



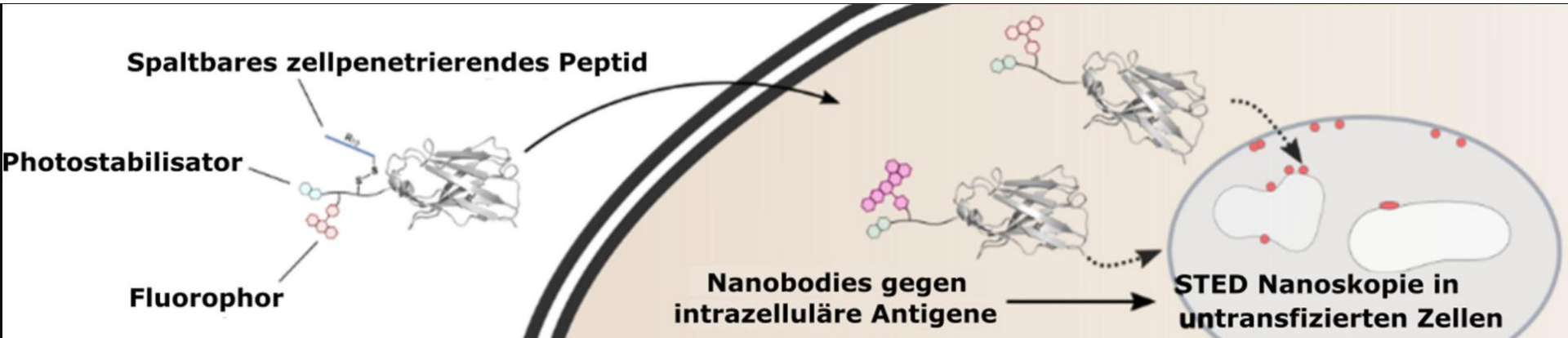
2021 Literature report I

Reporter: Li Zhifeng

Date: 2021-10-14

Cell-Permeable Nanobodies Allow Dual-Color Super-Resolution Microscopy in Untransfected Living Cells

Anselm F. L. Schneider, Laila S. Benz, Martin Lehmann, and Christian P. R. Hackenberger*



Author



Christian P.R. Hackenberge

1996-1998	Undergraduate studies and prediploma in Chemistry	Albert-Ludwigs-Universität ,Freiburg
1998-1999	Graduate studies and M.Sc. with Prof. Samuel H. Gellman	Univ. of Wisconsin/Madison, USA
2000-2003	Ph.D. research with Prof. Carsten Bolm (summa cum laude)	RWTH-Aachen
2003-2005	Postdoctoral work with Prof. Barbara Imperiali	Massachusetts Institute of Technology, USA
2004	Research stay with Prof. Sheena E. Radford	University of Leeds, UK
2005-2006	Junior group leader as Liebig-Scholar (FCI)	Freie Universität Berlin
2006-2011	Emmy-Noether-Group leader (DFG)	Freie Universität Berlin
2011	Habilitation and venia legendi in Organic Chemistry	Freie Universität Berlin
2011-2012	Associate Professor (W2) for Bioorganic Chemistry	Freie Universität Berlin
2012-	Leibniz-Humboldt-Professor (W3) for Chemical Biology	Humboldt Universität zu Berlin and LFMP

Research interest

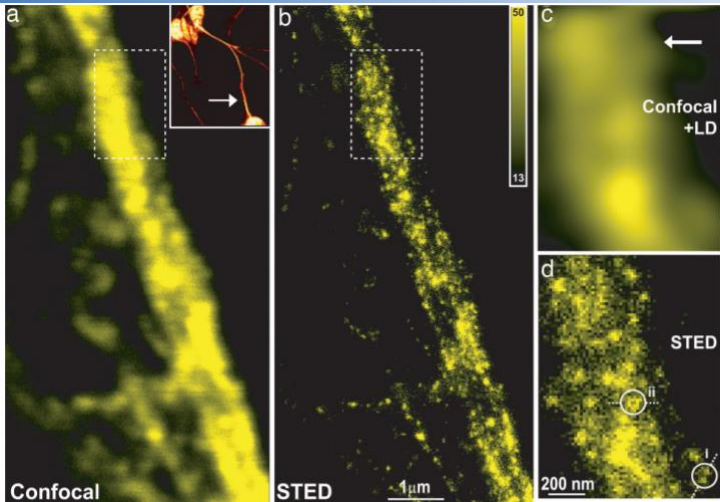
Development of ligation and modification strategies for the synthesis of functional proteins
Labeling strategies for antibody- and nanobody-conjugates, generation of antibody-drug-conjugates (ADCs)
Synthesis and proteomic analysis of labile phosphorylated peptides (pLys and pCys)
Intracellular delivery and targeting of functional proteins
Functional investigation of the Alzheimer-relevant Tau protein
Engineering of protein-based multivalent scaffolds, metabolic oligosaccharide engineering



