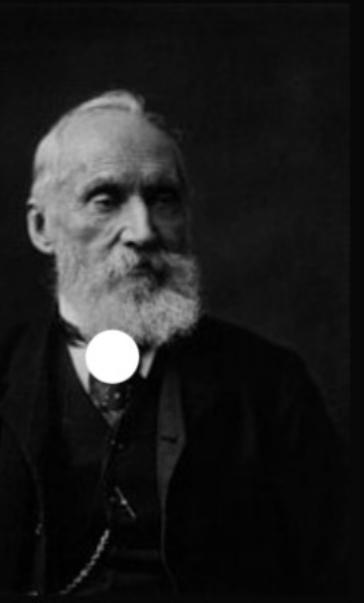


Introduction to Neuro-Genomics



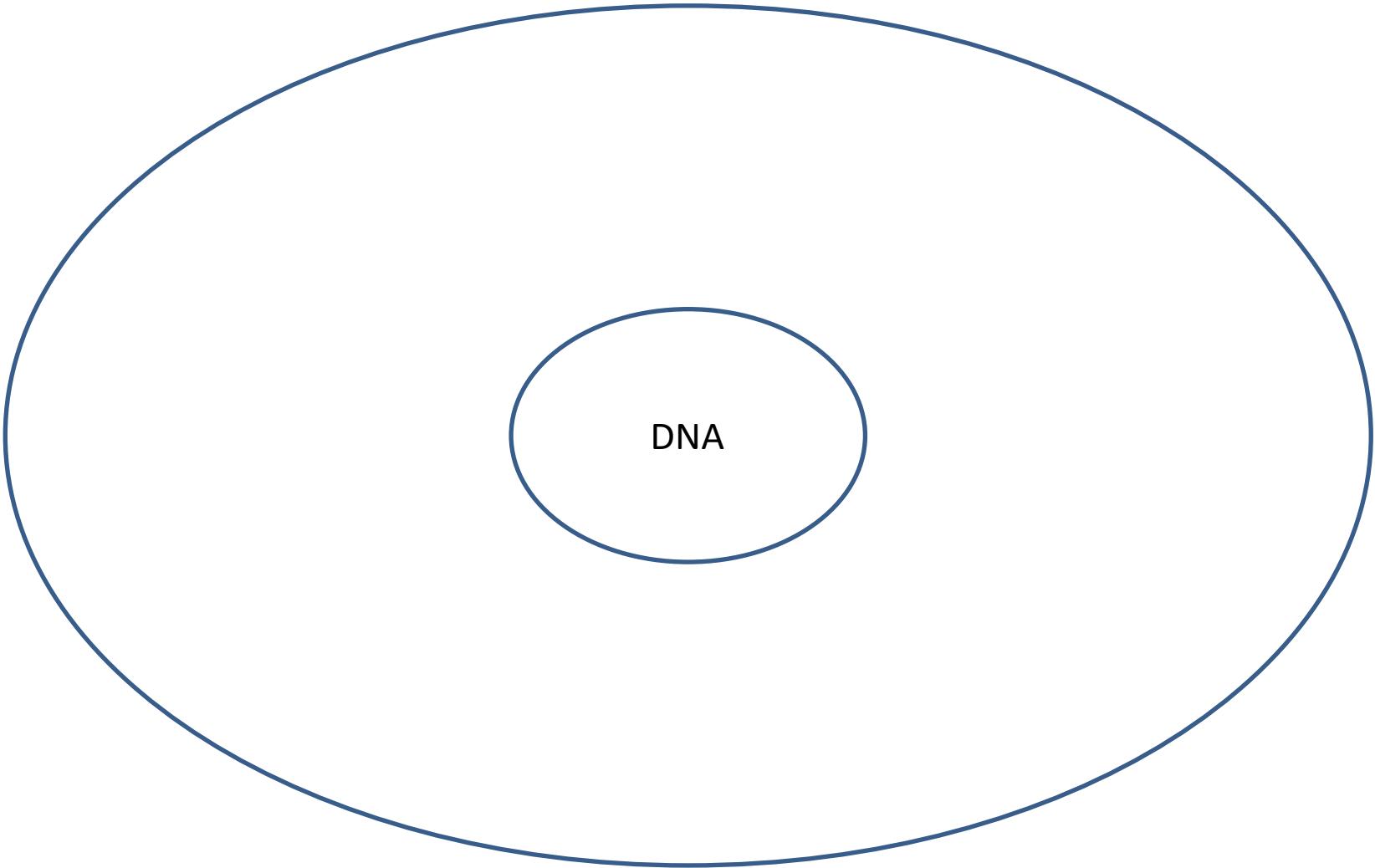


I often say that when you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind: it may be the beginning of knowledge, but you have scarcely, in your thoughts, advanced to the stage of science, whatever the matter may be.

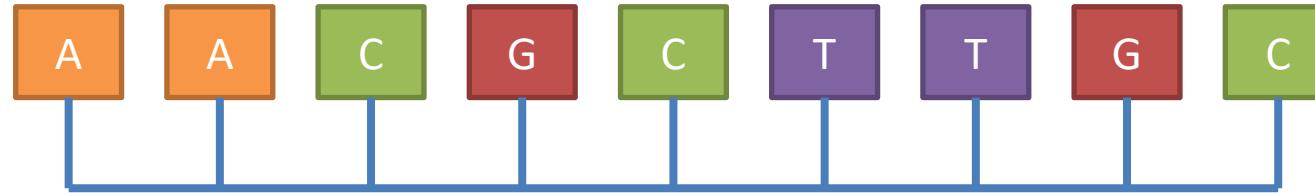
(Lord Kelvin)

Talk outline

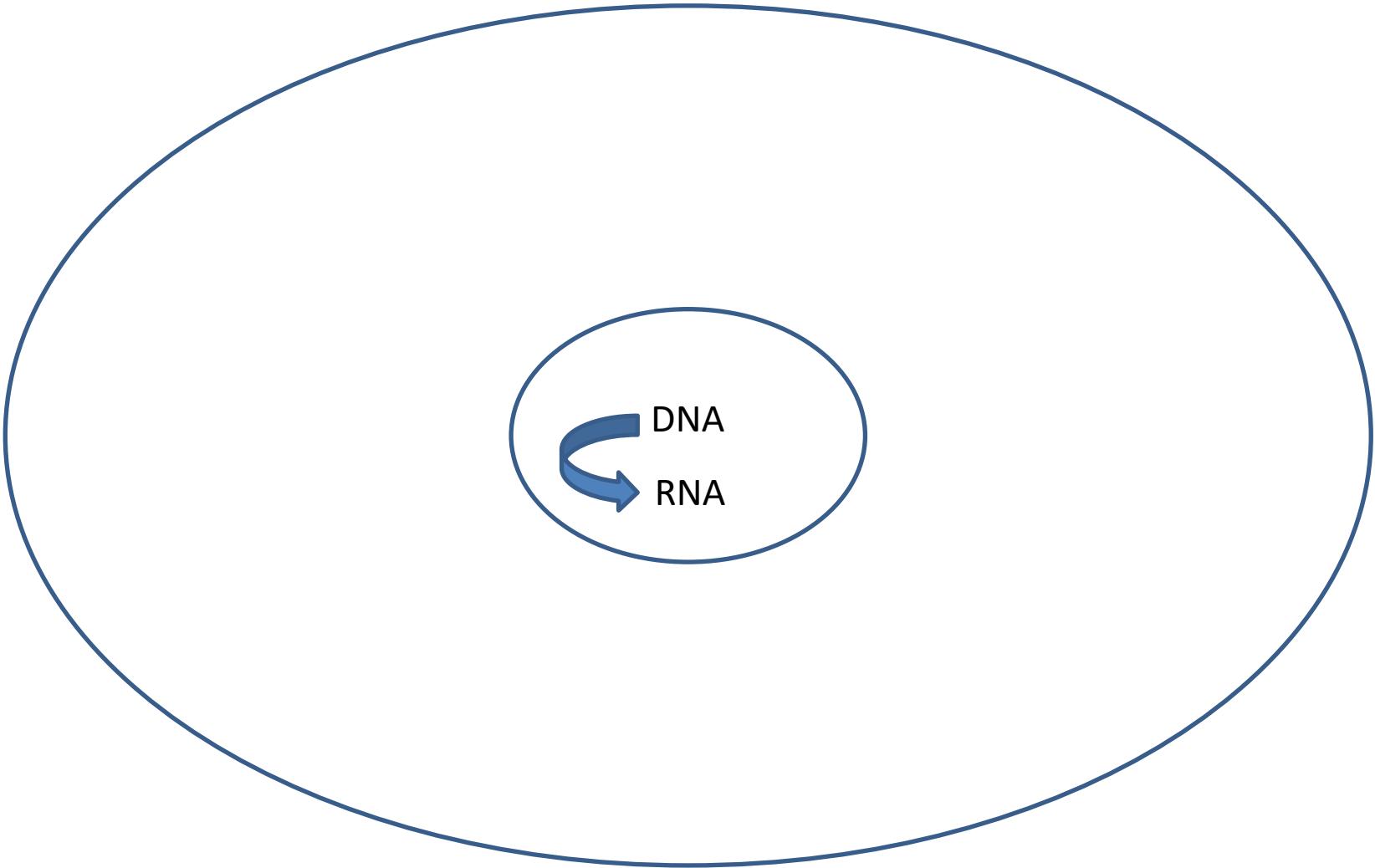
- The importance of RNA sequencing
- An example: what makes squid and octopus smart?
- Single cell genomics
- Spatially-resolved transcriptomics
- RNA content of brain tissues in super-resolution

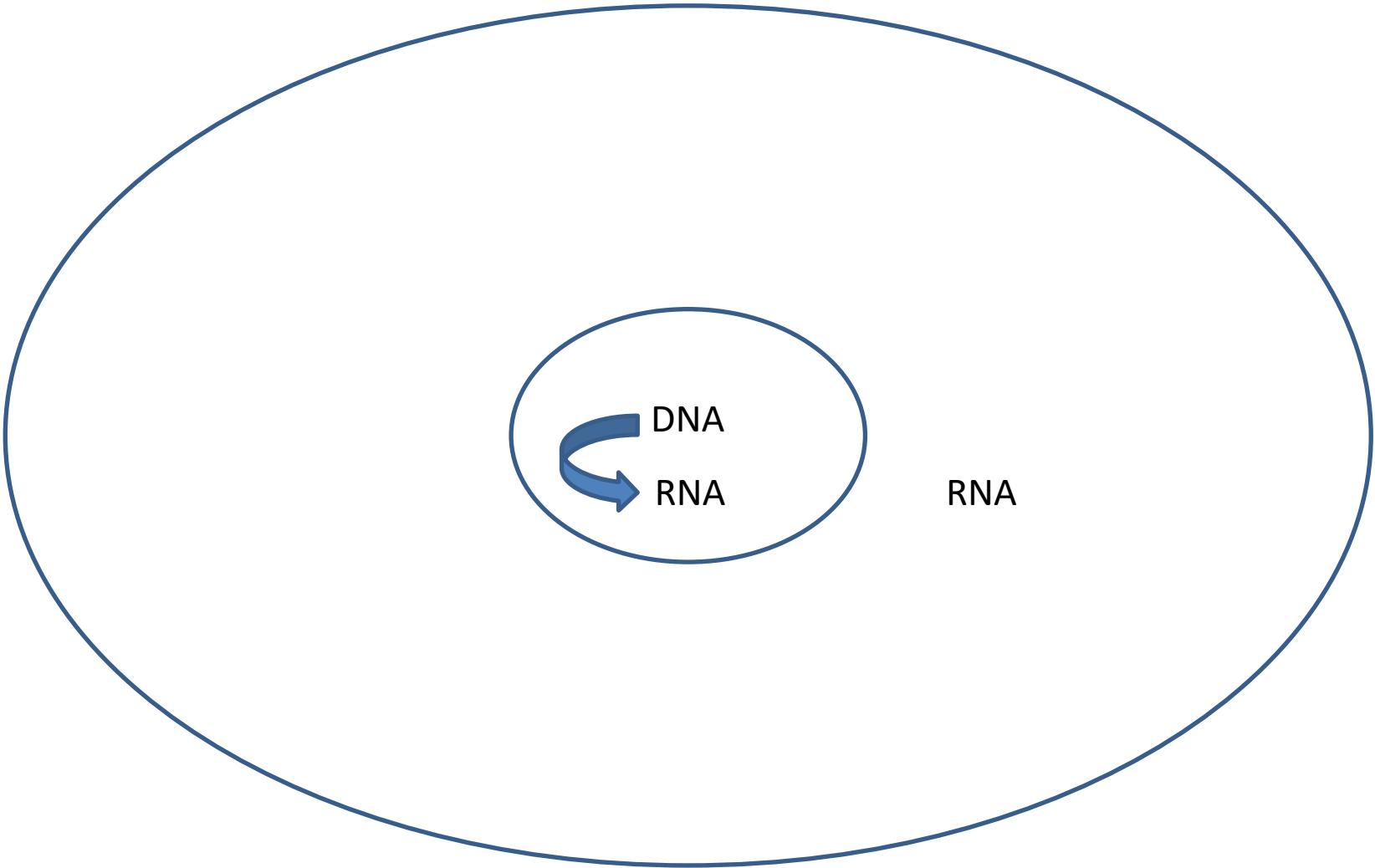


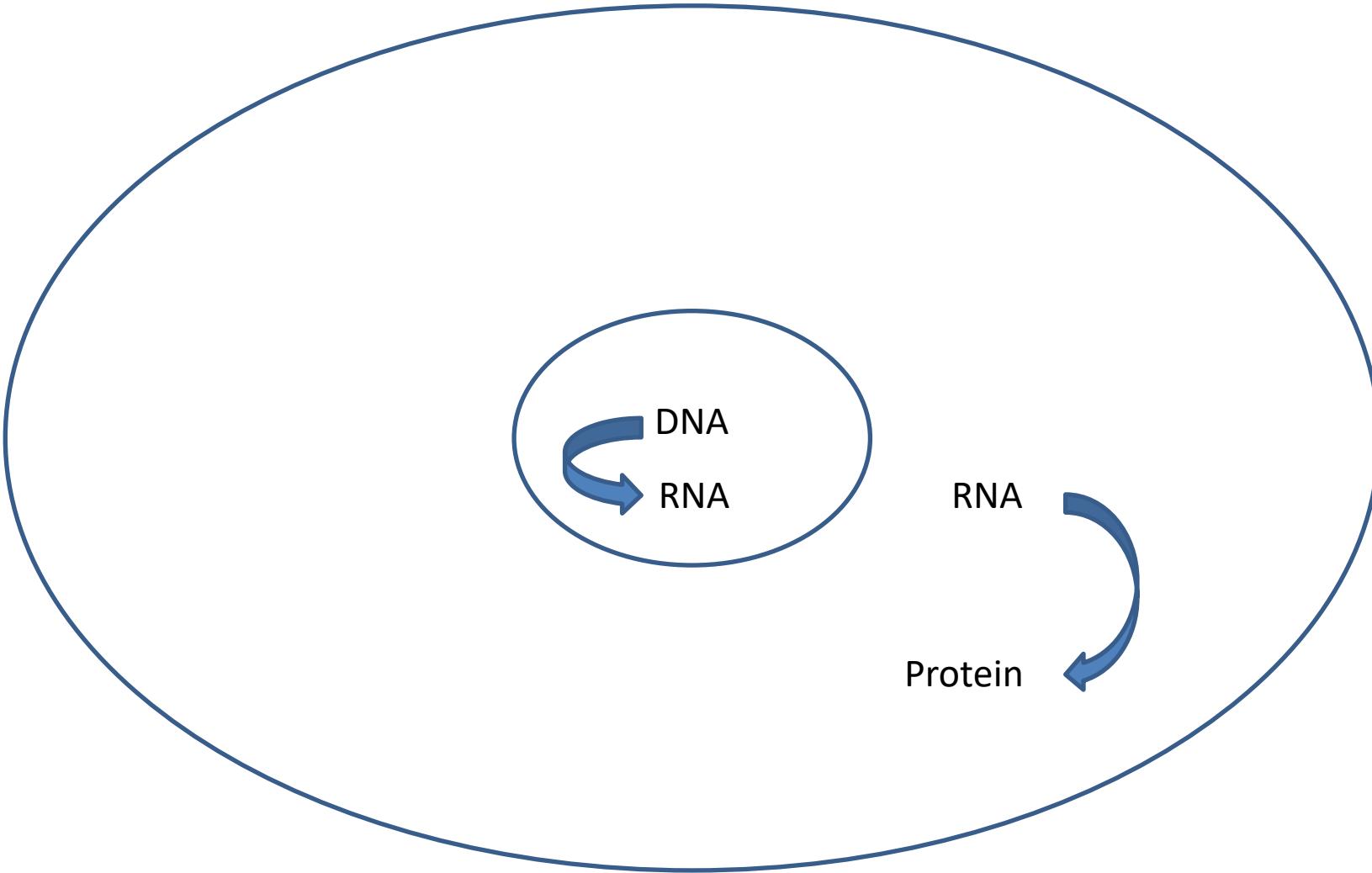
DNA



TTACTTATTGTTCTTGAAAGCATTGTTGACCACCGAGACA
AAGTTTAAGAAAATTGACTTTCTTCTTCATTTATAATGTC
TTTGACCATTGAGGGTCTTAGCAACTTCAGGAAATGTT
TCATGCAGCAACGACACAACGTATCAACTAAAGCAGCAA
AGAATCCAGGTTTGCATTTAACCTTCAGTTCCATAACG
CGGGCCCTTCCGAATCGGAAGGATAGGGACTTGACCCTGT
TCCTGGGCATCGCGCAGTTCCCCCGAAGATCACCCGAAC
TGAAGCGCCTTTAGGAAGAAAACCTCCAGTCGAGAAGTT
GTCACGAAAACCGGAGAACGAAATATTGGCGGTAAAGGAAG
AACAAATCAAAAAAGAATTATATATATACAAACCCGGTC
GACTTAA ...





