

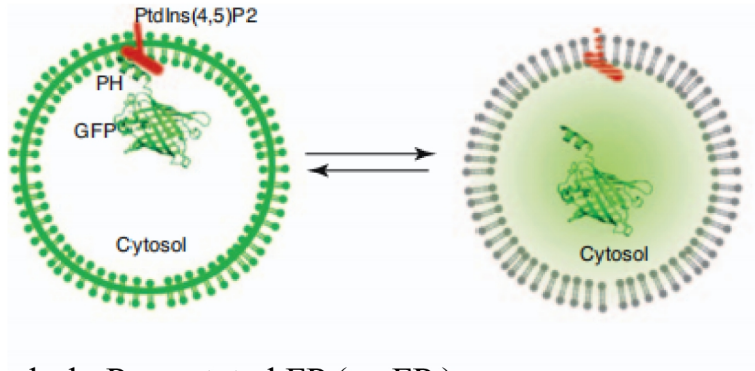
# Fluorescent protein-Based Hybrid Biosensors

**Reporter: Zhou Xuelian**

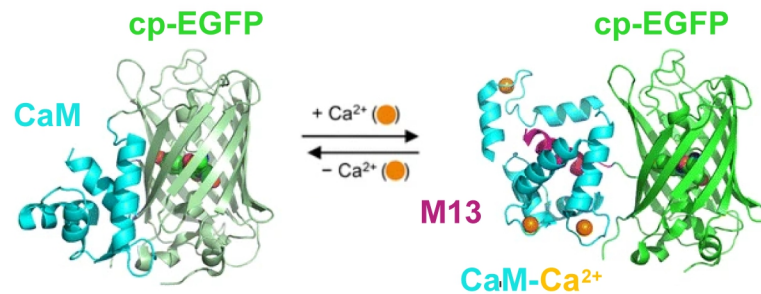
**Date: 2021-4-15**

# Fluorescent protein-based biosensors

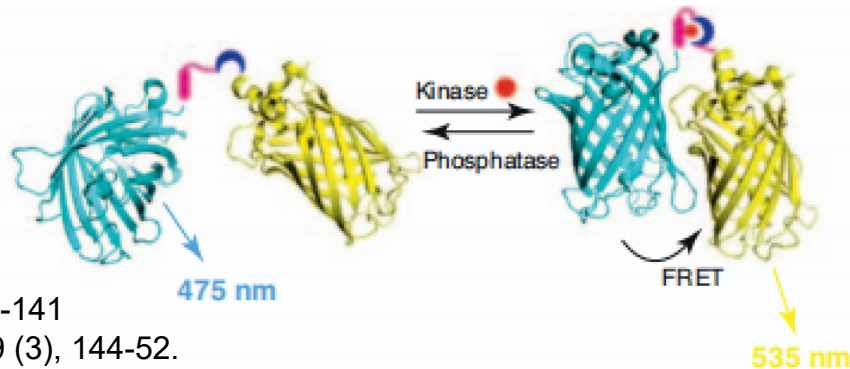
a) Single FP



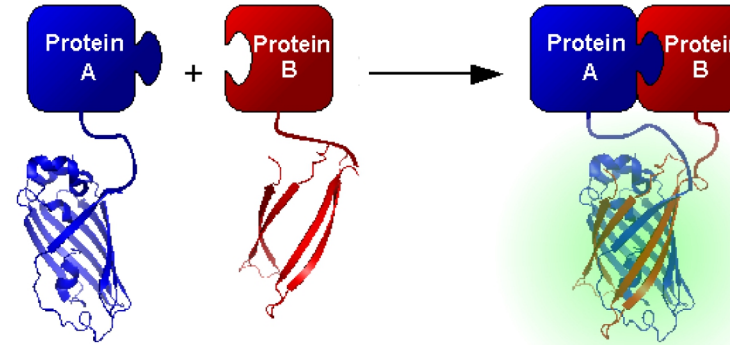
b) Circularly Permutated FP ( cpFP )



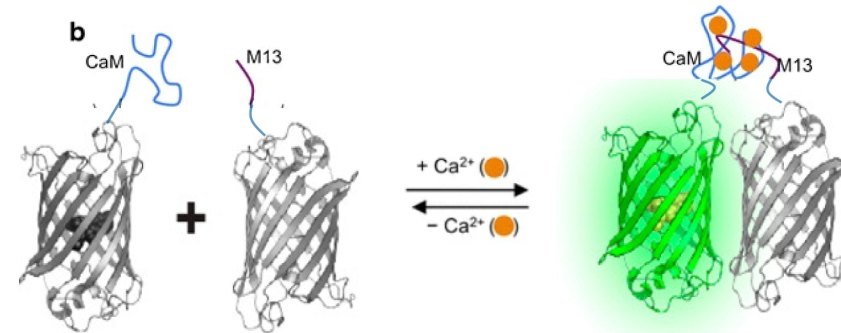
c) Fluorescent Resonance Energy Transfer ( FRET )



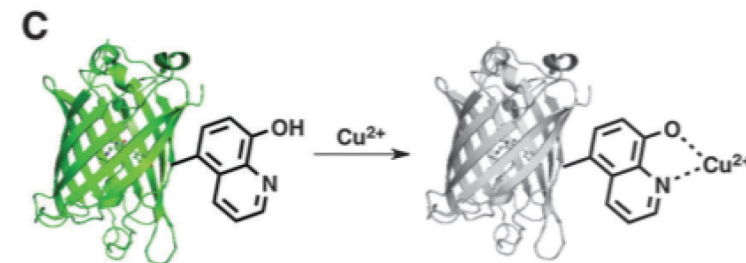
d) Bimolecular Fluorescence Complementation ( BiFC )



e) Dimerization-Dependent FP ( ddFP )

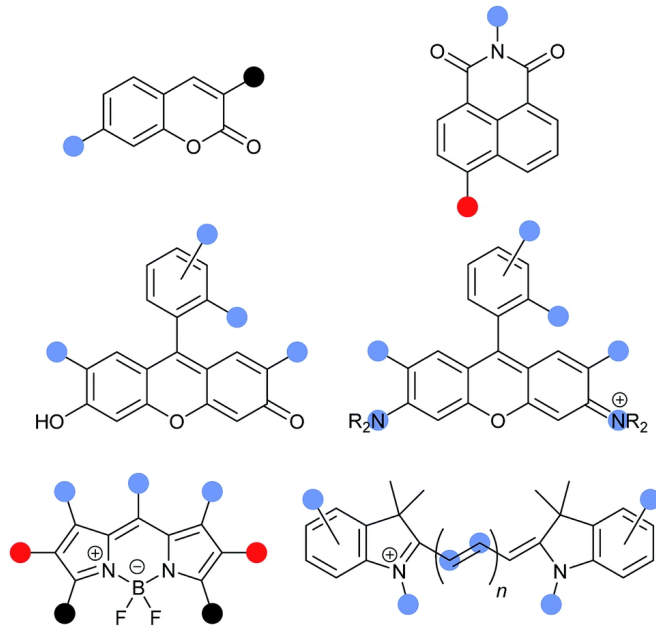


f) Genetic Incorporation of Unnatural Amino Acids ( UAAs )



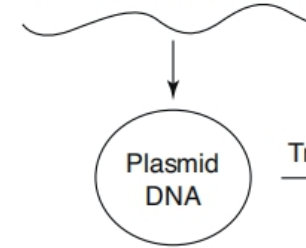
# Reasons for choosing hybrid biosensors

Synthetic fluorophore



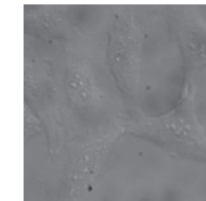
VS

Gene encoding sensor

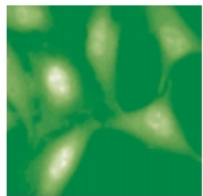


Transfection

genetic targetability



Transcription  
Translation



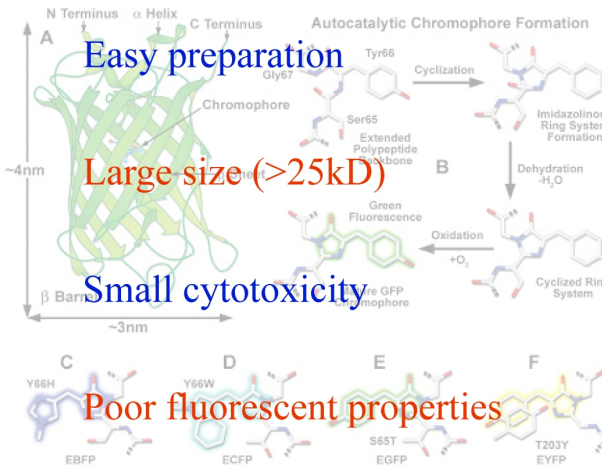
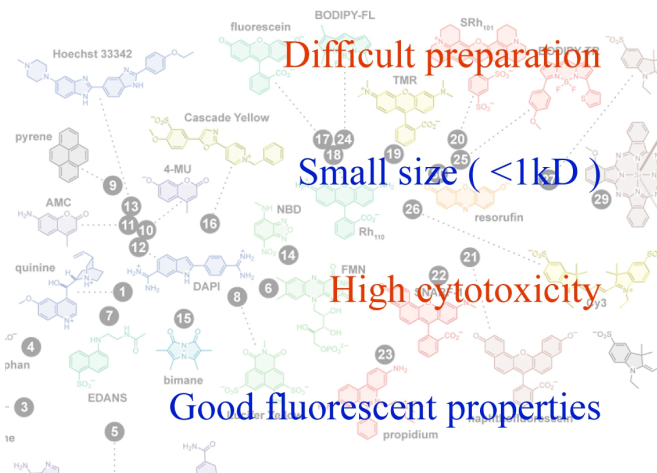
TRENDS in Biotechnology

