Literature Report

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ARTICLES

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Single-molecule imaging of glycan–lectin interactions on cells with Glyco-PAINT

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Sander I. van Kasteren

2001 MSc Chemistry & Medicinal Chemistry, Faculty of Chemistry, University of Edinburgh
2007 PhD, Faculty of Chemistry, University of Oxford
2007 – 2010 Sir Henry Wellcome Postdoctoral Fellow, University of Dundee
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Sander van Kasteren works on the interface between chemistry and immunology. He uses his background in organic synthesis and dendritic cell biology to study and manipulate the uptake and routing of antigen in dendritic cells.

Lorenzo Albertazzi

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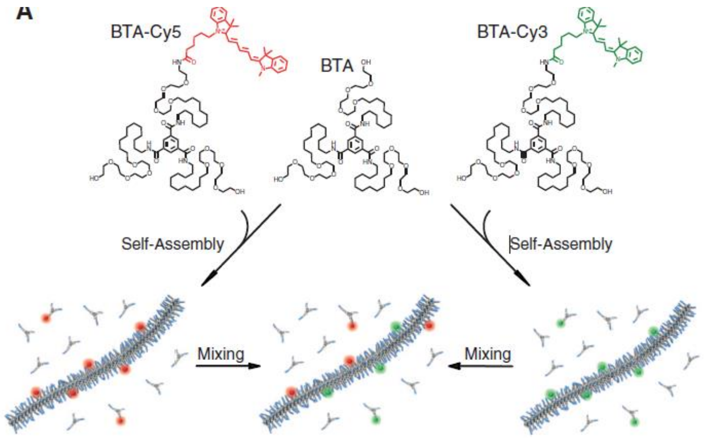
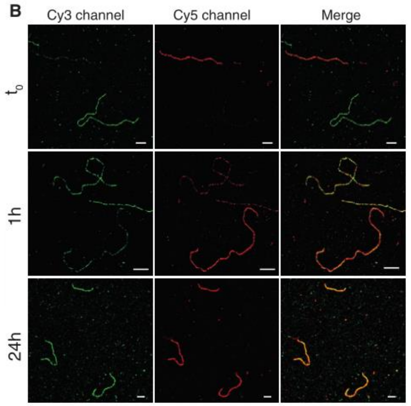
Group 2: Nanoscopy for Nanomedicine
Junior group leader, the Institute of Bioengineering of Catalonia
Develops technologies based on micro- and nanoparticles for monitoring patients and for treating diseases.
Use advanced microscopy techniques such as super-resolution imaging to understand the structure of synthetic nanomaterials in vitro and their biological interactions and to provide the design rules guiding the rational design of novel nanomedicines.

Corresponding author——Lorenzo Albertazzi

Science, 2014, 491

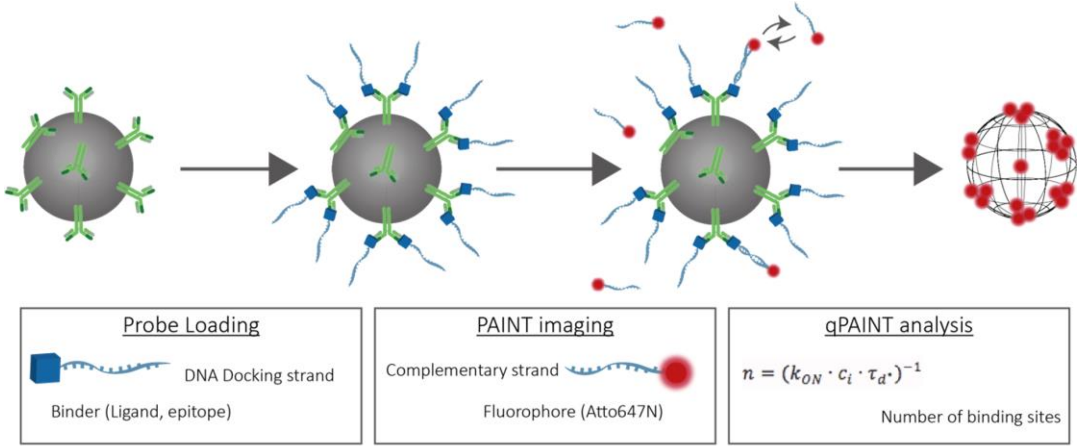
Probing Exchange Pathways in One-Dimensional Aggregates with Super-Resolution Microscopy.

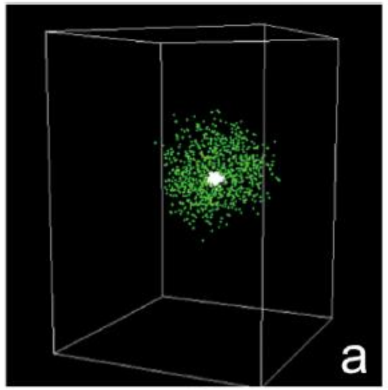


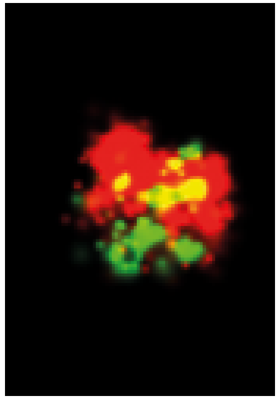


ACS Nano 2018, 762

Nanoscale Mapping Functional Sites on Nanoparticles by Points Accumulation for Imaging in Nanoscale Topography (PAINT).



