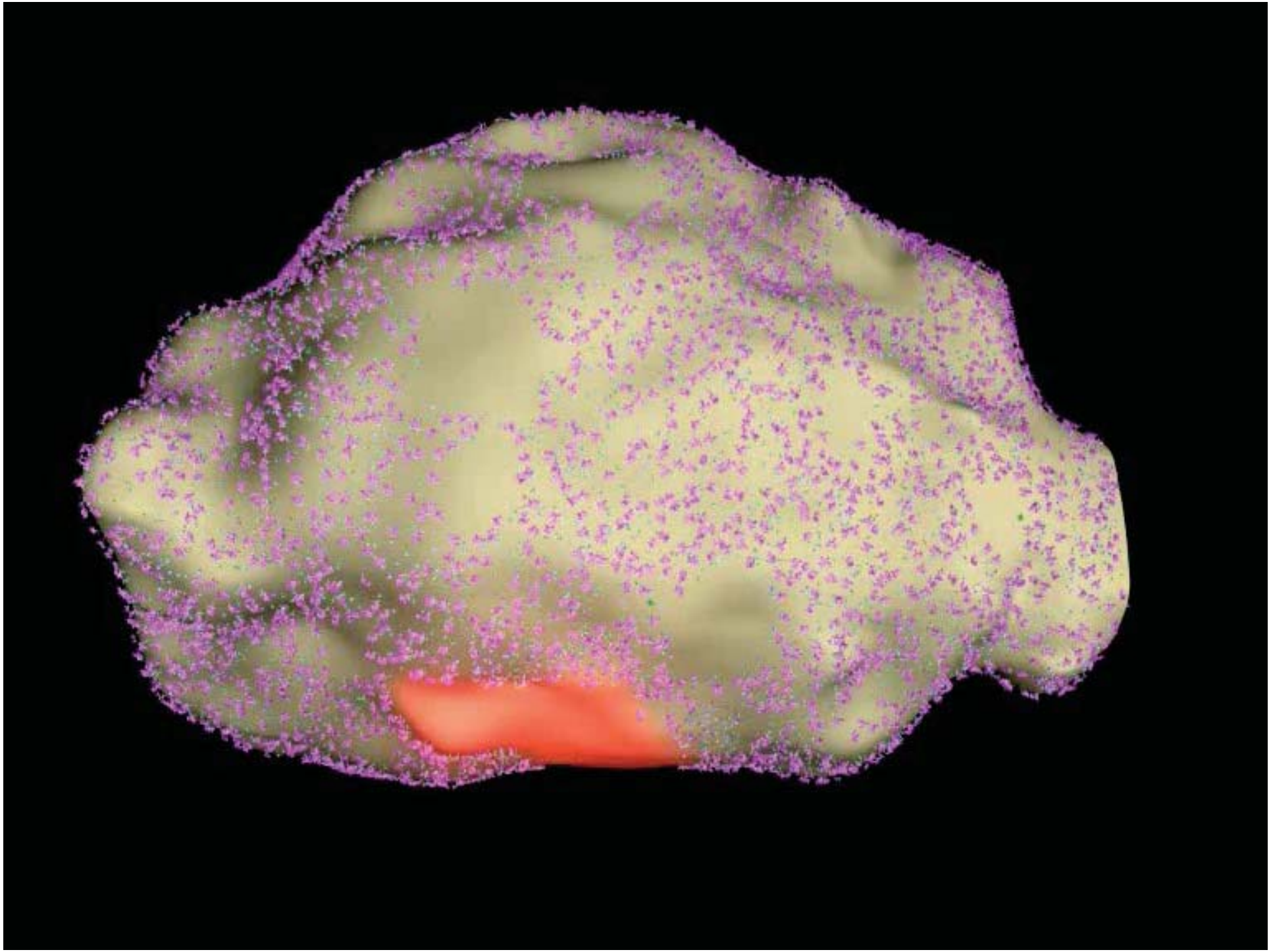
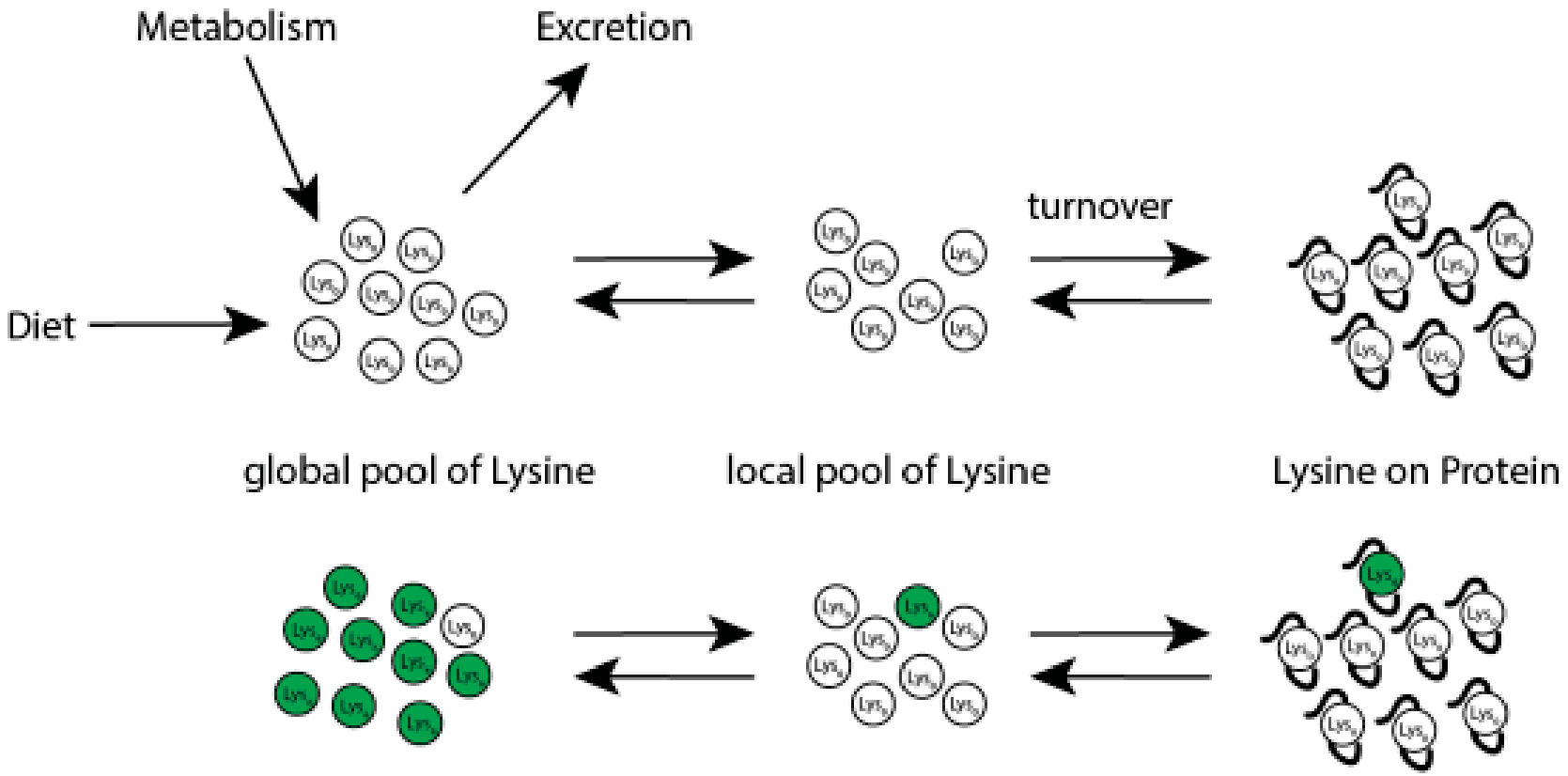


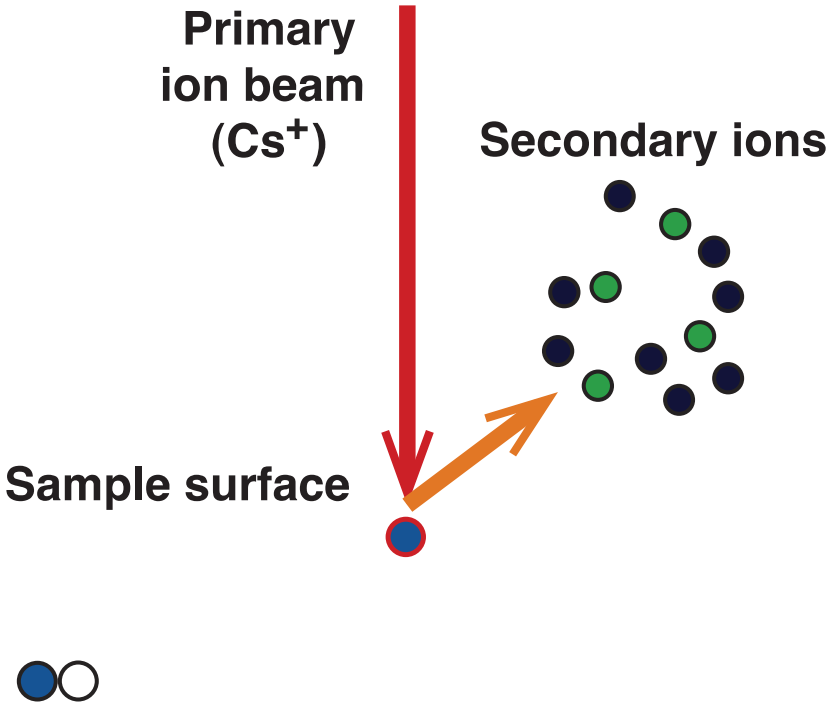
What about the temporal domain, looking at transformations that take hours, days or weeks?

Measure the incorporation of unusual atoms



adapted from Price, JC *et al*., (2010)

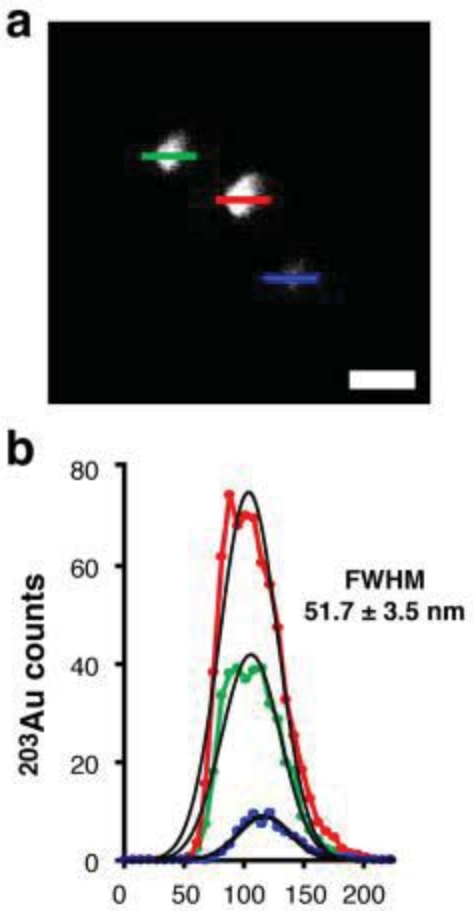
Ideal tool: nanoscale secondary ion mass spectrometry (NanoSIMS)



