Kinetics of Palladium(0)-Allyl Interactions in the Tsuji-Trost Reaction, derived from Single-Molecule Fluorescence Microscopy

Johannes A. Menges, [a] Alexander Grandjean, [a] Anne Clasen, [a] and Gregor Jung* [a]

In memoriam Prof. Marcus Motzkus, Heidelberg University.

Introduction

Over the last decade, single-molecule fluorescence spectroscopy (SMFS) emerged as a tool to bring insights into the microscopic behavior of molecular, chemical systems. [1–5] When ensemble measurements conceal uncommon events, single-molecule observation has the potential to provide a deeper understanding of chemical processes. [6–8] In these first studies, it was shown that even text book reactions can show ructions of reaction pathways, still leading to an identical product. [9] Hidden intermediates [10] or static disorder is revealed for single nanoparticles and enzymes. [11,12] Reactivity mapping of heterogeneous catalysts has been accomplished with spatial resolution. [13] Ligand exchange [14,15] and polymerization [16] reactions have been investigated in model systems for homogeneous catalysis. [17]

To further advance this field in direction of recent synthetic chemistry, we previously introduced multi-emissive probes for the so-called participant approach. [18–21] The Tsuji-Trost allylic substitution provides an example of a well-known and widely-used organometallic reaction. [22–24] We have chosen to study this reaction as only few components are involved and there are still some mechanistic details under discussion. The putative active catalyst is PdL2 (L = Ligand, e.g. PPh3), which is formed during dissociation from a Pd0.

Kinetics of Palladium(0)-Allyl Interactions in the Tsuji-Trost Reaction, derived from Single-Molecule Fluorescence Microscopy

Johannes A. Menges, [a] Alexander Grandjean, [a] Anne Clasen, [a] and Gregor Jung* [a]

In memoriam Prof. Marcus Motzkus, Heidelberg University.

Introduction

Over the last decade, single-molecule fluorescence spectroscopy (SMFS) emerged as a tool to bring insights into the microscopic behavior of molecular, chemical systems. [1–5] When ensemble measurements conceal uncommon events, single-molecule observation has the potential to provide a deeper understanding of chemical processes. [6–8] In these first studies, it was shown that even text book reactions can show ructions of reaction pathways, still leading to an identical product. [9] Hidden intermediates [10] or static disorder is revealed for single nanoparticles and enzymes. [11,12] Reactivity mapping of heterogeneous catalysts has been accomplished with spatial resolution. [13] Ligand exchange [14,15] and polymerization [16] reactions have been investigated in model systems for homogeneous catalysis. [17]

To further advance this field in direction of recent synthetic chemistry, we previously introduced multi-emissive probes for the so-called participant approach. [18–21] The Tsuji-Trost allylic substitution provides an example of a well-known and widely-used organometallic reaction. [22–24] We have chosen to study this reaction as only few components are involved and there are still some mechanistic details under discussion. The putative active catalyst is PdL2 (L = Ligand, e.g. PPh3), which is formed during dissociation from a Pd0.

[pd0l2 + o-r \rightleftharpoons pd0l2] (1)

Via this transient complex, a cationic \( \eta^1 \)-allyl-complex is yielded. Especially for this part of the reaction, the Pd-mediated deallylation, ambiguity exists regarding the progression of the oxidative addition of allylic substrates to Pd0. The latter step may proceed similar to a \( \Delta_2 \) reaction [31] with immediate release of the leaving group [eq. (2)].

[pd0l2 \rightleftharpoons pd0l2] (2)

Alternatively, the Pd-center may insert into the C–OR bond [30–32] (RO = leaving group). Here, an oxidative association with formation of either a \( \eta^1 \)-allylic \( \eta^1 \)-intermediate or a \( \eta^1 \)-Pd-complex [33,34] are proposed, with the leaving group temporarily bound in the ligand sphere. From these intermediates a consecutive dissociation step then is expected. [35] With the aim of obtaining new insights into the mechanisms of leaving group release, we studied the
interactions of Pd\(^0\) with our recently introduced, dual-emissive and immobilizable substrates\(^{[36,37]}\). During this reaction, tetrakis(triphenylphosphine)-palladium(0) (Pd\(^0\)) causes a strong bathochromic shift in substrate fluorescence (SI Figure S2).

