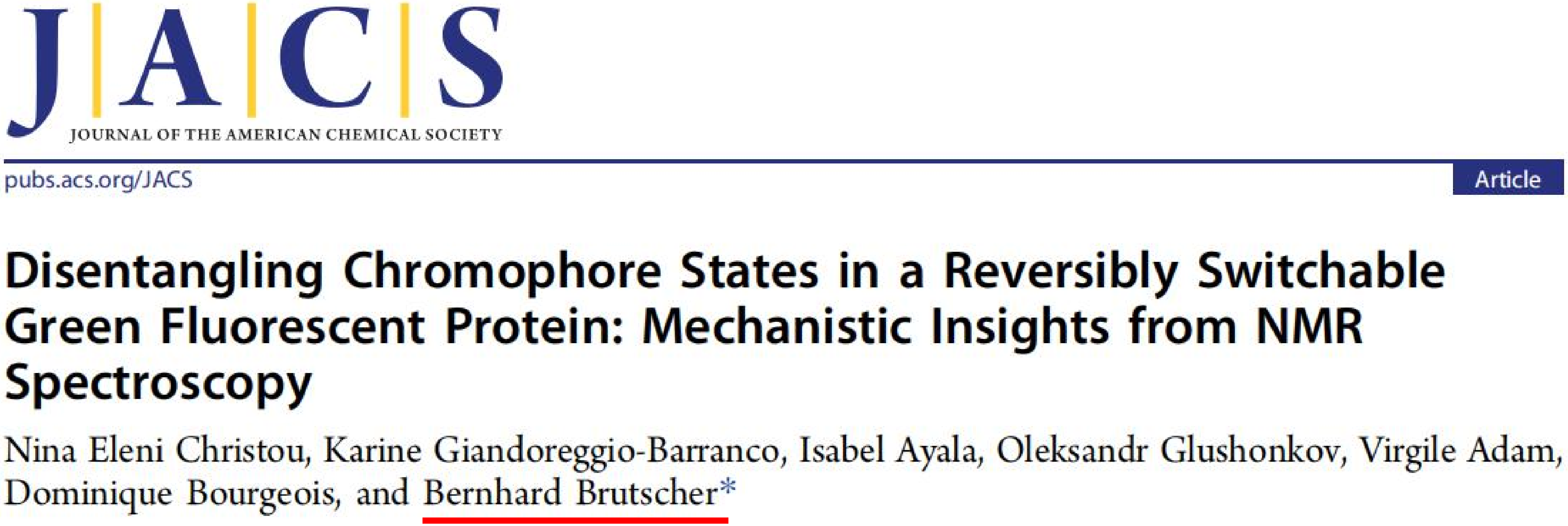
Literature Report

Reporter: Zhou Xuelian

Date: 2021-6-3



JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
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Article

Disentangling Chromophore States in a Reversibly Switchable Green Fluorescent Protein: Mechanistic Insights from NMR Spectroscopy

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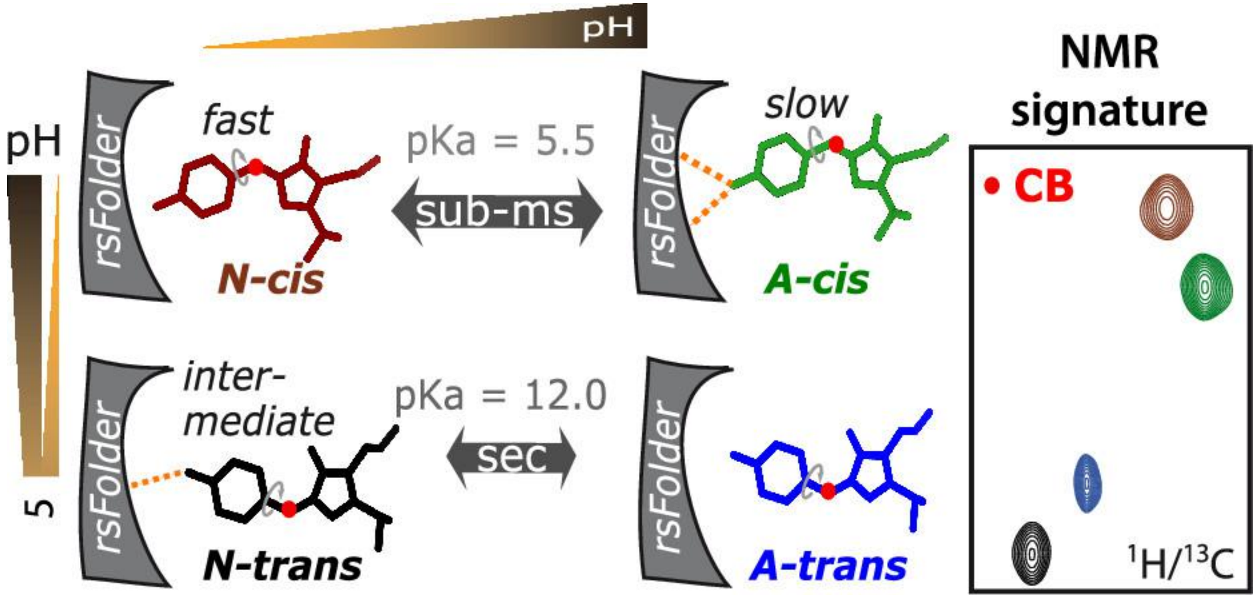
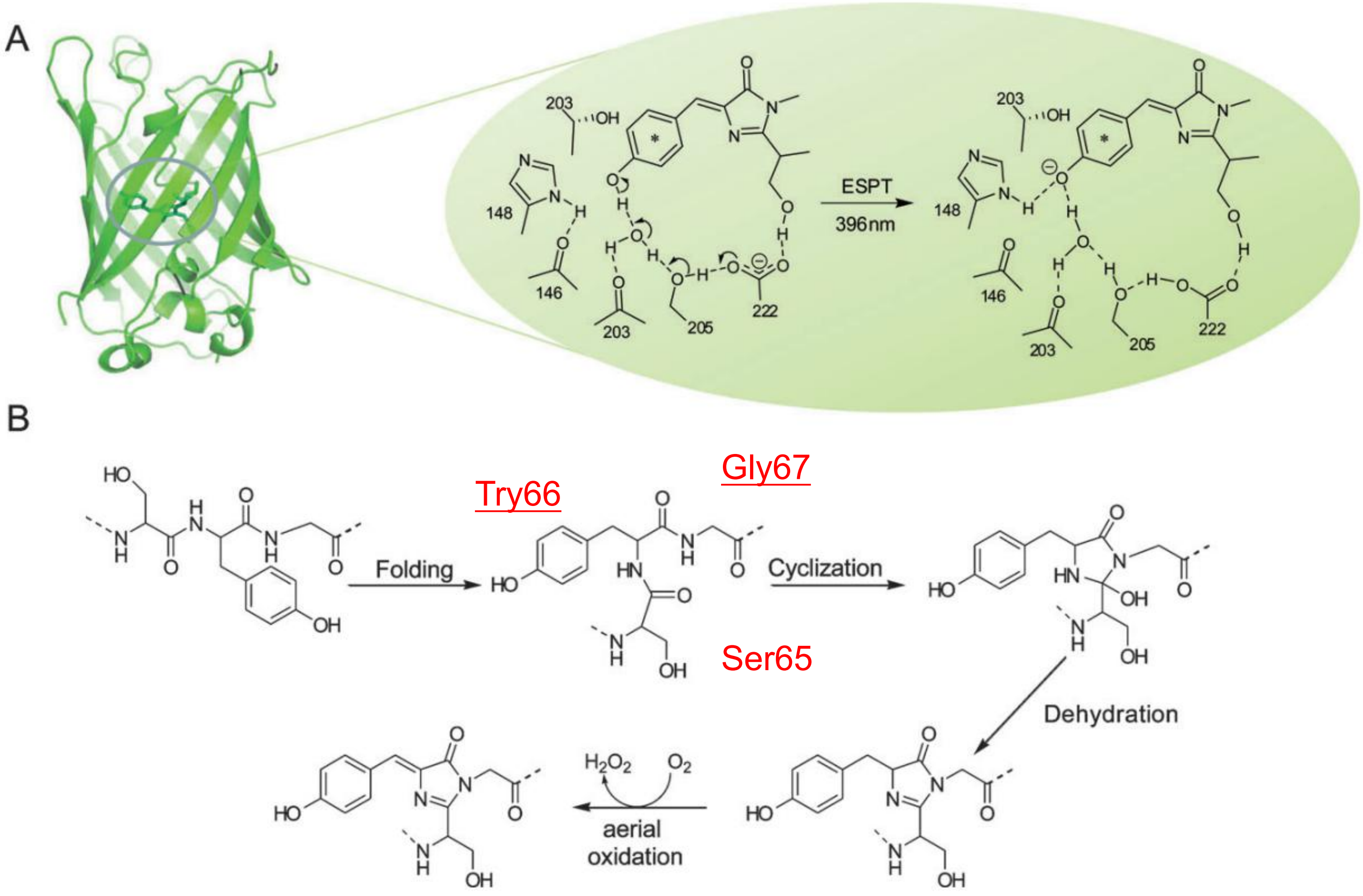
Institut de Biologie Structurale (IBS)