NanoSIMS 50 L
by Cameca

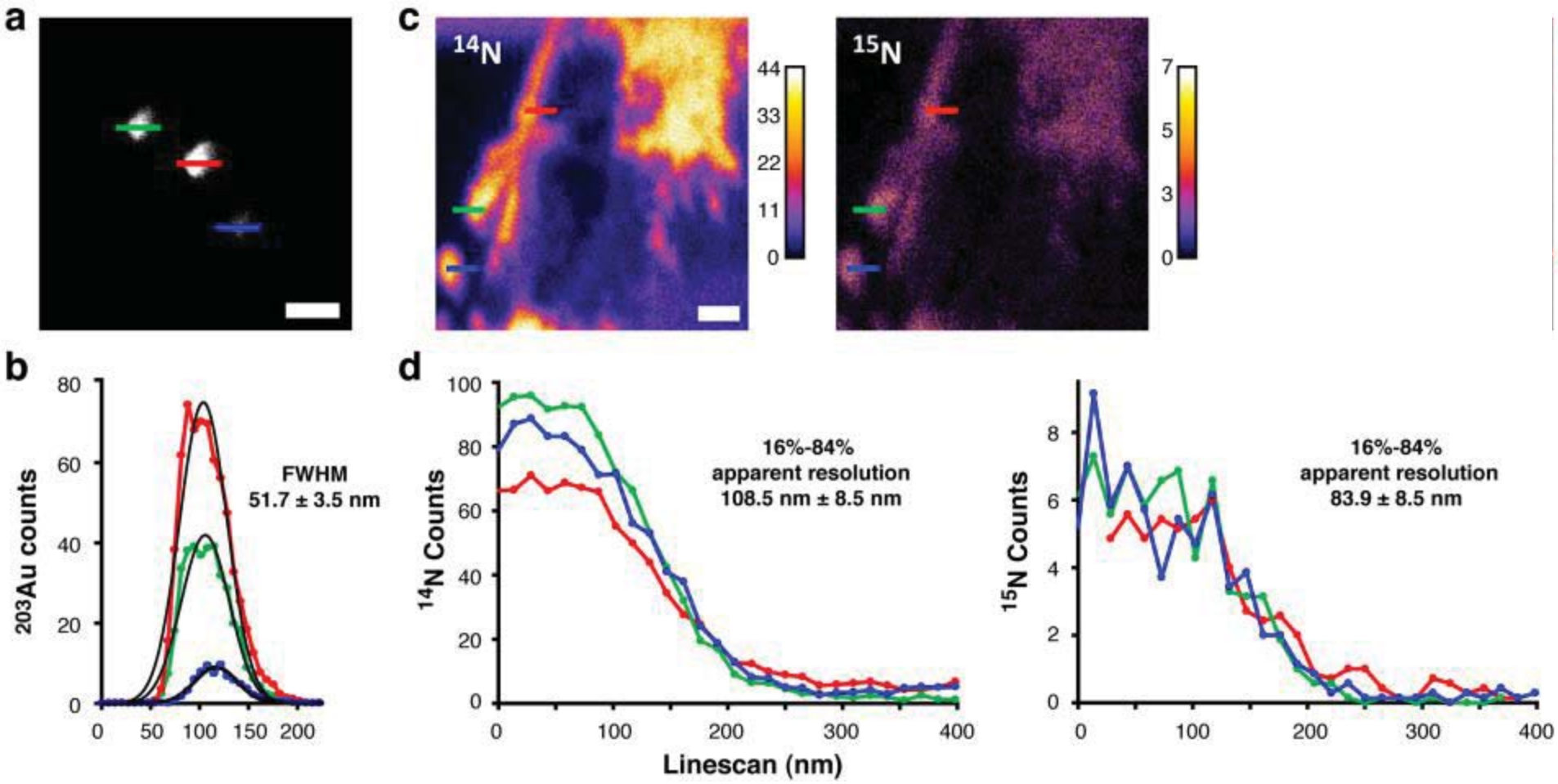
http://lgb.epfl.ch/page-78579-en.html

NanoSIMS resolution in neuronal samples



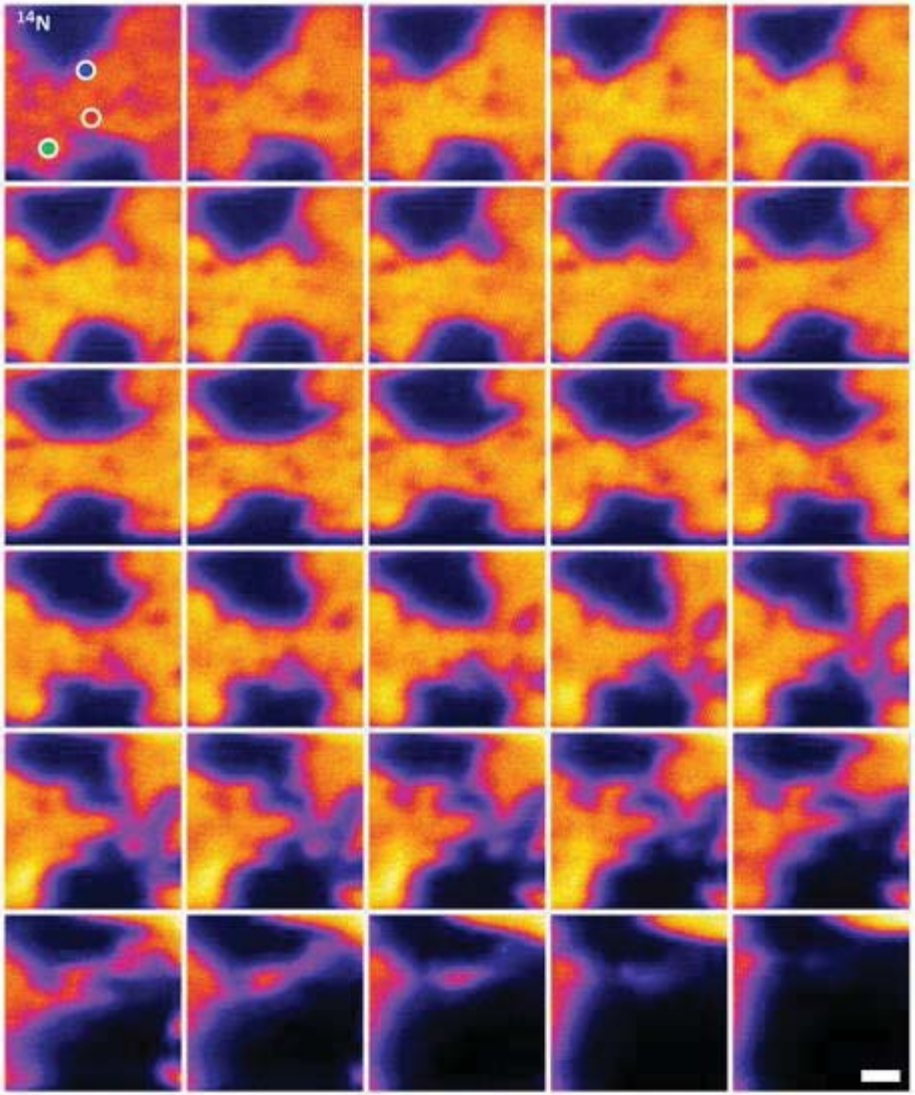
Saka et al. (2014) Nature Comm

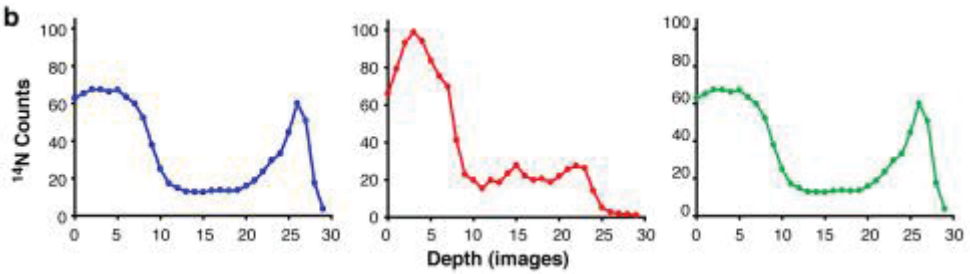
NanoSIMS resolution in neuronal samples



Saka et al. (2014) Nature Comm

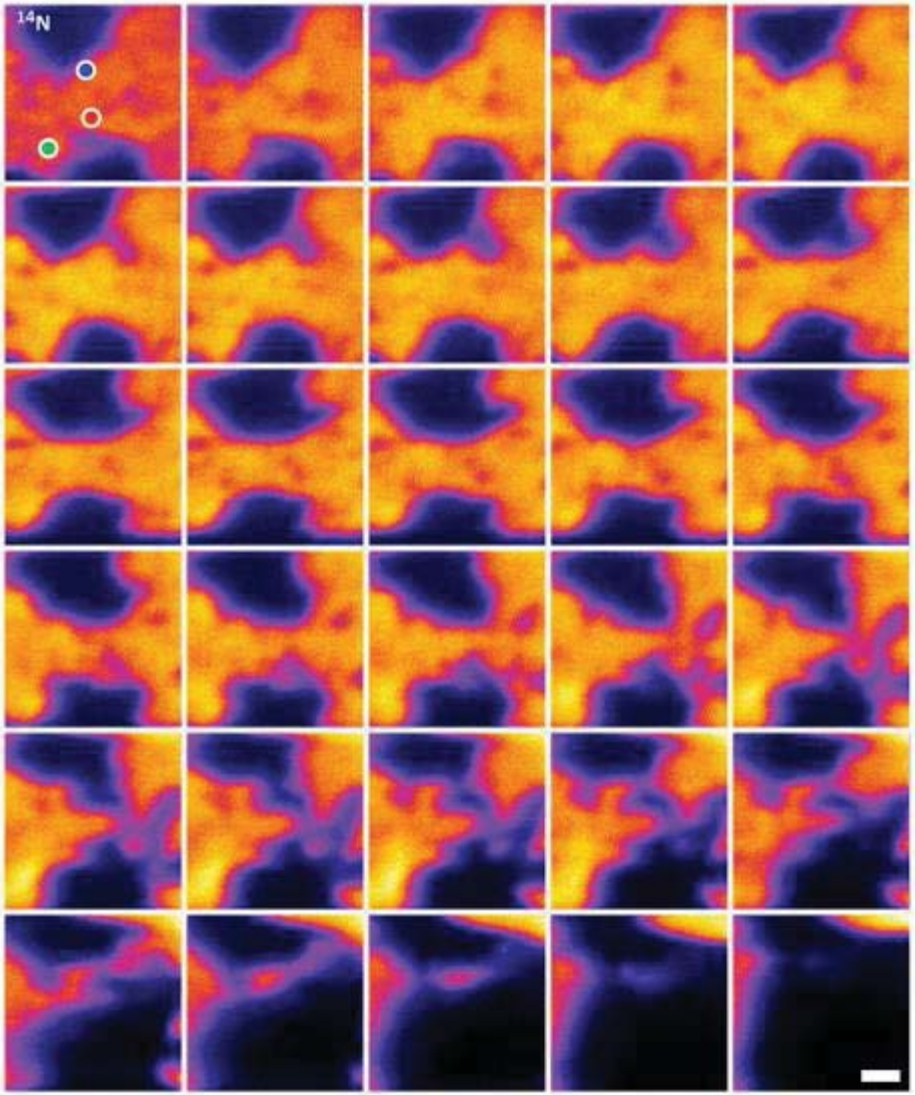
NanoSIMS resolution in neuronal samples

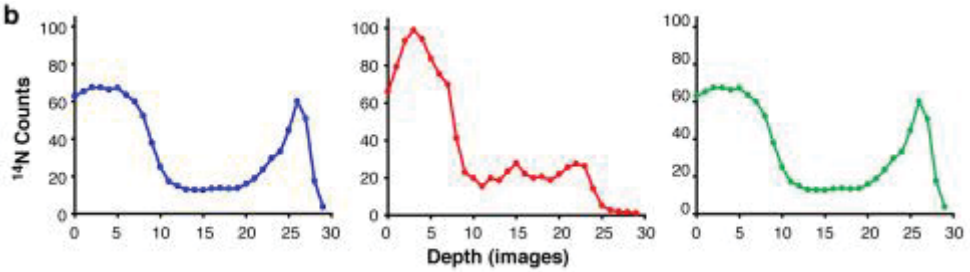




Saka et al. (2014) Nature Comm

NanoSIMS resolution in neuronal samples



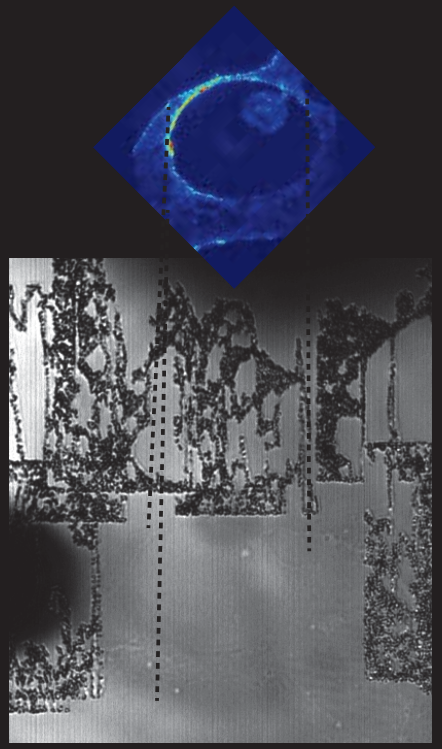


But how do we know what each structure is?

