

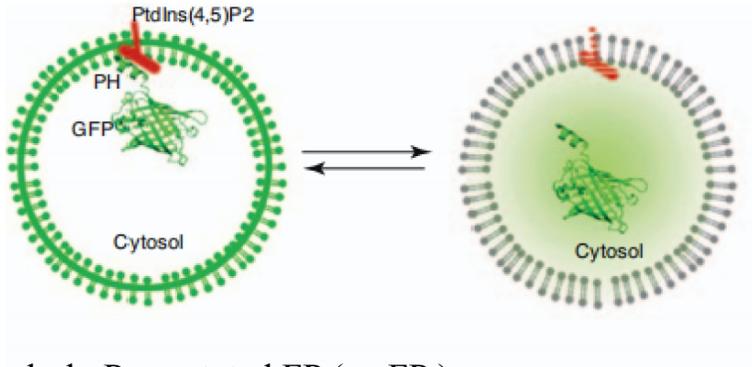
Fluorescent protein-Based Hybrid Biosensors

Reporter: Zhou Xuelian

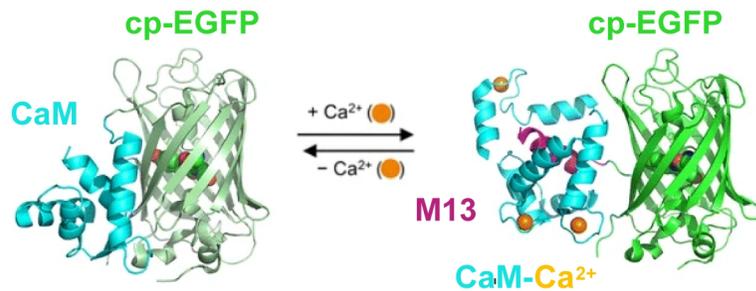
Date: 2021-4-15

Fluorescent protein-based biosensors

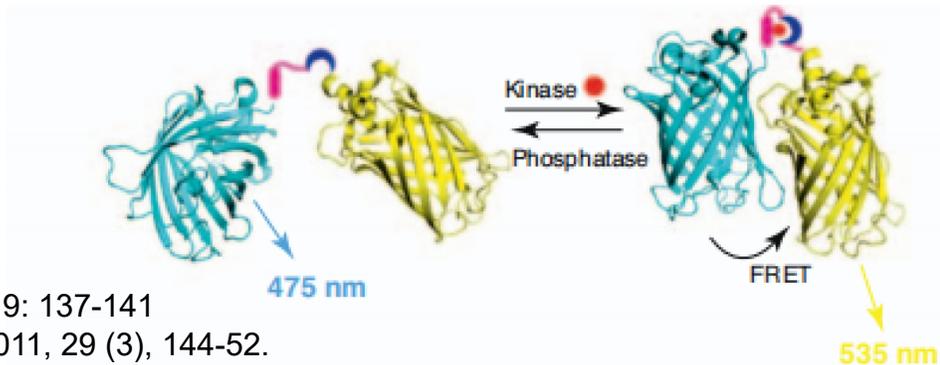
a) Single FP



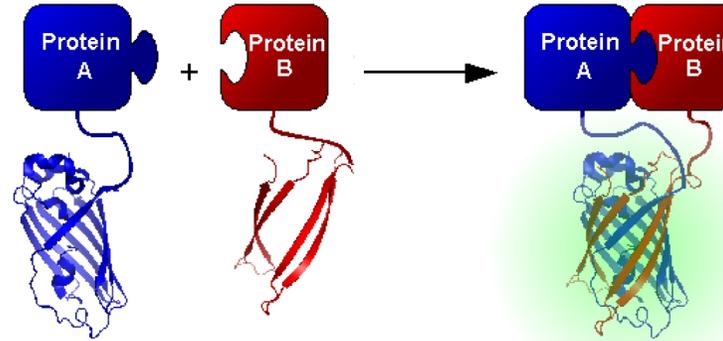
b) Circularly Permutated FP (cpFP)



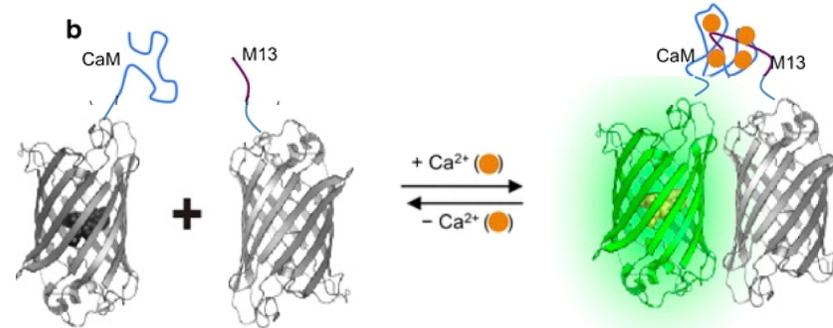
c) Fluorescent Resonance Energy Transfer (FRET)



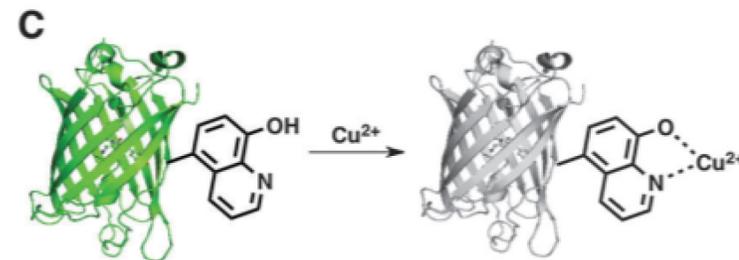
d) Bimolecular Fluorescence Complementation (BiFC)



e) Dimerization-Dependent FP (ddFP)

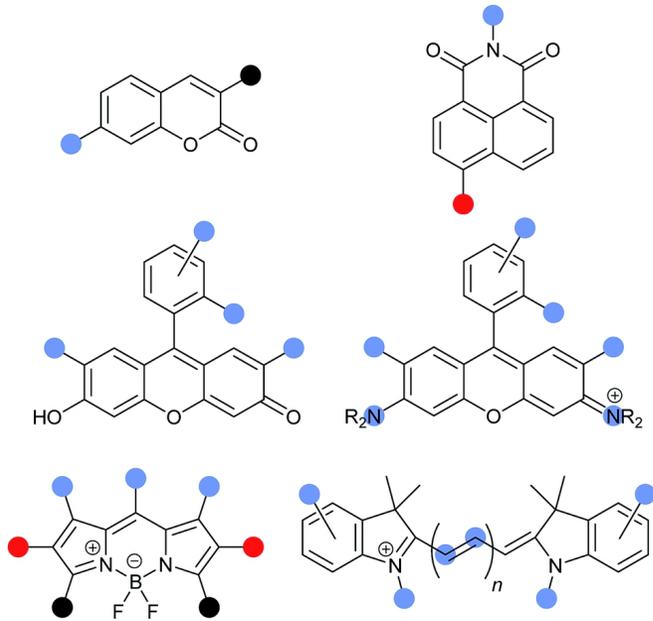


f) Genetic Incorporation of Unnatural Amino Acids (UAAs)



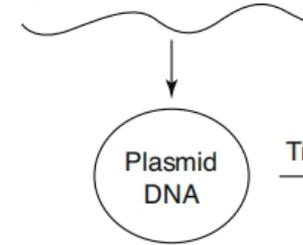
Reasons for choosing hybrid biosensors

Synthetic fluorophore



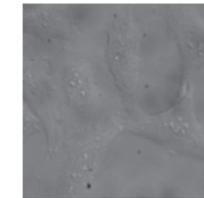
VS

Gene encoding sensor

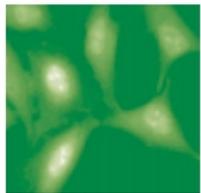


Transfection

genetic targetability



Transcription
Translation



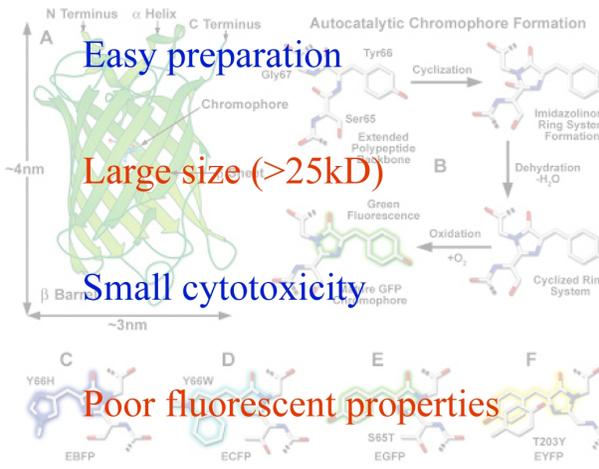
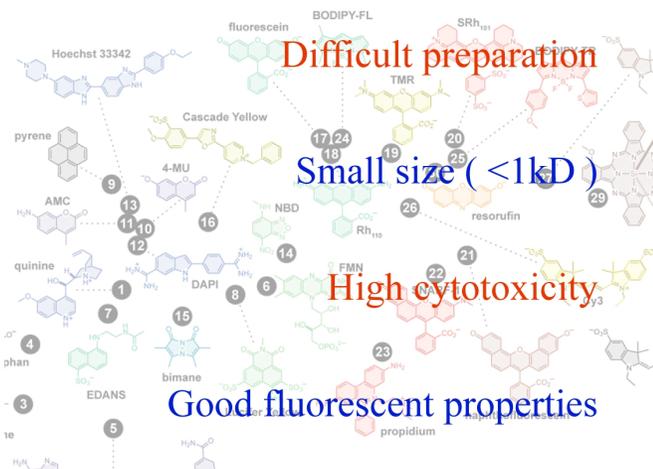
TRENDS in Biotechnology