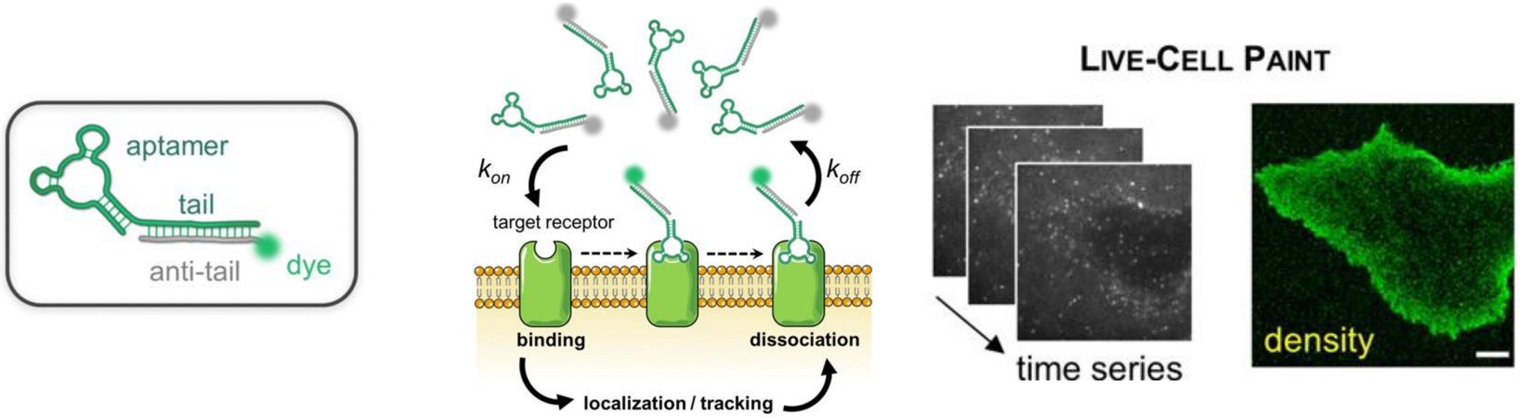




Corresponding author——Lorenzo Albertazzi

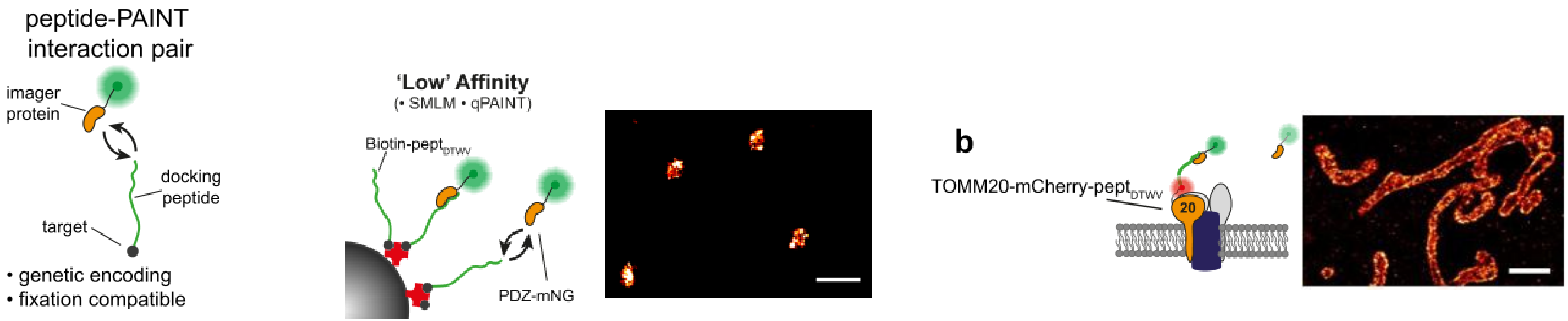
Angew. Chem. Int. Ed., 2020, 18546

Aptamers with Tunable Affinity Enable Single-Molecule Tracking and Localization of Membrane Receptors on Living Cancer Cells.



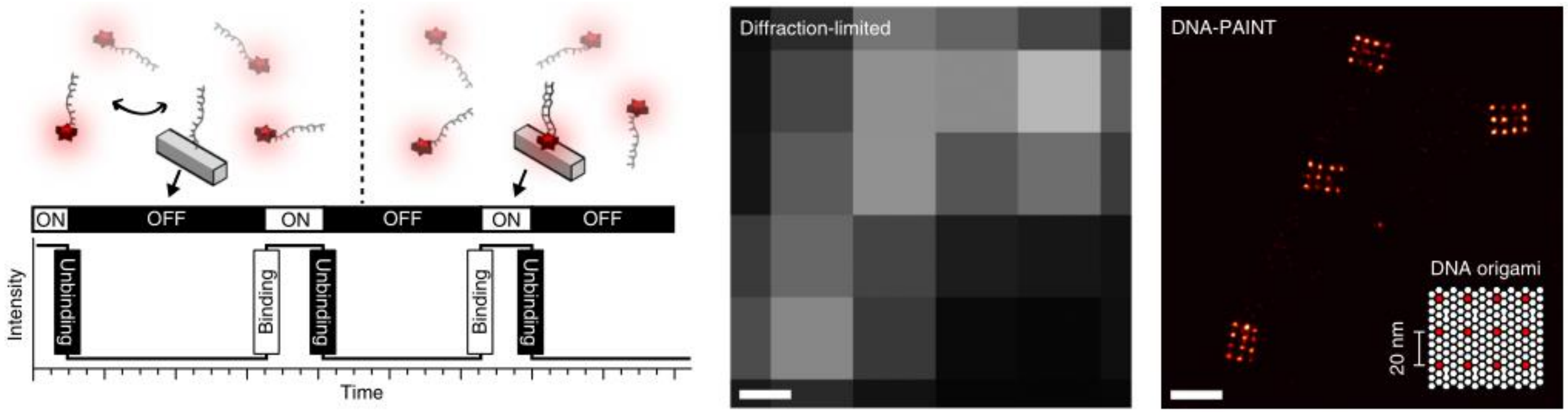
Nano Lett., 2021, 21, 9509−9516

Small Peptide−Protein Interaction Pair for Genetically Encoded, Fixation Compatible Peptide-PAINT.

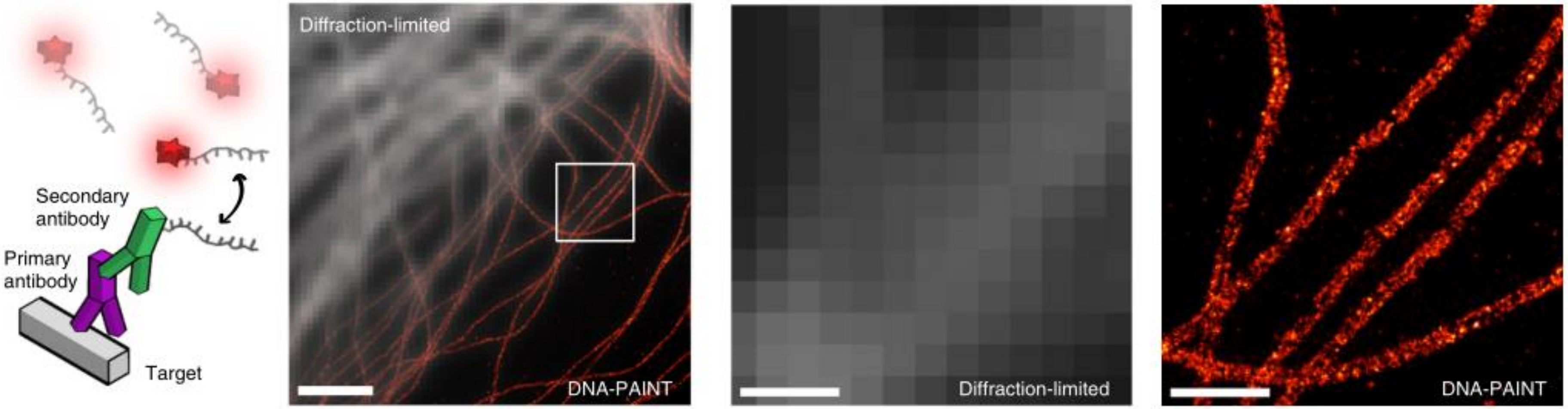


Points Accumulation for Imaging in Nanoscale Topography (PAINT)

