染料单分子荧光调控与成像

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超分辨成像突破了光学衍射极限，重定义了非侵入式光学显微成像的分辨率边界，变革了生命科学研究范式。超分辨成像的分子定位机制根源于染料分子的开关过程，基于动态的荧光开关切换，形成在时空中孤立的分子信号，从而通过单个分子信号的解码突破物理限制。然而，传统染料研究对单分子荧光光物理性质的忽视，已成为限制超分辨成像时空分辨率和生物医学分子诊断应用的瓶颈，**单分子荧光开关的时空涨落调控更成为关键的科学问题**，其难点在于提升单分子荧光光通量（N），以强化定位准确度（不确定度，$\sigma \propto 1/\sqrt{N}$）和调制单分子开关速率($k_r$)，以匹配活细胞成像时间分辨率（$t \propto 1/k_r$）需求。

围绕超分辨成像染料单分子结构、荧光性质和应用进行研究：基于季铵哌嗪诱导设计[1]，抑制分子扭曲电荷转移态非辐射跃迁，双倍提升光通量，提升单分子时空定位精度至8.6 nm；提出单分子荧光开关动力学理论[2]，基于螺环内氢键构筑和吸电子诱导效应强化[3-4]，发展具有自适应单分子开关速率切换的染料工具，实现活细胞快速分子定位成像（时间分辨率达到2 s）；发展蛋白标签标记系统，建立分子分布和运动双维度诊断体系，实现活细胞状态分子测量诊断[5]，为自免疫难诊断疾病建立模式细胞分子指标的诊断基础。

从染料分子结构本源出发，通过取代基诱导、氢键效应开发，基于单分子荧光开关的调制探索，为分子定位超分辨成像开发系统染料工具库，将进一步促进超分辨成像技术转化为生物医学中的分子诊断工具。

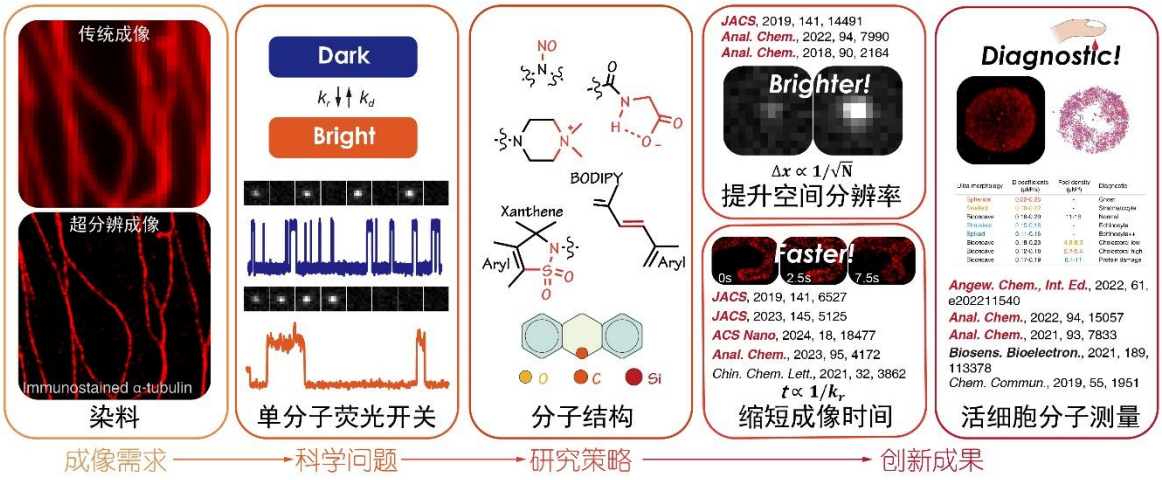

Fig. 1 Modulation of single-molecule fluorescence switching for enhaning spatial resolution and diagnostics in super-resolution imaging.

关键词：单分子荧光；单分子光物理性质；超分辨成像；荧光探针

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Dye single-molecule fluorescence modulation and imaging

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Super-resolution imaging breaks the optical diffraction limit, defines the resolution boundary of non-invasive optic microscopy, and revolutionizes the paradigm of life science researches. The mechanism of molecular localization in super-resolution imaging roots in the on-off switching of dye molecules. Through dynamic on-off transitions of fluorescence, molecule signals isolated in space and time could be individually decoded to break the physic limitations. However, traditional dye research ignores the single-molecule fluorescence photophysics prosperties, making a barrier for super-resolution imaging spatiotemporal resolution and biomedical molecular diagnostic applications. The modulation of single-molecule fluorescence switch in a spatiotemporal manner becomes the key scientific problem. The key points are improvement of single-molecule fluorescence photon flux (N) to enhance the localization accuracy (uncertainty, $\sigma \propto 1/\sqrt{N}$) and tuning single-molecule switching dynamics (k_r) to satisfy the time resolution ($t \propto 1/k_r$) required in living-cell imaging.

This speech focused on the study of dye single-molecule structure, fluorescence properties and applications: by designing quaternary piperazine induction effect[1], the molecular twisted intramolecular charge transfer and the consequent nonradiative decay is inhibited, doubly enhanced the photon flux and improved the single-molecule localization accuracy to 8.6 nm; through introduction of single-molecule fluorescence switch dynamic theory[2], adaptive switching speed dye toolboxes were developed to realize fast living-cell molecule localization imaging (time resolution up to 2 s)[3-4]; with the development of protein-tag labeling system, a molecular distribution and motion two-dimension diagnostic system was built to enable living-cell state molecular diagnostic[5], aiming for providing molecular distribution indicators for autoimmunity disease of high diagnostic difficulty.

From the basis of molecular structure, the design of substituent inductive and hydrogen-bonding capability and the exploration of single-molecule fluorescence switching modulation, a library of dye toolsets was developed for molecular localization super-resolution imaging, which would transform the super-resolution imaging techniques into biomedical molecular diagnostic tools.