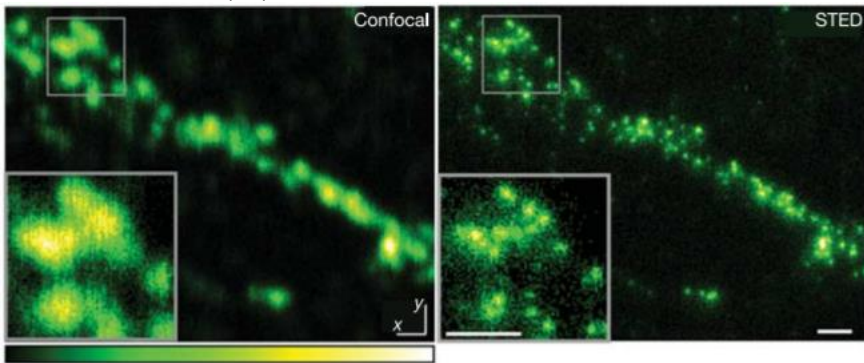
Dr. Anselm. F. L. Schneider

2011-2014	Bachelor of Science(B.Sc.) Biochemistry	Freie Universität Berlin
2014-2016	Master of Science(M.Sc.) Biochemistry	Freie Universität Berlin
2015.10-2016.6	Master's Thesis with Prof. Mark Howarth	University of Oxford
2016.05-2020.12	PhD in the lab of Prof. Christian Hackenberger	Freie Universität Berlin
2021.01-2021.06	Postdoctoral work	Leibniz-Forschungsinstitut für Molekulare Pharmakologie

