Novel nanobody-based tools for studying the synaptic vesicle life cycle

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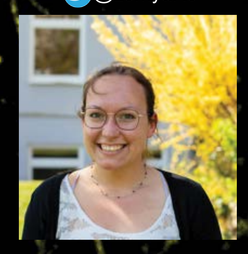


Ronja Rehm

QR: publication iNbSyt1

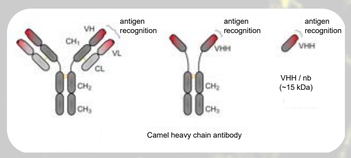
Abstract

Synaptic vesicles are important organelles in neurotransmission and their precise composition is essential for information transfer from neuron to neuron. While many aspects about their functions at the synapse are known, open questions remain regarding how these stoichiometrically highly-precise organelles are formed and maintained to mature to functional SVs that are able to release neurotransmitters at the synapse. To accurately study SV biogenesis, it is important to maintain the highly-precise stoichiometry of SV components in place and untouched. This renders experiments involving overexpression of a particular SV protein of difficult interpretation and instead requires endogenous expression.

One powerful approach to access endogenous localization, distribution and movement of proteins are intrabodies: small probes based on high-affinity nanobodies that can be expressed inside the cell. Here, I present the characterization of an intrabody against the SV Ca2+-sensor Synaptotagmin-1 (Syt1), that was named iNbSyt1. As shown in our recently published paper (QRcode in upper corner, https://doi.org/10.1002/smtd.202300218), this intrabody enables not only the direct live imaging of SVs (mScarlet-/mNeonGreen-iNbSyt1), but also allows to detect single action potentials thanks to a highly sensitive synaptically localized Ca2+-sensor, jGCaMP8s-iNbSyt1. Meanwhile, expression of iNbSyt1 does not affect vesicle mobility, synaptic localization, or fusion capacity, which makes this highly modifiable tool optimal to study synaptic processes in living neurons without genetic perturbation of the SV protein target. Likewise, we show that the underlying nanobody also is an ideal probe for several super-resolution imaging techniques, including STED microscopy, Structured Illumination Microscopy (SIM), DNA-PAINT and Expansion Microscopy.

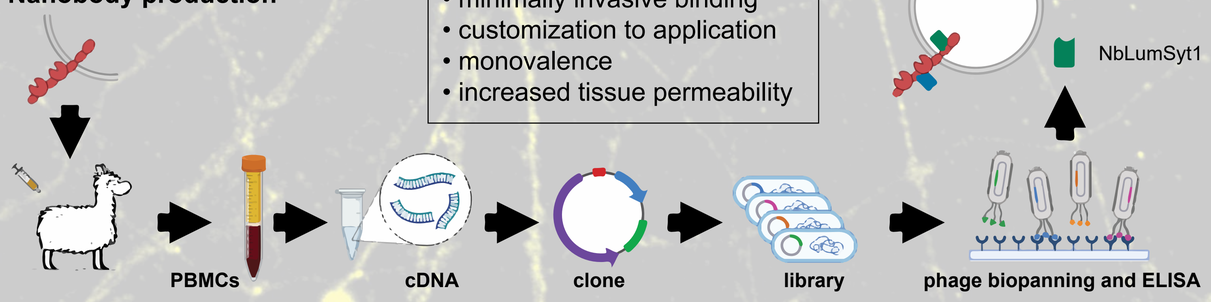
Overall, the NbSyt1 is a versatile small imaging probe that offers adaptation to divers experimental requirements and will find a broad use in the microscopy field.

Nb tools for studying the SV life cycle



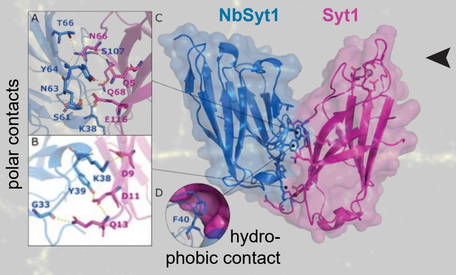
Nanobody production

Main advantages
- small size (15 kDa)
- minimally invasive binding
- customization to application
- monovalence
- increased tissue permeability



Epitope

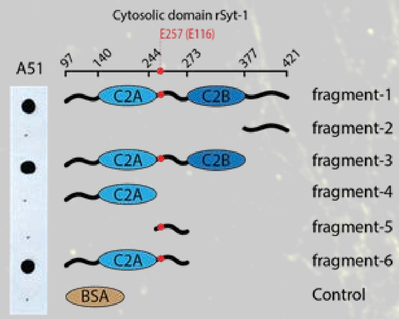
Crystal structure



The crystal structure of NbSyt1 indicates polar and hydrophobic interactions with its target protein Syt1.