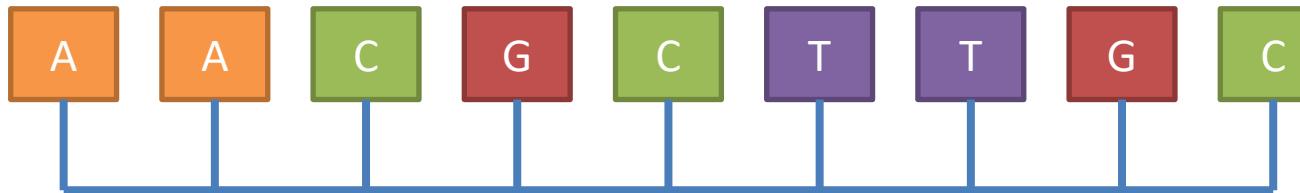


Gene = location in the DNA from which mRNA is transcribed, and later on a protein is translated

Human Genome Project



DNA



TTACTTATTGTTCTTGAAAGCATTGTTGACCACGGAGACA
AAGTTTAAGAAAATTGACTTTCTTCTTCATTATAATGTC
TTTGACCATTGAGGGTCTTAGCAACTTCAGGAAATGTT
TCATGCAGCAACGACACAACGTATCAACTAAAGCAGCAA
AGAATCCAGGTTTGCATTTAACCTTCAGTTCCATAACG
CGGGCCCTTCCGAATCGGAAGGATAGGGACTTGACCCTGT
TCCTGGGCATCGCGCAGTTCCCCCGAAGATCACCCGAAC
TGAAGCGCCTTTAGGAAGAAAATCCAGTCGAGAAGTT
GTCACGAAAACCGGAGAACGAAATATTGGCGGTAAAGGAAG
AACAAATCAAAAAAGAATTATATATATACAAACCCGGTC
GACTTAA ...

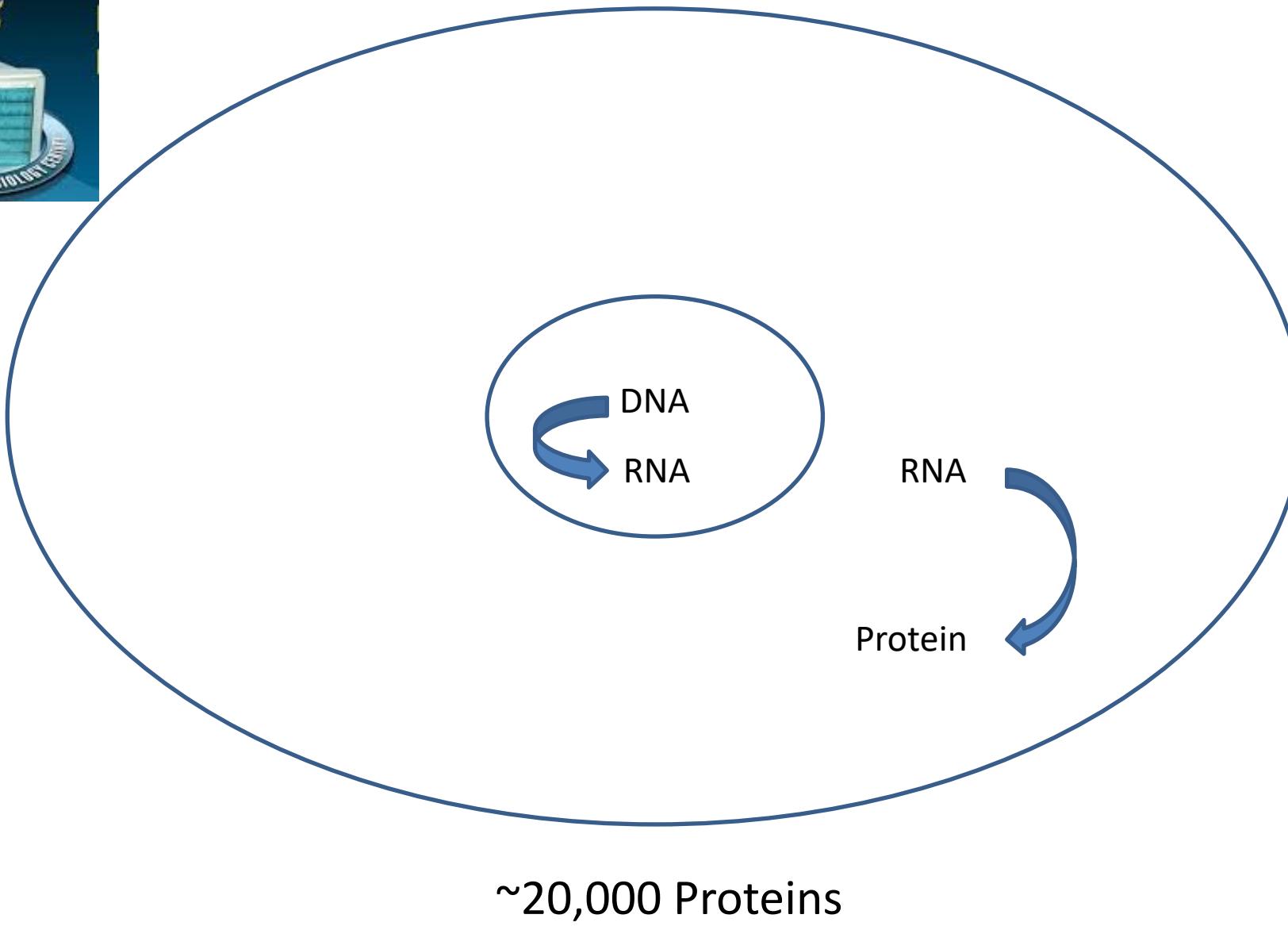
**Engineering problem – move from reading 1000 bases to reading 1 billion
bases**

(Manhattan project - from 0.5 watt reactor to 0.5MW reactor in a year)

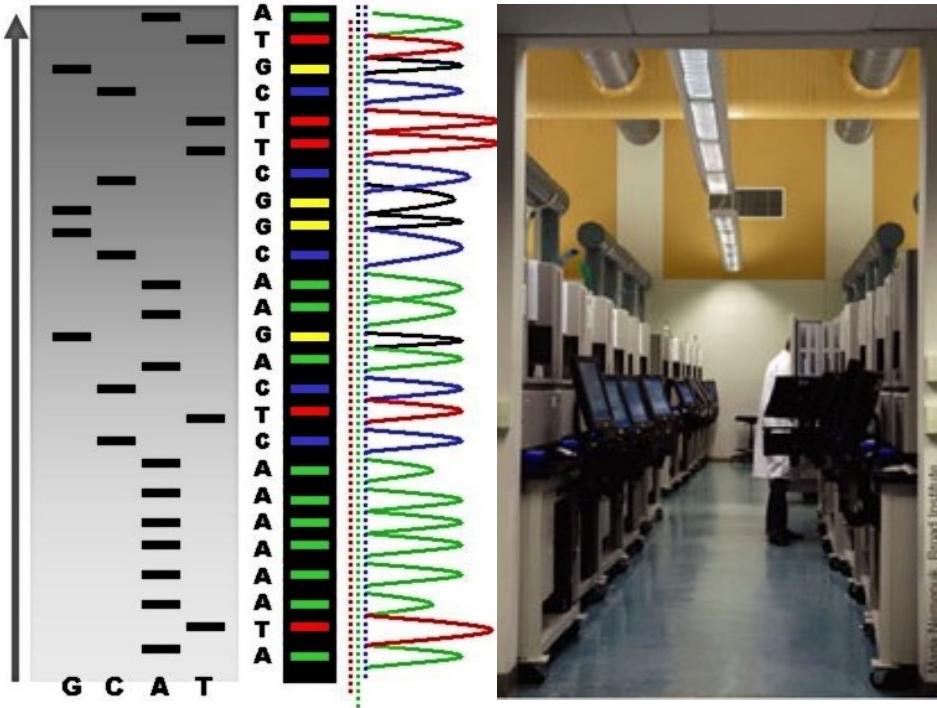
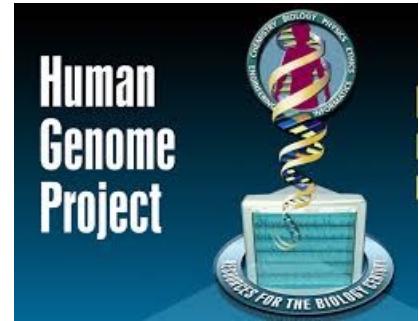
Human Genome Project



The direct outcome = the genome + RNA/proteins types

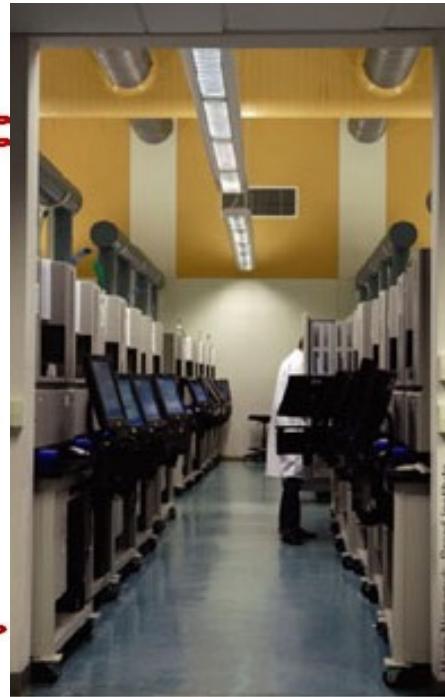
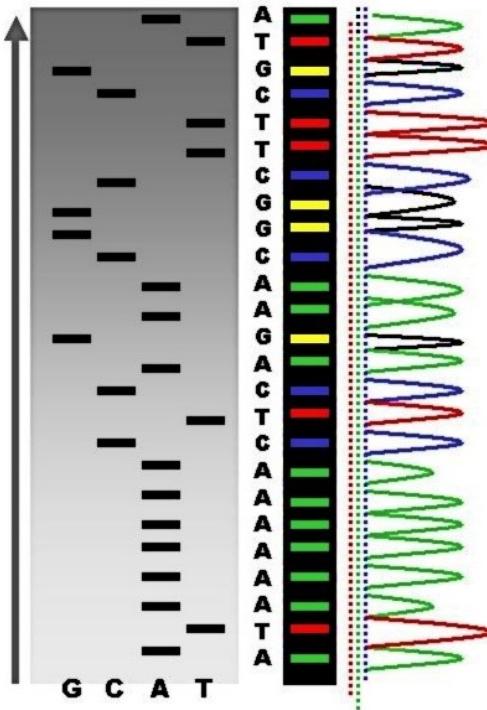
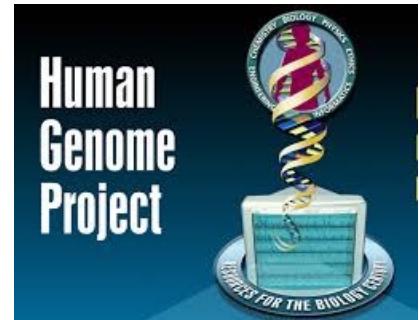


The indirect outcome = we are very good at sequencing DNA/RNA!



**~\$300,000,000
Hundreds of machines
9 months**

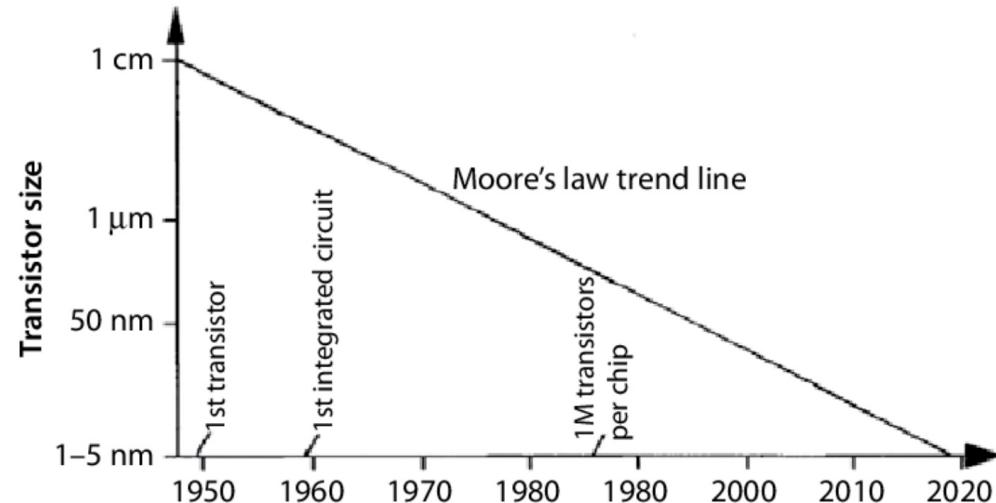
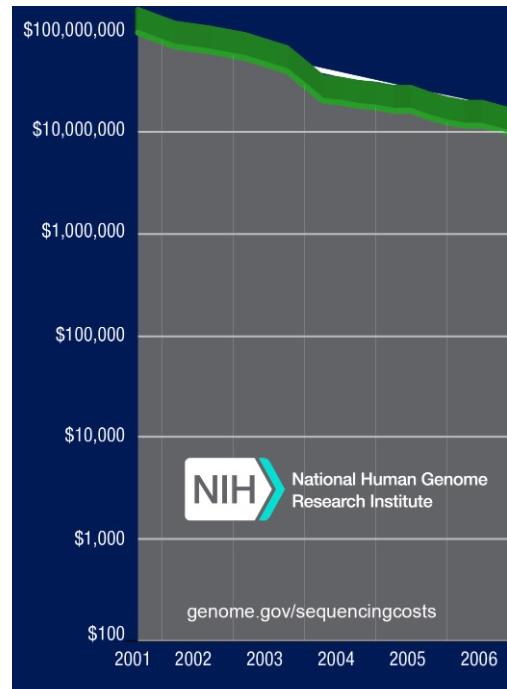
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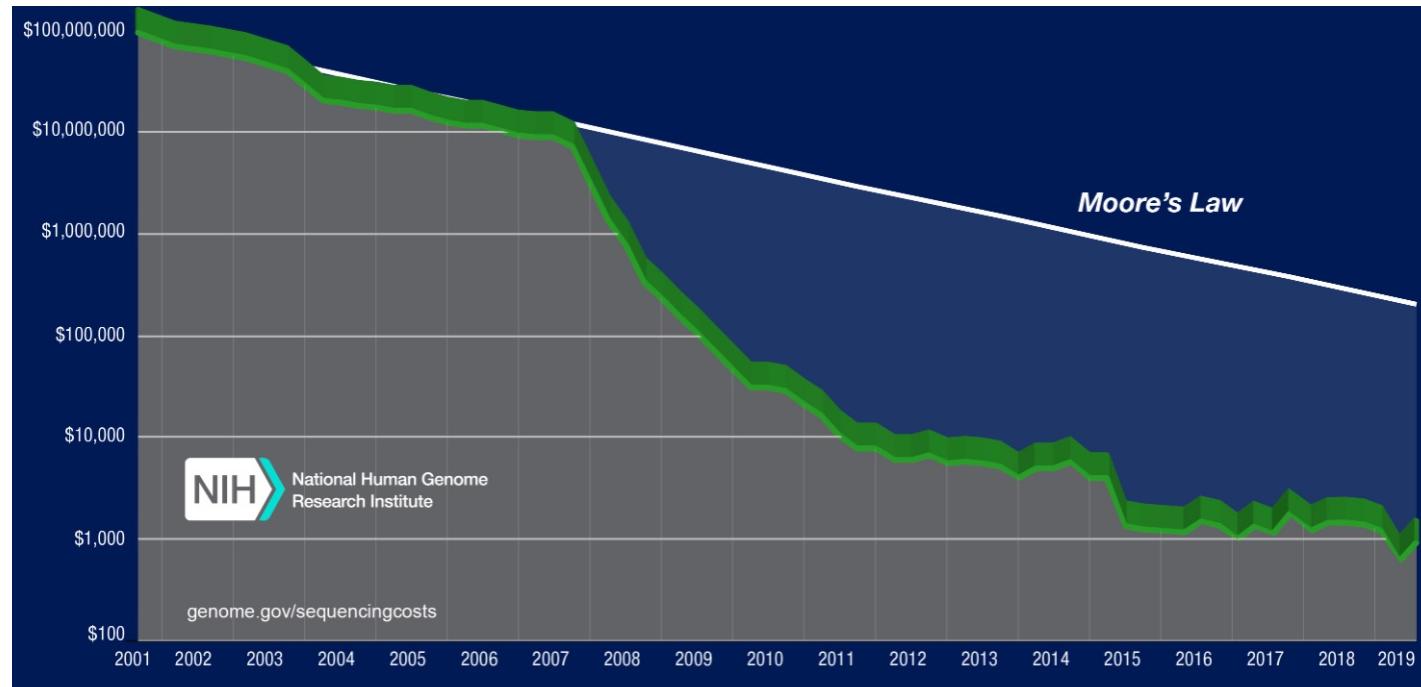
**~\$300,000,000
Hundreds of machines
9 months**