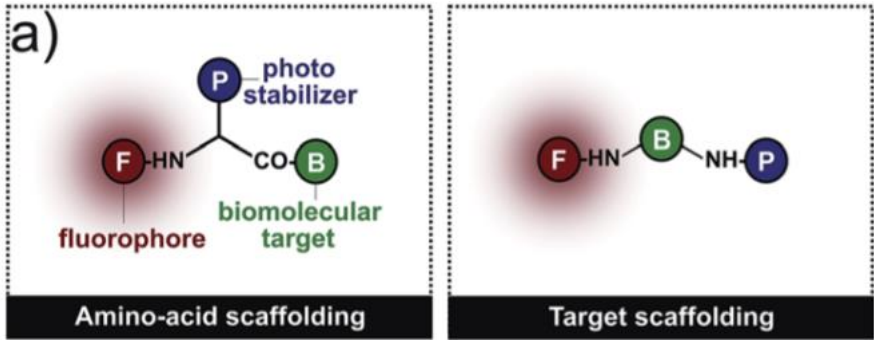
Introduction



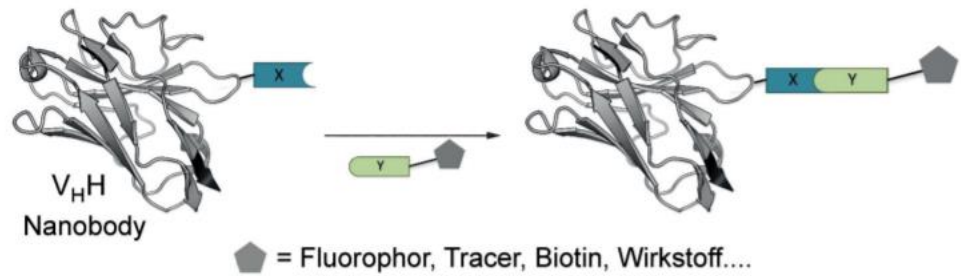
PNAS ,2006,103 (31),11440-11445



Nature ,2006,440, 935–939



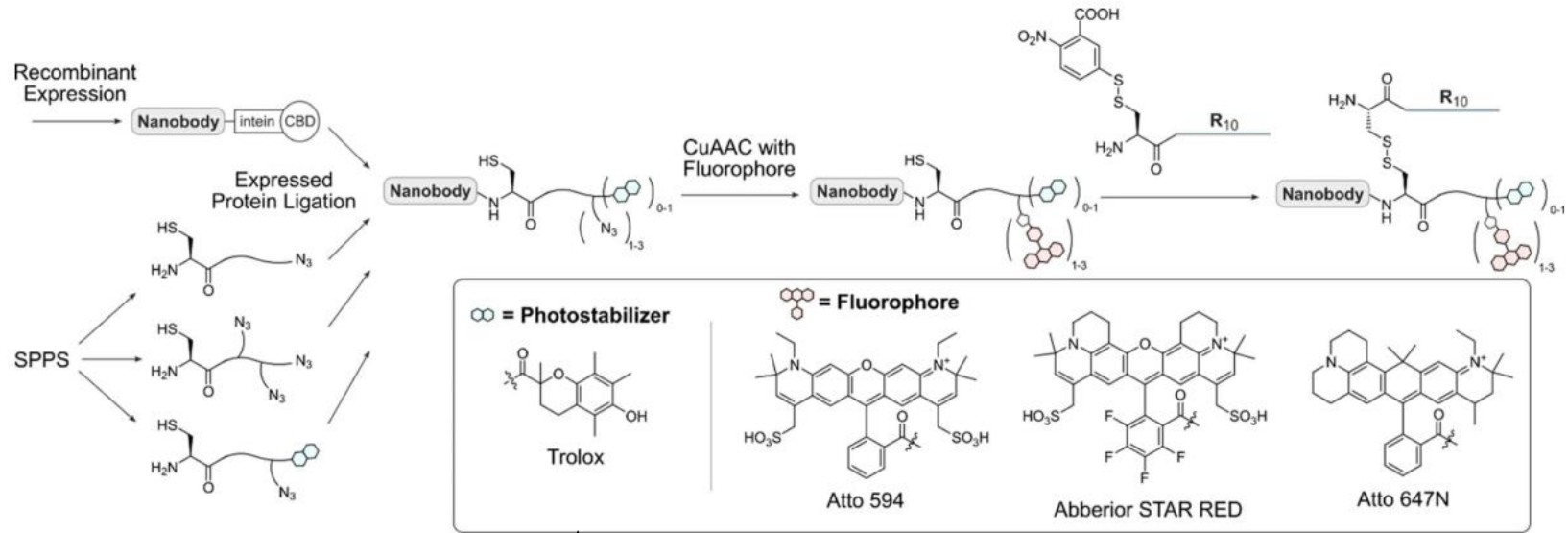
Faraday Discuss., 2015, 184, 221–235



