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Single-molecule electrical spectroscopy of organocatalysis



A general single-molecule electrical spectroscopy (SMES) of organocatalysis is directly established on an *in situ* label-free electrical detection platform to efficiently capture the hidden intermediates and precisely clarify the conversion relationship of each event in a simple (homo-condensation) or complex (crosscondensation) system of a benzoin reaction. Furthermore, a new thermodynamic and kinetic model is built to deduce the inventory of substrates around the individual catalyst, enabling single-molecule quantitative analysis. Chen Yang, Lei Zhang, Huiping Li, ..., Wenguang Zhu, Fanyang Mo, Xuefeng Guo

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Highlights

Universal single-molecule electrical spectroscopy to monitor complex reactions

Novel reaction dynamics and new intermediates discovered by correlation analysis

New thermodynamic/kinetic model offering quantitative analysis on a molecular basis



Development

Practical, real world, technological considerations and constraints

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Single-molecule electrical spectroscopy of organocatalysis

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SUMMARY

Conventional mechanistic studies based on detection of species concentration reveal the basic laws of matter conversion, but inherently display ensemble averages. Single-molecule detection provides a solution and direct visualization of reaction pathway rather than only detection of species. Here, we establish an in situ labelfree electrical detection platform and report universal single-molecule electrical spectroscopy (SMES) of organocatalysis to decipher the intrinsic mechanism of a benzoin reaction. This SMES is capable of monitoring each event in a simple (homo-condensation) or complex (cross-condensation) system. Both experimental and theoretical results consistently revealed accuracy of the spectroscopic attribution and proved its reliability. Furthermore, the correlation of adjacent events in the spectroscopy indicated the local repeated interaction between a catalyst and a substrate. Through a new thermodynamic and kinetic model, the inventory of substrates around the single-molecule catalyst can be reversely deduced, which enables quantitative analysis of the target single molecule.

INTRODUCTION

Catalysis enabled by small organic molecules, namely organocatalysis, has grown into a complementary mode of (transition metal) catalysis due to the discovery and realization of its prominent merits, including convenient availability and great robustness of the catalysts, mild reaction conditions, and extraordinary selectivity.^{1–3} In the golden age of organocatalysis, one of the most crucial aspects sought for further advance in this area is the identification of the relationships among different intermediates and important transformations rather than mere species detection via nuclear magnetic resonance or mass spectrometry, which warrants further progress.¹ The catalytic processes of organocatalysis are multifarious and complex in different types of organocatalysts. However, they share a uniform core, which is the periodic structural transformation of organocatalysts during the catalytic cycles. Therefore, an ideal solution to obtain insight into important transformations is the availability of efficient spectroscopic methods that can visualize *in situ* the dynamic changes of different organocatalysts at the single-event level.

In situ single-molecule real-time electrical monitoring technology meets these requirements. Real-time monitoring of only one organocatalyst provides the most intrinsic characteristics of organic reactions, such as discovery of hidden intermediates,^{4,5} tracking of the reaction trajectory,⁶ visualization of the reaction pathway,^{7,8} and description of the complete reaction mechanism. In addition, the thermodynamics and kinetics of elementary reactions,⁹ the correlation of adjacent reaction

Progress and potential

Analysis of reaction trajectories is a reliable and efficient method for the mechanistic investigation of chemical reactions. However, conventional macroscopic technologies to track the trajectories of chemical reactions provide the picture of species concentration variations versus time, which inherently display ensemble averages. In this work, we established an unprecedented, powerful singlemolecule electrical spectroscopy (SMES) approach on an in situ label-free electrical detection platform.

This SMES provides the real-time visualization of the reaction trajectories of only one molecule and more information in higher dimensions (such as correlation, time sequence, and dynamic interaction mode) hidden in ensemble averages. We also redefined the thermodynamics and kinetics at the singlemolecule level and achieved the inventory of the single-molecule substrates on this basis. This SMES opens up infinite possibilities to decipher the intrinsic reaction mechanism.



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events, and memory effects^{10,11} can also be derived from single-molecule electrical detection. In this work, we focus our research on the development of a general single-molecule electrical spectroscopy (SMES) methodology on an *N*-heterocyclic carbene (NHC) catalyzed benzoin reaction by employing this unique technology.

