CJC Chinese Journal of Chemistry 中国化学 - An International Journal

For submission: https://mc.manuscriptcentral.com/cjoc
For published articles: https://onlinelibrary.wiley.com/journal/16147065

Recent Advances

Cite this paper: *Chin. J. Chem.* **2023**, *41*, 2889—2907. **DOI: 10.1002/cjoc.202300259**

Monitoring Molecular Dynamics with Single-Molecule Electronic Devices and Fluorescence Techniques†

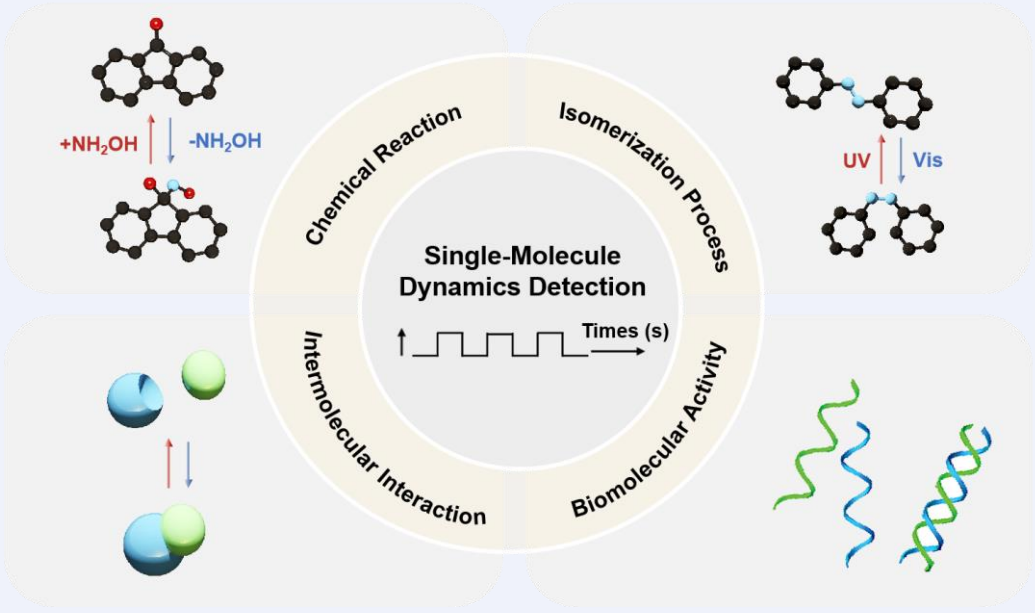
Lan Yang,[a] Zhizhuo Zhang,[a] Cong Zhao,[a] Yani Huo,[a] Enyu Zhang,[a] Suhang He,*[,a] Chuancheng Jia,*[,a,b] and Xuefeng Guo*[,a,b]

[a] *Center of Single-Molecule Sciences, Institute of Modern Optics, Tianjin Key Laboratory of Micro-scale Optical Information Science and Technology, Frontiers Science Center for New Organic Matter, College of Electronic Information and Optical Engineering, Nankai University, 38 Tongyan Road, Jinnan District, Tianjin 300350, China*
[b] *Beijing National Laboratory for Molecular Sciences, National Biomedical Imaging Center, College of Chemistry and Molecular Engineering, Peking University, 292 Chengfu Road, Haidian District, Beijing 100871, China*

Keywords

Single-molecule dynamics | Single-molecule junction | Fluorescence | Conformational change | Reaction mechanisms | Enzymes | Host-guest systems | Molecular electronics

Comprehensive Summary



Monitoring the dynamics of a single molecule provides unique insights into the fundamental physical and chemical properties of individual molecules. Over the past few decades, various approaches have been developed to enable the real-time and high-resolution detection at the single-molecule level. Among them, electrical and optical methods are the most promising tools, for the reason that electrical detection offers high resolution while optical technology provides non-invasive, targeted measurement with the added benefit of visualization. In this review, we summarized the current state-of-the-art electrical and optical techniques for single-molecule measurement and discussed their applications in detecting dynamic events such as conformational isomerizations, intermolecular interactions, chemical reactions, and biomolecular activities. In addition, we discussed the challenges and opportunities in this area and proposed possible directions for future development.

*E-mail: guoxf@pku.edu.cn; jiacc@nankai.edu.cn; phengites@163.com
† Dedicated to the Special Issue of Single-Molecule Measurement and Imaging.

Left to Right: Lan Yang, Zhizhuo Zhang, Cong Zhao, Yani Huo, Enyu Zhang, Suhang He, Chuancheng Jia, and Xuefeng Guo

Lan Yang is currently a Ph.D. candidate in Center of Single-Molecule Sciences, College of Electronic Information and Optical Engineering, Nankai University, with Prof. Chuancheng Jia and Prof. Xuefeng Guo. Her research is focused on single-molecule devices and dynamics.

Zhizhuo Zhang is now studying for his master's degree under the supervision of Prof. Chuancheng Jia and Prof. Xuefeng Guo in Center of Single-Molecule Sciences, College of Electronic Information and Optical Engineering, Nankai University. His research is focused on single-molecule devices and dynamics.

Cong Zhao is currently a Ph.D. candidate in Center of Single-Molecule Sciences, College of Electronic Information and Optical Engineering, Nankai University, with Prof. Chuancheng Jia and Prof. Xuefeng Guo. Her research is focused on single-molecule devices and dynamics.

Yani Huo is currently a Ph.D. candidate in Center of Single-Molecule Sciences, College of Electronic Information and Optical Engineering, Nankai University, with Prof. Chuancheng Jia and Prof. Xuefeng Guo. Her research is focused on single-molecule devices and dynamics.

Enyu Zhang is currently a Ph.D. candidate in Center of Single-Molecule Sciences, College of Electronic Information and Optical Engineering, Nankai University, with Prof. Chuancheng Jia and Prof. Xuefeng Guo. His research is focused on single-molecule devices and dynamics.

Suhang He is a visiting scholar in Center of Single-Molecule Sciences, Nankai University. She studied for a Ph.D. in the group of Prof. Xiaohai Zhou at Wuhan University and carried out postdoctoral research in the group of Prof. Werner Nau at Constructor University from 2019. Her research focuses on supramolecular chemistry and supramolecular electronics.

Chuancheng Jia received his Ph.D. degree in 2014 from the College of Chemistry and Molecular Engineering, Peking University, with Prof. Xuefeng Guo. From 2014 to 2020, he was a postdoc at the Institute of Chemistry, Chinese Academy of Sciences and University of California, Los Angeles. He joined the faculty as a professor at Nankai University in 2020. His research is focused on single-molecule devices and dynamics.

Xuefeng Guo received his B.S. degree in 1998 from Beijing Normal University and his Ph.D. degree in 2004 from the Institute of Chemistry, Chinese Academy of Sciences, Beijing. From 2004 to 2007, he was a Postdoc at the Columbia University Nanocenter. He joined the faculty as a Professor under the Peking '100-Talent' Program at Peking University in 2008. His current research is focused on single-molecule science and technology.

Contents

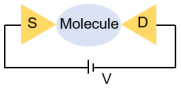
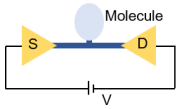
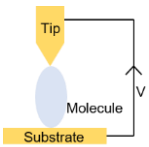
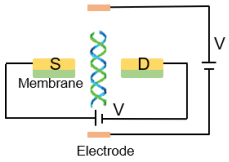
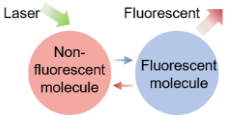
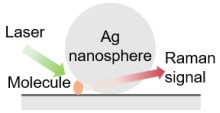
1. Introduction

The development of modern science and technology has facilitated the extension of both nanoscience and life science to the single-molecule scale. Single-molecule dynamics refer to the physical or chemical change of a single molecule, including conformational change, and structural changes induced by intramolecular, intermolecular interaction, or upon chemical reactions. The objective of single-molecule dynamics research is to probe the molecular behavior at the molecular level, thereby disclosing fundamental physical and chemical properties that are concealed in macroscopic experiments.[1-3]

To achieve this goal, a diverse range of single-molecule technologies have been developed and can be classified into two main categories. The first category is electrical-based approaches, which typically involve single-molecule junctions (SMJs),[2,4-6] one-dimensional-nanomaterial-based transistors,[7] scanning probe microscopes (SPM),[8-10] and nanopore sensors.[11-13] The second category is based on single-molecule optical detection, including single-molecule fluorescence spectroscopy[3,14-16] and single-molecule Raman scattering spectroscopy[17-19] (Table 1). This review predominantly focuses on the technologies that facilitate the prolonged monitoring of individual molecular dynamics processes within the *in situ* reaction environment in the liquid phase, specifically, single-molecule junctions (particularly graphene-based single-molecule junctions), one-dimensional-nanomaterial-based transistors and single-molecule fluorescence spectroscopy. While other electrical and optical technologies, such as surface probe microscopes (SPMs),[8-10] nanopore sensors,[12-13] and surface Raman scattering spectroscopy,[18-19] have also achieved significant advancements in recent years, they will not be covered in this review.

In electrical-based approaches (Figure 1a), the molecular dynamic process can be monitored by measuring current fluctuations, resulting from different conductivity values of various molecular states. The details of the molecular dynamic process can

Table 1 Comparison of different single-molecule detection technologies

Platform	Schematics	Advantages	Ref.
Single-molecule junctions (SMJs)		Applying electric fields and injecting electrons; multiple external control methods; high compatibility under different working conditions	2,4,5,6
One-dimensional-nanomaterial-based transistors		Multiple external control methods; integrating with other techniques; real-time measurement of currents	5,6
Scanning probe microscope		Dynamic processes are triggered by thermal annealing, light irradiation, tunnelling electrons or electric fields	8,9,10
Nanopore sensors		Able to continuously capture single molecules at a relatively high rate; DNA and RNA sequencing with long reads	12,13
Single-molecule fluorescence spectroscopy		Robust reaction conditions; multiple environmental control methods	3,14,15,16
Single-molecule Raman scattering spectroscopy		Multiple environmental control methods; provides vibrational signatures of chemical bonds	18,19

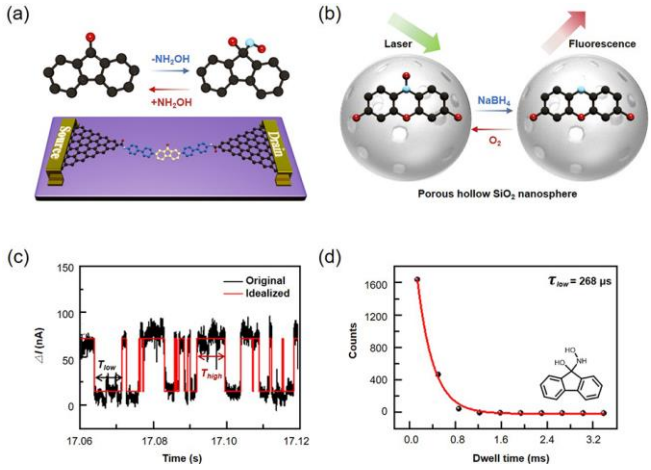


Figure 1 Detection methods and analysis methods of single-molecule dynamics. (a) Schematic diagram of single-molecule electrical methods. Reprinted with permission,[39] Copyright 2018, American Association for the Advancement of Science. (b) Schematic diagram of single-molecule optical detection. Reprinted with permission,[4] Copyright 2022, Springer Nature Limited. (c) Theoretical fitting of the current-time curves with two distinguishable states using a QuB software. (d) Mean duration time of the low-conductance states achieved by plotting and fitting the dwell times in step. Reprinted with permission,[39] Copyright 2018, American Association for the Advancement of Science.

then be deciphered using analysis method specially designed for single-molecule dynamics. In optical-based approaches (Figure 1b), detection of the optical signals emanating from a single fluorescent molecule necessitates the use of optical microscopes such as total internal reflection, confocal, and super-resolution microscopes. The optical signals garnered from these technologies provide a diverse array of multi-dimensional data pertaining to single-molecule dynamics and encompassing temporal, spectral, and spatial dimensions.[20] The two classes of single-molecule technologies offer unique insights into the dynamics of individual molecules from distinct perspectives. Single-molecule electrical technologies enable the detection of single-molecule events in real time with exceptional precision *in situ*, while single-molecule optical technologies possess benefits like high sensitivity, target specificity, and two-dimensional image metrology. Therefore, integration approaches that merge the benefits of both electrical and optical methodologies are gaining increasing popularity.

In this review, we provided an overview of single-molecule technologies, with a focus on graphene-based single-molecule junctions, one-dimensional nanomaterial-based transistors (*e.g.*, single polymer chains, carbon nanotubes, and silicon nanowires), and optical-based single-molecule technologies, particularly fluorescence. The recent advancements in these technologies have been highlighted. Additionally, we discussed integrated systems that combine both optical and electrical methods, and their potential applications in studying single-molecule dynamics.

2. Signal Analysis and Kinetic Parameters Extraction

Ample information about molecular dynamics can be obtained by electrical and optical monitoring of single-molecule dynamic processes, hence how to deal with a large number of signals associated with a particular reaction is essential. Since the time average is generally equal to the overall average, basic quantities can be redefined from the time domain.[4] For example, in real-time electrical measurement based on single-molecule junctions, switching between two or more states can produce a significant number of reproducible current fluctuations. The current value is initially analyzed by a one-dimensional histogram, yielding

a bimodal or multimodal current distribution that corresponds to the different structural characteristics of the molecule (Figure 1c). Next, the dwell time of each state can be extracted from the real-time conductivity curve (Figure 1d) using a QuB software.[21] Finally, the distribution of time can be fitted using a single exponential function:

$$P(\tau) = \frac{1}{\langle\tau\rangle}\exp\left(-\frac{\tau}{\langle\tau\rangle}\right).$$

Here, the mean duration time $\langle\tau\rangle$ can be determined by using the single exponential function, which can be further employed to calculate other reaction kinetic parameters. For example, the activation energy can be obtained through the Arrhenius equation:

$$K_{\mathrm{T}} = A(T)\exp\left(\frac{-E_{\mathrm{a}}}{RT}\right).$$

where K_{T} is the reciprocal of $\langle\tau\rangle$, $A(T)$ is the pre-exponential factor, R is gas constant and T is the temperature. The energy difference (ΔE) between two states can be derived according to Boltzmann statistics:[22-23]

$$\Delta E = -RT\ln\frac{\langle\tau_1\rangle}{\langle\tau_2\rangle}.$$

The Gibbs' free energy,[24] ΔG^0, can be obtained by:

$$\Delta G^0 = -RT\ln K^0,$$

where equilibrium constant K^0 should be redefined at the single-molecule level. In the case of first-order reactions, such as enzyme catalysis, it has the following characteristics: $\Delta G = \Delta E$, $K^0 = k_1/k_2$. In second-order reactions, such as intermolecular interactions, an extra correction is required on account of the dispersed reactant concentrations. In this case, according to the Langmuir isotherm model, the equivalent equilibrium constant K is corrected as:

$$K = \frac{\alpha}{1-\alpha}C,$$

where α is the proportion of one stable state of the immobilized molecule, and C refers to the concentration of other interacting molecules dispersed in solution.[25] In addition, enthalpy (ΔH) and entropy (ΔS) of the single-molecule reaction can be derived from:

$$\Delta G = \Delta H - T\Delta S.$$

In general, this method can determine the average duration of the transitional state and r kinetic parameters of the reaction for each pivotal step. It is worth mentioning that the parameters derived from this method are consistent with the theoretical computations,[7] which validates the trustworthiness of this methodology.

3. Single-Molecule Dynamics Based on Single-Molecule Junctions

Over the past decades, several fabrication methods for creating single-molecule junctions have been established. These methods include mechanically controlled break junction (MCBJ),[26-27] scanning-tunneling-microscope break junction (STM-BJ),[28-29] electromigration,[30-31] and lithography-defined cutting-based single-molecule junction. Through these techniques, it is possible to translate molecular state information into observable electrical signals, providing an exceptional platform for exploring the intrinsic characteristics of single molecules which are not accessible in ensemble-level experiments. MCBJ and STM-BJ have been utilized to investigate the dynamics of individual molecules.[32-35] However, these dynamic junctions have relatively short residence times, leading to information obtained that is primarily governed by statistical laws. Even when hovering mode is employed in STM-BJ, the duration is limited to a few hundred milliseconds (< 1 s). In contrast, graphene-based single-molecule junctions, fabricated through lithography-defined cutting, allow for the *in situ* monitoring of a molecule's properties for an extended period. This enables the acquisition of more comprehensive and detailed information. Besides, this approach is noteworthy because graphene is a two-dimensional carbon nanomaterial that has excellent stability and natural compatibility with biomolecules. Our group has developed Dash-line lithography (DLL) to fabricate graphene point contact arrays functionalized with carboxylic acid terminals.[36] The nanometer-scale gaps between these arrays serve as bridging sites for amino group-containing target molecules, forming stable graphene single-molecule junctions (SMJs) through amide linkages. These graphene SMJs can be measured *in situ* for extended periods. Notably, the size of the nanogaps can be precisely controlled through oxygen plasma etching, allowing only molecules with the same length as the nanogaps to be connected to the graphene electrodes. This ensures that kinetic information is detected at the single-molecule scale.[1,37] Thus, this technology can be used to study chemical reactions across a broad environmental range, spanning from room temperature to cryogenic temperatures, as well as from high vacuum to liquid phase.[4]

Graphene-based single-molecule junction presents a promising platform for real-time monitoring and analysis of single-molecule dynamics. Such an approach can offer valuable insights into the transient and intermediate states of chemical reactions, as well as the stochastic processes underlying them, by converting these events into detectable electrical signals. Furthermore, this technique enables external stimuli, such as light, chemicals, temperature, magnetic field, or electric fields, to be applied for regulating the reactivity and selectivity of chemical reactions. In this section, we present a detailed account of the research conducted on single-molecule dynamics using graphene-based single-molecule junctions, with a particular focus on the *in-situ* electrical detection of conformational changes, molecular interactions, and chemical reaction processes.