RSC Adv., 2018, 8, 29051-29061

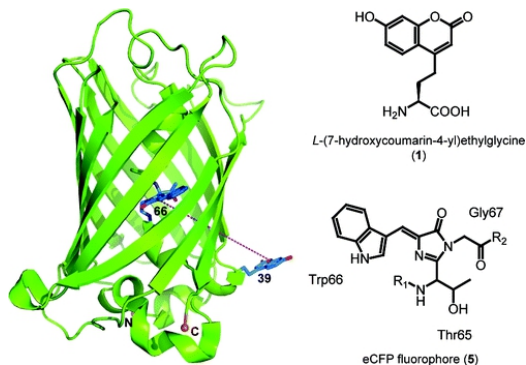
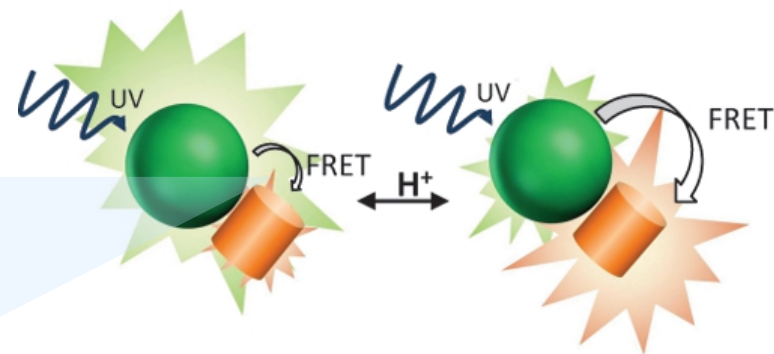
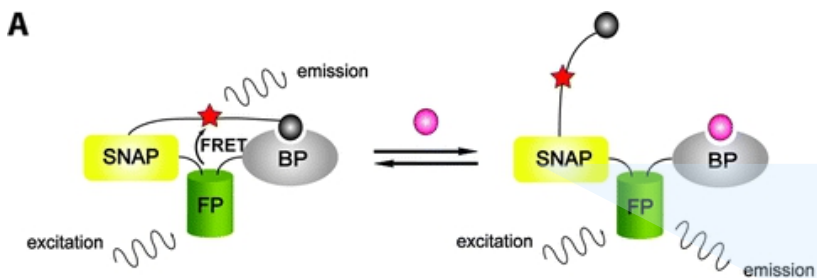
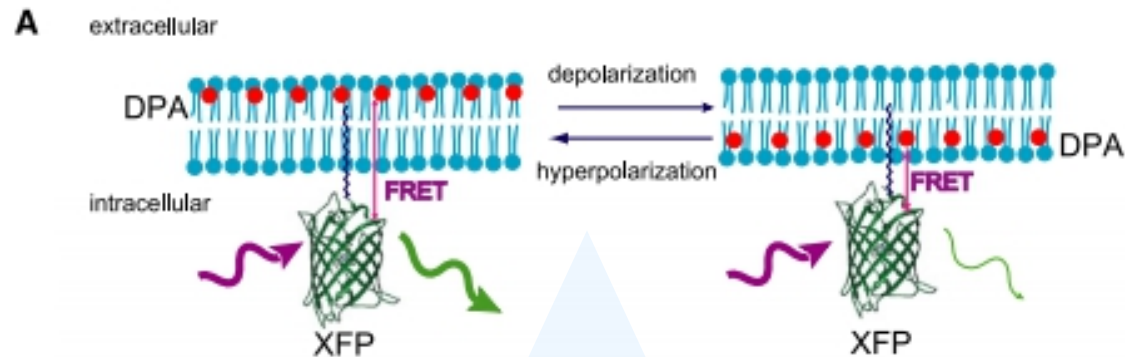
Trends Biotechnol 2011, 29 (3), 144-52

Synthetic fluorophore

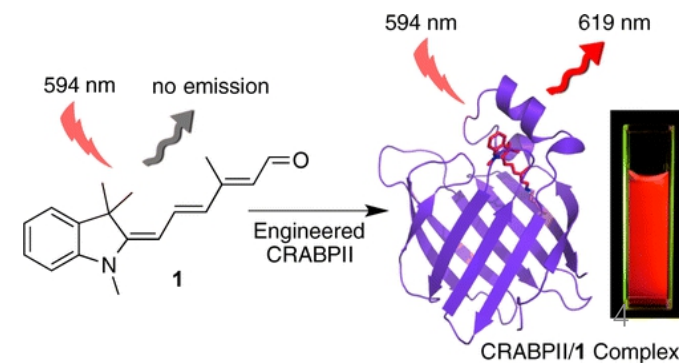
Fluorescent protein



# Fluorescent protein-based hybrid biosensors

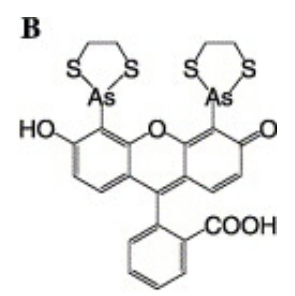
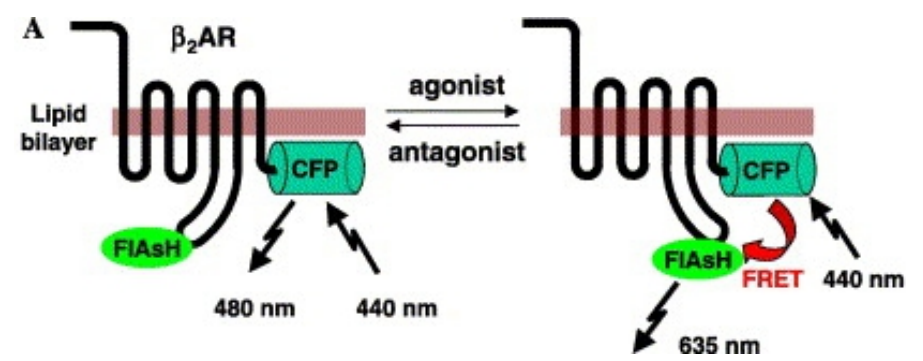


1. 遗传靶向性
2. 双荧光团系统
3. 比例定量分析
4. 传感器动态范围、灵敏度

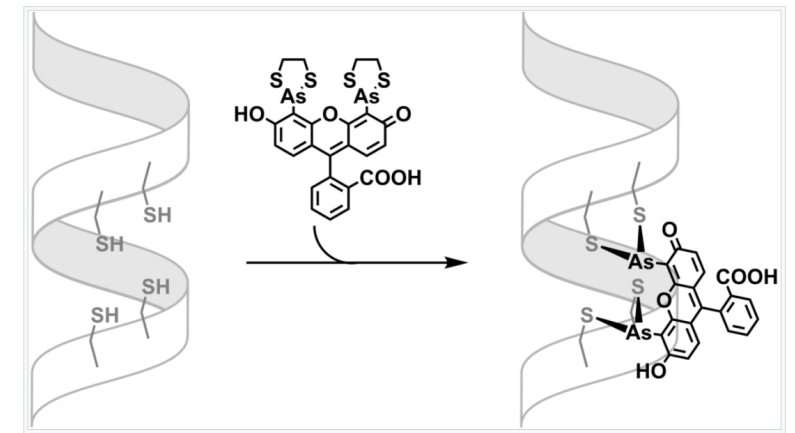


# Fluorescent protein-based hybrid biosensors

遗传编码支架蛋白——tag protein



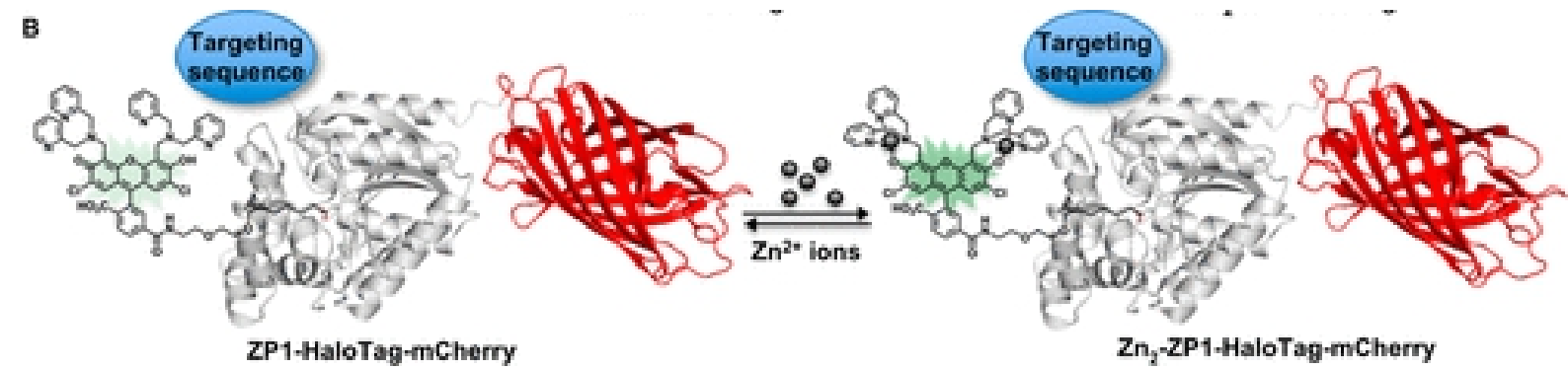
荧光素砷发卡标签  
FIAsh (fluorescein arsenical hairpin binder)



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

尺寸小，但具有细胞毒性

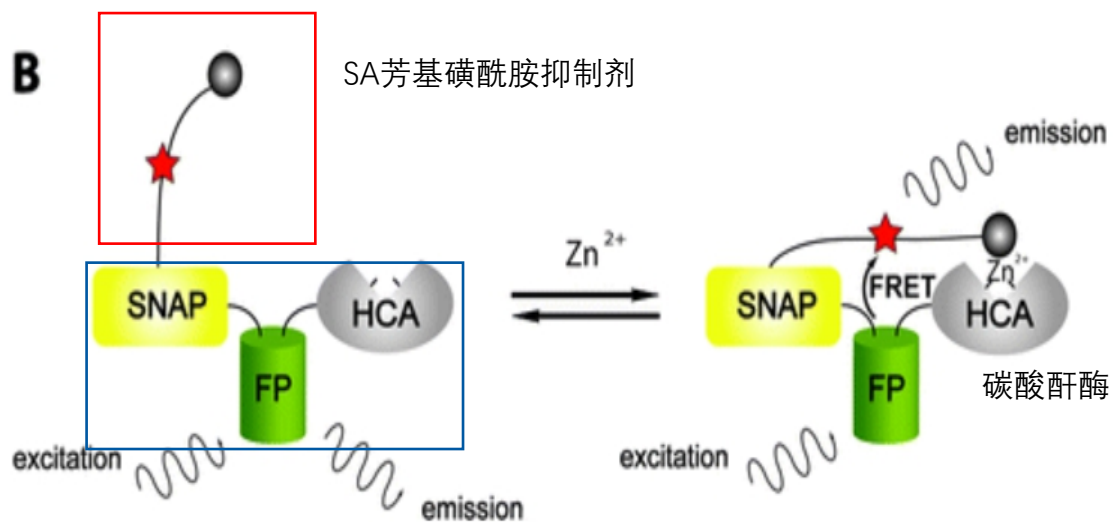
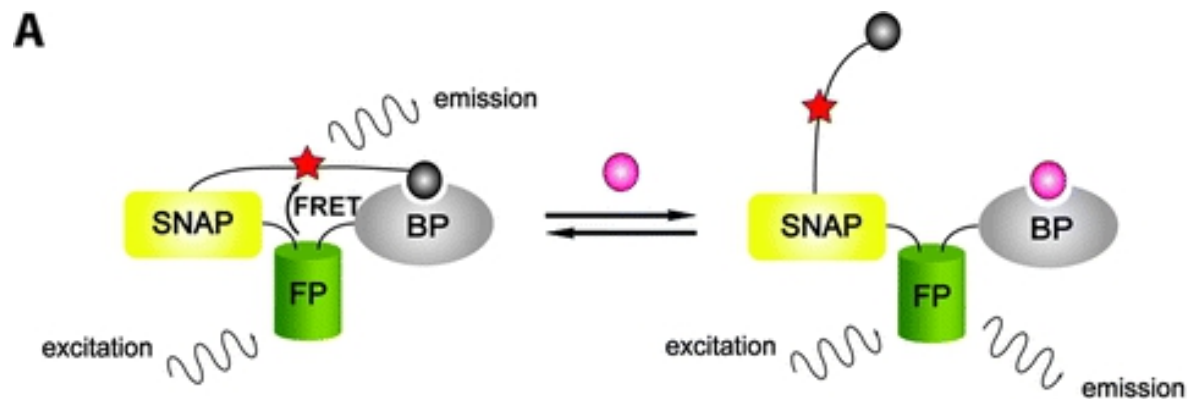
四半胱氨酸基序 (CCXXCC)



