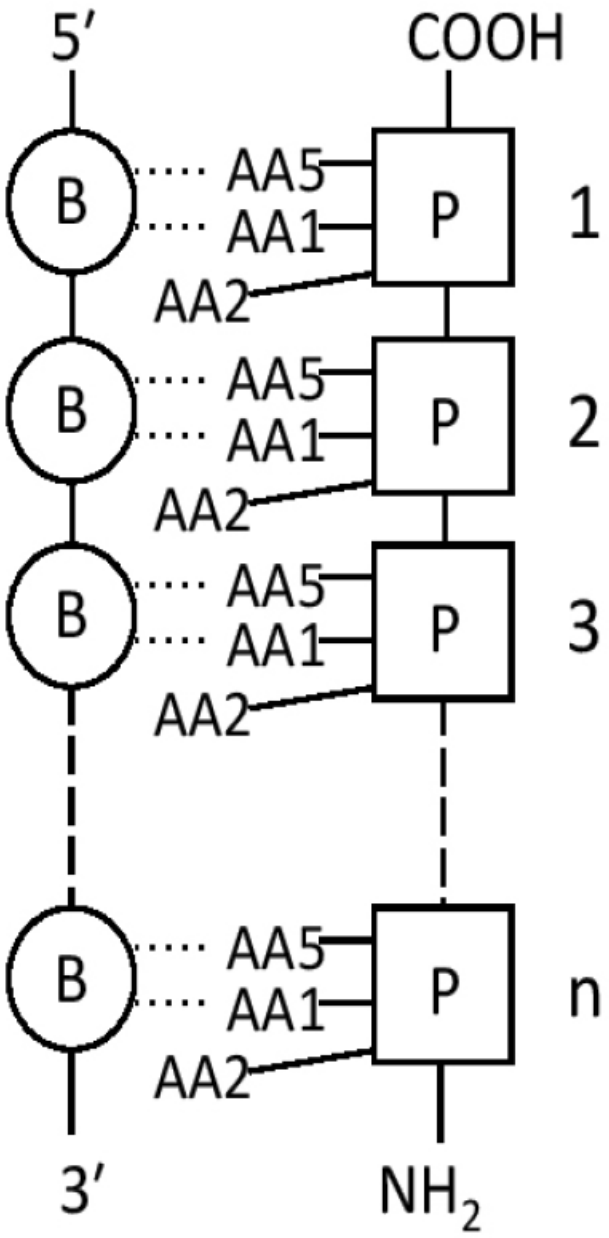
It's made of a series of **36 amino acid** repeats. Each **repeat** binds a single RNA base.

Each 36 amino acid binding unit is a helical structure with **three amino acids** involved in contact with RNA.

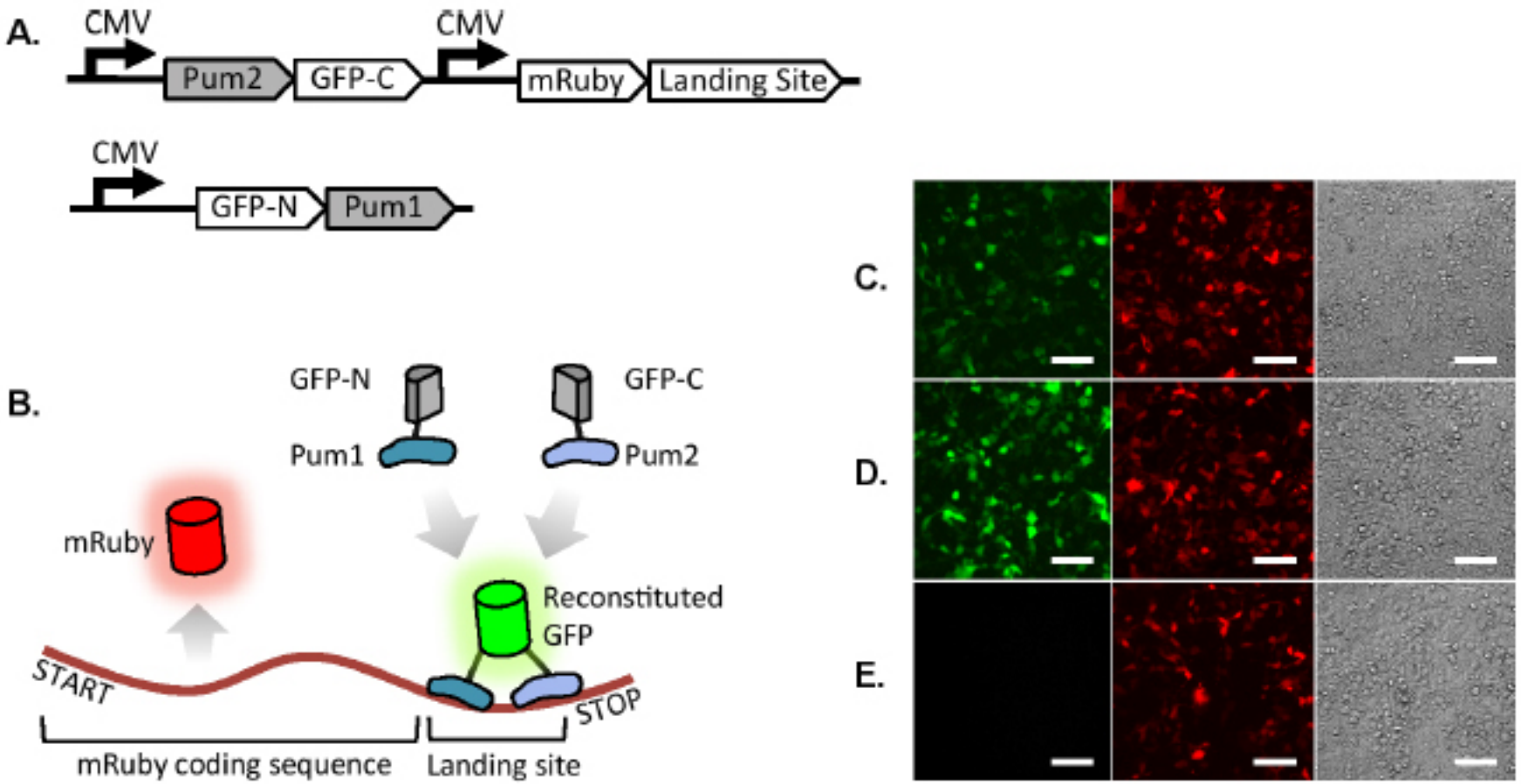


human Pumilio1 in complex with RNA; PDB 1M8X

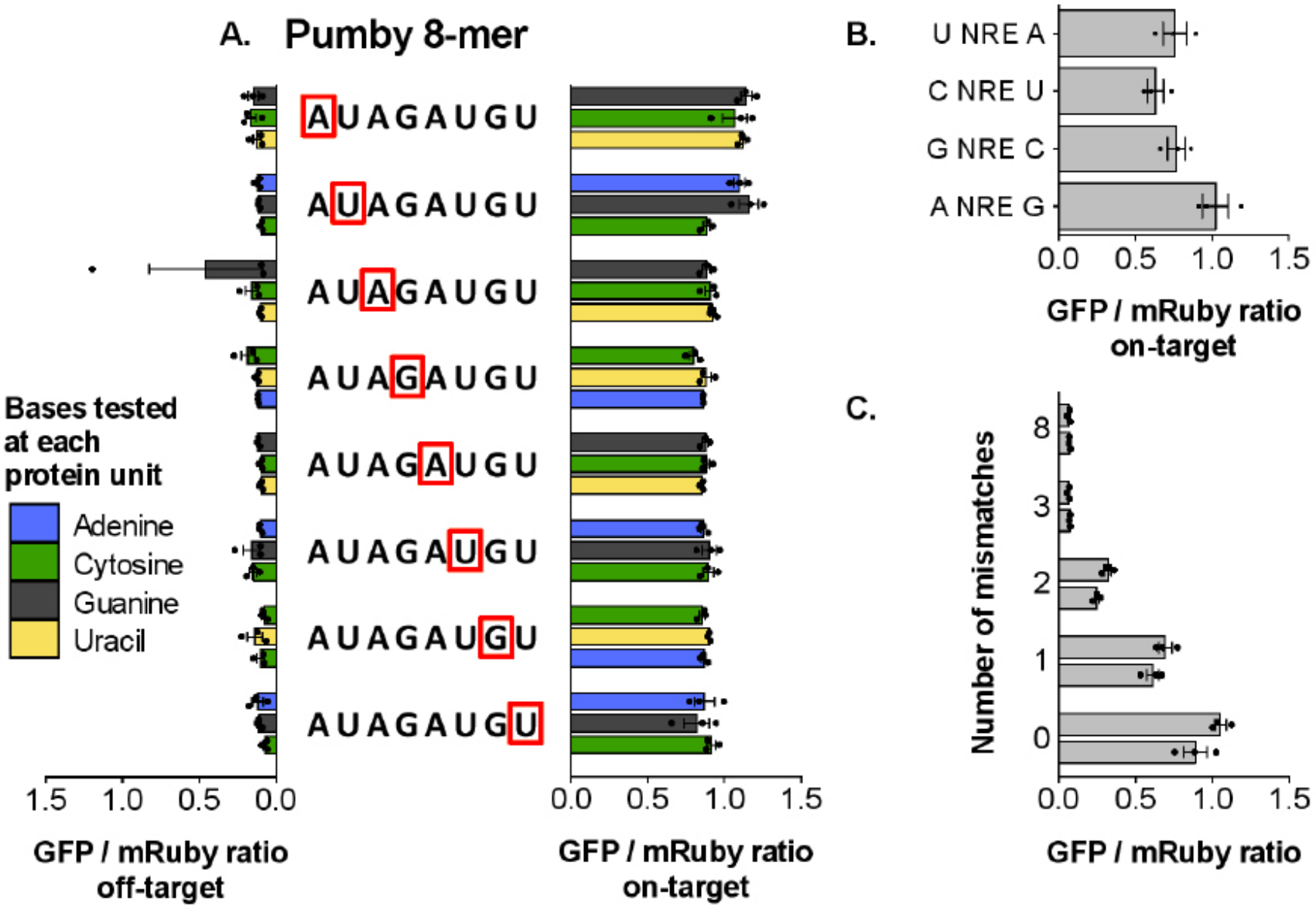
Adamala*, Martin-Alarcon*, Boyden (2016) in press.