DNA-PAINT



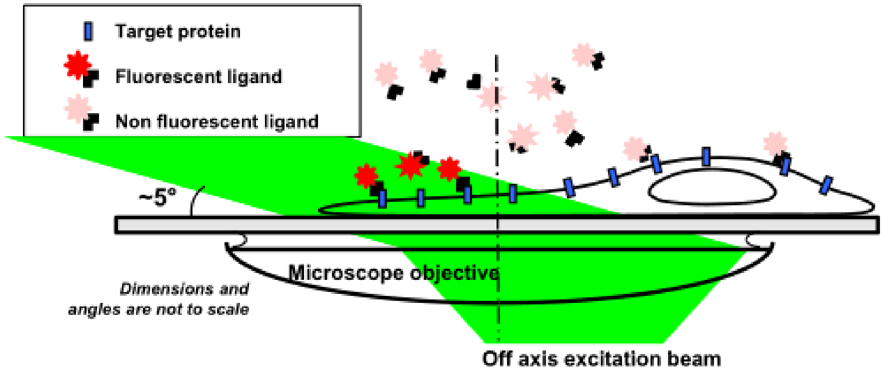
In situ protein-labeling strategy

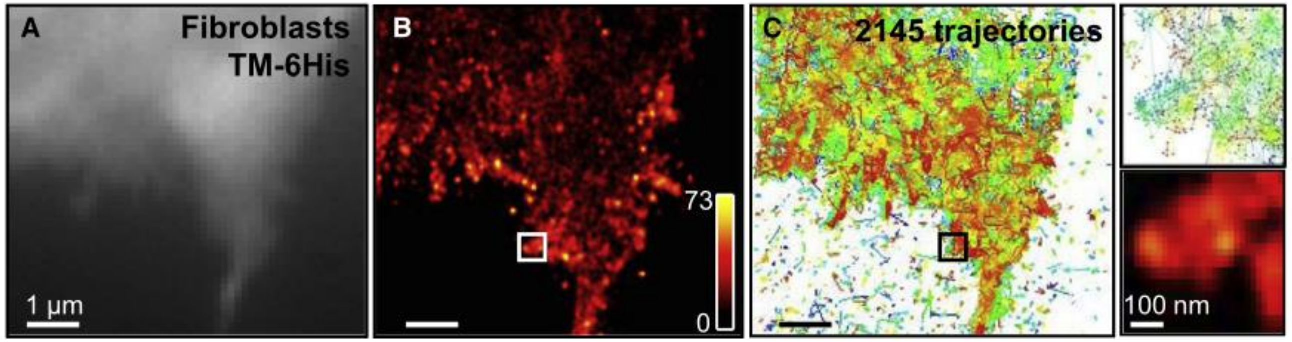


Nature Protocols, 2017, 1198

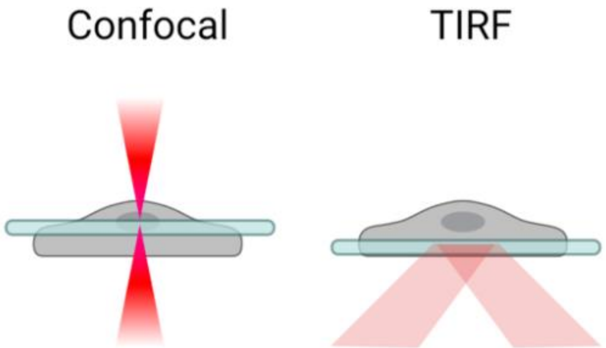
Universal Points Accumulation for Imaging in Nanoscale Topography (uPAINT)

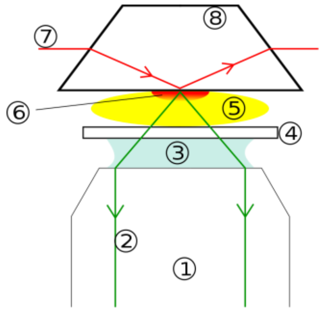
Biophysical Journal, 2010, 1303



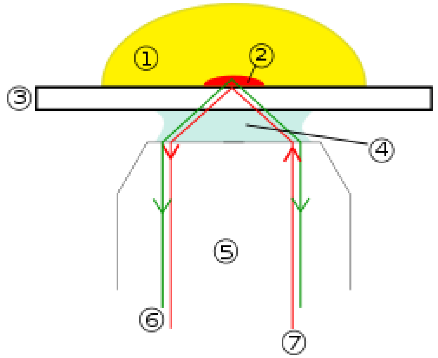


Total internal reflection fluorescence microscope (TIRFM)



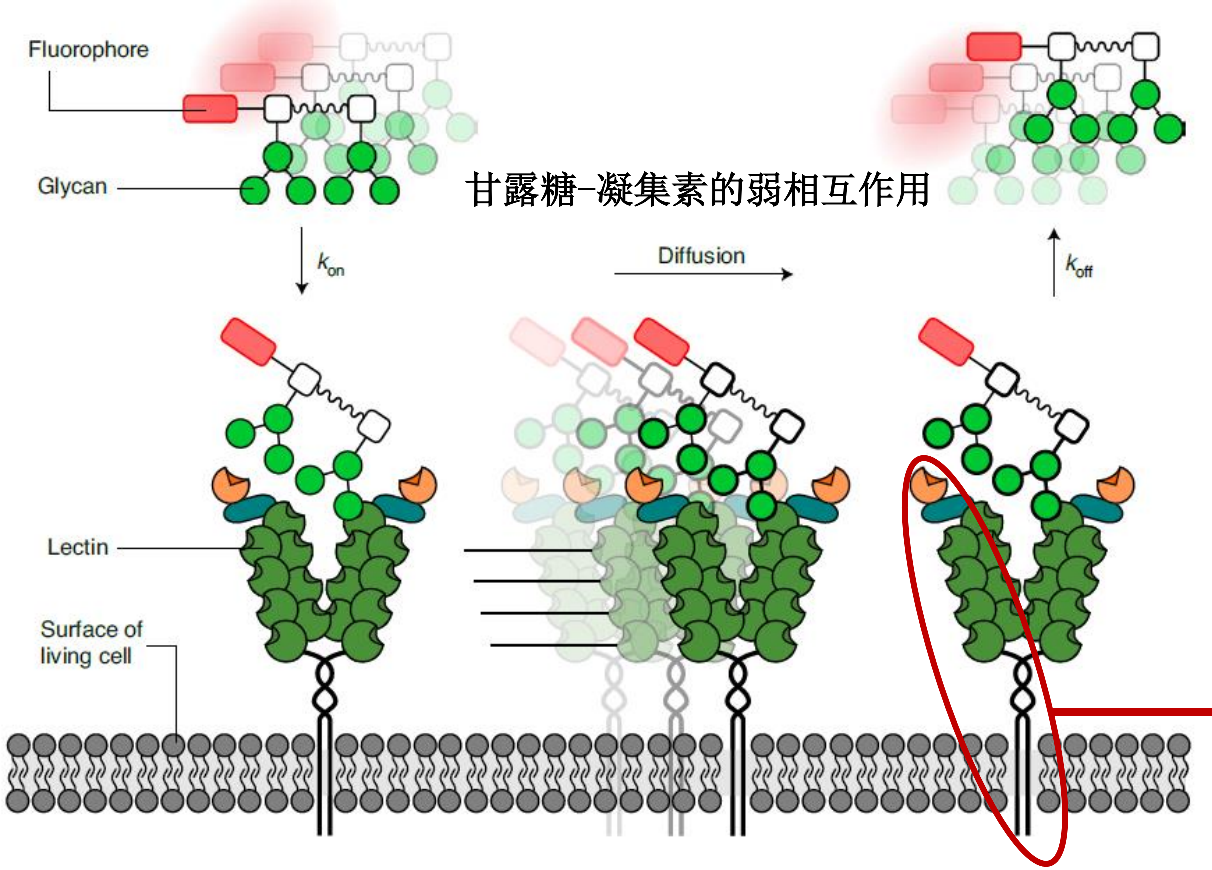


Prism-Based (Trans)



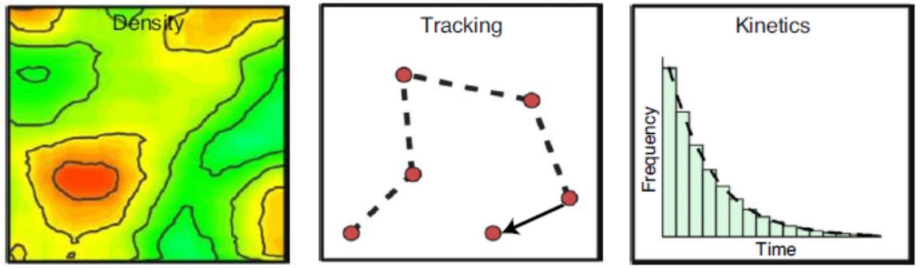
Objective-Based (Cis)