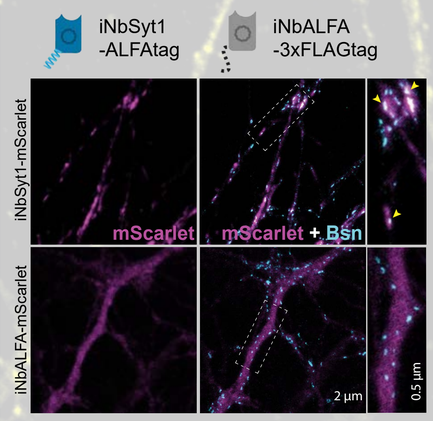
Epitope mapping and dot blot results for NbSyt1 demonstrates a targeting to a region around AA257, adjacent to the C2A-domain of Syt1.

Epitope mapping



Using intrabodies (iNbSyt1) for imaging native SVs

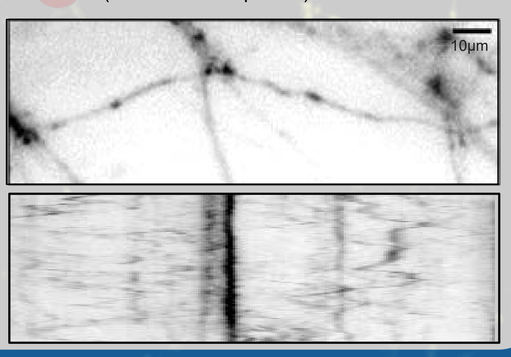
Synaptic localization of iNbSyt1 at direct expression



Expression of the iNbSyt1 demonstrates a synaptic-like localisation as validated by co-localisation with Bassoon (Bsn).

Expression of iNbSyt1 on SVs does not interfer with vesicle mobility or dynamics.

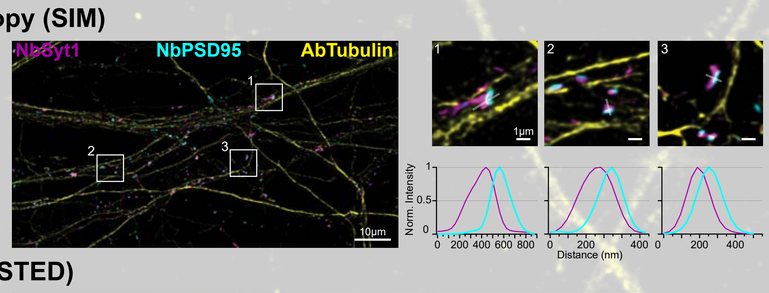
iNbSyt1 bound vesicles remain highly mobile



NbSyt1 in super-resolution microscopy

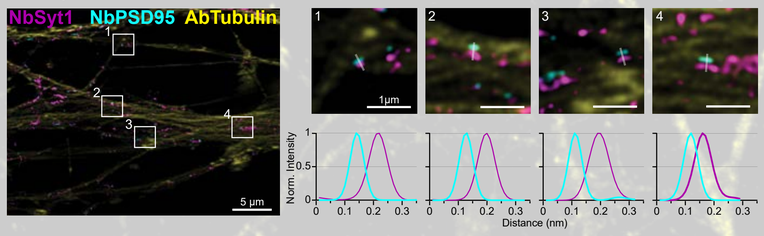
Structured Illumination Microscopy (SIM)

NbSyt1 / NbPSD95 directly fluorescently labelled

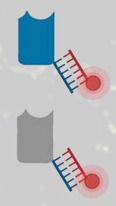


Stimulated Emission Depletion (STED)

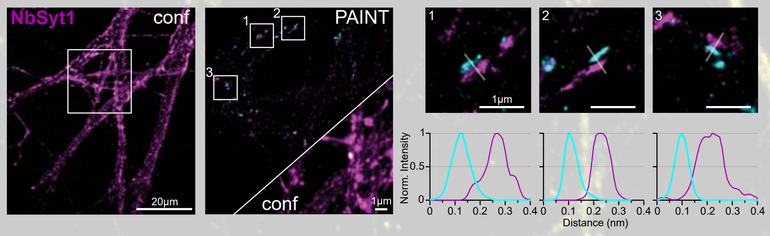
NbSyt1 / NbPSD95 directly fluorescently labelled