Angew. Chem. 2018, 130, 2336-2357

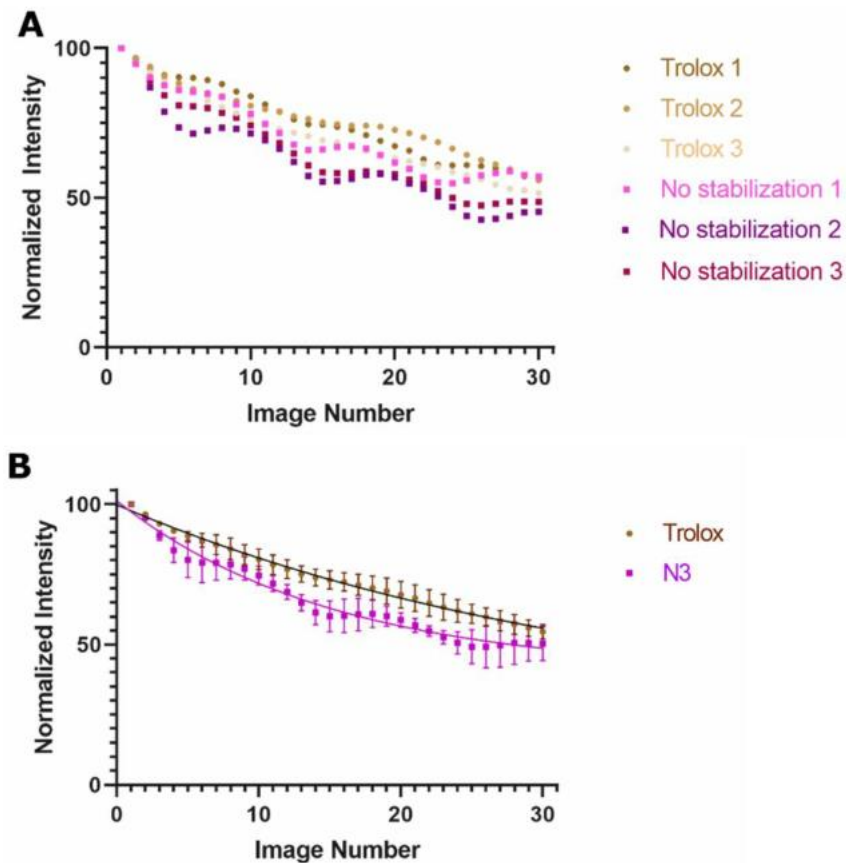
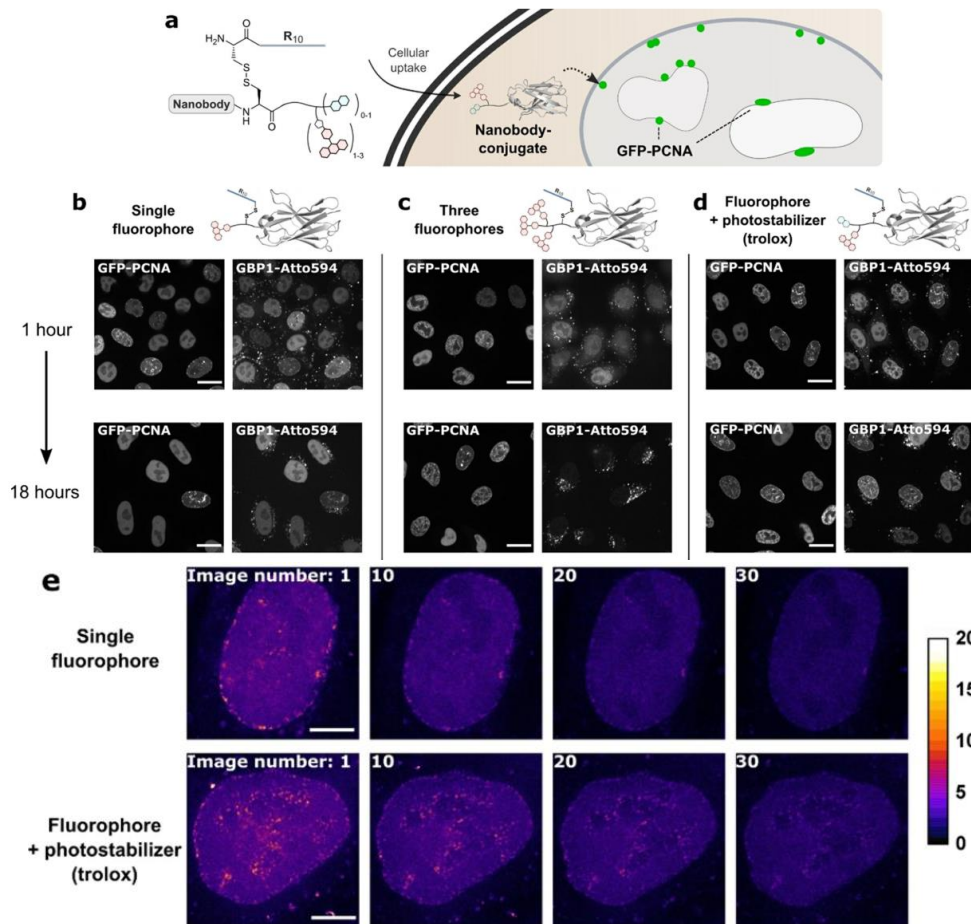
单域抗体 (V_HH)：高水溶性，高稳定性，靶向性。
 天然重链抗体及其进一步重组得到的纳米抗体在疾病诊断和治疗中具有靶向性，并较其他普通抗体具有更强的抗原结合能力，小到半抗原和肽，大到蛋白和病毒，纳米抗体也可对其表位进行识别。
纳米体：尺寸小，导致连接误差小，即靶标和标签之间的距离小。