NHCs are a type of versatile organic molecule, which possess a singlet carbene stabilized by neighboring heteroatoms.¹² Due to their special structure and nucleophilic property, NHCs have been widely applied in not only transition-metal catalysis as ligands^{13,14} but also organocatalysis as catalysts themselves.^{2,15} For example, the umpolung benzoin condensation is one of the most famous reactions catalyzed by NHCs and enables efficient C-C bond construction by using conveniently available aldehydes.¹⁶ In 1958, Breslow and coworkers put forth a mechanism for the NHCcatalyzed benzoin reaction,¹⁷ which has become widely accepted. In general, the precursor of a given NHC (for example imidazolium salt) is converted to the free carbene catalyst by deprotonation. This free carbene then attacks an aldehyde molecule to produce enaminol-type Breslow intermediates (BIs) upon proton transfer. The BI further reacts again with a second aldehyde molecule to furnish the resulting intermediate, which finally eliminates the benzoin products and the NHC catalyst. Specifically, Berkessel et al. synthesized and characterized the crucial BIs by adopting catalysts with low reactivity or aldehydes with high electrophilicity,^{18,19} which strengthened Breslow's proposed mechanism. However, as a result of their high reactivity, the dynamic processes toward key intermediates of the benzoin reaction have not yet been thoroughly investigated, and thus remain a huge challenge. To this end, on the basis of single-molecule nanocircuits formed from an individual NHC catalyst covalently sandwiched between graphene electrodes, we demonstrated an unprecedented SMES whose main advantages are high time resolution and faithful synchrony between electrical signals and the structural change of the NHC catalyst. More importantly, the SMES is entirely unactivated on side reactions involving substrates only, for example aldol condensations, and thus helps to decipher the detailed mechanism of the NHC-catalyzed benzoin reaction.

RESULTS AND DISCUSSION

Device fabrication and characterization of homo-condensation

Considering the reactivity¹⁸ and the sensitivity²⁰ toward reaction conditions, we chose an NHC catalyst with an unsaturated backbone, namely 1,3-bis(2,6-diisopropylphenyl) imidazol-2-ylidene (IPr), as the center of the molecular bridge. Two alkyl spacers were connected to the IPr center to decouple the catalyst from the electrodes.²¹ The azide functional groups at both ends of the molecular bridge were designated to anchor the NHC catalyst onto graphene electrodes. Thus, the traceless Staudinger ligation between the azide groups and the activated carboxyl terminated graphene electrodes forges two stable amide bonds, resulting in the formation of graphene-molecule-graphene single-molecule junctions (Figure 1A and supplemental information). The successful connection was confirmed by the responses of current-voltage (*I-V*) curves (Figure 1B; other devices are provided in Figure S1). Under the optimized conditions, nearly 11% with ~10 of 92 devices on the same silicon chip showed a response, indicating that the probability of connecting only one catalyst is ~95% and demonstrating reproducibility (see supplemental information).

The changes in the catalyst structure are reflected in current fluctuation, which can be monitored in real time under a constant bias voltage (300 mV). By treatment with a base solution of diazabicycloundecene in ultra-dry toluene under anhydrous ¹Beijing National Laboratory for Molecular Sciences, State Key Laboratory for Structural Chemistry of Unstable and Stable Species, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, P. R. China

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Figure 1. Single-molecule electrical spectroscopy of homo-condensation

(A) Schematic diagram of the single-molecule electrical monitoring platform.

(B) Current responses to the voltages before and after connection.

(C) Real-time electrical spectroscopy of benzaldehyde homo-condensation.