To probe the early events during the interaction of Pd\(^0\) with the substrate, i.e. the equilibrium of complex formation, we used time-correlated single photon counting (TCSPC)\(^{[37]}\) and analyzed the quenching behavior by a Stern-Volmer (SV)-analysis. Monitoring fluorescence emission of individual reactive chromophores with two-channel detection in total-internal reflection fluorescence microscopy (TIRFM)\(^{[38]}\) should then enable conclusions about the role of Pd during chemical transformation. In addition, we investigated several data analysis routines for adaption to irreversible reactions. Hidden Markov modelling\(^{[39]}\) or Bayesian statistical analysis\(^{[40]}\), very well suited for the analysis of Förster resonance energy transfer (FRET) trajectories in equilibria, allow for extracting kinetic or mechanistic data from two channel SM fluorescence trajectories. Problems may occur when applied to a single reaction step with superimposed fluorescence quenching. We therefore searched for an unbiased statistical analysis of obtained single-molecule data. At first, we pursued noise-cancelling auto- and cross-correlation analyses of single particle emission\(^{[41]}\) for extraction of substrate decay and product rise. Subsequently, we compared the outcome with the results from analyzing the histograms of substrate fluorescence termination and product fluorescence formation events. With these methods, kinetic information of the reaction steps in equation (1) and (2) could be extracted, but photochemical bypass reactions were deciphered as well.

### Results and Discussion

We synthesized pyrene-based, reactive fluorophores 2\(a\), 2\(b\) and 2\(c\) from 1\(a\), 1\(b\) and 1\(c\) according to existing procedures\(^{[36,37]}\) (scheme 1). The benzylated probes 3\(a\), 3\(b\) and 3\(c\) were synthesized in a similar fashion (SI pp.: 4–7). Compounds 2\(a\), 2\(b\) and 3\(a\), 3\(b\) were used for kinetic experiments in cuvette whereas 2\(c\) and 3\(c\) were used for immobilization on quartz surfaces prior to SM visualization.\(^{[42]}\) All probes 2\(a\), 2\(b\), 3\(a\) and 3\(b\) were stable in ethanol at standard conditions in the time span of these experiments. In steady-state experiments with 3\(a\) and 3\(b\), no product formation at RT was noticed, neither in presence of Pd\(^0\), nor in combination with Pd\(^0\) and the nucleophiles ethylmalonate and acetoacetate\(^{[28]}\) (SI Figure S3). Due to the lacking reactivity at RT towards Pd\(^0\), the benzylated derivatives served as control probes in this study. Electronic spectra and photophysical properties of all compounds were determined and are compiled in table S1 (SI Figure S1).

We performed steady-state and time resolved measurements in solution for characterizing the Pd\(^0\) interaction with the substrate prior to the reaction. With addition of various amounts of Pd\(^0\) up to a saturated solution (150 \(\mu\)M), fluorescence emission of unreactive 3\(a\), 3\(b\) and 1\(b\) was quenched (SI Figure S4). Association constants \(K_{SV}\) of \(\sim 10^7 \text{M}^{-1}\) were obtained from a SV plot\(^{[42]}\) (Figure 1a). Fluorescence lifetimes of 2\(a\), 2\(b\) and 3\(a\), 3\(b\), on the other hand, remained unchanged shortly after addition of Pd\(^0\) (SI Figure S5). These findings indicate static quenching by Pd\(^0\), i.e. complex formation.\(^{[43]}\)

![Scheme 1. Synthesis of reactive (2a,b,c) and reference probes (3a,b,c) for the Tsuji-Trost deallylation reaction\(^{[36,37]}\) performed in acetone or dimethylformamide.](image-url)
Fluorescence Correlation Spectroscopy (FCS)

FCS was performed in oxygen-free ethanol to further determine the underlying kinetics (SI pp. 17). We used compounds 3b and 1b as control probes, with enough brightness and photostability for FCS under short wavelength excitation ($\lambda_{exc} = 445$ nm and 488 nm; SI Figure S7). Moreover, reactive 2b was investigated as well, shortly after addition of Pd$^0$. As the measurement time was limited due to the consumption of 2b, we exploited high excitation intensities for obtaining reasonable FCS curves (Figure 1b). Autocorrelation curves revealed a dark state in the presence of Pd$^0$, the ligand PPh$_3$ (1 mM) and only in the absence of oxygen. Consequently, strong fluorescence fluctuations cannot unambiguously be traced back to the interaction with the catalyst.