ACS Chem. Biol. 2020, 15, 2, 396–406

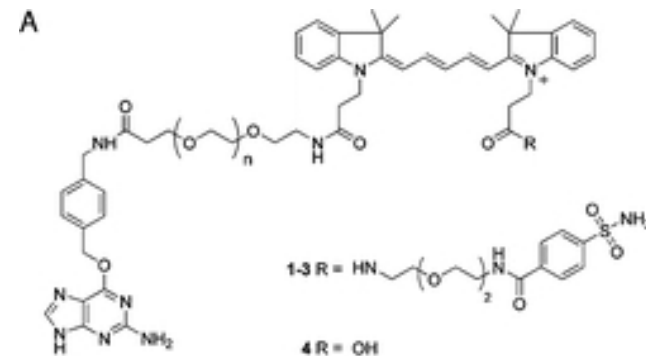
# Fluorescent protein-based hybrid biosensors

遗传编码支架蛋白——tag protein



Snifits

合成部分: BG-PEG<sub>n</sub>-Cy5-SA

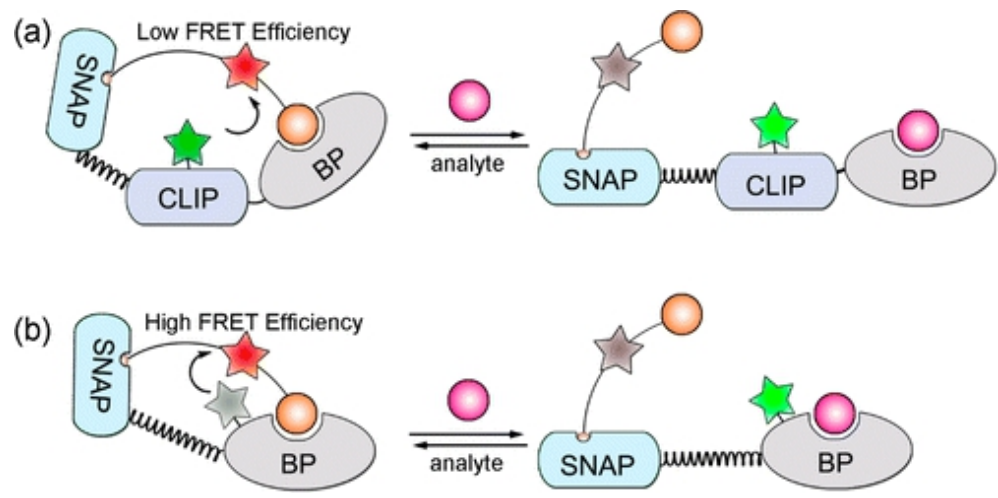


融合蛋白: SNAP\_mCherry\_HCA

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

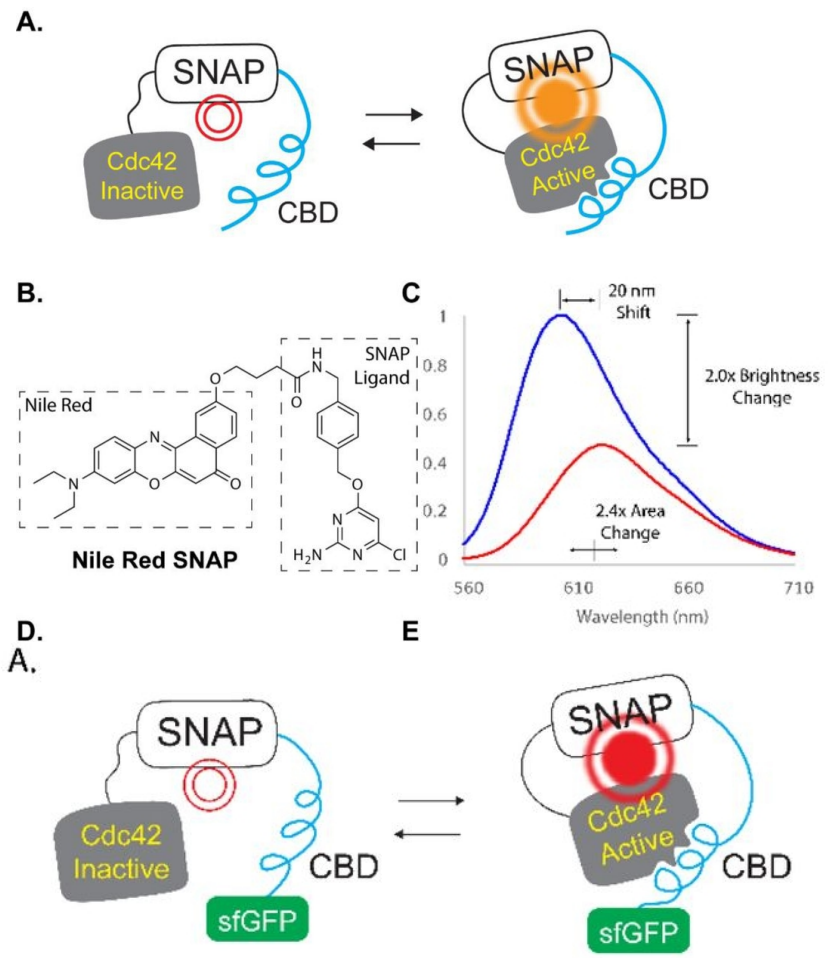
# Fluorescent protein-based hybrid biosensors

遗传编码支架蛋白——tag protein



uSnifit

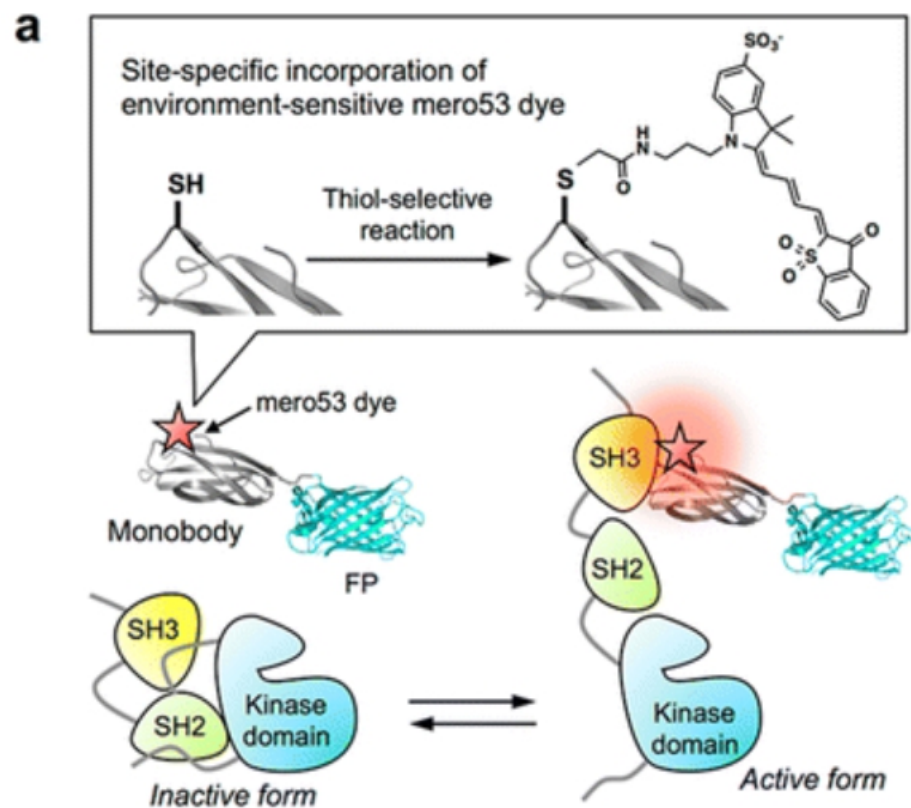
J. Am. Chem. Soc. 2016, 138, 16, 5258–5261



BioRxiv, 2020, 206748

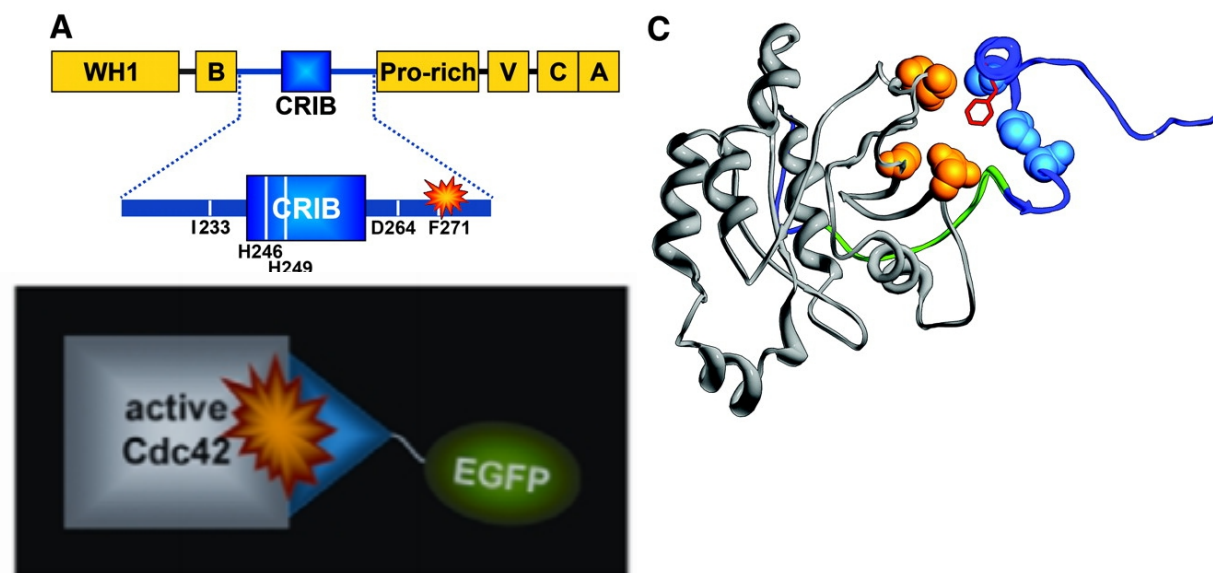
# Fluorescent protein-based hybrid biosensors

遗传编码支架蛋白——Biological protein



Src家族激酶 (SFK)