Fast NMR techniques in combination with other biophysical methods to kinetic events

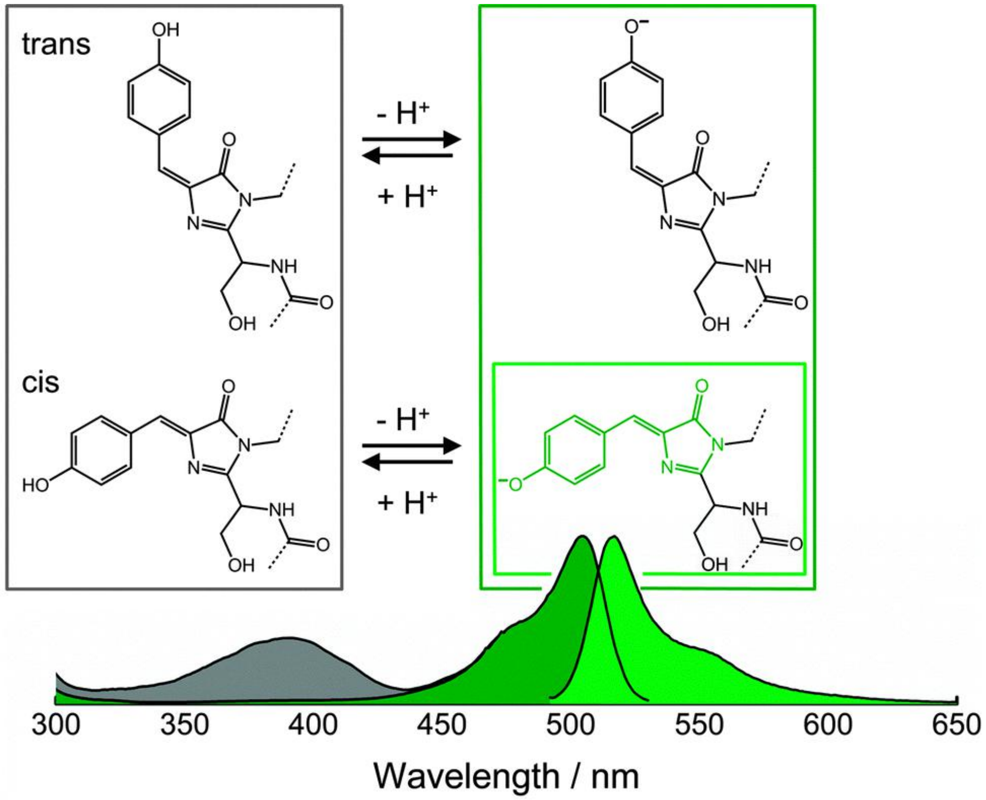
- the folding of proteins and nucleic acids;
- the structural and dynamic characterization of transiently populated excited states;
- the influence of chaperone proteins on the folding kinetics;
- light-induced protein conformational dynamics in light-sensing proteins.



Chromophore of fluorescent protein like GFP

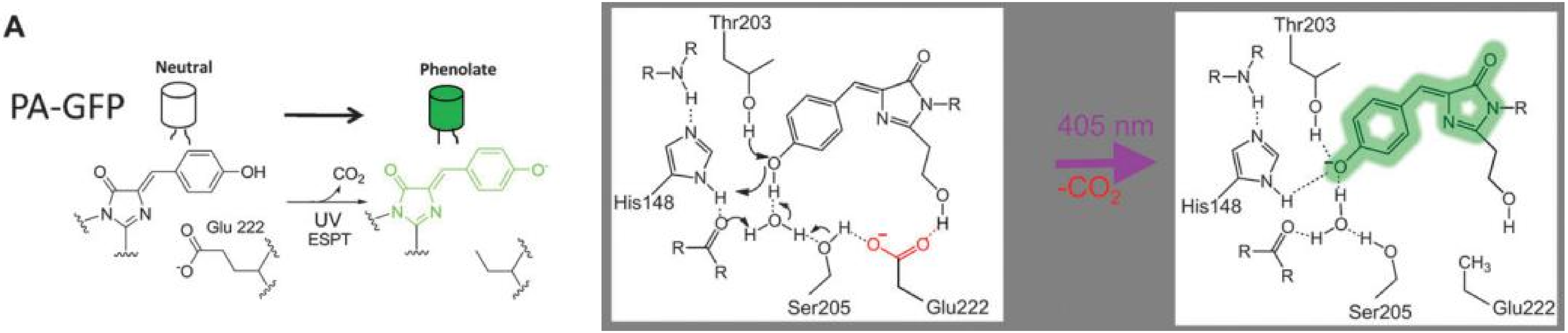


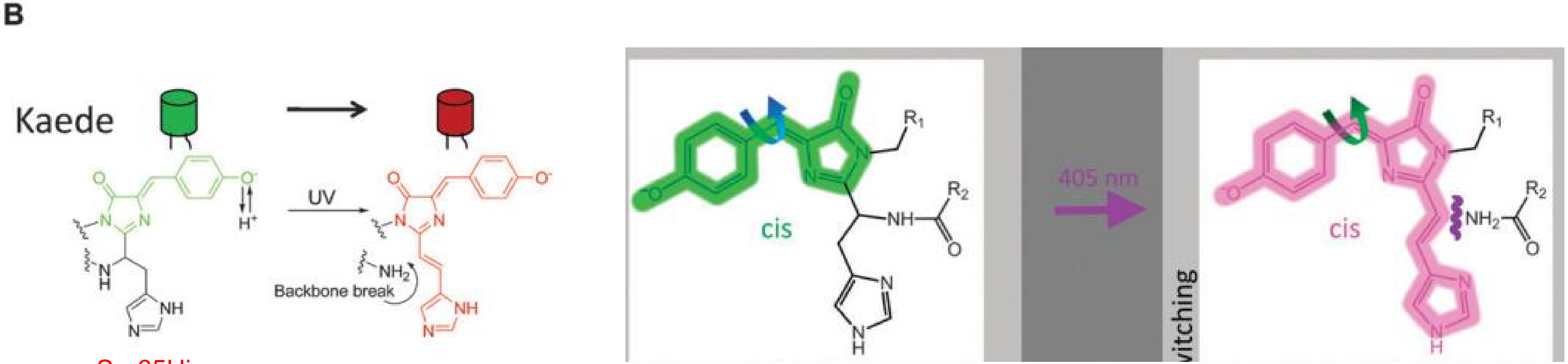
4-(**p**-**h**ydroxy**b**enzylidene)-5-**i**midazolinone **(p-HBI)**