Exchange DNA-PAINT

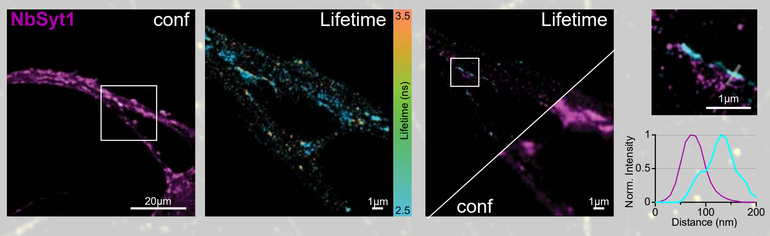


NbSyt1 / NbPSD95 fused to the DNA docking strand + imager strands harbouring a common fluorophore

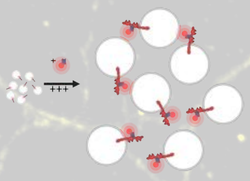


Lifetime DNA-PAINT

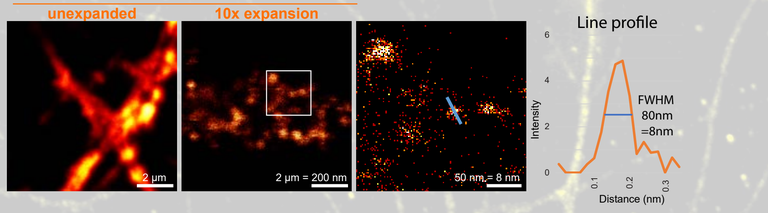
NbSyt1 / NbPSD95 fused to the DNA docking strand + imager strands harbouring fluorophores of slightly different spectra



Expansion microscopy



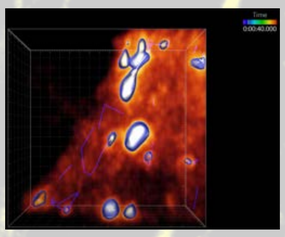
NbSyt1 fused to expansion cassettes to improve anchoring to the gel and enabling fluorescence detection



The NbSyt1 is a powerful choice as a small binder for a broad variety of imaging techniques by its ability to be customized to the needs of a special technique by creation of a fusion construct ranging from a direct fluorescent label to a sensor of synaptic activity.

Outlook and future directions

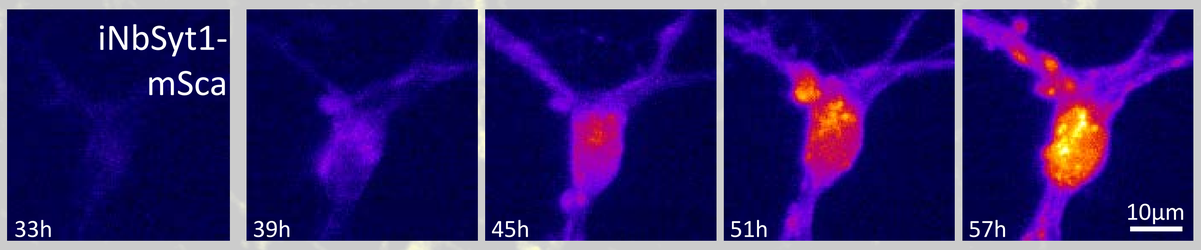
- We thouroughly characterized this nanobody iNbSyt1 targeting the cytosolic domain of the SV protein Syt1.
- Tools based on this nanobody can be used to study the SV life cycle in various directions to:
  - resolve SVs by several nanoscopy techniques;
  - modulate and follow SV formation;
  - investigate calcium-transience;
  - reveal new SV-associated proteins;
- Currently, we are using these tools to study the biogenesis, maturation and recycling capability of newly formed SVs in developing neurons.



Possible analysis approach

Imaging and modulating SV biogenesis

mSca-iNbSyt1 expression in developing neurons allows following its distribution inside the soma



ca. 30h after plating, mSca-signal starts to appear and increases inside soma, first as rather diffused signal. Throughout the timecourse, a more punctate signal develops, before/while a synaptic like pattern becomes visible along the processes.