Glyco-PAINT



甘露糖-凝集素的弱相互作用

单分子结合动力学



甘露糖受体（**Mannose Receptor, MR**）

一种凝集素，参与免疫反应

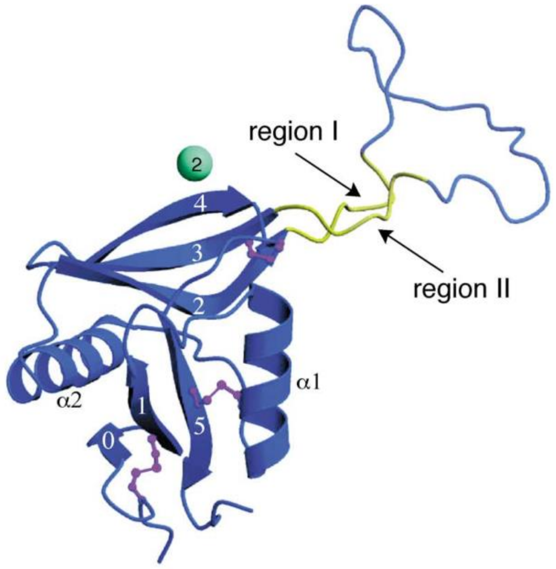
富含半胱氨酸的结构域 **(CRD)**
结合硫酸化碳水化合物

FNII结构域
结合胶原蛋白

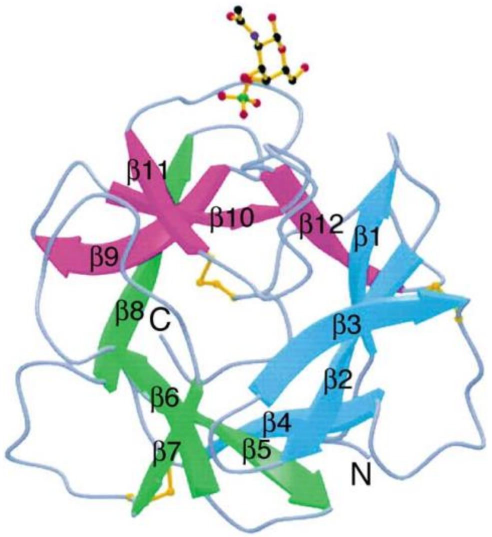
8个**C**型凝集素结构域 **(CTLD)**
结合甘露糖、海藻糖、**GlcNAc ……**

Journal of Leukocyte Biology, 2012, 1177

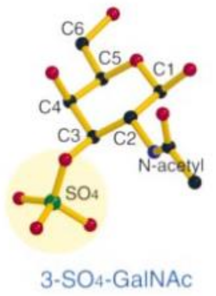
Glycan–Lectin Interactions

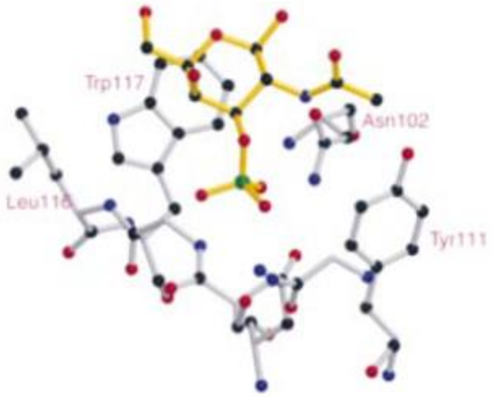


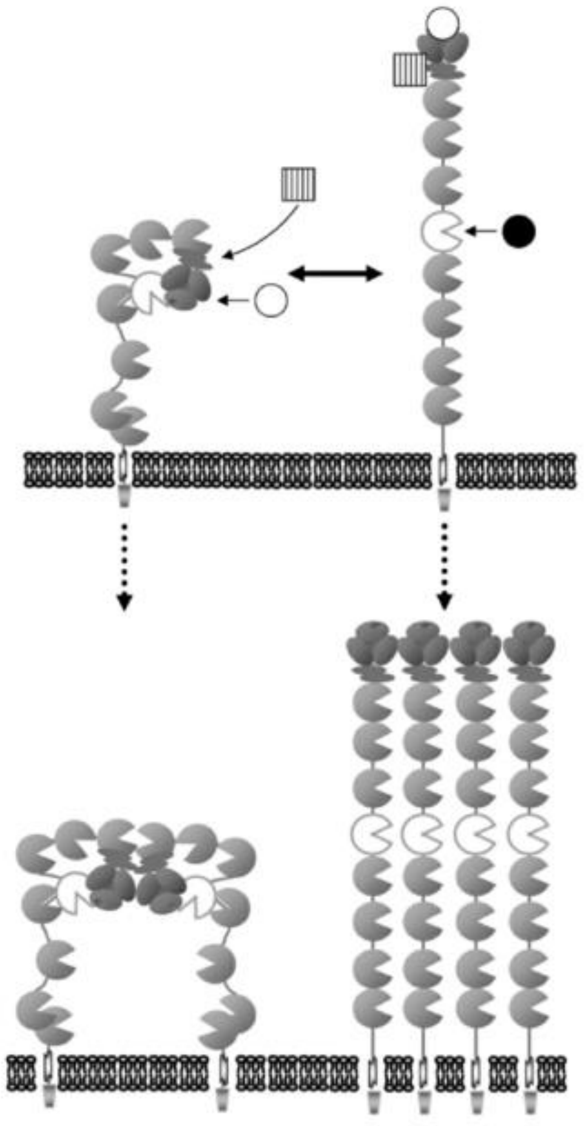
CTLD 4



CRD







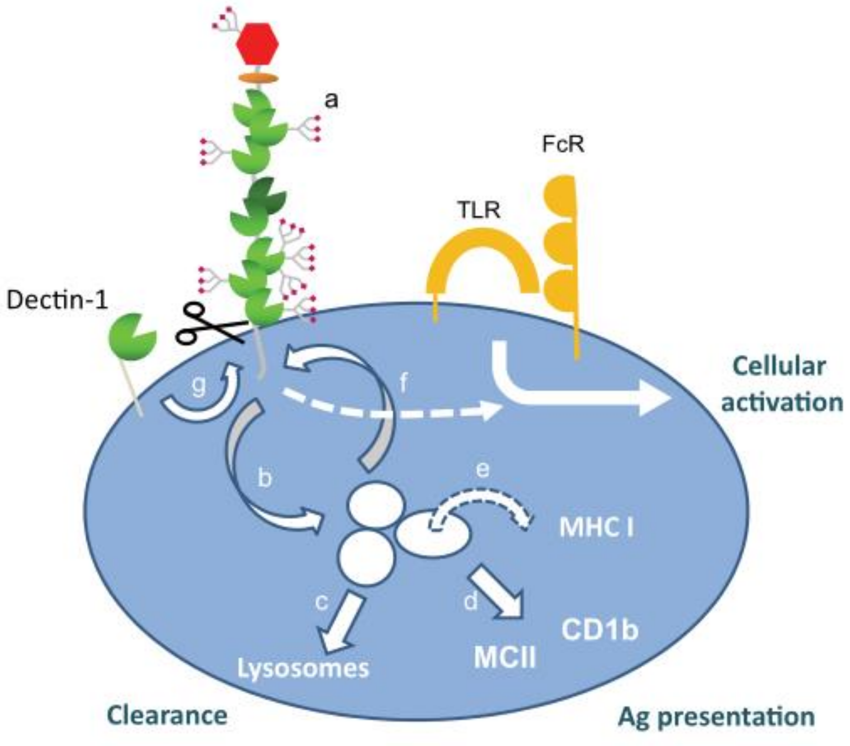
Biochimica et Biophysica Acta, 2002, 364
The Journal of Experimental Medicine, 2000, 1105