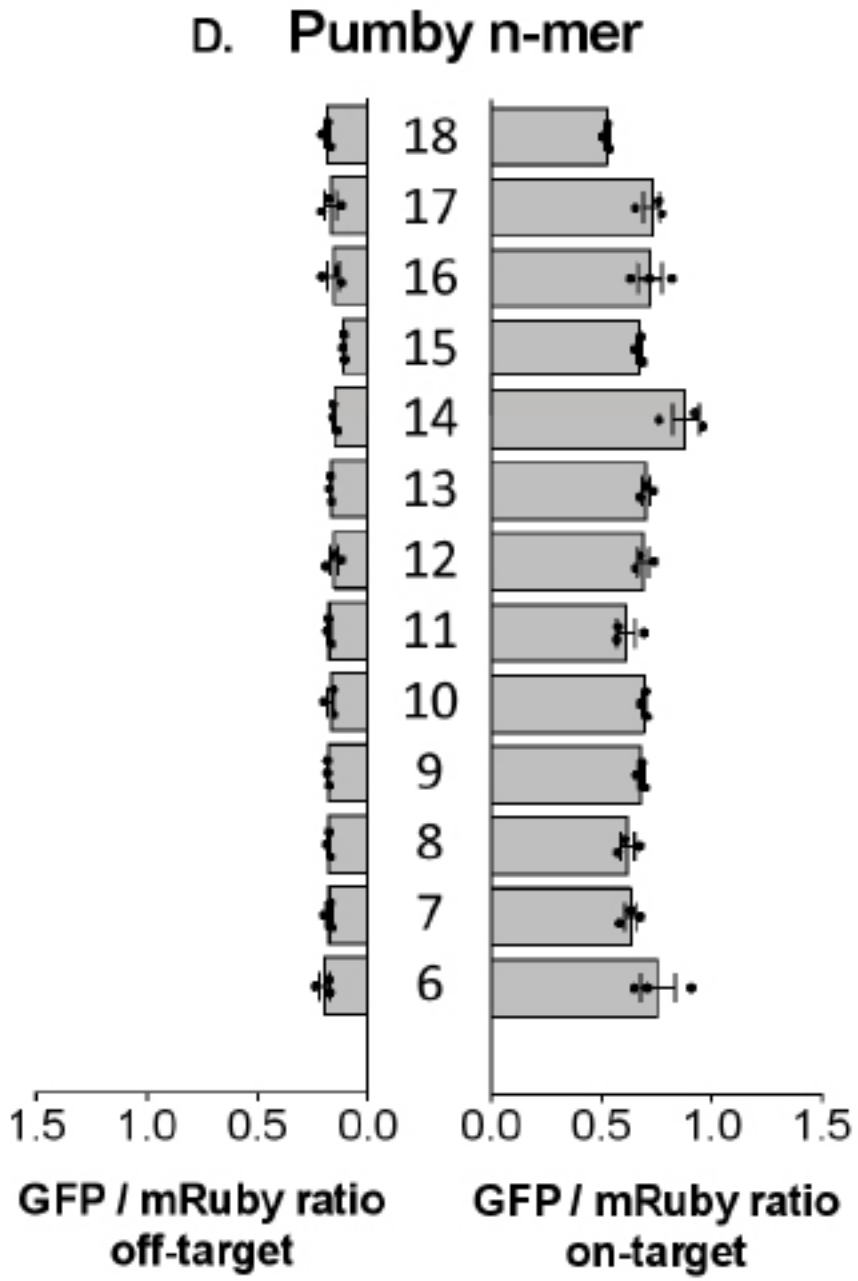
Adamala*, Martin-Alarcon*, Boyden (2016) in press.



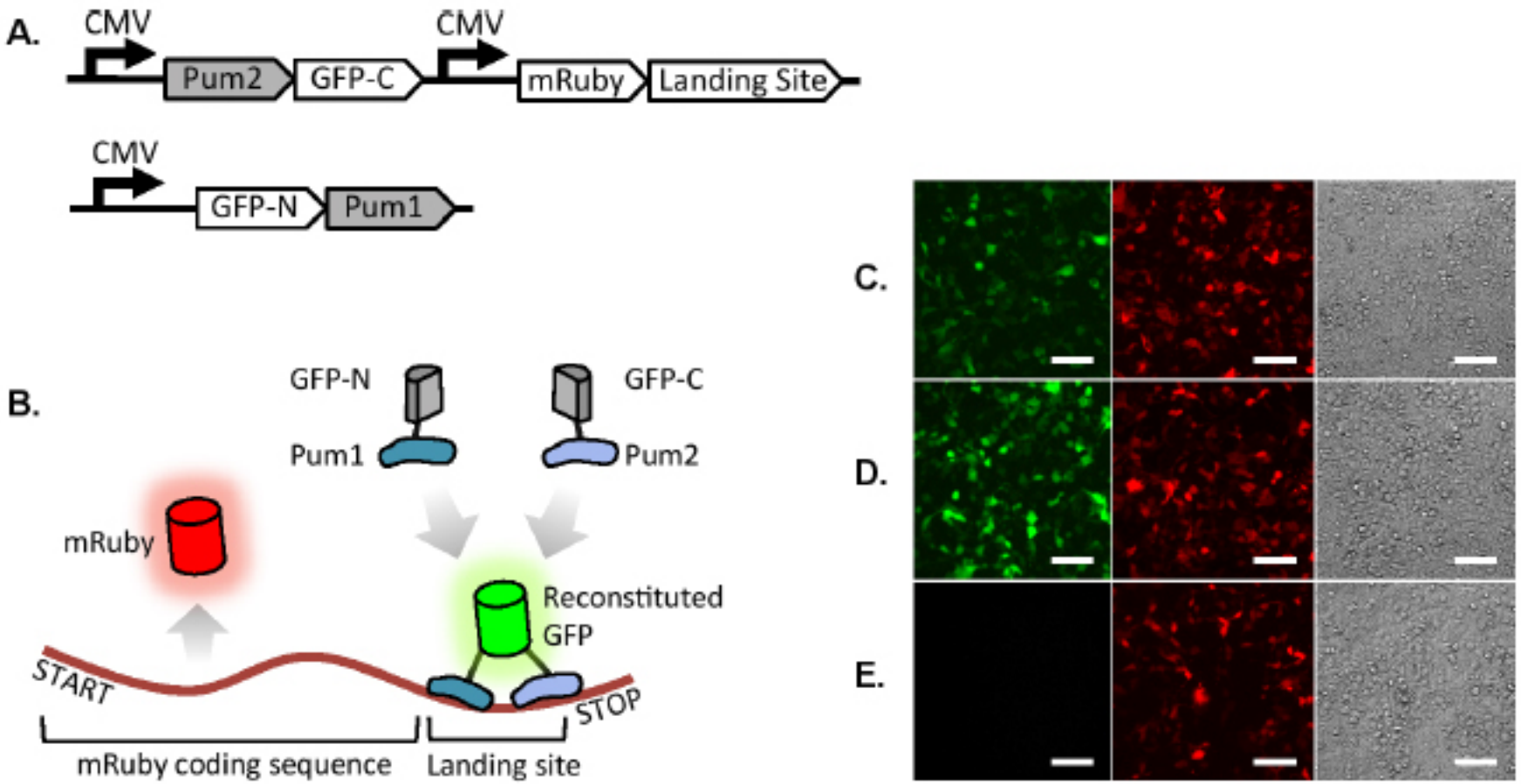
Adamala*, Martin-Alarcon*, Boyden (2016) in press.