Chem. Soc. Rev., 2009,38, 2852-2864.
Chem. Soc. Rev., 2014, 43, 1088--1106

Irreversible photoswitching in fluorescent proteins



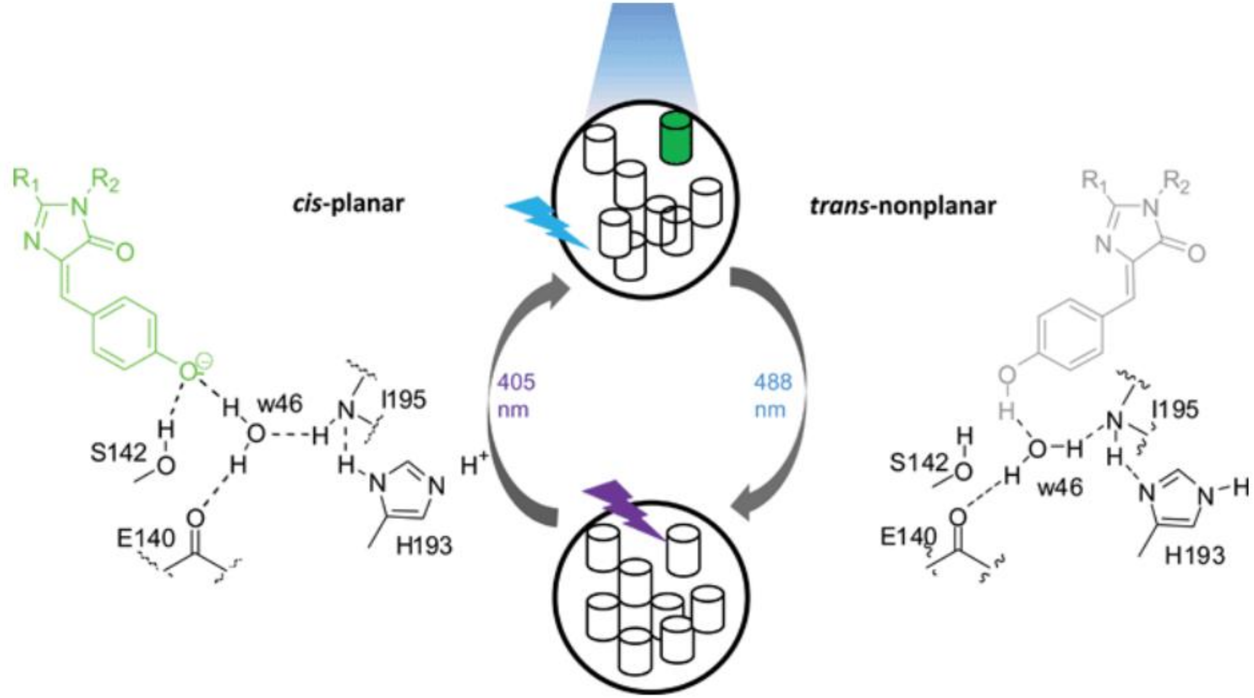


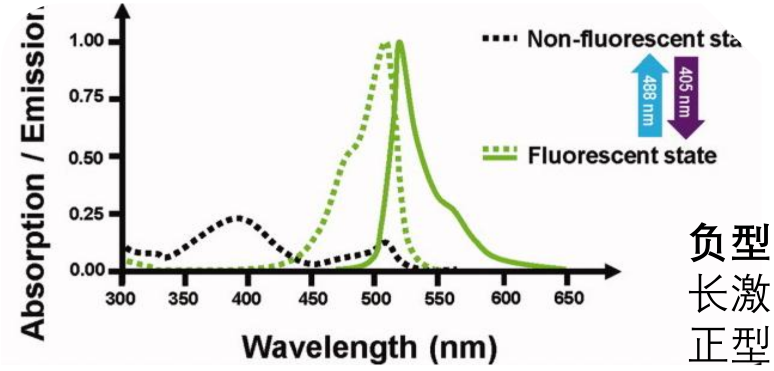
Ser65His

Chem. Soc. Rev., 2009,38, 2852-2864.
Chem. Soc. Rev., 2014, 43, 1088--1106

Reversible photoswitching in fluorescent proteins

Dronpa

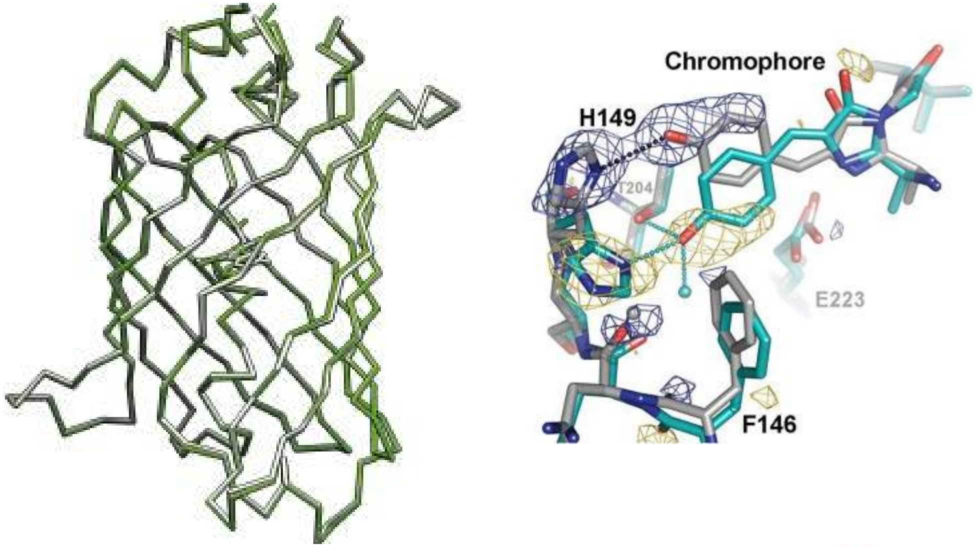


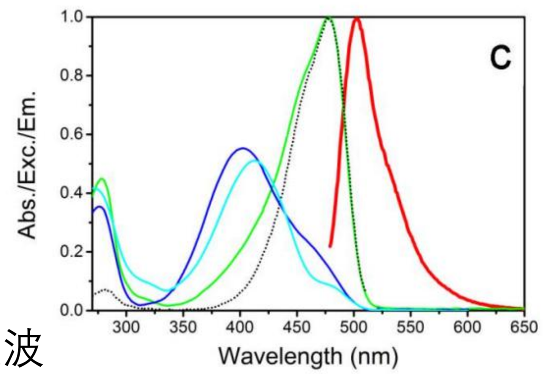


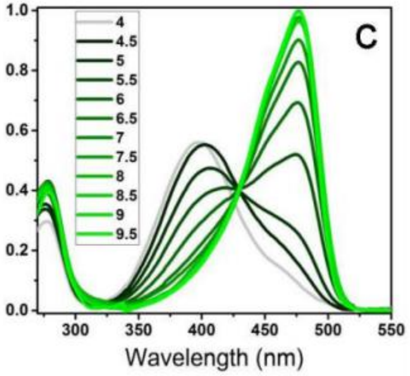
负型RSFP：由on态吸收波长激发RSFP的关闭
正型RSFP：由on态吸收波长激发RSFP的开启