Moreover, since the amplitude and the contrast of the fast fluctuations depend on illumination intensities, these observations point to typical light driven processes. However, photochemical release of a phosphine ligand could not be excluded. Equation (3) therefore represents the kinetic description of a reversible dark state formation from autocorrelation curves.

$$G(t) = \frac{1}{<N<T>} \left( \frac{1}{1 + \frac{t}{\tau_{diff}}} \right) \left( 1 + \frac{k_{dark\ eff}}{k_{bright}} \cdot e^{-\left(\frac{t}{\tau_{dark\ eff} + \tau_{bright}}\right)} \right)$$

(3)

Through application of equation (3), effective dark state formation and decomposition rates $k_{dark\ eff}$ and $k_{bright}$ in the $\mu$s-time regime were obtained. While on the $\mu$s-time scale hardly any significant differences between 3b and 2b are found in FCS, we noticed a distinct reduction of apparent diffusion time $t_{diff}$ from 160 ($\pm$36) $\mu$s to < 40 ($\pm$4) $\mu$s upon addition of Pd$^0$ only in 2b. Although the interpretation of apparent $t_{diff}$ requires caution, we conclude that the extracted $k \approx 1.5 \cdot 10^4$ s$^{-1}$, that is $k_1$ or $k_2$ in equation (2), appears as the upper limit for the reaction of 2b with Pd$^0$L$_4$. Still, this upper limit is several orders of magnitude above the rate constants of the conversion in cuvette experiments ($k \approx 2 \cdot 10^2$ s$^{-1}$), and thus may not reflect the putative $S_2$-like short cut in equation (2). In summary, the only specific interaction with Pd$^0$, which is not seen in the control experiment, is the reduction of $t_{diff}$. Due to resulting limited observation time window, that is the diffusion time through the detection volume, FCS in solution can neither clearly distinguish between the pathways of fluorescence loss in eq. (2) nor does provide the kinetics of eq. (1) in its present configuration.

TIRF-microscopy

For exploring the Pd$^0$ interaction during chemical conversion, the deallylation reaction was visualized on the SM level. Simultaneous observation of substrate and product with low background was achieved with a prism-based TIR$^{[38]}$ excitation in a custom built setup$^{[36,47]}$ and dual-channel detection. Excitation and emission spectra of silanes 1c and 2c in solution as well as utilized laser lines and detection channels are shown in Figure 2a. Dyes 1c, 2c and 3c were immobilized on a, with methylsilane passivated quartz surface, as described before$^{[36]}$, leading to 1imm (product), 2imm (reactive substrate), and 3imm (reference substrate) (SI Figure S10). Movies (100 ms/frame) of immobilized substrates 2imm and 3imm were recorded in ethanol before, during and after addition of 100 $\mu$m Pd$^0$. With 2imm a clear shift of emitting molecules from the “blue” (417–477 nm) to the “orange” (500–600 nm) detection channel was observed, which was initiated by Pd$^0$(PPh$_3$)$_4$ (Figure 2b). Single-molecule fluorescence trajectories with apparent noise were extracted with the selection criterion of single bleaching steps. Finally, 1741 SM-trajectories from 9 samples of 3 independent preparations of 2imm from movies during addition of Pd$^0$ were collected (amount of all trajectories: see SI p. 24).