Cell. Mol. Life Sci., 2008, 1302

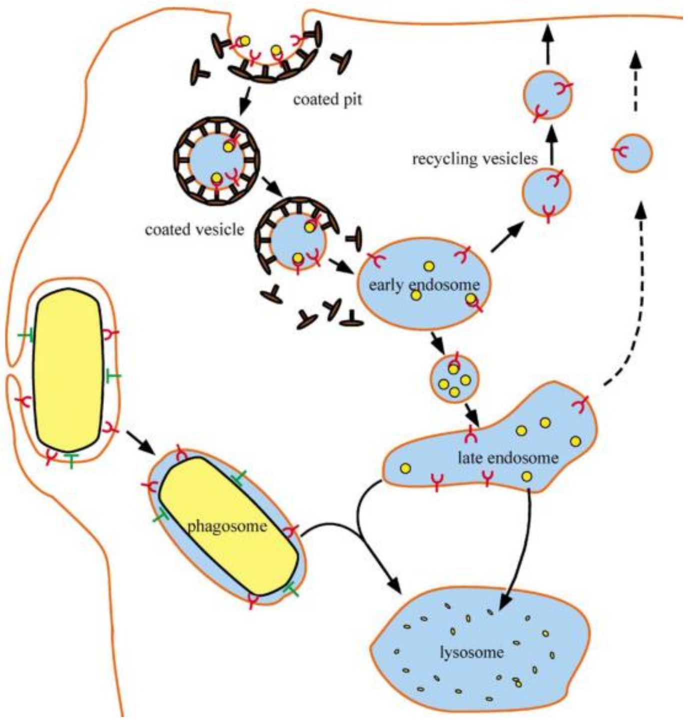
Glycan–Lectin Interactions

MR 改善抗原（交叉）呈递
- 识别病原体，诱导免疫细胞成熟
- MR介导的内吞作用导致病原体向早期内含体的运输

利用活细胞表面聚糖-MR动力学信息、MR内化率研究Glycan–Lectin相互作用

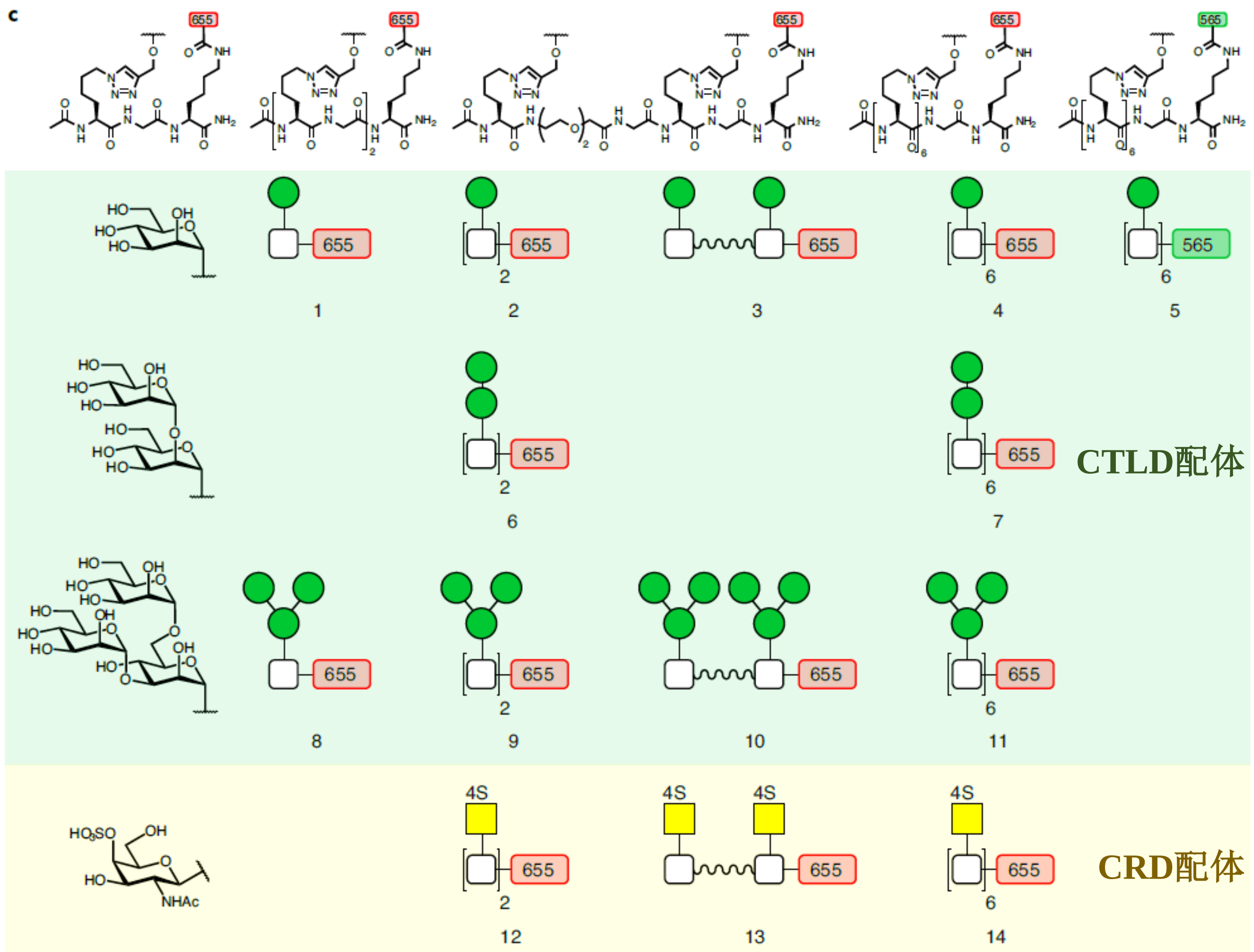


Journal of Leukocyte Biology, 2012, 1177



Biochimica et Biophysica Acta, 2002, 364

Glycan Probes



探针配体设计

- 构型：甘露糖苷单体，α1,2-二甘露糖苷，α1,3-α1,6-三甘露糖苷
- 价态：不同的化学计量数（一个、两个或六个聚糖）
- 聚糖之间间距不同（一个或四个氨基酸）