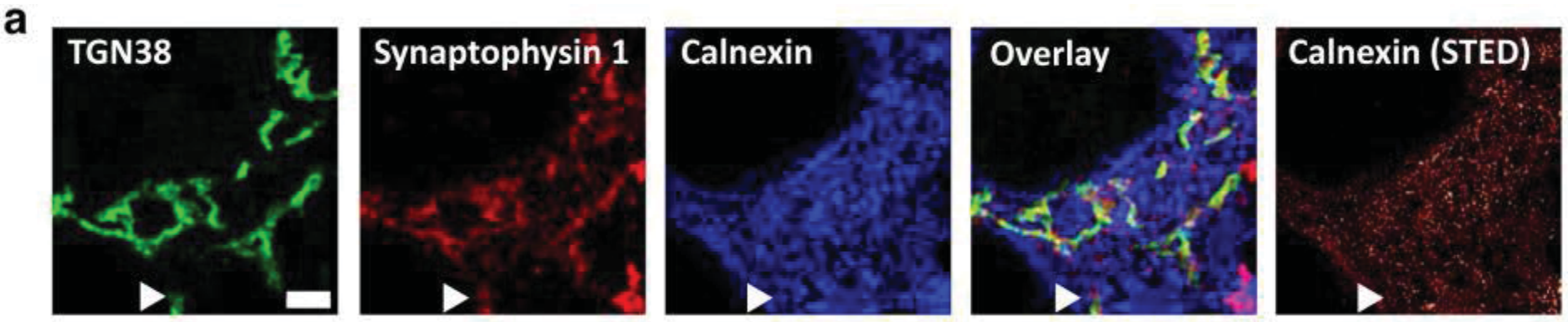
Saka et al. (2014) Nature Comm

Correlated optical and isotopic nanoscopy (COIN)

Grow cells in presence of an isotopically labeled metabolite	N15-Leucine incorporation
Label cells for fluorescence imaging	Immuno-staining, click labeling
Embed samples in plastic resin	LR White embedding
Cut thin sections using an ultramicrotome, place sections on silicone wafer	50-200 nm
Image the samples using light microscopy	Confocal and STED imaging
Mark the imaged regions	Burning with Mai Tai laser at 750 nm
Image same regions using SIMS	NanoSIMS 50L



COIN examples



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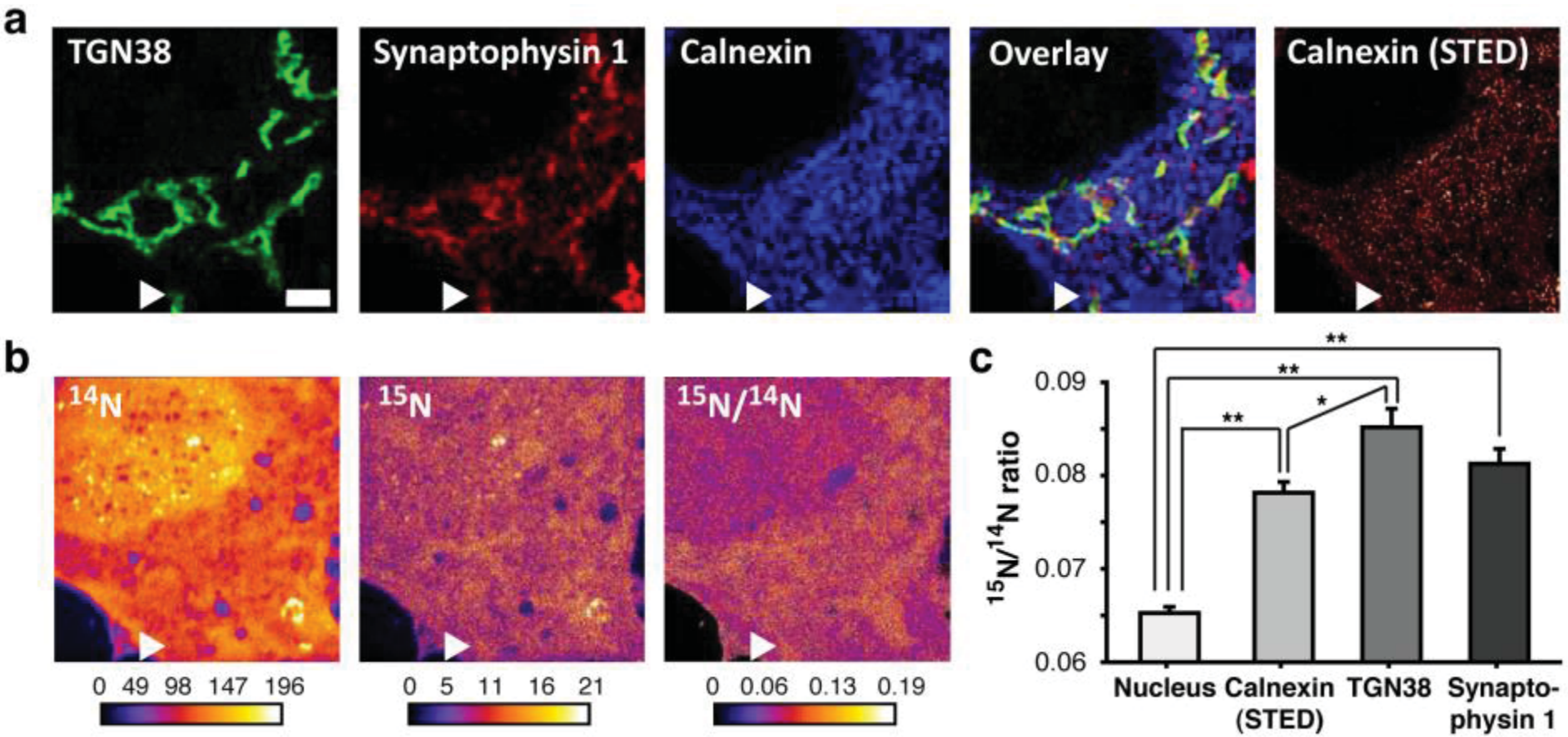
COIN examples



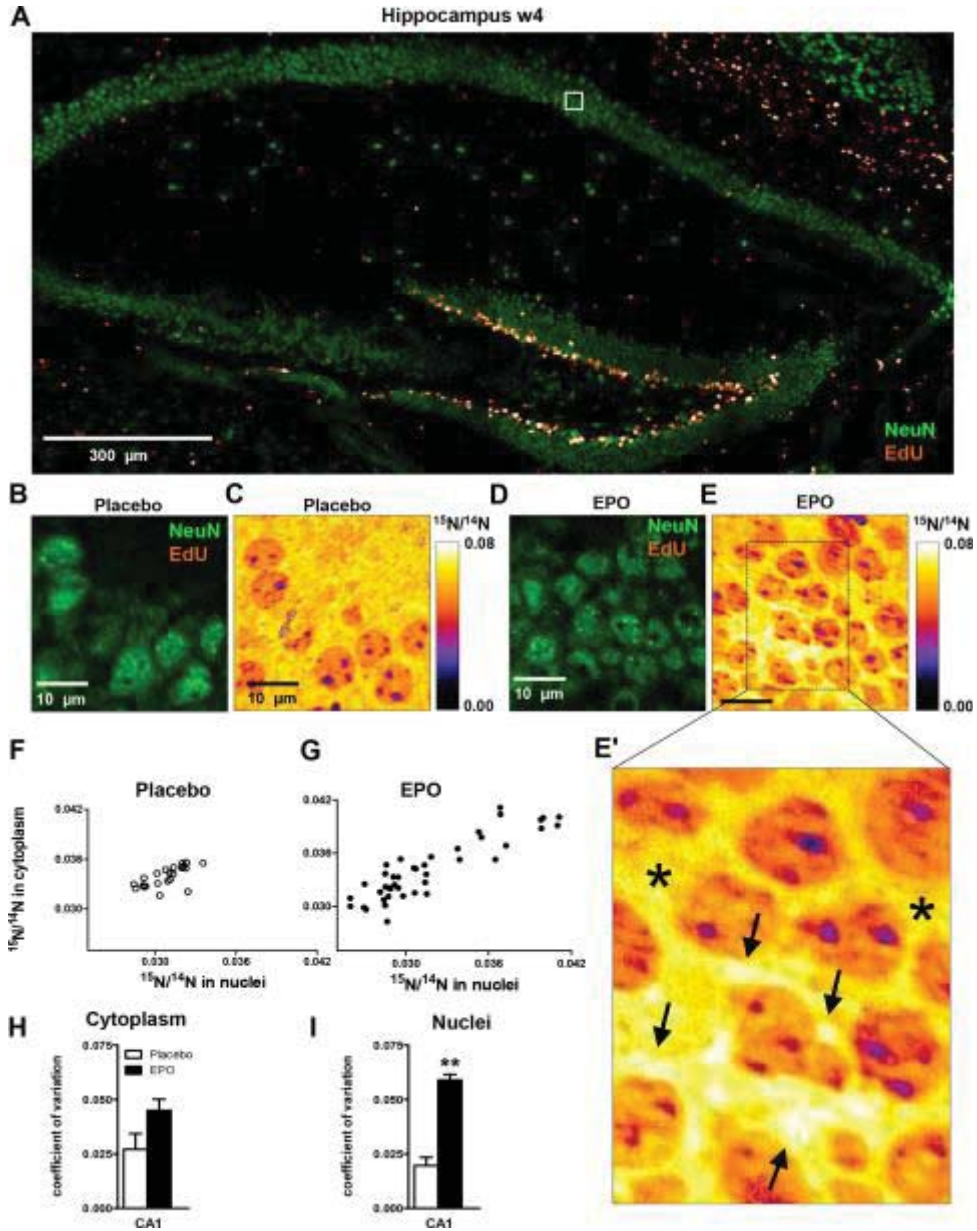
Saka et al. (2014) Nature Comm

Potential applications of these tools:

Relying solely on the ability to **measure isotopes** (for example, ^{14}N, ^{15}N, ^{12}C, ^{13}C):

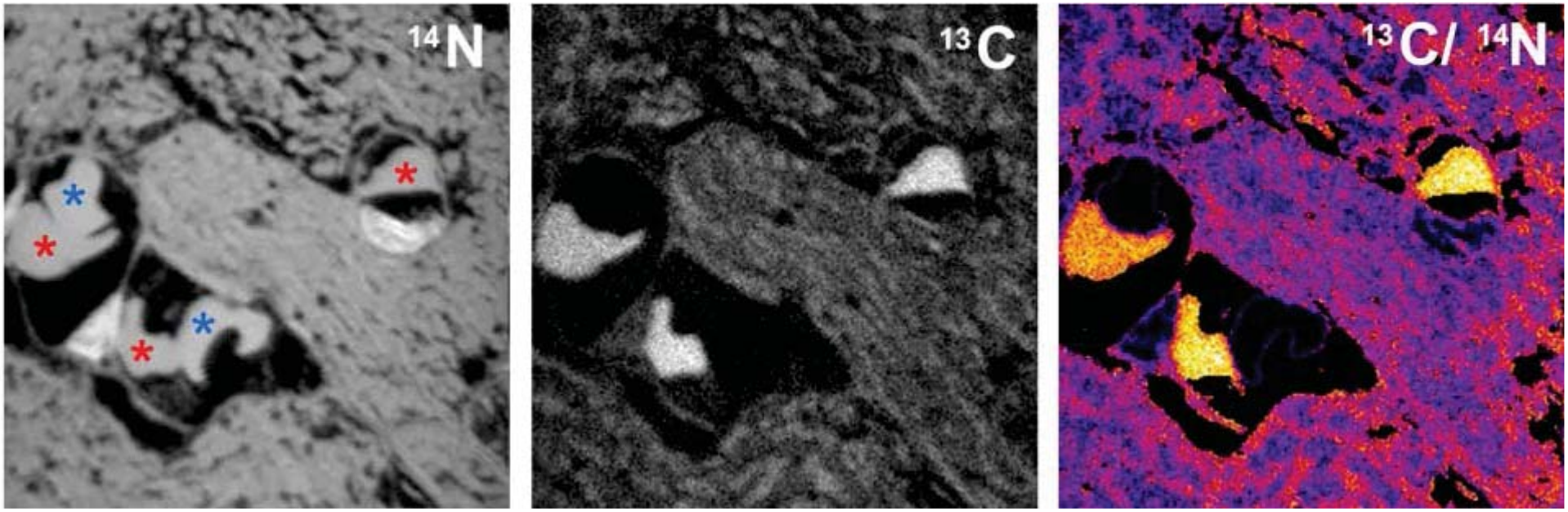
- Cellular metabolism and turnover
- Development of organisms
- Plasticity, *in vitro* and *in vivo*
- Metamorphosis (insects)
- Metal ion distributions in tissue

Measuring the formation of new neurons after doping with erythropoetin (EPO)



Hassouna et al. (2016)
Molecular Psychiatry

Looking at new and old cells in the body



Richter et al. (2017) Neurophotonics

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- Metal ion distributions in tissue

Relying on ability to **measure isotopes at high resolution**:

- The age and metabolism of specific protein complexes and aggregates
- The turnover of membranes, organelles, or sub-organelle parts
- Protein assemblies/aggregates from human samples (blood), for diagnostics

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Exploit the high resolution fully for molecular studies