3.1. Monitoring chemical reactions with SMJs

In the realm of chemical reaction mechanisms, the acquisition of precise pathways through macroscopic experiments is typically challenging. One promising approach is by integrating the substrate structures into a graphene-based single-molecule junction to analyze the electrical signals which are caused from the molecular structural change and other reaction related events. With the approach, a wide range of chemical reactions, including nucleophilic, rearrangement, and catalytic reactions, have been studied.

The nucleophilic substitution reaction with a S_N1 pathway is a two-step process which comprises the heterolysis of the reactant, resulting in the formation of a carbocation intermediate and the subsequent recombination process in which the carbocation intermediate combines with a nucleophile to generate the product. Monitoring the nucleophilic substitution reaction can be achieved using an electrical platform based on graphene-based SMJs (Figure 2a).[38] To create stable graphene-molecule-graphene SMJs, a molecular wire containing a functional center of 9-phenyl-9-fluorenol was covalently linked to the graphene electrode nanogaps. In this manner, any reaction related molecular structural change will cause fluctuations in the current, and can be recorded in the form of electrical signals. This method provides a real-time kinetical monitoring of the formation of individual carbocation intermediates with strong solvent dependence in nucleophilic substitution reactions.

Graphene-based SMJs have also shown the potential for monitoring the nucleophilic addition reaction. Specifically, a junction was constructed by covalently connecting a molecular wire containing 9-fluorenone as the functional center and amino groups on both ends, to graphene electrodes.[39] Real-time single-molecule electrical measurements were used to monitor the reversible reaction process during the nucleophilic addition of NH_2OH to the carbonyl group in solution. This resulted in two distinct signal

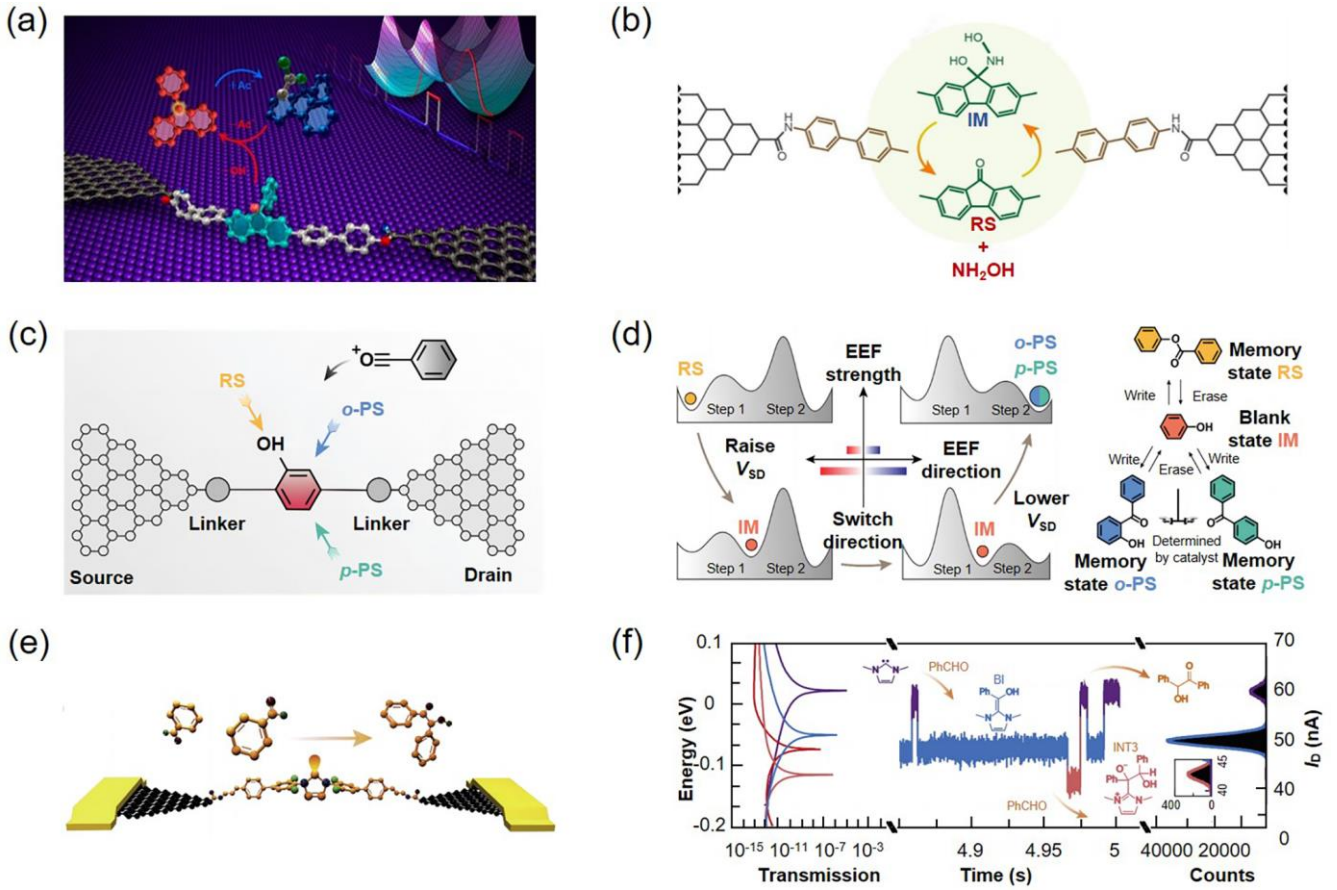


Figure 2 Detection of chemical reactions based on single-molecule junction. (a) Schematic diagram of GMG-SMJs that shows the formation dynamics of 9-phenyl-9-fluorenyl cations in a S_N1 reaction. Reprinted with permission,[38] Copyright 2018, American Chemical Society. (b) Schematic diagram of a single-molecule model reaction to detect solvent effects. Reprinted with permission,[40] Copyright 2021, American Chemical Society. (c) Schematic diagram of the resistance switching via single-molecule Fries rearrangement. (d) Regulation of the energy profile and manipulation of the species by EEFs (external electric fields) and Schematic of the write-erase progress on single-molecule memristors. Reprinted with permission,[41] Copyright 2022, Wiley-VCH GmbH. (e) Schematic diagram of single-molecule electrical platform to monitor the homo-condensation. (f) Three relative stable structures derived from NHC catalysts during the Benzoin reaction. Reprinted with permission,[42] Copyright 2021, Elsevier Inc.

oscillations within a time scale of a few microseconds. Catalyzed in a basic solution, the reaction proceeded through the nucleophilic addition of NH_2OH to the carbonyl group of 9-fluorenone (reactant state (RS)) to form a shallow intermediate state (IS') via a transition state. IS' was then transformed to a stable intermediate state (IS) in water by fast proton transfer. Both experimental results and theoretical simulations indicate that the high and low conductance states are attributed to the corresponding RS and IS. Furthermore, single-molecule measurements revealed that the nucleophilic addition was highly solvent-dependent, which may be applied to study solvent effects. For example, a single-molecule electrical nanocircuit was used to directly detect the microscopic heterogeneity of the solution and compare interactions in solvent networks (Figure 2b).[40] Experimental results showed that RS and intermediate (IM) have opposite solvent polarity preference, with IM tending to be stable in high-polarity solvents while RS tends to be stable in low-polarity solvents. As solvent polarity increased, the dwell time τ (kinetics) of IM gradually increased and contributed a major proportion of the conductance states (thermodynamics).

The Fries rearrangement, which involves the reconfiguration of the acyl moiety of the phenol center, can be controlled by applying an external electric field (EEF) to a covalently integrated phenol molecule in the nanogap between graphene electrodes (Figure 2c).[41] The rearrangement event could be split into two simple elementary reactions: the conversion of the phenyl benzoate state (RS) to the phenol intermediate (IM), and the generation of the product state from IM (Figure 2d). Research shows that a positive external electric field can accelerate step 1 by lowering the energy barrier, whereas step 2 is inhibited. The strength of EEF also modulates the occupancy of RS and IM in step 1. Conversely, a negative EEF preferentially facilitates the reaction in step 2. Thus, this single-molecular platform can work as a useful tool to separately control each elemental step of a multi-step reaction.

In another study, the efficacy of *N*-heterocyclic carbene (NHC) catalyzed benzoin reaction was analyzed through single-molecule electrical spectroscopy (SMES).[42] In this approach, individual *N*-heterocyclic carbene (NHC) catalysts are covalently sandwiched between graphene electrodes to form single-molecule nanocircuits, which resulted in unprecedented SEMS (Figure 2e). One of the significant advantages of SMES is its high temporal resolution and the reliable synchronicity between the electrical signals and the structural changes in the catalysts. Specifically, real-time current fluctuations at a constant bias voltage can be used to decipher changes in the NHC catalyst structure. The energy profile showed that NHC has the highest corresponding current level owing to its close proximity to the graphene Fermi level of perturbed frontier molecular orbitals, followed by Breslow intermediates (BI), the first addition intermediate (pre-BI), and finally, the second addition intermediate (INT3) (Figure 2f).

3.2. Monitoring conformational isomerizations with SMJs

The conformational changes of molecules are closely related to the physical and chemical properties of materials. In recent years, the investigation of single-molecule isomerization has gained increasing importance in fields of organic chemistry,

biochemistry, pharmaceutical chemistry, and molecular electronics. By using the graphene-based single-molecule junction platform with ultra-high temporal resolution, molecular conformational nuances can be detected. For example, the stereoelectronic effect of aromatic chains, specifically, the variation of twisting angles and corresponding π–π overlaps between phenyl rings, which have the potential to cause changes in conductance, can be detected.[43-44] The hexaphenyl aromatic chain molecule, as the functional molecule in a graphene-based single-molecule junction, provides a typical illustration of this concept.[43] The obtained current signals demonstrated that the twisting of the two ends of the benzene ring provokes a change in the dihedral angle between the phenyls, which can lead to varying degrees of conjugation and, in turn, produce current fluctuations (Figures 3a, b). Furthermore, the detection of finer isomeric states in molecular junctions can be achieved through the incorporation of substituent side groups. To this end, researchers have employed a subtle approach involving the introduction of an azobenzene side group into the triphenyl central ring (Figure 3c).[45] By utilizing either light or an electric field, they were able to control the *cis-trans* isomerization of the azobenzene side group.[46] This isomerization process was found to affect the rotation barrier of the phenyl ring located near the substituted position, while having minimal impact on the rotation barrier of the phenyl rings that are away. Apart from the observed isomerism arising from the rotation of single bonds, isomerism resulting from the rotation of double bonds, phenyls, as well as further molecular cyclization process were also detected based on graphene-based single-molecule junction (Figure 3d).[44]

Investigations have also been conducted on macromolecules with complex structures, for instance, a cyclic peptide containing an embedded photoswitch moiety,[47] revealing that the cyclic peptide molecule exists in three distinct states following photo-induced *cis-trans* isomerization. This phenomenon arises from the combined effects of multiple chemical bond rotations within the cyclic peptide molecule. Among them, the photoisomerization of azobenzene causes significant current fluctuations, whereas isomers arising from the rotation of other multiple chemical bonds produce smaller current fluctuations. The conductivity observed in the *cis* isomer is generally higher than that of the *trans* counterpart. This is because the electron density of the *cis* isomer is continuous and well-spanned, while that of the *trans* isomer is discontinuous.

The conformational isomerism of molecules can be regulated by external conditions like temperature or electric field.[43,46] Experiments about temperature-dependent and voltage-dependent on three tetraphenyl-ethylene (TPE)-based derivatives have been conducted.[44] The result showed the conformational states of TPE-2A and the average lifetime of each conformation change regularly with the increase of electric field or temperature (Figures 3e, f). According to the data analysis method in the previous section, the mean duration time of each conductance state can be derived from the corresponding residence time plots of the corresponding states. Subsequently, thermodynamic parameters for the rotation of chemical bonds can be obtained from temperature-dependent experiments using the Van't Hoff equation. Combining with theoretical analysis, it has been demonstrated that conformational isomerism is regulated by temperature and electric field, specifically, temperature provides sufficient energy for conformational isomerism to occur, while electric fields reduce activation energy. Most recently, the phenomenon of proton transfer-induced tautomerization in a single porphyrin-based single-molecule junction has been reported.[48] Such tautomerization can also be activated and well-controlled over a wide range of large temperature and bias voltage values range.

3.3. Monitoring intermolecular interactions with SMJs

The hydrogen bond is a weak but important interaction force that is ubiquitous in nature and has a pivotal role in determining the shapes, properties, and functions of biological molecules. Due to the unique properties of hydrogen bonds, they can exploit for molecular recognition purposes, particularly in biomolecular systems. For instance, functionalizing probes with 4-mercaptobenzamide, which contains both the hydrogen-bond donor and acceptor sites, enables the recognition of individual bases in DNA on a platform such as the scanning tunneling microscope (Figure 4a).[49] When the target base is captured by a 4-mercaptobenzamide

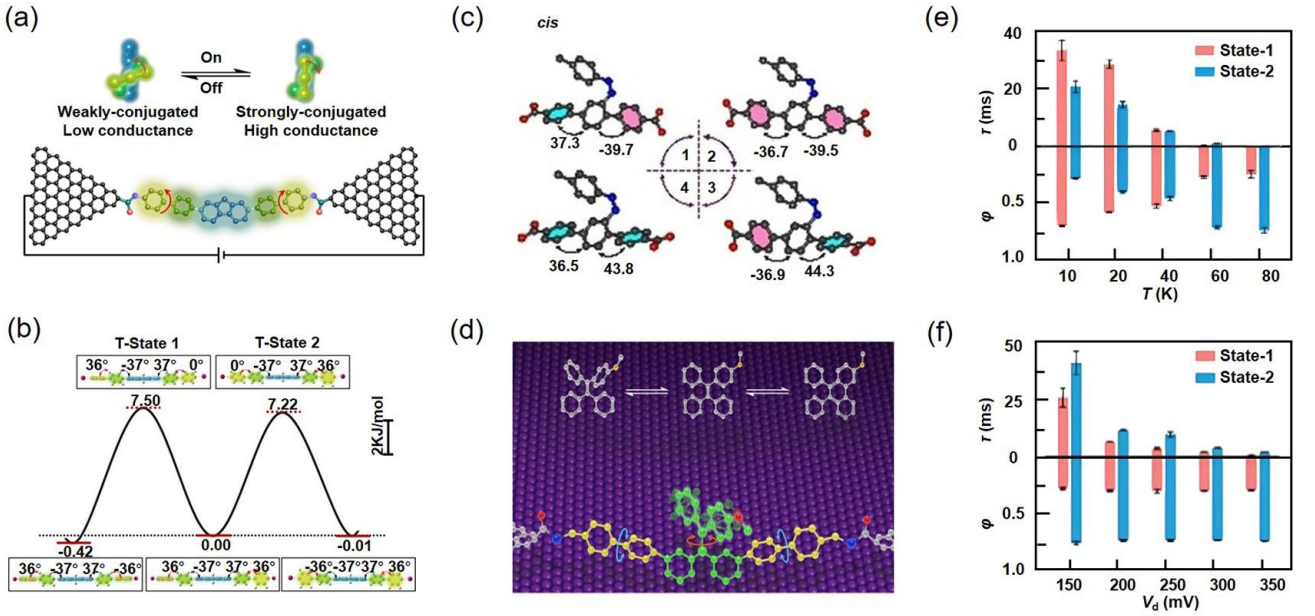


Figure 3 Detection of molecular conformational isomerizations based on single-molecule junction. (a) Schematic diagram of hexaphenyl aromatic chain single-molecule junctions used to observe conformational transitions of triphenyl units. (b) Electron energy diagram of three stable conformations and corresponding transition states for the hexaphenyl molecule. Reprinted with permission,[43] Copyright 2017, American Chemical Society. (c) The molecular structure of PR_L (the phenyl ring away from the N=N bond) orientation in *cis* form. Reprinted with permission,[45] Copyright 2021, Wiley-VCH GmbH. (d) Schematic of the device for detecting the dynamic stereoscopic structure of a single molecule. (e) Lifetime and occurrence proportion changes of two conductance states of the device connected with TPE-2A at different temperatures. (f) Lifetime and occurrence proportion changes of two conductance states of the device connected with TPE-2A at different voltages. Reprinted with permission,[44] Copyright 2022, Elsevier Inc.