**~\$1,000
1 machine
3 days**

Sequencing the human genome by following Moore's Law

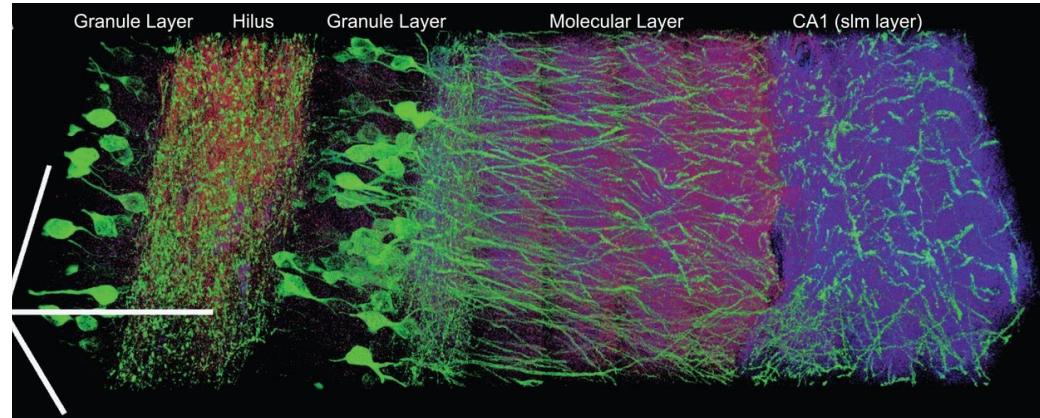
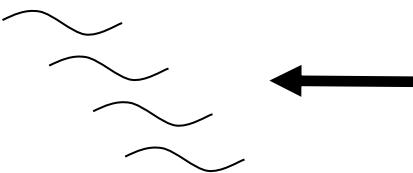


Breaking Moore's Law: modern bio-engineering



The power of RNA sequencing

RNA extraction

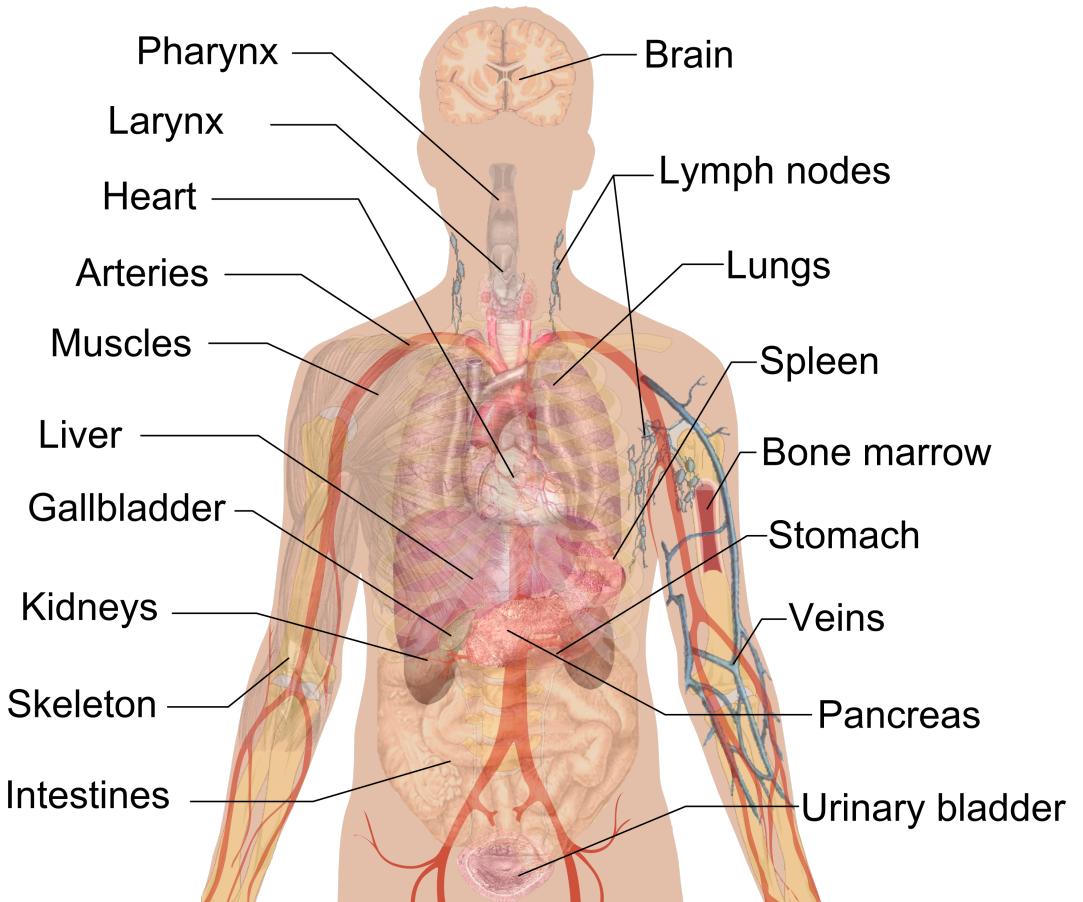


A photograph of a NextSeq 500 RNA sequencing instrument, which is a benchtop sequencer.

> Seq #1
ACTAGTCAACCTCGGACCAGAGCACACGGGCAGGAATCA
> Seq #2
TGTGACGTACACGTCACATGTGTCACAGATAGACATGACG
> Seq #3
TTGGCTCCACACTGCCGCATGCATGCGTGCGATCGAGCC
> Seq #4
AAACACACGTGCGTGCACGTCTTCTCAGGAGATCTCGAC
...
> Seq #10,000,000
...

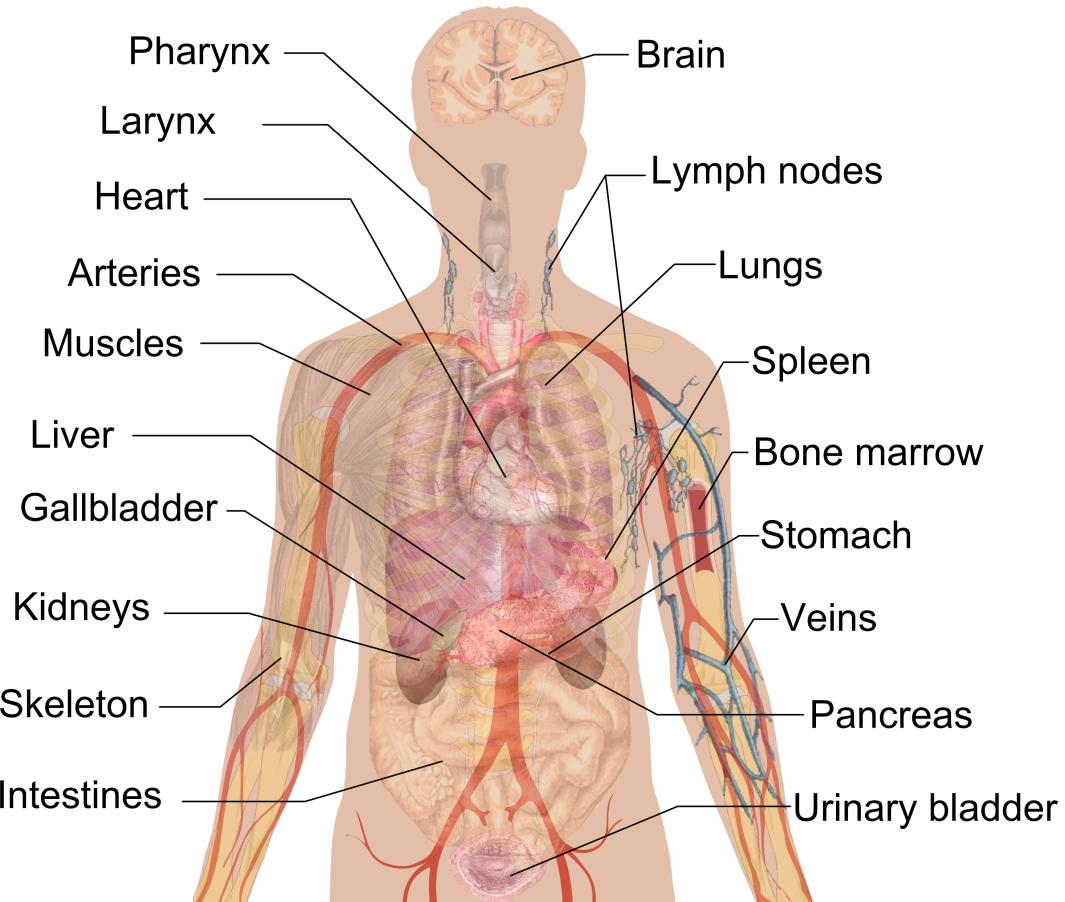
RNA
sequencing

Next generation sequencing is transforming biology and medicine

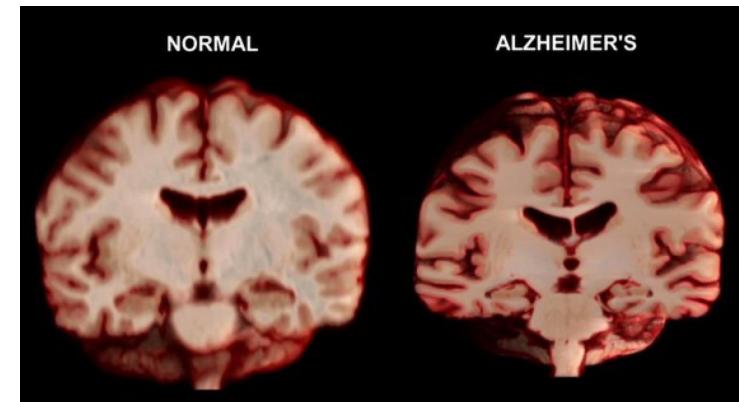


Sequencing machine

Next generation sequencing is transforming biology and medicine

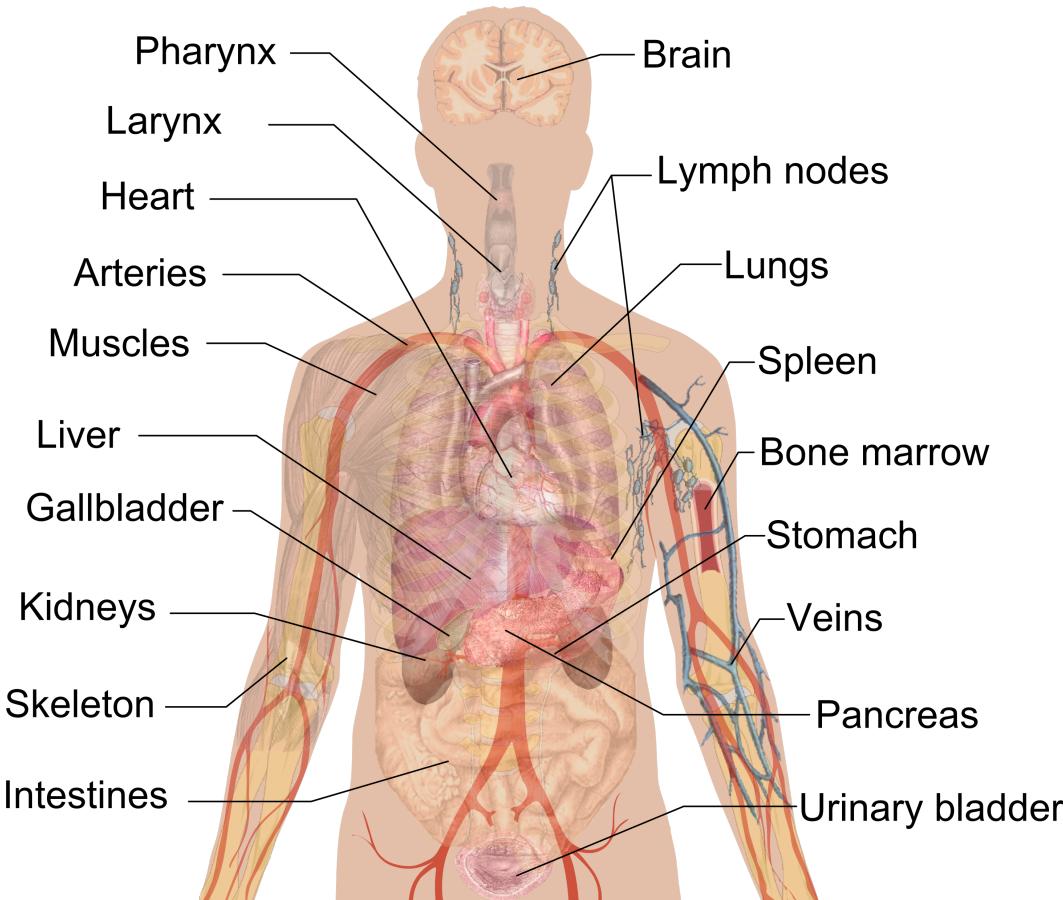


Sequencing machine

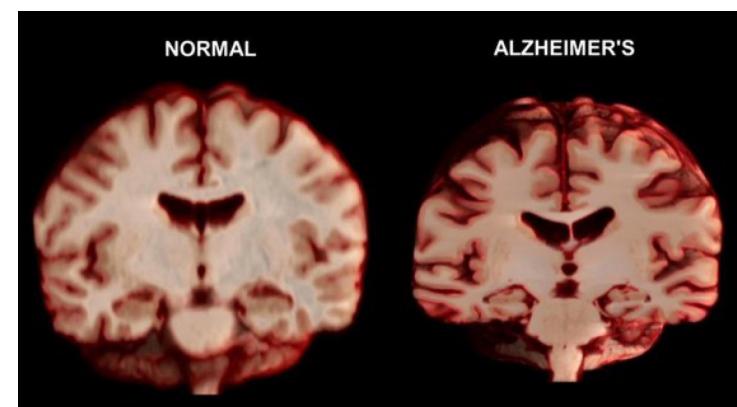


Genomics = study of the genome, including the DNA itself, and all the genes and their interactions

Transcriptomics = study of all the RNA



Sequencing machine



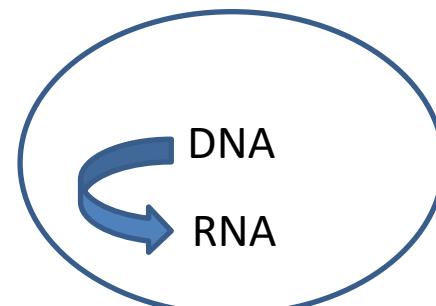
~100,000
Single-celled
Trichomonas vaginalis