Introduction

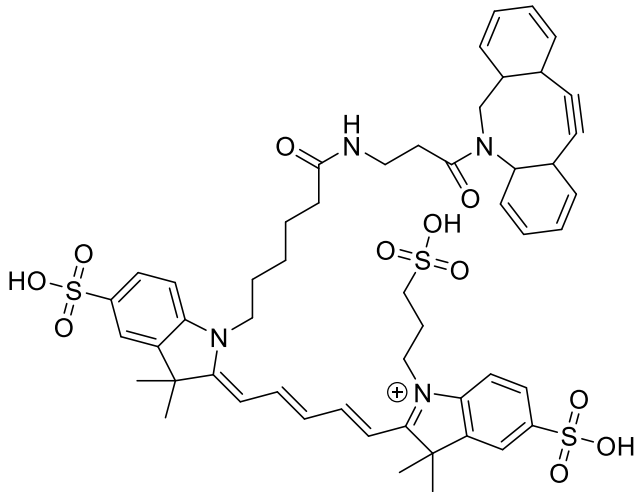


Trolox: 水溶性维生素E。一种细胞通透或水溶性的VE类似物，具有抗氧化的性质。可作为一种有效的三线态淬灭剂可以改善荧光分子的光物理性质。

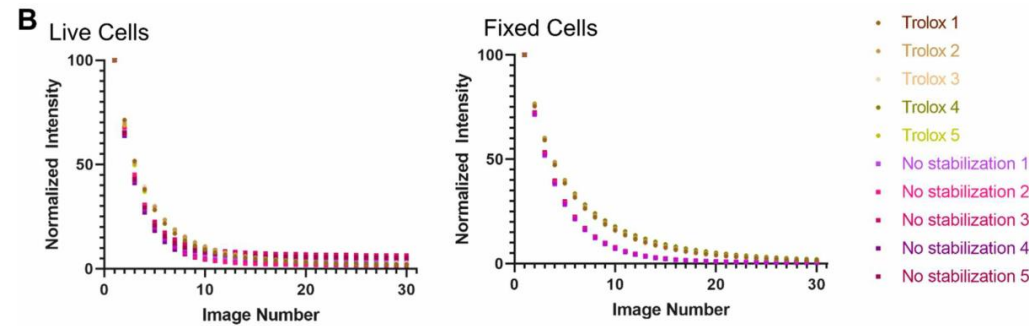
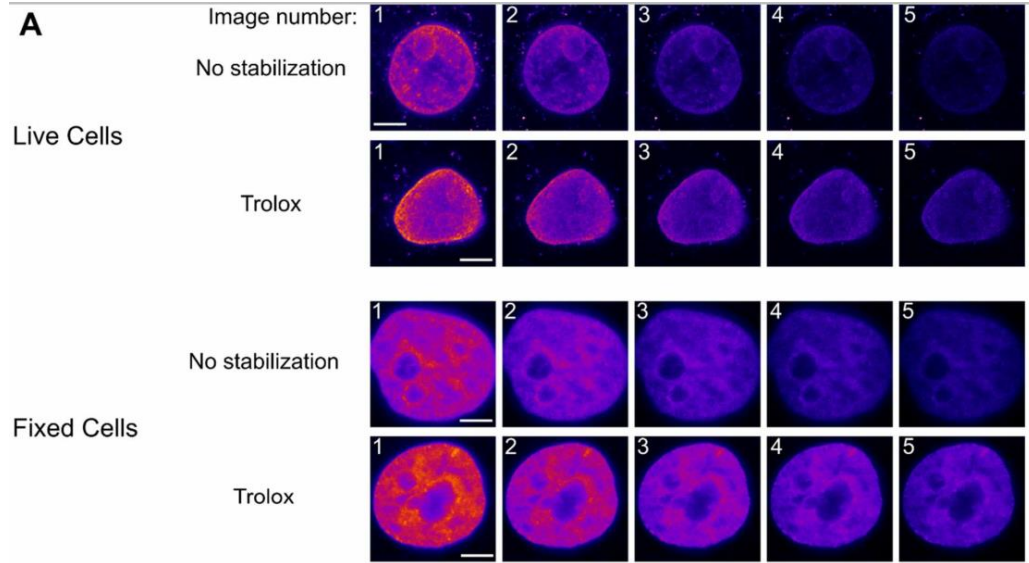
纳米体-荧光团-光稳定剂偶联物的评价



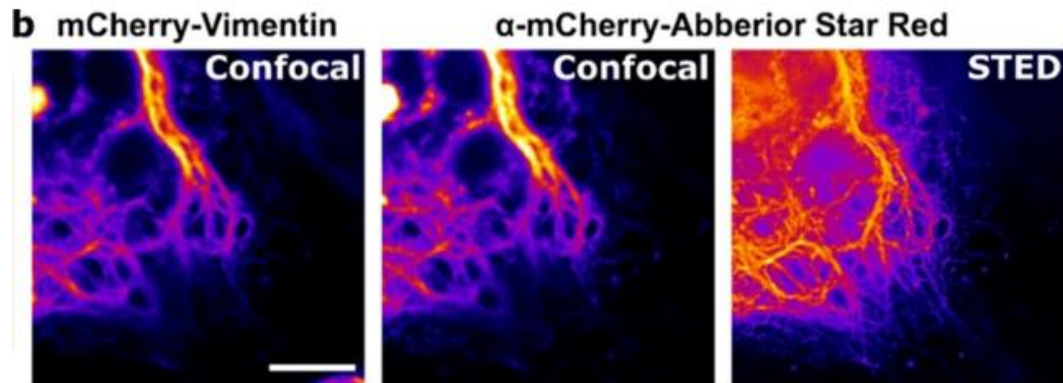
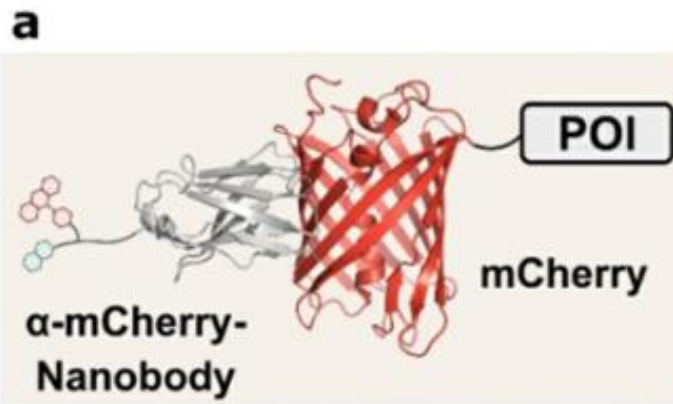
纳米体-荧光团-光稳定剂偶联物的评价