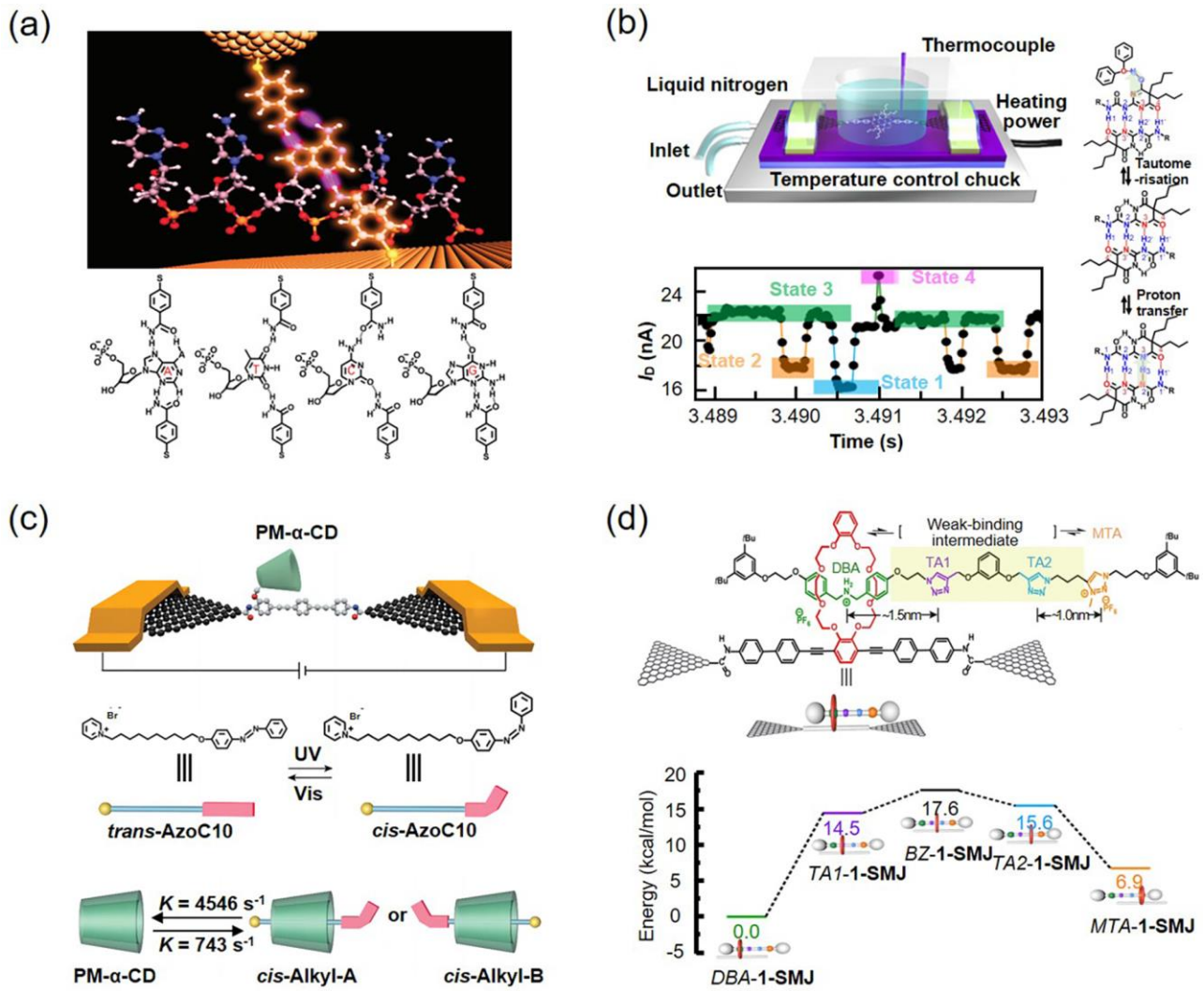


Figure 4 Detection of molecular dynamics based on single-molecule junctions. (a) Schematic diagram of an electronic tunnel identifying a single base in a DNA oligomer. Reprinted with permission,[49] Copyright 2010, Macmillan Publishers Limited. (b) Measurements setup of hydrogen bond dynamics based on hydrogen bond-bridged single-molecule junctions (HBB-SMJs). Reprinted with permission,[50] Copyright 2018, Springer Nature Limited. (c) Direct monitoring of the stochastic binding dynamics of a host-guest complex. Reprinted with permission,[54] Copyright 2022, Wiley-VCH GmbH. (d) Real-time observation of the dynamics of an individual rotaxane molecular shuttle using a single-molecule junction. Reprinted with permission,[56] Copyright 2021, Elsevier Inc.

molecule on the probe via a hydrogen bond, a characteristic current burst is observed. Different bases generate their characteristic currents, allowing for the establishment of a 'fingerprint' database of bases.

Hydrogen bond dynamics are also commonly investigated through spectroscopic methods, such as infrared and Raman spectroscopy, with hydrogen/deuterium substitution being a prevalent technique. However, such methods are ensemble experiments and do not provide information on the hydrogen bond dynamics of a single molecule. Recent advancements in the field of graphene-based single-molecule junctions have enabled direct measurements of hydrogen bond dynamics at the single-molecule and single-event level. By constructing quadruple hydrogen bond supramolecular molecular junctions, it is possible to detect the real-time hydrogen bond rearrangement process concerning temperature and solvent.[50] This study revealed that the current signal characteristics are heavily influenced by the solvent (diphenyl ether and TeCA). In addition, the dynamic process of metastable structures was also identified, which was induced by intermolecular proton transfer and lactam-lactim tautomerism (Figure 4b).

The host-guest complexation is another important intermolecular event that falls under the purview of supramolecular chemistry. The host-guest interaction is typically distinguished by its high specificity, and thus, it constitutes the fundamental basis of molecular recognition. In recent years, there has been growing interest in host-guest dynamics, particularly at the single-molecule level, using macrocyclic molecules, such as cucurbiturils, cyclodextrins, crown ethers, and others.

Cyclodextrins are cyclic oligosaccharides composed of six to eight *D*-glucose units linked by α-1,4-glucose bonds.[51] These molecules have a unique circular cavity that offers promising applications in molecular recognition.[52] A recent study employed a molecule containing a rigid conjugated organic framework and a permethylated-β-cyclodextrin (PM-β-CD) side arm, highlighting the potential of these structures in sensing and biosensing applications.[53] By covalently connecting target molecules to graphene point contacts, the association and dissociation of the amino acid-cyclodextrin complex can be monitored in real time via electrical signals. The method even enables the identification of amino acids with different charge states and enantiomers. Furthermore, the interaction between a photoswitchable guest and a cyclodextrin host in a graphene-based single-molecule junction was investigated by designing a pseudorotaxane structure using permethylated-α-cyclodextrin (PM-α-CD) as a cavity and 1-[10-(4-phenylazophenoxy)decyl] pyridinium bromide (AzoC10) as an axle (Figure 4c).[54] The current fluctuations observed in the devices were at-

tributed to the association and dissociation of AzoC10 and PM-α-CD. When switching from visible to ultraviolet light, the Azoc10 guest underwent a conformational change which was accompanied by the disappearance of a high conductance state and appearance of the low conductance state. This suggests that the dynamic process of the host-guest interaction between PM-α-CD and AzoC10 molecules, as well as the photoswitchable behavior of AzoC10, was detected. Furthermore, the host-guest dynamics of crown ether and methyl viologen[55] and rotaxane-based molecular shuttle[56] in graphene-based single-molecule junctions have also been studied. As shown in Figure 4d, real-time measurements of graphene-based SMJs on an individual [2]rotaxane molecule reveal the dynamic shuttling process of an individual [2]rotaxane molecule, unveiling a previously unidentified weak-binding intermediate.[56] The [2]rotaxane-based molecular shuttle 1 is constructed using the well-established crown-ether-dibenzylammonium-triazolium system. Molecular shuttle 1 consists of a dibenzylammonium motif (DBA) and a methyl triazolium group (MTA) serving as the primary binding sites, connected by a spacer containing two triazole rings (TA1 and TA2) and a 1,3-phenyl diether group. By monitoring changes in the electrical current, the shuttle process can be detected and described in three steps: the dibenzo-24-crown-8 crown ether moves from the DBA site to the TA1/TA2 group, and subsequently to the MTA group or returns to the original DBA site.

4. Single-Molecule Dynamics on One-Dimensional Nanomaterials-Based Field-Effect Transistors

One-dimensional (1D) nanomaterials (such as polymer chains, carbon nanotubes, and silicon nanowires) exhibit extremely high surface-to-volume ratio, excellent electrical properties, and fast signal response, making them ideal candidates for the development of ultrasensitive single-molecule electronic biosensors. Among them, field-effect biosensors are widely used, which use the electrostatic potential of the target molecules to modulate the electron fluxes of the conductive channel, ultimately leading to signal detection.[57] In the molecularly gated sensing approach, chemical interactions and physical conformational changes of target molecules are translated into real-time readable electrical signals. This enables the detection of single-molecule dynamics of organic small molecules and biological macromolecules, such as DNA and proteins.

4.1. Single-molecule dynamics based on single polymer chains

Single polymer chains are chain structures composed of biopolymers, usually consisting of double-stranded DNA (dsDNA) helices or α-helical peptides.[58-60] Field-effect transistors based on single polymer chains have the potential to be mass-produced and integrated into complementary metal-oxide semiconductor (CMOS) chips, making them an attractive option for a wide range of applications, particularly for the detection of single-molecule dynamics.

Recently, a single-molecule sensor has been proposed, which is composed of a probe molecule precisely conjugated to a molecular wire.[61] The molecular wire is a 25-nm-long α-helical peptide with a specific conjugation site on the amino acid side chain attached at the middle of the peptide, which is further connect with the probe molecules. Additionally, metal-specific conjugation groups were introduced at the ends of the peptide chain to self-assemble on the metal nanoelectrodes (Figure 5a).[62] In such single-polymer-chain transistors (SPCTs), the interaction between the probe molecule and the target molecule in the liquid phase induces a change of resistance, which can be observed through the current pulse, providing dynamic information of a single molecule.

Single-molecule dynamics, including DNA oligomers, aptamers, antibodies and antigens, and enzymes, can be detected through the utilization of SPCTs.[61] For example, in the case of antibody-antigen sensor, a fluorescein dye molecule (as antigen) is mounted on the molecular bridge of an SPCT (Figure 5b). By titrating antifluorescein antibody, the dynamic process of antibody-antigen binding can be monitored via current fluctuations. In addition, the molecular bridge of an SPCT can also be conjugated with a single Phi29 DNA polymerase molecule,[63] and the real-time binding of the polymerase with nucleotides can be converted into electrical signals, thus enabling the monitoring of polymerase activity at the single-molecule level (Figure 5c). Furthermore, detailed analysis of single-molecule dynamics and the signal pulse characteristics of the polymerase replication template holds potential for DNA sequencing.

4.2. Single-molecule dynamics based on SWCNTs

Single-walled carbon nanotubes (SWCNTs) are a form of nanomaterials consisting of carbon atoms that demonstrate compatibility with organic/biological molecules and exhibit remarkable electronic properties, high stability, and chemical versatility. These characteristics make SWCNTs one of the most popular one-dimensional nanomaterials, particularly in the field of single-molecule dynamics detection, especially in the biological field. Moreover, SWCNTs have a simple chemical composition and are easy to prepare. Chemical vapor deposition (CVD) is a prevalent technique for synthesizing high-quality SWCNTs with minimal defects. During the preparation process, it is necessary to select a high melting point catalyst that can effectively control the chirality of SWCNTs. Furthermore, the catalyst concentration should be appropriately diluted based on the SWCNT density before dispersing it onto the growth substrate to ensure the production of high-quality SWCNTs.[64]. Following the growth stage, separation and purification steps are necessary to obtain SWCNTs with high purity for subsequent fabrication of high-performance devices.

4.2.1. Point-functionalization of SWCNTs. To facilitate the attachment of probe molecules to the surface of SWCNTs, it is necessary to functionalize the interfaces of the latter. This is due to the perfect lattice structure of the original surfaces of SWCNTs, which lack reactive site. Functionalization can be achieved through three main strategies: electrochemical oxidation, free radical reaction controlled by the gate, and non-covalent π-π interaction. Electrochemical oxidation involves the use of electrochemical method to oxidize the carboxylate groups, resulting in the generation of carboxyl groups on SWCNTs (Figure 5d).[65] These carboxyl groups can then form covalent bonds with probe molecules, enabling point functionalization. By controlling the chemical reaction using electronically controlled electrochemical potentials, single-point functionalization can be achieved. The second method involves exposing the SWCNT-based devices to 4-formylbenzene diazonium hexafluorophosphate (FBDP),[66] and then adjusting the Fermi level (EF) of the SWCNTs by applying a gate voltage. When the gate voltage is positive relative to the surface of SWCNTs, EF is shifted up, providing electrons to the positively charged diazonium of the FBDP to form an aryl radical, and promoting the generation of sp^3 defects in the aryl radical. The degree of functionalization is influenced by the electron density near the Fermi level of SWCNTs. When a negative gate voltage is applied, the Fermi level is lowered, which leads to a decrease in the population of electrons in the vicinity of the Fermi level and a cessation of the functionalization reaction. One approach to controlled functionalization involves connecting sp^3 defects on the SWCNT surface with probe molecules, resulting in diazonium point functionalization (Figure 5e).[67] The third method can be achieved by adhering pyrene molecules to SWCNTs through non-covalent π-π interactions (Figure 5f). These interactions provide dilute anchor points that can be used for subsequent functionalization.[68-69] By bolting the individual molecule onto SWCNTs using these dilute anchor points, it is possible to achieve single-molecule level detection.

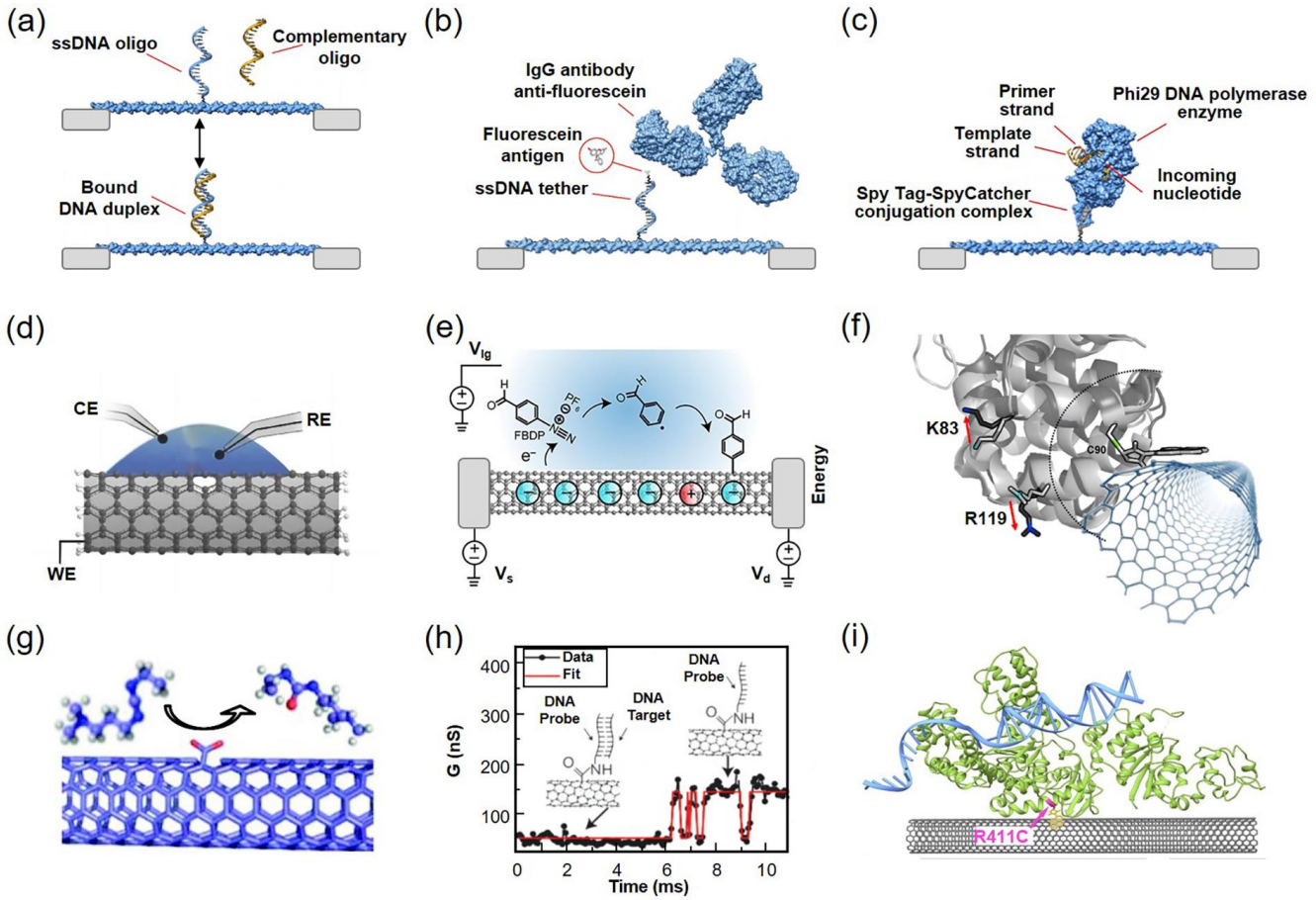


Figure 5 Detection of single-molecule dynamics based on single polymer chains and carbon nanotubes. (a) Schematic diagram of a single-polymer-chain transistor for detecting DNA oligo hybridization. (b) Antibody-antigen sensors. (c) DNA polymerase activity sensor. Reprinted with permission,[62] Copyright 2022, National Academy of Science. (d) Functionalization of SWCNT through electrochemical oxidation of carboxylates. Reprinted with permission,[65] Copyright 2017, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (e) Functionalization of SWCNTs through V_{lg}-induced diazonium reaction. Reprinted with permission,[67] Copyright 2018, American Chemical Society. (f) Functionalization of SWCNT through non-covalent π-π interaction. Reprinted with permission,[73] Copyright 2013, American Chemical Society. (g) Schematic diagram of dynamic process between an EDC molecule and a single-molecule probe covalently attached on the nanotube. Reprinted with permission,[27] Copyright 2008, American Chemical Society. (h) The detection of DNA hybridization kinetics based on SWCNT. Reprinted with permission,[71] Copyright 2011, Macmillan Publishers Limited. (i) The detection of Taq DNA polymerase dynamics based on a SWCNT. Reprinted with permission,[76] Copyright 2022, American Association for the Advancement of Science.