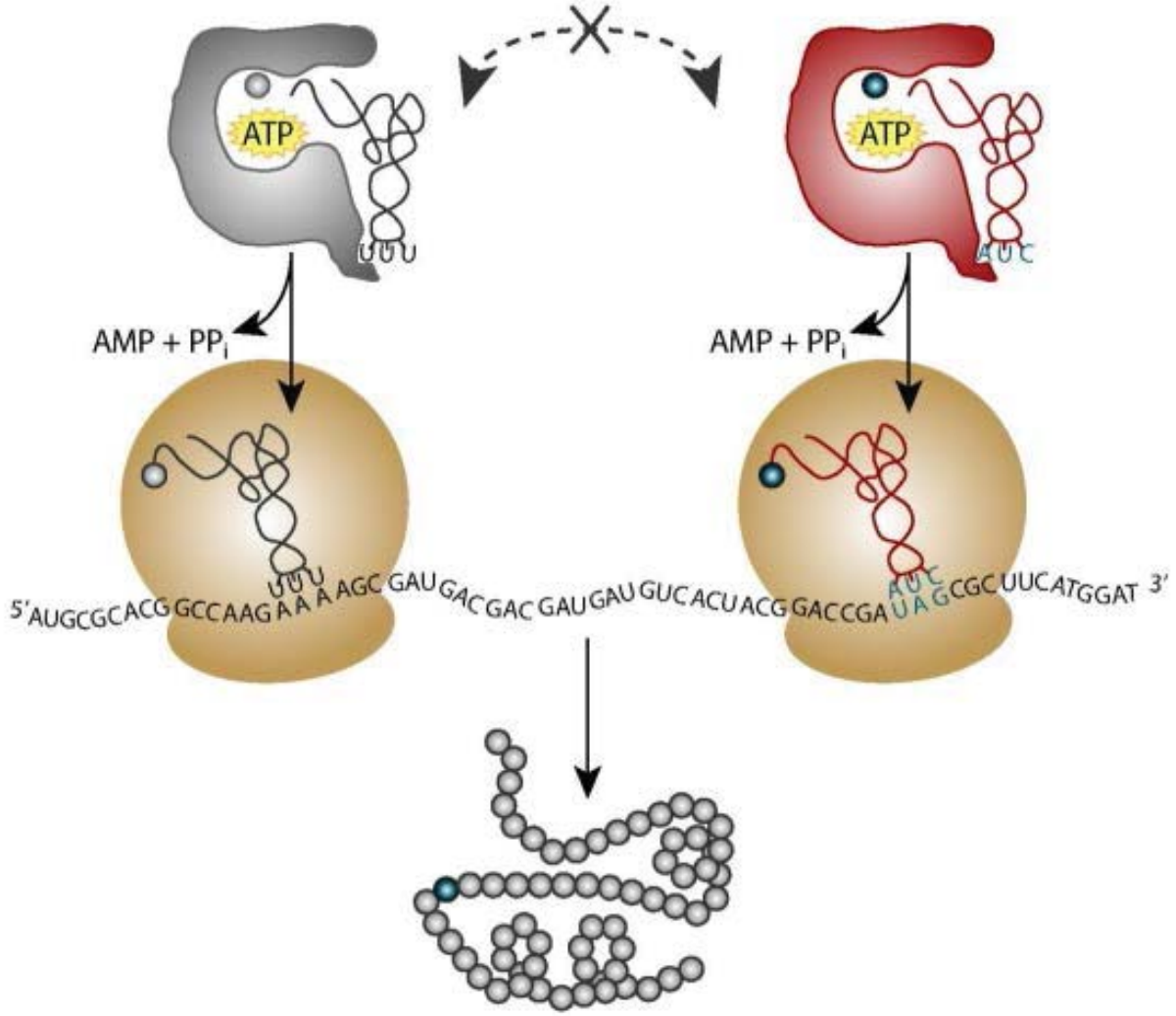
- New probes and ways of labeling proteins for SIMS are needed

Specific labeling using unnatural amino acids (UAAs)

Incorporation of non-canonical amino acids (ncAA/UAA) into proteins

- Uses a bio-orthogonal **synthetase-tRNA** couple, mutagenized

- The protein of interest sequence containing an **Amber stop** codon



Ingrid Vreja

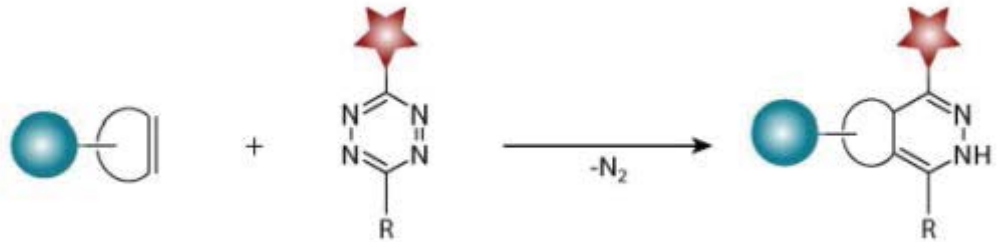
Specific labeling using unnatural amino acids (UAAs)

Huisgen cycloaddition

Azide + alkyne or alkene

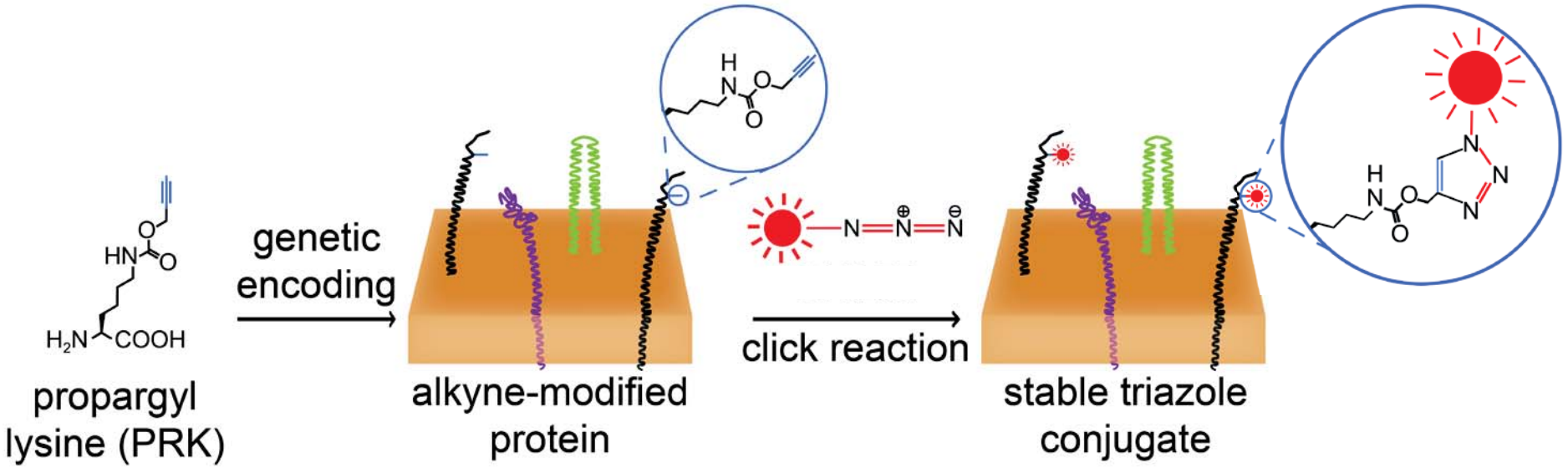
SPIEDAC reaction

Strained alkene or alkyne + tetrazine



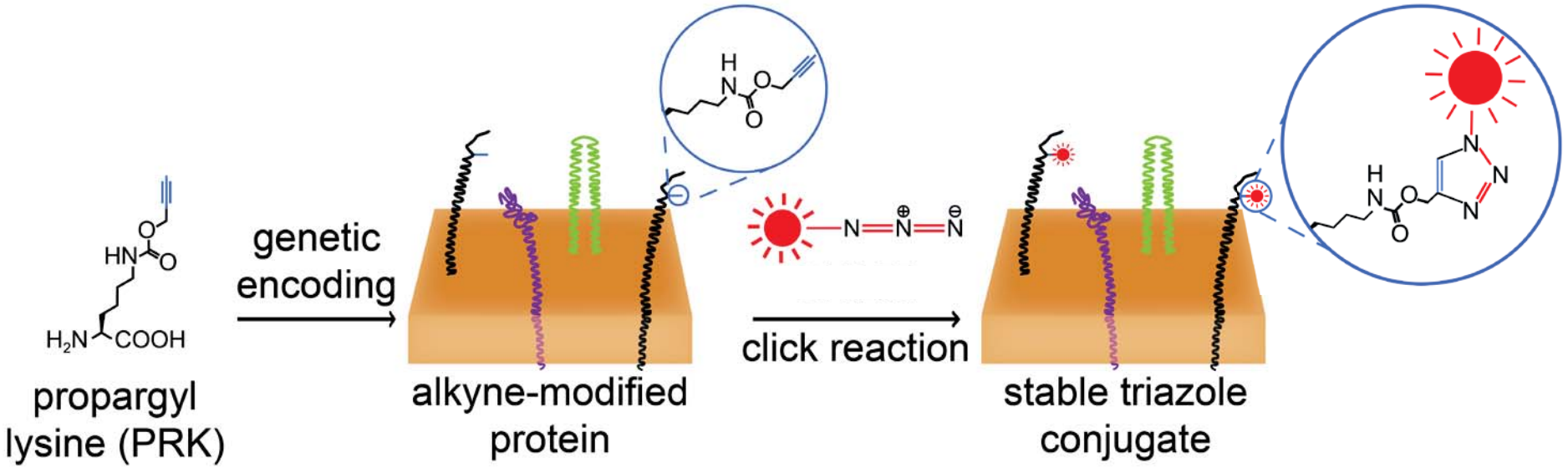
Ingrid Vreja

Specific labeling using unnatural amino acids (UAAs)



Vreja et al. (2015) Angewandte Chemie

Specific labeling using unnatural amino acids (UAAs)



Advantages:

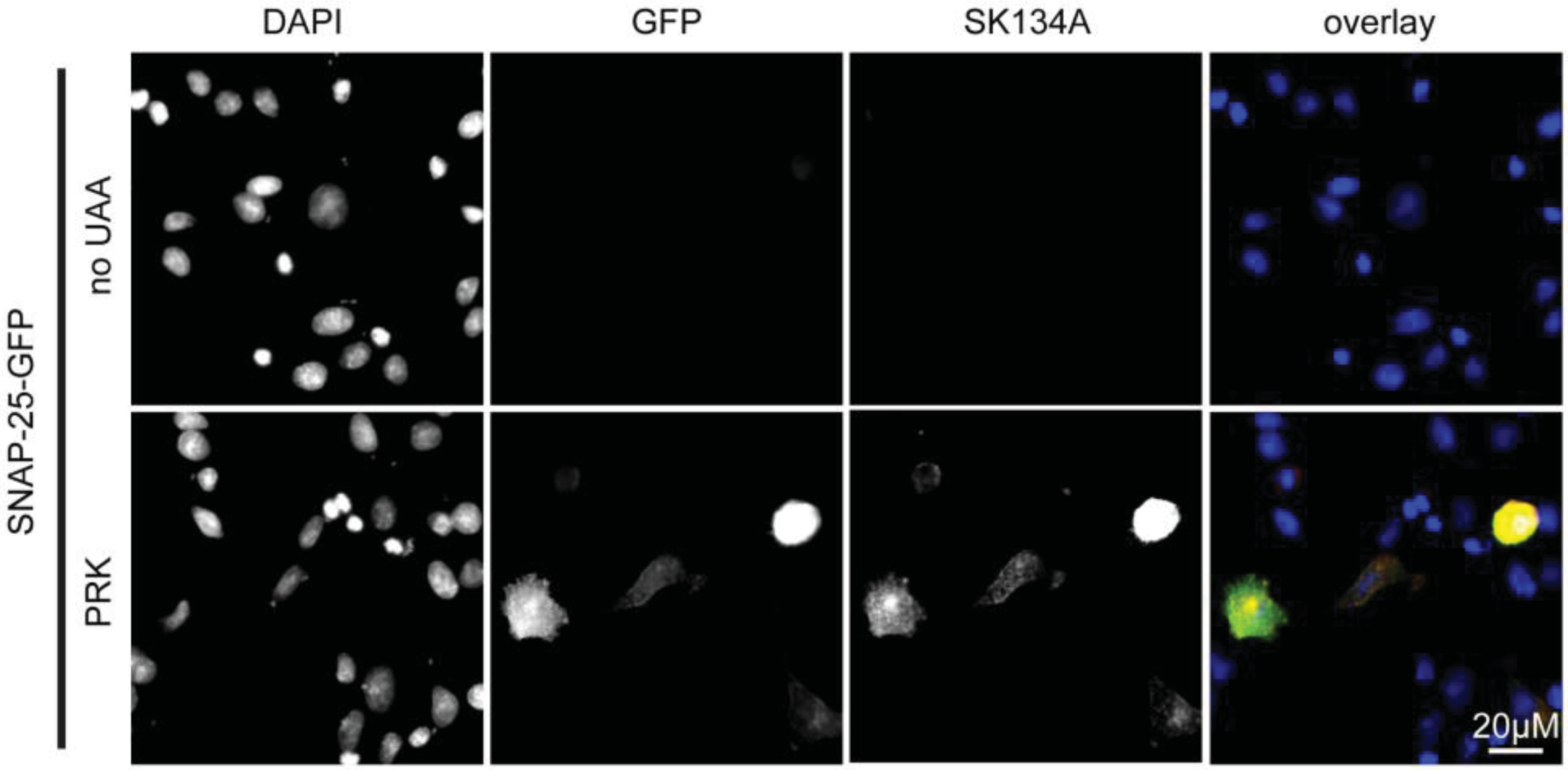
Wide choice of labels for different imaging techniques