RSC Adv., 2018, 8, 29051-29061

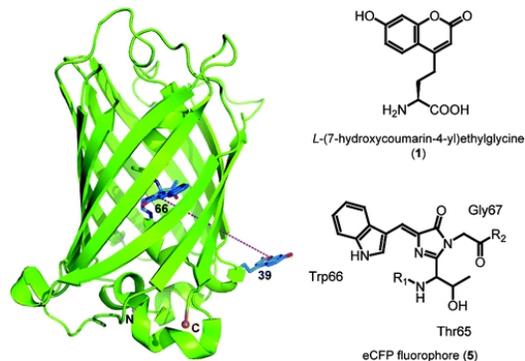
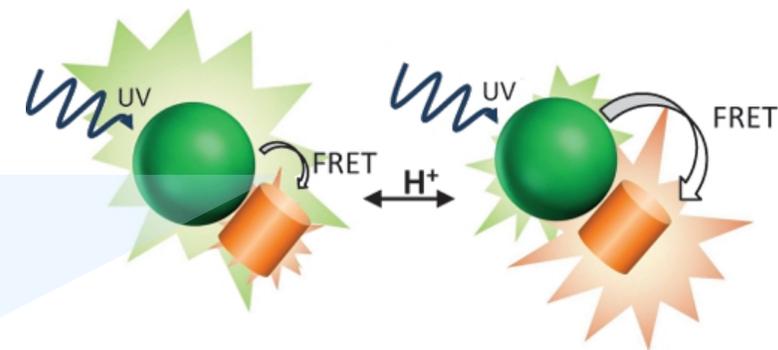
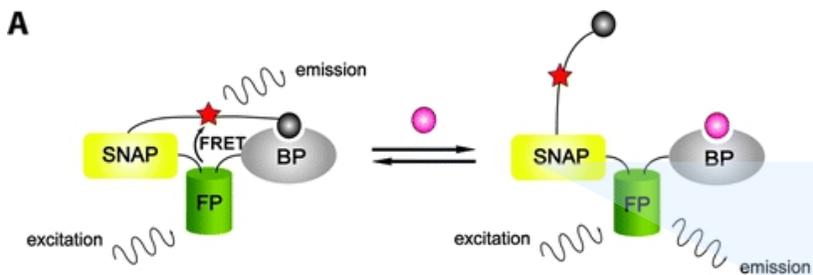
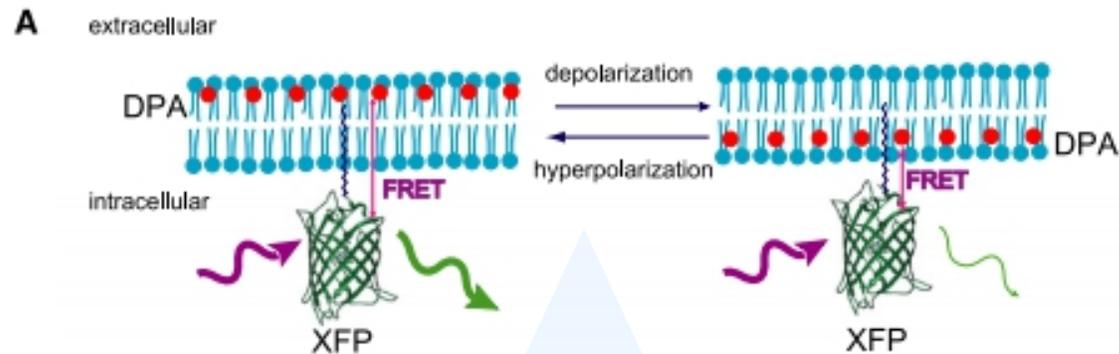
Trends Biotechnol 2011, 29 (3), 144-52

Synthetic fluorophore

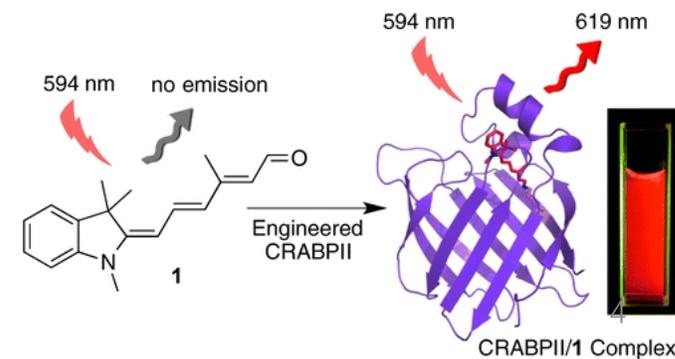
Fluorescent protein



Fluorescent protein-based hybrid biosensors

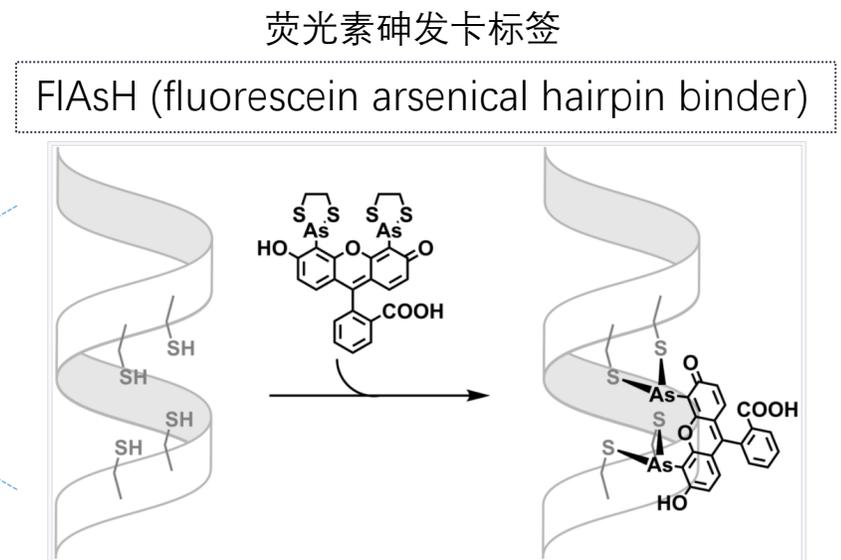
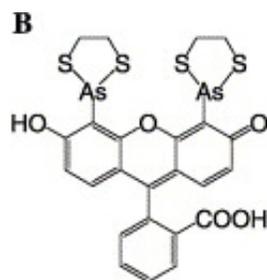
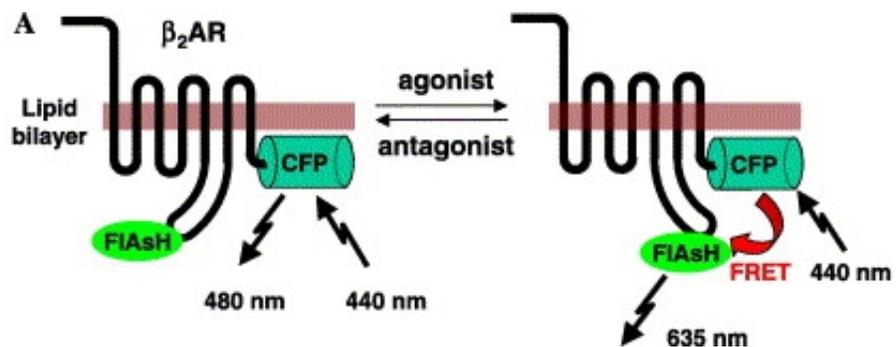


1. 遗传靶向性
2. 双荧光团系统
3. 比例定量分析
4. 传感器动态范围、灵敏度



Fluorescent protein-based hybrid biosensors

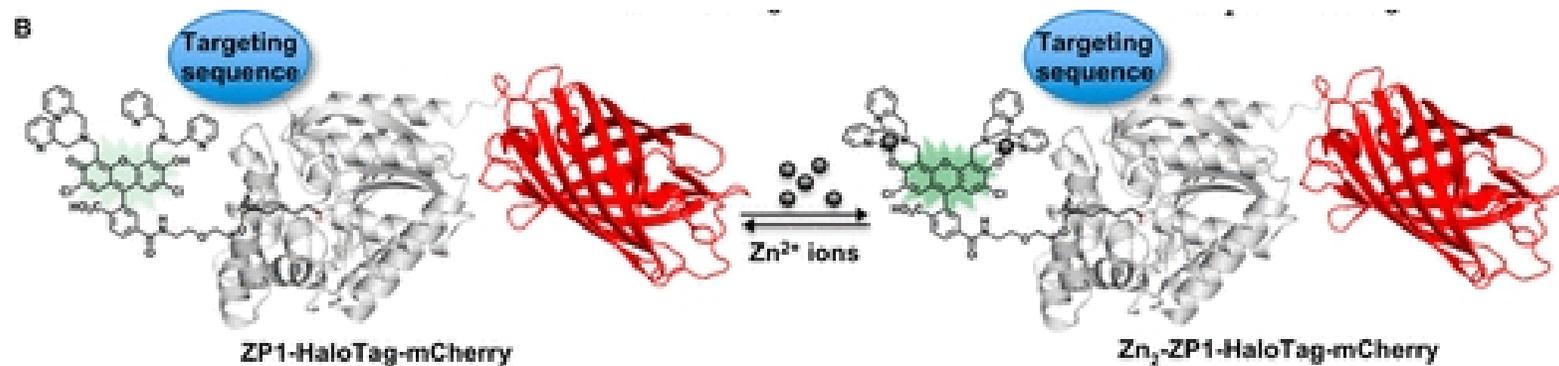
遗传编码支架蛋白——tag protein



Biochem Biophys Res Commun 2006, 343 (4), 1191-6.