rsFolder

cis-A ⇄ trans-N

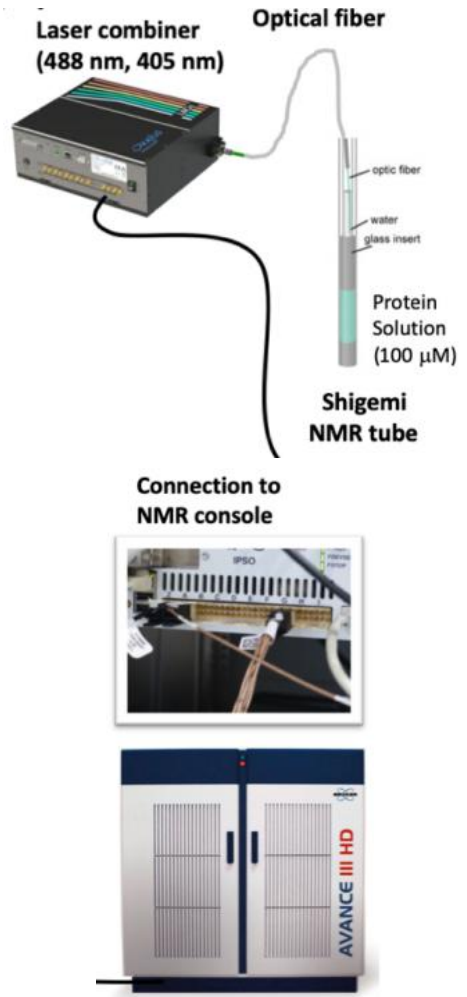


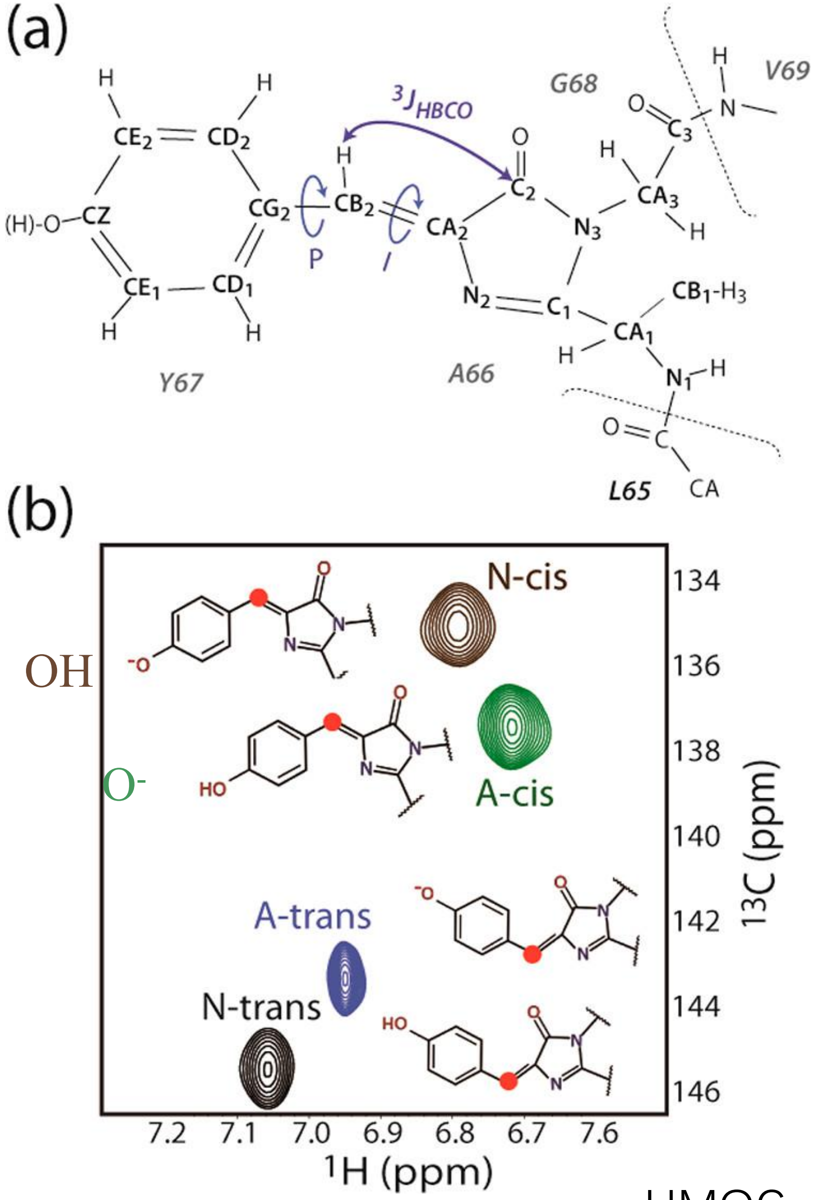




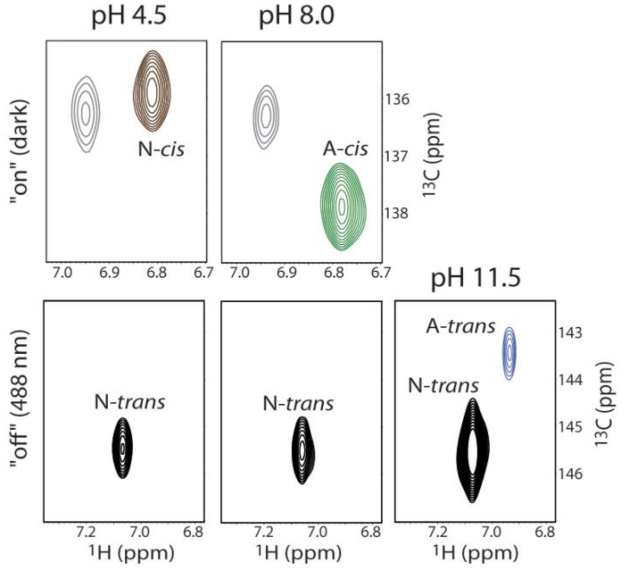
Sci Rep. 2016; 6: 18459.
Biophys J. 2019; 117(11): 2087–2100.