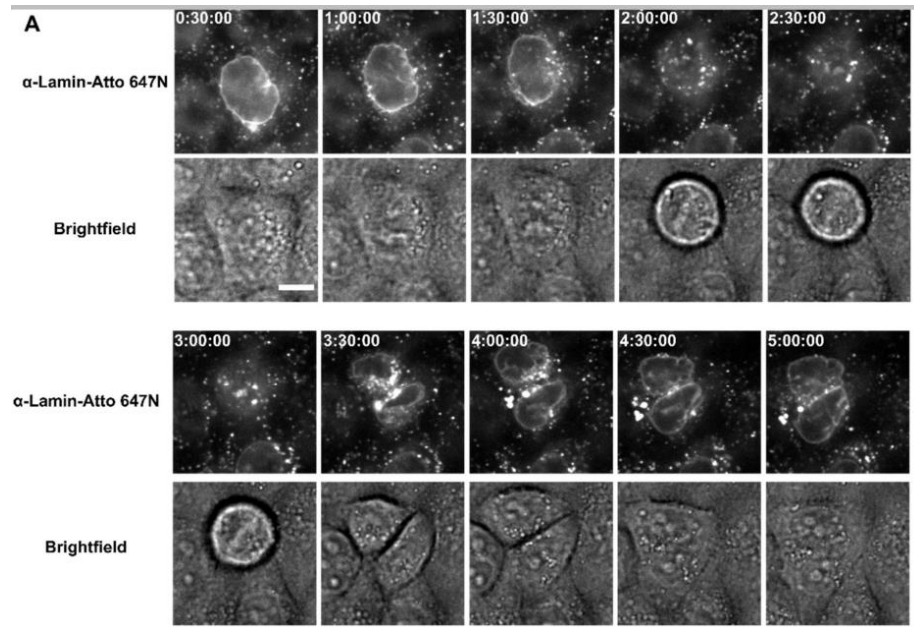
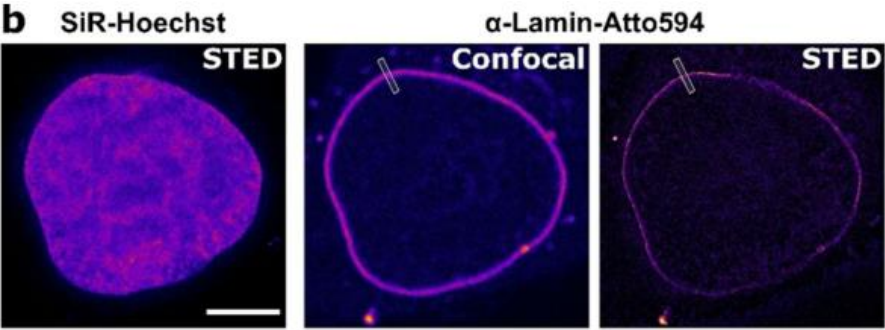
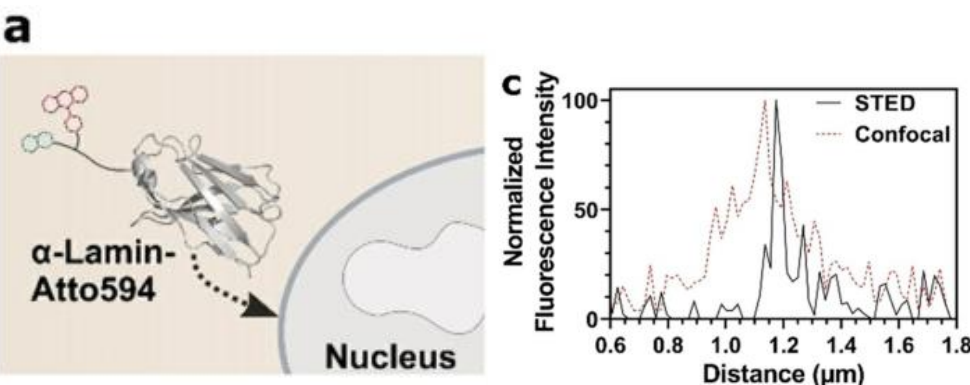
GBP1-Sulfo-Cy5 conjugates were taken up into HeLa Kyoto cells expressing nuclear EGFP fused to histone H2B.