(D) Left: transmission spectra of four species, where the dominated transmission orbitals (p-FMOs) are displayed. Center: enlargement of spectroscopy from (C). Right: corresponding statistical histograms of (C).

and oxygen-free conditions, the weakly acidic imidazolium salt was converted to the targeted NHC molecule²² (Figure S2, the current level was transferred from ~53 nA to ~60 nA). After the addition of benzaldehyde, the benzoin reaction catalyzed by the single organocatalyst proceeded smoothly at 298 K (Figure 1A). Note that the unactivated catalyst cannot catalyze the reaction (Figure S3). Remarkably, periodic transitions of the currents were observed by the nanoampere-level current sampled at 57,600 Sa/s (Figure 1C, enlarged in Figure 1D). It was convenient to distinguish three current levels, which correspond to three relative stable structures derived from the NHC catalyst, through statistical analysis (Figure 1D, right). The I-t curve shows that the free NHC state in the catalytic cycle has the highest current level (~60 nA) (Figure S2). The other two states should be attributed to intermediates during subsequent reactions. In addition, the middle state corresponds to an earlier intermediate along the reaction coordinate than the lowest state due to the sequence relationship (Figure 1D, center). To ascribe these conductance states, we calculated the transmission spectra (Figures 1D [left] and S4) of various possible intermediates in the energy profile (Figure 2A). Because the transmission mechanism is dominated

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Figure 2. Temperature-dependent measurements

(A) Energy profile of homo-condensation along the reaction coordinate.

(C) Lifetimes of the four intermediates under corresponding temperatures. The error scales were derived from the corresponding single exponential fittings based on the statistics of five different devices. (D and E) Plots of the thermodynamic and kinetic parameters (InQ versus 1,000/T and Ink versus 1,000/T) deduced from temperature-dependent measurements. ΔH of the two additions were derived from the Van 't Hoff equation. The activation energies of the forward and reverse reactions of the two additions were obtained by the Arrhenius equation.

by electrons tunneling assisted by perturbed frontier molecular orbitals (*p*-FMOs), the matching degree between *p*-FMO and the graphene Fermi level determines the species conductance. Therefore, NHC corresponds to the highest current level

⁽B) Catalysis spectroscopy under different temperatures.

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because of the highest proximity between its p-FMO and the Fermi level of graphene, followed by BI, then the first addition intermediate (pre-BI), and finally the second addition intermediate (INT3). Notably, the direct conversion between pre-BI and BI (proton shuttle) was not favorable at 298 K due to a high energy barrier (~46.40 kcal/mol, Figure 2A). However, the presence of H_2O facilitated a more feasible pathway (the energy barrier is substantially reduced to ~4.28 kcal/mol). The trace of H₂O in ultra-dry solvent and substrate could be involved in the reaction at the single-molecule level. No obvious change of conductance states was observed after adding extra $H_2O(1_{00})$ to the system (Figure S5), which supports the notion that the protic solvent assisted the proton shuttle mechanism, in line with the macroscopic experiment (see supplemental information). Due to the high reactivity of pre-BI to BI presented by the energy profile (Figure 2A), the conductance state of pre-BI cannot be detected in the ~17-µs time resolution at 298 K. Therefore, according to previous analysis of transmission spectra, the middle conductance (~50 nA) was assigned to BI and the lowest state (~43 nA) was assigned to INT3.

The species of pre-BI was expected to be monitored at low temperatures. As the temperature gradually decreased, fewer reaction events were presented in the 500-ms windows (Figure 2B; long-term *I*-t curves are provided in Figure S6), which means a decrease in the reaction rates (*r*). When the temperature was dropped to 238 K, a new conductance state (red) appeared and showed more frequently at 218 K. The new conductance state had a strong correlation with the appearance of other conductance states, which were generated by NHC or BI and returned to them after the end. In particular, the sequence from NHC through the new state to BI can be observed (inset of Figure 2B). In contrast, no relationship between this state and INT3 was found in the *I*-t curves. According to the time sequence among these states, the new state should be assigned to pre-BI. The transmission spectrum of pre-BI in Figure 1D (left) is consistent with the order of the experimental conductance levels, which also supports our assignment, thus providing a complete reaction scenario.