In addition to the above method of point-functionalization, the introduction of probes can also be realized through the creation of nanowells using nano-fabrication technologies. For instance, the chemical reactions could be limited to a specific point on individual SWCNTs by using lithographically patterned nanowells, thereby creating a single-molecule probe.[70] Stable and isolated functional groups at predetermined positions on SWCNTs can be formed using high-yield covalent chemistry and confinement within nanowells patterned in a thin polymer layer. This approach allows for the observation of consecutive chemical reactions, molecular interactions, and molecular conformational changes on a single molecule, enabling various successive, secondary reactions and interactions with single-molecule resolution.

4.2.2. Monitoring chemical reactions with SWCNT-based devices. The point-functionalization of SWCNTs results in devices that exhibit high sensitivity to chemical reactions on their surfaces due to their exceptionally high surface-to-volume ratios. This property enables the detection of single-molecule chemical reactions, such as the interaction between a single carboxylate group and its environment, through the conductance fluctuation of SWCNTs with point functionalization.[23] In the experiment, the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) molecules in a solution led to obvious spikes in conductance and broad noise spectrum, showing in a two-level fluctuation. EDC acts as an activator and dehydrator, leading to the formation of acyl isourea derivatives of carboxylic acid. These intermediate species are rapidly hydrolyzed in the presence of water, demonstrating that EDC combined with the nucleophilic catalytic site provided by carboxylates in solution and formed a transient and reactive intermediate species: *O*-acylisourea. The hydrolysis of *O*-acylisourea generates a mixed urea, which can free the carboxylate to bind another EDC molecule. The conductance fluctuations of SWCNTs occur due to the formation of *O*-acylisourea complexes at the point-functionalized site of SWCNTs. The generation and hydrolysis of these complexes strongly affect the conductance of the SWCNTs, leading to the observed two-level fluctuations. Consequently, the electronic transduction of SWCNTs conductance can be utilized to monitor the reaction process between carboxylates and EDC (Figure 5g). It was also possible to monitor the reaction between EDC and carboxylates by the SWCNT-based devices which are point-functionalized by nanowell-confined chemistry.[70] Experimental analysis revealed that the conductance of exposed devices decreased by an average of 20% in an EDC solution environment due to the formation of single COOH/EDC complex, which significantly alters the conductance.

4.2.3. Monitoring single DNA dynamics with SWCNT-based devices. The investigation of DNA dynamics is a fundamental aspect of many genomic diagnostic technologies. Single DNA measurements, which provide insights into the intramolecular dynamics and conformational changes of DNA, can be accomplished using point-functionalized SWCNT-based devices. The devices can

also be employed to examine the hybridization dynamics and thermodynamics of two different types of duplex DNAs.[25] In an experimental setting, immersing the device in a buffer containing complementary target DNA resulted in a large-amplitude two-level fluctuation in conductance. The low-conductance state is attributed to the binding of the target DNA to a covalently attached DNA probe,[71] which led to increased scattering and charge transfer at the defect. Conversely, the high-conductance state corresponds to a device with unbound probe DNA (Figure 5h). Furthermore, the two conductance states exhibit a strong temperature dependence. Building upon the previous findings, the research group further studied the dynamics of DNA hybridization by SWCNT-based devices and discovered that the electric field could regulate the hybridization and melting of DNA.[72] During the experiment, a 300 mV gate bias produced a repulsive electrostatic force which decreased the rate constants of the DNA hybridized state and increased the rate constants of the DNA melted state. The results show that voltage control could be used to study the hybridization and melting dynamics and thermodynamics, instead of temperature. Furthermore, a sequence-dependent calibration between applied bias and temperature could be established.

SWCNTs possess the capability to detect not only the hybridization dynamics of DNA but also its molecular conformational dynamics. Covalent modification of the primary single-molecule probe on the SWCNTs with 5' amino-modified DNA oligomers enables real-time measurement of the conformational dynamics of a single DNA G-quadruplex.[70] The conductance of SWCNT-based devices shows fluctuations between low-conductance (low-G) and high-conductance (high-G) states in the presence of K^+ or Na^+ ions, which correspond to the two configurations of DNA. The low-G state corresponds to the folded conformation of DNA, while the high-G state corresponds to the unfolded conformation of DNA. In addition, the lifetime of DNA folding conformation in the K^+ solution is significantly longer than that in the Na^+ solution. These findings suggest that SWCNTs have promising applications in real-time detection and monitoring of DNA conformational dynamics.

4.2.4. Monitoring protein dynamics with SWCNT-based devices. Proteins constitute the fundamental organic components of cells and serve as the cornerstone of biological life. Understanding their intricate structural and functional properties is of utmost importance, as it can yield significant insights into the underlying mechanisms of diseases. SWCNT-based devices have emerged as a valuable label-free platform for examining protein dynamics with exceptional sensitivity. They have been extensively used in the detection of protein dynamics, exemplified by their use in analyzing lysozyme and DNA polymerase.[22,73-76]

Lysozyme is an alkaline enzyme capable of catalyzing the hydrolysis of mucopolysaccharides in bacteria and neutralizing viruses by directly binding to their negatively charged protein. As a result, lysozyme finds widespread applications in the fields of medicine, food, and bioengineering. When a single lysozyme molecule is immobilized onto the SWCNT-based device, a stable high-bandwidth sensor for protein motion is produced. The motion of lysozyme molecules generates changes in electrostatic potentials, which can be converted into dynamically changing electron fluxes with shot noise limitations.[22] Thus, the catalytic mechanism and activity of lysozyme can be studied by analyzing the electronic signals.

DNA polymerase is a crucial enzyme that accurately replicates and repairs DNA with extremely low error rates. In recent years, there has been significant interest in investigating the dynamics of DNA polymerase using SWCNT-based devices. For example, electronic nanocircuits were bioconjugated with individual DNA polymerase I Klenow fragment (KF) molecules, allowing the enzyme's function and dynamic changes to be monitored using electrical signals.[74] The data obtained from these experiments can be used to statistically determine the key dynamic parameters governing the enzyme's open and closed conformations. Notably, the method also confirmed that the rate-limiting step for catalysis occurred during the enzyme's open state, providing novel insights into the mechanism of KF-catalyzed bond formation. Based on the above work, researchers further investigated the accommodation of deoxyribonucleoside triphosphate (dNTP) analogs using KF molecules. The dynamics and conformational changes during the process of native and analog dNTP incorporation were studied.[75] Specifically, DNA polymerization was monitored using dNTP analogs with phosphodiester or nucleobase modifications and compared with native dNTP. Statistical analysis was then employed to examine the differences in incorporation dynamics and conformations. The results indicate that the dNTP analog changed the time required for nucleotide recognition but not the dynamics of KF's closed conformation. In addition to the KF molecule related research, a recent study on the dynamics of Thermus aquaticus (Taq) DNA polymerase has received considerable attention.[76] In the experiment, the Taq molecule was attached to the SWCNT-based device using the same method (Figure 5i), the current signal in the activity buffer that contains mismatched dNTPs remained featureless, whereas the presence of matched dNTPs resulted in the emergence of two-level switching. The current signal of 0 corresponds to the Taq's open conformation, whereas the offset current state corresponds to the Taq's closed conformation.

4.3. Single-molecule dynamics based on silicon nanowires

Silicon nanowires (SiNWs) are a novel class of one-dimensional semiconductor nanomaterials. Typically, they have a wire diameter of approximately 10 nm and consist of a monocrystalline silicon inner core covered by a SiO_2 outer layer sheath.[77] CVD is a common method for synthesizing SiNWs with a diameter smaller than 20 nm and a length exceeding 1 μm. Subsequently, these SiNWs are transferred to receptor substrates for further device fabrication. Recently, a novel technique called direct mechano-sliding transfer (DMST) has been reported, enabling direct transfer of CVD-grown SiNWs without the need for solvents, fluids, or lubricants.[78] DMST capitalizes on the direct physical interaction between SiNWs and the substrate, allowing for precise control of SiNW density on the receptor substrates through the application of pressure. Due to their compatibility with contemporary semiconductor technology, SiNWs exhibit tremendous potential for integration into nanocircuitry. The adjustable electrical properties and surface chemistry of SiNWs make them an ideal choice for constructing biosensors. For example, they can be used to monitor DNA hybridization dynamics and protein dynamics with ultrasensitivity.

4.3.1. Point-functionalization of SiNWs. To proceed with point-functionalization, one method is the use of high-resolution electron beam lithography to create a narrow window on the surface of a spin-cast layer of PMMA.[79] Subsequently, the silicon core of SiNWs is exposed by chemical wet etching to form a hydrogen-terminated silicon trench at the nanoscale level. The resulting gap is sufficiently narrow to accommodate a single analyte molecule, thereby ensuring single-molecule detection. Then, the end of the nanowire is modified with an aldehyde group through photochemical hydrosilylation, a well-established approach for surface functionalization (Figure 6a). Finally, the biological probe, such as an antibody, is integrated into the circuit to complete the real-time label-free bio-detecting device. Another method of point-functionalization is achieved through the Ni^{2+} coordination links.[80] Specifically, the devices are fabricated by chemical reactions on the aminated surface of SiNWs, with phenylene1,4-diisothiocyanate, Nα,Nα-bis(carboxymethyl)-*L*-lysine hydrate, and $NiCl_2$, sequentially. After Ni^{2+} chelation, functional Ni-NTA end groups are formed, and the target substance, such as a protein, is attached to the Ni^{2+} ions through coordination bonds. This

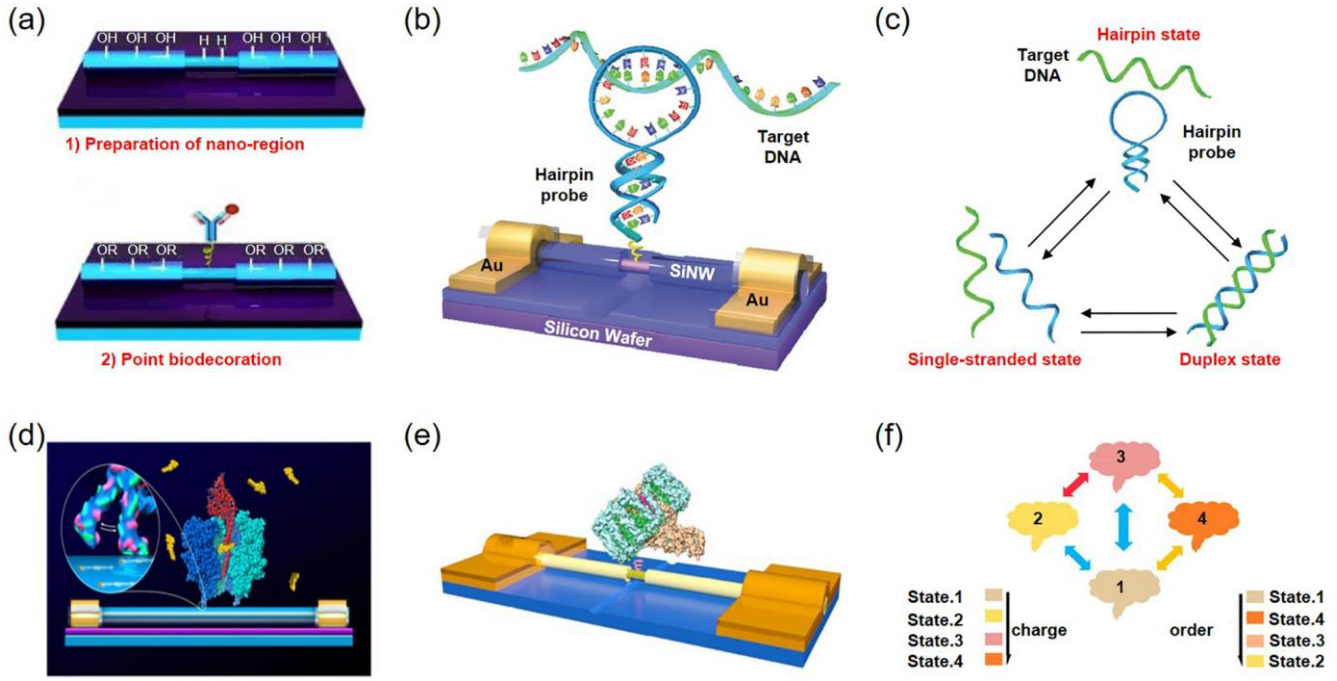


Figure 6 Detection of single-molecule dynamics based on SiNWs. (a) The fabrication process of SiNW-based single-molecule electrical transistors. Reprinted with permission,[79] Copyright 2014, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (b) Schematic diagram of the interactions between hairpin probes and target DNA. Reprinted with permission. (c) Schematic demonstration of three-phase transitions during hairpin DNA hybridization with the complementary target. Reprinted with permission,[82] Copyright 2017, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (d) Schematic diagram of the hydrolysis dynamic process of F1-ATPase (F1) based on SiNWs. Reprinted with permission,[83] Copyright 2017, American Chemical Society. (e) Schematic diagram of the detection of a single LH1-RC dynamics based on SiNWs. (f) Conversion relationship of four states of a single LH1-RC. Reprinted with permission,[85] Copyright 2021, American Chemical Society.

method avoids surface modification, leading to high sensitivity and selectivity. When biological molecules, such as DNAs or proteins, are attached to the SiNWs device, their conformational changes or interactions induce an additional gate, thus affecting the conductance of the SiNW-based device and enabling corresponding dynamic detection.

4.3.2. Monitoring DNA dynamics with SiNW-based devices. Similar to the SWCNT-based devices, SiNW-based single-molecule electronic biosensors can also provide label-free and ultra-sensitive detection of single DNA dynamics, for example, the internal hybridization and dissociation process of DNA.[81] In this study, two-level current oscillations with strong temperature dependence were observed, revealing the thermodynamic and dynamic properties of DNA hybridization at the hairpin site. The current fluctuation was due to different conformations of DNA, with the low-conductance state corresponding to the hairpin formation and the high-conductance state corresponding to the unfolded coil structure. With the same device, a follow-up study used the hairpin structure as a DNA probe to monitor the hybridization event with another target DNA molecule[82] (Figure 6b). To achieve this, a single DNA strand has been attached to a high-gain field-effect silicon nanowire, the device was then exposed to a solution containing a complementary target DNA. As expected, a three-level fluctuation behavior in time-averaged current changes was observed. This fluctuation of current reflects the dynamic hybridization of two single DNA strands (Figure 6c). Specifically, the low-conductance state represents the probe-target duplex conformation, the intermediate-conductance state represents the hairpin conformation, and the high-conductance state represents the single-strand conformation.