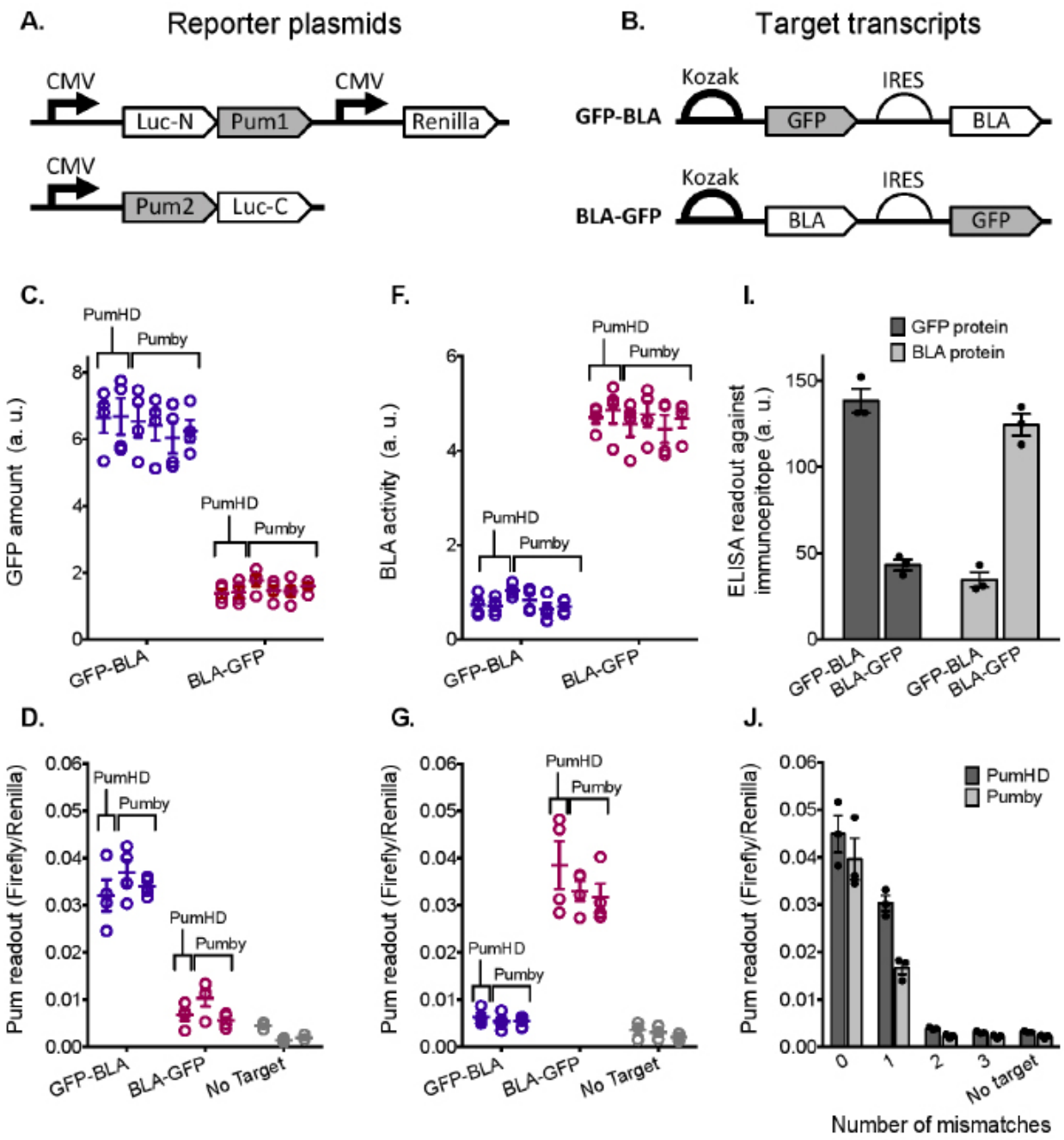
Adamala*, Martin-Alarcon*, Boyden (2016) in press.



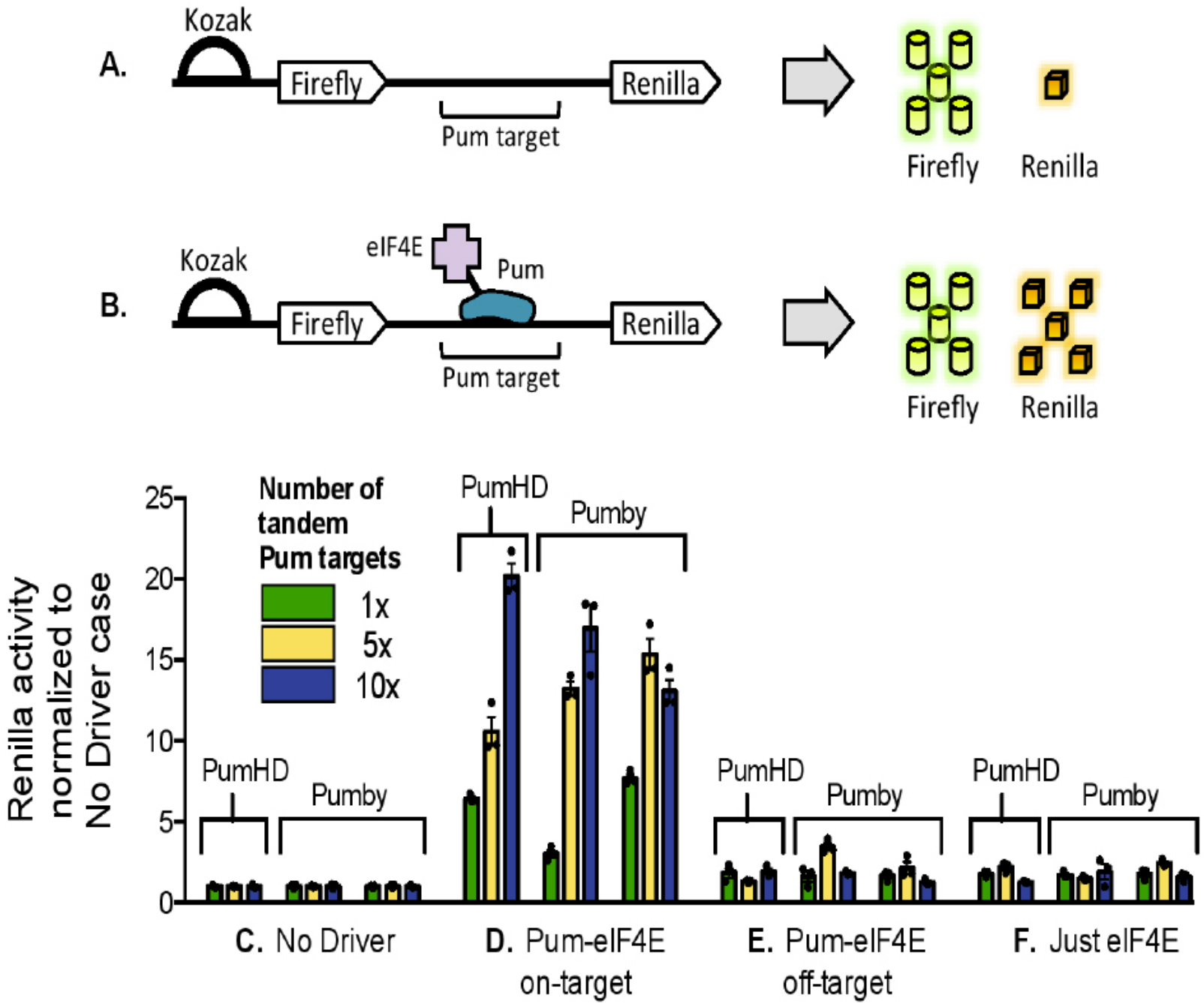
Adamala*, Martin-Alarcon*, Boyden (2016) in press.



Adamala*, Martin-Alarcon*, Boyden (2016) in press.