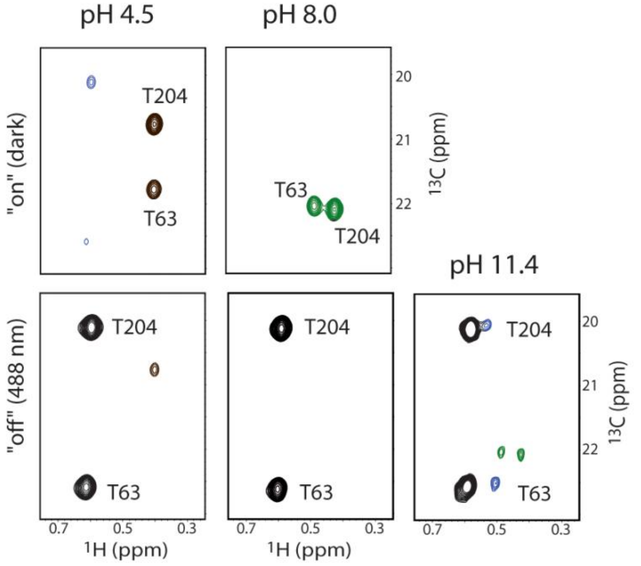
rsFolder 发色团 NMR 谱特征



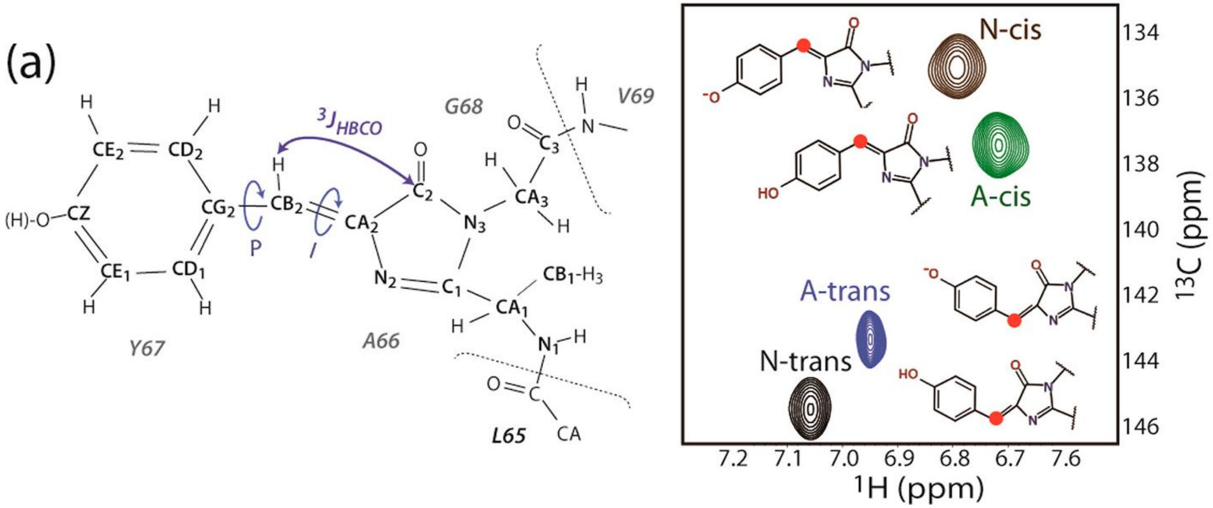


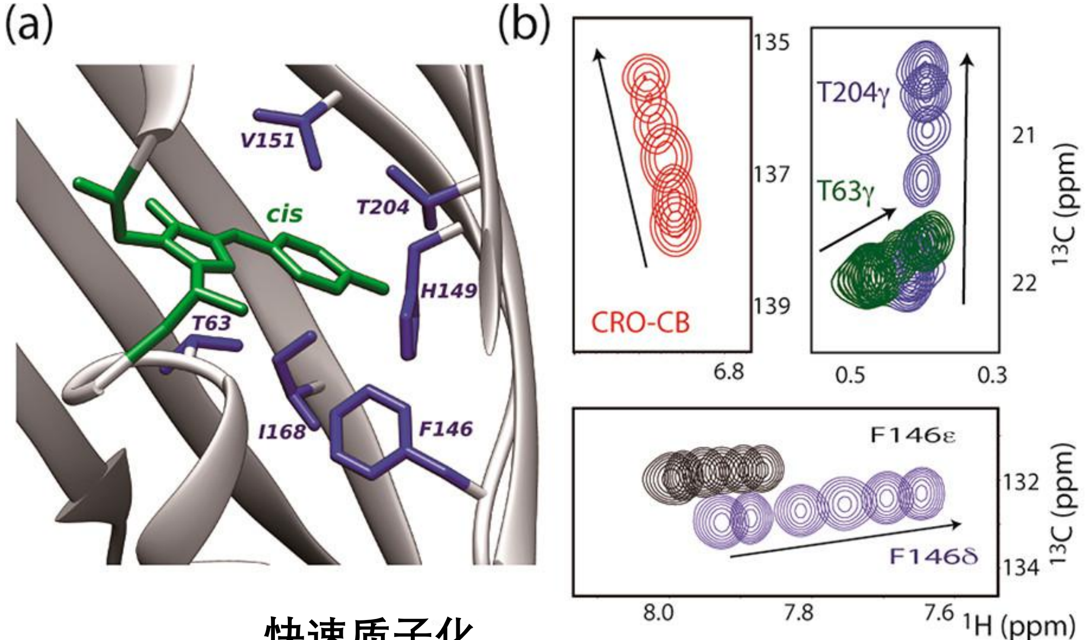
HMQC





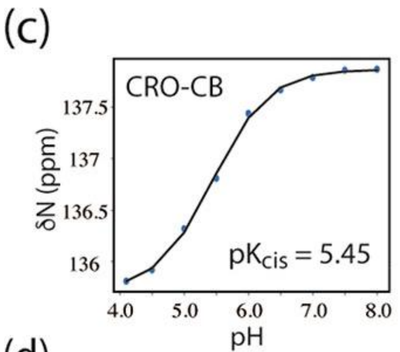
顺式发色团质子化平衡和环翻转动力学

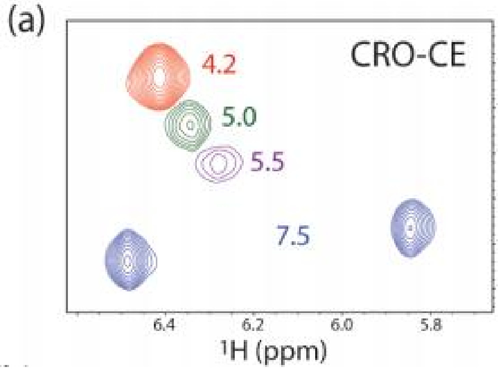




kex≫1000s^{-1} 快速质子化交换动力学

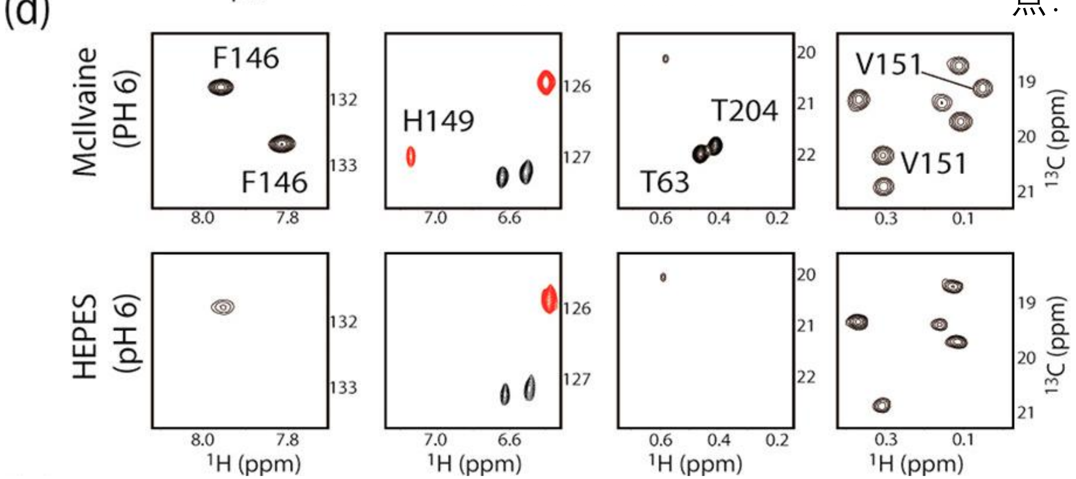
pH 7.5→4.2



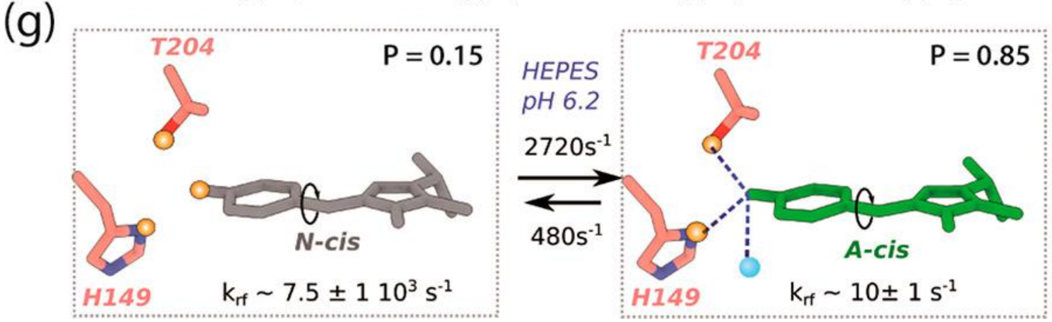


A-cis 态的环翻转动力学缓慢

化学不等价位点：CE1、CE2

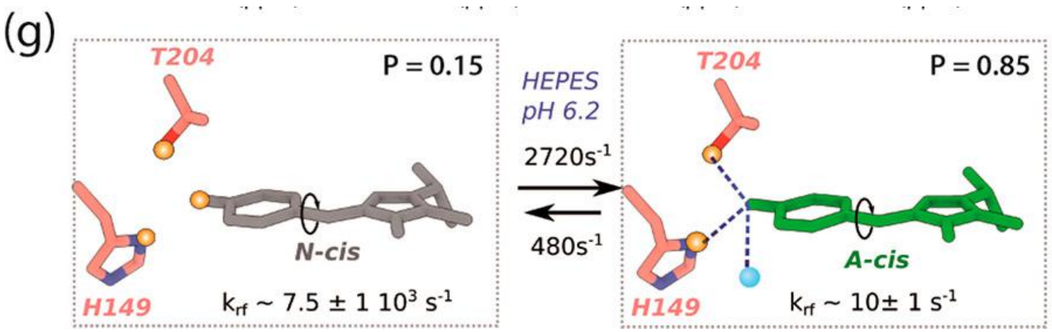


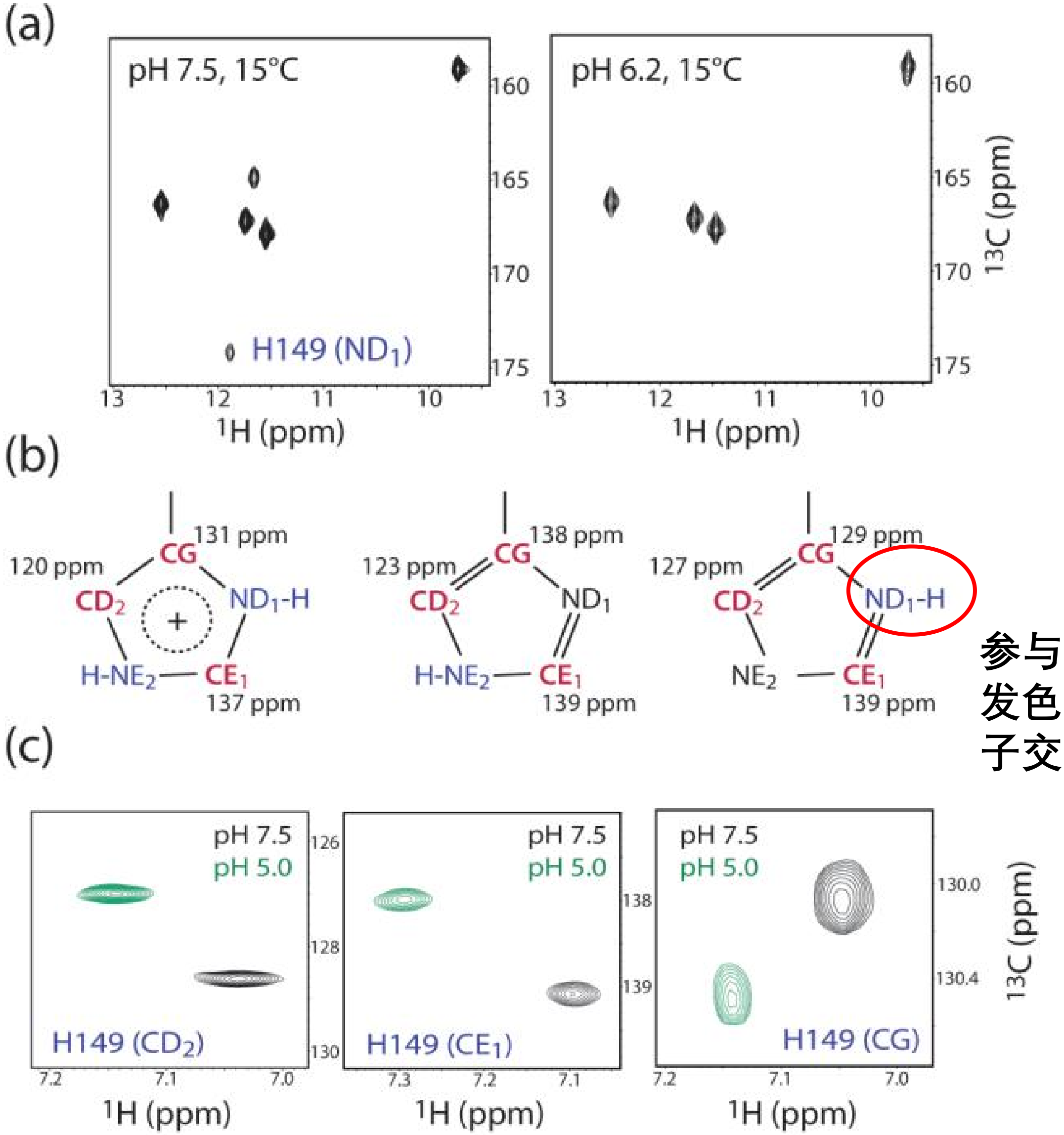