尺寸小，但具有细胞毒性

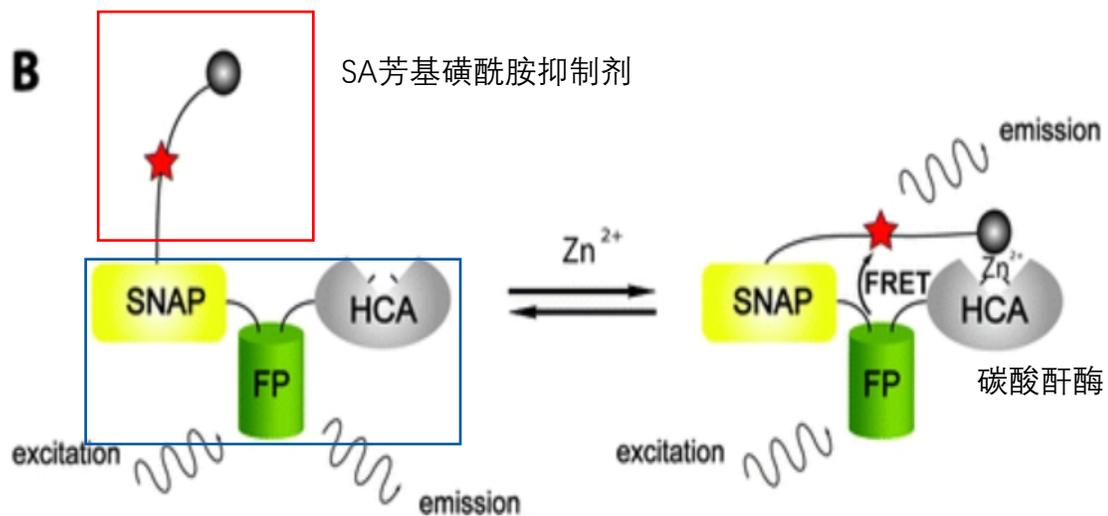
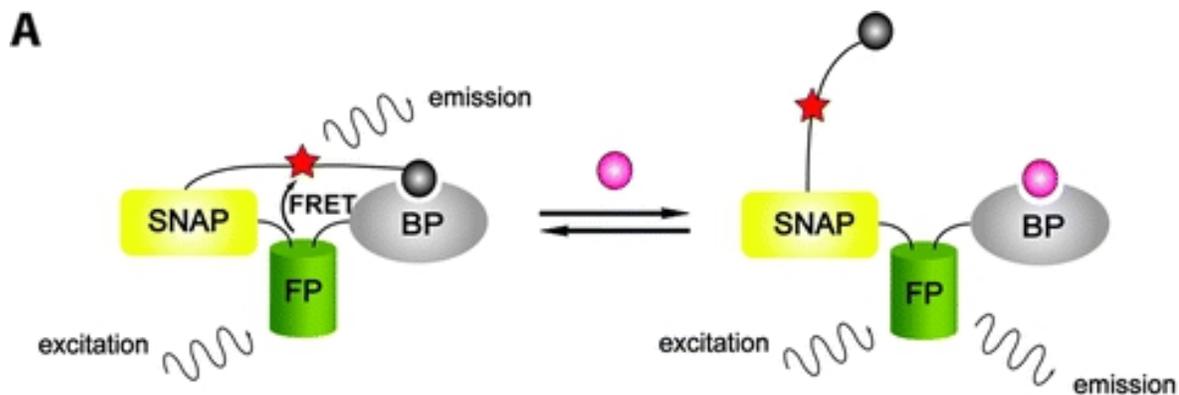
四半胱氨酸基序 (CCXXCC)



ACS Chem. Biol. 2020, 15, 2, 396–406

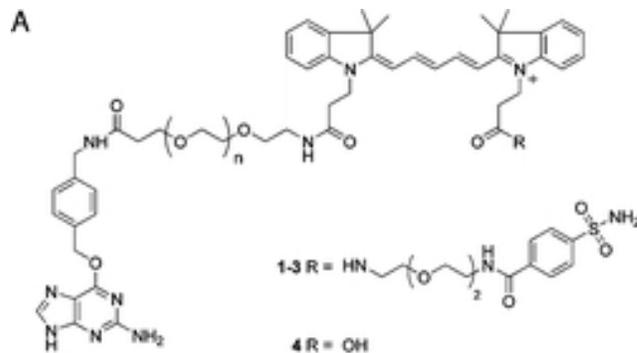
Fluorescent protein-based hybrid biosensors

遗传编码支架蛋白——tag protein



Snifits

合成部分: BG-PEG_n-Cy5-SA

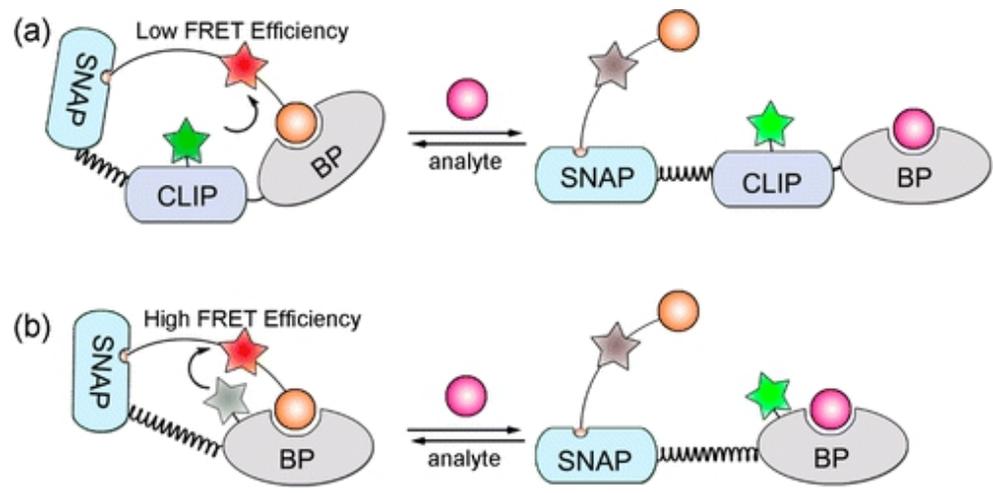


融合蛋白: SNAP_mCherry_HCA

不需要强制改变结合蛋白质的构象变化,即可改变两个荧光团之间的FRET效率;灵活的选择和调整不同特性的荧光团;动态范围受限

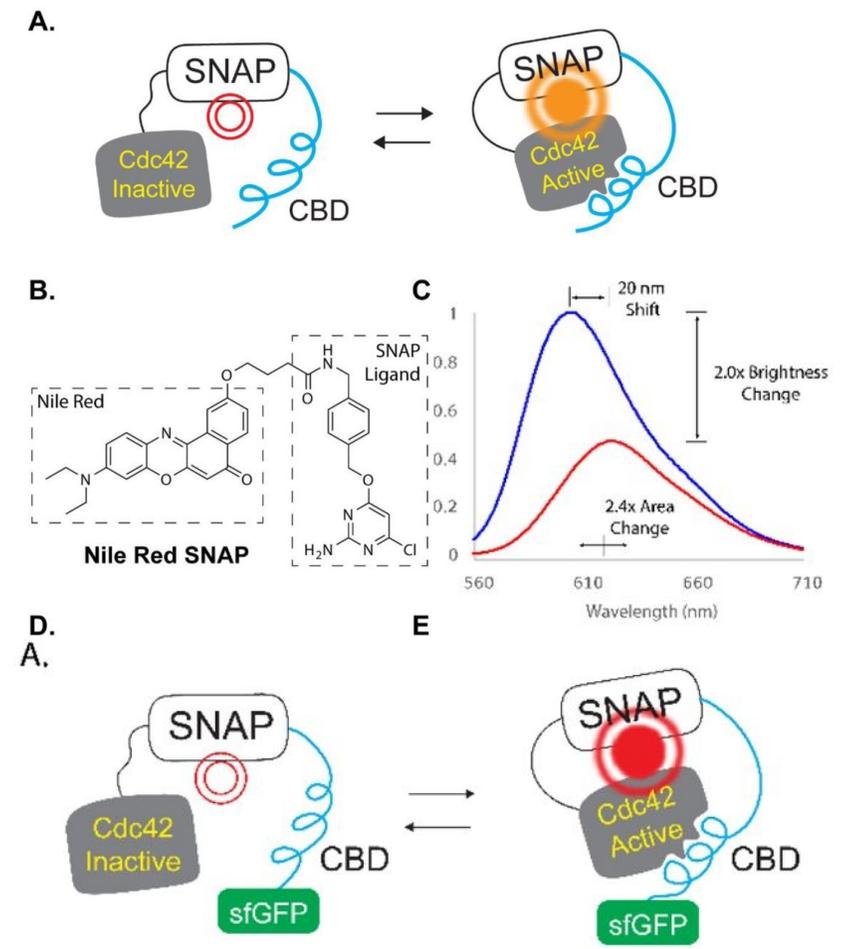
Fluorescent protein-based hybrid biosensors

遗传编码支架蛋白——tag protein



uSnifit

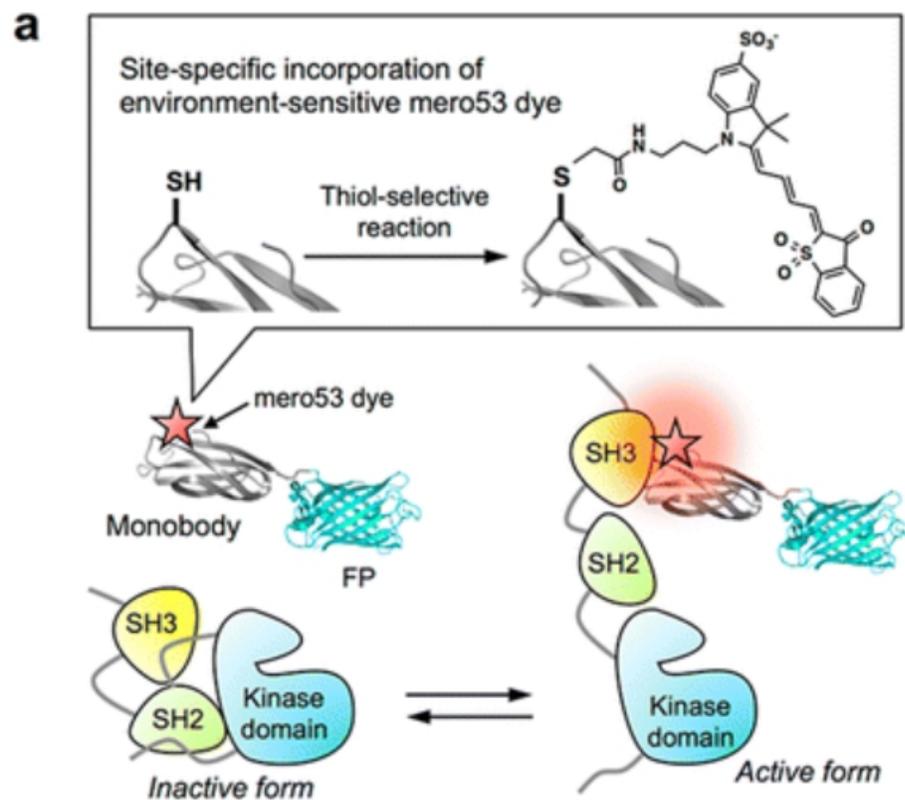
J. Am. Chem. Soc. 2016, 138, 16, 5258–5261