~20,000
C. elegans



~20,000 Proteins in humans



RNA
Protein



~25,000
Arabidopsis thaliana



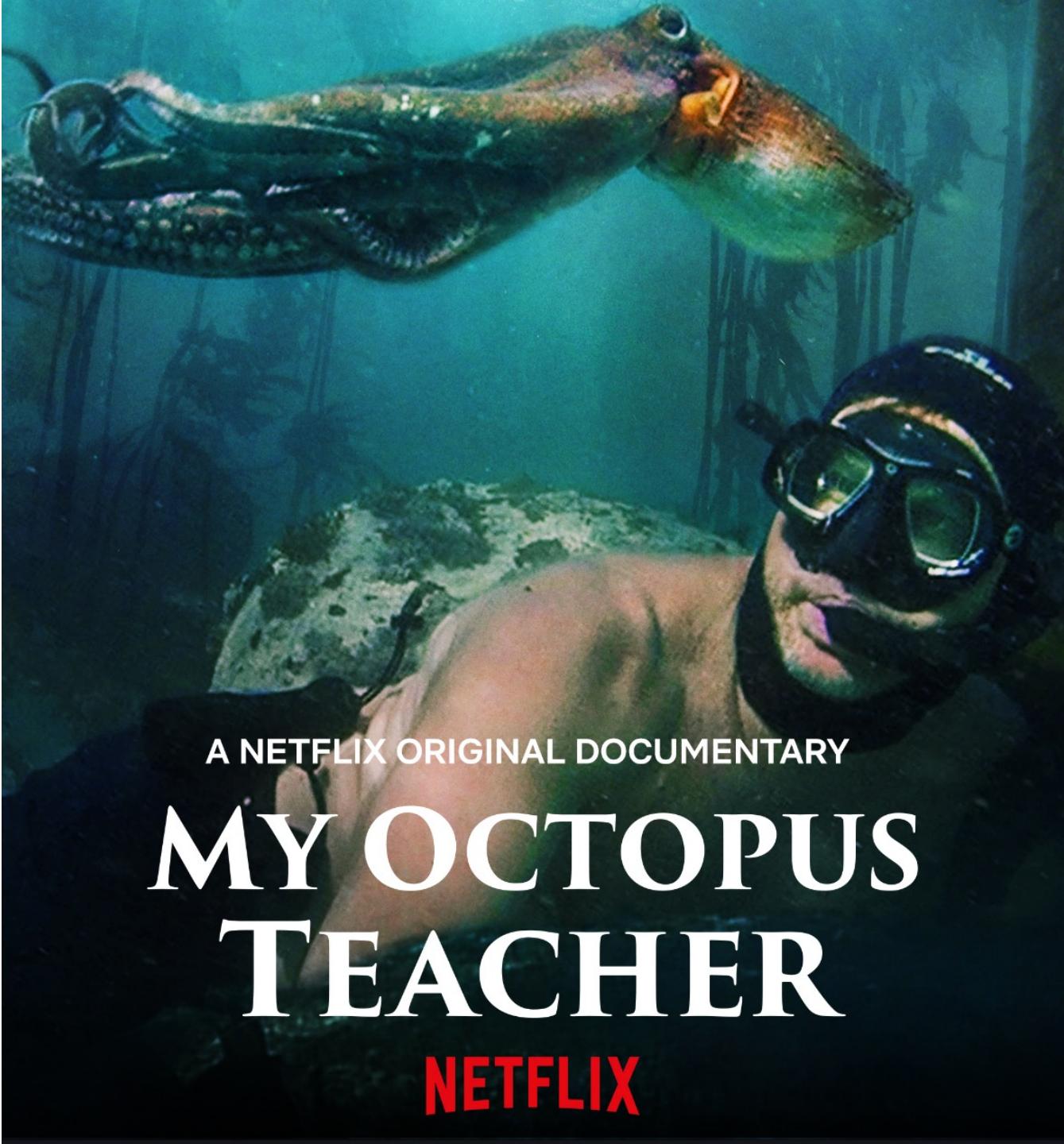
~50,000
Rice

How can we explain complexity with such a small number of genes?



Talk outline

- The importance of RNA sequencing
- An example: what makes squid and octopus smart?
- Single cell genomics
- Spatially-resolved transcriptomics
- RNA content of brain tissues in super-resolution



A NETFLIX ORIGINAL DOCUMENTARY

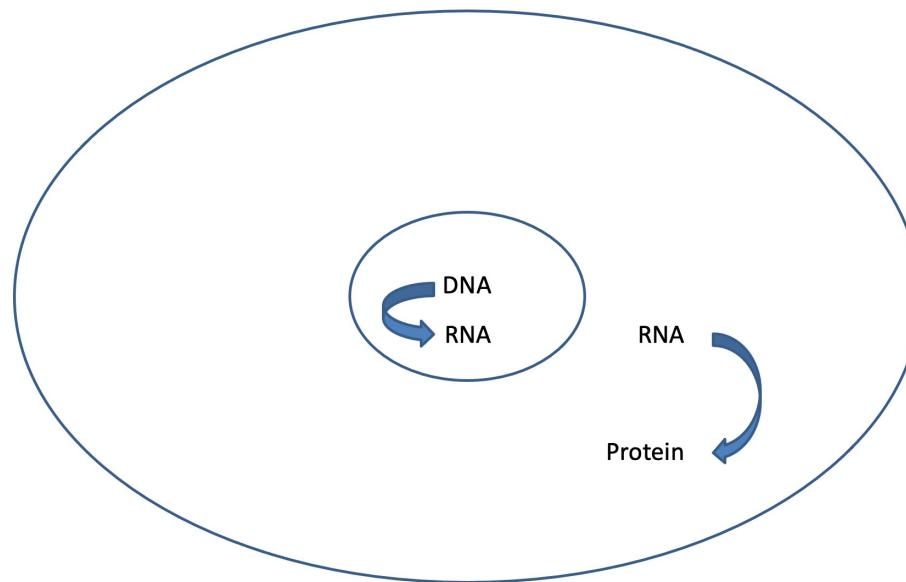
MY OCTOPUS TEACHER

NETFLIX

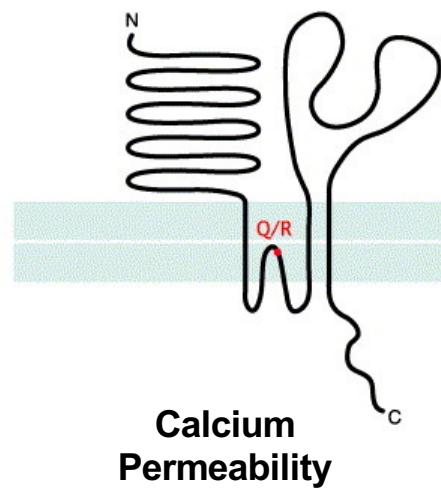
What makes cephalopods smart?



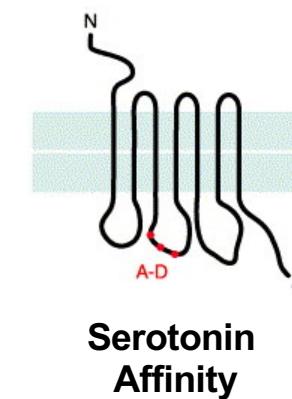
RNA editing adds complexity to the mRNAs in the human brain



Glutamate receptors: GluR2, GluR5, GluR6



Calcium Permeability



Serotonin Affinity

Serotonin 5HT2C receptors

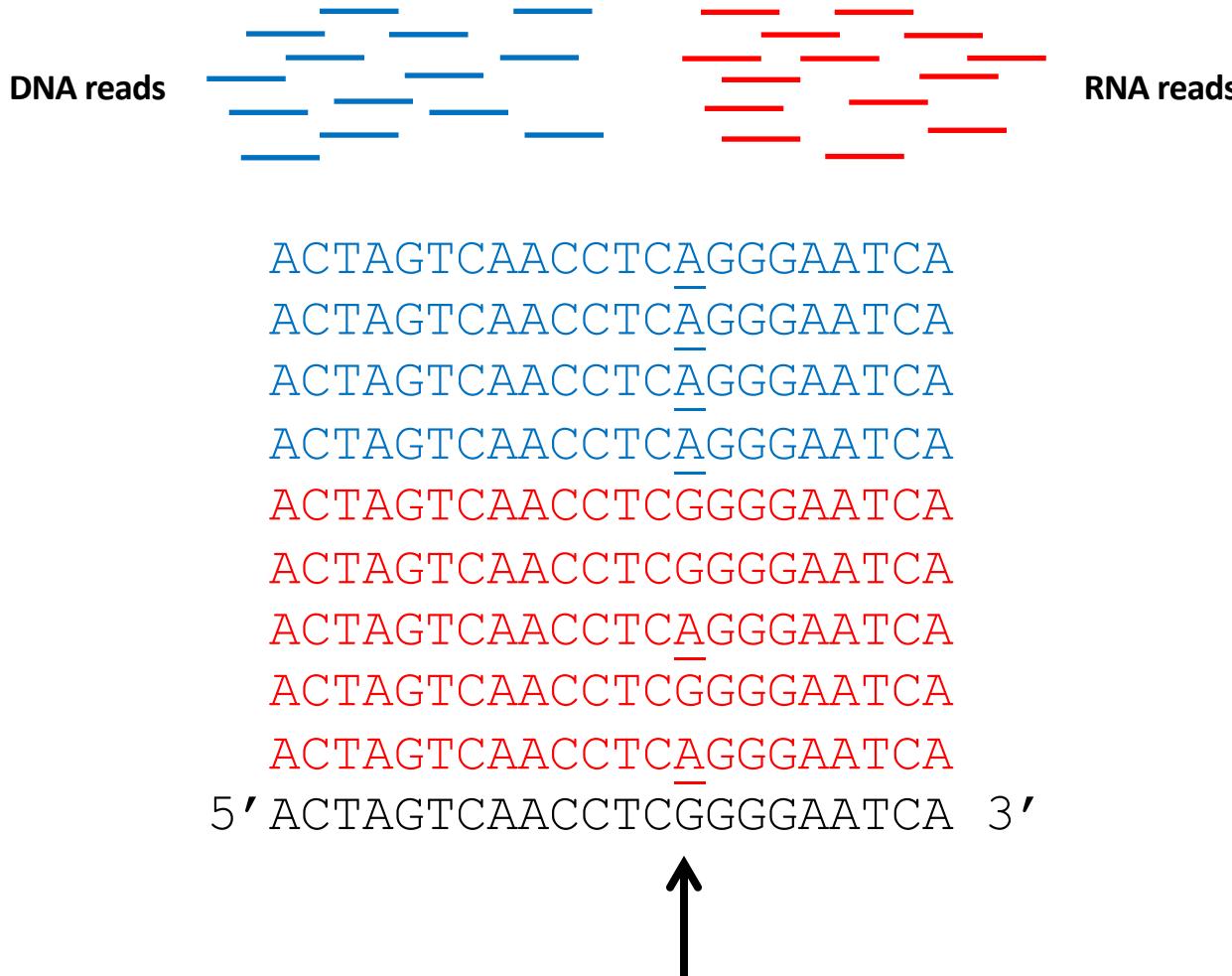
Molecular characterization of the squid



Loligo pealei,
the Atlantic
squid

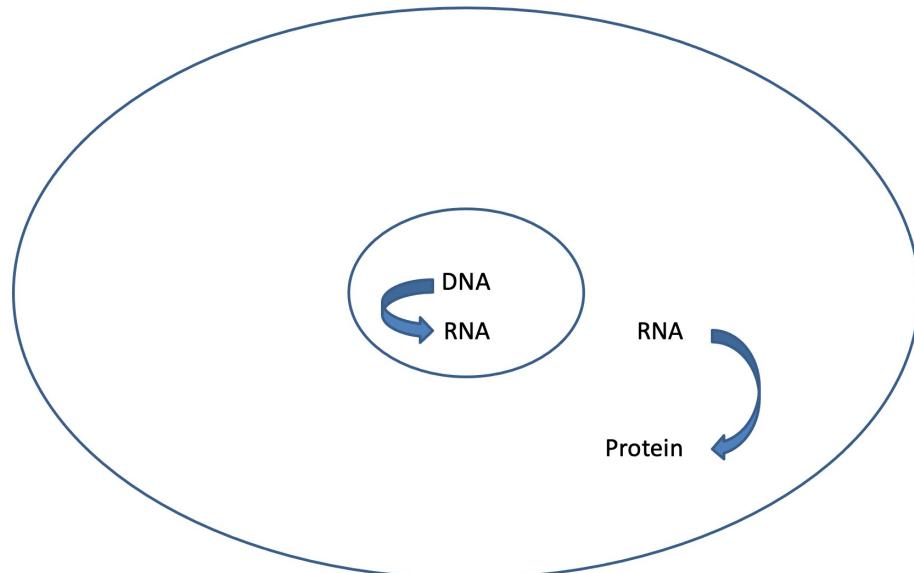
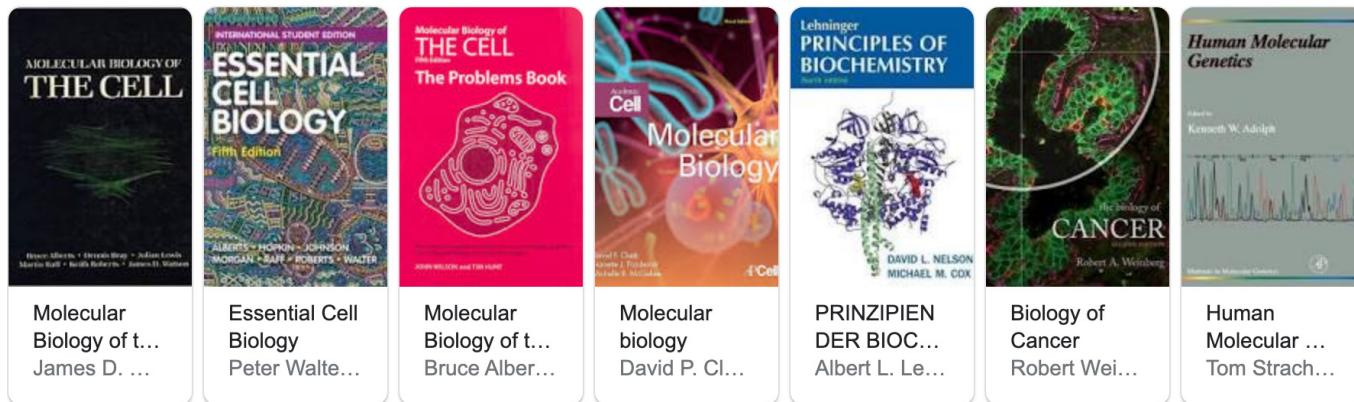


Transforming biological question into a data analysis question



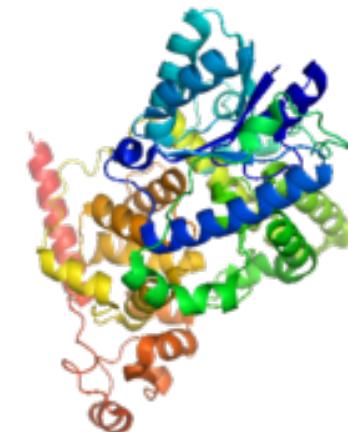
RNA editing is widespread in almost all the genes in the squid & octopus brain

Textbooks / Molecular biology



RNA=DNA??

One protein with 2^{10} variations



Science

Octopuses and squids can rewrite their RNA. Is that why they're so smart?



EVOLUTION Curiouser and Curiouser-- Octopus's Evolution Is Even Stranger Than Thought

nature

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nature > research highlights > article

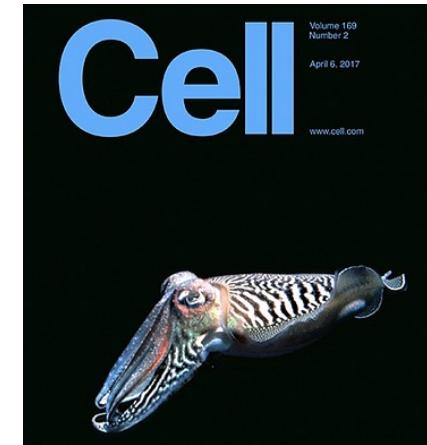


Octopuses (*Octopus vulgaris*) extensively edit their RNA to diversify the proteins they make. Claudio Contreras/NPL

MOLECULAR BIOLOGY · 06 APRIL 2017

Octopus smarts may come from RNA edits

Compared with other species, complex cephalopods stray more from the instructions written in their genomes.