The kinetic and thermodynamic parameters can be derived from the temperaturedependent measurements. After idealization of the *I-t* curves (Figure S7), the time intervals of each state are extracted and the lifetimes (τ) (Figure 2C) can be derived from single exponential fittings of their distributions (Figure S8). As the temperature decreases, the increase in all states' lifetimes indicates that the frequency of transitions from one state to another is becoming lower. According to $r = 1/\tau$ and the Arrhenius equation, the activation energy of the two main addition processes (NHC to BI and BI to INT3) can be obtained through the linear fitting of lnr versus 1,000/T (concentration was constant and the rate constant k could be replaced by r [Figures 2D and 2E]). The kinetic properties of the reaction can be derived conveniently by analyzing the time-dependent spectroscopy. In addition, the ratio (Q) of the species appearance during long-term measurements reflected the equilibrium (thermodynamic properties) of the reaction. Similarly, ΔH of two main processes can be derived according to the Van 't Hoff equation $(-RT\ln(K) = \Delta H - T\Delta S)$. Negative changes of enthalpy indicate the exothermic property of the overall catalysis cycle, which is consistent with theoretical studies (Figure S9). More thermodynamics and kinetics parameters are provided in Table S1.

Characterization of cross-condensation

As a general condensation reaction catalyzed by NHCs, the benzoin reaction leads to not only the homo-condensation of aromatic aldehydes but also the

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Figure 3. Single-molecule electrical spectroscopy of cross-condensation at 298 K (A) Schematic diagram of two independent homo-condensations.

(B) Electrical spectroscopy of two independent homo-condensations catalyzed by the same single-molecule catalyst device.

(C) Schematic diagram of cross-condensation.

(D) Electrical spectroscopy of cross-condensation and corresponding statistical histogram.

(E) Enlargement of data in (D) showing four kinds of reaction trajectories.

homo-condensation of alkyl aldehydes²³ and their cross-condensations.²⁴ Next, we sought to further demonstrate the power of our method in interpreting the reaction mechanism by studying the cross-condensation reaction. Figure 3 shows both homo- and cross-condensations of benzaldehyde and octylaldehyde at 298 K. As

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expected, each reaction displayed three clear current levels (Figure 3B). The highest current levels of both reactions were the same and represented the free NHC state, which in turn confirmed our assignment in the above experiments. The current levels of BI (blue color for benzaldehyde homo-condensation and green color for octylaldehyde homo-condensation) were similar but distinguishable for both homo-condensations. The lowest current levels of both reactions were assigned to INT3 accordingly. With the attributions of homo-condensation in hand, we carried out the cross-condensation of benzaldehyde and octylaldehyde (Figure 3C). By comparing the current levels (centers of statistical histogram peaks) in Figure 3D with those in Figure 3B, current levels related to homo-condensation could be identified easily (Figure 3E). The remaining current levels were located between the current levels of NHC- γ - γ (INT3 of octylaldehyde homo-condensation) and NHC- α - α (INT3 of benzaldehyde homo-condensation) and were attributed to NHC- γ - α and NHC- α - γ (INT3 of cross-condensations), respectively, according to the reaction trajectory (Figure 3D). Therefore, its SMES is remarkably versatile and reliable for different benzoin reactions, including complex crossed benzoin reactions (see Figure S10 for another set of different cross-condensation experiments).

In addition to trajectory tracking, more details of the benzoin reactions could be obtained from these electrical spectroscopies. The dwell times (Figure 3E) and number of counts (Figure 3D) for each current level reflected the stability of different intermediates during the reaction progress. The free NHC and BI possessed a long dwell time and dominant signal counts, meaning relatively high stability, consistent with the separable ability of free NHCs²⁵ and BIs.^{18,19,26,27} The difference in signal counts in the cross-condensation means varying selectivity. The higher counts for the BI of benzaldehyde than that of octylaldehyde indicate the preference for forming the conjugated structure. By comparing the proportion of four kinds of INT3 (Figure 3D, right), it was found that both formed BI intermediates preferred to react with benzaldehyde instead of octylaldehyde. This peculiarity originates from dominant interactions between catalyst and substrate rather than electrophilicity of substrates. In short, benzaldehyde molecules have a stronger intermolecular force with aromatic groups of IPr than octylaldehyde, which shortens the spatial distance between benzaldehyde molecules near IPr. This characteristic selectivity of the condensation is also consistent with the macroexperiments (Figure S11 and Table S2).