磷酸根离子加快质子化交换动力学



N-cis非荧光性

H149 在质子转移中的作用





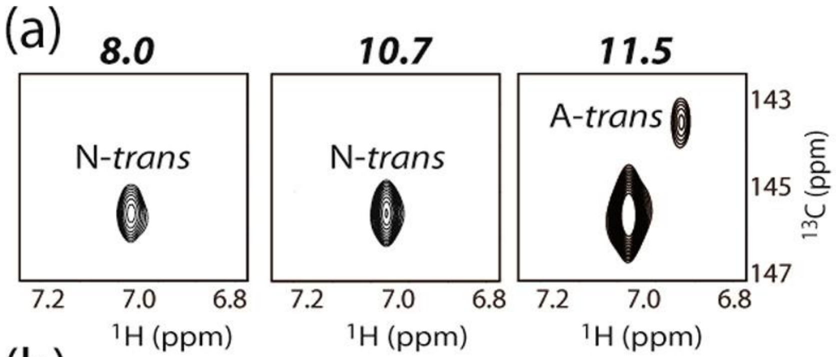
参与了顺式发色团的质子交换

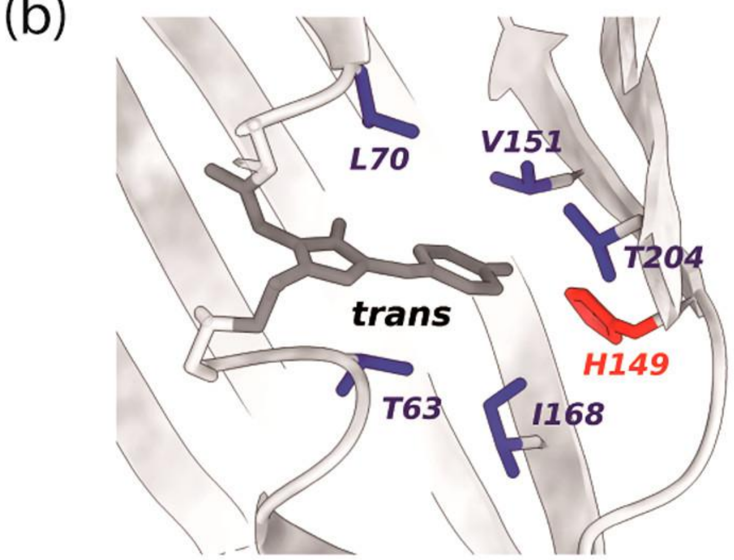
His的互变异构状态受质子转移反应途径的影响很小，主要维持在ND_1-H 构型

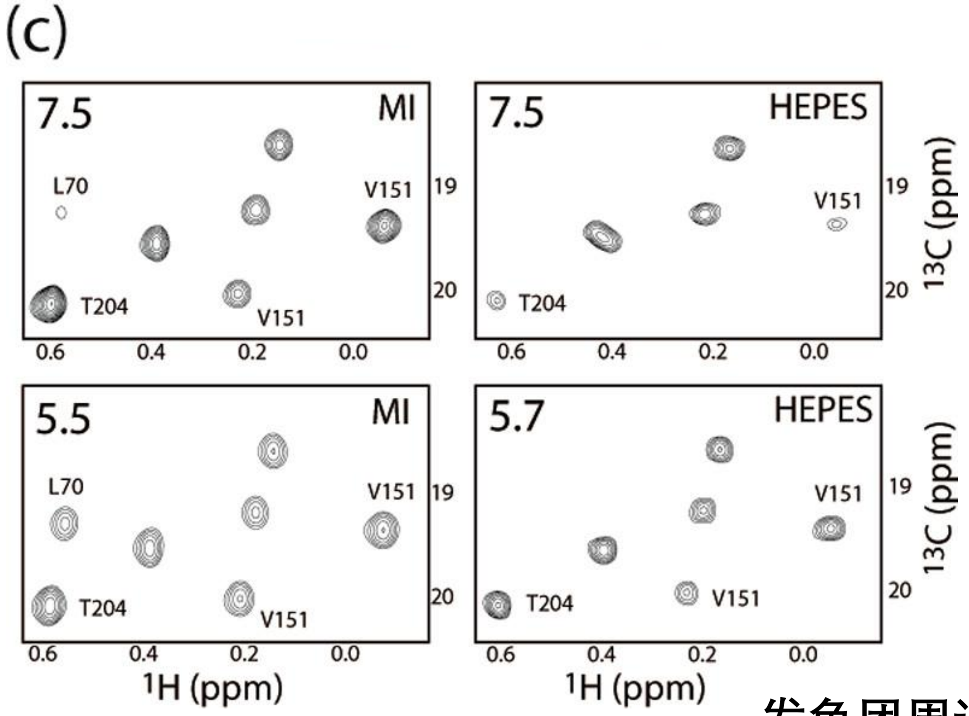
反式发色团质子化平衡和环翻转动力学

kex ≪ $1000\mathrm{s}^{-1}$

慢速质子化
交换动力学



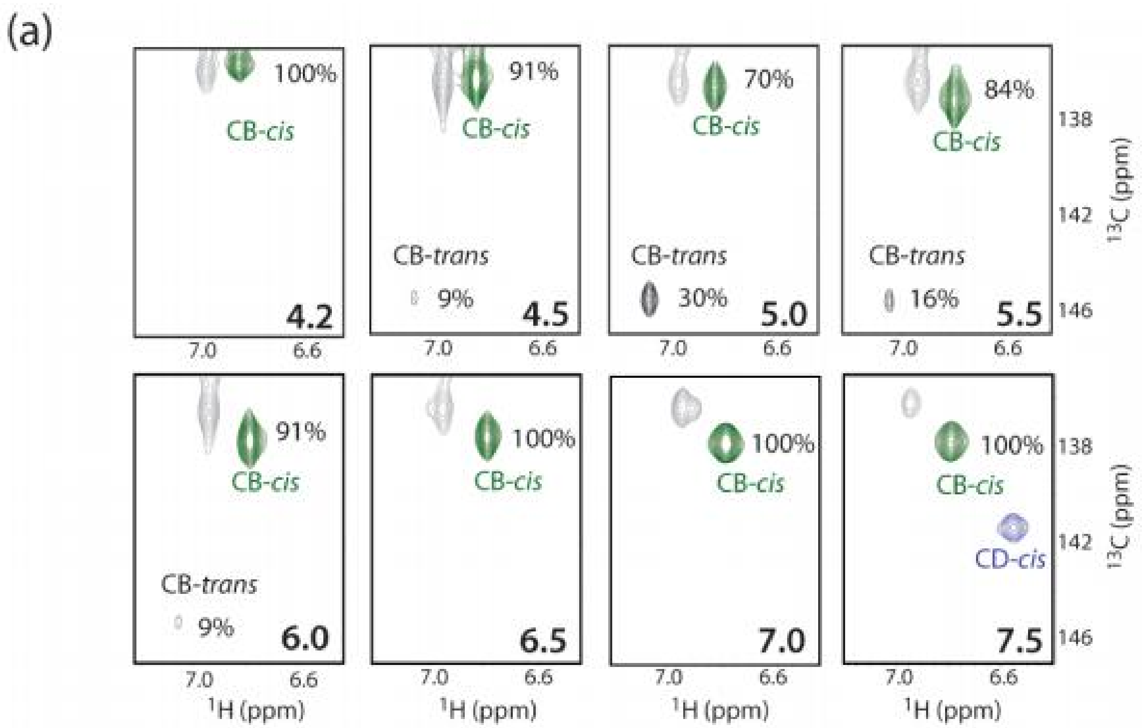


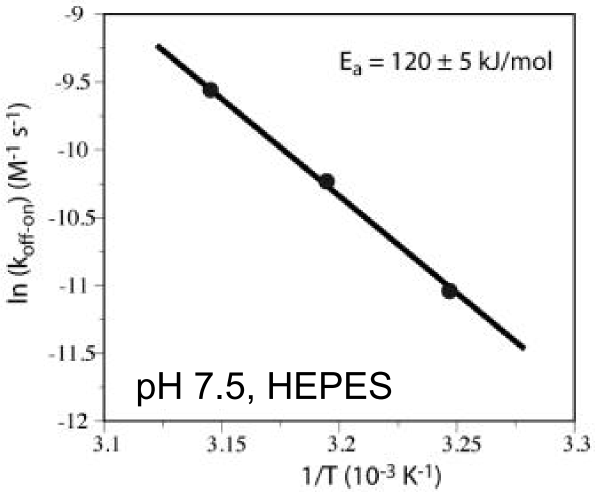


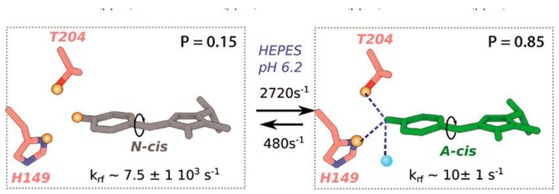
谱线展宽

发色团周边其他残
基的二次质子化

黑暗中顺反式发色团异构化的热力学和动力学

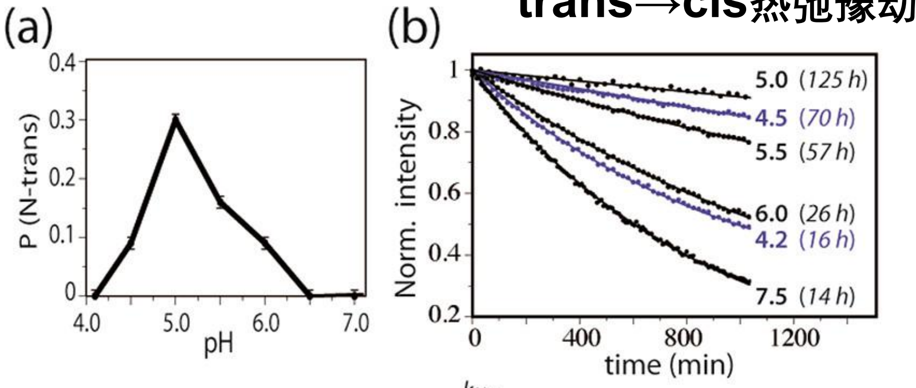