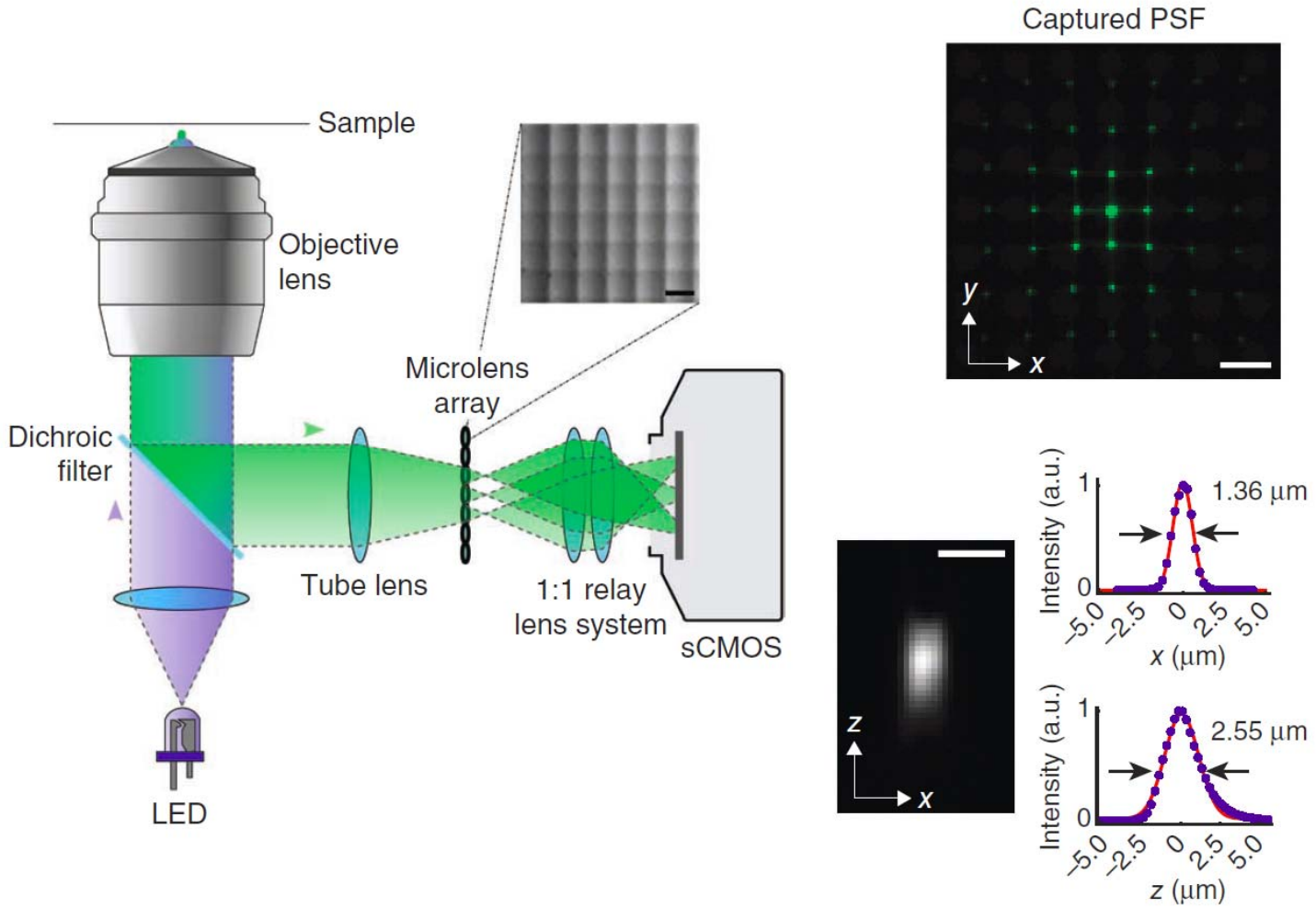


Adamala*, Martin-Alarcon*, Boyden (2016) in press.



Adamala*, Martin-Alarcon*, Boyden (2016) in press.

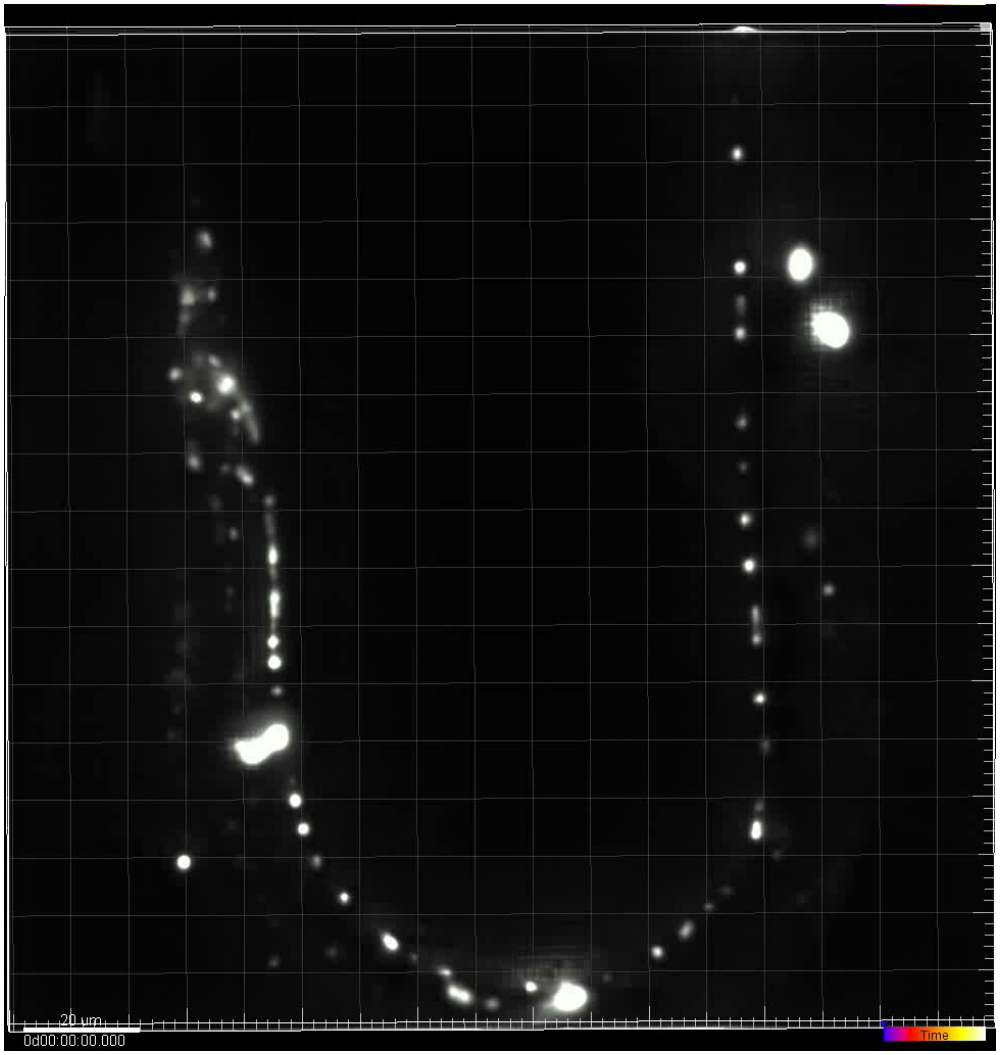
Simultaneous, whole-animal, 3-D microscopy: light-field imaging



Prevedel*, Yoon*, et al. (2014) *Nature Methods* 11:727-730.

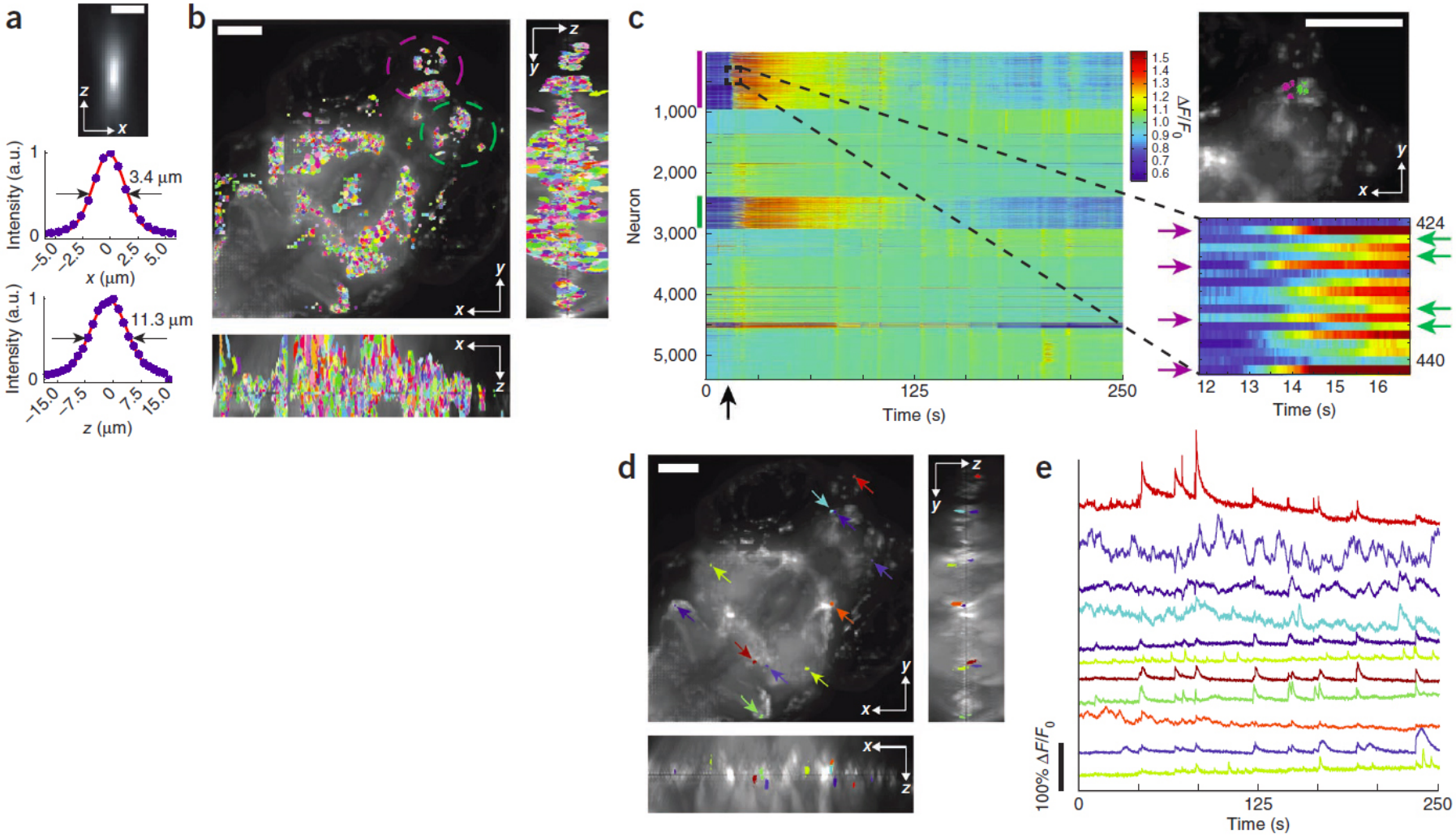
Plans, parts lists, software at http://lightfieldscope.org

Imaging neural activity throughout organism with known connectome



Prevedel*, Yoon*, et al. (2014) *Nature Methods* 11:727-730.