Correlation analysis

This useful electrical spectroscopy has the potential to decipher deeper dynamics beyond ensemble. To obtain more dynamic insight into the benzoin reaction, we designed and conducted concentration-dependent experiments (Figure 4A and another set of data in Figure S12). When the substrate (benzaldehyde) concentration was extremely low (5 \times 10⁻⁹ mol/L), the NHC state dominated the *I*-t curve due to the low collision probability between catalyst and substrate. Only a few catalytic cycles were monitored along with frequent reversible transitions between NHC and BI. With increasing concentration, the dominant current level was gradually shifted to BI and the appearance of INT3 also increased, indicating that more catalytic events took place. These tendencies were obvious, as shown by the statistical results in Figure 4B. In addition, the distribution of different current levels tended to be constant when the concentration was higher than ~5 \times 10⁻⁷ mol/L. This means that the reaction was limited under diffusion at low substrate concentrations, while at high substrate concentrations the reaction rate was limited by the energy barrier of nucleophilic attack of NHC to the substrate (the same holds true for the conversion from BI to INT3). Considering that these phenomena shared common kinetic characteristics of enzymes, we built a model with single substrate and two consecutive catalytic



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Figure 4. Concentration-dependent measurements at 298 K

(A) Catalysis spectroscopies under different concentrations of the substrate.

(B) Corresponding statistical histograms of (A).

(C) Column chart of product molecules during 10 s at different concentrations fitted by macroscopic Michaelis-Menten equation.

(D) Autocorrelation function of adjacent reaction events at low and high concentrations.

(E) Schematic diagram of the interaction between one catalyst and substrates at low and high concentrations, respectively.

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processes, and calculated the product formation rates versus concentration using the Michaelis-Menten equation (Equation 1; the detailed derivation process is provided in supplemental information). The yield of product molecules in experiments can be obtained by counting direct conversion from INT3 to NHC in a given time sequence. The relationship between the yield in constant intervals (10 s) and concentration can be fitted perfectly by our model (Figure 4C). As the concentration of the substrate increases, the rate of product formation increases until saturation, which corresponds to the maximum turnover rate (~2 s⁻¹) of the catalyst. The single-molecule behavior obtained by long-term statistics is consistent with the universal ensemble catalysis. Additionally, the maximum working efficiency of a single catalyst is higher than that of the ensemble due to the exclusion of ineffective collisions of catalyst molecules themselves, indicating a better account of the catalytic process:

$$r = \frac{V_{\max} \times [A]^2}{[A]^2 + \left(\frac{k_3 + k_{-2}}{k_2} + \frac{k_3}{k_1}\right) \times [A] + \frac{(k_3 + k_{-2})k_{-1}}{k_1 k_2}}.$$
 (Equation 1)

More interestingly, details presented in the *I*-t curves provided novel dynamic characteristics. As shown in the inset of Figure 4A, scattered or clustered signals (conversion between NHC and BI) appeared at low concentrations, while dense and stochastic signals appeared at high concentrations. To describe these characteristics guantitatively, we recorded the reaction events at given intervals and analyzed the correlation properties of adjacent intervals by an autocorrelation function. We selected 10 ms as a fixed interval, which involves approximately one reversible event in each case. The autocorrelation functions of low (5 \times 10⁻⁹ mol/L, orange line) and high (5 \times 10⁻⁶ mol/L, red line) concentrations are presented in Figure 4D (the cases of other concentrations are provided in Figure S13). At low concentrations, in ~1-4 successive events, the reaction has some correlation rather than being completely random at high concentrations. This implied the existence of repeated interaction between catalyst and substrate at low concentrations. The memory effect (dynamic disorder) of the catalyst can be characterized through the autocorrelation functions of the dwell times in Figure S14. No correlation between the dwell times of NHC and BI excluded the changes in the domain of the catalyst itself, such as enzyme or nanoparticle catalysts. Therefore, the novel dynamic characteristics indeed resulted from the number of substrates in the microenvironment around the single-catalyst site. The repeated interaction may be attributed to the electron-rich nature of the catalyst and electron-deficient nature of the aromatic ring of the substrate as well as π - π stacking between the molecular bridge and the aromatic aldehyde. A controlled experiment of alkyl aldehyde as the substrate is provided in Figure S15. No correlation was observed during adjacent events, which further supports this explanation. Therefore, the clustered properties of signals resulted from the repeated interaction between catalyst and substrate, and the scattered properties among clustered signals originated from the long diffusion distance of another substrate at low concentrations. On the contrary, the reactions controlled by kinetics at high concentrations did not show the obvious correlation among adjacent events. Collectively, in addition to the identification of multiple intermediates and the visualization of the complex cross-coupling process, the electrical spectroscopy also indicates the interaction between catalyst and substrate at the core space beyond ensemble average.