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



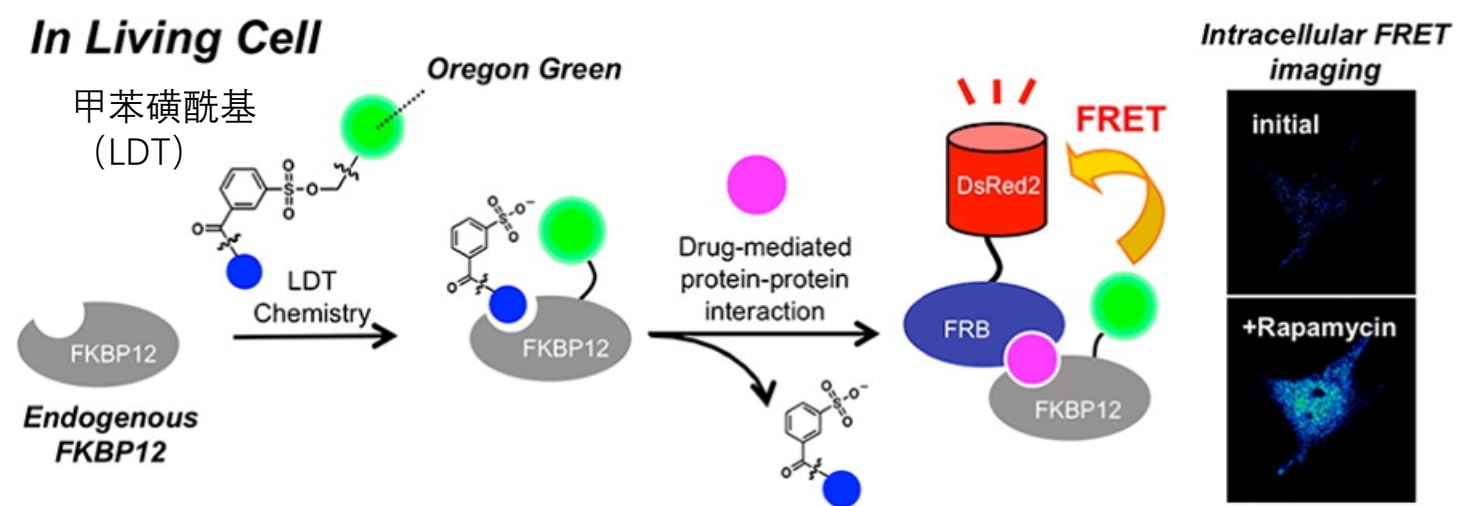
体积小，并且可以定制以通过高通量筛选来结合不同的靶标。

Science. 2004, 305, 5690, 1615-1619

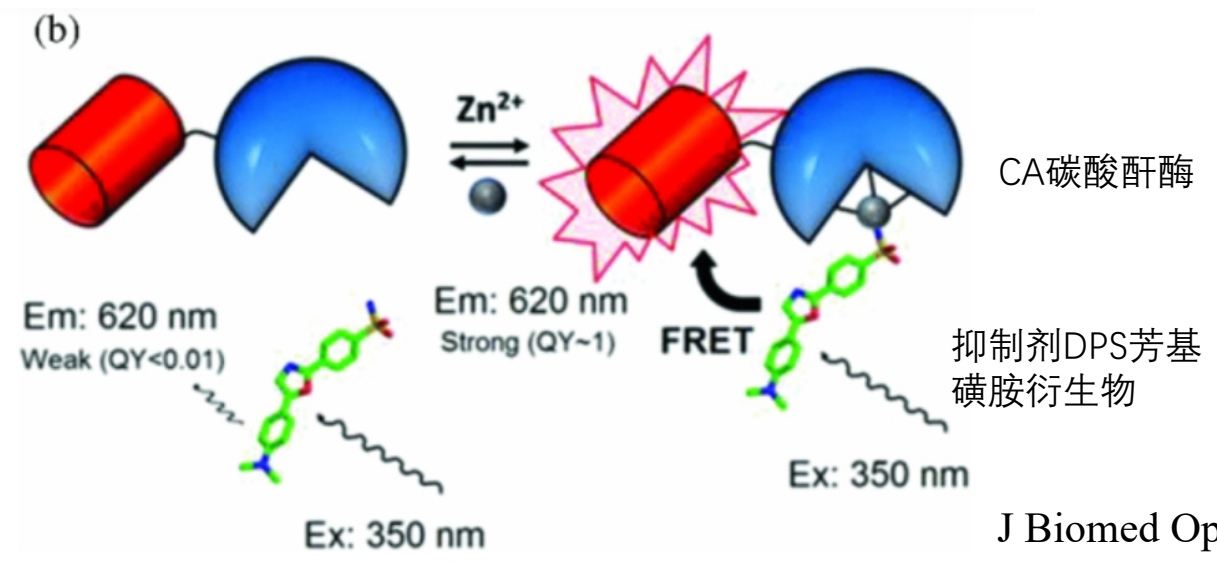


# Fluorescent protein-based hybrid biosensors

遗传编码支架蛋白——Biological protein



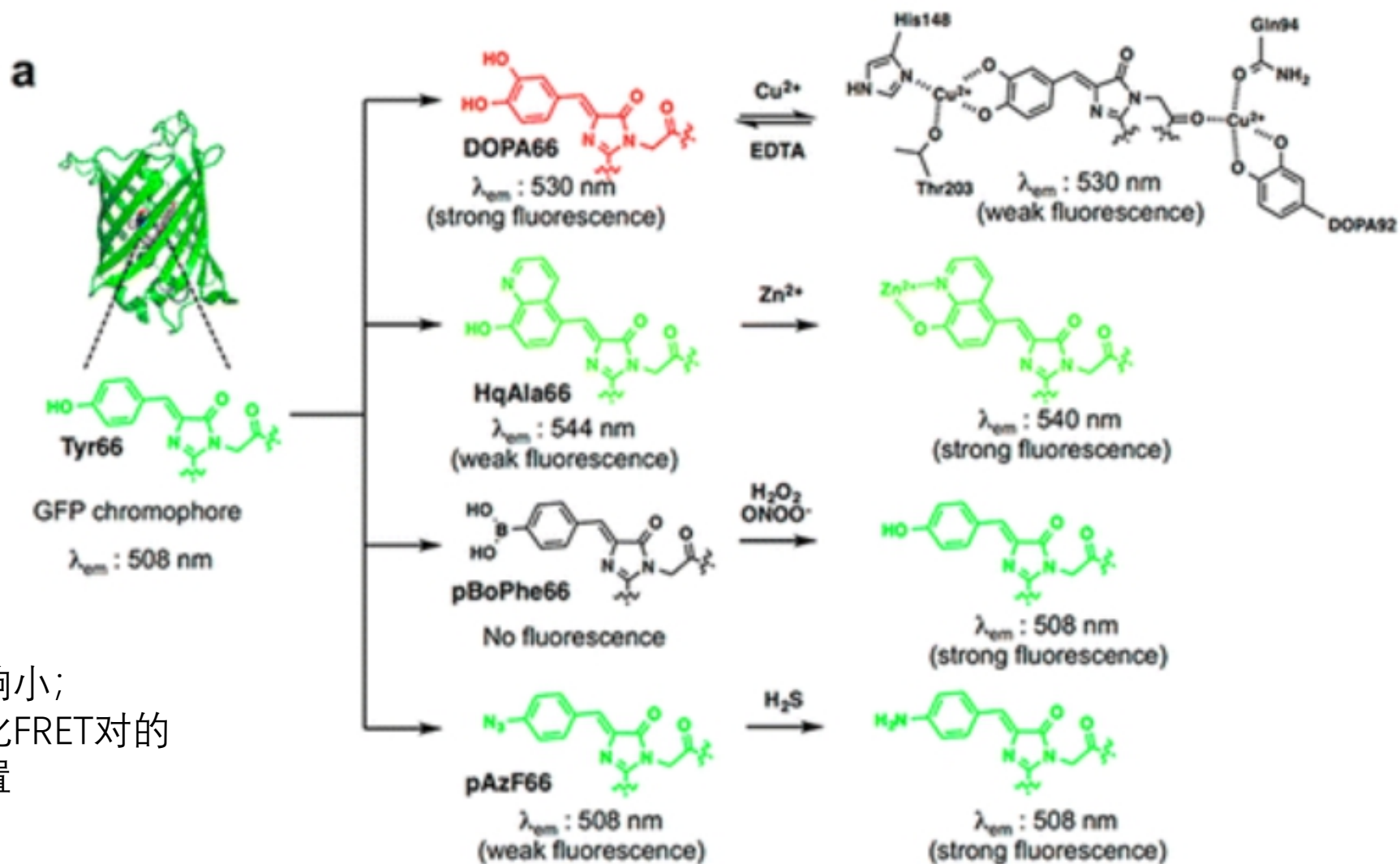
J. Am. Chem. Soc. 2013, 135, 18, 6782–6785



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

# Fluorescent protein-based hybrid biosensors

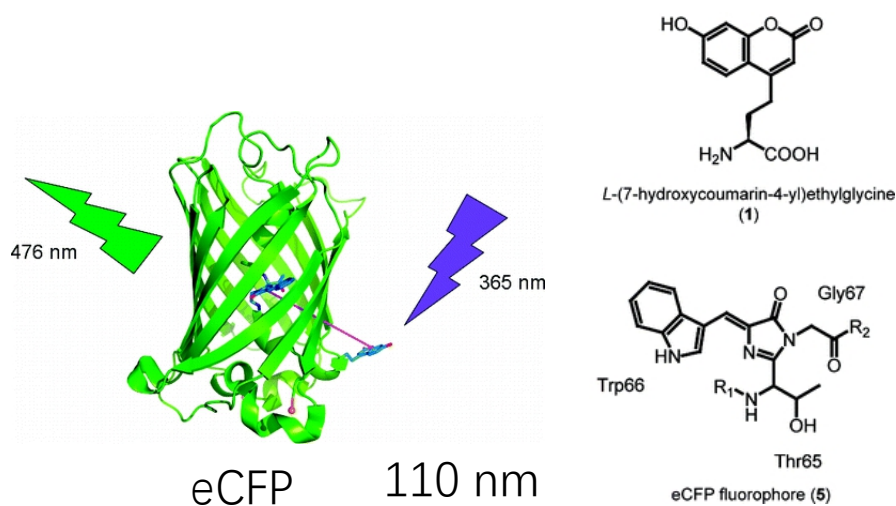
非天然氨基酸



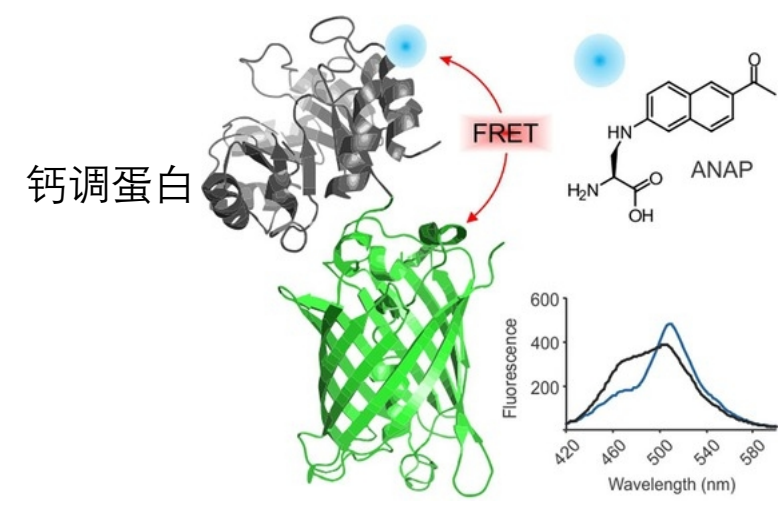
蛋白影响小;  
方便优化FRET对的  
相对位置

# Fluorescent protein-based hybrid biosensors

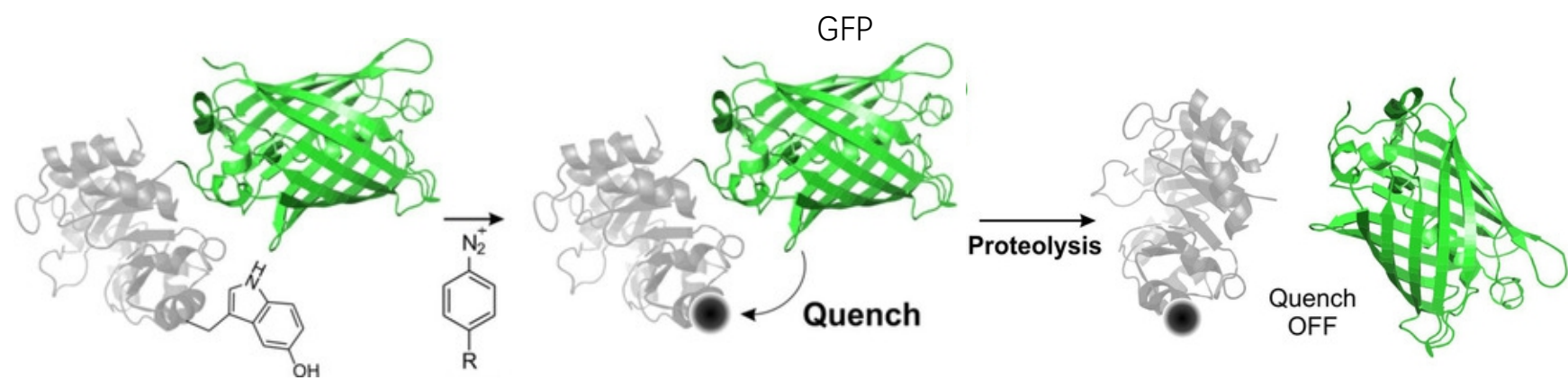
## 非天然氨基酸荧光团



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



ChemBioChem 2017, 18, 511.