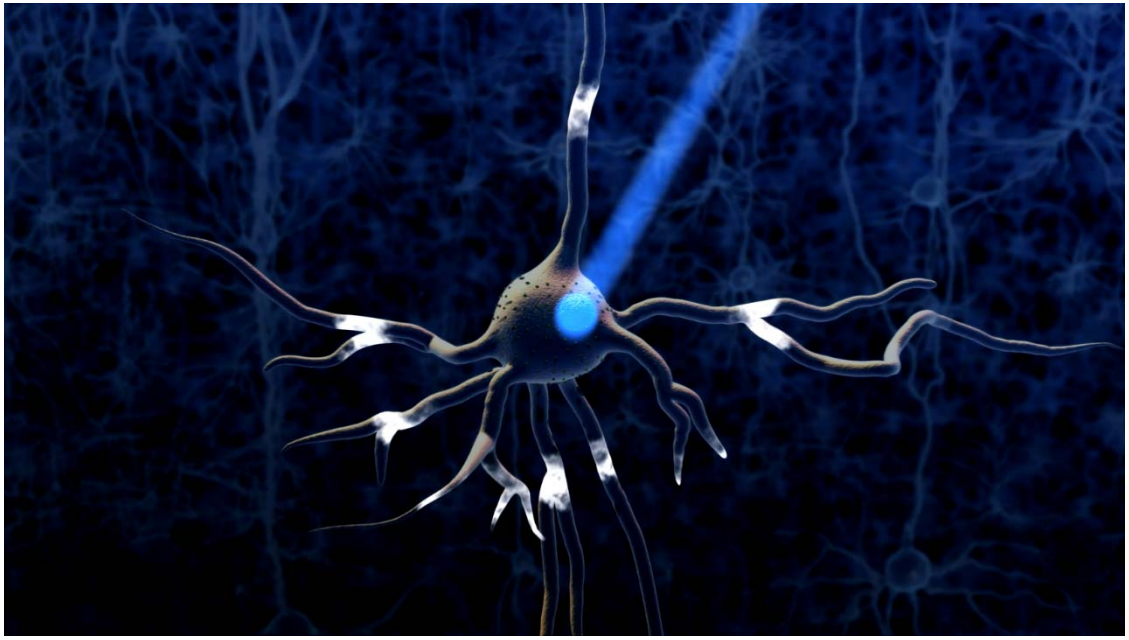
Imaging zebrafish neural activity in 3-D (at 20 Hz, below)

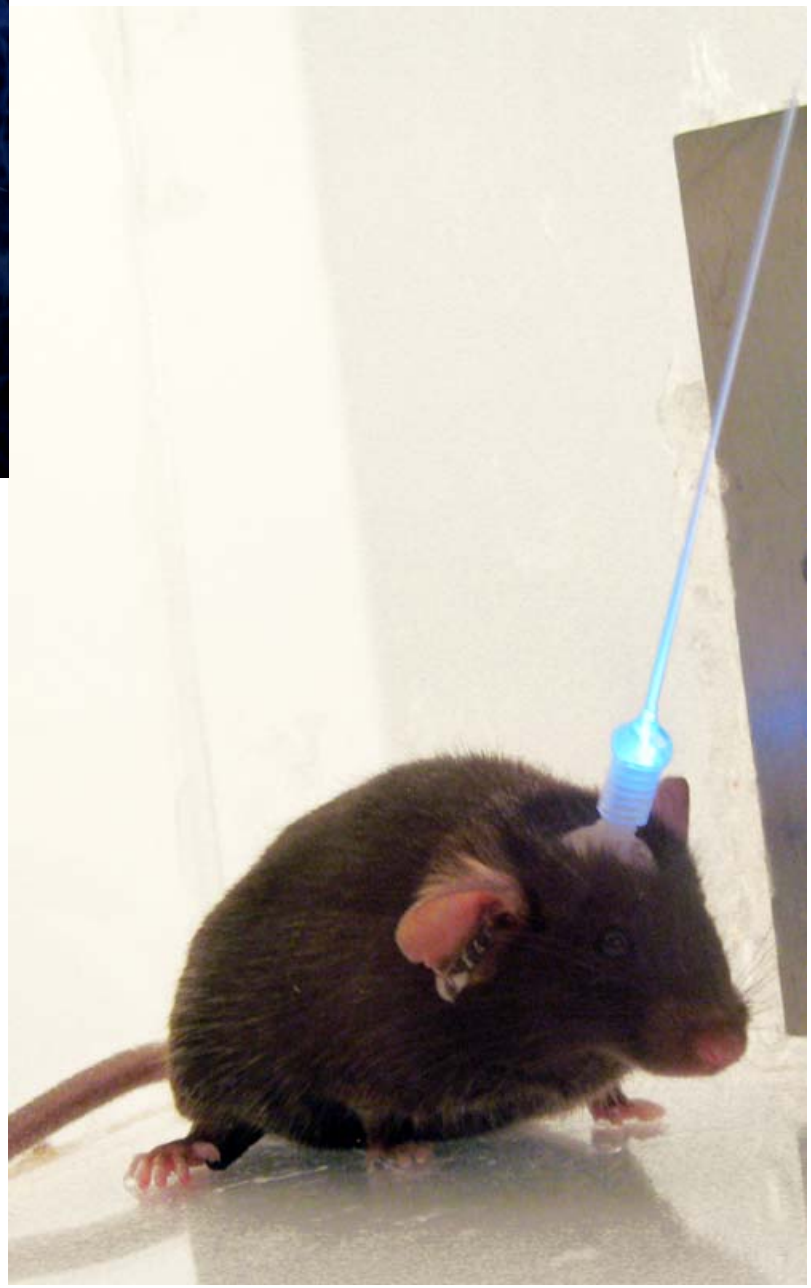


Prevedel*, Yoon*, et al. (2014) *Nature Methods* 11:727-730.

Control: neural activity

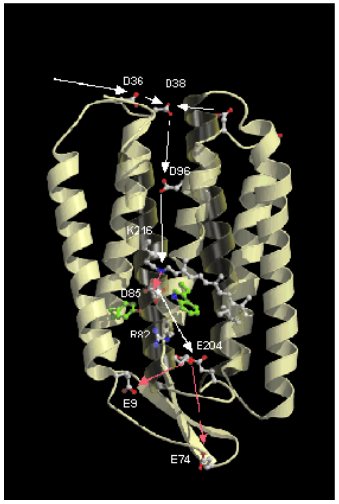
How can we control high-speed neural electrical activity with single-cell, millisecond-timescale precision?





Bacteriorhodopsins: Light-driven proton pumps

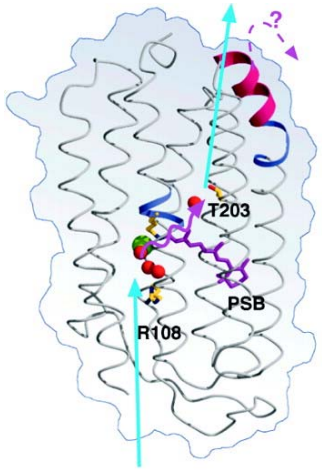
http://www.genome.duke.edu/genomelife/2011/03/systems-under-stress/



http://www.biochem.mpg.de/523002/Protein_BR

D. Oesterhelt and W. Stoeckenius (1971) Rhodopsin-like Protein from the Purple Membrane of Halobacterium halobium. Nature New Biology 233:149-152.

Halorhodopsins: Light-driven chloride pumps



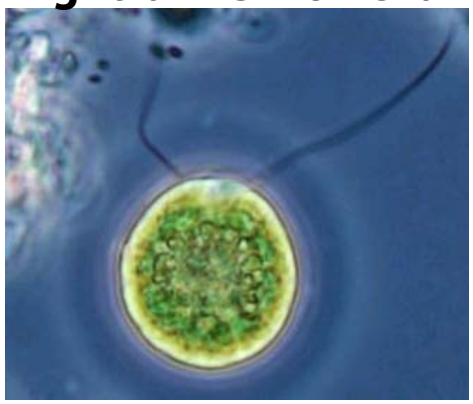
http://www.sciencemag.org/content/288/5470/1390.full

Matsuno-Yagi A, Mukohata Y (1977) Two possible roles of bacteriorhodopsin; a comparative study of strains of Halobacterium halobium differing in pigmentation. Biochem Biophys Res Commun 78:237-43.

