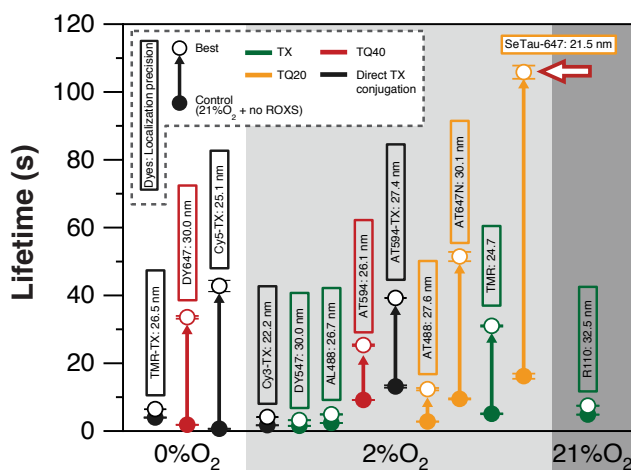


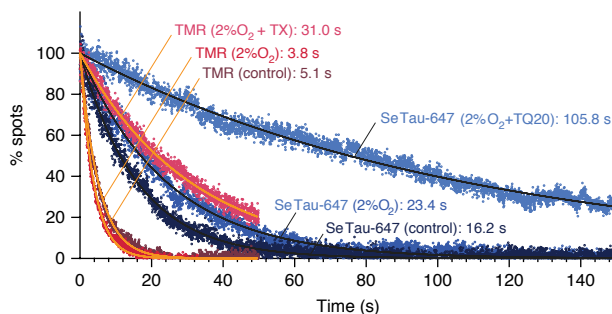
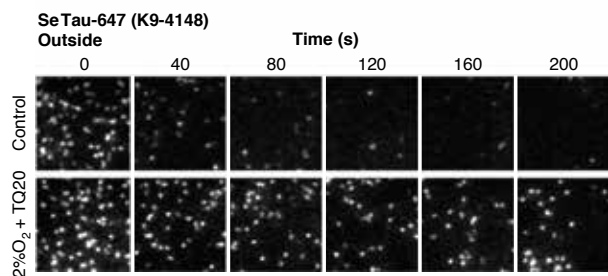
# Longer Single Molecule Tracking with SeTau and Seta Dyes

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%O<sub>2</sub>. **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).