**Science** Contents ▾ News ▾ Careers ▾ Journals ▾

RNA EDITING

Squid are hyper-editors when it comes to RNA

The New York Times**TRILOBITES**

A Genetic Oddity May Give Octopuses and Squids Their Smarts

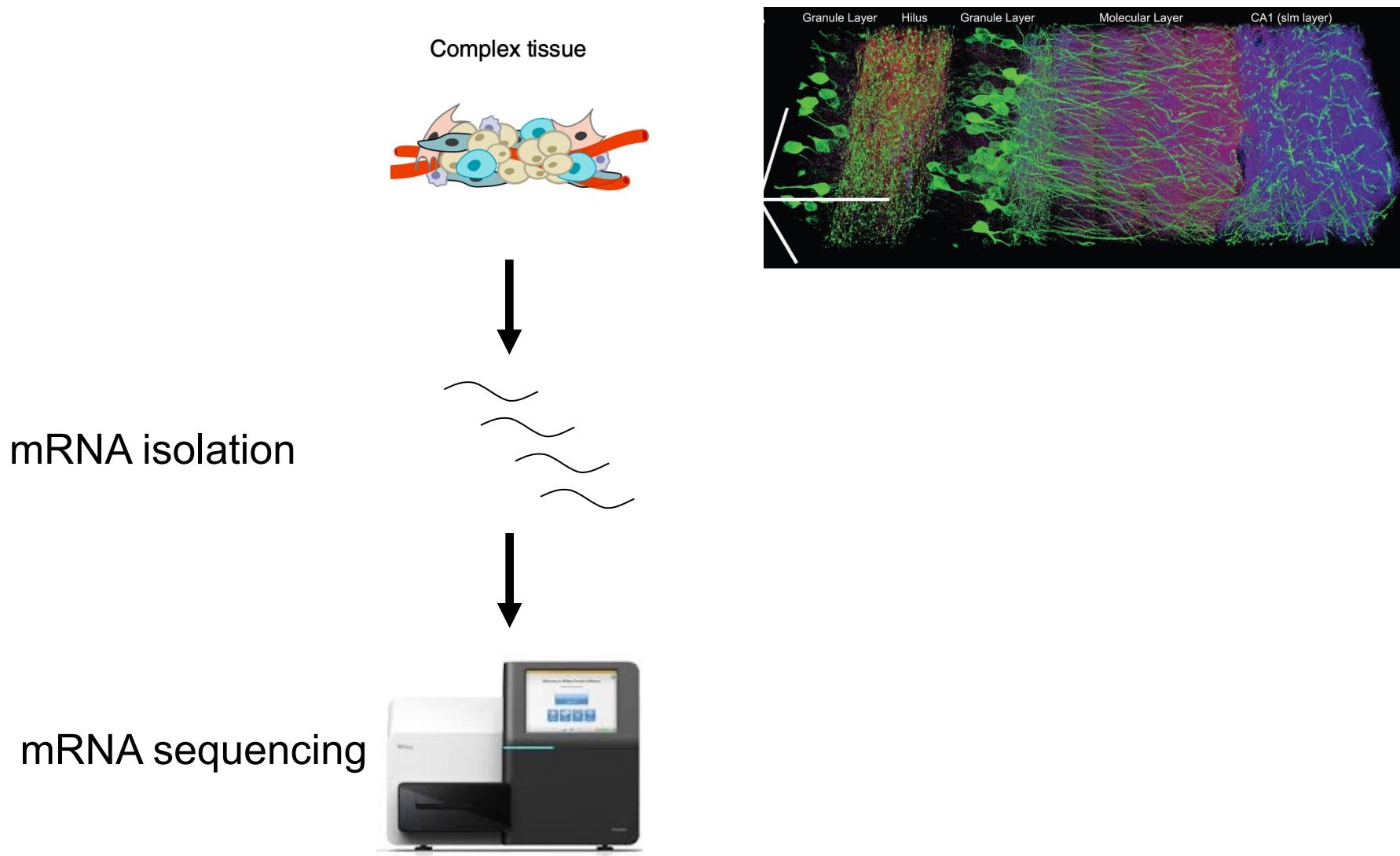
Which one is the exception?



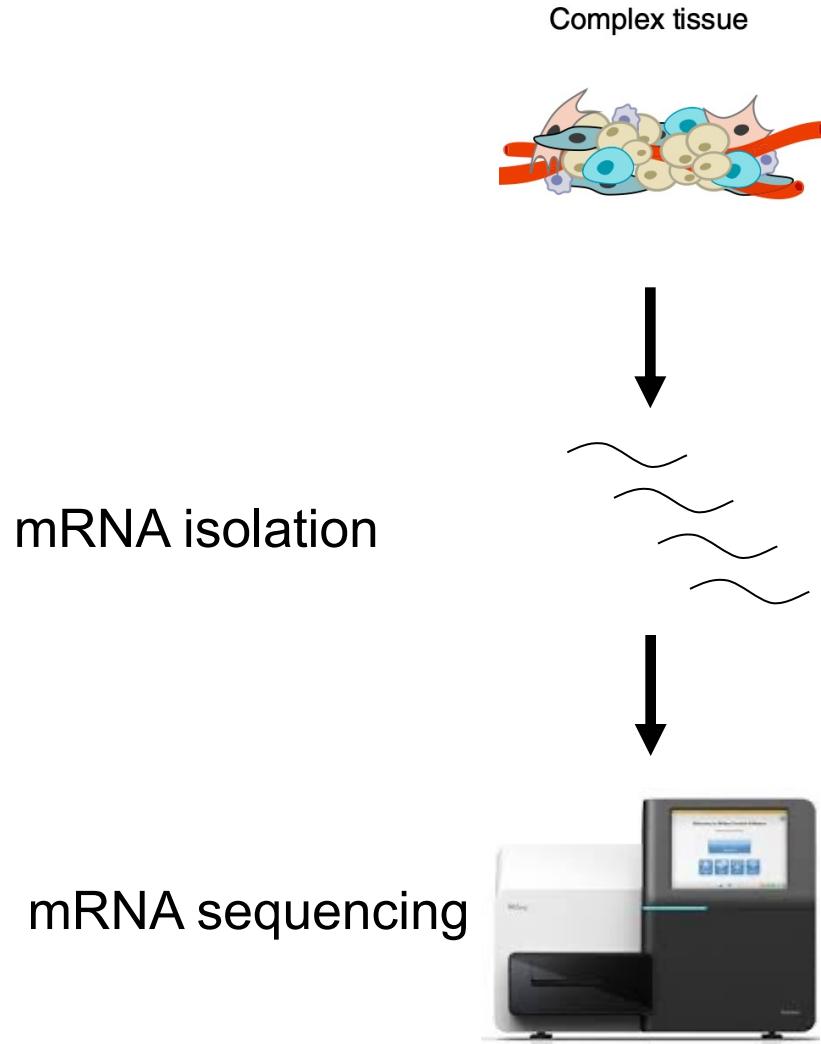
Talk outline

- The importance of RNA sequencing
- An example: what makes squid and octopus smart?
- Single cell genomics
- Spatially-resolved transcriptomics
- RNA content of brain tissues in super-resolution

What do we lose in bulk sequencing?



What do we lose in bulk sequencing?

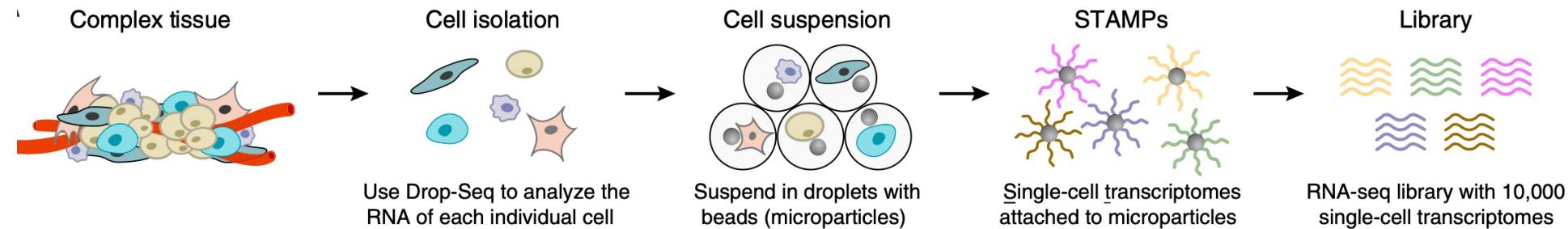


Single cell RNA sequencing



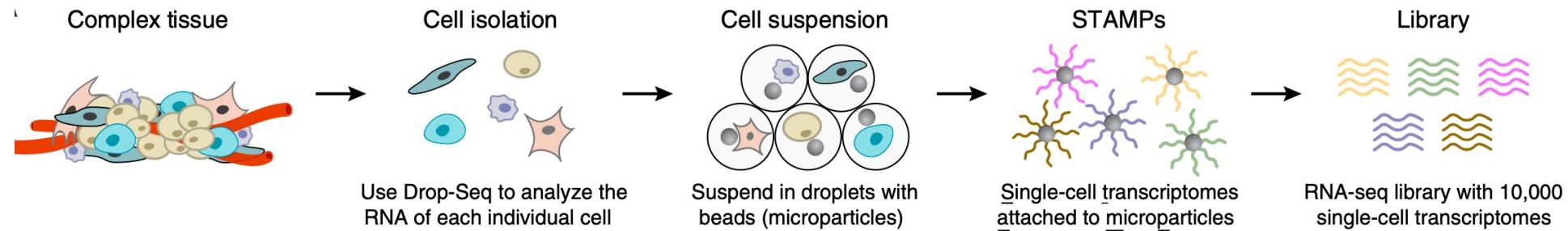
Macosko et al., Cell, 2015

Single cell RNA sequencing



Macosko et al., Cell, 2015

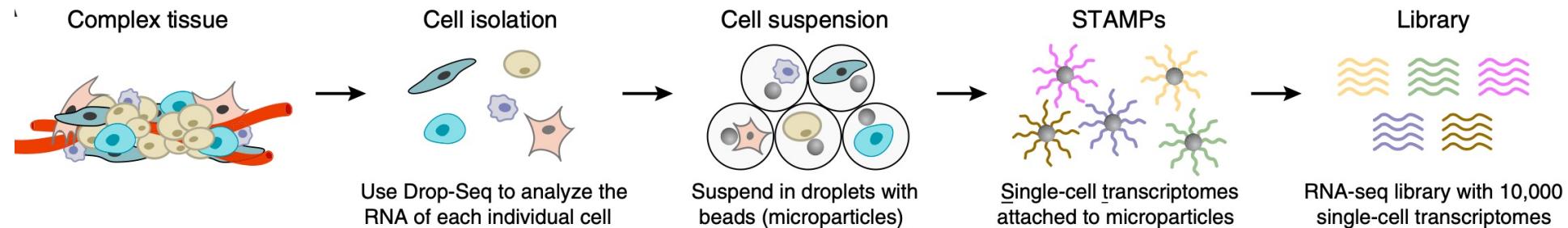
Single cell RNA sequencing



Macosko et al., Cell, 2015

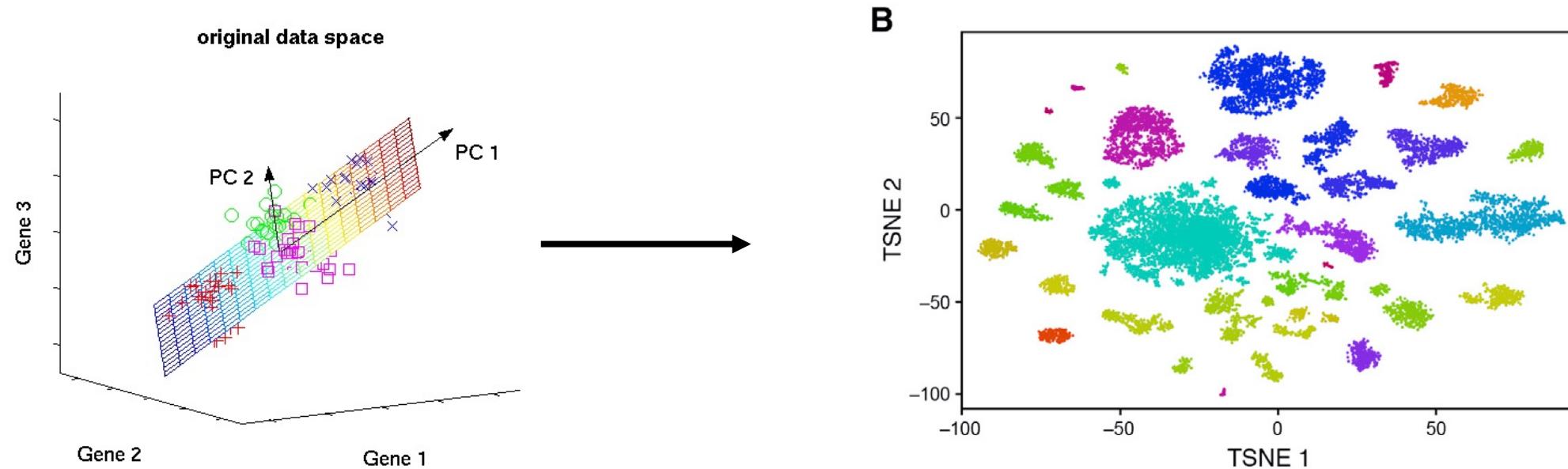
- How can we compare cells when we have ~20,000 dimensions?

Single cell RNA sequencing

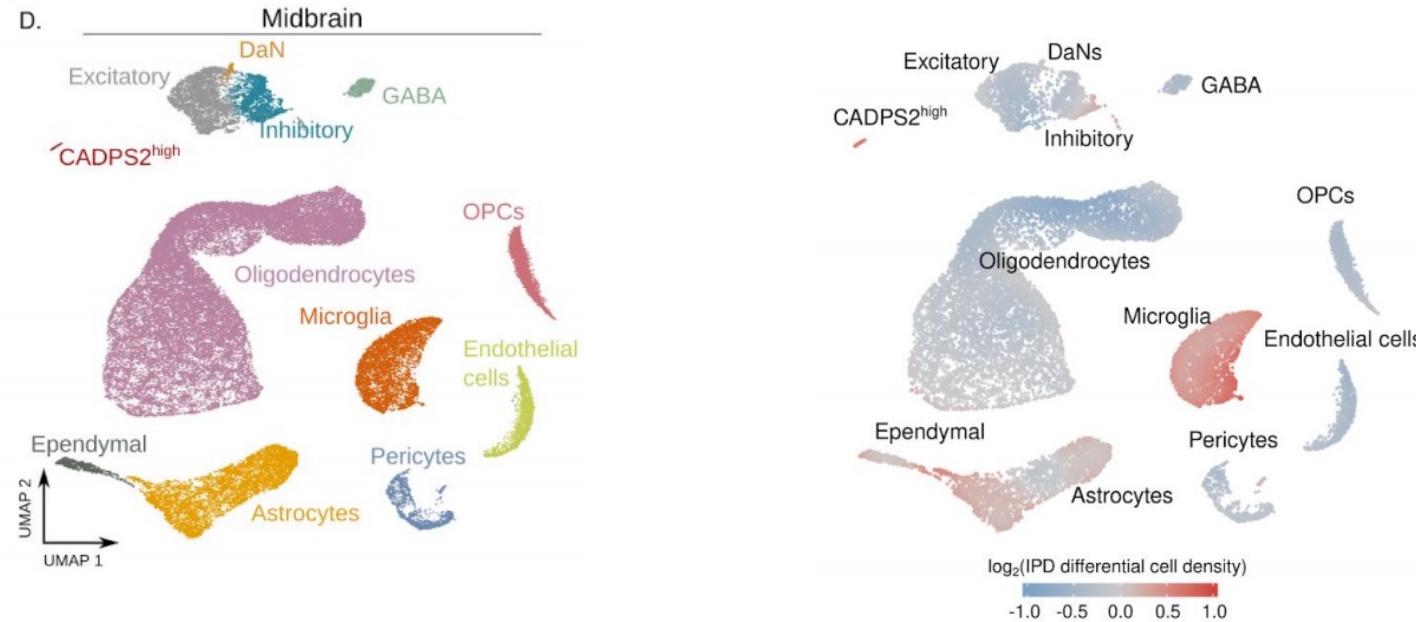


Macosko et al., Cell, 2015

- How can we compare cells when we have ~20,000 dimensions?