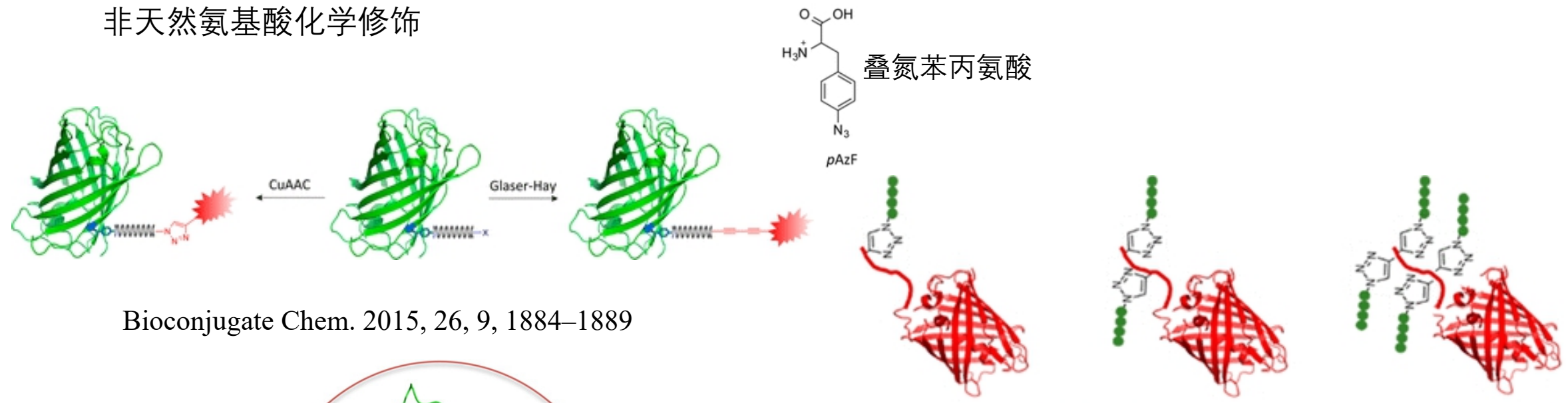


5-羟基色氨酸

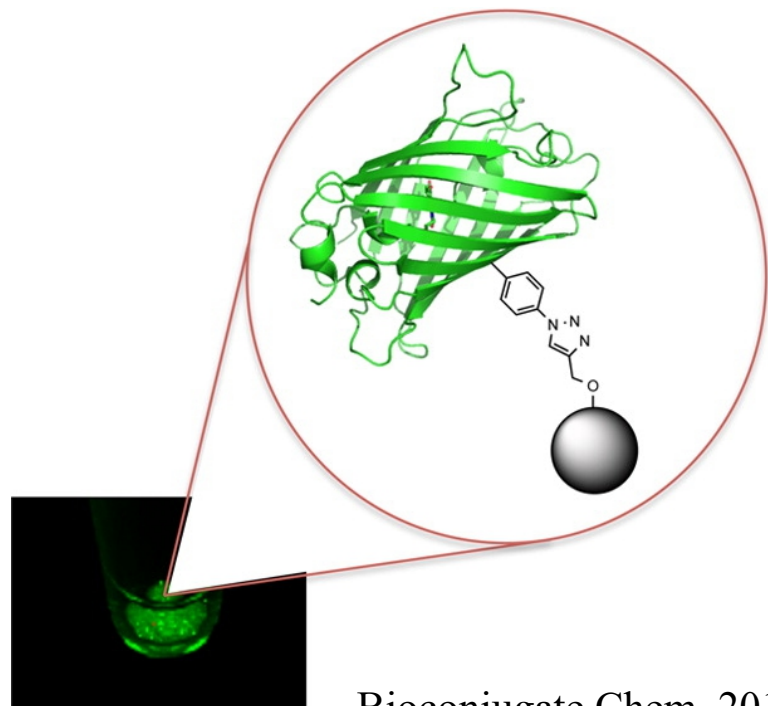
ChemBioChem 2019, 20, 1659.

# Fluorescent protein-based hybrid biosensors

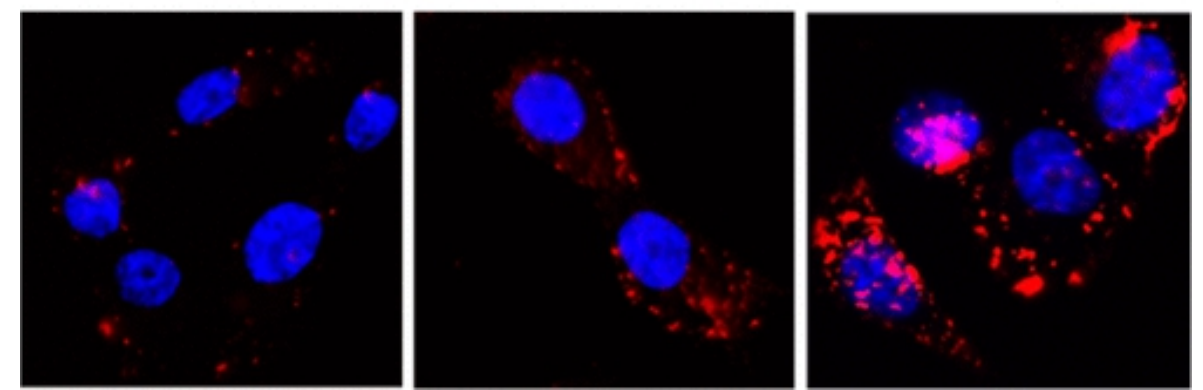
非天然氨基酸化学修饰



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



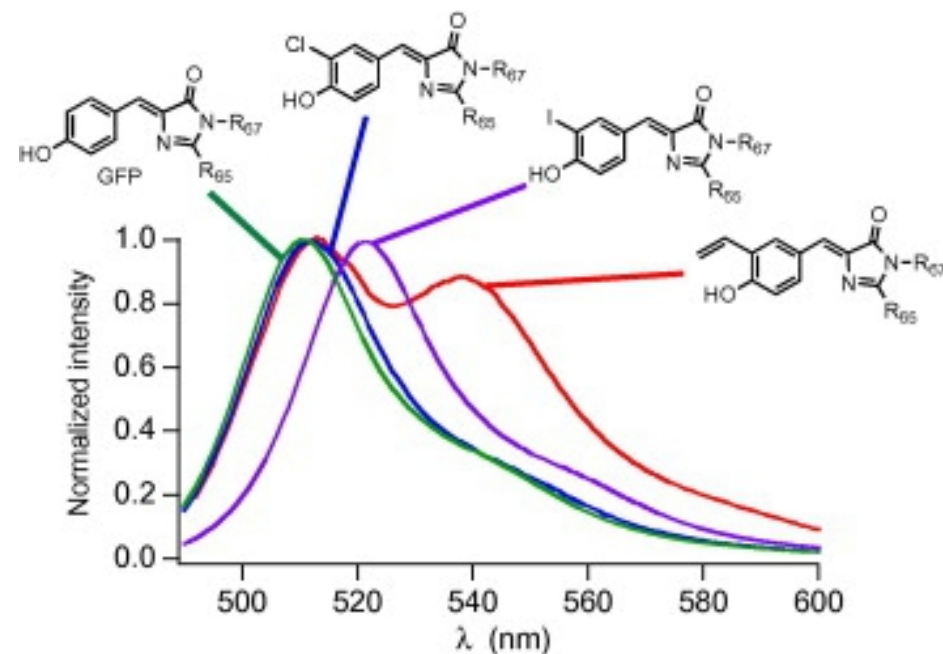
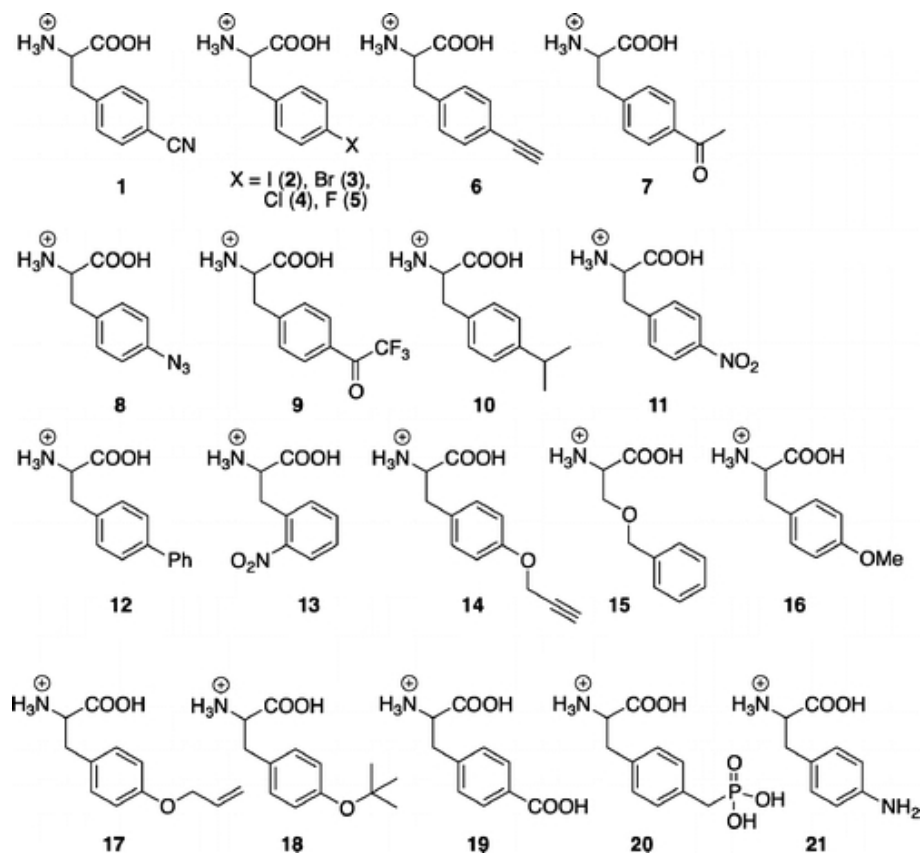
Bioconjugate Chem. 2014, 25, 11, 1916–1920