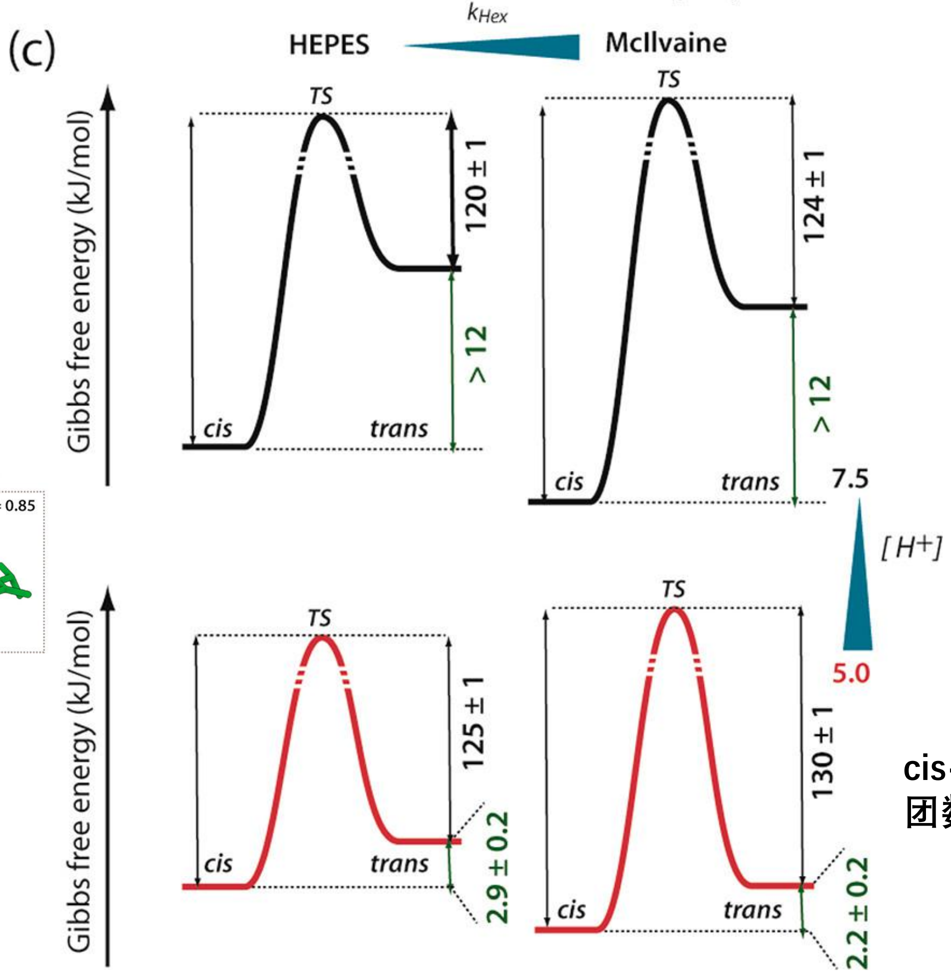


$$lnk = \frac{-E_a}{RT} + lnA$$

trans→cis热弛豫动力学

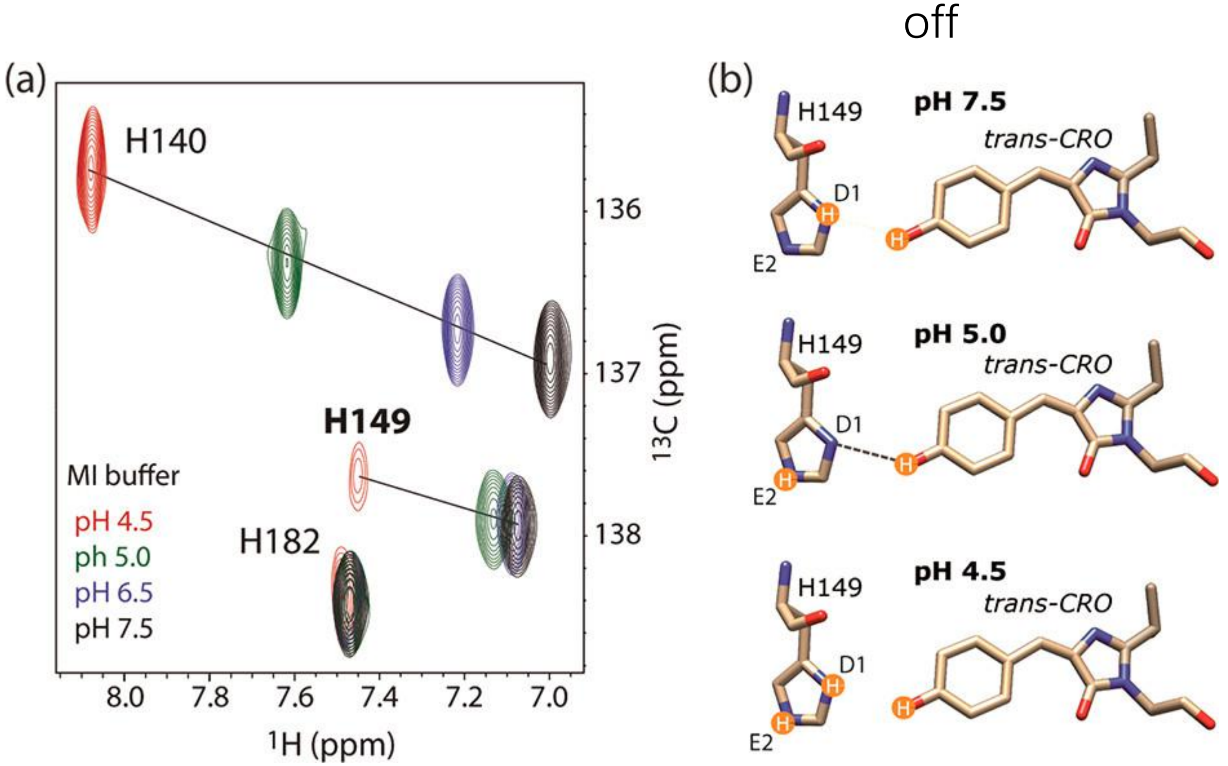


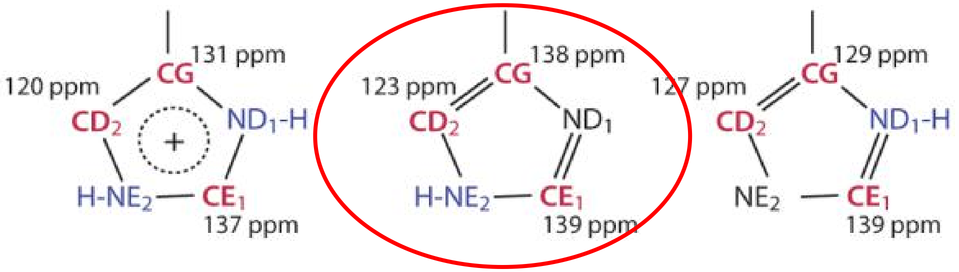
MI



cis-N发色
团数量增加

H149 对稳定反式生色团的潜在作用

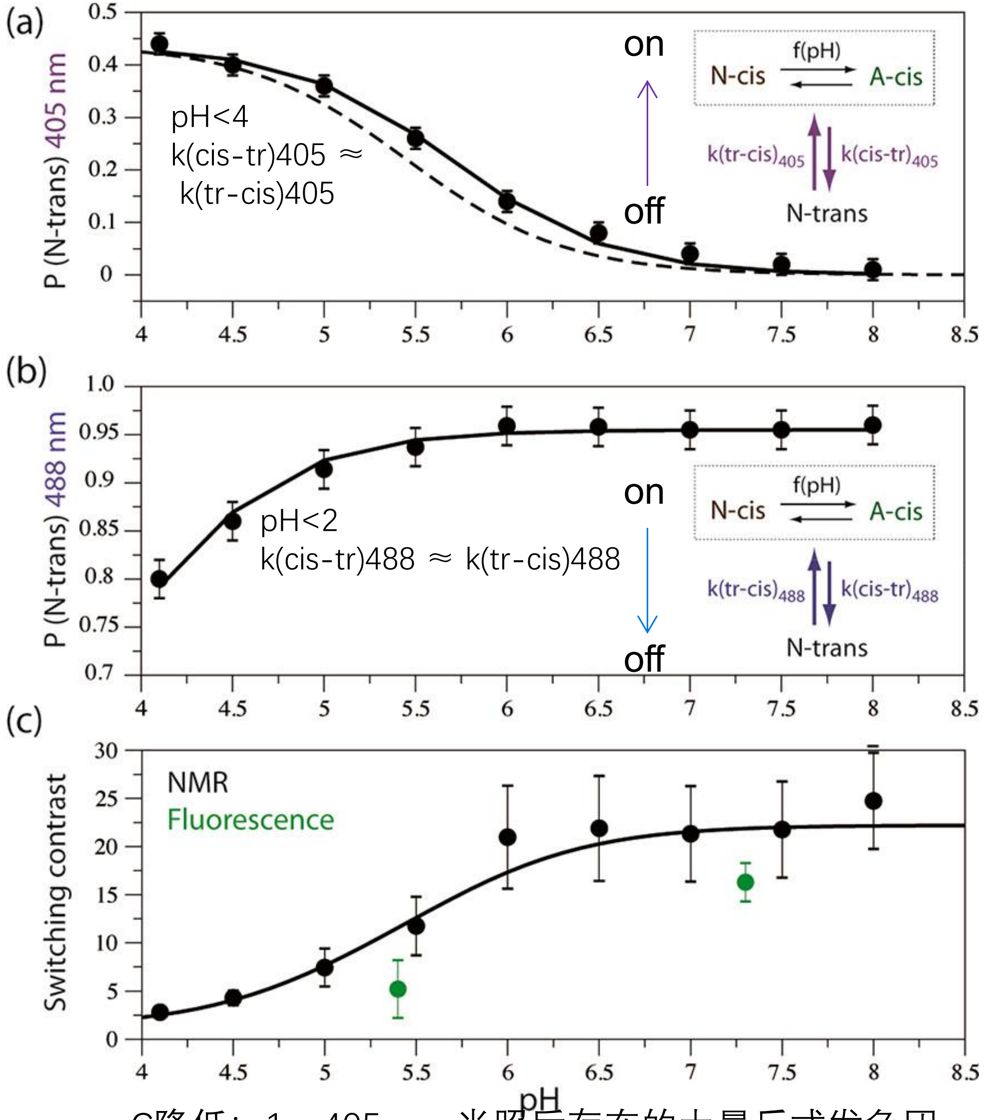




pH 5.0 时， His 形成的 NE_2-H 互变异构态更有利于与反式发色团形成 H 键使其稳定。

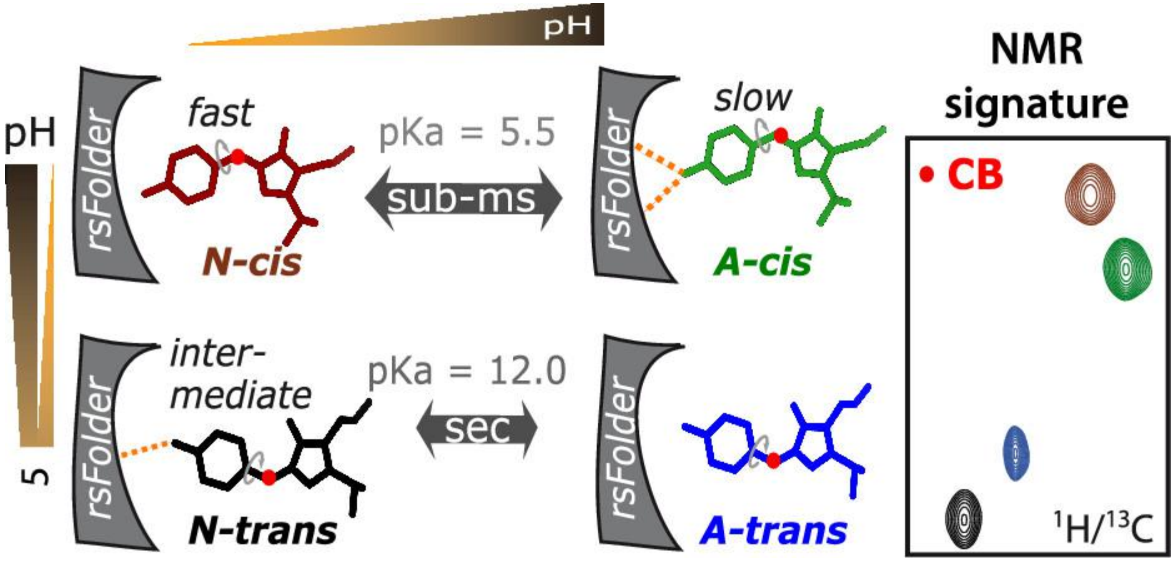
光照条件下的发色团状态群与开关对比度

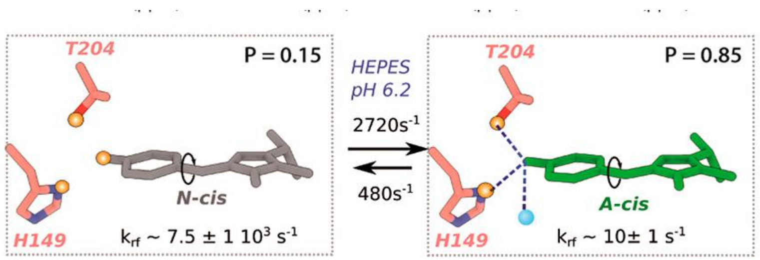
$$C = P(\text{A-}cis)_{405}/P(\text{A-}cis)_{488}.$$