BioRxiv, 2020, 206748

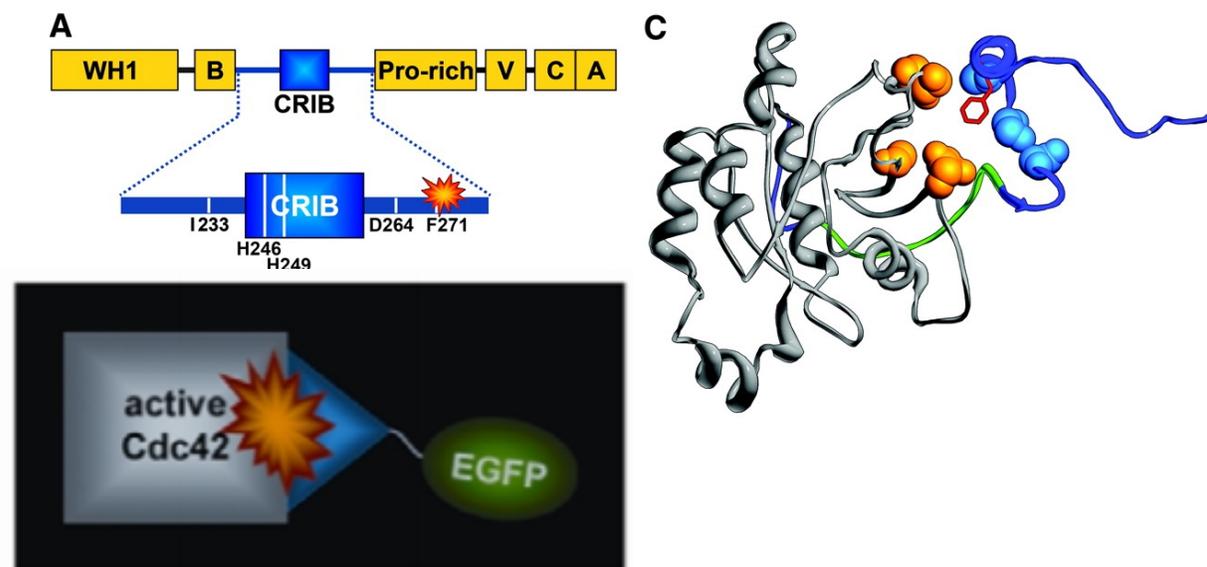
Fluorescent protein-based hybrid biosensors

遗传编码支架蛋白——Biological protein



Src家族激酶 (SFK)