→ with flourophores, with isotopes, **or combined**

Very small tag size (less influence on proteins)

Vreja et al. (2015) Angewandte Chemie

The specificity of this reaction

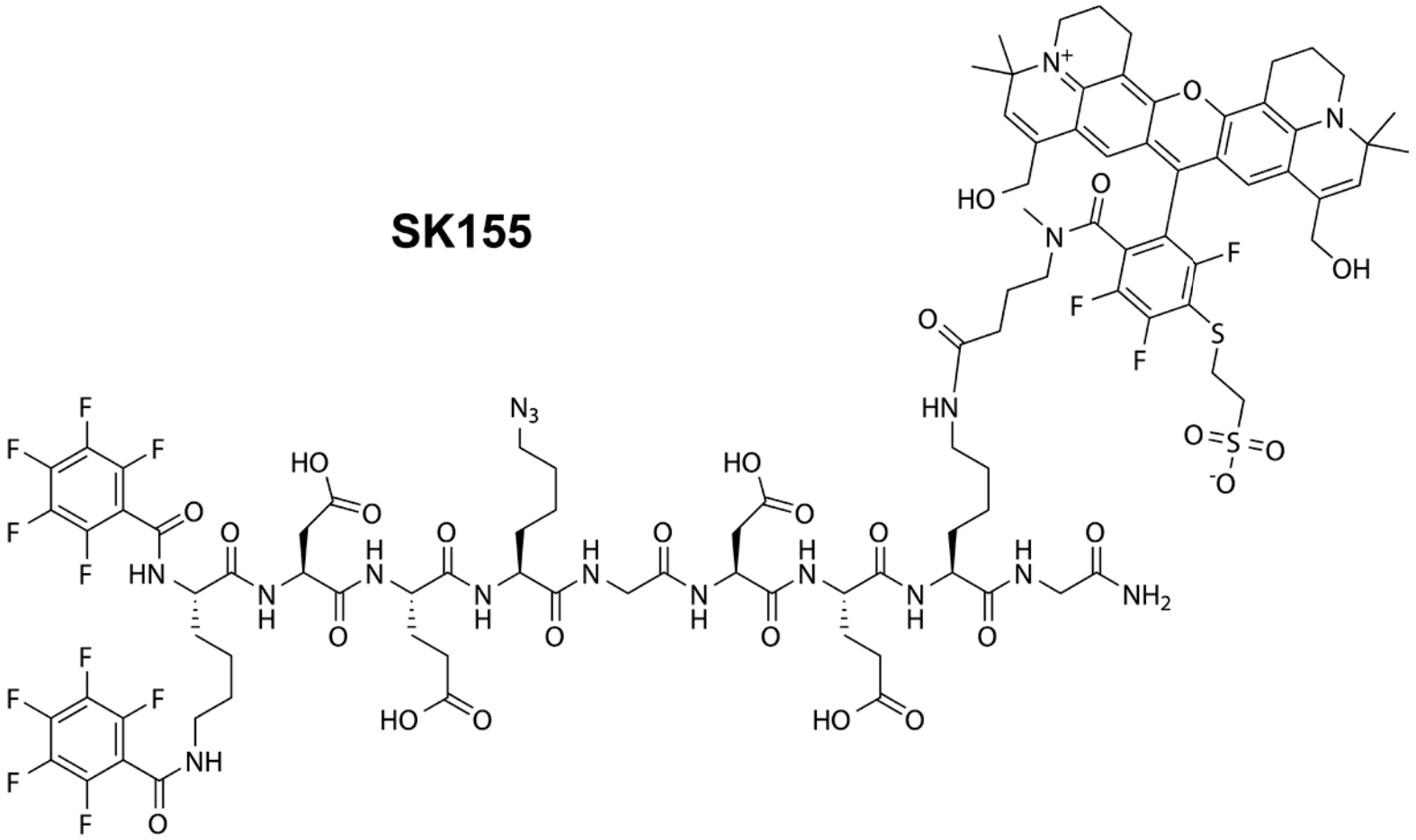


Ingrid Vreja

Dual probe for fluorescence and NanoSIMS

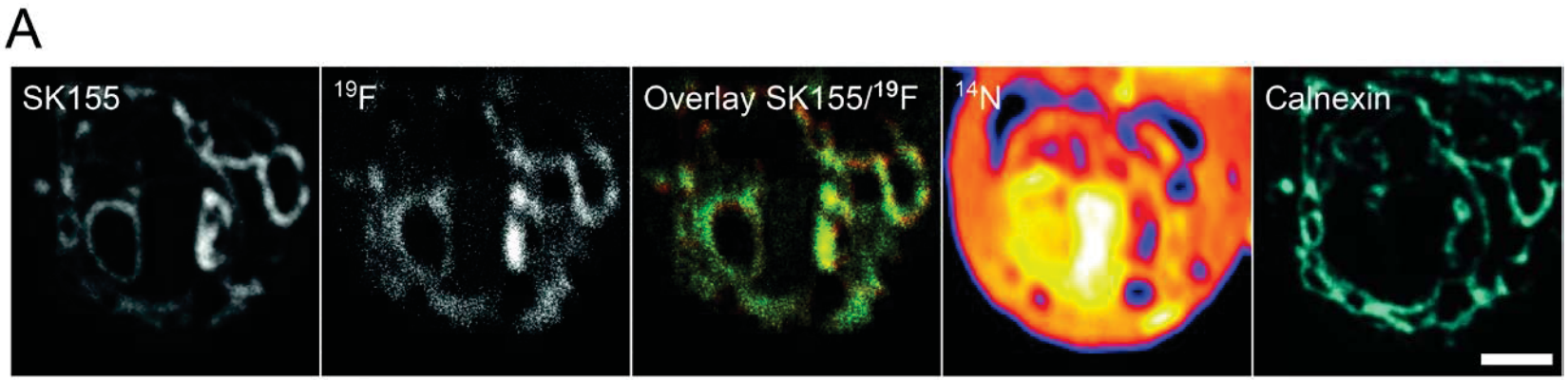
In collaboration with Prof. Ulf Diederichsen, University of Göttingen

SK155



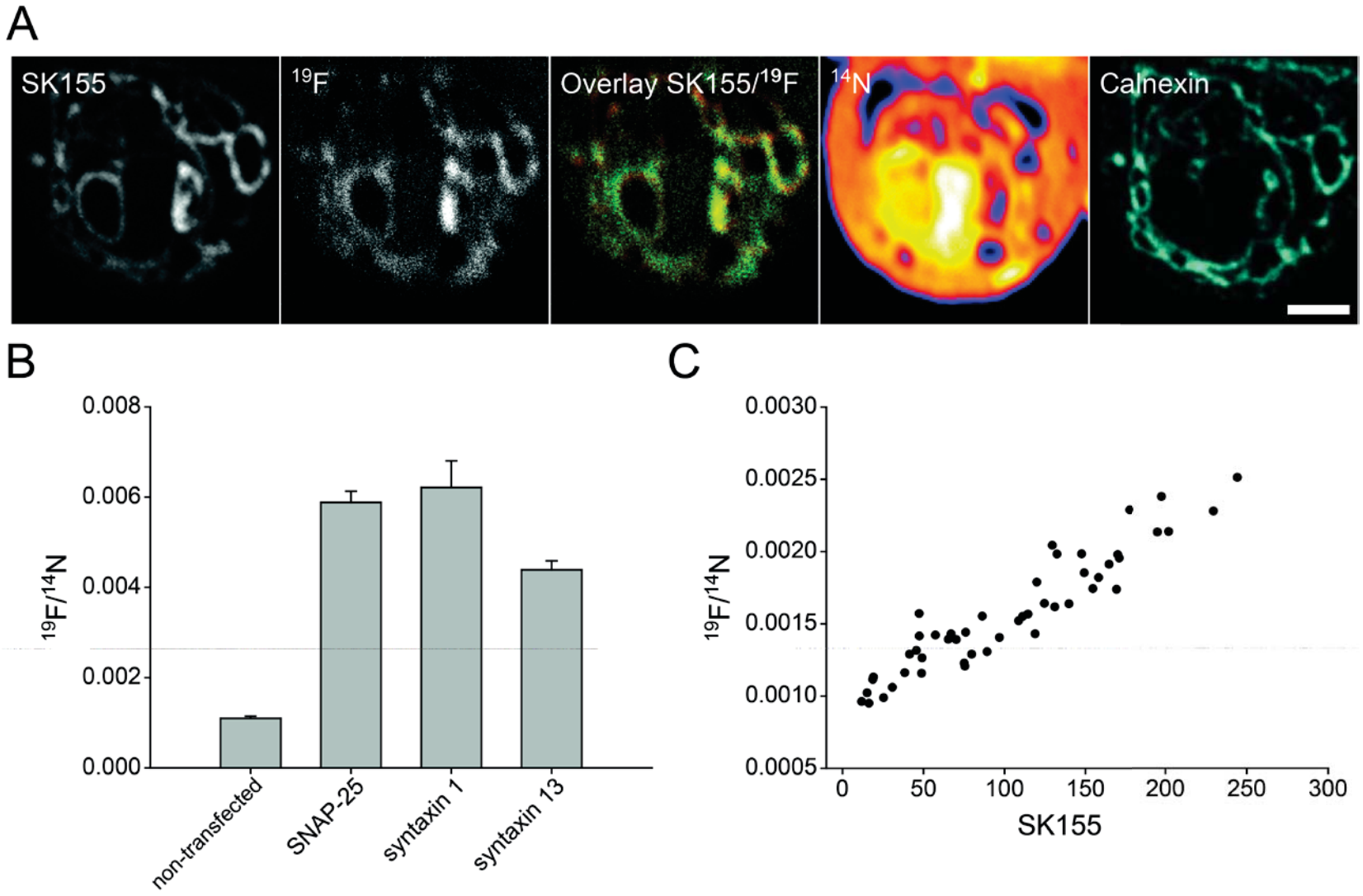
Vreja et al. (2015) Angewandte Chemie

COIN imaging using SK155 (^{19}F)



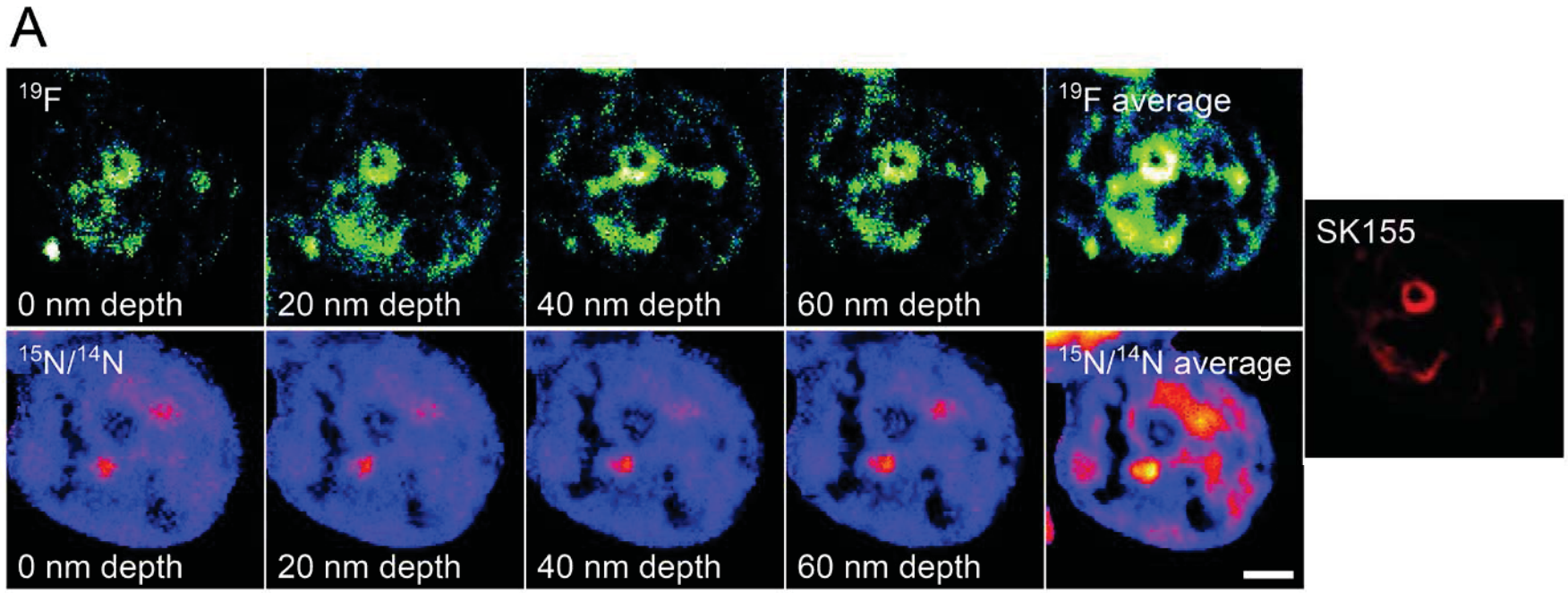
Vreja et al. (2015) Angewandte Chemie

COIN imaging using SK155 (^{19}F)