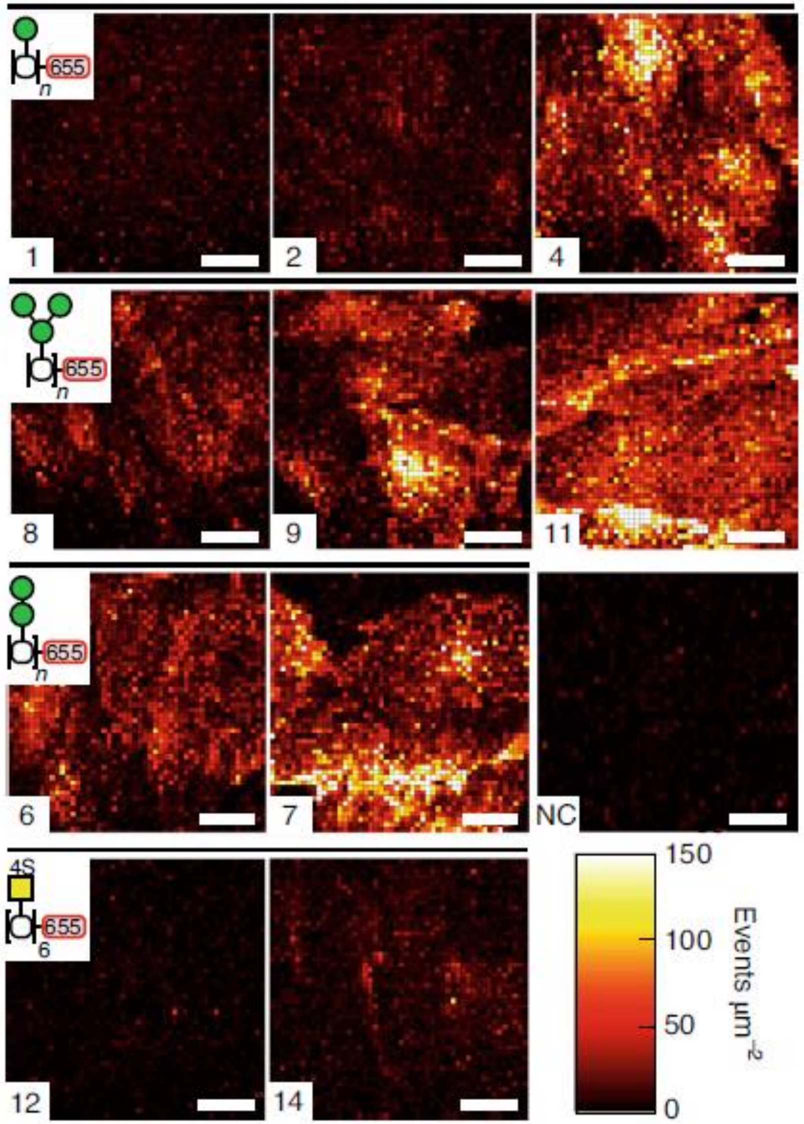
单分子水平的三个定量数据

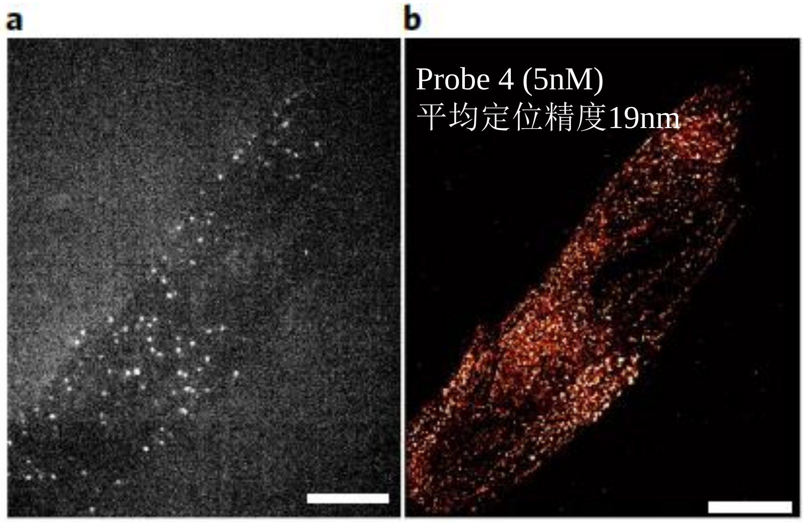
- 探针-MR结合的密度和空间分布
- 探针-MR复合物的扩散
- 探针在 MR 上的停留时间

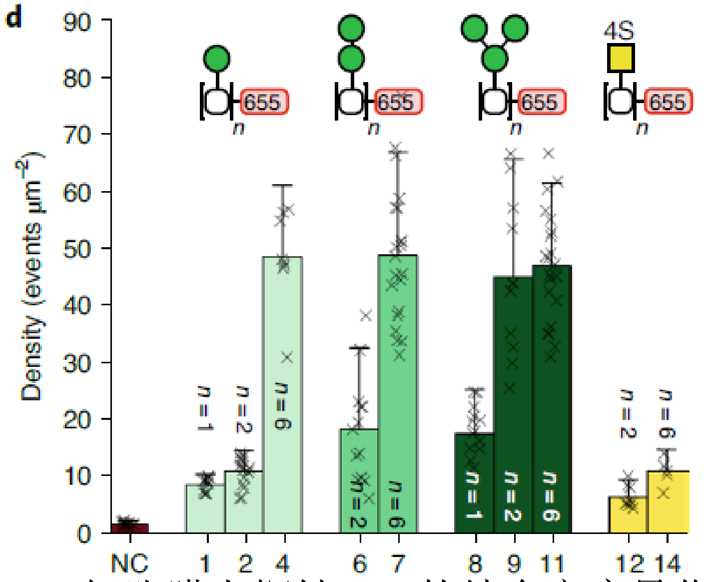
确定相对结合动力学常数（k_{on} 和 k_{off}）

探索多价作用对Glycan–Lectin结合动力学的影响

Live-cell sugar–lectin binding rate measurements.