4.3.3. Monitoring protein dynamics with SiNW-based devices. In recent years, there have also been many explorations in studying of the dynamic process of proteins using SiNW-based device at the single-molecule or single-event level. For example, the hydrolysis dynamic process of an F1-ATPase (F1) has been monitored in real-time using a high-gain SiNW-based field-effect transistor circuit (Figure 6d).[83] In the experiment, large two-level current fluctuations were observed, where the high current state corresponded to the binding dwell of F1, while the low current state corresponded to the two catalytic dwell: ATP hydrolysis and Pi release. In another study, conformational dynamics of protein has been detected by immobilizing a single LH1-RC complex on the surface of a SiNW (Figure 6e).[84] The results showed that the LH1-RC complex exhibits thermally activated structural vibration, as evidenced by four-level current oscillations observed at temperatures ranging from 15 to 55 °C.[84-85] These oscillations correspond to the four distinct conformations of the LH1-RC complex, which is strongly temperature dependent (Figure 6f). The conformational changes of the protein are primarily driven by anharmonic vibrational modes that are induced by temperature fluctuations, resulting in a transition from a well-ordered state with weak bond energies to a disordered state with stronger bond energies. At the optimum temperature, the LH1-RC complex is predominantly in state 2 or 3. The conformational change of protein mainly emerges as anharmonic vibration modes, which are conducive to photon acquisition and heat transmission.

5. Single-Molecule Fluorescence Techniques

The ability to detect and analyze the optical signals emitted by individual molecules is crucial to the development of nanoscience. In recent decades, fluorescence-based optical technologies have made remarkable progress in single-molecule detection, including the conformational changes of small molecules and catalytic reaction kinetics. Moreover, single-molecule fluorescence technology plays a critical role in macromolecules analysis, particularly in biological applications. The significant advantage of single-molecule fluorescence is its ability to detect individual molecule with high spatial and temporal resolution. As such, it can directly explore the hidden heterogeneity of biological molecules even un-

der close physiological conditions, such as conformational changes and dynamic transformation between different states of a single biomolecule.

5.1. Principles of single-molecule fluorescence detection

Fluorescence was first observed by Herschel in 1845,[86] and later in 1852, Stokes provided a more comprehensive description of the phenomenon and coined the term fluorescence.[87] The generation of fluorescence involves the absorption of energy by an electron from incident light, which transitions the electron from the ground state to the excited state. Subsequently, as the electron returns to the ground state, the electron emits lower-frequency light, known as fluorescence.

Single-molecule technology requires detection of the fluorescence signal of a single target molecule within the field of view. This necessitates the use of high-resolution microscopic imaging techniques such as total internal reflection microscopes, confocal microscopes, and super-resolution microscopes, which are essential for the success of single-molecule technology.

The total internal reflection fluorescence (TIRF) microscope is a type of wide-field microscope that utilizes the evanescent wave generated by the total internal reflection of light at an interface for illumination, which results in a limited light volume. The evanescent wave intensity decreases exponentially with increasing distance from the interface, thus restricting the illuminated area to the adjacent region of the interface (within 100 nm).[88-89] This property effectively suppresses the background lighting, leading to a higher signal-to-noise ratio that is essential to enable single molecule detection. The prism-type total internal reflection microscope and objective-type total internal reflection microscope are two classical methods of TIRF. The latter is generally more popular for single-molecule detection. The prism-type TIRF microscope features separate excitation and collection equipment on opposite sides of the sample, which necessitates thin samples and increases the working distance. In contrast, the objective-type total internal reflection microscope avoids these problems and is easier to operate (Figure 7a). In the objective-type TIRF microscope, the excitation light is focused on the rear focal plane at the edge of the objective lens, creating an evanescent field on the side of the objective lens.[14]

The confocal microscope is an imaging tool that utilizes a pinhole to selectively filter out-of-focus light and to limit the observation volume (Figure 7a), thereby enabling the detection of single molecules.[90] With this approach, the spatial and axial resolution has been improved, as compared to traditional wide-field imaging, resulting in more detailed imaging. The resolution of the confocal microscope is determined by the size of the pinhole, which corresponds to the size of the Airy disk. The design restricts the observation volume to the femtoliter range. In addition, confocal microscope permits point observations, enabling the detection of only one molecule as the main fluorescence source in the limited observation area.[14]

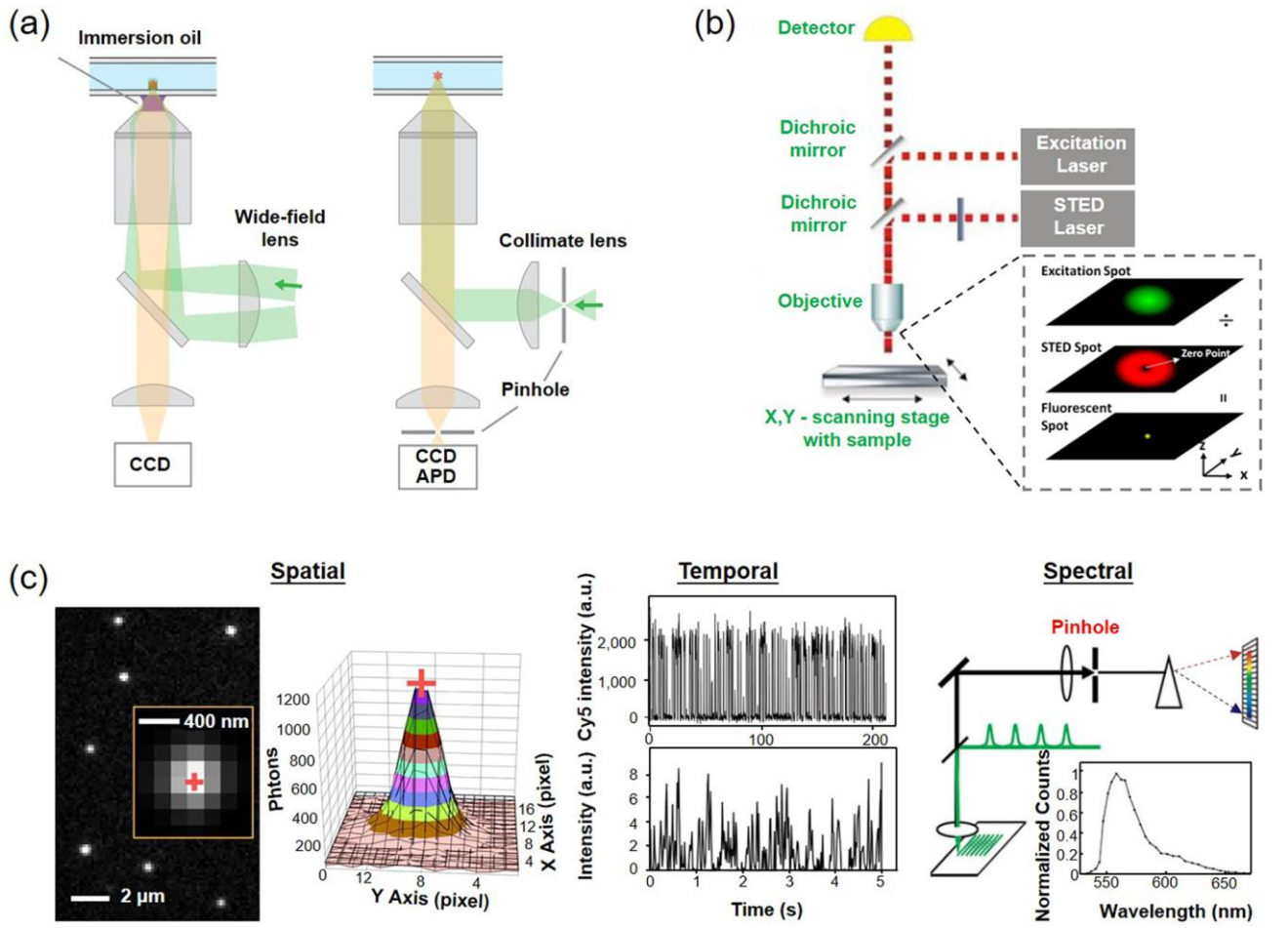


Figure 7 Single-molecule fluorescence techniques. (a) Schematic of total internal reflection microscope (left) and confocal microscope (right). Reprinted with permission,[14] Copyright 2017, American Chemical Society. (b) Schematic of the STED microscopy. Reprinted with permission,[94] Copyright 2017, American Chemical Society. (c) Spatial, temporal, and spectral dimensions of single-molecule fluorescence. In spatial dimension, illustrated as a widefield fluorescence image of a single AF647 molecule (left) and the intensity distribution of a single Cy3 molecule (right); in temporal dimension, illustrated as laser-induced reversible fluorescence photoswitching of Cy3-Cy5 dyes (up) and binding/unbinding of individual Nile Red molecules to a lipid vesicle (down); in spectral dimension, illustrated as scanning-based single-location spectroscopy of single-molecule. Reprinted with permission,[20] Copyright 2018, American Chemical Society.

Super-resolution microscopes are a type of advanced technique that can produce images with higher spatial resolution than conventional microscopes. Based on the location of the detector from the sample, super-resolution microscopes can be classified into two categories: near-field microscope and far-field microscope. The near-field scanning optical microscope (NSOM or SNOM), which uses an aperture or a tip with sub-diffraction size, operates in a very close range to the sample and captures the evanescent wave carrying near-field super-resolution information.[91] However, due to its invasive nature, this technique is primarily limited to imaging surfaces. On the other hand, far-field super-resolution microscopes are capable of imaging three-dimensional structures and have several types such as stimulated emission depletion (STED), structured illumination microscopy (SIM), photo-activated localization microscopy (PALM), and stochastic optical reconstruction microscopy (STORM). Among them, the STED microscope was the first to be invented and most widely used. This technique was proposed by Hell and his colleagues in 1994,[92] and was experimentally verified in 2000.[93] A typical STED microscopic system utilizes two beams of illumination.[94] The first beam, referred to as the excitation light, is focused into a diffraction-limited focal spot to stimulate molecules located in the area. The second beam, known as annular light with zero central intensity, is used to illuminate the fluorescence molecules that are in the excited state. After the two beams are superimposed, the depletion light will exhaust the excited fluorescence molecules in the surrounding region via the excited radiation process, resulting in the quenching of fluorescence molecules in the surrounding region. Consequently, only the fluorescence molecules in the center of the focal spot remain (Figure 7b). This technique enables obtaining a fluorescent luminescent point that is smaller than the diffraction limit, which is advantageous for collecting single-molecule fluorescence.

The integration of various fluorescence microscopy techniques has led to the development of noninvasive and target-specific single-molecule fluorescence detection methods. These methods enable the acquisition of extensive information in the spatial, temporal, and spectral dimensions of single molecules (Figure 7c).[20] In the spatial dimension, the position of a single molecule can be determined with nanometer precision. In terms of the temporal dimension, time trajectories of fluorescence intensity, especially the on-off intensity dynamics of fluorescence, enables ultra-high time resolution in tracking single molecule dynamics, typically at millisecond scales. Furthermore, single-molecule fluorescence also provides spectral information, which is useful for molecular recognition.

5.2. Monitoring reaction dynamics with single-molecule fluorescence

Single-molecule fluorescence is a highly informative technique that enables the detection and analysis of the molecular dynamics of enzymes as well as small molecules. Notably, it has been demonstrated that this technology is well-suited for the investigation of catalytic reaction kinetics.

The application of single-molecule spectroscopy in the study of enzyme kinetics marked a new phase in single-molecule enzymology in the late 1990s. One important study focused on cholesterol oxidase (COx) in *Brevibacterium sp.*, a 53 kDa monomeric protein. By detecting the fluorescence of the enzyme reaction center, changes in the oxidation-reduction states during enzyme-catalyzed reactions were observed.[95-96] Flavin adenine dinucleotide (FAD), a coenzyme, binds non-covalently to the active site of the protein. During the enzyme turnover cycle in cholesterol oxidation, FAD undergoes a reduction to $FADH_2$ followed by subsequent re-oxidation to FAD. Notably, the oxidized form of FAD exhibits fluorescence, whereas the reduced form, FADH, does not. Each enzyme turnover cycle involves the switching of FAD between its fluorescent oxidized form and the non-fluorescent reduced form, thereby causing the quenching or recovering of the single-molecule fluorescence. These switching cycles correspond to enzyme turnovers and provide detailed information about the chemical kinetics. The study revealed the presence of both static and dynamic disorder in enzyme-catalyzed reaction kinetics, as seemingly identical COx molecules exhibited significant variations in reaction rates, reflecting static disorder during FAD reduction. Additionally, individual enzymes displayed noticeable fluctuations in their reaction rates, contributing to dynamic disorder in reaction kinetics.

Single-molecule fluorescence spectroscopy is not limited to enzymatic studies, it also offers insights into other dynamic processes of small molecules. For instance, the monitoring of isomerization processes using single-molecule fluorescence spectroscopy has been demonstrated.[97] In the study, the spiropyran molecule was excited using a laser, which initiated its ring-opening reaction, producing the fluorescent merocyanine molecule that exists in two stable isomerization states (TTC and TTT, see Figure 8a). The dynamic interconversion, as well as the proportions of the two isomers, can be distinguished and monitored from the fluorescence signals, such as the single-step jumps in the time traces of spectral means.

Fluorescent probes, such as 3'-(*p*-aminophenyl) fluorescein (APF), resazurin or Amplex Red, are commonly used to produce high-fluorescence products via catalytic redox reactions. These reactions can be monitored and recorded with ultra-high temporal resolution using an electron-multiplying charge-coupled device camera. By monitoring the fluorescence intensity of a single catalyst, such as nanoparticles or nanorods, the catalytic rate can be determined. Additionally, the position of each fluorescent molecule can be precisely identified with nanometer accuracy by fitting its fluorescence image using Gaussian analysis. This enables the accumulation of a large number of fluorescence reaction events to generate a distribution of reaction activity on a single catalyst.[96] For instance, the conversion of non-fluorescent Amplex Red to the fluorescent resorufin is catalyzed by gold nanorods in H_2O_2 solution, the relationship between catalytic efficiency and the structures and defects of gold nanorods can be elucidated using this strategy (Figure 8b).[98] Moreover, researchers have developed a model nanocatalyst platform that comprises of platinum nanoparticles (NPs) sandwiched between a solid SiO_2 core and a mesoporous SiO_2 shell (Figure 8c). This platform can be used to investigate the catalytic reaction dynamics of Amplex Red conversion within nanopores under *in situ* conditions.[99]

Researchers have also studied photocatalytic reactions based on semiconductors using single-molecule fluorescence techniques, in order to better understand the reactivities, distributions, and local nanostructure of active sites on a single particle. Semiconductors possess unique electrical characteristics that allow electrons in the valence band to be excited to the conduction band by lasers, resulting in the generation of electron-hole pairs. The electrons or holes then migrate to the surface of the semiconductor catalyst where they initiate the reduction or oxidation of molecules. Through the single-molecule fluorescence reaction of photo-generated hole-induced Amplex Red oxidation and electron-induced resazurin reduction, researchers successfully investigated the reaction rates of the aforementioned reactions, and revealed that they were non-uniform along single rutile titanium oxide (TiO_2) nanorods (Figure 8d).[100] This suggests that the activity at surface structural defects may be higher than that at the (100) facet sites of TiO_2 nanorods. Interestingly, the hole-induced and electron-induced activities were found to exhibit a strong spatial correlation on individual nanorods, as also revealed on BiOBr nanoplate (Figure 8f).[101] This correlation is attributed to the separate distribution of electrons and holes in the area with rich defects, allowing them to be captured by APF and resazurin separately.