ACS Chem. Biol. 2014, 9, 12, 2708–2717

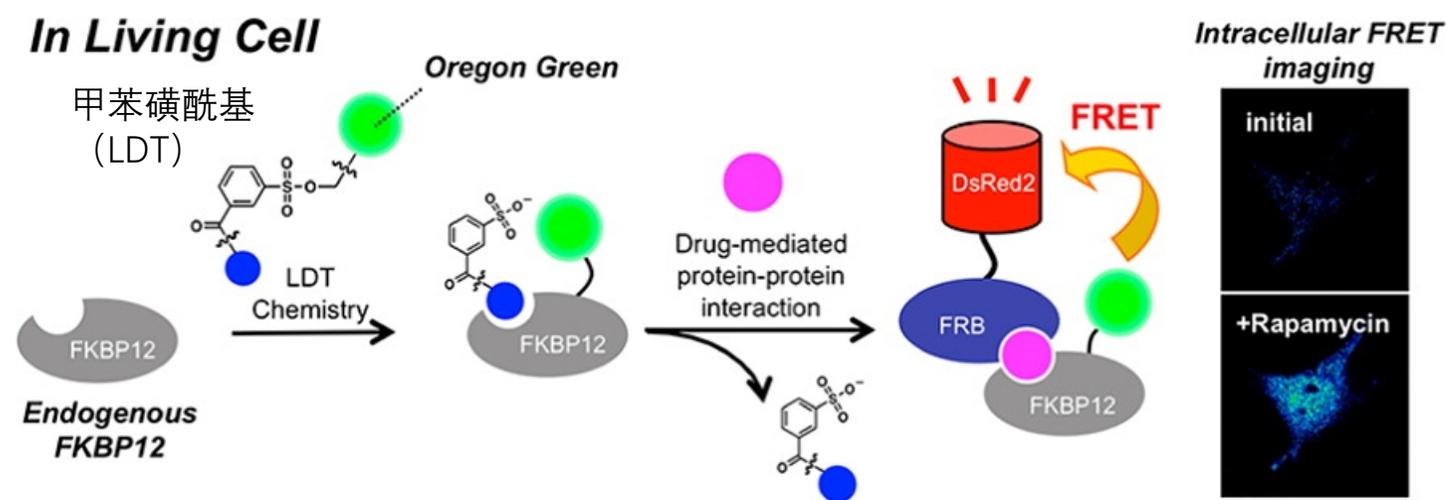


体积小，并且可以定制以通过高通量筛选来结合不同的靶标。

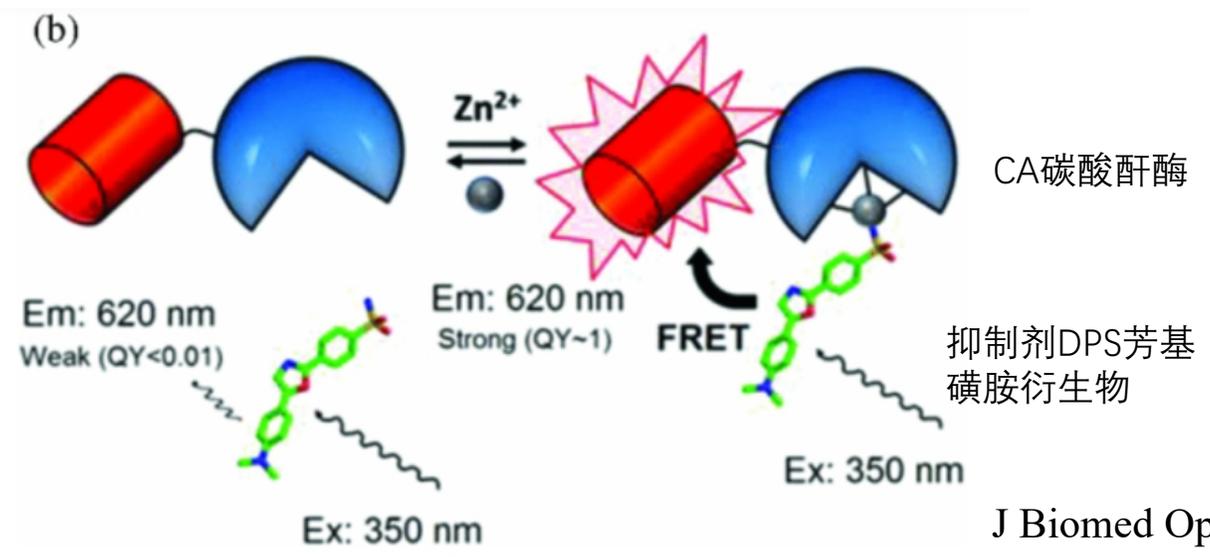
Science. 2004, 305, 5690, 1615-1619

Fluorescent protein-based hybrid biosensors

遗传编码支架蛋白——Biological protein



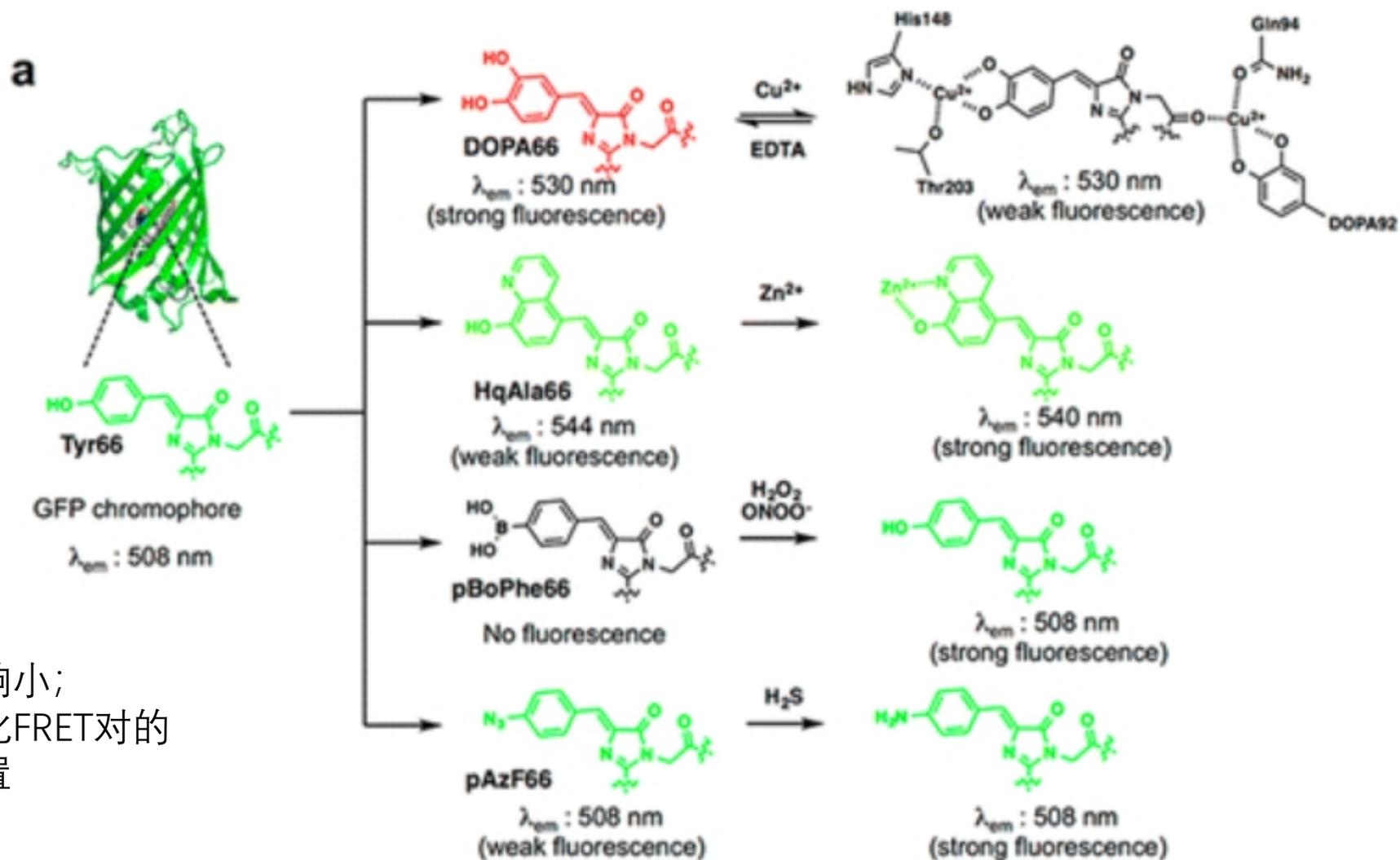
J. Am. Chem. Soc. 2013, 135, 18, 6782–6785



J Biomed Opt. 2011. 16(8): 087014.

Fluorescent protein-based hybrid biosensors

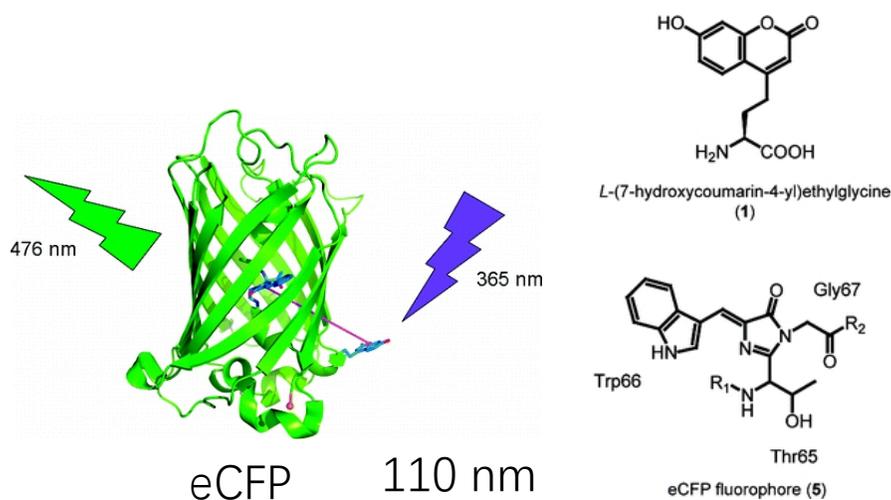
非天然氨基酸



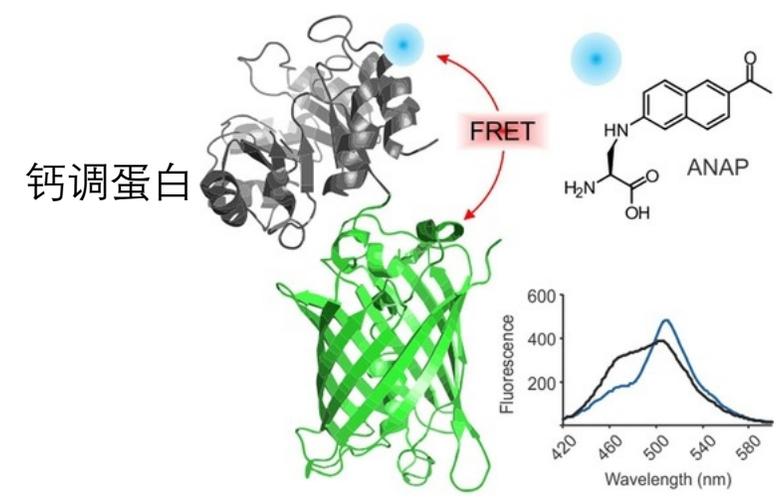
蛋白影响小;
方便优化FRET对的
相对位置

Fluorescent protein-based hybrid biosensors

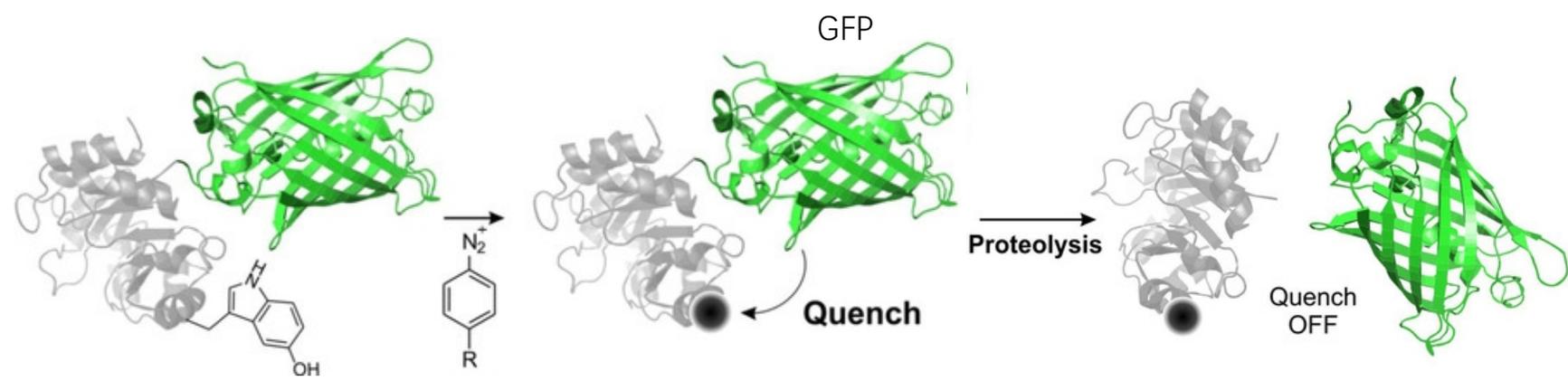
非天然氨基酸荧光团



J. Am. Chem. Soc. 2011, 133, 11, 3708–3711



ChemBioChem 2017, 18, 511.

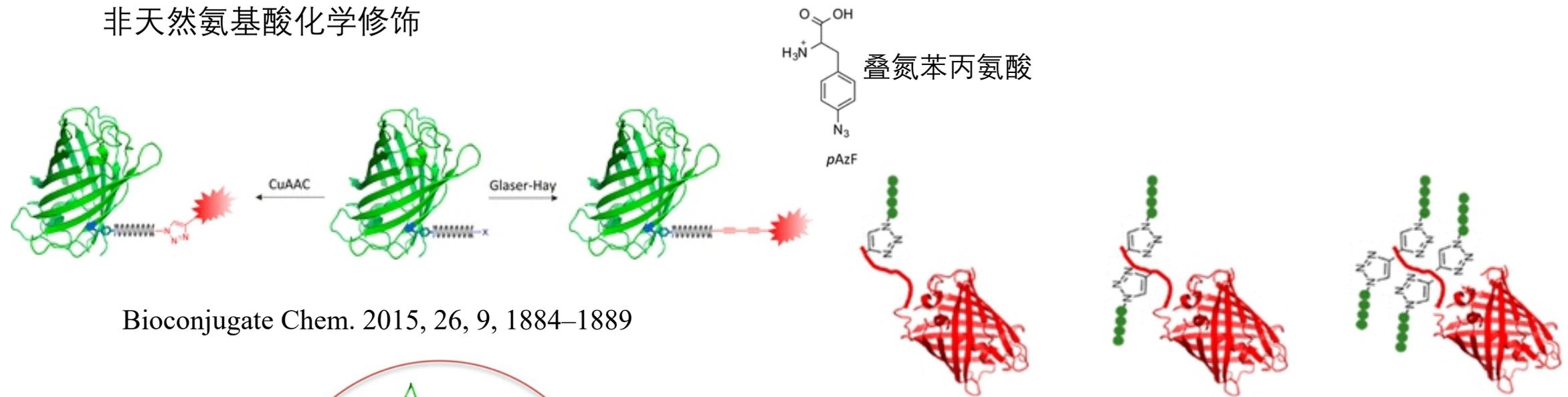


5-羟基色氨酸

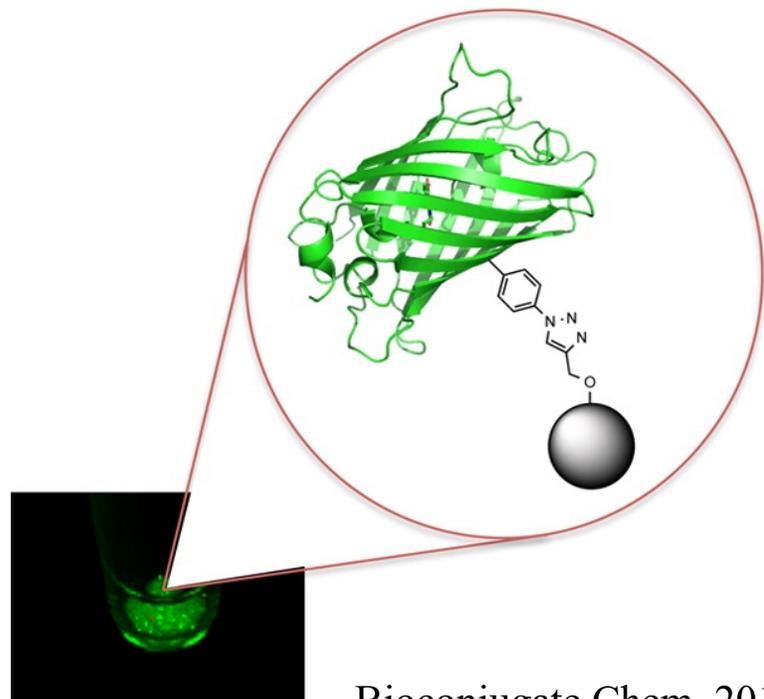
ChemBioChem 2019, 20, 1659.