具有细胞渗透性的mCherry纳米体用于活细胞中mCherry融合蛋白的STED观察

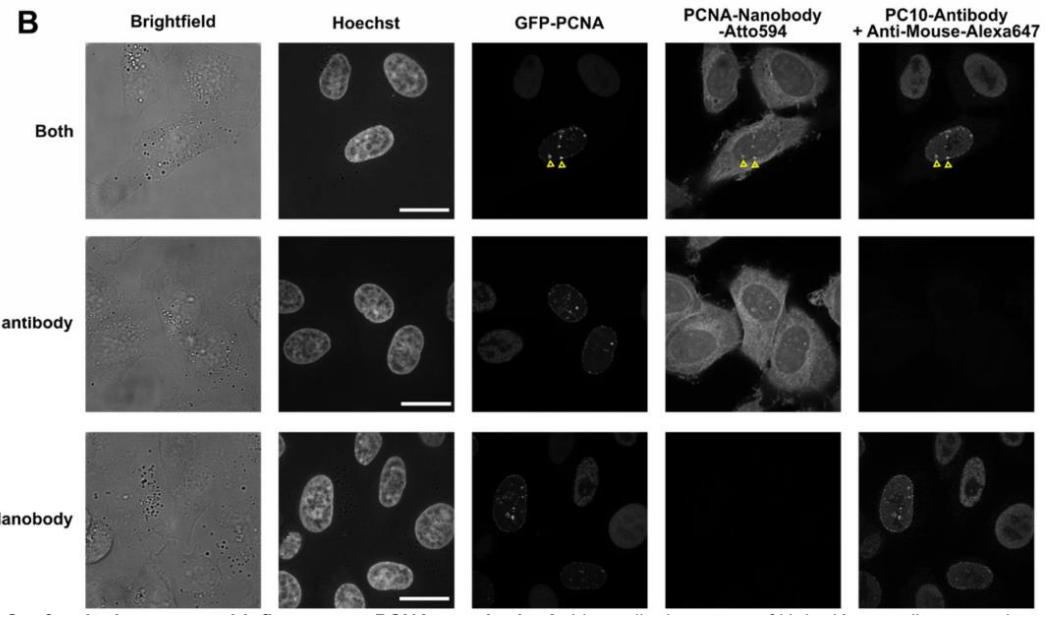
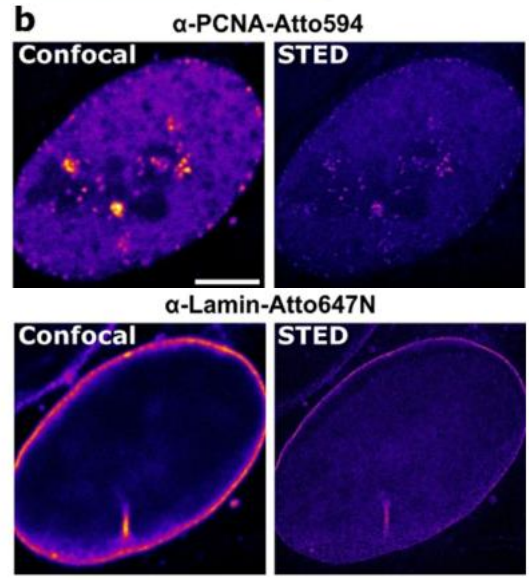
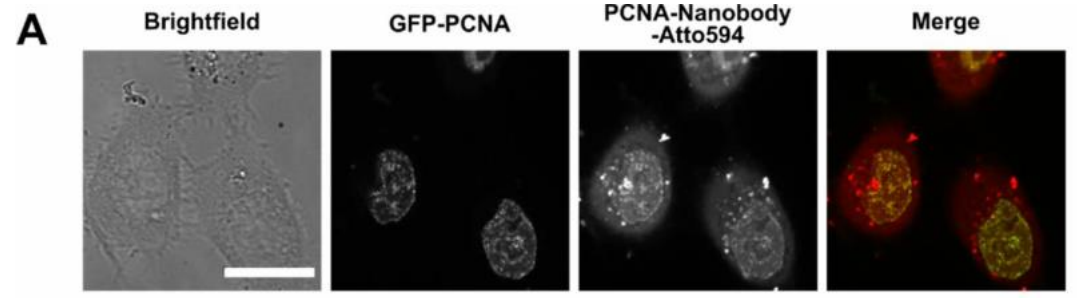
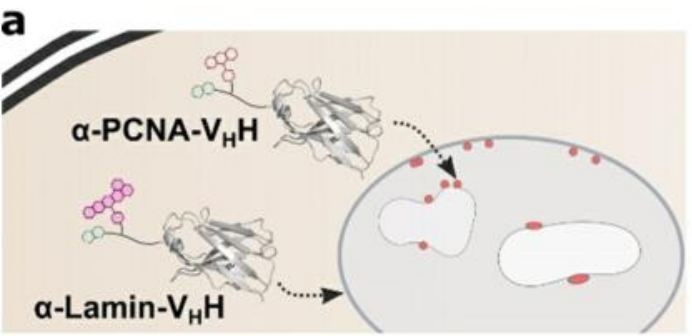