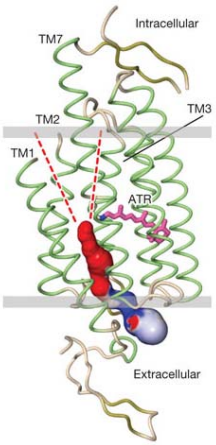
Matsuno-Yagi A, Mukohata Y (1980) ATP synthesis linked to lightdependent proton uptake in a rad mutant strain of Halobacterium lacking bacteriorhodopsin. Arch Biochem Biophys, 199:297-303.

Schobert B, Lanyi JK (1982) Halorhodopsin is a light-driven chloride pump. J Biol Chem, 257:10306-13.

Channelrhodopsins: Light-driven ion channels



http://starcentral.mbl.edu/microscope/portal.php?pagetitle=assetfactsheet&imageid=3245

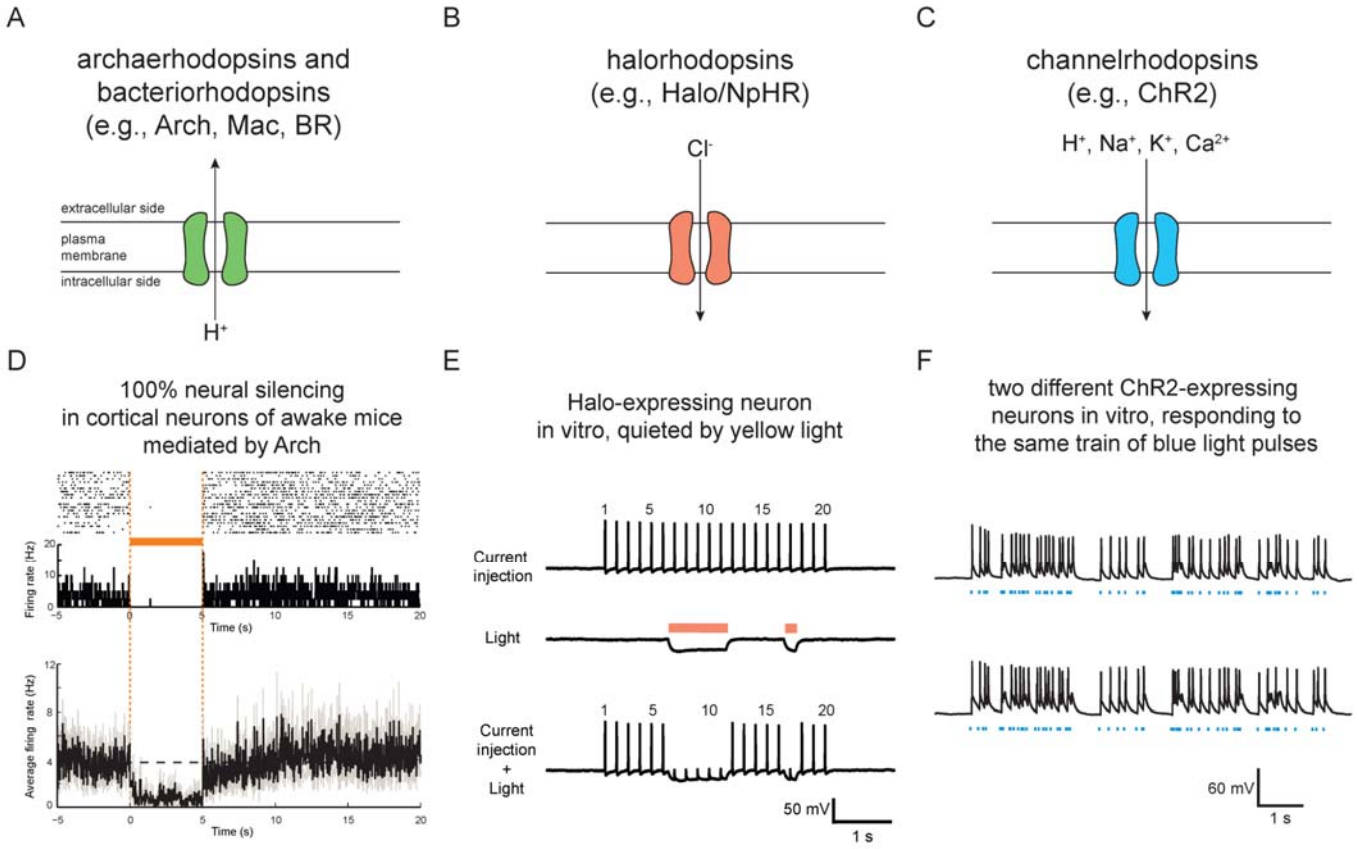


http://www.nature.com/nature/journal/v482/n7385/full/nature10870.html

Nagel G, Ollig D, Fuhrmann M, Kateriya S, Musti AM, Bamberg E, Hegemann P (2002) Channelrhodopsin-1: a light-gated proton channel in green algae. Science, 296:2395-8.

Nagel G, Szellas T, Huhn W, Kateriya S, Adeishvili N, Berthold P, Ollig D, Hegemann P, Bamberg E (2003) Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. Proc Natl Acad Sci U S A, 100:13940-5.

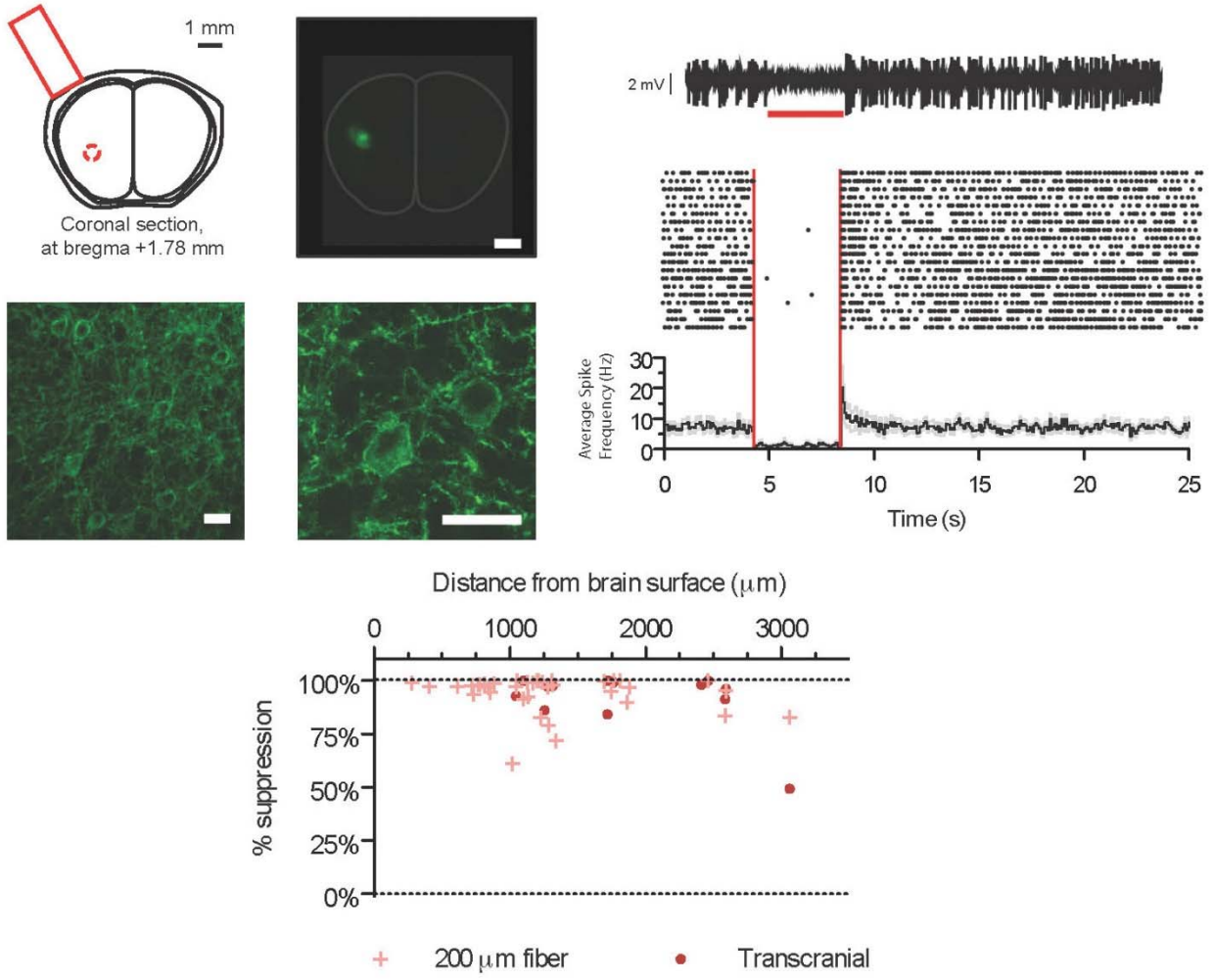
Optogenetics: microbial opsins, seven-transmembrane proteins, binding endogenous all-trans-retinal