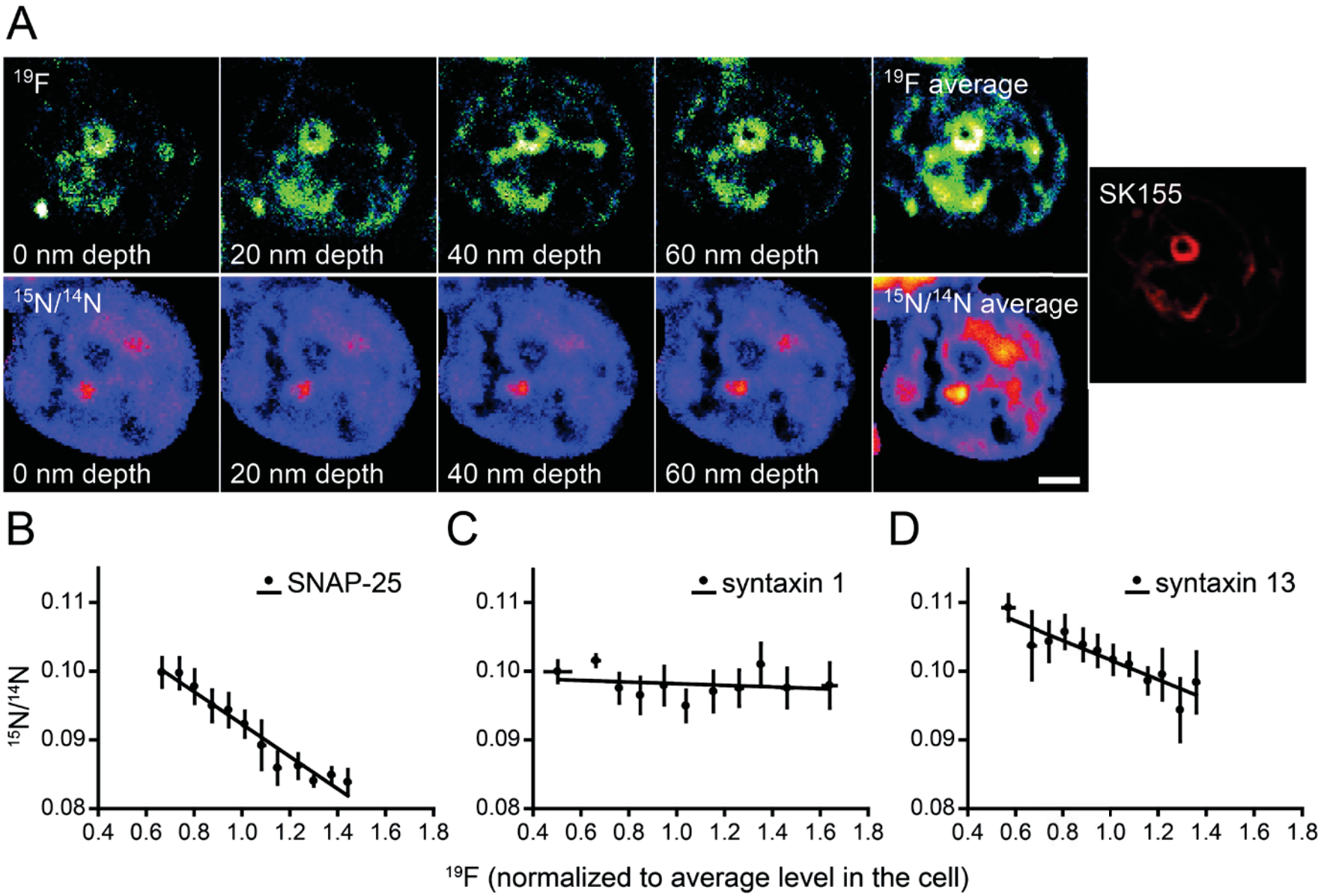
Vreja et al. (2015) Angewandte Chemie

COIN imaging using SK155 (^{19}F)



Vreja et al. (2015) Angewandte Chemie

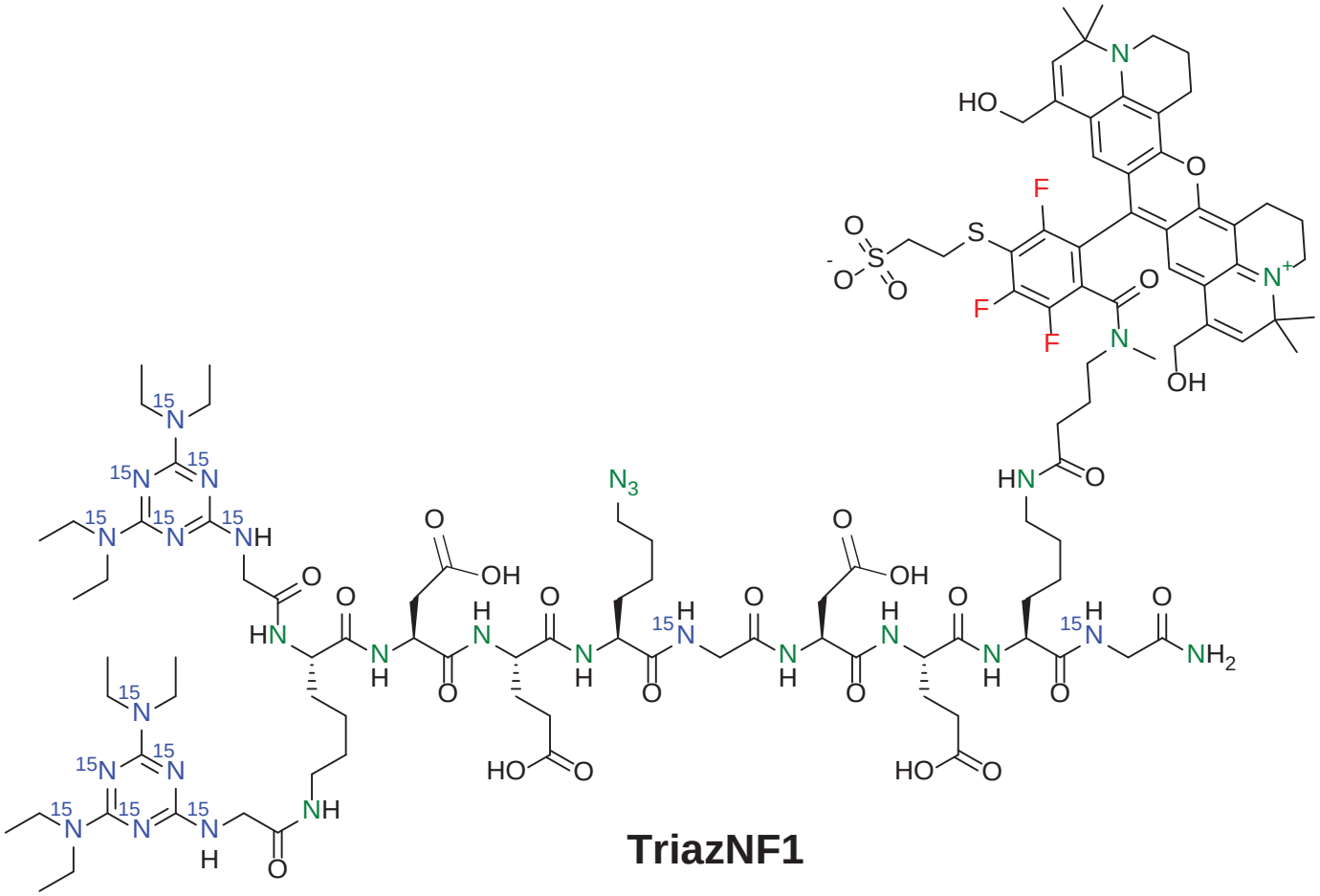
COIN imaging using SK155 (^{19}F)



Vreja et al. (2015) Angewandte Chemie

Another example probe:

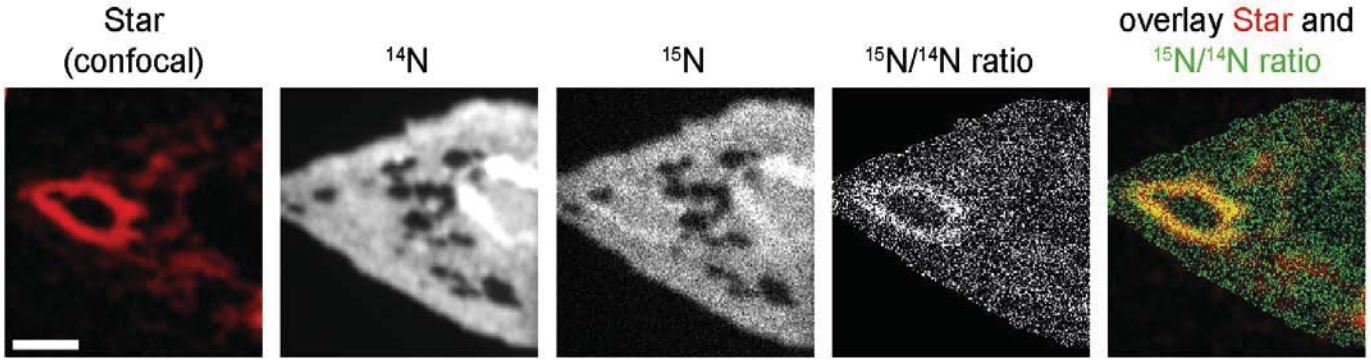
In collaboration with Prof. Ulf Diederichsen, University of Göttingen



TriazNF1

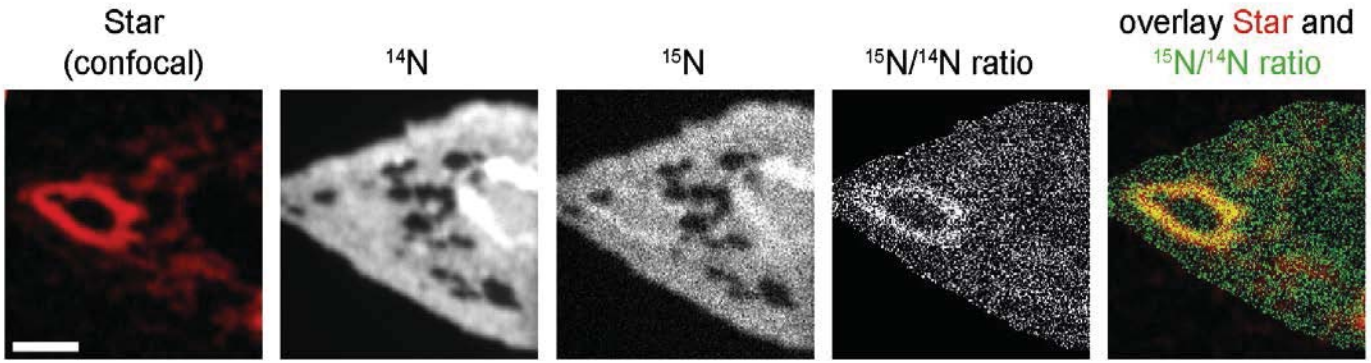
Kabatas et al. (2015) Chem Comm

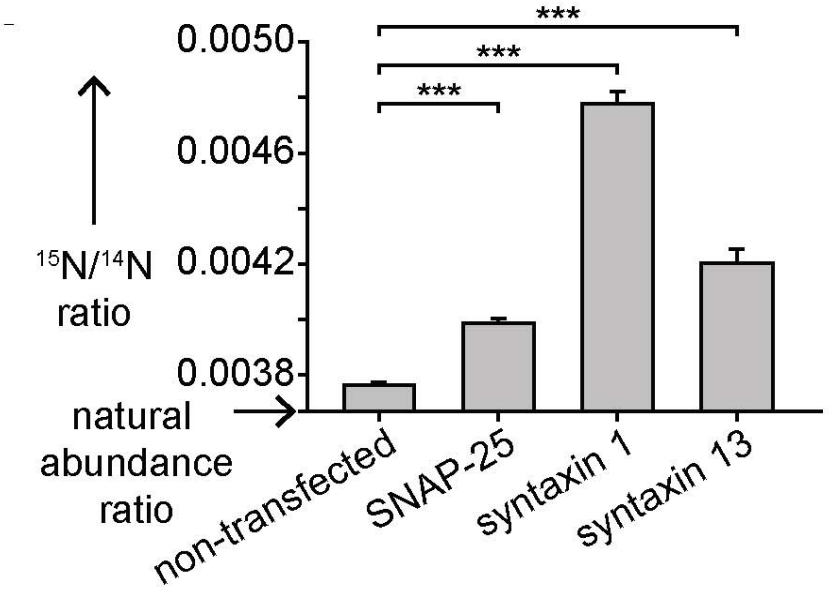
COIN imaging using TriazNF1 (^{15}N)