Single cell RNA sequencing in Parkinson's Disease (PD)



Smajić et al., MedRxiv, 2020

- Substantia nigra and midbrain of postmortem human donors

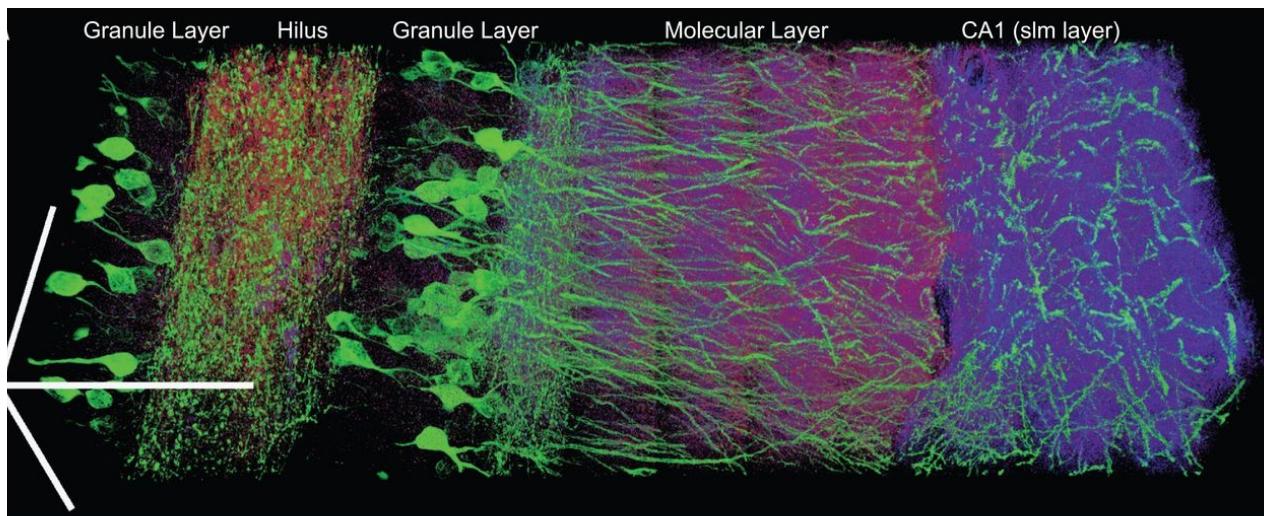
Talk outline

- The importance of RNA sequencing
- An example: what makes squid and octopus smart?
- Single cell genomics
- Spatially-resolved transcriptomics
- RNA content of brain tissues in super-resolution

What kind of information is lost with next-generation sequencing?



Sequencing
machine

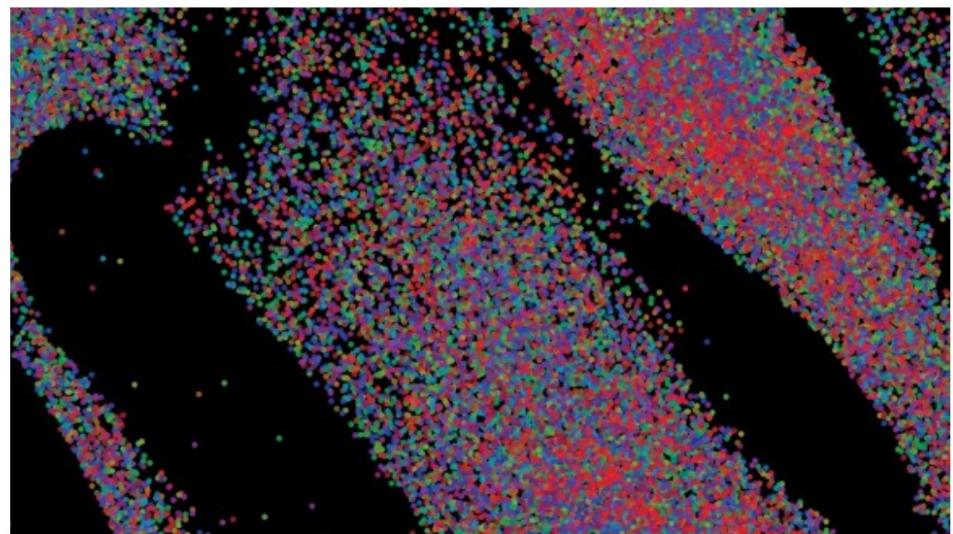


nature > nature methods > focus

FOCUS | 06 JANUARY 2021

Method of the Year 2020: spatially resolved transcriptomics

Spatially resolved transcriptomics is our Method of the Year 2020, for its ability to provide valuable insights into the biology of cells and tissues while retaining information about spatial context.

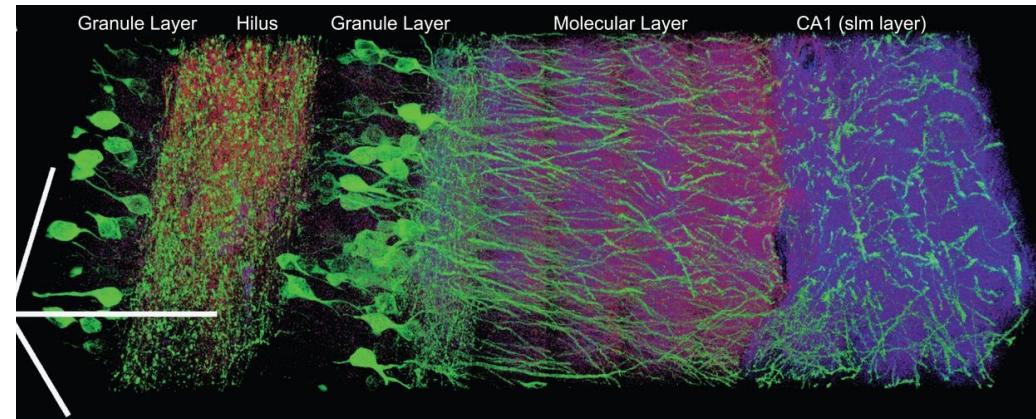


The gap between imaging and sequencing



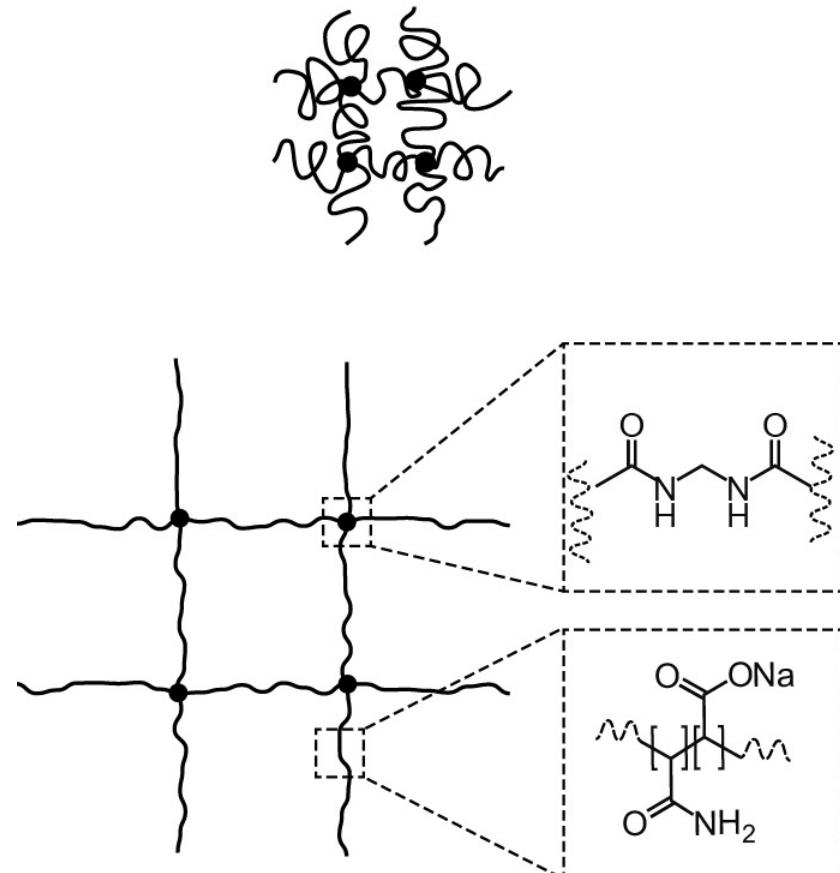
RNA
sequencing

The concept of *in situ* sequencing (Lee *et al.*, 2014, Science)

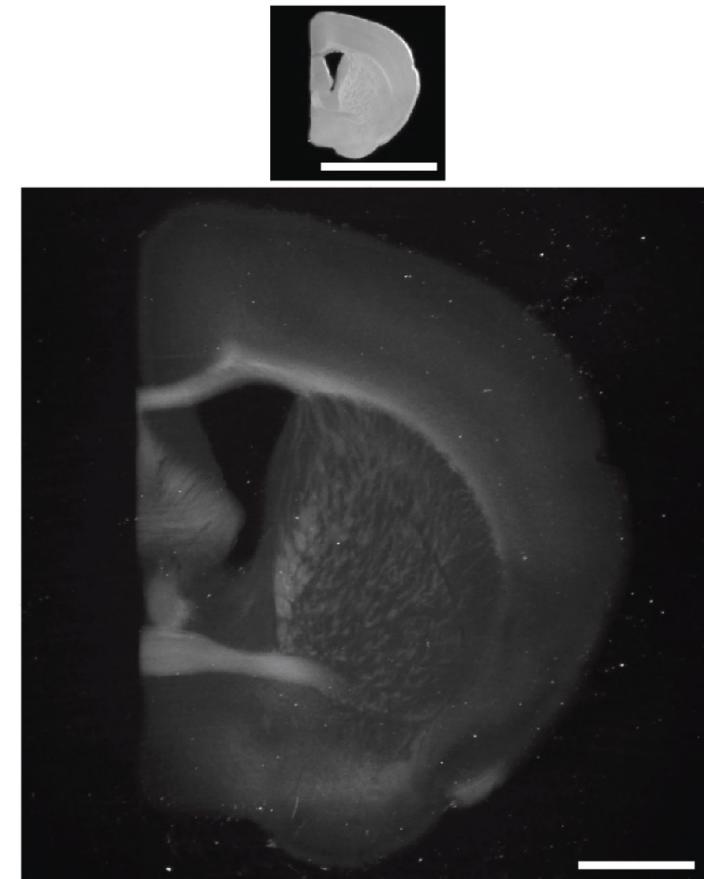


Physical magnification of objects with expansion microscopy

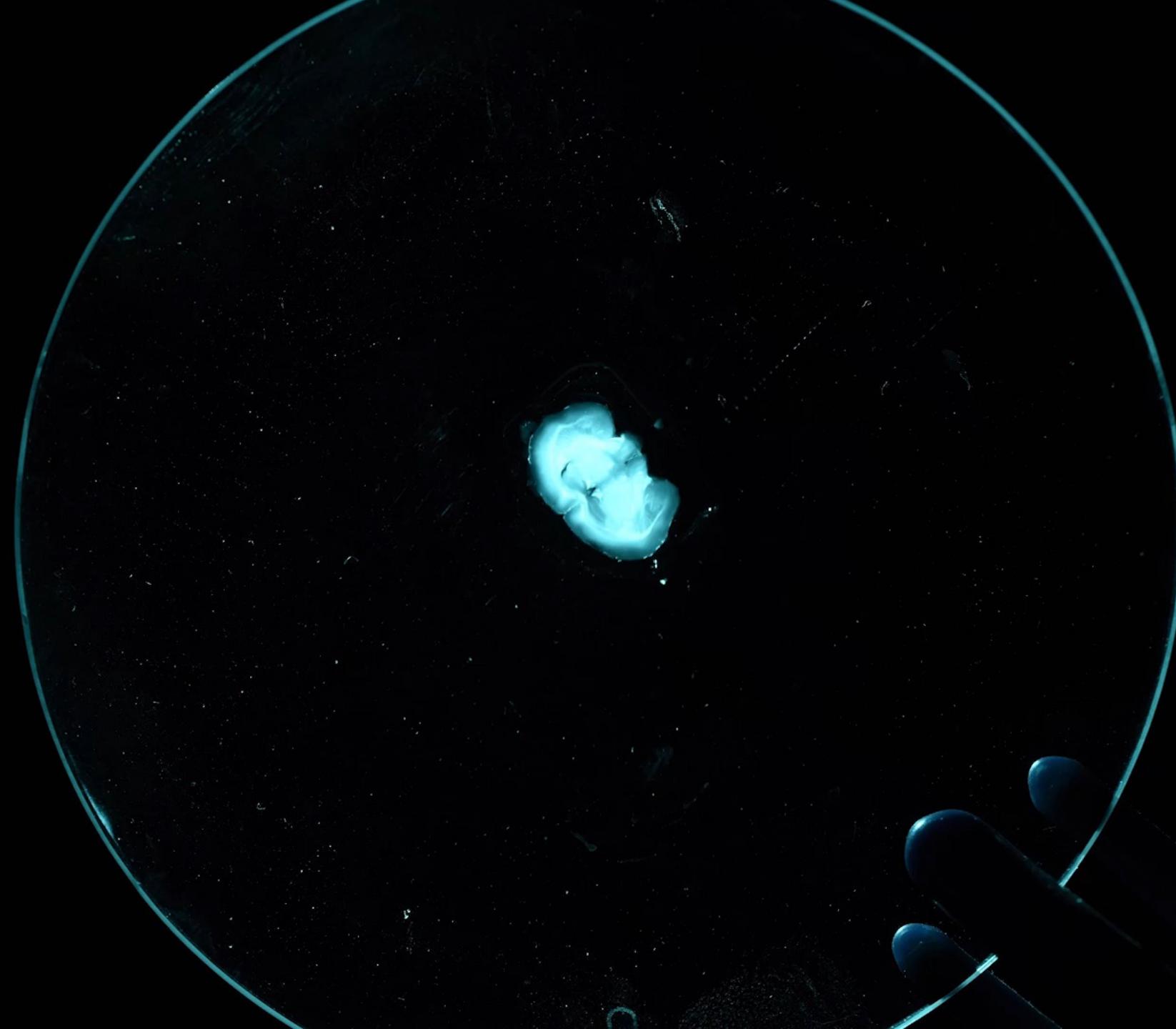
Sodium polyacrylate:
polymer that swells in water
(found in baby diapers)