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

# Fluorescent protein-based hybrid biosensors

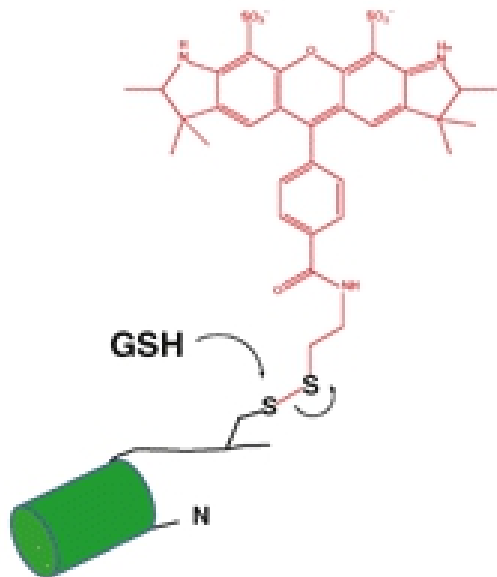
引入UAA——非特异性aaRS pCNF (对氰基苯丙氨酸)



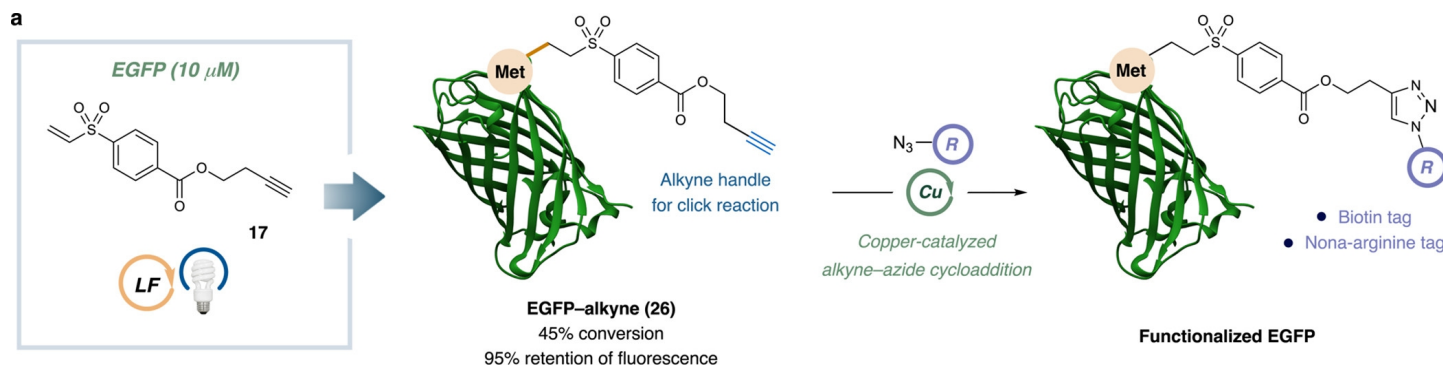
对GFP66位发色团位点进行非天然氨基酸改造来改变光谱，只需要调整不同的氨基酸底物，可以通过使用单个aaRS迅速实现这一目标，而无需使用多个不同的UAA / aaRS系统进行表达。

# Fluorescent protein-based hybrid biosensors

## 天然氨基酸化学修饰

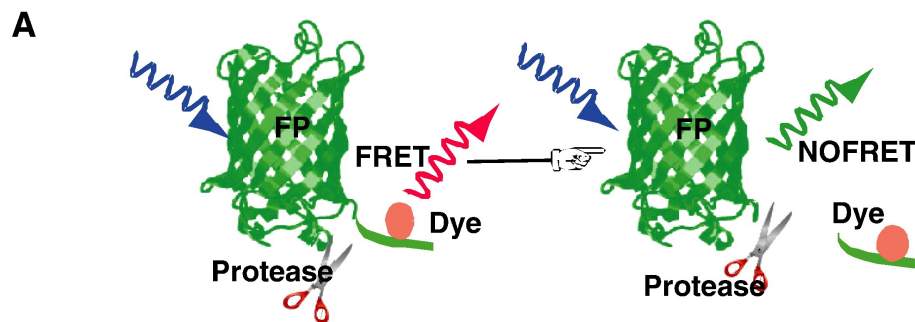


Chemistry Letters 34, 6 (2005) 766-767



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

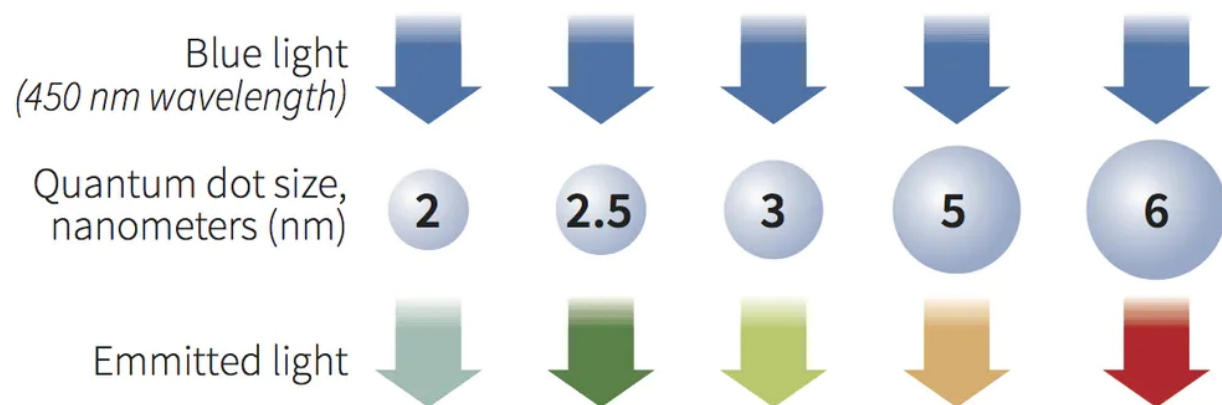
Biochimica et Biophysica Acta 1823 (2012) 215–226

# Fluorescent protein-based hybrid biosensors

## Quantum dots

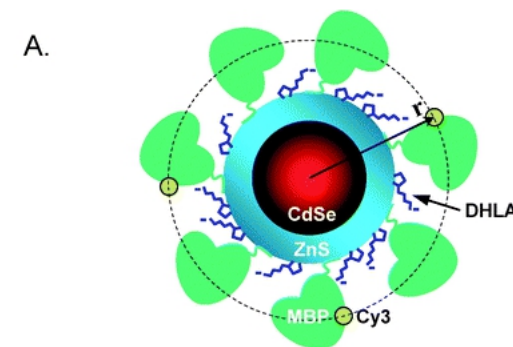
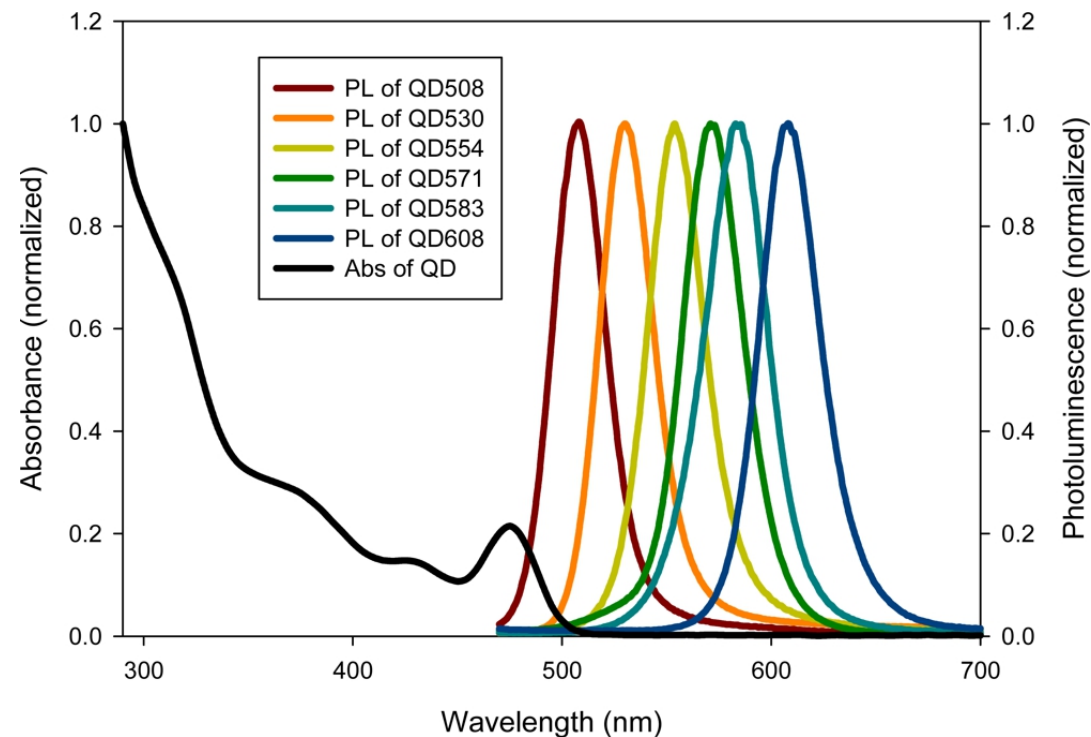
### What are quantum dots

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

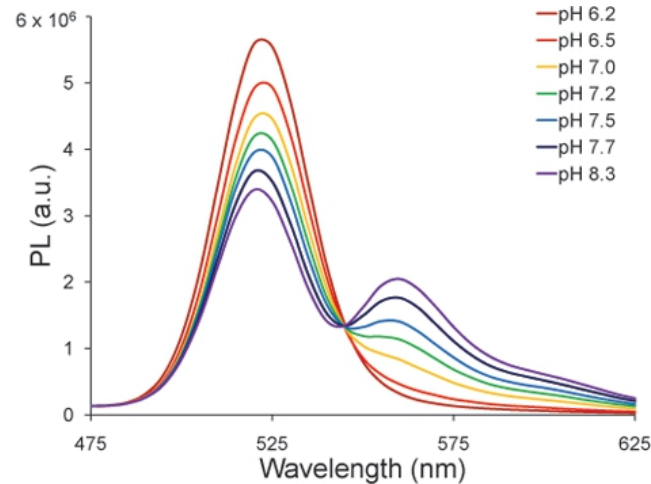
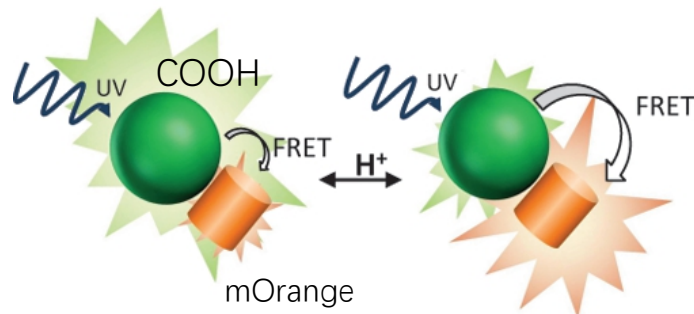
Nanoscale Adv 2019, 1, 3424 - 3442