To attain the temporal evolution from the starting material to product without any data preselection (except
the mentioned single step in the blue channel), correlation analyses\(^{[41]}\) were conducted. Autocorrelation decays provide the time constant for molecule disappearance (due to reaction or photobleaching) as in FCS but also do not distinguish between these processes. Cross-correlation analysis, in contrast, is specific for the reaction and yields the time constant for product formation once a substrate molecule is identified. Moreover, artifacts and random noise are cancelled out in correlation functions\(^{[48,50]}\) and the time resolution of the acquisition frequency (10 Hz \(\pm\)100 ms) is sustained in contrast to our later analyses based on binning. Emission trajectory pairs of each measurement were aligned into single paired data series. Subsequently, the overall correlation curves were calculated, which were corrected for spectral cross-talk according to the method described by Bacia et al.\(^{[49]}\) (SI pp. 25). The obtained corrected cross-correlation \(G(r,t)\) curves of measurements with introduction of Pd\(^6\) show positive amplitudes (Figure 3a). In the conversion of \(2_{\text{imm}}\) by Pd\(^6\) a pronounced maximum is seen, while corresponding curves of \(3_{\text{imm}}\) only show a weak maximum (Figure 3a and SI Figure S11b). Kinetic constants were derived from mono- or biexponential fits of these curves, in which the rising component is interpreted as product formation. A time constant of \(t_{\text{imm, orange}}\) of 15.7 (\(\pm\)1.5) s of \(2_{\text{imm}}\) with presence of Pd\(^6\) results from this analysis (SI Figure S16). This time constant is considerably lower, by a factor 4, than the previously determined time constant of product formation in a fluorogenic approach, that is when only the formed product molecules were excited.\(^{[36]}\) However, if we take the competing photobleaching of the substrate \(2_{\text{imm}}\) into account (i.e. the decay of the blue autocorrelation functions of control \(3_{\text{imm}}\) and \(2_{\text{imm}}\) without Pd\(^6\), see Figure 4 and SI Figure S11), then a reduced time constant \(t_{\text{red}} = 6.2 (\pm 1.9)\) s is expected (SI p. 34). The significant retardation from the expected 6 to the observed 16 s putatively points to an intermediate, specific to an interaction with Pd\(^6\), before \(1_{\text{imm}}\) is produced in the reaction. However, no conclusions about its nature or any reaction branching as inferred from eq. (2) can be drawn from these correlation analyses. If fluorescent intermediates with a different emission spectrum were present, then signal-pair histograms\(^{[48,50]}\) (SI Figure S14), created from the paired emission trajectories, could suggest their presence. However, such an analysis provided no unambiguous indication for spectrally shifted intermediates specific to the presence of Pd\(^6\). We therefore interpret any putative intermediate as dark state in the reaction from \(2_{\text{imm}}\) to \(1_{\text{imm}}\). Please note that even the cross-correlation curves of \(2_{\text{imm}}\) without Pd\(^6\) shows

Figure 2. a) Fluorescence excitation (dashed lines) and emission (solid lines) spectra of soluted compounds 2c (purple, blue) and 1c (green, orange) in ethanol. Also, the utilized laser lines at 405 nm (purple) and 488 nm (cyan) are shown and spectral ranges of collected emission light from 417–477 nm (blue channel) and from 500–600 nm (orange channel); b) Images of visualized surface areas with immobilized \(2_{\text{imm}}\), top: blue channel, bottom: orange channel, (1) with initiation of the reaction through Pd\(^6\), (2) after 5 s with Pd\(^6\) solution, (3) after 7 s with Pd\(^6\) solution, binned for 1 s (10 frames) (product molecules in first frame formed through photochemical reaction); c) Dual-Channel fluorescence trajectories of \(2_{\text{imm}}\) with Pd\(^6\) with visible color change; d) Dual-Channel fluorescence trajectories of \(2_{\text{imm}}\) without Pd\(^6\) with generation of „orange” fluorescence (solid lines represent sliding average of fluorescence trajectories with time resolution of 1 s).
a rising component (as does $3_{\text{imm}}$ with Pd$^0$). These latter findings are in contrast to the cuvette experiments, where only Pd$^0$ was able to convert 2 into 1. A photochemical side reaction is the most likely explanation.