Fluorescent protein-based hybrid biosensors

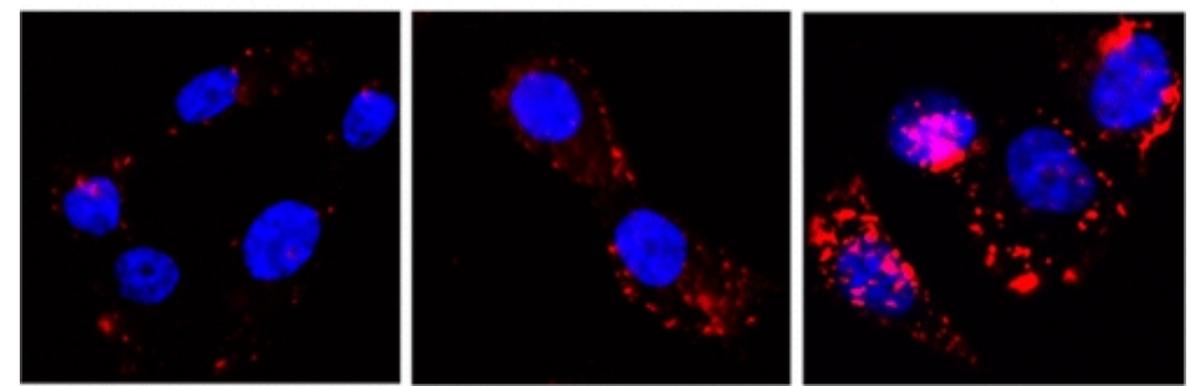
非天然氨基酸化学修饰



Bioconjugate Chem. 2015, 26, 9, 1884–1889



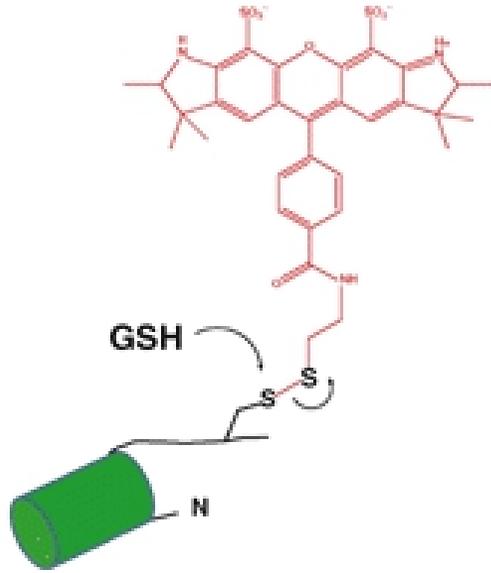
Bioconjugate Chem. 2014, 25, 11, 1916–1920



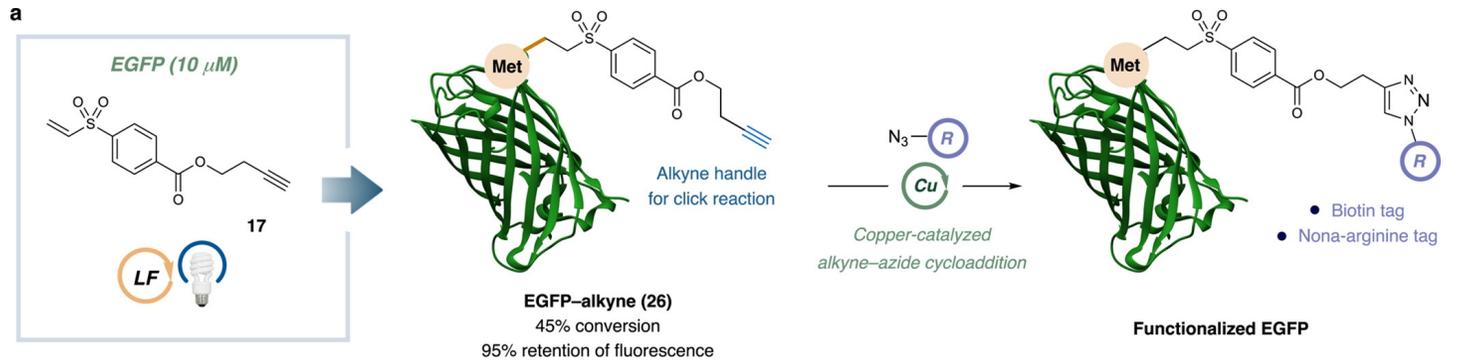
Bioconjugate Chem. 2019, 30, 2, 432–442

Fluorescent protein-based hybrid biosensors

天然氨基酸化学修饰

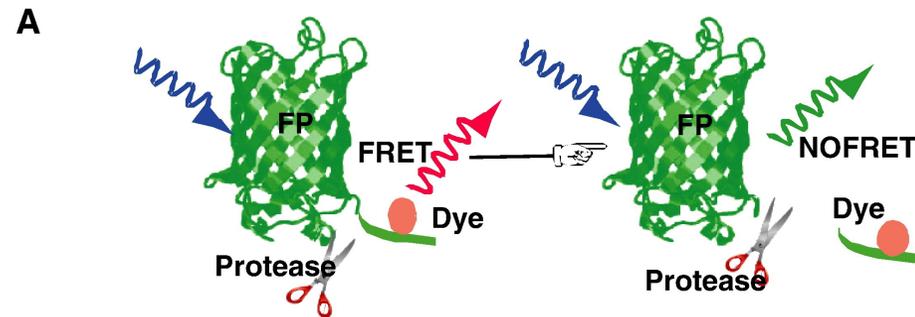


Chemistry Letters 34, 6 (2005) 766-767



J. Am. Chem. Soc. 2020, 142, 51, 21260–21266

半胱氨酸：低丰度、高亲核性硫醇基团，是与亲电试剂选择性反应的经典靶标。定点诱变、单个半胱氨酸突变体



其他低丰度氨基酸：赖氨酸、酪氨酸、色氨酸

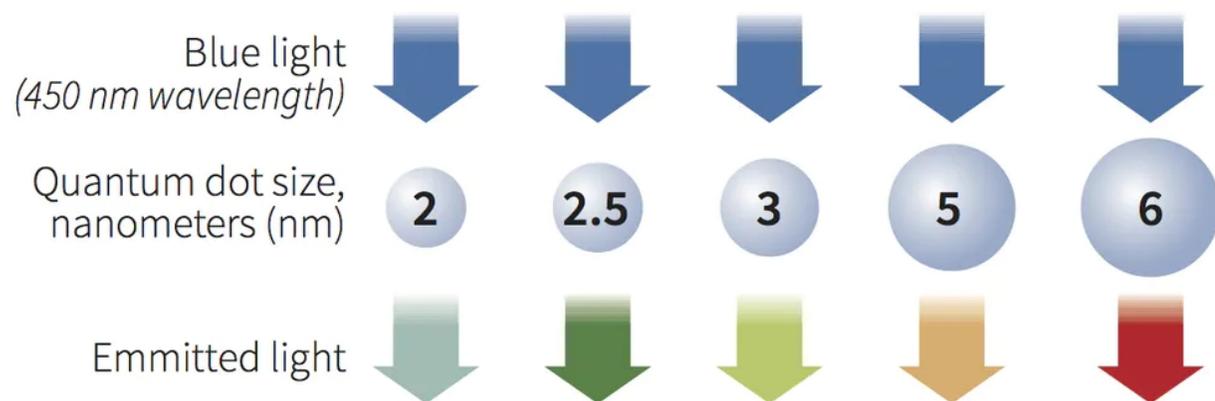
Biochimica et Biophysica Acta 1823 (2012) 215–226