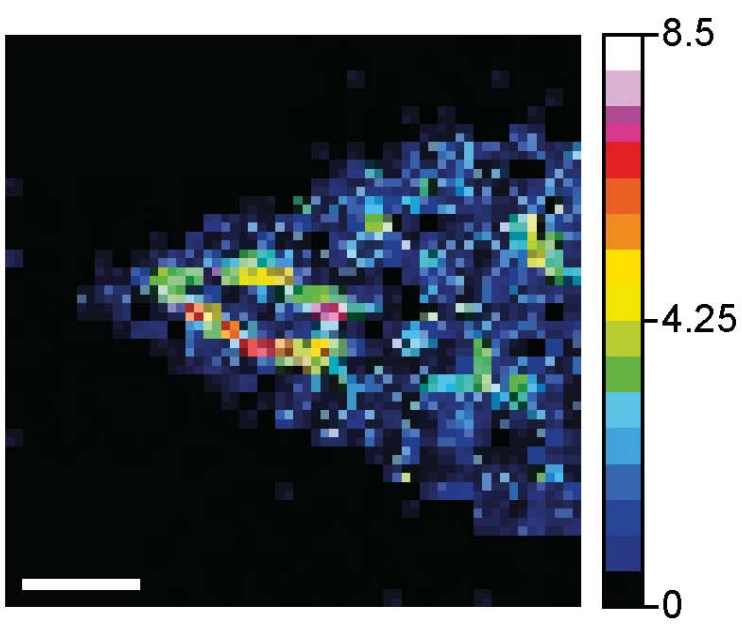


Kabatas et al. (2015) Chem Comm

COIN imaging using TriazNF1 (^{15}N)

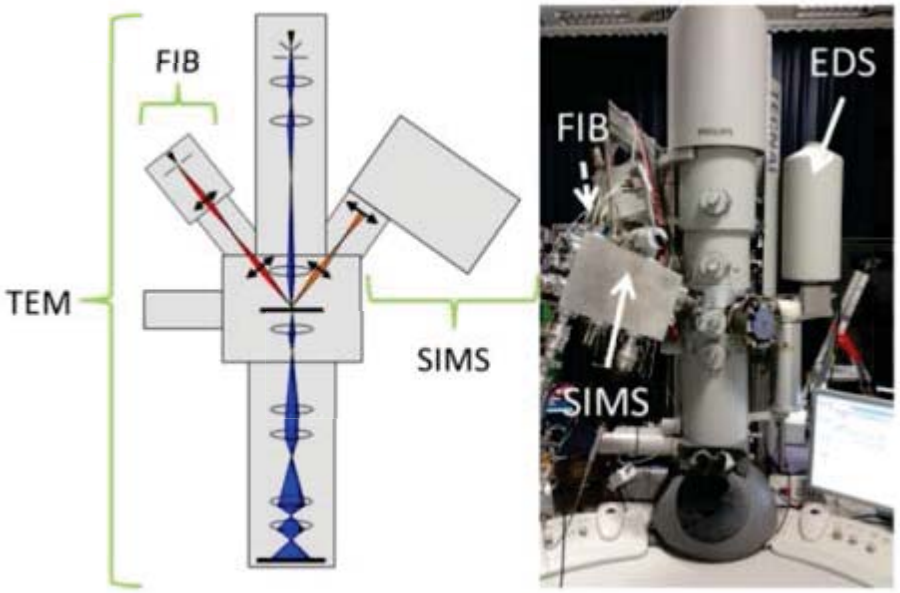


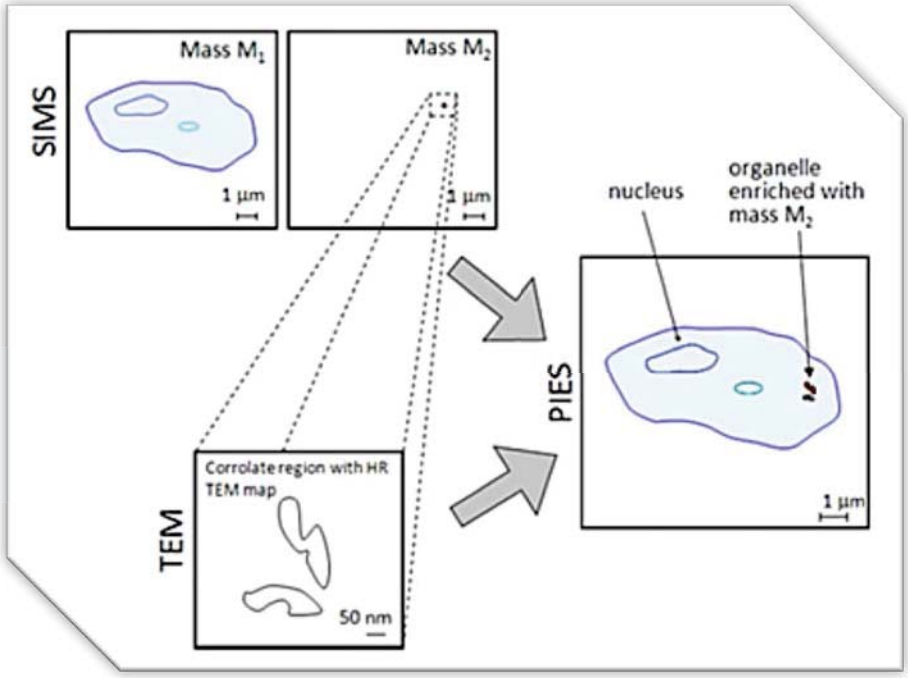




Kabatas et al. (2015) Chem Comm

Future applications: Fluorescence/SIMS/ESI/other high-resolution microscopy techniques = Boron



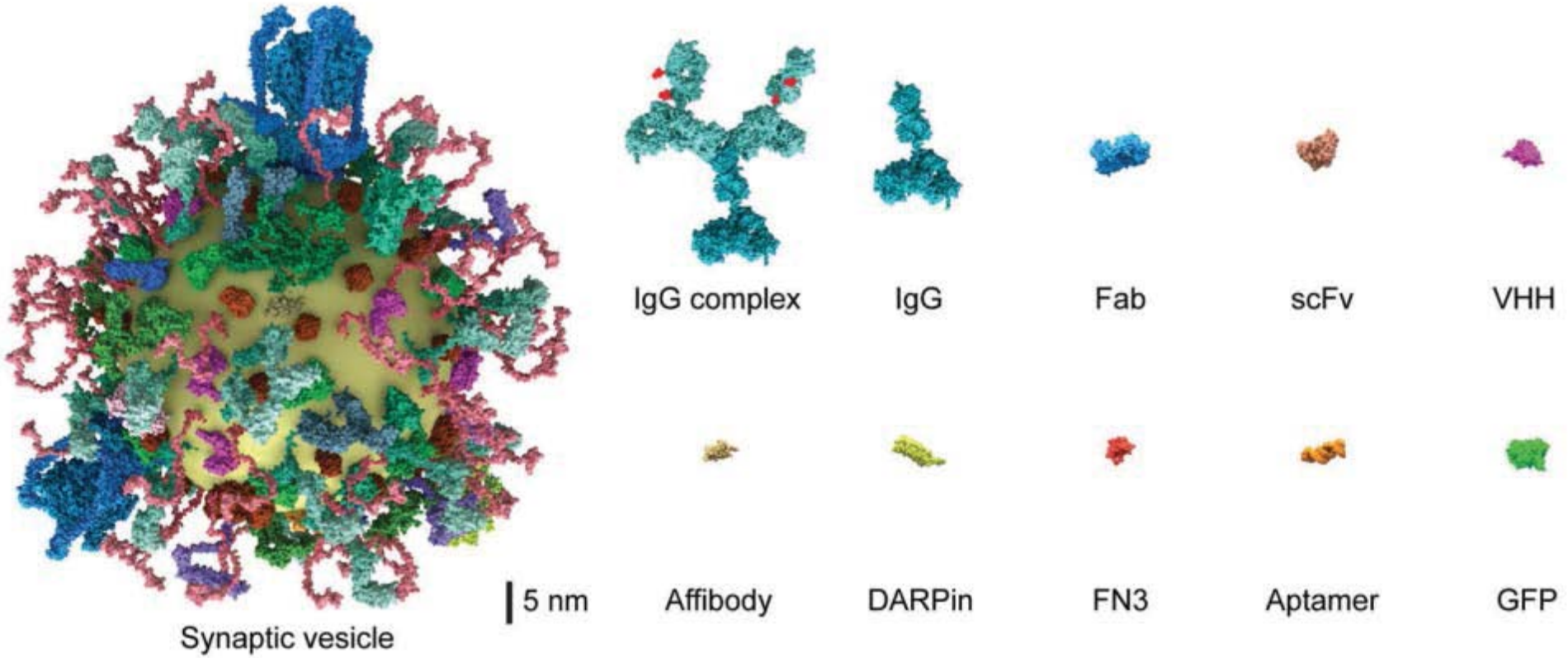


T. Wirtz et al. (2015) Nanotechnology

Future applications: Fluorescence/SIMS/ESI/other high-resolution microscopy techniques = Boron