The interaction between substrates and the single catalyst can also be semi-quantitatively analyzed. The autocorrelation functions were almost equal at the concentrations below 10^{-8} mol/L (Figure S13), showing that the decrease in concentration only extends the time interval between clusters (Figure S12). Thus, we can further define that the correlation among adjacent events can be used as a criterion for the



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repeated interaction (one catalyst with one substrate) in the microenvironment. The kinetic and thermodynamic model in a microenvironment with only one catalyst molecule and one substrate molecule may have new definitions. When the reaction dynamics were between diffusion control and dynamic control (i.e., the correlation among just-appeared adjacent events), the concentration of the substrate can be simplified as "1" and the ratio (Q) of NHC and BI can be derived from the statistical histogram during a long-term reaction (Figures 1D and S12). The phenomenological thermodynamics and kinetics can be correspondingly converted to numerology (statistical treatment) in single-molecule systems. Accordingly, the equilibrium (K) can be equal with Q/1 rather Q/C in the Langmuir absorption equation, where C is the concentration of the substrate. This correction can more intrinsically reflect the interactions at the single-molecule level in the microenvironment. Additionally, ΔG can be derived from $\Delta G = -RT \ln K$ (see Table S1). The concentration of the substrate did not affect these thermodynamic parameters (ΔG and K). Therefore, the effective concentration under dynamic control can be obtained through adverse deduction $(K = Q_{high-concentration}/C_{effective} = Q_{low-concentration}/1)$, affording that the effective concentration under maximum turnover was ~32, which reflects the inventory of the substrates via the thermodynamic method on the single-molecule detection platform.

Similarly, the kinetic method can also be used for the inventory of substrates. The law of mass action in phenomenological kinetics may also have new definitions at the single-molecule level. The reaction rate catalyzed by one catalyst is $r = 1/\tau =$ $k \times 1 \times 1$ when the substrate concentration is 1. The effective concentration (~14) at high concentrations can also be obtained through adverse deduction $(k = r_{high-concentration}/(C_{effective} \times 1) = r_{low-concentration}/(1 \times 1))$, which reflects the inventory of the substrates via the kinetic method at the single-molecule detection platform. Two methods provide the same magnitude number of molecules surrounding the single catalyst at the high concentration, indicating the feasibility of statistical thermodynamics and kinetics with single-molecule insight. The errors of the two methods result from the idealization of the just-appeared autocorrelation process: definition of the concentration as "1" at any time interval. Note that occasionally the catalyst might interact with multiple molecules at the same time intervals under diffusion control. Therefore, different from fluorescence correlation spectroscopy approach of counting the number of molecules in the microenvironment,²⁸ the electrical spectroscopy on our single-molecule platform provides a new strategy to semi-quantitatively measure the number of molecules that have effective interaction with the target single molecule.

Conclusions

This work establishes an unprecedented SMES approach to comprehensively analyze the benzoin reaction process and detect the highly reactive intermediate before and after the **BI**. In addition, unique dynamics beyond ensemble average was observed and a new thermodynamic and kinetic model at the single-molecule level was developed, which provides a new perspective from which to resolve complex organocatalysis as well as infinite opportunities for deciphering unknown mechanisms in organic chemistry.

EXPERIMENTAL PROCEDURES

Resource availability

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Xuefeng Guo (Guoxf@pku.edu.cn).





Materials availability

The materials can be produced following the procedures in the section on synthesis and characterization in the supplemental information.

Data and code availability

This study did not generate any datasets.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.matt. 2021.05.024.

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AUTHOR CONTRIBUTIONS

X.G., F.M., and W.Z. conceived and designed the experiments. C.Y., Y.G., and C.J. fabricated the devices and performed the device measurements. L.Z. carried out the molecular synthesis. H.L. built and analyzed the theoretical model and performed the quantum transport calculation. X.G., F.M., C.Y., and L.Z. analyzed the data and wrote the paper. All authors discussed the results and commented on the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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