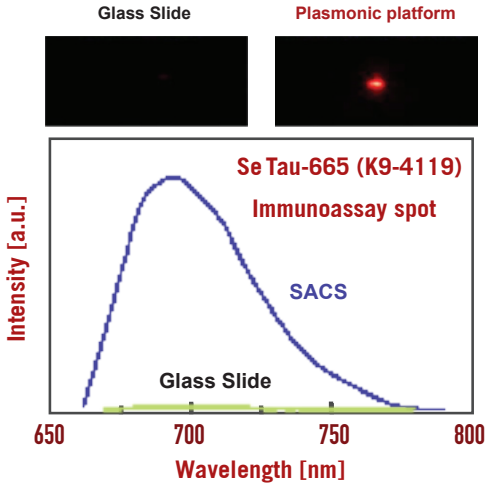
Longer Single Molecule Tracking with SeTau and Seta Dyes

The photophysical properties of **SeTau-665** (K9-4119) were investigated on a plasmonic platform of self-assembled colloidal structures (SACS) of silver prepared on a semitransparent silver film and a SeTau-665-based immunoassay was performed on this platform and a control glass slide.

The fluorescence properties of **SeTau-665** substantially change due to plasmonic interactions. While the average brightness increase of **SeTau 665** in ensemble measurements was about 70-fold , fluorescence enhancements up to 400-times were observed on certain "hot spots" for single molecule measurements. The intensity increase is strongly correlated with a simultaneous decrease in fluorescence lifetime in these "hot spots". The high increase in brightness allowed to reduce the excitation power resulting a reduced background and increased photostability.



Emission spectra of a SeaTau-665 (K9-4119) immunoassay spot (ensemble concentration) on a glass and on an SACS surface. Top panels show photographs taken with 635 nm excitation and observed through a 695 nm long-pass filter [2].

The remarkable fluorescence enhancement observed when using squaraine rotaxanes such as **SeTau 665** on plasmonic platforms should allow not only reducing the detection limits in sensing devices but also enable single molecule measurements which were previously impossible.

			Excitation Light Sources					Characteristics					
Product Number (Spec Sheet)	Product Name (Product Info)	Target Group	488	635	650	680	700	Medium	λ abs [nm]	ε [M^{-1}. cm^{-1}]	λ em [nm]	QY [%]	FLT [ns]
K9-3152 NEW	SeTau-488-NHS	NH_2	•					PB 7.4	486	78000	533	30	
K9-4119	SeTau-665-NHS	NH_2			•	•	•	PB 7.4	664	160000	712	53	3.1
K9-4142	SeTau-647-di-NHS	NH_2		•	•			PB 7.4	650	200000	694	65	3.2
K9-4145	SeTau-633-Ethyl-Ester			•	•			$CHCl_3$	634	105000	683	68	
K9-4148	Seta-647-Maleimide	SH		•	•			PB 7.4	648	200000	692	45	3.2
K9-4149	SeTau-647-NHS	NH_2		•	•			PB 7.4	649	200000	695	61	3.2
K9-4150	SeTau-647			•	•			PB 7.4	647	211000	693	59	3.1
K9-4159 NEW	SeTau-660-NHS	NH_2		•	•	•		PB 7.4	663	240000	694	50	3.3
K9-4169 NEW	SeTau-670-NHS	NH_2		•	•	•		PB 7.4	673	275000	694	36	1.6
K9-4179 NEW	SeTau-680-NHS	NH_2		•	•	•		PB 7.4	683	215000	705	58	2.9

[2] Luchowski R., Single molecule immunoassay on plasmonic platforms. Curr. Pharm. Biotech. 11, 96-102 (2010).

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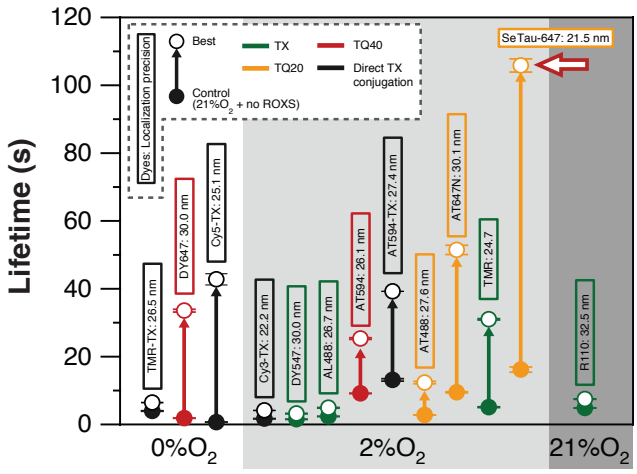
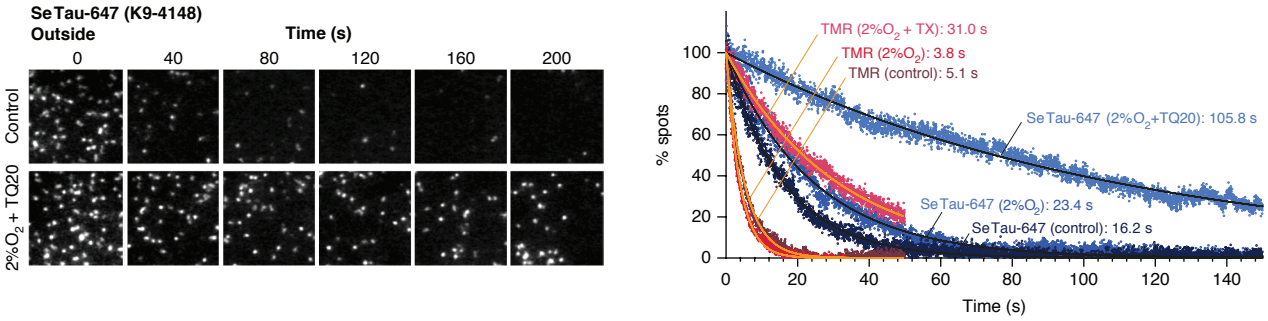
Longer Single Molecule Tracking with SeTau and Seta Dyes

The photobleaching rates of 13 fluorescent dyes (see below), 4 of them conjugated to trolox, were investigated for single-molecule measurements at 37°C in living cells. For this purpose they were linked to a tag protein that was fused to CD47.

In this study SeTau-647 (**K9-4149 and K9-4148**), a squaraine rotaxane dye, exhibited the best photobleaching performance with an exponential lifetime of 105.8 sec and the best localization precision with 21.5 nm. As a membrane-impermeable dye it allowed for the observation of up to 12,000 frames, which is the **longest single-fluorescent-molecule tracking ever reported** (see images below and paper in Nature Chem. Biol. [1]).



Photobleaching lifetimes of 13 dyes (including Atto-647N, Atto-594, DY-647, TMR, Cy3-Tx and Cy5-Tx) under controlled conditions and conditions for slowest photobleaching [1]. Most dyes including **SeTau-647** (arrow) exhibited the longest photobleaching time at 2%O2. **SeTau-647** exhibited the best photobleaching performance with an exponential lifetime of 105.8 sec and the best localization precision with 21.5 nm.



TIRF microscopic images (left) of a time series for **SeTau-647** linked to ACP-CD47 (fluorophore is located on the outer surface of a T24 epithelial cell). Time-dependent reductions of the numbers of fluorescent spots found in each 33-ms frame for TMR and **SeTau-647 (K9-4148)** on the extracellular surface (right) [1].

[1]. Tsunoyama, T.A. et al. Super-long single-molecule tracking reveals dynamic-anchorage-induced integrin function. Nat.Chem.Biol. 14, 497-506 (2018).

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