Fluorescent protein-based hybrid biosensors

Quantum dots

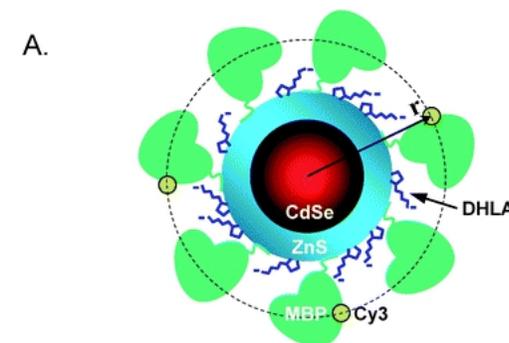
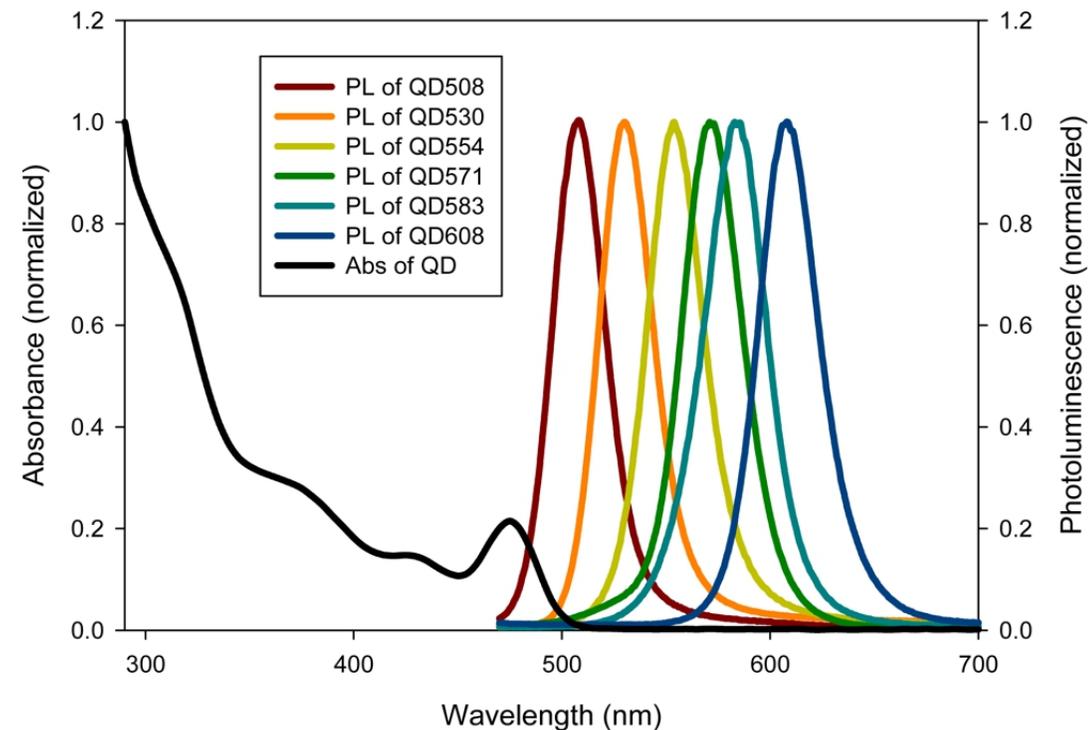
What are quantum dots

Tiny man-made crystals that have the ability to convert a spectrum of light into different colours



高亮度、高QY、耐光漂白、宽激发、窄发射、尺寸可控光谱、结合多受体

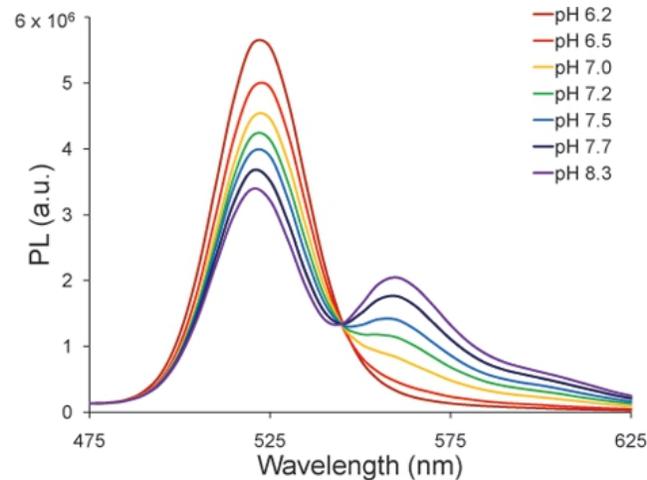
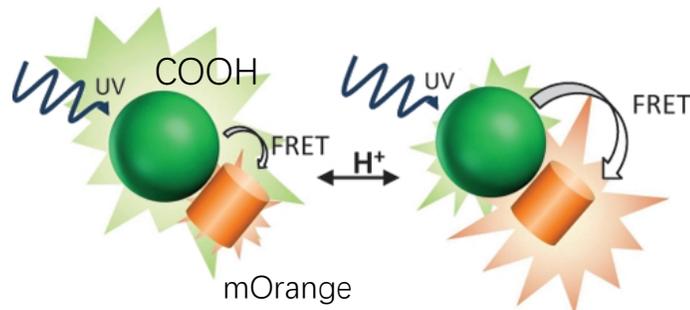
Nanoscale Adv 2019, 1, 3424 - 3442



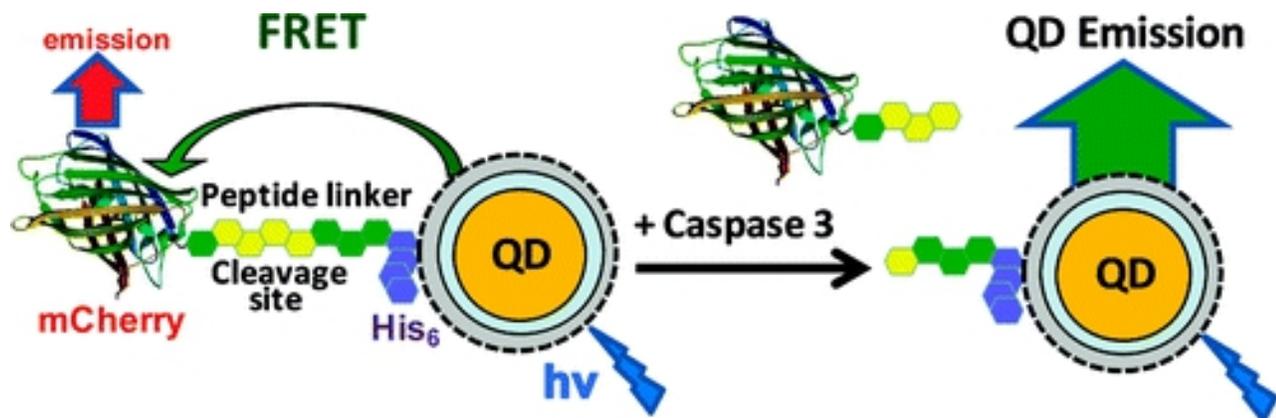
Sensors 2011, 11(12), 11036-11055

Fluorescent protein-based hybrid biosensors

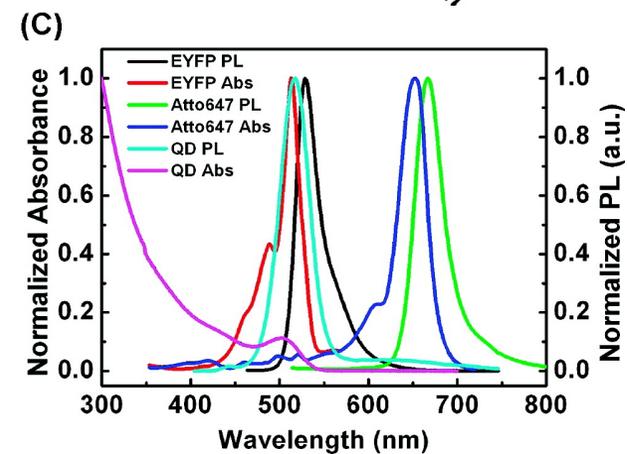
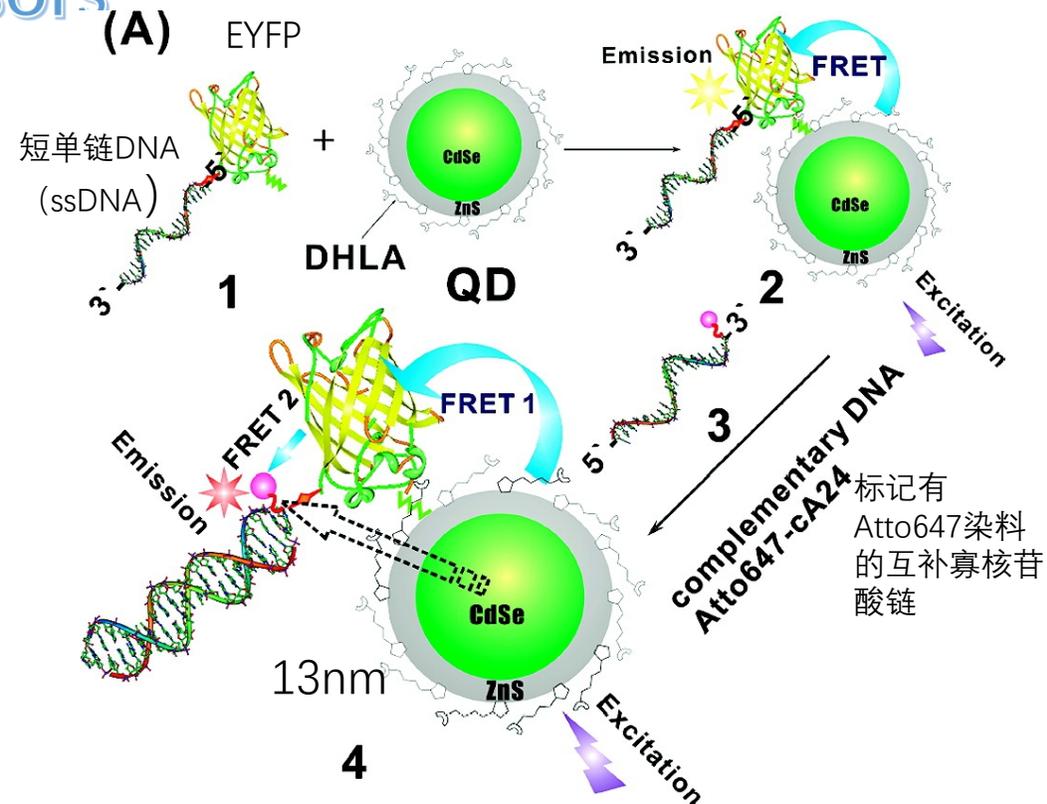
Quantum dots