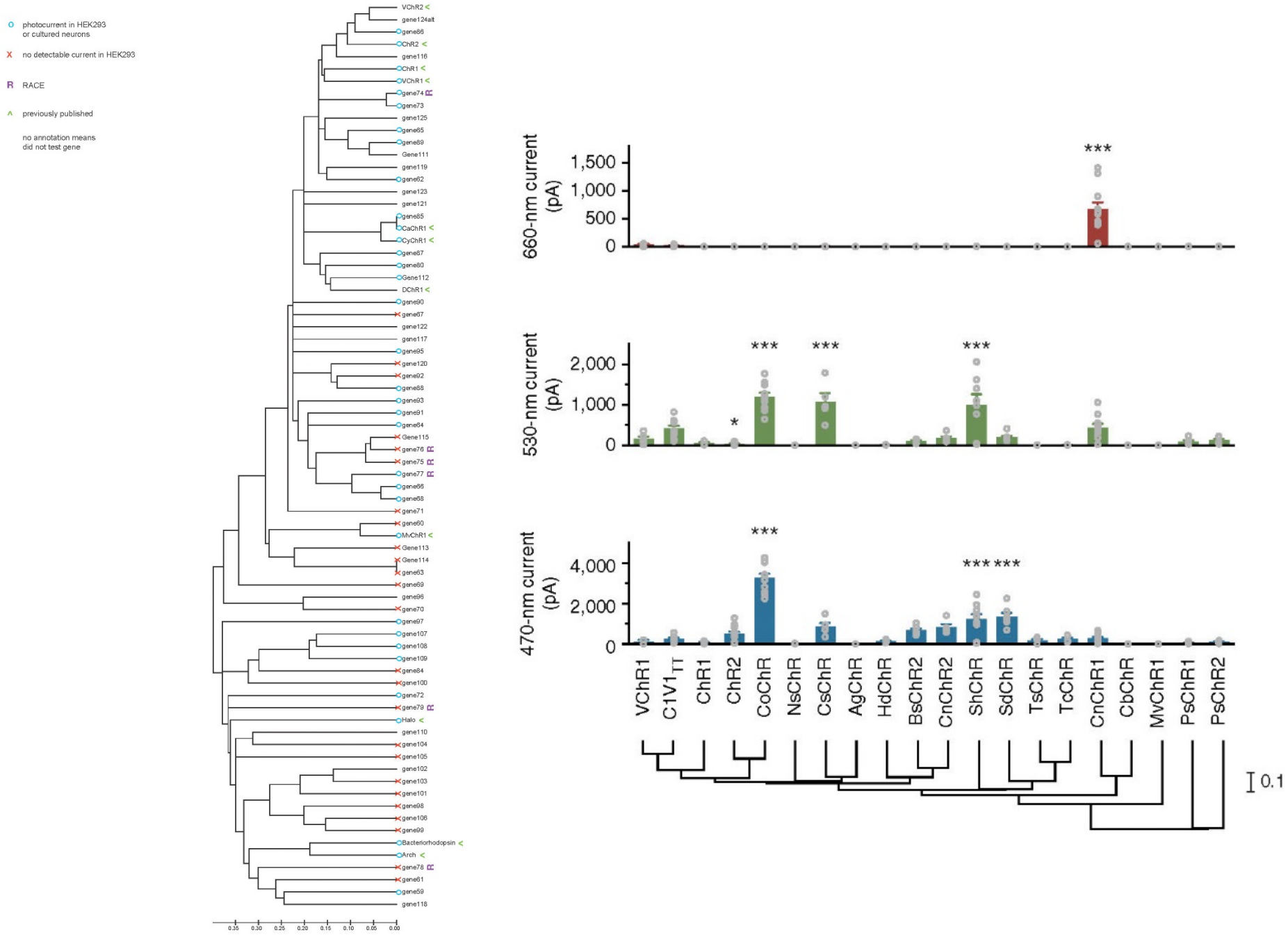
Boyden (2011) *Faculty of 1000 Biology Reports* 3:11.

Sequences, plasmids, links to DNA/virus providers, at http://syntheticneurobiology.org/protocols

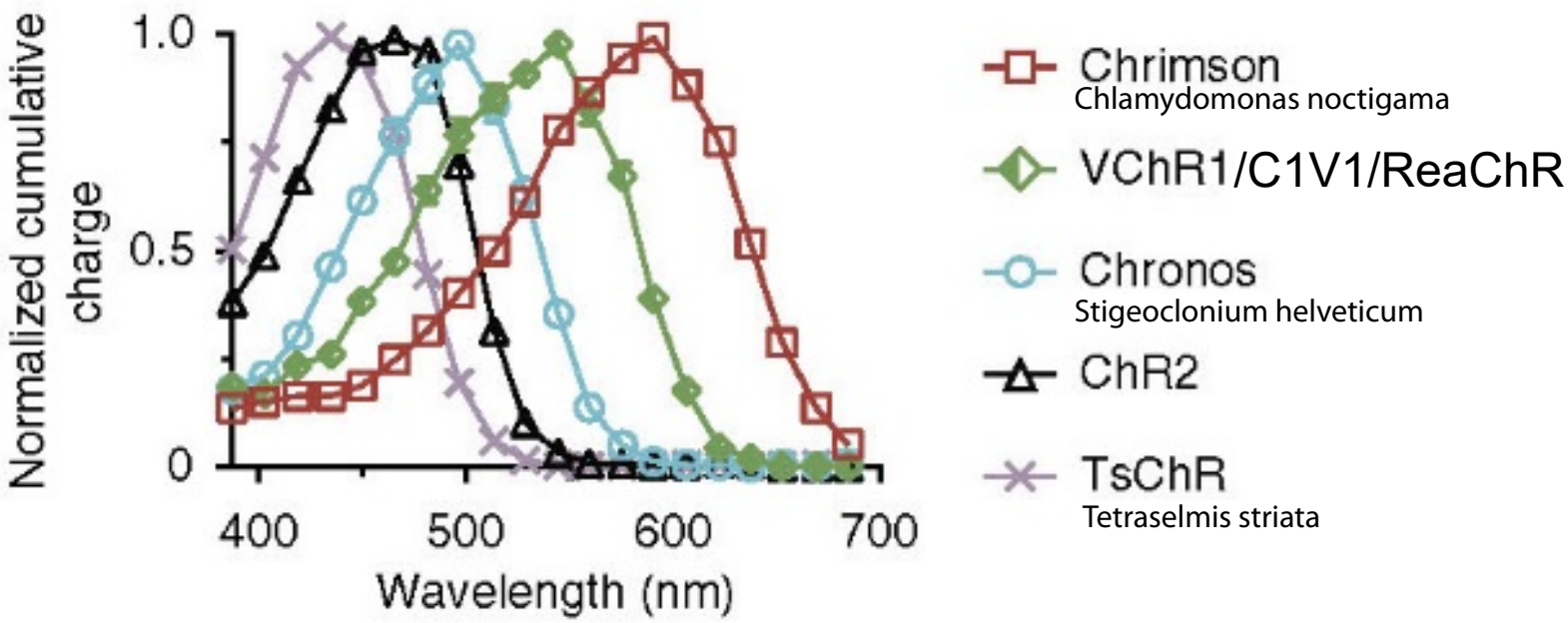
Noninvasive optogenetic neural silencing: Jaws



Chuong et al. (2014) *Nature Neuroscience* 17:1123-1129.