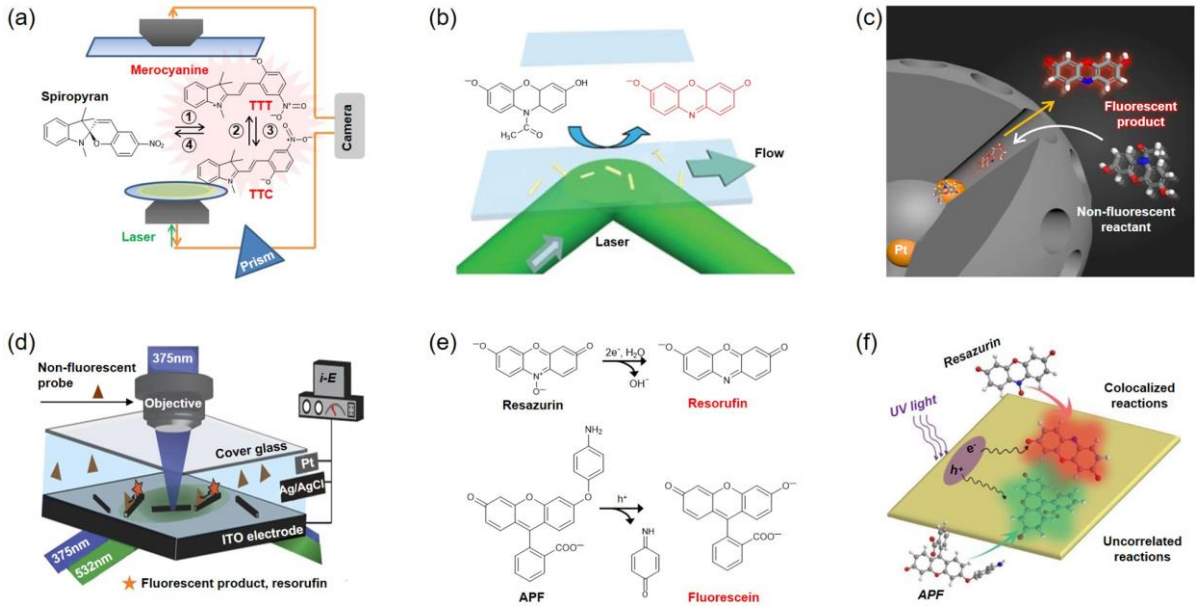


Figure 8 Monitoring of reaction dynamics with the single-molecule fluorescence technology. (a) Schematic of the isomerization process of TTC and TTT. Reprinted with permission,[97] Copyright 2017, American Chemical Society. (b) Schematic of the fluorogenic deacetylation reaction of Amplex Red to resorufin catalyzed by individual Au@mSiO$_2$ nanorods,[98] Copyright 2012, Springer Nature Limited. (c) Schematic of the single-particle catalysis of Amplex Red to resorufin catalyzed in the confined environment in nanopores. Reprinted with permission,[99] Copyright 2018, Springer Nature Limited. (d) Wide-field single-molecule fluorescence imaging of the photoelectrocatalysis via two-laser total internal reflection excitation. Reprinted with permission,[100] Copyright 2016, Springer Nature Limited. (e) The chemical reaction of reductive deoxygenation of resazurin by photogenerated electrons to produce highly fluorescent resorufin and oxidative cleavage of the aminophenyl group of 3′-(*p*-aminophenyl)-fluorescein (APF) by photogenerated holes to produce fluorescein. (f) Schematic diagram of photocatalytic reduction and oxidation reactions on BiOBr nanoplates using fluorogenic probes. Reprinted with permission,[101] Copyright 2021, American Chemical Society.

5.3. Monitoring biomolecular dynamics with sm-FRET

Single-molecule Förster resonance energy transfer (sm-FRET) is a widely used and versatile technology for investigating biomolecular dynamics. The theoretical underpinnings of FRET were first described by Theodor Förster in 1948, and the process relies on the dipole-dipole interaction between two fluorophores meeting special requirements. Specifically, these fluorophores must have emission spectra that overlap to a certain extent, and they must be within an appropriate distance of each other (less than 10 nm). Under these conditions, the energy of the excited donor electron is transferred to the acceptor molecule, causing the donor to return to the electronically ground state and the acceptor to transition to the electronically excited state. The efficiency of FRET between the donor and acceptor is inversely proportional to the sixth power of the distance between them:

$$E_{\mathrm{FRET}} = \frac{1}{1 + (R / R_0)^6},$$

where R is the distance separating the donor and acceptor, and R_0 is the critical distance at which fifty percent of the energy transfer via FRET is achieved.

Because of its high sensitivity to the distance between a fluorophore pair, sm-FRET is regarded as a powerful technique that is able to study the conformational changes and the molecular interactions involving biomolecules. This technique has been widely employed in the study of various biomolecular dynamics, such as DNA replication and repair, transcription, translation, enzymatic reactions, molecular motors, membrane proteins, nucleic acids, protein and RNA folding, and many others.[102]

Studying multi-molecular complex systems presents a significant challenge due to their complex nature and large size. The emergence of FRET technology has provided an opportunity to overcome these challenges. For instance, by combining TIRF technology and FRET technology, the dynamics of human Argonaute 2 (hAgo2) and hAgo2-RNA complexes have been studied by introducing a site-specific donor and acceptor fluorophores in natural hAgo2 (Figure 9a).[103] The FRET efficiency histogram has multiple peaks, each corresponding to a specific conformation, thereby reflecting dynamic information.

While single-molecule FRET (sm-FRET) has mainly been used *in vitro*, recent studies have shown that it can also monitor conformational dynamics and heterogeneity in living cells.[104] For example, the in-cell sm-FRET method was used to investigate agonist-induced conformational dynamics in a single metabotropic glutamate receptor dimer (Figure 9b).

The temporal resolution provided by total internal reflection microscopy is often inadequate to monitor most dynamic processes of proteins, which usually occur faster than 20 milliseconds.[105] In this regard, several strategies have been explored to enhance the temporal resolution of single-molecule fluorescence techniques. One of such methods is two-dimensional fluorescence lifetime correlation spectroscopy (2S FLCS), which can capture dynamic events on a microsecond timescale (Figures 9c, d).[106] Another attempt involves the combination of sm-FRET and confocal multi-parameter fluorescence detection (MFD), enabling measurements on the order of microseconds or even nanoseconds.[107] With this technology, it is possible to monitor the conformational changes and interconversion kinetics of chromatin fibers with great precision at the microsecond scale.[108]

Sm-FRET provides only partial dimension information of biological molecules. Therefore, the integration of other biophysical technologies to acquire additional dimension information has garnered increasing attention. To this end, a self-assembled nanoscopic force clamp constructed from DNA has been proposed to expand the scope of single molecular force spectroscopy technology.[109] This approach enables the exertion of defined and tunable force on the molecular system by exploiting the entropy elasticity property of single-stranded DNA fragments. Conformational changes of molecules are monitored by single-molecule Förster resonance energy transfer, whereby a four-way Holliday junction

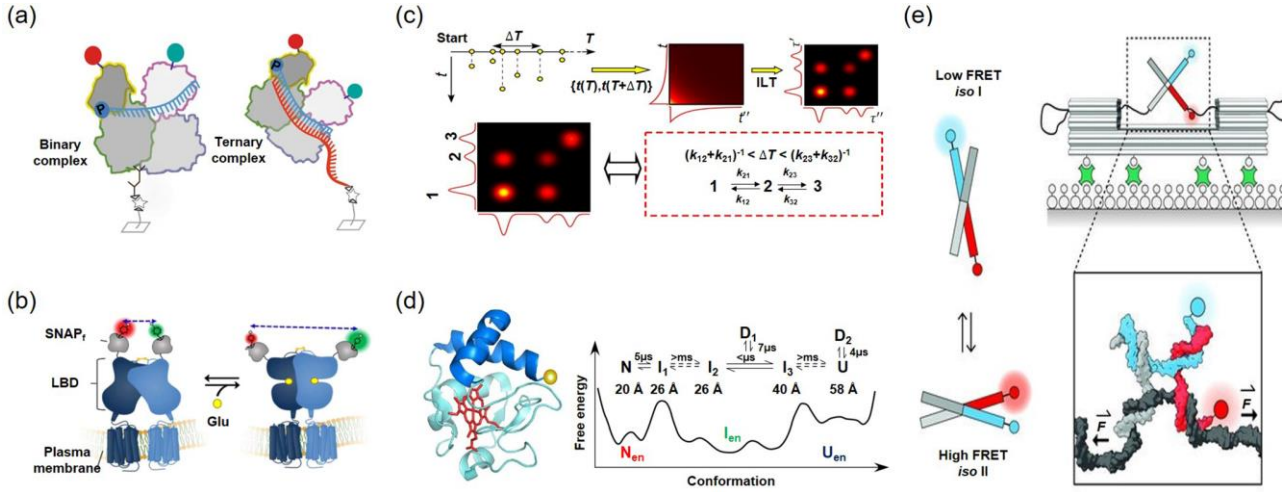


Figure 9 Detection of Biomolecular Dynamics by sm-FRET. (a) Detection of the conformational changes accompanying the stepwise target binding process by FRET. Reprinted with permission,[103] Copyright 2022, Springer Nature Limited. (b) Monitoring the conformational dynamics in Sf-mGluR2 dimers by FRET. Reprinted with permission,[104] Copyright 2021, Springer Nature Limited. (c) Concept of two-dimensional fluorescence lifetime correlation spectroscopy. (d) Conformational transition of cytochrome. Reprinted with permission,[106] Copyright 2015, Springer Nature Limited. (e) Detection of Holliday junction conformer transitions under force by FRET. Reprinted with permission,[109] Copyright 2016, American Association for the Advancement of Science.

(HJ) is linked to the nano-force clamp. The HJ continuously switches between two stacked conformations (isoI and isoII) (Figure 9e). This process can be effectively detected by a pair of fluorophore FRET located on the two walls of the HJ. The outcome indicates that the isoII conformation increasingly dominates with the increase of force. Additionally, the dynamic process of DNA bending induced by the TATA-binding protein under force was also monitored.

6. Electrical and Optical Combined Techniques for Monitoring Single-Molecule Dynamics

Single-molecule fluorescence technology has gained wide acceptance in the study of biomolecules due to its ability to capture dynamic processes occurring on the order of microseconds, which is well-suited to biomolecular dynamics. However, for ultrafast dynamic processes such as chemical reactions, single-molecule electrical detection is better suited due to its ultra-high temporal resolution, which can reach a hundred picoseconds level. Additionally, single-molecule electrical detection can provide real-time and unmarked single-molecule measurements with easier integration capabilities. Combining optical and electrical technologies can provide comprehensive understanding of single-molecule dynamics, including the position information, electrical and optical properties of target molecules. Such a multi-dimensional approach holds great promise for the next-generation of single-molecule detection methods.

Single-molecule electrical detections using solid-state devices such as SMJs[110-113] and SiNWs[84,114] have recently been augmented with optical method, such as the electrical and optical combined system as illustrated in Figure 10a.[115] The incorporation of optical measurements has enabled the verification of the

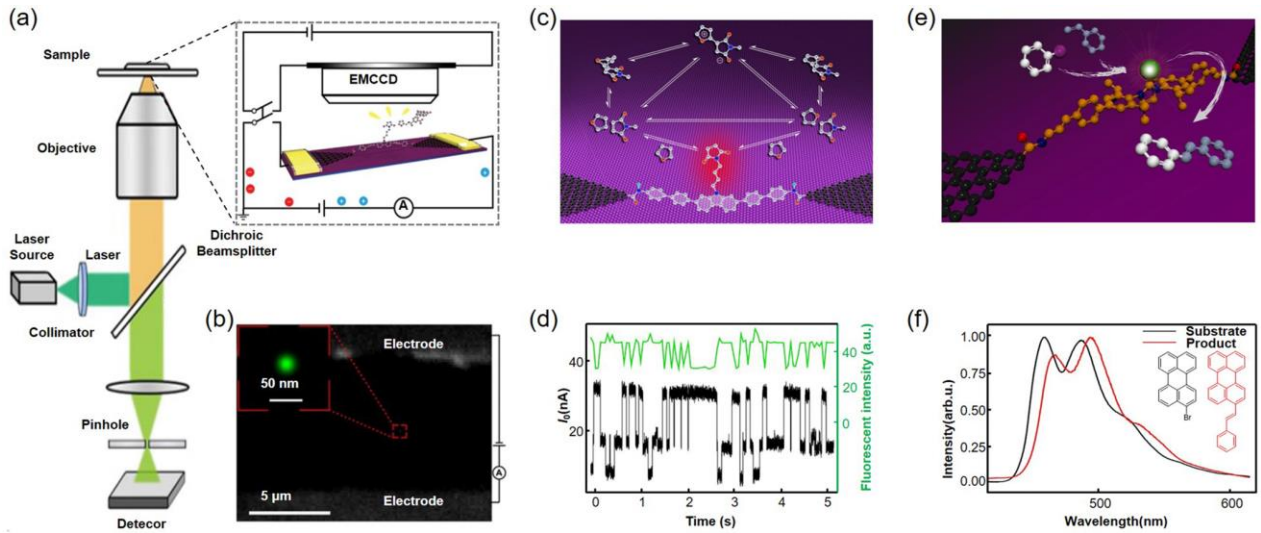


Figure 10 Single-molecule photoelectric combination technology. (a) Schematic of photoelectric combination system, in which electrical signals are obtained through single-molecule junctions and optical signals are obtained through electron-multiplying charge-coupled device (EMCCD). Reprinted with permission,[115] Advanced Sensor Research published by Wiley-VCH GmbH. (b) Visualization of a single-molecule connection by fluorescent signal. (c) Schematic diagram of the single-molecule Diels-Alder reaction dynamics. (d) Synchronous monitoring of current and fluorescent signals during the 5-s reaction of the single-molecule Diels-Alder reaction. Reprinted with permission,[111] Copyright 2021, American Association for the Advancement of Science. (e)Schematic diagram of the single-molecule Mizoroki-Heck reaction. (f) Normalized fluorescence emission spectra of 3-bromoperylene and its Mizoroki–Heck reaction product in macroscopic experiments. Reprinted with permission,[110] Copyright 2022, Springer Nature Limited.

presence of only a single molecule in the electrical detection apparatus. The Diels-Alder reaction between fluorescein substituted furan and maleimide, using a graphene-based single-molecule junction, exemplifies this approach (Figure 10c). The super-resolution microscopic imaging of the device revealed only one bright spot imaged between the graphene electrode arrays, indicating only one single molecular connection was sited (Figure 10b).[111] In addition, this combined system allowed simultaneous collection of fluorescence intensity and electrical signal data, which shows a strong correlation (Figure 10d). Specifically, the high conductivity state corresponds to fluorescence quenching, whereas the low conductivity corresponds to the highest intensity fluorescence. This consistency confirmed that the molecular bridge was indeed the reaction center.

As another example, the dynamic process of palladium-catalyzed Mizoroki-Heck reaction was studied using electrical and optical combined technology (Figure 10e).[110] A single-molecule junction was formed by connecting an *N*-heterocyclic carbene-palladium (NHC-Pd) between two graphene point electrodes. During the Mizoroki-Heck reaction of styrene and fluorescence 3-bromoperylene, blinking at the site of NHC-Pd catalyst was observed. This was due to the transfer of energy/charge from the catalytic center to graphene electrodes. The synchronization of the recorded optical and electrical signal changes demonstrated the dynamic process of the single-molecule reaction. Moreover, by comparing the fluorescence spectra before and after the reaction, it was further confirmed that the functional center of the single molecular junction (NHC-Pd) catalyzes the Mizoroki-Heck reaction (Figure 10f).

The combined system enables the simultaneous detection of the optical and electrical signatures of a single molecule. The interdependence of these signatures, originating from the same molecule, improves detection reliability and provides additional information for further analysis.

7. Summary and Outlook

In summary, we present a comprehensive overview of single-molecule measurement techniques using electrical and optical methods. These technologies offer great opportunities to reveal a wealth of dynamic information at the single-molecule level, including conformational isomerism, host-guest interactions, chemical reactions (such as nucleophilic substitution/addition, rearrangement, and catalysis), as well as molecular activities involved in biological functions (such as protein detection and DNA hybridization). Furthermore, the integration of both electrical and optical techniques holds great potential for acquiring multimodal molecular information and properties, making it a highly promising approach for single-molecule dynamic detection.

While current techniques have demonstrated success in studying various molecular dynamics and providing valuable insights into the underlying mechanisms, there are still limitations. For instance, they are insufficient in trapping intermediates/transition states of dynamic events or exploring the quantum mechanisms of reactions. Therefore, integrating other cutting-edge techniques such as ultrafast optics, ultracooling, and super-resolution technology, could be a promising direction towards achieving transient structures of excited states of molecules or capturing dynamic processes of charge/energy transfer at the pico/femtosecond timescale. Additionally, to obtain a comprehensive understanding of the kinetics involved in various processes, it is crucial to gather multidimensional information by integrating techniques from diverse fields including electronics, optics, mechanics, and magnetism. Consequently, the development of multimodal standard techniques becomes imperative. Furthermore, there is a growing demand to expand the applications of single-molecule devices in studying dynamics. These devices can be utilized to monitor chemical reaction processes in industrial production, enable precise molecular diagnostics in the field of healthcare, and facilitate the sequencing of biomolecules like DNA, proteins, and polysaccharides. By harnessing the potential of these advancements, we can unlock new avenues for scientific exploration and technological innovation.

Acknowledgement

We acknowledge primary financial supports from the National Key R&D Program of China (2021YFA1200102, 2021YFA1200101, and 2022YFE0128700), the National Natural Science Foundation of China (22173050, 22150013, 21727806, and 21933001), the Fundamental Research Funds for the Central Universities (63223056), the Tencent Foundation through the XPLORER PRIZE, "Frontiers Science Center for New Organic Matter" at Nankai University (63181206), the Natural Science Foundation of Beijing (2222009), and Beijing National Laboratory for Molecular Sciences (BNLMS202105).