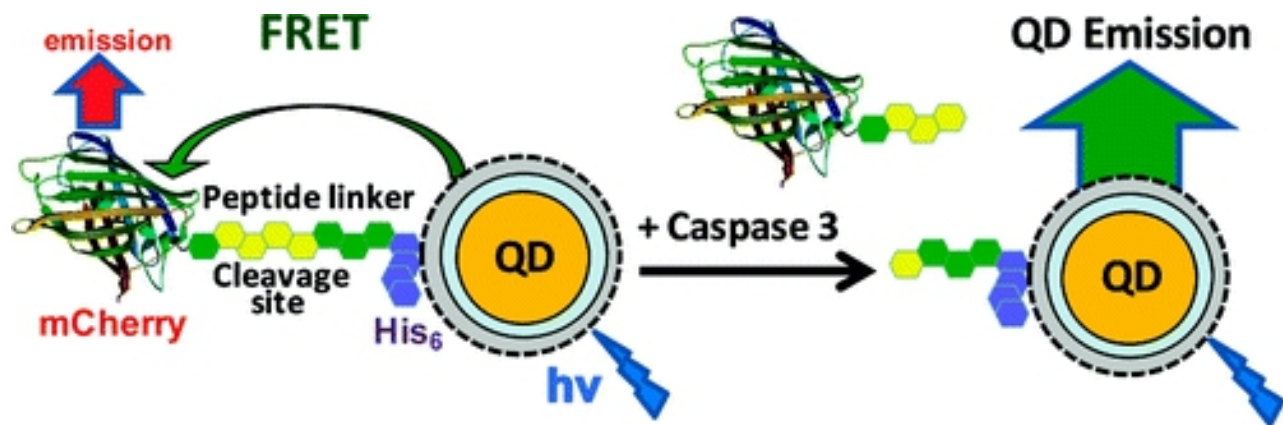
Sensors 2011, 11(12), 11036-11055

# Fluorescent protein-based hybrid biosensors

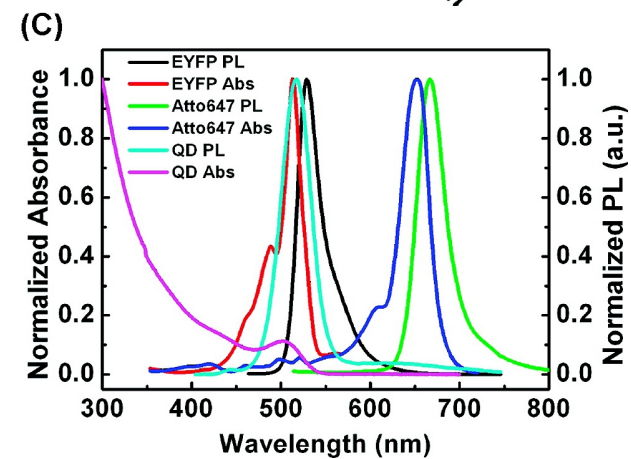
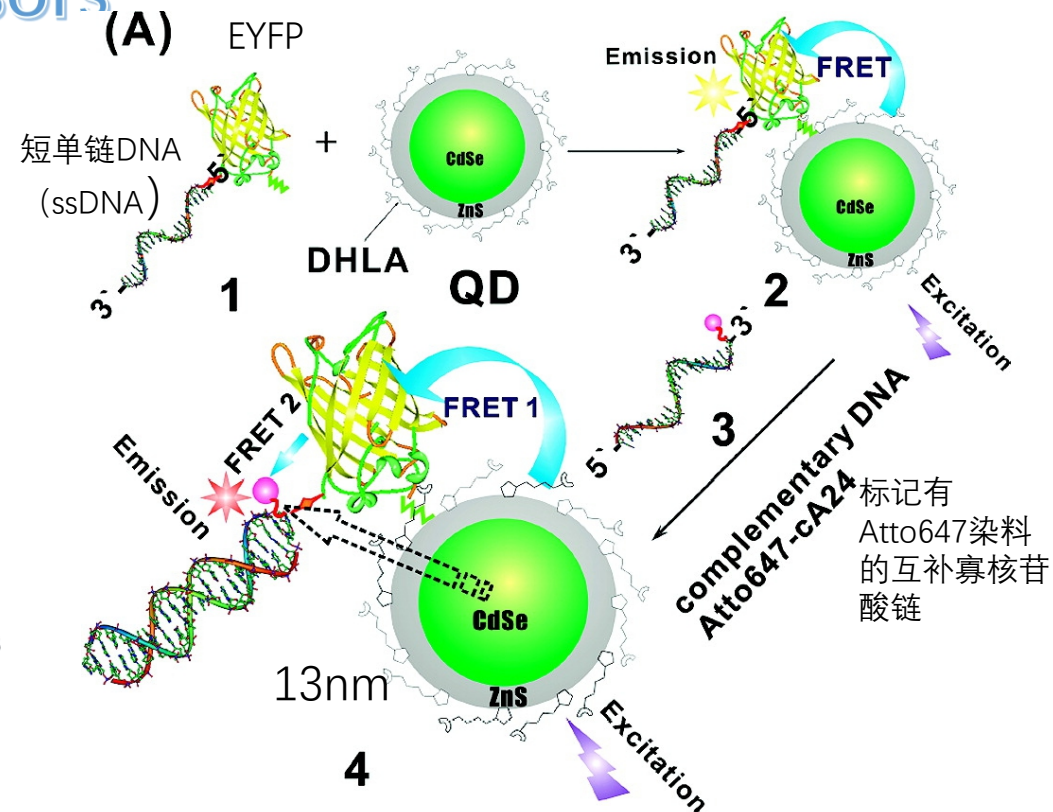
Quantum dots



ACS Nano 2012, 6, 4, 2917–2924



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

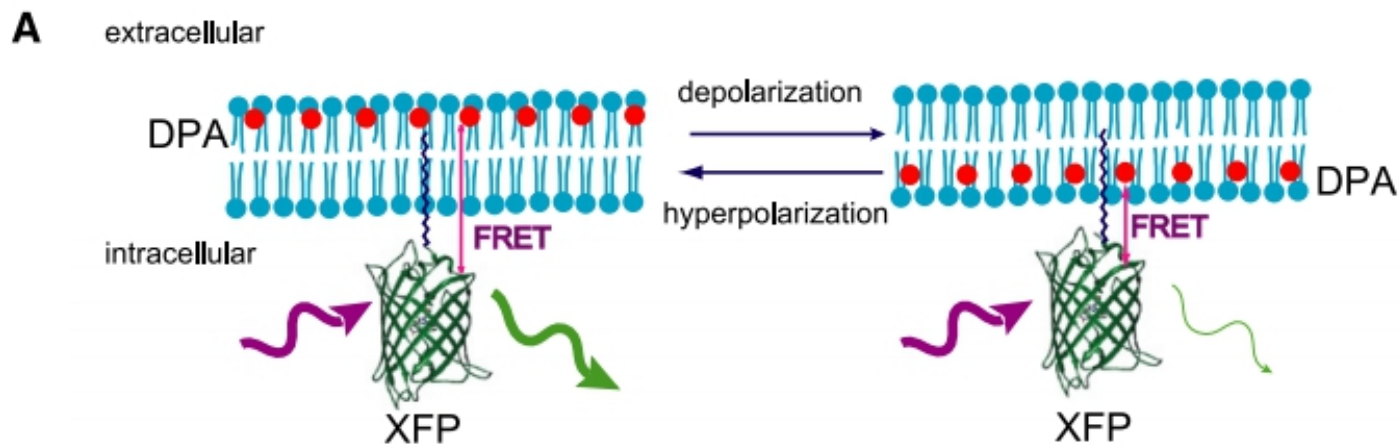


J. Am. Chem. Soc., 2008, 130, 4815.

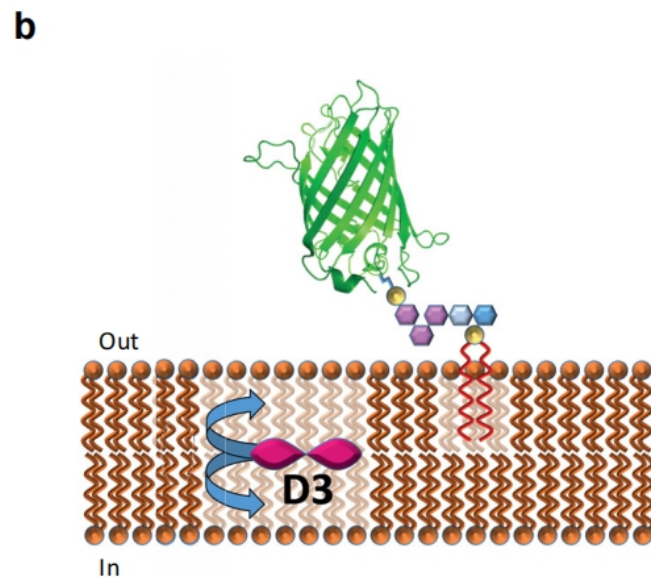
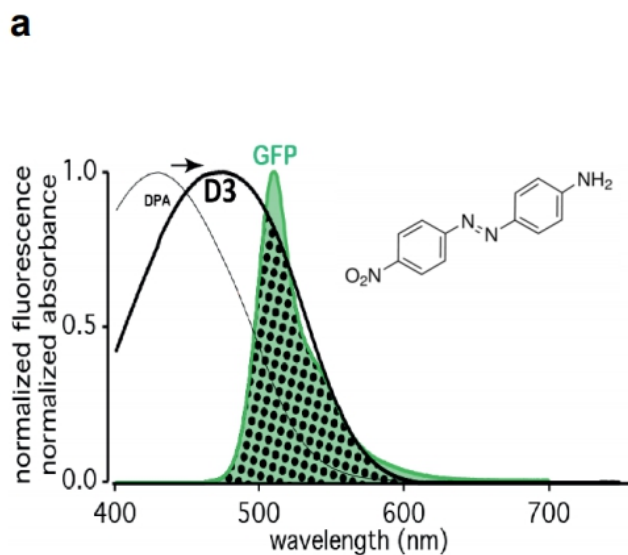


# Fluorescent protein-based hybrid biosensors

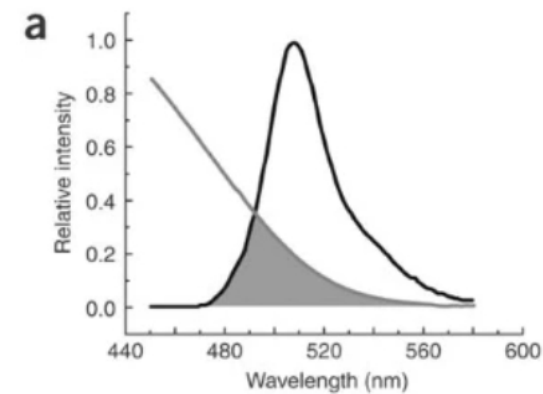
膜电位传感器——hVOS



Biophys. J. 2010, 99 (7), 2355–2365

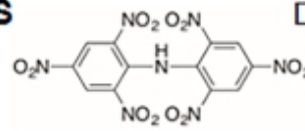


Proc Natl Acad Sci U S A 2021, 118 (6).