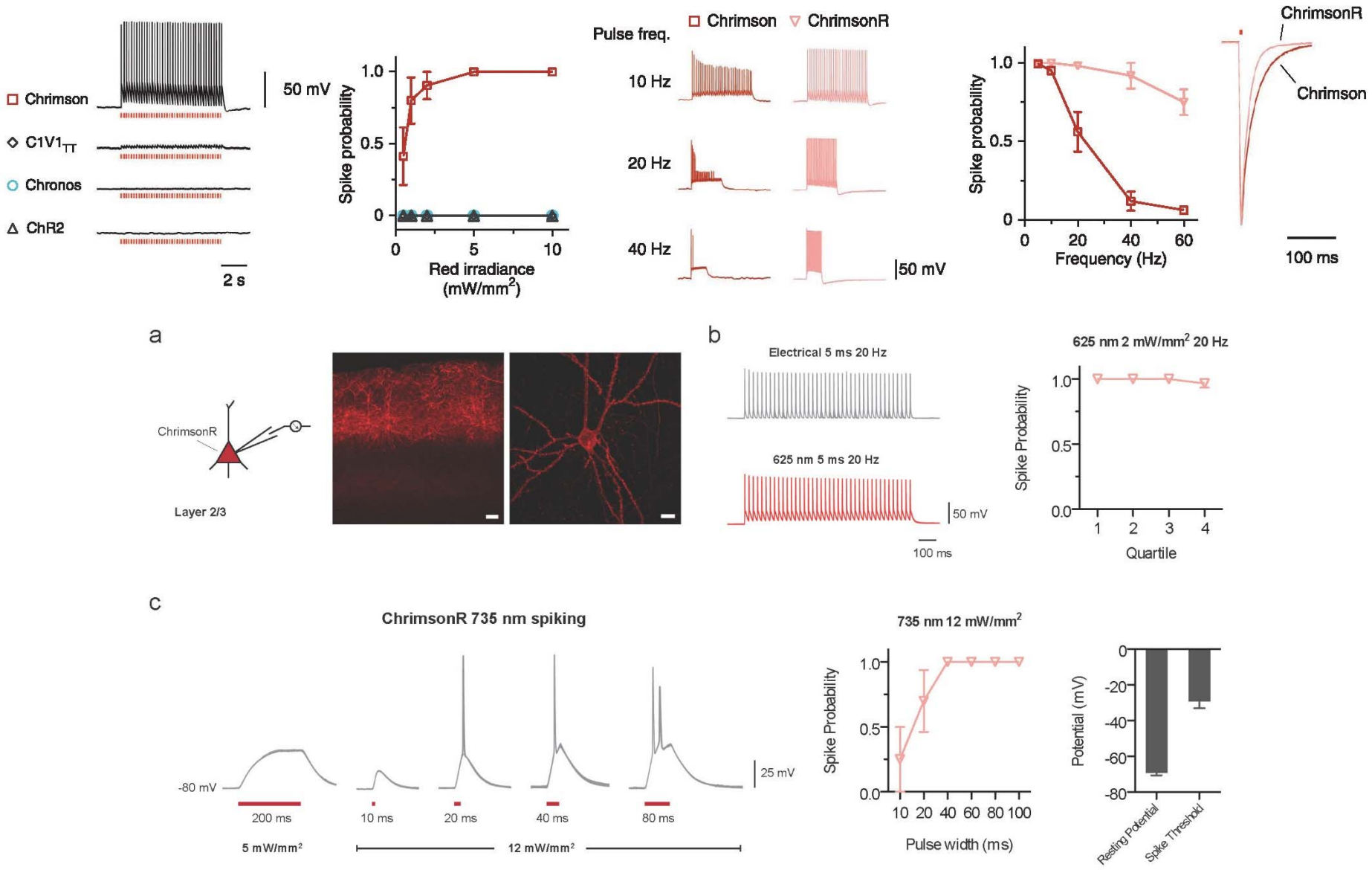


Klapoetke et al. (2014) *Nature Methods* 11:338–346.



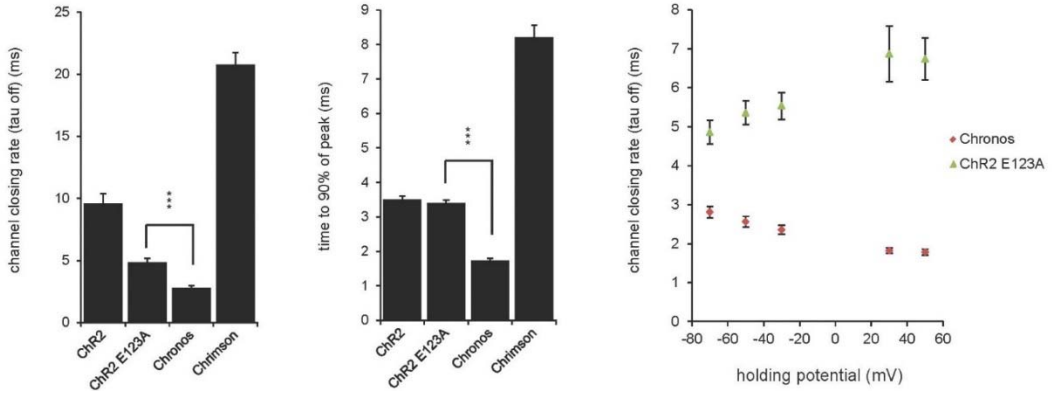
Klapoetke et al. (2014) *Nature Methods* 11:338–346.

Chrimson: quasi-infrared neural stimulation

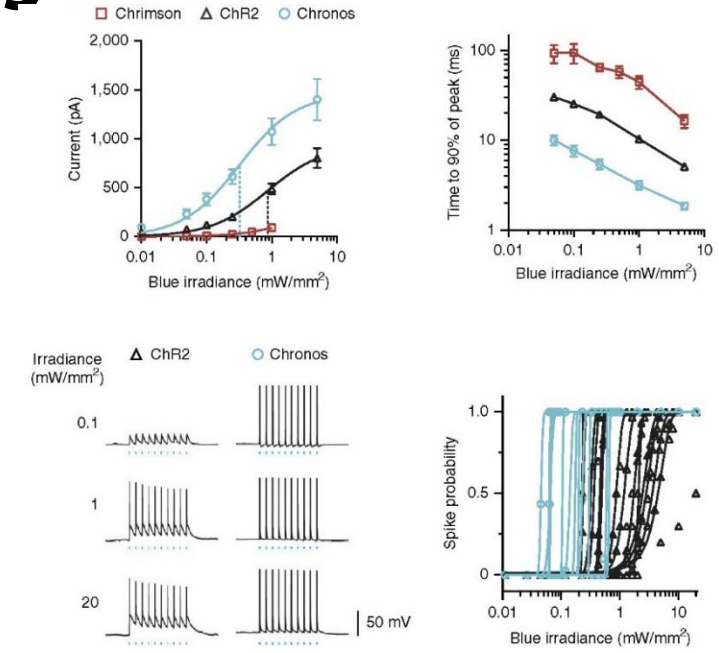


Klapoetke et al. (2014) *Nature Methods* 11:338–346.

Chronos: a very fast channelrhodopsin…



…that is also very light sensitive!



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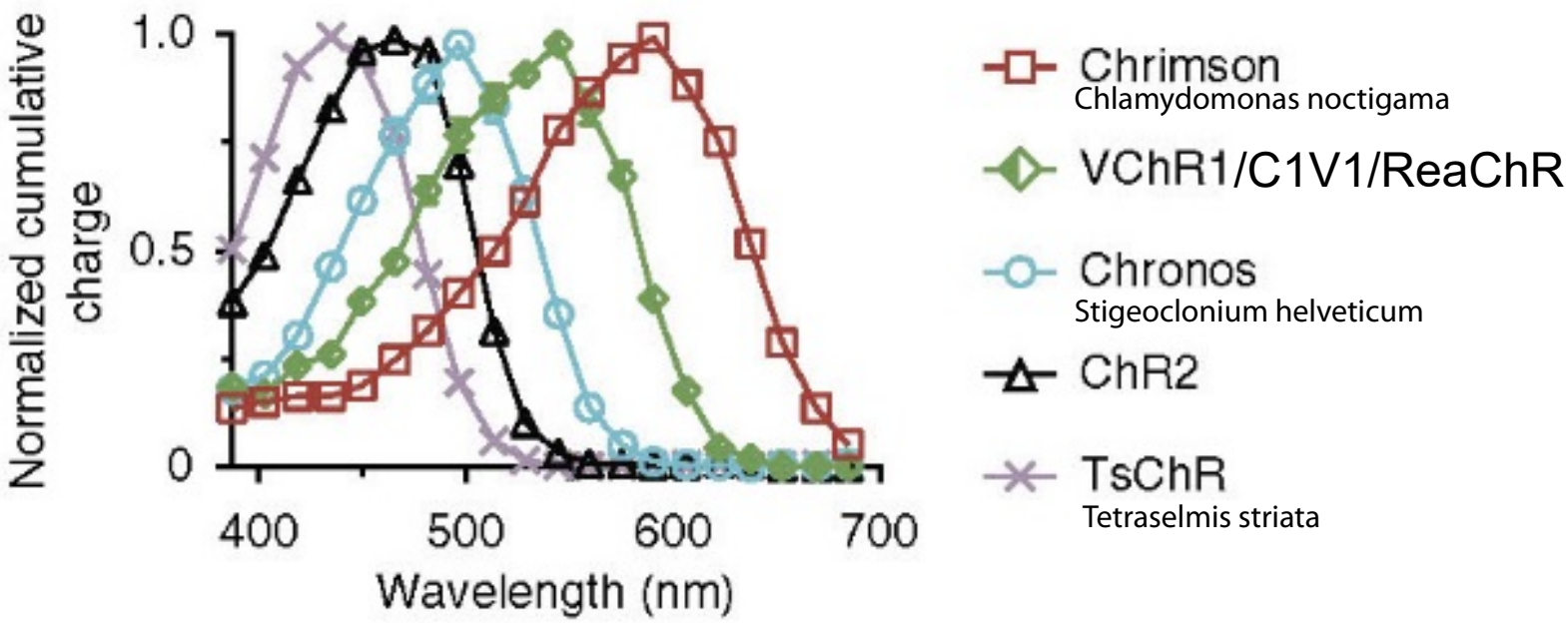

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http://syntheticneurobiology.org/

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Ticker tapes: George Church, Keith Tyo, Konrad Koerding

Transgenics: Hongkui Zeng

Conflict of Interest Statement

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