具有细胞渗透性的荧光Lamin纳米体用于内源性核纤层蛋白的超分辨成像

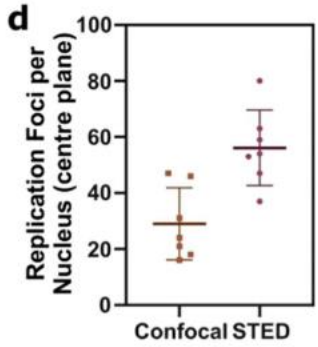
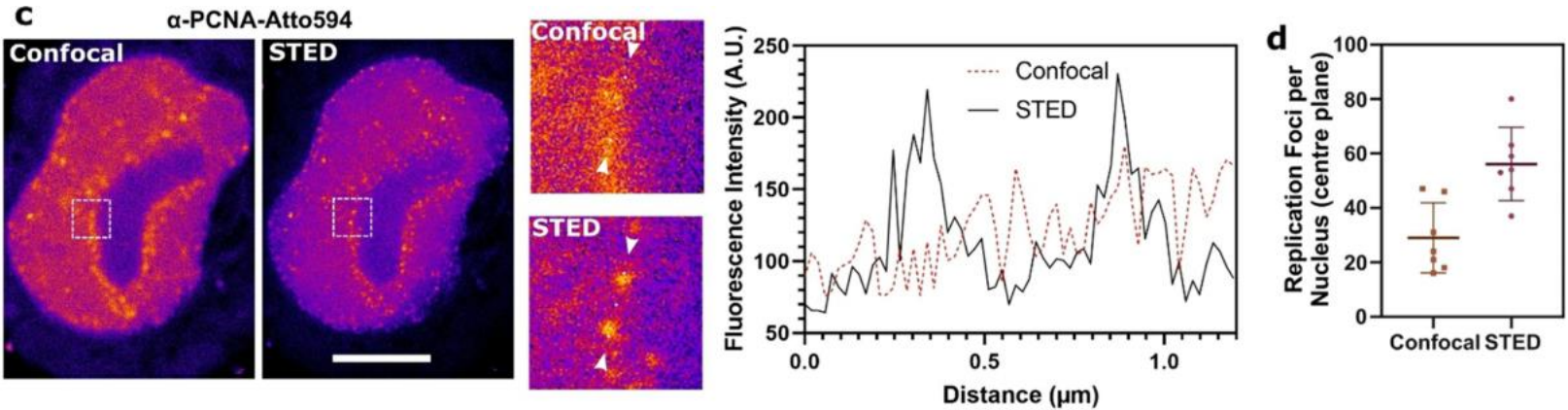


1 μM α -Lamin nanobody conjugated to trolox and Atto 647N
 HeLa Kyoto cells
 every 30 minutes at 37° C

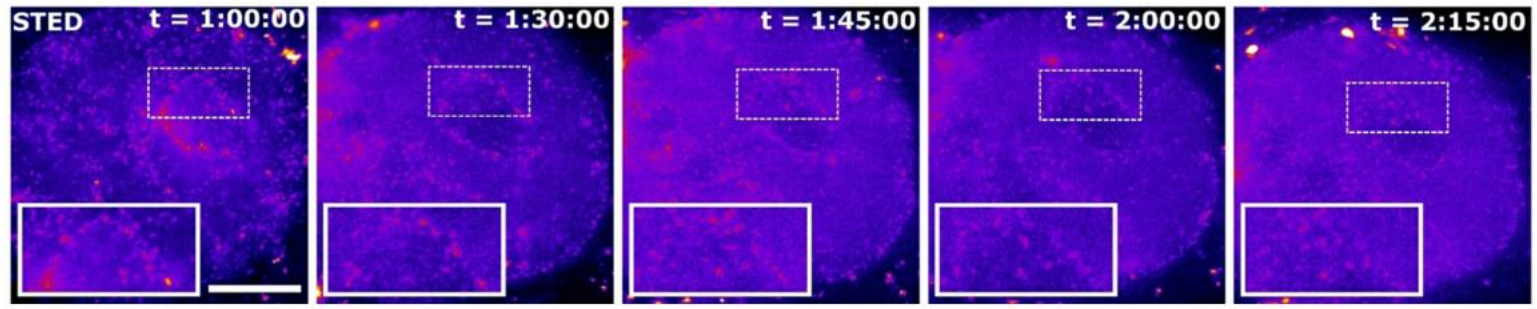
具有细胞渗透性的PCNA纳米体用于复制位点的超分辨延时成像



具有细胞渗透性的PCNA纳米体用于复制焦点的超分辨延时成像



e α -PCNA-Abberior Star Red



Summary

- 本文介绍了其所知的首次分子内光稳定剂在活细胞成像中的应用
- 通过将光稳定剂和荧光团偶联到具有细胞渗透性的纳米体上，证明该光稳定剂变体比含有多个荧光团的纳米体表现更好
- 通过递送两种不同的纳米体证明了细胞内目标物可同时双色染色
- 将这些发现应用到改造的具有细胞渗透性的PCNA纳米体，对细胞内的复制位点有了前所未有的了解