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Easy visualization of inhibitory synapses in cell culture and tissue with a versatile peptidic probe

Inhibitory synapses in neuron cultures and brain tissue can now be visualized with ease and with high contrast. The newly developed synthetic affinity probe Sylite can be applied both for widefield and confocal 3D volumetric synapse visualization, for in-tissue inhibitory circuits mapping and for super-resolution imaging of synapses.

Vladimir Khayenko and Noah Nordblom from the research group of Dr. Hans Michael Maric at the Rudolf Virchow Center (RVZ) of the University Würzburg (Germany) have developed Sylite, a short peptide-based probe for the visualization of inhibitory synapses in neuronal cultures and tissue. Sylite is an activity related probe that beats antibodies in selectivity and simplicity and versatility of application. The development of Sylite and its application in conventional and super-resolution microscopy and further in cellular and circuit level studies, was recently published in Angewandte Chemie.

Synapse visualization and limitations

Inhibitory synapses in the central nervous system are either glycinergic or GABAergic and are commonly identified and visualized using gephyrin, the pan-inhibitory synapse marker. Gephyrin is a scaffolding protein that stabilizes inhibitory receptors at the postsynaptic density, and its concentration closely correlates with the number of receptors and synaptic strength.

Visualization of inhibitory synapses can be achieved with genetic tagging, this, however, is not always applicable or wanted since induced secondary gephyrin expression can have morphological and/or functional effects. An alternative approach is immunostaining of endogenous proteins, but it requires protocol tailoring for different sample types and imaging techniques.

Sylite reveals inhibitory synapse ultrastructure and brain connectivity

”Starting from an endogenous ligand of gephyrin, a universal marker of the inhibitory synapse, we developed a short peptidic binder and dimerized it, introducing antibody-like avidity and significantly increasing affinity and selectivity,“ explains Maric. “The probe with the best correlation and overlap with recombinant gephyrin in mammalian cells was the dimeric peptidic probe that we called Sylite, a short fluorescent peptidic probe with low nanomolar affinity,” says Maric.

Sylite is a functional probe designed to target receptor binding gephyrin isoforms, i.e., isoforms that exhibit functional roles in neurons. In the laboratory of Dr. Christian Specht (INSERM, Paris, France) Khayenko confirmed the linear correlation of the probe to neuronal gephyrin, whereas the gold standard gephyrin antibody exhibited non-linear scaling. Together with Specht, Khayenko visualized the synapse with super resolution microscopy and determined the ultrastructure with nanoscopic precision. „I was inspired by the outstanding performance of Sylite in neurons and keen

on exploring its potential in tissue imaging, as the probe had even more potential, since the small size could facilitate tissue penetration," describes Khayenko. This was the reason why the team reached out to Prof. Philip Tovote and his student Sara Reis from the Institute of Clinical Neurobiology at the University of Würzburg. Together they performed multiple brain tissue stainings, where Sylite outperformed gephyrin antibodies in every aspect. Sylite achieved synapse visualization with no staining artifacts, penetrating 50 µm thick brain sections within just one hour, while antibody penetration was only partial after 24 hours and after 72 hours significant staining artifacts where visible. Recognizing the power of the probe they studied the synaptic connectivity and profiled inhibitory inputs and synapse sizes of excitatory and inhibitory neurons in the midbrain.

Future applications and availability of Sylites

"I am currently working on Sylite derivatives that will enable us to visualize inhibitory post-synapses and explore the modulation of inhibitory signaling in living cells," says Khayenko. Maric concludes, "Compact synthetic probes, such as Sylite open new research avenues in neuroscience. They enable better localization precision, resolution and excellent performance in tissue and numerous key components of the nervous system remain to be addressed in the same way."

Sylite is commercially available through NanoTag Biotechnologies GmbH (Cat.# P4001).

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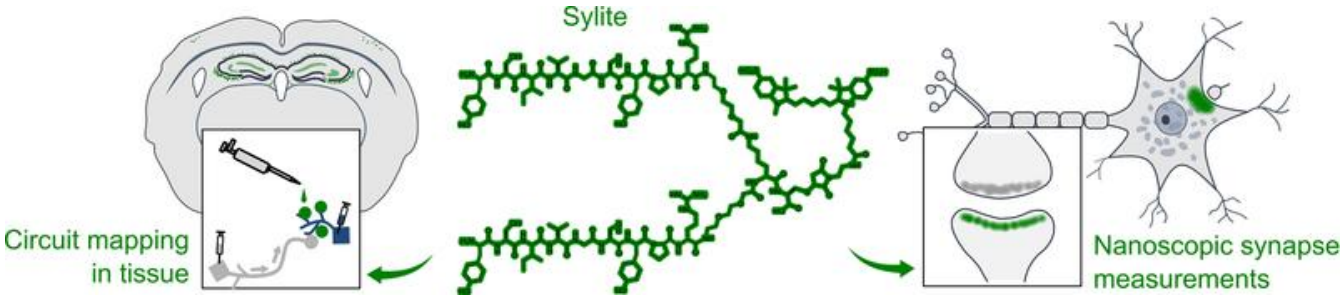

Sylite enables accurate nanoscopic measurements in primary neurons (right), simplifies and accelerate tissue staining, and can be used for brain circuit mapping and synapse profiling (left). s,
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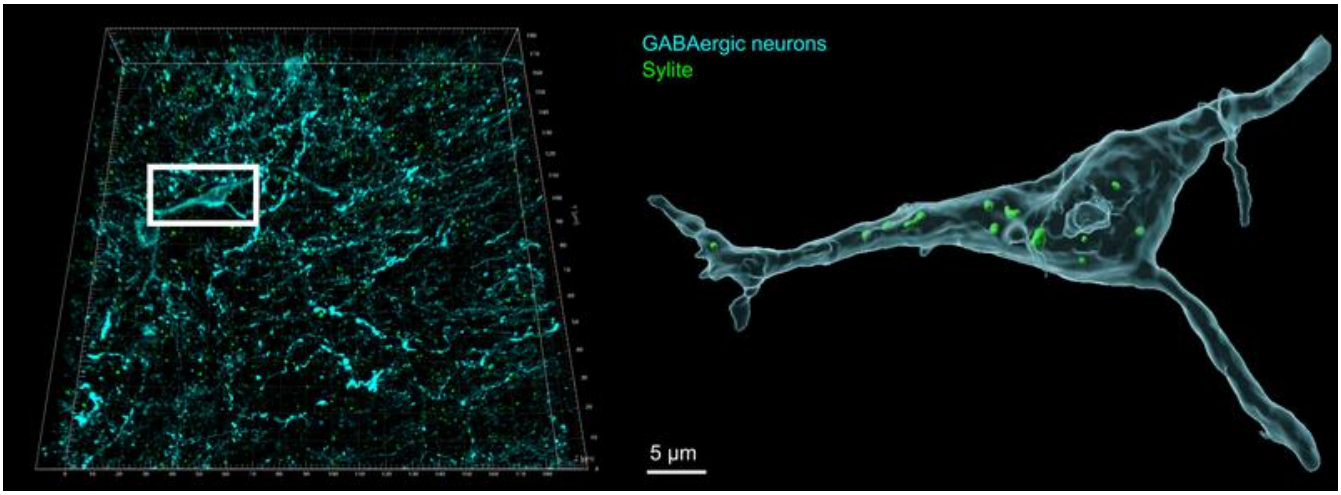

Sylite staining of a periaqueductal gray midbrain section, GABAergic neurons are visualized in light blue. 3D volumetric reconstruction of single GABAergic neuron cell body from brain section.
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