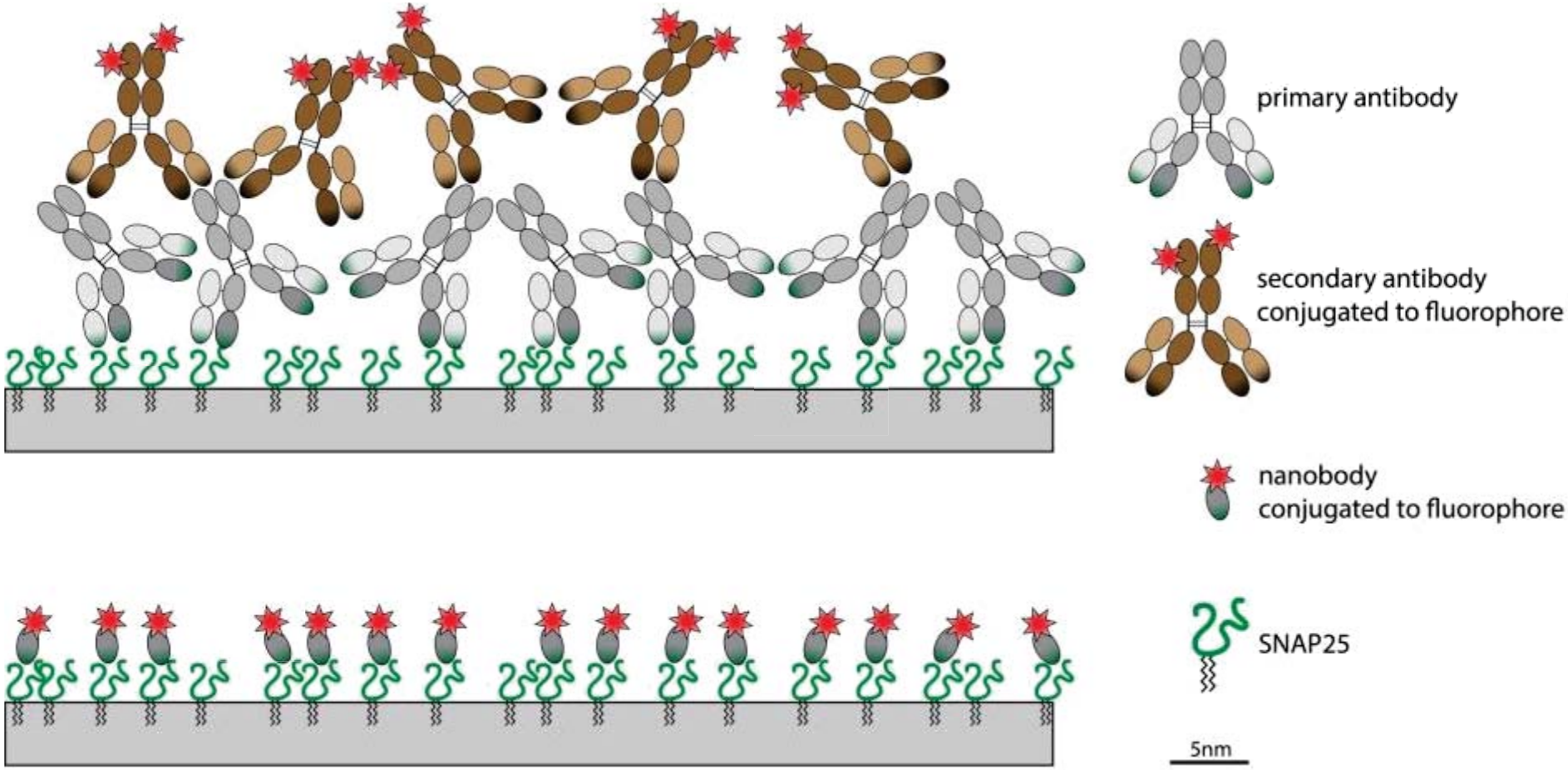
Selda Kabatas

More flexibility in labeling: GFP nanobodies coupled to specific isotopes

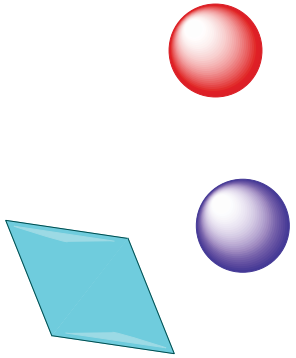


Felipe Opazo, Selda Kabatas

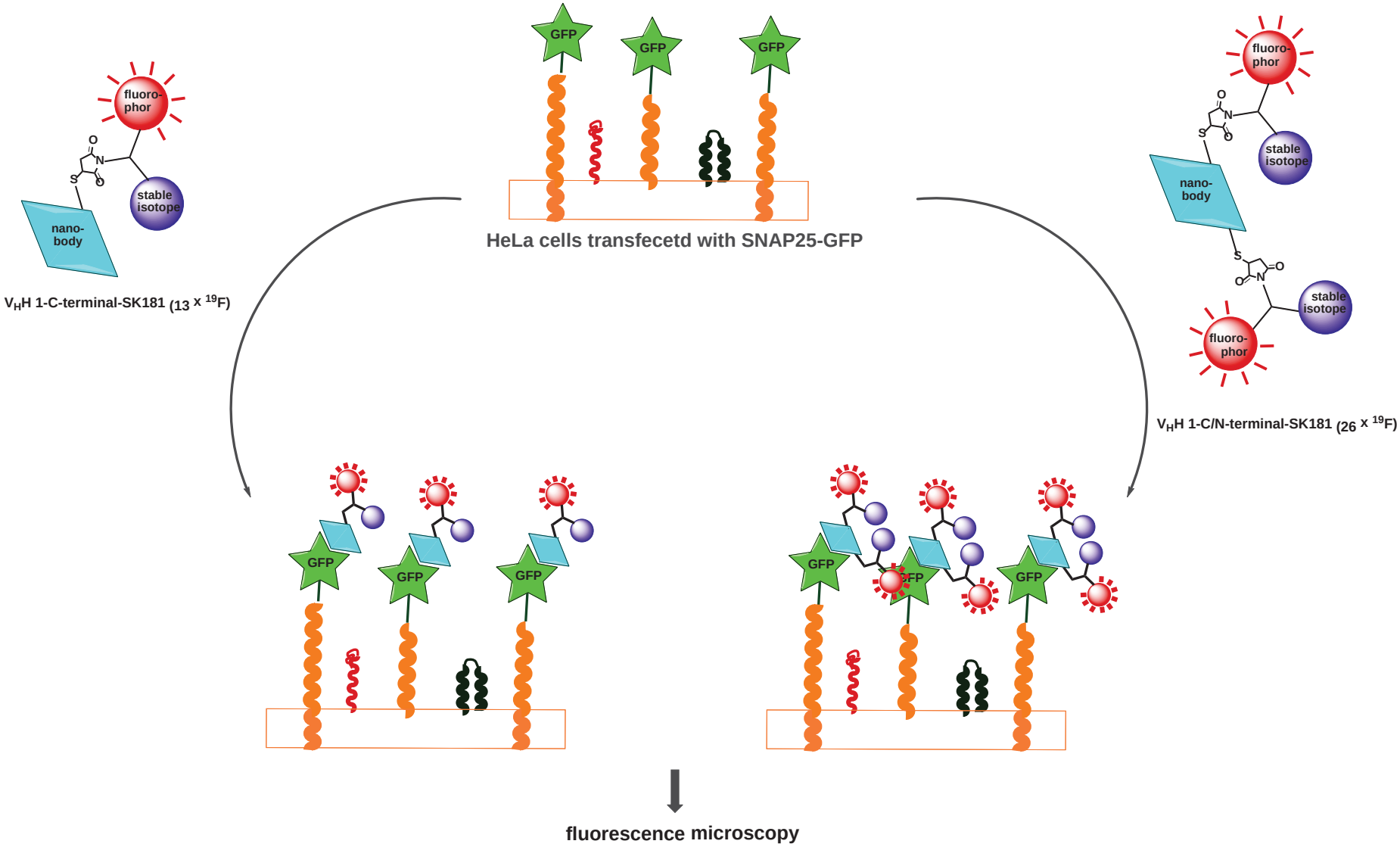
More flexibility in labeling: GFP nanobodies coupled to specific isotopes



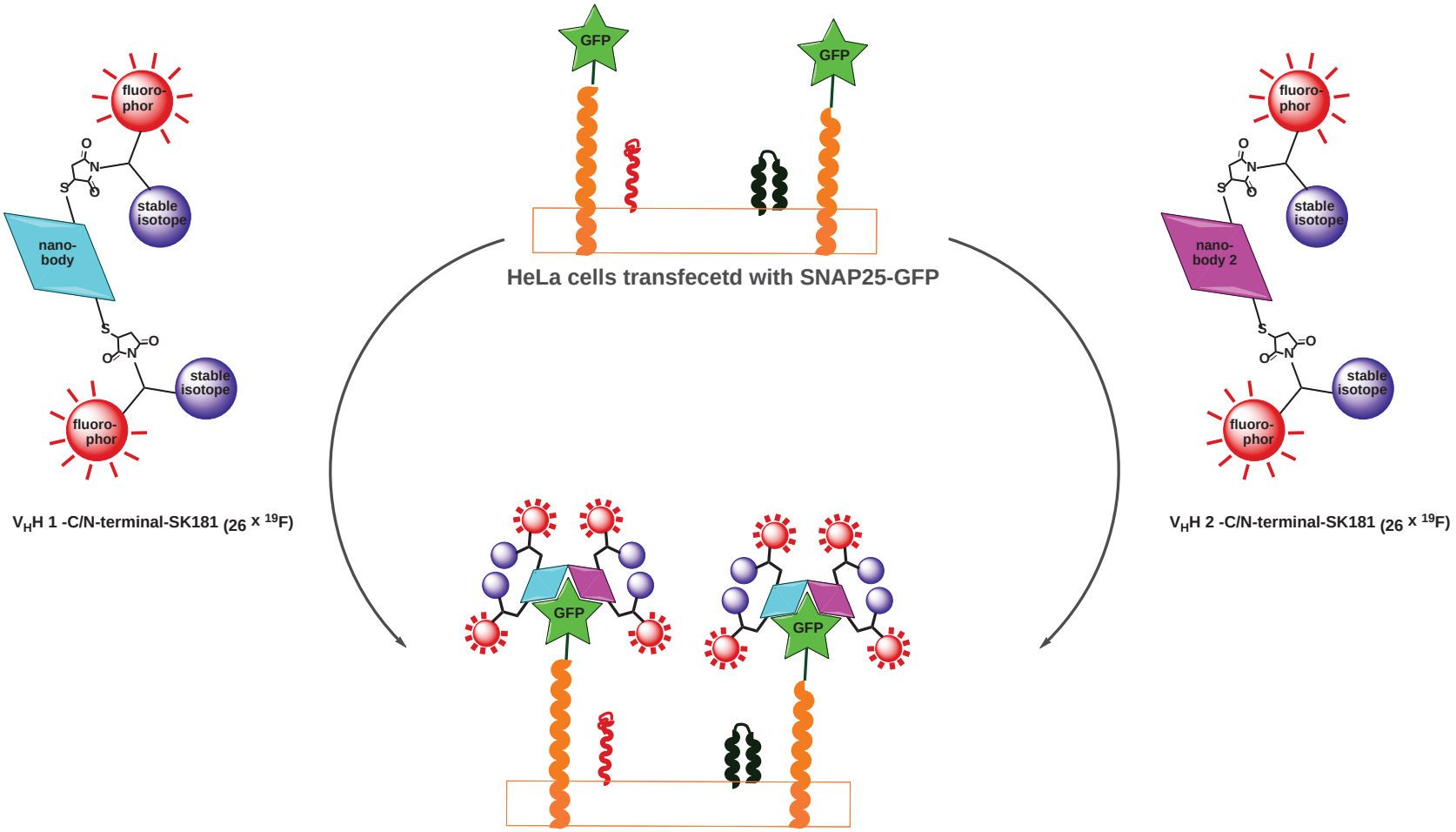
Felipe Opazo, Selda Kabatas



Labeling GFP with single nanobodies



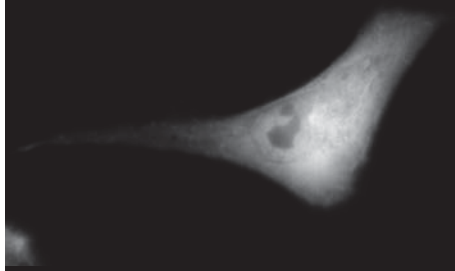
Labeling GFP with two nanobodies simultaneously



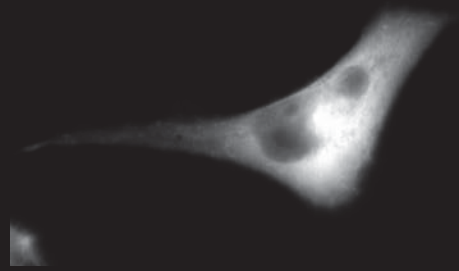
→ With combination of nanobodies and SK181, **52 x Fluor, or 80 x Boron** in a single GFP-protein! High signal-to-noise ratio in SIMS.

Works fine in fluorescence (SIMS measurements coming soon)

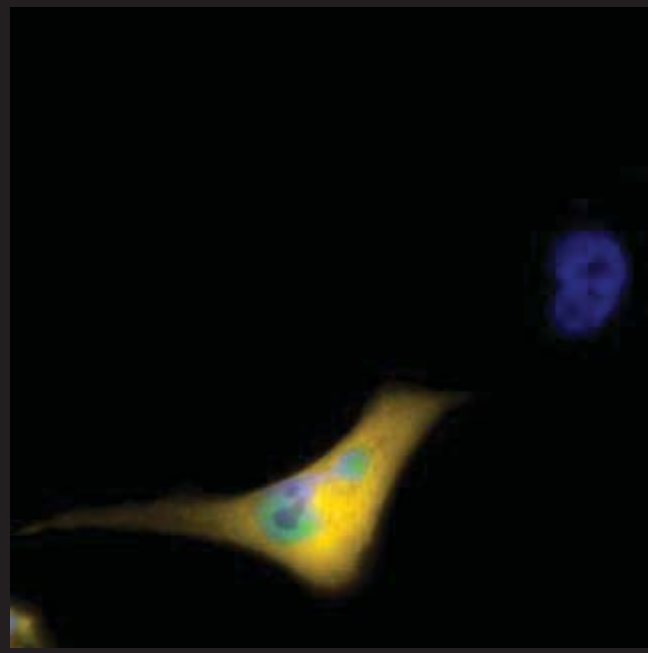
GFP



Star635



Overlay (DAPI, GFP, Star635)

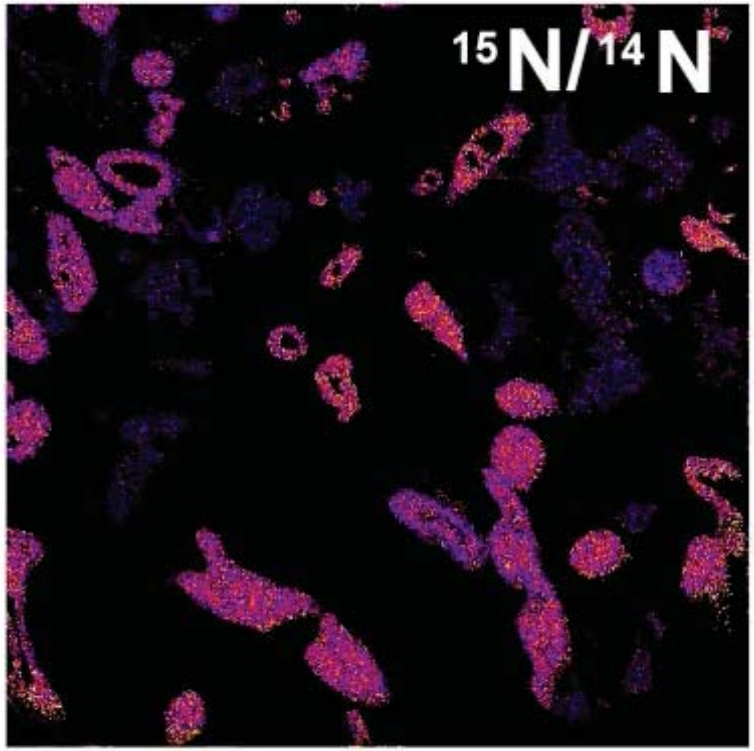


UNIVERSITÄTSMEDIZIN GÖTTINGEN UMG

Ingrid Vreja
Dr. Selda Kabatas
Dr. Sinem Saka
Sebastian Jähne
Katharina Kröhnert

GEORG-AUGUST-UNIVERSITÄT GÖTTINGEN

Prof. Dr. Ulf Diederichsen



LEIBNIZ-INSTITUT FÜR OSTSEEFORSCHUNG WARNEMÜNDE

Dr. Angela Vogts

TECHNISCHE UNIVERSITÄT MÜNCHEN

Dr. Carmen Höschen

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