细胞膜上探针-MR的结合密度量化

重构密度图–结合动力学

$$r = k_{\mathrm{on}} * [R] * [L]$$

r: 结合反应二级速率

k_{on}: 结合速率常数

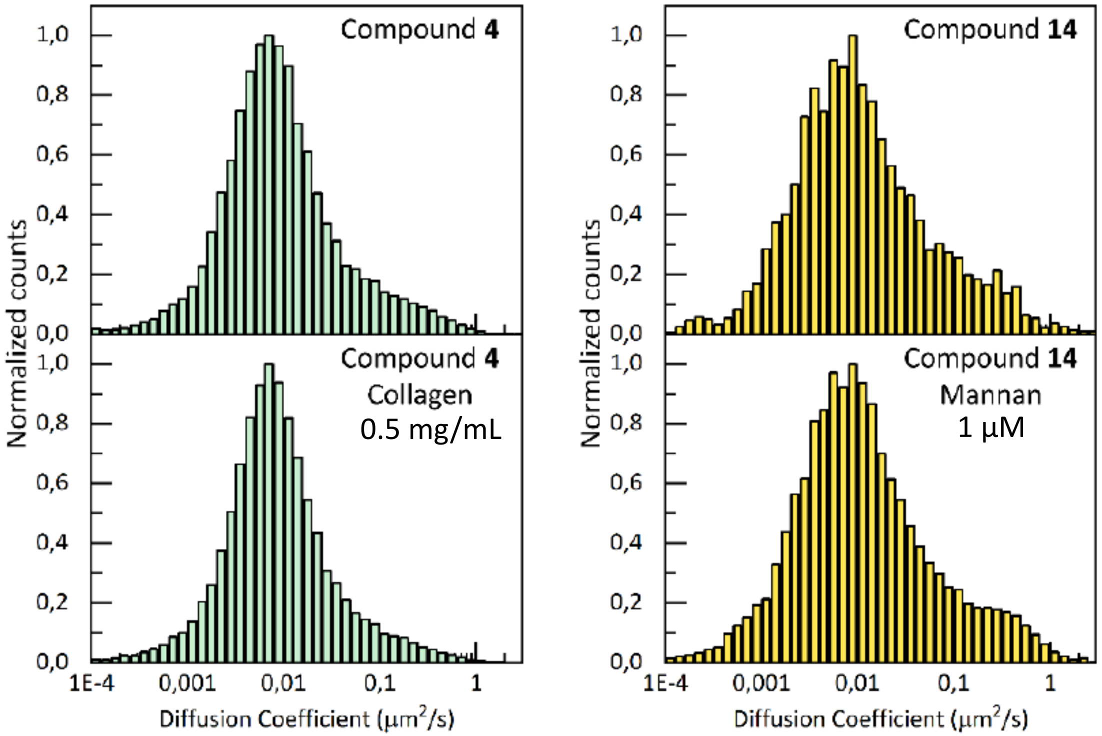
$[R]$: 细胞受体密度

$[L]$: 探针的浓度

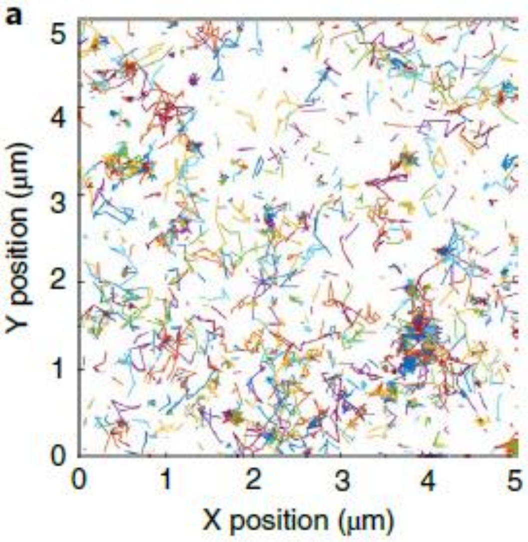
结合密度- k_{on}相对值

- 多价结合
- 甘露糖构型
- MR受体结构域

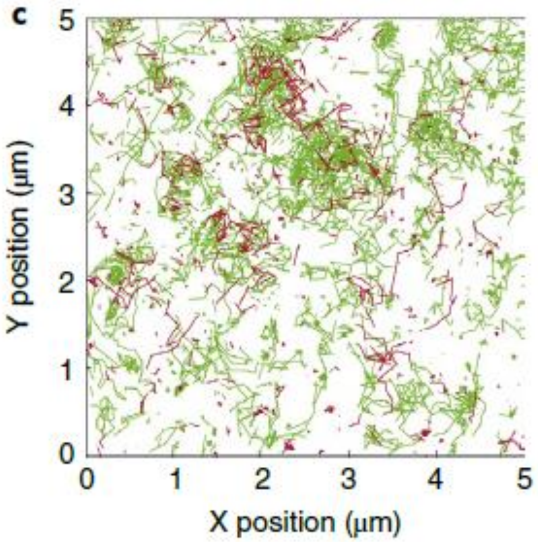
Lateral diffusion of the MR on the cell membrane



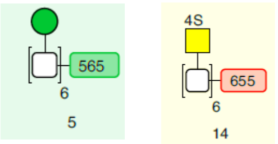
Multivalent mannoside probes do not promote MR clustering.