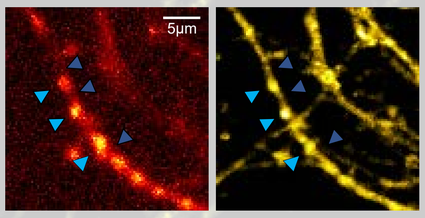
Timecourse network

Timecourse soma

Calcium activity

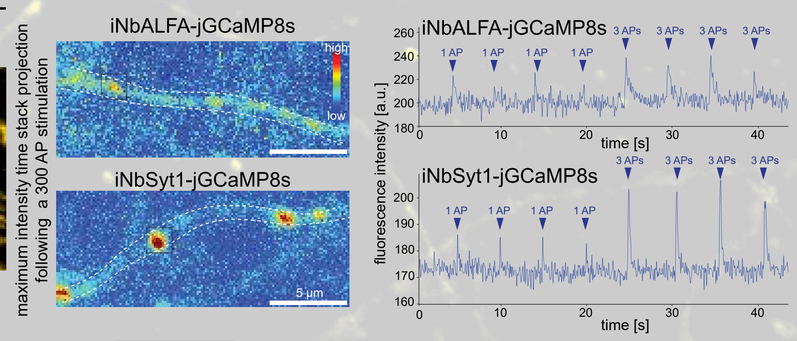
iNbSyt1 fused to jGCaMP8s (Ca^{2+} sensor) serves as a highly sensitive tool to follow SV activity both timely and locally

Fusion of the Ca^{2+}-sensor jGCaMP8s to iNbSyt1 presents a novel tool that enables highly sensitive detection of neuronal activity directly localised at the synapse.

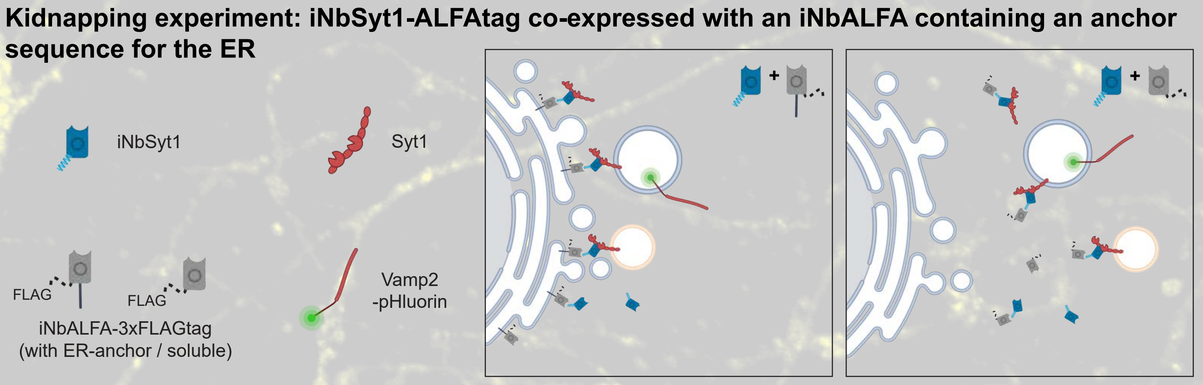


iNbSyt1 fused to jGCaMP8s with bound Ca2+ (on-state)

iNbSyt1 fused to jGCaMP8s without Ca2+ (off-state)



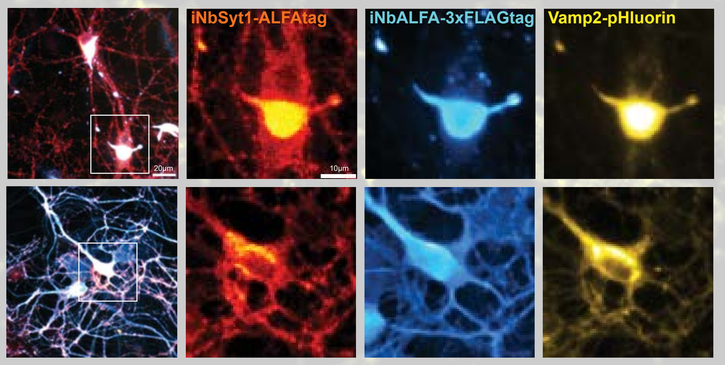
Kidnapping experiment: iNbSyt1-ALFAtag co-expressed with an iNbALFA containing an anchor sequence for the ER



Co-expression of the iNbSyt1-ALFAtag with an iNbALFA that it-self is fused to an ER-anchor sequence (iNbALFA-ER) leads to recruitment of the iNbSyt1-ALFAtag to the ER and with it its target protein Syt1. Interestingly, Vamp2 also accumulates in the neuronal soma.

iNbSyt1-ALFAtag + iNbALFA-ER anchor

iNbSyt1-ALFAtag + iNbALFA-ER as soluble protein



SCHRAM-STIFTUNG T0287/35359/2020

Acknowledgements

SFB 1286 Quantitative Synaptology

DFG FO 1342/1-3