References

[1] Li, Y.; Yang, C.; Guo, X. Single-Molecule Electrical Detection: A Promising Route toward the Fundamental Limits of Chemistry and Life Science. *Acc. Chem. Res.* **2020**, *53*, 159–169.
[2] Xie, X.; Li, P.; Xu, Y.; Zhou, L.; Yan, Y.; Xie, L.; Jia, C.; Guo, X. Single-Molecule Junction: A Reliable Platform for Monitoring Molecular Physical and Chemical Processes. *ACS Nano* **2022**, *16*, 3476–3505.
[3] Li, Y.; Zhao, L.; Yao, Y.; Guo, X. Single-Molecule Nanotechnologies: An Evolution in Biological Dynamics Detection. *ACS Appl. Bio. Mater.* **2020**, *3*, 68–85.
[4] Chen, H.; Jia, C.; Zhu, X.; Yang, C.; Guo, X.; Stoddart, J. F. Reactions in Single-Molecule Junctions. *Nat. Rev. Mater.* **2022**, *8*, 165–185.
[5] Chen, Y.; Huang, L.; Chen, H.; Chen, Z.; Zhang, H.; Xiao, Z.; Hong, W. Towards Responsive Single-Molecule Device. *Chin. J. Chem.* **2021**, *39*, 421–439.
[6] Li, P.; Jia, C.; Guo, X. Structural Transition Dynamics in Carbon Electrode-Based Single-Molecule Junctions. *Chin. J. Chem.* **2021**, *39*, 223–231.
[7] Gu, C.; Jia, C.; Guo, X. Single-Molecule Electrical Detection with Real-Time Label-Free Capability and Ultrasensitivity. *Small Methods* **2017**, *1*, 1700071.
[8] Zhang, Y.; Du, S.; Hirakawa, K. Deep-Nanometer-Scale Terahertz Spectroscopy Using a Transistor Geometry with Metal Nanogap Electrodes. *Light Sci. Appl.* **2021**, *2*, 460–472.
[9] Stone, I.; Starr, R. L.; Zang, Y.; Nuckolls, C.; Steigerwald, M. L.; Lambert, T. H.; Roy, X.; Venkataraman, L. A Single-Molecule Blueprint for Synthesis. *Nat. Rev. Chem.* **2021**, *5*, 695–710.
[10] Sweetman, A.; Champness, N. R.; Saywell, A. On-Surface Chemical Reactions Characterised by Ultra-high Resolution Scanning Probe Microscopy. *Chem. Soc. Rev.* **2020**, *49*, 4189–4202.
[11] Jia, W.; Hu, C.; Wang, Y.; Gu, Y.; Qian, G.; Du, X.; Wang, L.; Liu, Y.; Cao, J.; Zhang, S.; Yan, S.; Zhang, P.; Ma, J.; Chen, H. Y.; Huang, S. Programmable Nano-Reactors for Stochastic Sensing. *Nat. Commun.* **2021**, *12*, 5811.
[12] Xue, L.; Yamazaki, H.; Ren, R.; Wanunu, M.; Ivanov, A. P.; Edel, J. B. Solid-State Nanopore Sensors. *Nat. Rev. Mater.* **2020**, *5*, 931–951.
[13] Cao, C.; Long, Y. T. Biological Nanopores: Confined Spaces for Electrochemical Single-Molecule Analysis. *Acc. Chem. Res.* **2018**, *51*, 331–341.
[14] Kondo, T.; Chen, W. J.; Schlau-Cohen, G. S. Single-Molecule Fluorescence Spectroscopy of Photosynthetic Systems. *Chem. Rev.* **2017**, *117*, 860–898.
[15] Li, P.; Chen, Y.; Wang, B.; Li, M.; Xiang, D.; Jia, C.; Guo, X. Single-Molecule Optoelectronic Devices: Physical Mechanism and Beyond. *Opto-Electron. Adv.* **2022**, *5*, 210094.
[16] Xiao, Y.; Xu, W. Single-Molecule Fluorescence Imaging of Nanocatalysis. *Chin. J. Chem.* **2021**, *39*, 1459–1470.
[17] Li, C. Y.; Duan, S.; Yi, J.; Wang, C.; Radjenovic, P. M.; Tian, Z. Q.; Li, J. F.

Real-Time Detection of Single-Molecule Reaction by Plasmon-Enhanced Spectroscopy. *Sci. Adv.* **2020**, *6*, eaba6012.

[18] Deckert-Gaudig, T.; Taguchi, A.; Kawata, S.; Deckert, V. Tip-Enhanced Raman Spectroscopy - From Early Developments to Recent Advances. *Chem. Soc. Rev.* **2017**, *46*, 4077–4110.

[19] Wang, X.; Huang, S. C.; Huang, T. X.; Su, H. S.; Zhong, J. H.; Zeng, Z. C.; Li, M. H.; Ren, B. Tip-Enhanced Raman Spectroscopy for Surfaces and Interfaces. *Chem. Soc. Rev.* **2017**, *46*, 4020–4041.

[20] Yan, R.; Moon, S.; Kenny, S. J.; Xu, K. Spectrally Resolved and Functional Super-resolution Microscopy via Ultrahigh-Throughput Single-Molecule Spectroscopy. *Acc. Chem. Res.* **2018**, *51*, 697–705.

[21] Nicolai, C.; Sachs, F. Solving Ion Channel Kinetics with the QuB Software. *Biophys. Rev. Lett.* **2014**, *8*, 191–211.

[22] Choi, Y.; Moody, I. S.; Sims, P. C.; Hunt, S. R.; Corso, B. L.; Perez, I.; Weiss, G. A.; Collins, P. G. Single-Molecule Lysozyme Dynamics Monitored by an Electronic Circuit. *Science* **2012**, *335*, 319–324.

[23] Goldsmith, B. R.; Coroneus, J. G.; Kane, A. A.; Weiss, G. A.; Collins, P. G. Monitoring Single-Molecule Reactivity on a Carbon Nanotube. *Nano Lett.* **2008**, *8*, 189–194.

[24] Quílez-Pardo, J. Do the Equilibrium Constants have Units? A Discussion on how General Chemistry Textbooks Calculate and Report the Equilibrium Constants. *Int. J. Physc. Chem. Ed.* **2019**, *11*, 73–83.

[25] Sorgenfrei, S.; Chiu, C. Y.; Gonzalez, R. L., Jr.; Yu, Y. J.; Kim, P.; Nuckolls, C.; Shepard, K. L. Label-Free Single-Molecule Detection of DNA-Hybridization Kinetics with a Carbon Nanotube Field-Effect Transistor. *Nat. Nanotechnol.* **2011**, *6*, 126–132.

[26] Reed, M. A.; Zhou, C.; Muller, C. J.; Burgin, T. P.; Tour, J. M. Conductance of a Molecular Junction. *Science* **1997**, *278*, 252–254.

[27] Xiang, D.; Jeong, H.; Lee, T.; Mayer, D. Mechanically Controllable Break Junctions for Molecular Electronics. *Adv. Mater.* **2013**, *25*, 4845–4867.

[28] Xu, B.; Tao, N. J. Measurement of Single-Molecule Resistance by Repeated Formation of Molecular Junctions. *Science* **2003**, *301*, 1221–1223.

[29] Venkataraman, L.; Klare, J. E.; Nuckolls, C.; Hybertsen, M. S.; Steigerwald, M. L. Dependence of Single-Molecule Junction Conductance on Molecular Conformation. *Nature* **2006**, *442*, 904–907.

[30] Park, J.; Pasupathy, A. N.; Goldsmith, J. I.; Chang, C.; Yaish, Y.; Petta, J. R.; Rinkoski, M.; Sethna, J. P.; Abruna, H. D.; McEuen, P. L.; Ralph, D. C. Coulomb Blockade and the Kondo Effect in Single-Atom Transistors. *Nature* **2002**, *417*, 722–725.

[31] Nesvorny, D.; Bottke, W. F., Jr.; Dones, L.; Levison, H. F. The Recent Breakup of an Asteroid in the Main-belt Region. *Nature* **2002**, *417*, 720–771.

[32] Aragones, A. C.; Haworth, N. L.; Darwish, N.; Ciampi, S.; Bloomfield, N. J.; Wallace, G. G.; Diez-Perez, I.; Coote, M. L. Electrostatic Catalysis of A Diels-Alder Reaction. *Nature* **2016**, *531*, 88–91.

[33] Li, Y.; Haworth, N. L.; Xiang, L.; Ciampi, S.; Coote, M. L.; Tao, N. Mechanical Stretching-Induced Electron-Transfer Reactions and Conductance Switching in Single Molecules. *J. Am. Chem. Soc.* **2017**, *139*, 14699–14706.

[34] Huang, X.; Tang, C.; Li, J.; Chen, L. C.; Zheng, J.; Zhang, P.; Le, J.; Li, R.; Li, X.; Liu, J.; Yang, Y.; Shi, J.; Chen, Z.; Bai, M.; Zhang, H. L.; Xia, H.; Cheng, J.; Tian, Z. Q.; Hong, W. Electric Field-Induced Selective Catalysis of Single-Molecule Reaction. *Sci. Adv.* **2019**, *5*, eaaw3072.

[35] Zhang, A.; Zhuang, X.; Liu, J.; Huang, J.; Lin, L.; Tang, Y.; Zhao, S.; Li, R.; Wang, B.; Fang, B.; Hong, W. Catalytic Cycle of Formate Dehydrogenase Captured by Single-Molecule Conductance. *Nat. Catal.* **2023**, *6*, 266–275.

[36] Cao, Y.; Dong, S.; Liu, S.; He, L.; Gan, L.; Yu, X.; Steigerwald, M. L.; Wu, X.; Liu, Z.; Guo, X. Building High-Throughput Molecular Junctions Using Indented Graphene Point Contacts. *Angew. Chem. Int. Ed.* **2012**, *51*, 12228–12232.

[37] Xiang, D.; Wang, X.; Jia, C.; Lee, T.; Guo, X. Molecular-Scale Electronics: From Concept to Function. *Chem. Rev.* **2016**, *116*, 4318–4440.

[38] Gu, C.; Hu, C.; Wei, Y.; Lin, D.; Jia, C.; Li, M.; Su, D.; Guan, J.; Xia, A.; Xie, L.; Nitzan, A.; Guo, H.; Guo, X. Label-Free Dynamic Detection of Single-Molecule Nucleophilic-Substitution Reactions. *Nano Lett.* **2018**, *18*, 4156–4162.

[39] Guan, J.; Jia, C.; Li, Y.; Liu, Z.; Wang, J.; Yang, Z.; Gu, C.; Su, D.; Houk, K. N.; Zhang, D.; Guo, X. Direct Single-Molecule Dynamic Detection of Chemical Reactions. *Sci. Adv.* **2018**, *4*, 2177.

[40] Guo, Y.; Yang, C.; Jia, C.; Guo, X. Accurate Single-Molecule Indicator of Solvent Effects. *J. Am. Chem. Soc. Au* **2021**, *1*, 2271–2279.

[41] Guo, Y.; Yang, C.; Zhou, S.; Liu, Z.; Guo, X. A Single-Molecule Memristor based on an Electric-Field-Driven Dynamical Structure Reconfiguration. *Adv. Mater.* **2022**, *34*, 2204827.

[42] Yang, C.; Zhang, L.; Li, H.; Guo, Y.; Jia, C.; Zhu, W.; Mo, F.; Guo, X. Single-Molecule Electrical Spectroscopy of Organocatalysis. *Matter* **2021**, *4*, 2874–2885.

[43] Xin, N.; Wang, J.; Jia, C.; Liu, Z.; Zhang, X.; Yu, C.; Li, M.; Wang, S.; Gong, Y.; Sun, H.; Zhang, G.; Liu, Z.; Zhang, G.; Liao, J.; Zhang, D.; Guo, X. Stereoelectronic Effect-Induced Conductance Switching in Aromatic Chain Single-Molecule Junctions. *Nano Lett.* **2017**, *17*, 856–861.

[44] Yang, C.; Shen, P.; Ou, Q.; Peng, Q.; Zhou, S.; Li, J.; Liu, Z.; Zhao, Z.; Qin, A.; Shuai, Z.; Tang, B. Z.; Guo, X. Complete Deciphering of the Dynamic Stereostructures of a Single Aggregation-Induced Emission Molecule. *Matter* **2022**, *5*, 1224–1234.

[45] Meng, L.; Xin, N.; Wang, J.; Xu, J.; Ren, S.; Yan, Z.; Zhang, M.; Shen, C.; Zhang, G.; Guo, X.; Meng, S. Atomically Precise Engineering of Single-Molecule Stereoelectronic Effect. *Angew. Chem. Int. Ed.* **2021**, *60*, 12274–12278.

[46] Meng, L.; Xin, N.; Hu, C.; Wang, J.; Gui, B.; Shi, J.; Wang, C.; Shen, C.; Zhang, G.; Guo, H.; Meng, S.; Guo, X. Side-group Chemical Gating via Reversible Optical and Electric Control in a Single Molecule Transistor. *Nat. Commun.* **2019**, *10*, 1450.

[47] Chen, X.; Yeoh, Y. Q.; He, Y.; Zhou, C.; Horsley, J. R.; Abell, A. D.; Yu, J.; Guo, X. Unravelling Structural Dynamics within a Photoswitchable Single Peptide: A Step Towards Multimodal Bioinspired Nanodevices. *Angew. Chem. Int. Ed.* **2020**, *59*, 22554–22562.

[48] Yan, Z.; Li, X.; Li, Y.; Jia, C.; Xin, N.; Li, P.; Meng, L.; Zhang, M.; Chen, L.; Yang, J.; Wang, R.; Guo, X. Single-Molecule Field Effect and Conductance Switching Driven by Electric Field and Proton Transfer. *Sci. Adv.* **2022**, *8*, 3541.

[49] Huang, S.; He, J.; Chang, S.; Zhang, P.; Liang, F.; Li, S.; Tuchband, M.; Fuhrmann, A.; Ros, R.; Lindsay, S. Identifying Single Bases in a DNA Oligomer with Electron Tunnelling. *Nat. Nanotechnol.* **2010**, *5*, 868–873.

[50] Zhou, C.; Li, X.; Gong, Z.; Jia, C.; Lin, Y.; Gu, C.; He, G.; Zhong, Y.; Yang, J.; Guo, X. Direct Observation of Single-Molecule Hydrogen-Bond Dynamics with Single-Bond Resolution. *Nat. Commun.* **2018**, *9*, 807.

[51] Chen, Y.; Huang, F.; Li, Z.-T.; Liu, Y. Controllable Macrocyclic Supramolecular Assemblies in Aqueous Solution. *Sci. China Chem.* **2018**, *61*, 979–992.

[52] Mikhail V. Rekharsky 1, Y. I. Complexation Thermodynamics of Cyclodextrins. *Chem. Rev.* **1998**, *98*, 1875–1918.

[53] Liu, Z.; Li, X.; Masai, H.; Huang, X.; Tsuda, S.; Terao, J.; Yang, J.; Guo, X. A Single-molecule Electrical Approach for Amino Acid Detection and Chirality Recognition. *Sci. Adv.* **2021**, *7*, eabe4365.

[54] Su, D.; Zhou, S.; Masai, H.; Liu, Z.; Zhou, C.; Yang, C.; Li, Z.; Tsuda, S.; Liu, Z.; Terao, J.; Guo, X. Stochastic Binding Dynamics of a Photoswitchable Single Supramolecular Complex. *Adv. Sci.* **2022**, *9*, 2200022.

[55] Wen, H.; Li, W.; Chen, J.; He, G.; Li, L.; Olson, M. A.; Sue, A. C. H.; Stoddart, J. F.; Guo, X. Complex Formation Dynamics in a Single-Molecule Electronic Device. *Sci. Adv.* **2016**, *2*, 1601113.

[56] Chen, S.; Su, D.; Jia, C.; Li, Y.; Li, X.; Guo, X.; Leigh, D. A.; Zhang, L. Real-Time Observation of the Dynamics of an Individual Rotaxane Molecular Shuttle Using a Single-Molecule Junction. *Chem* **2022**, *8*, 243–252.

[57] Wanekaya, A. K.; Chen, W.; Myung, N. V.; Mulchandani, A. Nanowire-Based Electrochemical Biosensors. *Electroanalysis* **2006**, *18*, 533–550.

[58] Porath, D.; Bezryadin, A.; de Vries, S.; Dekker, C. Direct Measurement of Electrical Transport Through DNA Molecules. *Nature* **2000**, *403*, 635–638.