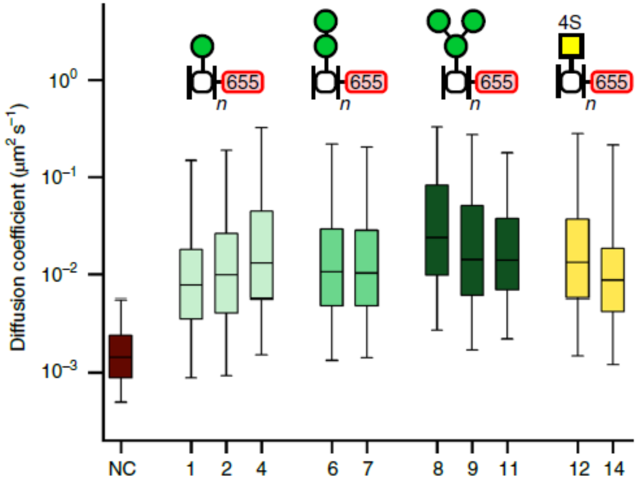


探针4的运动轨迹



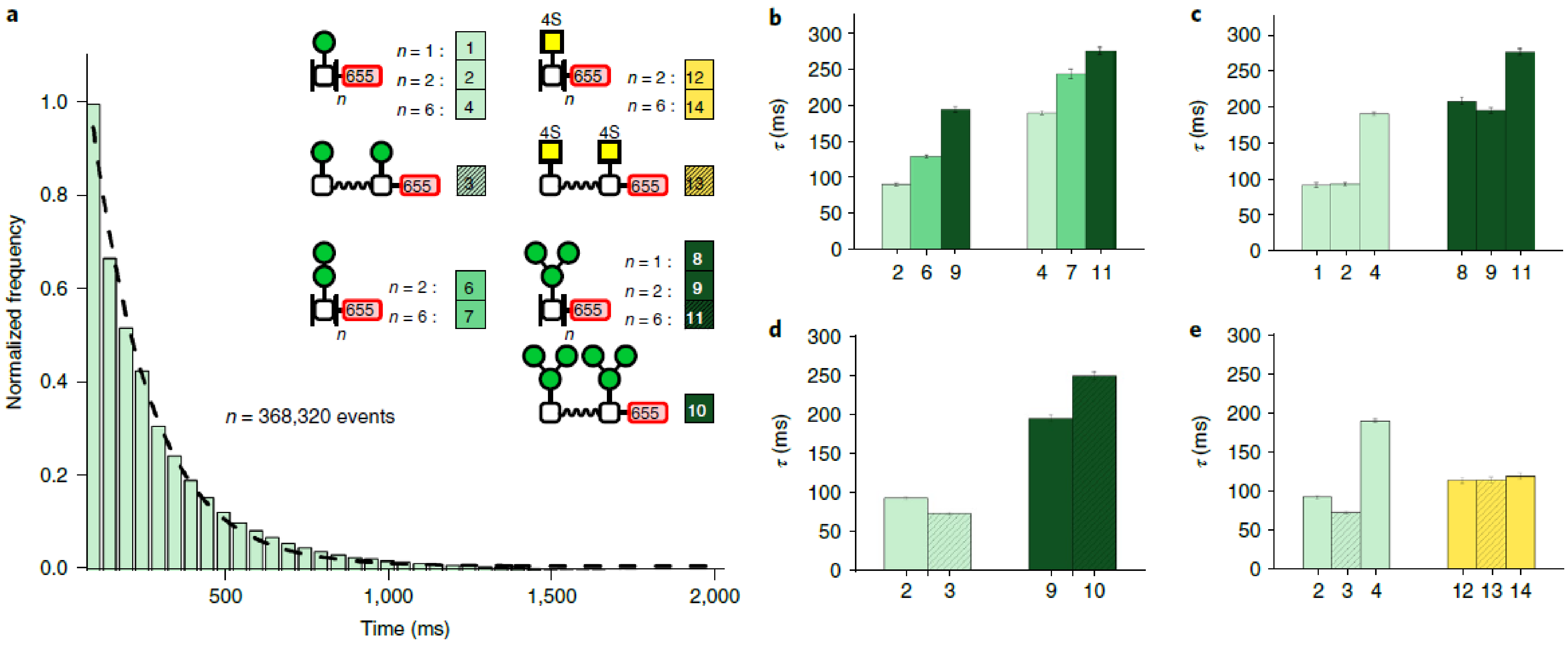
探针5、探针14的运动轨迹





Diffusion coefficient
~ 0.01 µm²/s

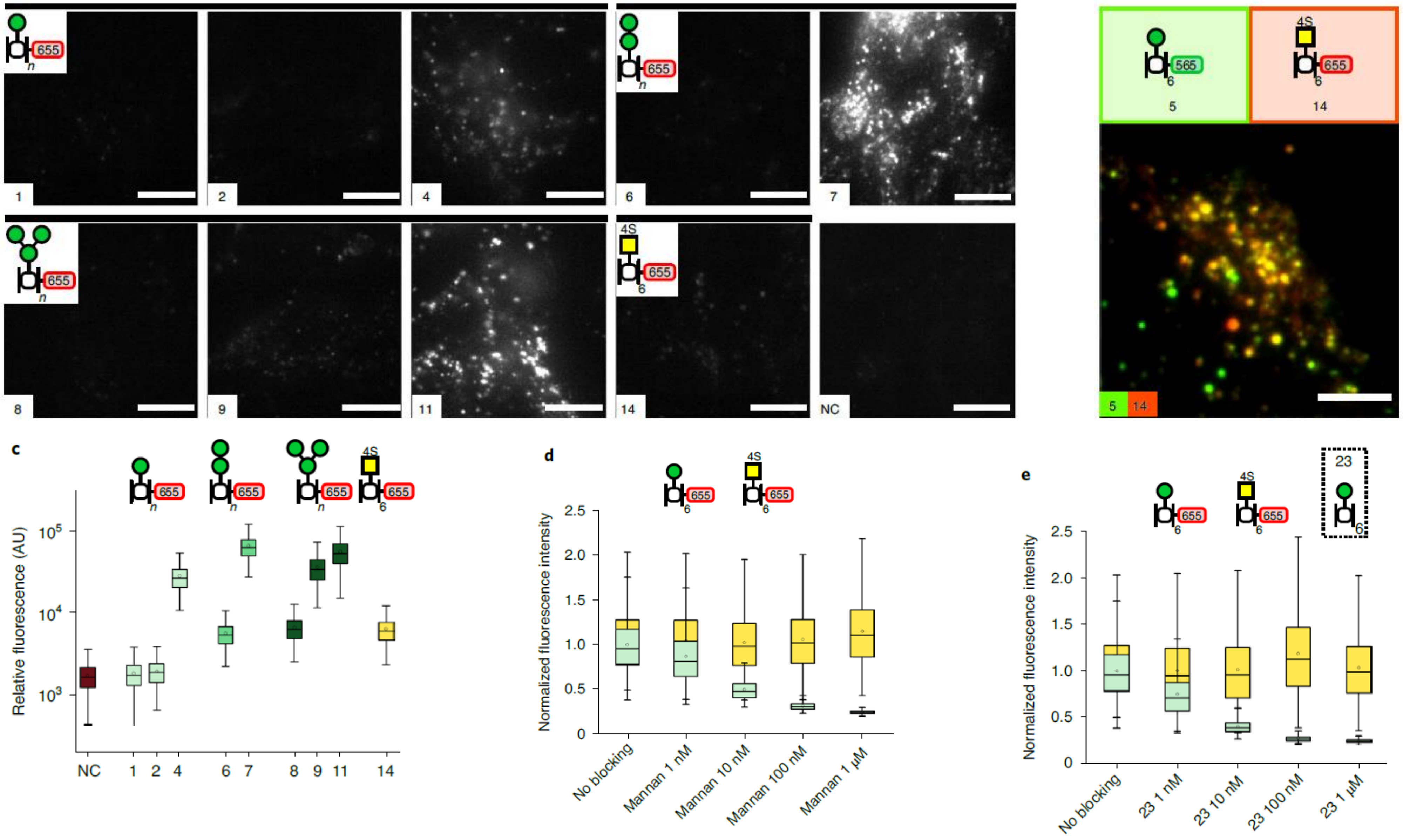
Effect of multivalency on binding time and k_{off}



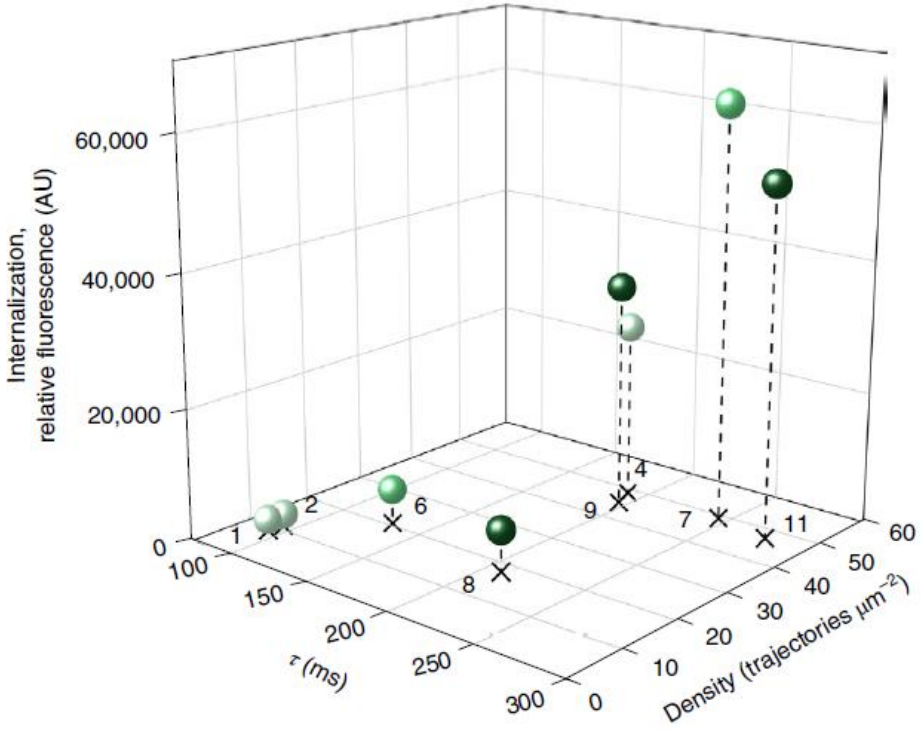
探针驻留时间 τ $y = y_0 + A_1 e^{-x/\tau}$

探针解离速率常数 k_{off} $\tau = 1/k_{off}$

Affinity-dependent cellular uptake



Summary



Glycan–Lectin结合动力学参数与细胞摄取的相关性

- 细胞表面结合密度（相对k_{on}）
- 探针驻留时间τ（τ=1/ k_{off}）
- 探针内化水平

- 利用Glycan–Lectin的弱相互作用开发了Glyco-PAINT。
- 定量研究聚糖价态、构型、间距对活细胞中甘露糖-MR在细胞膜上的动力学结合常数k_{on}（相对值）、解离常数k_{off}和细胞摄取。
- k_{off}对聚糖化学计量数和几何结构的微小变化更敏感，是调节Glycan–Lectin相互作用的最合适参数。
- Glyco-PAINT 够将聚糖的细胞摄取与活细胞中的不同结合参数相关联，多价聚糖有较长的停留时间和较高的摄取率，MR的CTLD结构域的结合明显强于CRD域。
- 通过Glyco-PAINT协助设计和验证 MR 的新治疗靶点，并扩展到活细胞上其他相关凝集素 - 碳水化合物相互作用的研究，阐明凝集素 - 碳水化合物相互作用在生物学功能中的确切作用，并用于相关药物靶向研究。