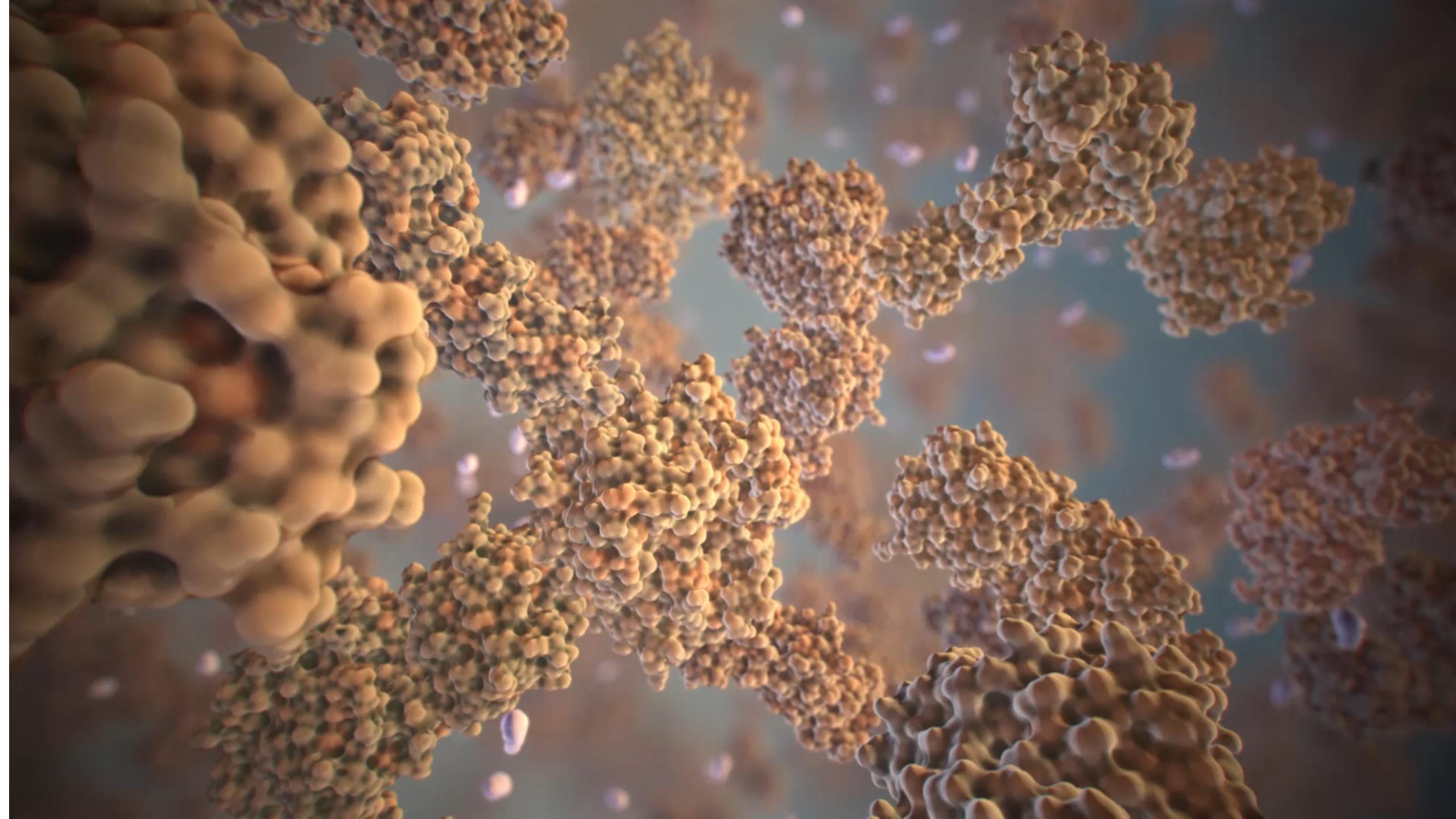


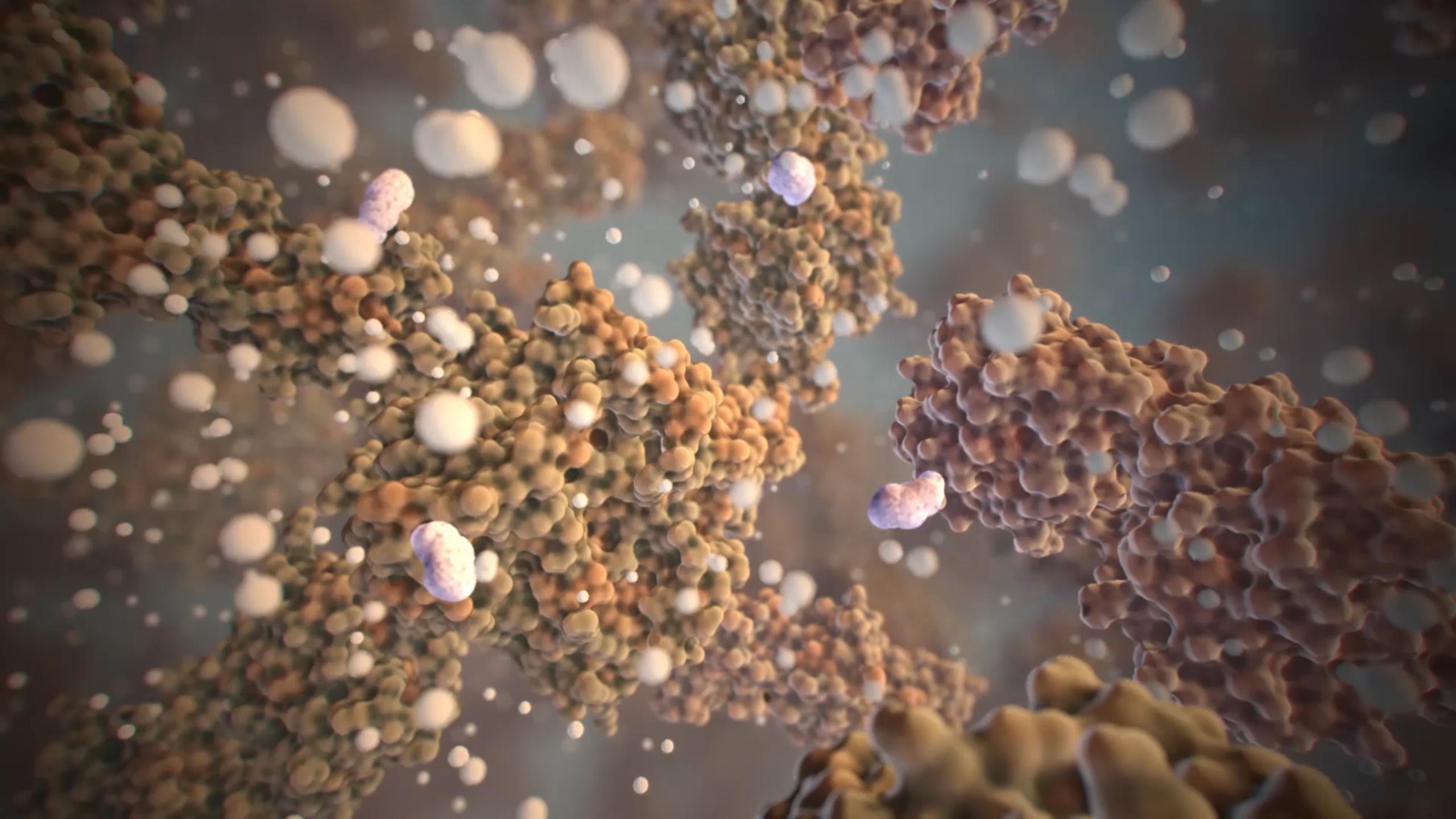
Embed brain
in polyacrylate network, digest the specimen,
then add water

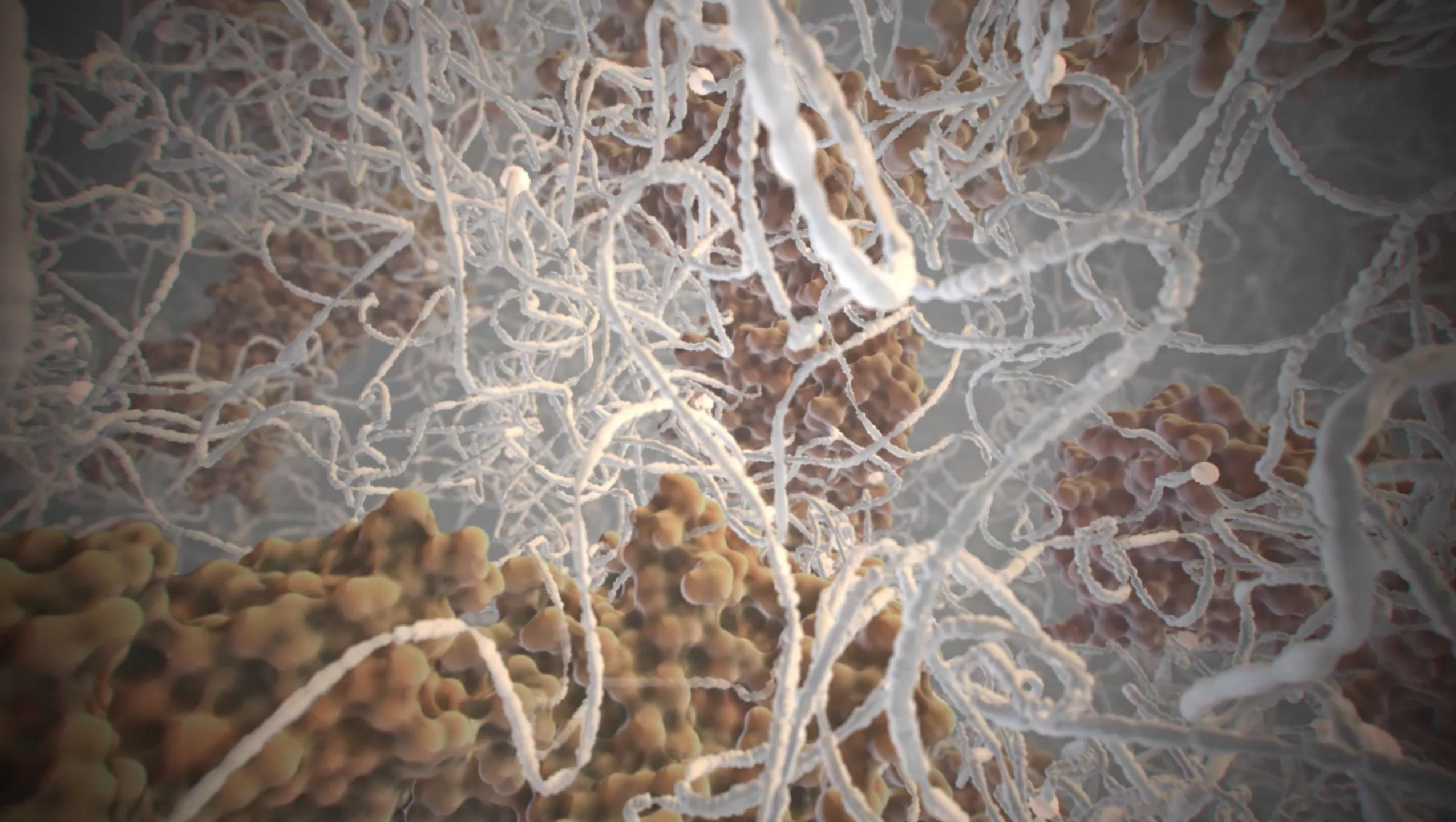


Scale bars: 5 mm









Talk outline

- The importance of RNA sequencing
- An example: what makes squid and octopus smart?
- Single cell genomics
- Spatially-resolved transcriptomics
- **RNA content of brain tissues in super-resolution**

What is the distribution of RNA in neurons?

Local protein synthesis is required for synaptic plasticity

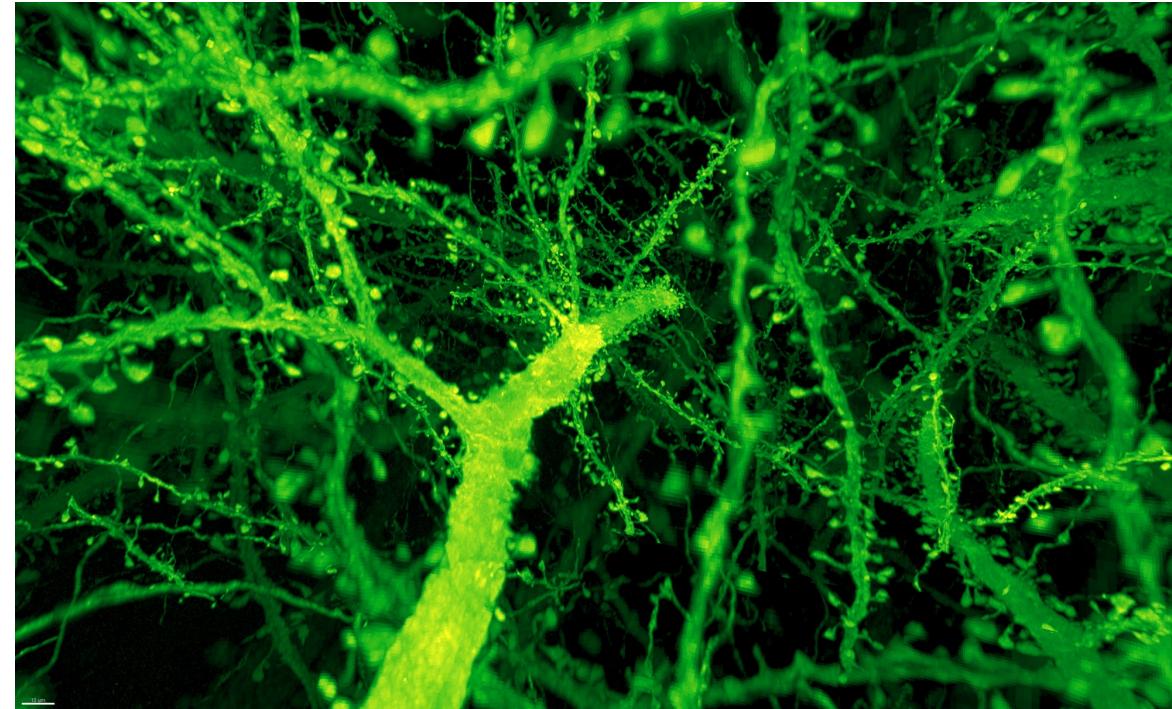
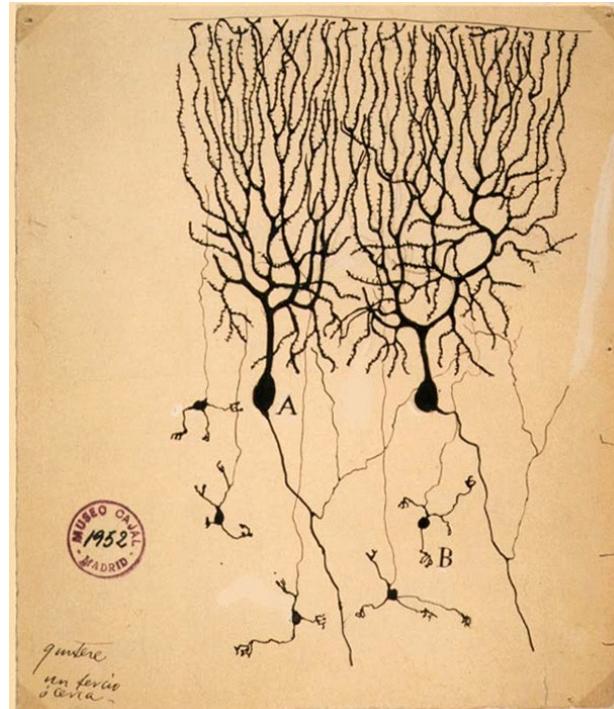
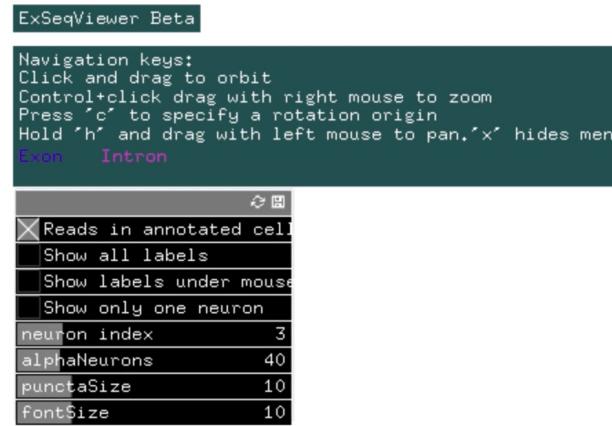
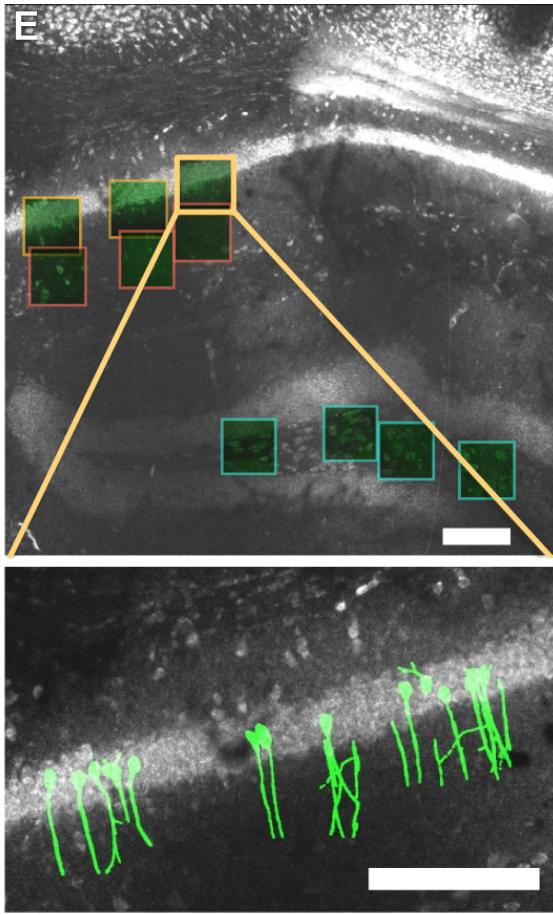