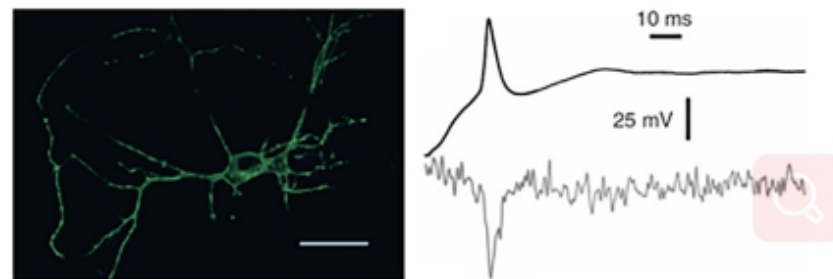
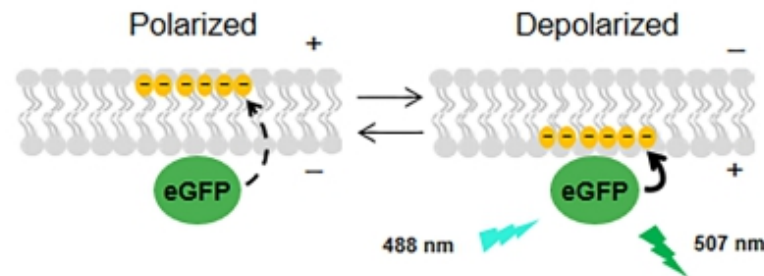


**B** hVOS Dipicrylamine

电压响应的膜  
移动猝灭剂



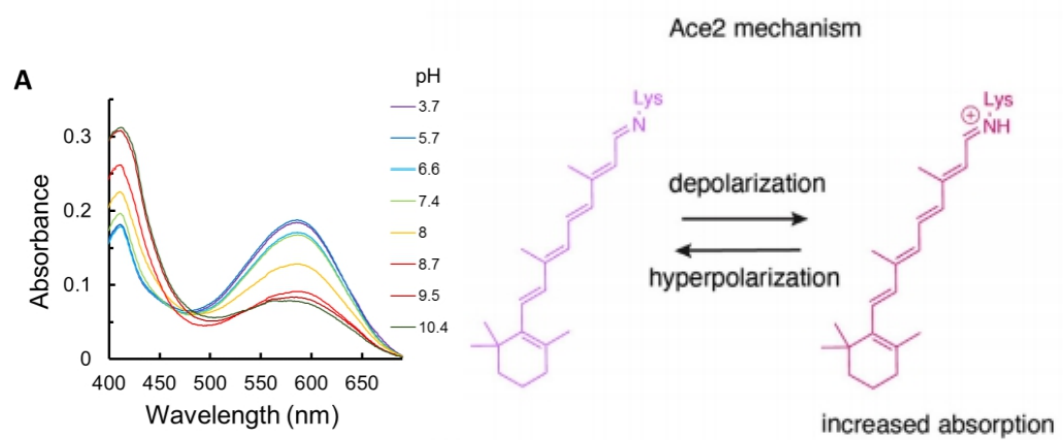
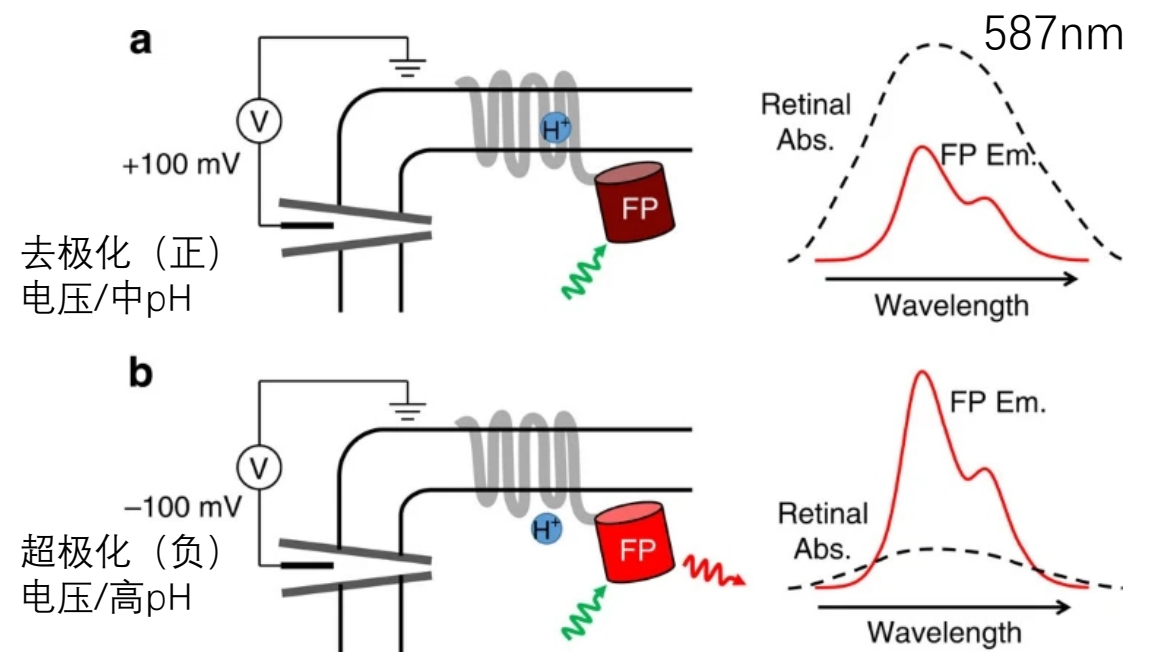
亲脂性阴离子染料  
双吡啶胺 (DPA)



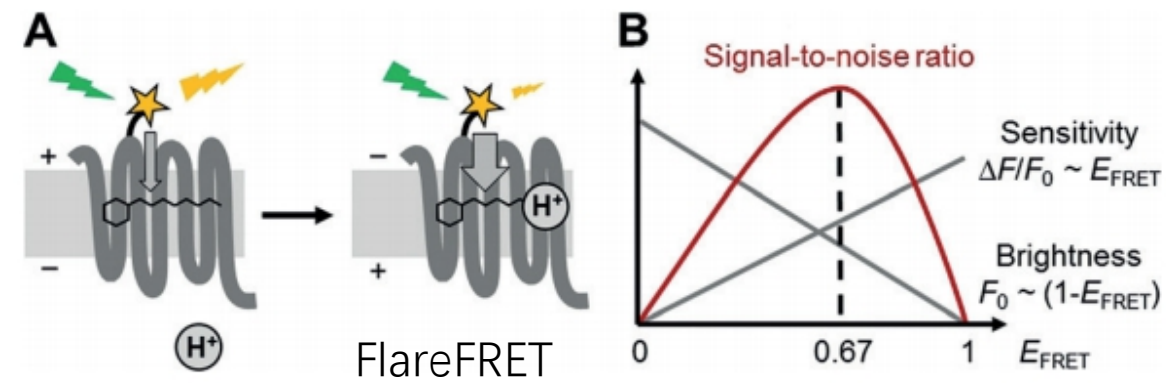
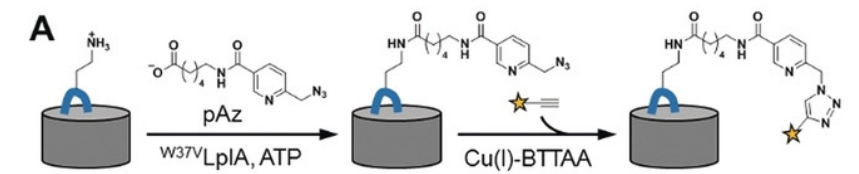
Nature Neuroscience 8, 1619–1626(2005)

# Fluorescent protein-based hybrid biosensors

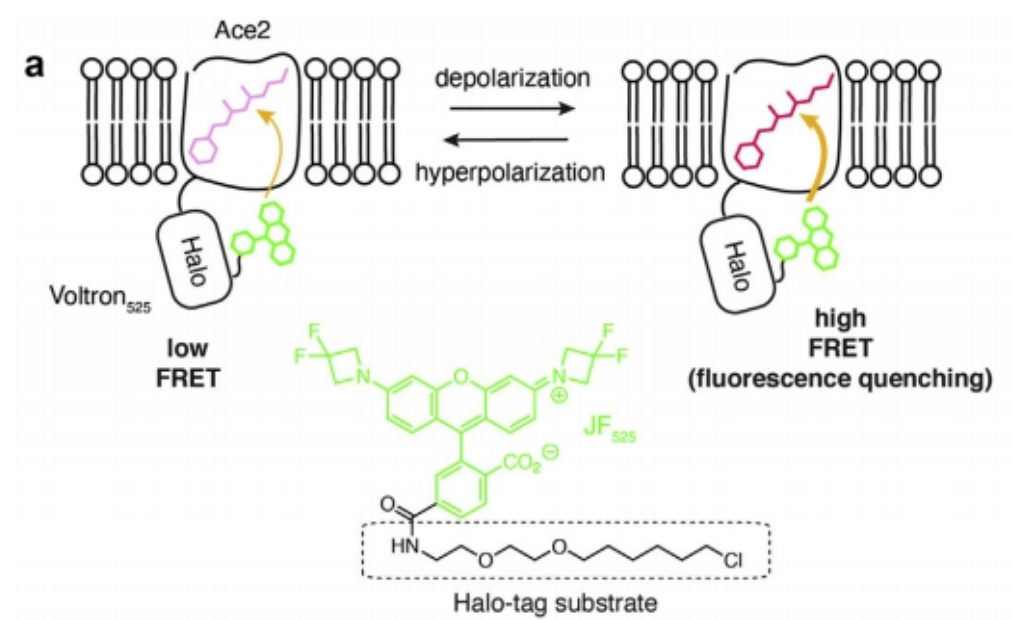
## 膜电位传感器——视紫红质



Nature Communications 5, 4625 (2014)



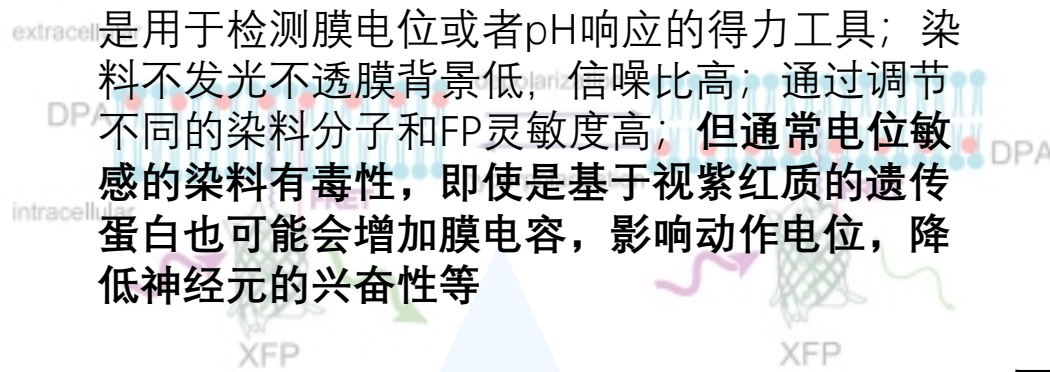
Angew. Chem. Int. Ed. 2018, 57, 3949–3953



Curr Opin Chem Biol 2020, 57, 58-64.

# 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



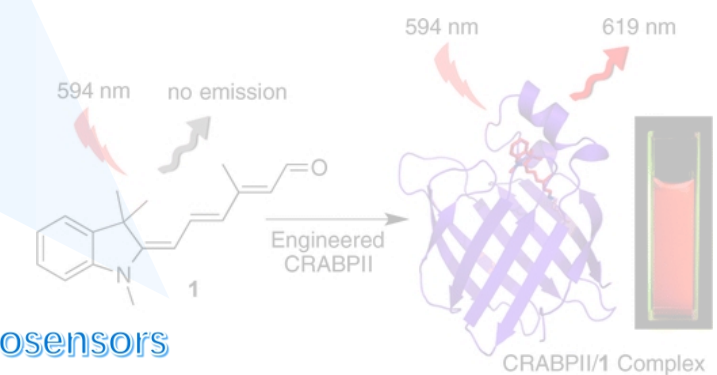
tag: 特异性更高，生物相容性好，混合标签的传感方式具有通用性，不受限于具有特定构象变化的蛋白；**体积大**  
Blo: 体积较小，与环境敏感染料联用可以极大提高传感器灵敏度；**大部分需要化学修饰结合小分子。**

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**



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



## Fluorescent protein-based hybrid biosensors

**THANKS!**