[59] Slinker, J. D.; Muren, N. B.; Renfrew, S. E.; Barton, J. K. DNA Charge Transport over 34 nm. *Nat. Chem.* **2011**, *3*, 228–233.
[60] Arikuma, Y.; Nakayama, H.; Morita, T.; Kimura, S. Electron Hopping Over 100 A Along an Alpha Helix. *Angew. Chem. Int. Ed.* **2010**, *49*, 1800–1804.
[61] Fuller, C. W.; Padayatti, P. S.; Abderrahim, H.; Adamiak, L.; Alagar, N.; Ananthapadmanabhan, N.; Baek, J.; Chinni, S.; Choi, C.; Delaney, K. J.; Dubielzig, R.; Frkanec, J.; Garcia, C.; Gardner, C.; Gebhardt, D.; Geiser, T.; Gutierrez, Z.; Hall, D. A.; Hodges, A. P.; Hou, G.; Jain, S.; Jones, T.; Lobaton, R.; Majzik, Z.; Marte, A.; Mohan, P.; Mola, P., 2nd; Mudondo, P.; Mullinix, J.; Nguyen, T.; Ollinger, F.; Orr, S.; Ouyang, Y.; Pan, P.; Park, N.; Porras, D.; Prabhu, K.; Reese, C.; Ruel, T.; Sauerbrey, T.; Sawyer, J. R.; Sinha, P.; Tu, J.; Venkatesh, A. G.; VijayKumar, S.; Zheng, L.; Jin, S.; Tour, J. M.; Church, G. M.; Mola, P. W.; Merriman, B. Molecular Electronics Sensors on a Scalable Semiconductor Chip: A Platform for Single-molecule Measurement of Binding Kinetics and Enzyme Activity. *Proc. Natl. Acad. Sci.* **2022**, *119*, e2112812119.
[62] Seker, U. O.; Demir, H. V. Material Binding Peptides for Nanotechnology. *Molecules* **2011**, *16*, 1426–1451.
[63] Blanco, L.; Bernad, A.; Lázaro, J. M.; Martín, G.; Garmendia, C.; Salas, M. Highly Efficient DNA Synthesis by the Phage φ29 DNA Polymerase. *J. Biol. Chem.* **1989**, *264*, 8935–8940.
[64] Gul, O. T.; Pugliese, K. M.; Choi, Y.; Sims, P. C.; Pan, D.; Rajapakse, A. J.; Weiss, G. A.; Collins, P. G. Single Molecule Bioelectronics and Their Application to Amplification-Free Measurement of DNA Lengths. *Biosensors* **2016**, *6*, 29.
[65] Goldsmith, B. R.; Coroneus, J. G.; Khalap, V. R.; Kane, A. A.; Weiss, G. A.; Collins, P. G. Conductance-Controlled Point Functionalization of Single-Walled Carbon Nanotubes. *Science* **2007**, *315*, 77–81.
[66] Gavrilyuk, J.; Ban, H.; Nagano, M.; Hakamata, W.; Barbas, C. F. 3rd Formylbenzene Diazonium Hexafluorophosphate Reagent for Tyrosine-Selective Modification of Proteins and the Introduction of a Bioorthogonal Aldehyde. *Bioconjug. Chem.* **2012**, *23*, 2321–2328.
[67] Lee, Y.; Trocchia, S. M.; Warren, S. B.; Young, E. F.; Vernick, S.; Shepard, K. L. Electrically Controllable Single-Point Covalent Functionalization of Spin-Cast Carbon-Nanotube Field-Effect Transistor Arrays. *ACS Nano* **2018**, *12*, 9922–9930.
[68] Chen, R. J.; Zhang, Y.; Wang, D.; Dai, H. Noncovalent Sidewall Functionalization of Single-Walled Carbon Nanotubes for Protein Immobilization. *J. Am. Chem. Soc.* **2001**, *123*, 3838–3839.
[69] Zhao, Y. L.; Hu, L.; Stoddart, J. F.; Grüner, G. Pyrenecyclodextrin-Decorated Single-Walled Carbon Nanotube Field-Effect Transistors as Chemical Sensors. *Adv. Mater.* **2008**, *20*, 1910–1915.
[70] Bouilly, D.; Hon, J.; Daly, N. S.; Trocchia, S.; Vernick, S.; Yu, J.; Warren, S.; Wu, Y.; Gonzalez, R. L., Jr.; Shepard, K. L.; Nuckolls, C. Single-Molecule Reaction Chemistry in Patterned Nanowells. *Nano Lett.* **2016**, *16*, 4679–4685.
[71] Star, A.; Tu, E.; Niemann, J.; Gabriel, J. C. P.; Joiner, C. S.; Valcke, C. Label-free Detection of DNA Hybridization Using Carbon Nanotube Network Field-Effect Transistors. *Proc. Natl. Acad. Sci.* **2006**, *103*, 921–926.
[72] Vernick, S.; Trocchia, S. M.; Warren, S. B.; Young, E. F.; Bouilly, D.; Gonzalez, R. L.; Nuckolls, C.; Shepard, K. L. Electrostatic Melting in a Single-Molecule Field-Effect Transistor with Applications in Genomic Identification. *Nat. Commun.* **2017**, *8*, 15450.
[73] Choi, Y.; Olsen, T. J.; Sims, P. C.; Moody, I. S.; Corso, B. L.; Dang, M. N.; Weiss, G. A.; Collins, P. G. Dissecting Single-Molecule Signal Transduction in Carbon Nanotube Circuits with Protein Engineering. *Nano Lett.* **2013**, *13*, 625–631.
[74] Olsen, T. J.; Choi, Y.; Sims, P. C.; Gul, O. T.; Corso, B. L.; Dong, C.; Brown, W. A.; Collins, P. G.; Weiss, G. A. Electronic Measurements of Single-Molecule Processing by DNA Polymerase I (Klenow fragment). *J. Am. Chem. Soc.* **2013**, *135*, 7855–7860.
[75] Pugliese, K. M.; Gul, O. T.; Choi, Y.; Olsen, T. J.; Sims, P. C.; Collins, P. G.; Weiss, G. A. Processive Incorporation of Deoxynucleoside Triphosphate Analogs by Single-Molecule DNA Polymerase I (Klenow Fragment) Nanocircuits. *J. Am. Chem. Soc.* **2015**, *137*, 9587–9594.
[76] Turvey, M. W.; Gabriel, K. N.; Lee, W.; Taulbee, J. J.; Kim, J. K.; Chen, S.; Lau, C. J.; Kattan, R. E.; Pham, J. T.; Majumdar, S.; Garcia, D.; Weiss, G. A.; Collins, P. G. Single-Molecule Taq DNA Polymerase Dynamics. *Sci. Adv.* **2022**, *8*, eabl3522.
[77] Morales, A. M.; Lieber, C. M. A Laser Ablation Method for the Synthesis of Crystalline Semiconductor Nanowires. *Science* **1998**, *279*, 208–211.
[78] Yin, D.; Li, J.; Feng, J.; Liu, W.; Yang, Z.; Li, S.; Li, M.; Li, L.; Guo, X. Direct Mechano-Sliding Transfer of Chemical Vapor Deposition Grown Silicon Nanowires for Nanoscale Electronic Devices. *J. Mater. Chem. C* **2022**, *10*, 469–475.
[79] Wang, J.; Shen, F.; Wang, Z.; He, G.; Qin, J.; Cheng, N.; Yao, M.; Li, L.; Guo, X. Point Decoration of Silicon Nanowires: An Approach toward Single-Molecule Electrical Detection. *Angew. Chem. Int. Ed.* **2014**, *53*, 5038–5043.
[80] Li, J.; He, G.; Ueno, H.; Jia, C.; Noji, H.; Qi, C.; Guo, X. Direct Real-Time Detection of Single Proteins Using Silicon Nanowire-Based Electrical Circuits. *Nanoscale* **2016**, *8*, 16172–16176.
[81] He, G.; Li, J.; Ci, H.; Qi, C.; Guo, X. Direct Measurement of Single-Molecule DNA Hybridization Dynamics with Single-Base Resolution. *Angew. Chem. Int. Ed.* **2016**, *55*, 9036–9040.
[82] He, G.; Li, J.; Qi, C.; Guo, X. Single Nucleotide Polymorphism Genotyping in Single-Molecule Electronic Circuits. *Adv. Sci.* **2017**, *4*, 1700158.
[83] Li, J.; He, G.; Hiroshi, U.; Liu, W.; Noji, H.; Qi, C.; Guo, X. Direct Measurement of Single-Molecule Adenosine Triphosphatase Hydrolysis Dynamics. *ACS Nano* **2017**, *11*, 12789–12795.
[84] Yang, Z.; Qi, C.; Liu, W.; Yin, D.; Yu, L.; Li, L.; Guo, X. Revealing Conformational Transition Dynamics of Photosynthetic Proteins in Single-Molecule Electrical Circuits. *J. Phys. Chem. Lett.* **2021**, *12*, 3853–3859.
[85] Lórenz-Fonfría, V. A.; Furutani, Y.; Ota, T.; Ido, K.; Kandori, H. Protein Fluctuations as the Possible Origin of the Thermal Activation of Rod Photoreceptors in the Dark. *J. Am. Chem. Soc.* **2010**, *132*, 5693–5703.
[86] Herschel, J. F. W. On a Case of Superficial Colour Presented by a Homogeneous Liquid Internally Colourless. *Phil. Trans. R. Soc.* **1845**, *135*, 143–145.
[87] Stokes, G. G. On the Change of Refrangibility of Light. *Philos. Trans. R. Soc. London* **1852**, *142*, 463–562.
[88] Axelrod, D. Cell-Substrate Contacts Illuminated by Total Internal Reflection Fluorescence. *J. Cell Biol.* **1981**, *89*, 141–145.
[89] Axelrod, D.; Burghardt, T. P.; Thompson, N. L. Total Internal Reflection Fluorescence. *Annu. Rev. Biophys. Bioeng.* **1984**, *13*, 247–268.
[90] Nwaneshiudu, A.; Kuschal, C.; Sakamoto, F. H.; Anderson, R. R.; Schwarzenberger, K.; Young, R. C. Introduction to Confocal Microscopy. *J. Invest. Dermatol.* **2012**, *132*, e3.
[91] Ash, E. A.; Nicholls, G. Super-Resolution Aperture Scanning Microscope. *Nature* **1972**, *237*, 510–512.
[92] Hell, S. W.; Wichmann, J. Breaking the Diffraction Resolution Limit by Stimulated Emission: Stimulated-Emission-Depletion Fluorescence Microscopy. *Opt. Lett.* **1994**, *19*, 780–782.
[93] Klar, T. A.; Jakobs, S.; Dyba, M.; Egner, A.; Hell, S. W. Fluorescence Microscopy with Diffraction Resolution Barrier Broken by Stimulated Emission. *Proc. Natl. Acad. Sci.* **2000**, *97*, 8206–8210.
[94] Chen, T.; Dong, B.; Chen, K.; Zhao, F.; Cheng, X.; Ma, C.; Lee, S.; Zhang, P.; Kang, S. H.; Ha, J. W.; Xu, W.; Fang, N. Optical Super-Resolution Imaging of Surface Reactions. *Chem. Rev.* **2017**, *117*, 7510–7537.
[95] Xie, X. S.; Lu, H. P. Single-molecule Enzymology. *J. Biol. Chem.* **1999**, 274, 15967–15970.
[96] Lu, H. P.; Xun, L.; Xie, X. S. Single-Molecule Enzymatic Dynamics. *Science* **1998**, *282*, 1877–1882.
[97] Kim, D.; Zhang, Z.; Xu, K. Spectrally Resolved Super-Resolution Microscopy Unveils Multipath Reaction Pathways of Single Spiropyran Molecules. *J. Am. Chem. Soc.* **2017**, *139*, 9447–9450.
[98] Zhou, X.; Andoy, N. M.; Liu, G.; Choudhary, E.; Han, K. S.; Shen, H.; Chen, P. Quantitative Super-Resolution Imaging Uncovers Reactivity Patterns on Single Nanocatalysts. *Nat. Nanotechnol.* **2012**, *7*, 237–241.
[99] Dong, B.; Pei, Y.; Zhao, F.; Goh, T. W.; Qi, Z.; Xiao, C.; Chen, K.; Huang,

W.; Fang, N. In Situ Quantitative Single-Molecule Study of Dynamic Catalytic Processes in Nanoconfinement. *Nat. Catal.* **2018**, *1*, 135–140.

[100] Sambur, J. B.; Chen, T. Y.; Choudhary, E.; Chen, G.; Nissen, E. J.; Thomas, E. M.; Zou, N.; Chen, P. Sub-particle Reaction and Photocurrent Mapping to Optimize Catalyst-Modified Photoanodes. *Nature* **2016**, *530*, 77–80.

[101] Shen, M.; Ding, T.; Rackers, W. H.; Tan, C.; Mahmood, K.; Lew, M. D.; Sadtler, B. Single-Molecule Colocalization of Redox Reactions on Semiconductor Photocatalysts Connects Surface Heterogeneity and Charge-Carrier Separation in Bismuth Oxybromide. *J. Am. Chem. Soc.* **2021**, *143*, 11393–11403.

[102] Lerner, E.; Cordes, T.; Ingargiola, A.; Alhadid, Y.; Chung, S.; Michalet, X.; Weiss, S. Toward Dynamic Structural Biology: Two Decades of Single-Molecule Forster Resonance Energy Transfer. *Science* **2018**, *359*, eaan1133.

[103] Willkomm, S.; Jakob, L.; Kramm, K.; Graus, V.; Neumeier, J.; Meister, G.; Grohmann, D. Single-Molecule FRET Uncovers Hidden Conformations and Dynamics of Human Argonaute 2. *Nat. Commun.* **2022**, *13*, 3825.

[104] Asher, W. B.; Geggier, P.; Holsey, M. D.; Gilmore, G. T.; Pati, A. K.; Meszaros, J.; Terry, D. S.; Mathiasen, S.; Kaliszewski, M. J.; McCauley, M. D.; Govindaraju, A.; Zhou, Z.; Harikumar, K. G.; Jaqaman, K.; Miller, L. J.; Smith, A. W.; Blanchard, S. C.; Javitch, J. A. Single-Molecule FRET Imaging of GPCR Dimers in Living Cells. *Nat. Methods* **2021**, *18*, 397–405.

[105] Quast, R. B.; Margeat, E. Single-Molecule FRET on Its Way to Structural Biology in Live Cells. *Nat. Methods* **2021**, *18*, 344–345.

[106] Otosu, T.; Ishii, K.; Tahara, T. Microsecond Protein Dynamics Observed at the Single-Molecule Level. *Nat. Commun.* **2015**, *6*, 7685.

[107] Sisamakis, E.; Valeri, A.; Kalinin, S.; Rothwell, P. J.; Seidel, C. A. Accurate Single-Molecule FRET Studies Using Multiparameter Fluorescence Detection. *Methods Enzymol.* **2010**, *475*, 455–514.

[108] Kilic, S.; Felekyan, S.; Doroshenko, O.; Boichenko, I.; Dimura, M.; Vardanyan, H.; Bryan, L. C.; Arya, G.; Seidel, C. A. M.; Fierz, B. Single-Molecule FRET Reveals Multiscale Chromatin Dynamics Modulated by HP1alpha. *Nat. Commun.* **2018**, *9*, 235.

[109] Nickels, P. C.; Wünsch, B.; Holzmeister, P.; Bae, W.; Kneer, L. M.; Grohmann, D.; Tinnefeld, P.; Liedl, T. Molecular Force Spectroscopy with a DNA Origami-Based Nanoscopic Force Clamp. *Science* **2016**, *354*, 305–307.

[110] Zhang, L.; Yang, C.; Lu, C.; Li, X.; Guo, Y.; Zhang, J.; Lin, J.; Li, Z.; Jia, C.; Yang, J.; Houk, K. N.; Mo, F.; Guo, X. Precise Electrical Gating of the Single-Molecule Mizoroki-Heck Reaction. *Nat. Commun.* **2022**, *13*, 4552.

[111] Yang, C.; Liu, Z.; Li, Y.; Zhou, S.; Lu, C.; Guo, Y.; Ramirez, M.; Zhang, Q.; Li, Y.; Liu, Z.; Houk, K. N.; Zhang, D.; Guo, X. Electric Field-Catalyzed Single-Molecule Diels-Alder Reaction Dynamics. *Sci. Adv.* **2021**, *7*, 0689.

[112] Yang, C.; Zhang, L.; Lu, C.; Zhou, S.; Li, X.; Li, Y.; Yang, Y.; Li, Y.; Liu, Z.; Yang, J.; Houk, K. N.; Mo, F.; Guo, X. Unveiling the Full Reaction Path of the Suzuki-Miyaura Cross-Coupling in a Single-Molecule Junction. *Nat. Nanotechnol.* **2021**, *16*, 1214–1223.

[113] Guo, Y.; Yang, C.; Li, H.; Zhang, L.; Zhou, S.; Zhu, X.; Fu, H.; Li, Z.; Liu, Z.; Jia, C.; Liu, Z.; Zhu, W.; Mo, F.; Zhang, D.; Guo, X. Accurate Single-Molecule Kinetic Isotope Effects. *J. Am. Chem. Soc.* **2022**, *144*, 3146–3153.

[114] Liu, W.; Li, J.; Xu, Y.; Yin, D.; Zhu, X.; Fu, H.; Su, X.; Guo, X. Complete Mapping of DNA-Protein Interactions at the Single-Molecule Level. *Adv. Sci.* **2021**, *8*, 2101383.

[115] Chang, X.; Huo, Y.; Zhao, C.; Sun, W.; Song, Z.; Qi, Z.; Wang, J.; Jia, C.; Guo, X. Single-Molecule Electronic Biosensors: Principles and Applications. *Adv. Sensor Res.* **2023**, *2*, 2200084.

Manuscript received: April 28, 2023
Manuscript revised: June 8, 2023
Manuscript accepted: June 21, 2023
Accepted manuscript online: June 22, 2023
Version of record online: August 8, 2023