Figure: Gao *et al.*, 2019, Science

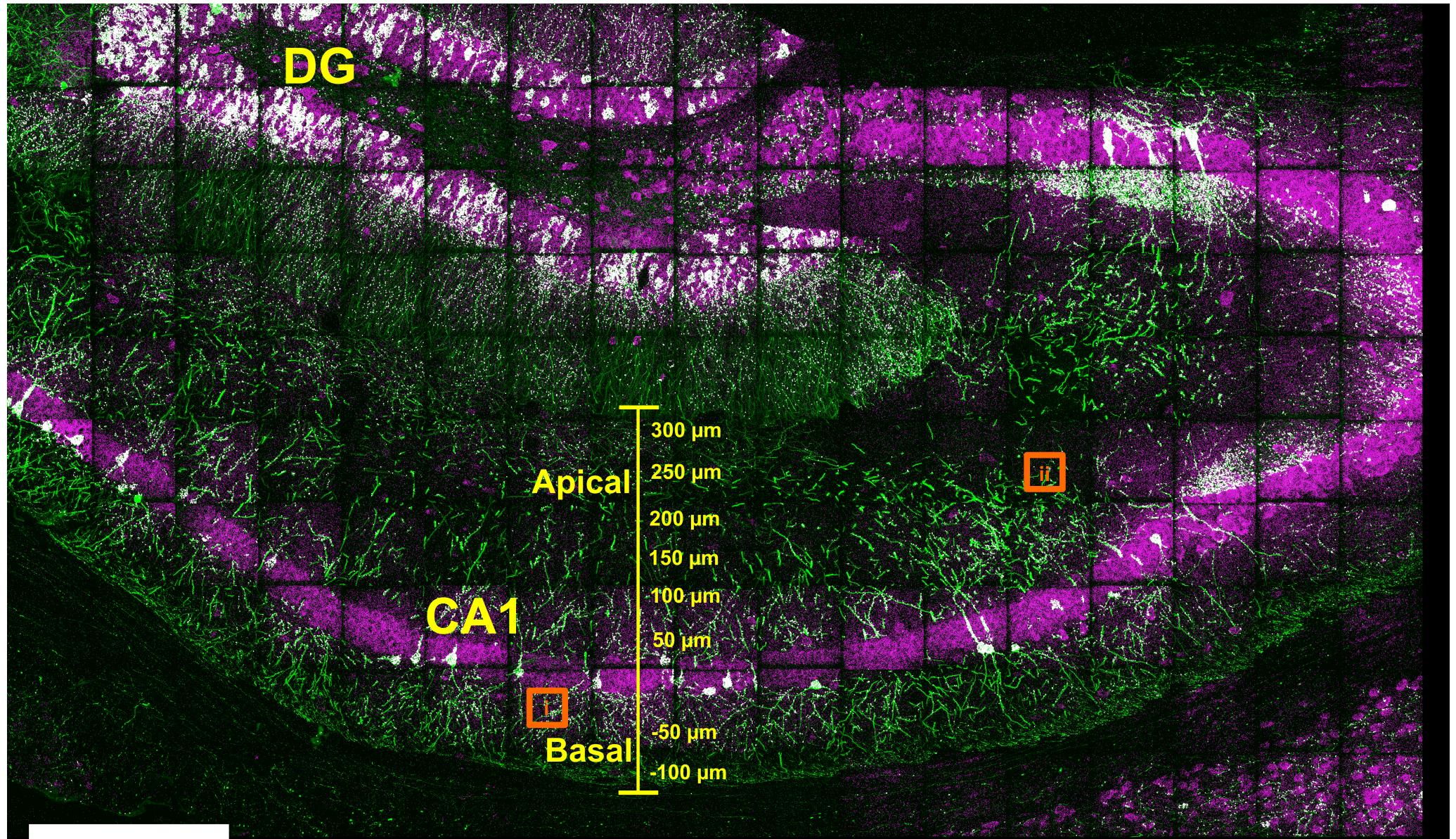
Expansion sequencing

Mouse Hippocampus

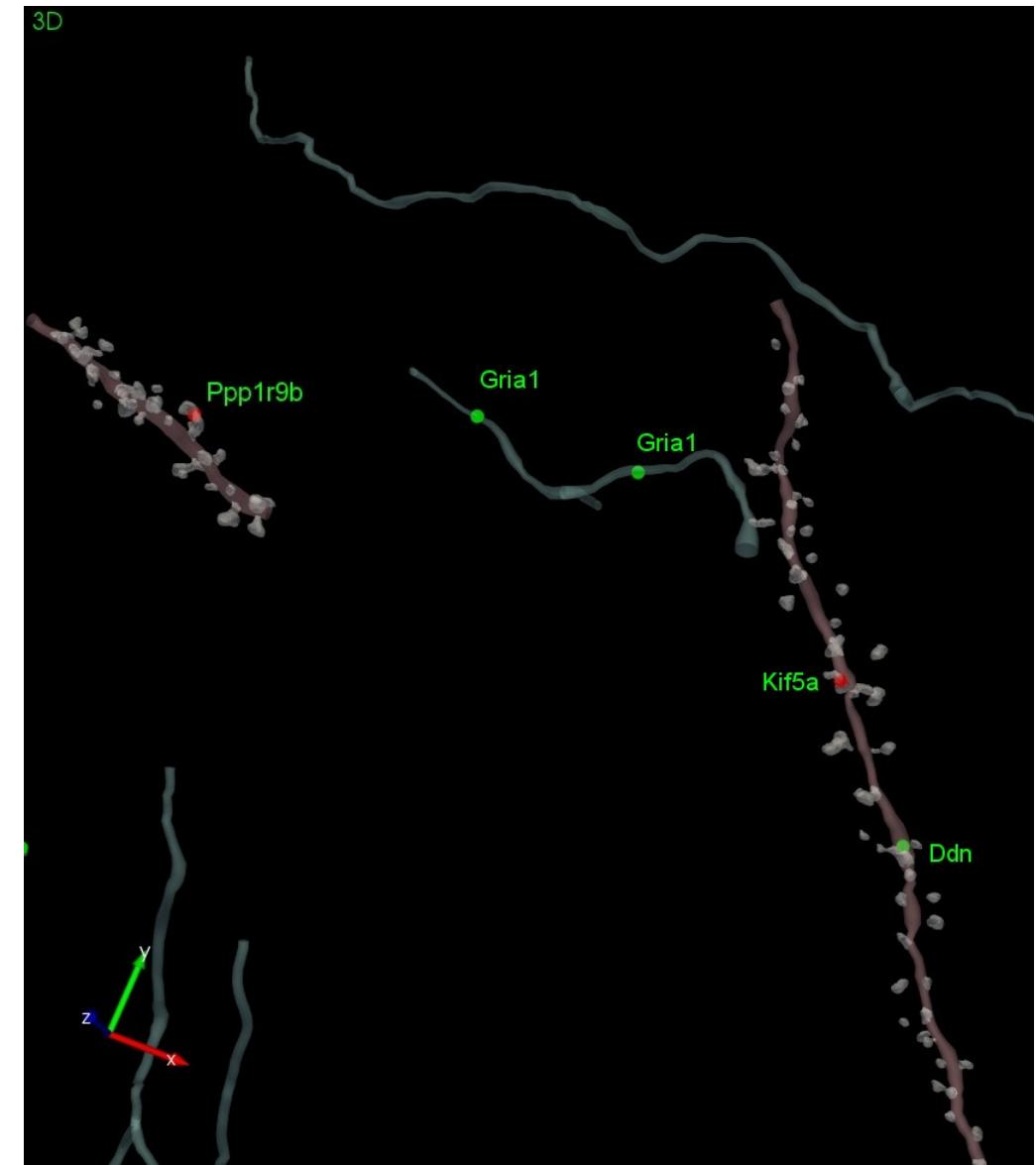
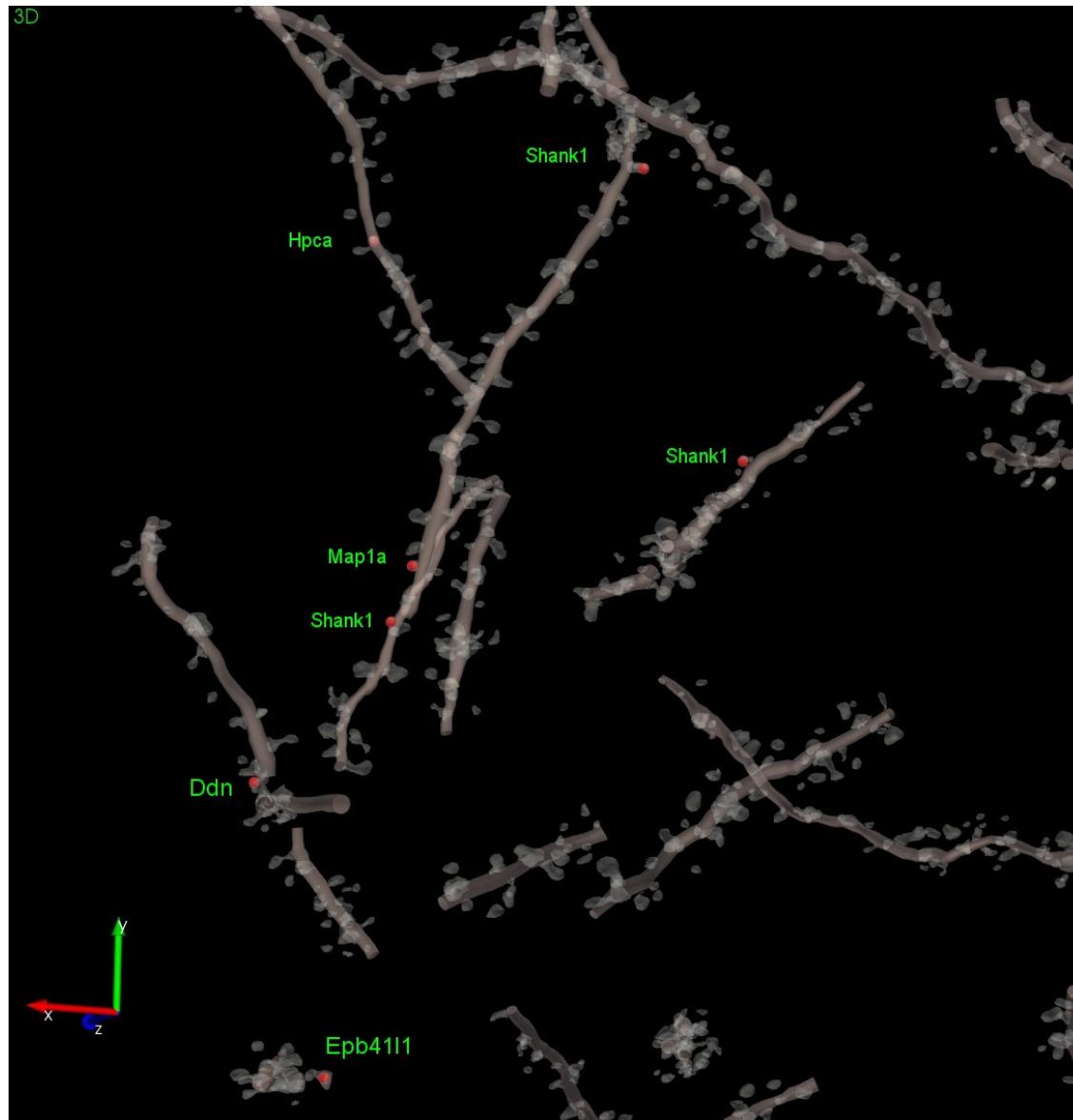


Mapping the mouse hippocampus with nanoscale resolution

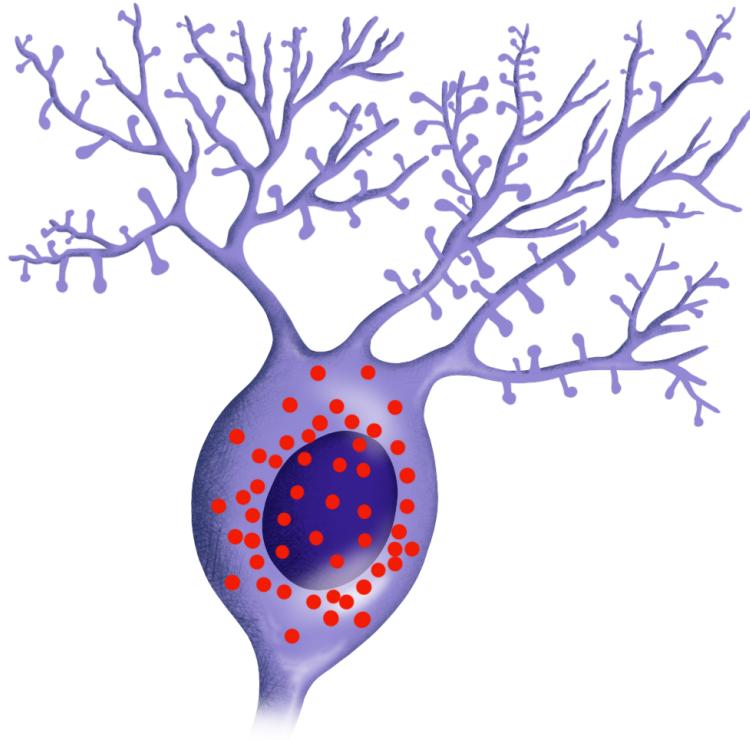
170 fields of view,
1 week of imaging,
1 week of image
analysis
1.2 million reads,
34 different mRNAs,
10TB of data
106,000 individual
spines segmented



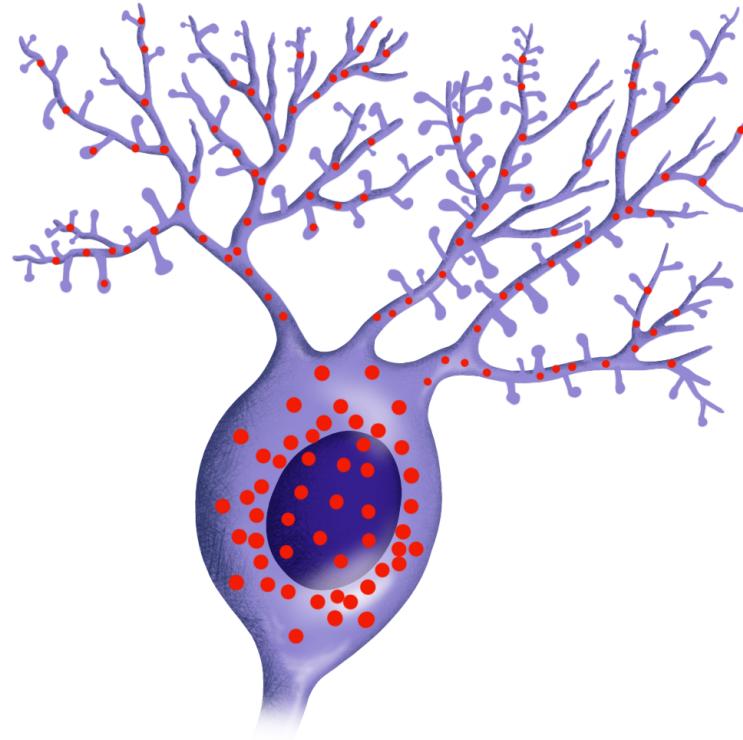
Mapping the mouse hippocampus with nanoscale resolution



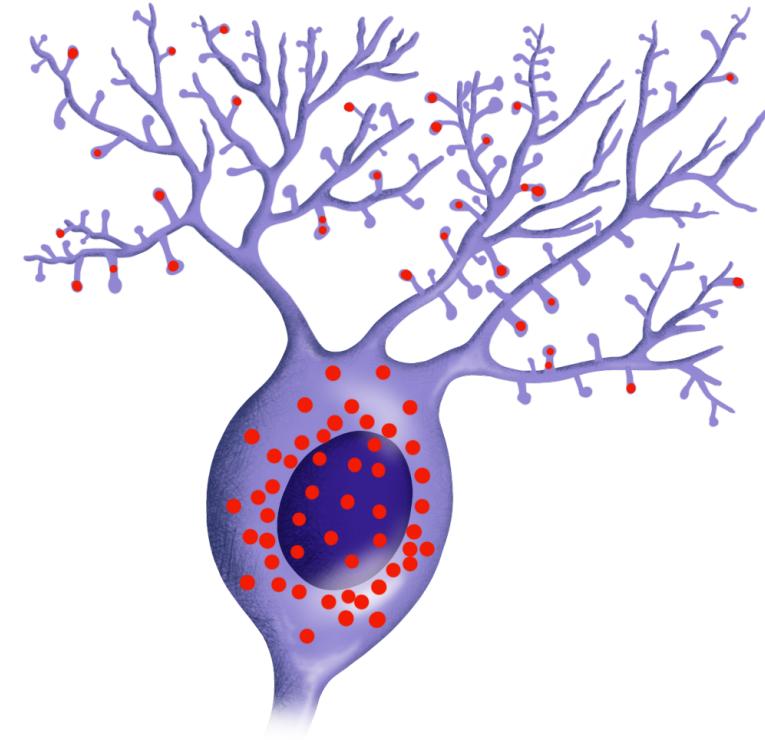
RNA expression patterns in neurons



Pattern I



Pattern II



Pattern III