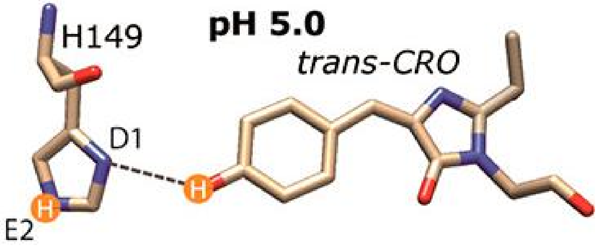


C降低：1、405 nm 光照后存在的大量反式发色团
2、488 nm 照明下的 cis→trans 转换效率较低

SUMMARY







- rsFolder的N-cis 状态由于不受氢键的限制，环翻转速率快，在光激发后经历非辐射弛豫，使其成为非荧光物质。相反，顺式阴离子发色团通过与周围残基（β桶上）的氢键作用得以稳定，导致发色团环翻转率降低，大大降低了其质子化转换的构象动力学。这种键合作用促进顺式阴离子发色团构象的稳定，表明该 H 键而是促进rsFolder提高荧光亮度的重要因素。
- 反式质子化发色团显示出依赖于 pH 的稳定性（在 pH 5 左右最为稳定），我们将其归因于与 H149 的咪唑啉环的瞬时 H 键形成，这种稳定性显著调节了黑暗下的顺反异构化，会降低光转换对比度。
- 荧光和非荧光发色团状态之间以复杂的 pH 依赖性方式变化，溶液 NMR 光谱是区分和探测光开关荧光蛋白中发色团状态和动力学变化的有力工具，帮助用来研究环境条件或蛋白质突变的影响，来合理设计具有改进特性的新近蛋白质变体。

THANKS!