ACS Nano 2012, 6, 4, 2917–2924



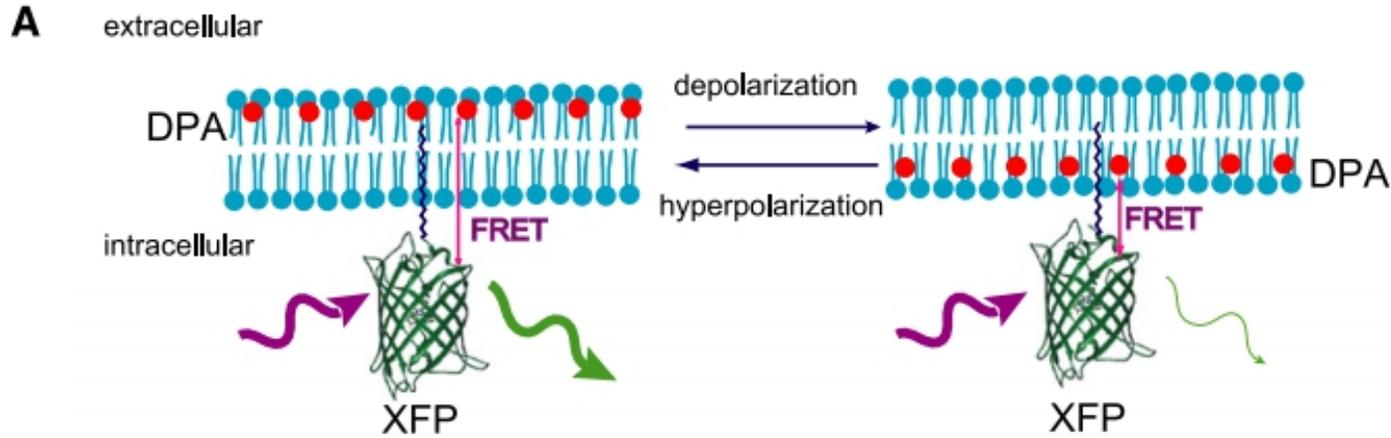
J. Am. Chem. Soc. 2009, 131, 11, 3828–3829



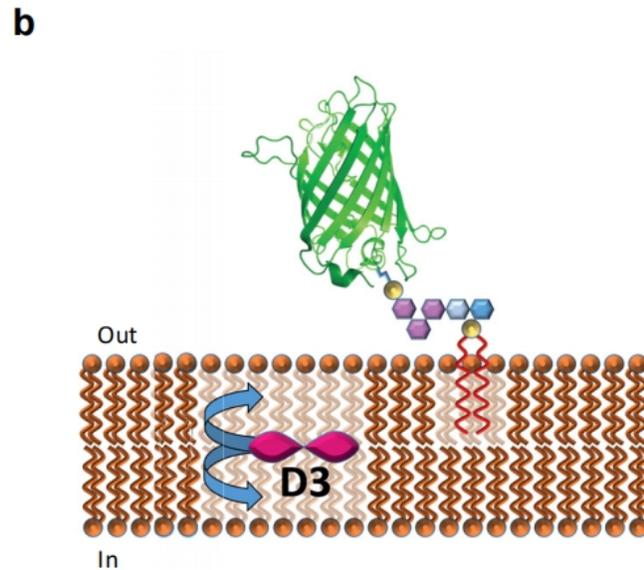
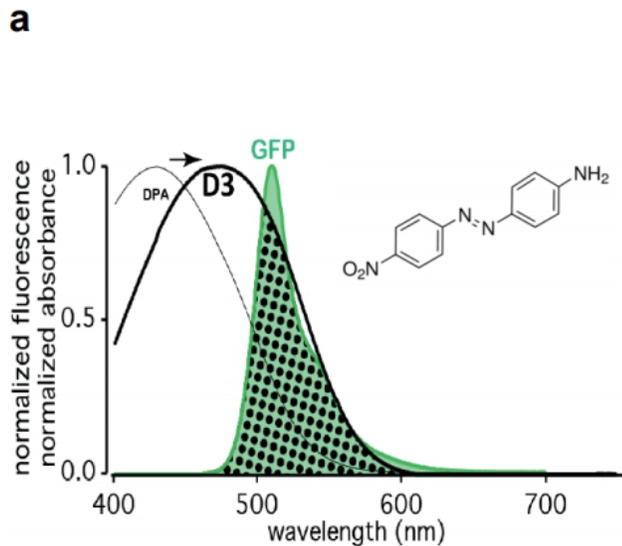
J. Am. Chem. Soc., 2008, 130, 4815.

Fluorescent protein-based hybrid biosensors

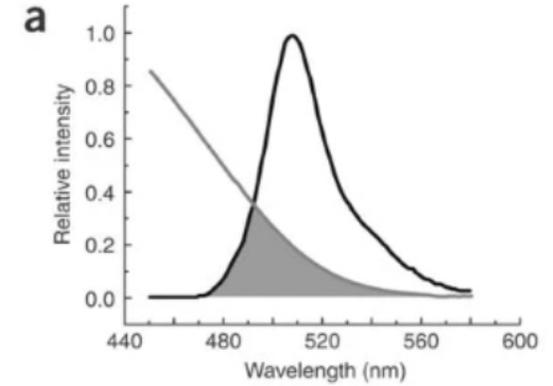
膜电位传感器——hVOS



Biophys. J. 2010, 99 (7), 2355–2365

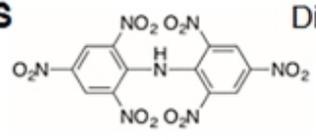


Proc Natl Acad Sci U S A 2021, 118 (6).

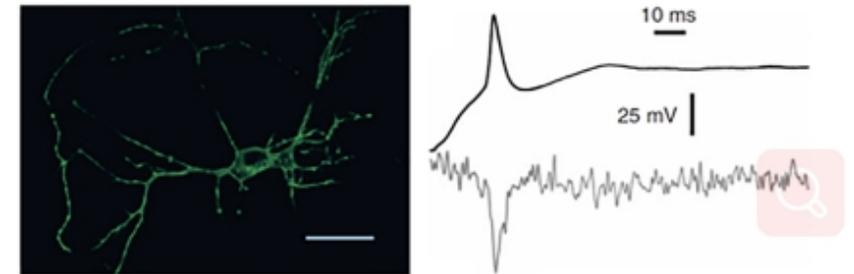
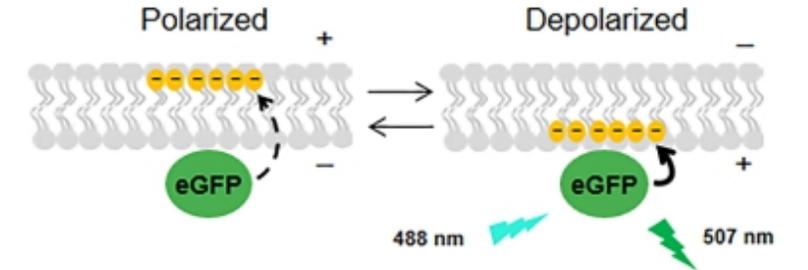


B hVOS Dipicrylamine

电压响应的膜
移动猝灭剂



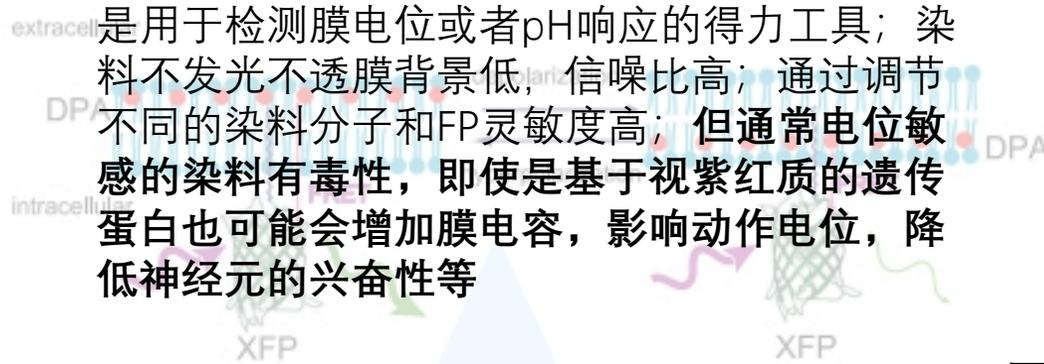
亲脂性阴离子染料
双嘧啶胺 (DPA)



Nature Neuroscience 8, 1619–1626(2005)

Summary

A 是用于检测膜电位或者pH响应的得力工具；染料不发光不透膜背景低，信噪比高；通过调节不同的染料分子和FP灵敏度高；但通常电位敏感的染料有毒性，即使是基于视紫红质的遗传蛋白也可能会增加膜电容，影响动作电位，降低神经元的兴奋性等



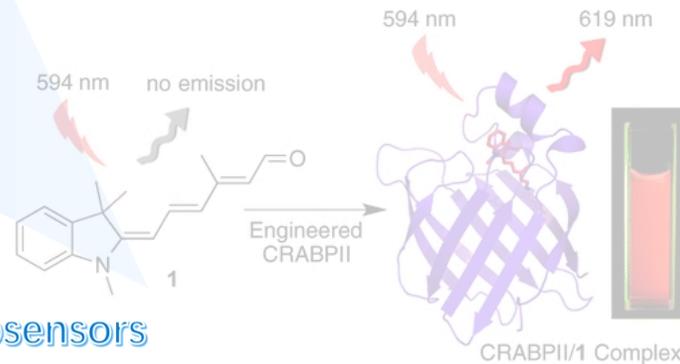
tag: 特异性更高，生物相容性好，混合标签的传感方式具有通用性，不受限于具有特定构象变化的蛋白；**体积大**
Blo: 体积较小，与环境敏感染料联用可以极大提高传感器灵敏度；**大部分需要化学修饰结合小分子。**
FRET效率低，动态范围低。

体积小，FRET效率高；方便优化FRET对的相对位置；**需要化学修饰的方法要考虑到细胞毒性；UAA、aaRS的选择受限**



Fluorescent protein-based hybrid biosensors

不受限于FRET对的选择，吸收光谱宽，可以很容易选择合适的发射波长避免直接激发的受体发射；并且只需要调整尺寸大小即可灵活调整光谱重叠面积，**提高FRET效率**；结合受体分子多，FRET效率高；尺寸相比小分子荧光团大；由于不可避免的竞争结合多个受体分子，**检测限低**；由于宽发射，**作为FRET受体有局限**



THANKS!