We subsequently chose an analysis method for extracting information out of individual trajectories, finally yielding conventional histograms. In contrast to the former analyses, any dark state is directly detected as discontinuity of fluorescence to agree with the outcome of the former analyses. A simple algorithm was used to sort trajectories into groups of reactive and non-reactive molecules. At first, normalized intensity trajectories were transformed into a binary signal via a threshold to exclude 99% of data points, which may correspond to zero emission (SI Figure S12). Then, for recognition of product formation, the orange intensity had to exceed the corresponding threshold for at least one second (Figure 2c, d). Consecutively, all trajectories showing conversion were selected and the number of reactive molecules was determined. Roughly 28% of trajectories of $2_{\text{imm}}$ with Pd$^0$ was deemed reactive with a significant rise in orange fluorescence during the observation window (Figure 2c), whereas the rest of molecules were considered to photo-bleach. We certainly lost a wealth of trajectories by these strict criteria and, therefore, overestimated photobleaching. In the first control experiment, that is $2_{\text{imm}}$ in ethanol, but without Pd$^0$, however, also around 12% of reactive molecules was determined. Roughly 28% of trajectories of $2_{\text{imm}}$ (≈487 of 1741 trajectories) with Pd$^0$ was deemed reactive with a significant rise in orange fluorescence during the observation window (Figure 2c), whereas the rest of molecules were considered to photo-bleach. We certainly lost a wealth of trajectories by these strict criteria and, therefore, overestimated photobleaching. In the first control experiment, that is $2_{\text{imm}}$ in ethanol, but without Pd$^0$, however, also around 12% of reactive molecules was determined. Roughly 28% of trajectories of $2_{\text{imm}}$ (≈487 of 1741 trajectories) with Pd$^0$ was deemed reactive with a significant rise in orange fluorescence during the observation window (Figure 2c), whereas the rest of molecules were considered to photo-bleach. We certainly lost a wealth of trajectories by these strict criteria and, therefore, overestimated photobleaching. In the first control experiment, that is $2_{\text{imm}}$ in ethanol, but without Pd$^0$, however, also around 12% of reactive molecules was determined. Roughly 28% of trajectories of $2_{\text{imm}}$ (≈487 of 1741 trajectories) with Pd$^0$ was deemed reactive with a significant rise in orange fluorescence during the observation window (Figure 2c), whereas the rest of molecules were considered to photo-bleach.
The timepoints of substrate emission termination \( t_i \) and product fluorescence formation \( t_j \) were resolved from the binary fluorescence trajectories and accumulated (Figure 3b). The resulting histograms were subsequently fitted with monoeponential functions (SI p. 33 and Figure 3c, extracted from Figure S13). The kinetics of the decay of \( 2_{\text{imm}} \) expressed by equation (4) shows a faint acceleration by Pd\(^0\) as expected (Figure 4).

\[
\tau_{\text{blue decay}} = \frac{1}{k_{\text{blue decay}}}
\]  

Equation (4)

The influence of Pd\(^0\) on \( 3_{\text{imm}} \) is less clear, as the error margins hide any measurable effect. Altogether, the obtained rate constants are in a similar range like those of the correlation analysis (Figure 4). Conversion of \( 2_{\text{imm}}/3_{\text{imm}} \) to the fluorescent product support a photochemical bypass reaction for both substrates to \( 1_{\text{imm}} \) with an apparent quantum yield of about \( \Phi_{\text{Pd,rea}} \approx 5.5 \cdot 10^{-3} \) and \( 2.8 \cdot 10^{-4} \) for \( 2_{\text{imm}} \) and \( 3_{\text{imm}} \) respectively. The most likely interpretation therefore is a photoinduced deprotection reaction, presumably via homolytic C–O cleavage\({}^{[51]}\) or photoionization reaction\({}^{[52]}\) (SI Scheme S2 p. 36). Its competition to the metal-organic reaction of \( 2_{\text{imm}} \) (SI p. 34) causes the seeming acceleration compared to the experiments in a fluorogenic approach\({}^{[36]}\).

More relevant for mechanistic insight is the analysis of the lag time \( \Delta t \) between the disappearance of the blue fluorescence and appearance of orange fluorescence, that is, the lifetime of the dark intermediate (Figure 3b,d). To perform an unbiased analysis of dark state evolution in regard to its mechanism, an empirical biexponential fit of \( \Delta t \) was utilized.

Firstly, the relative amplitudes of a fast \( (A_{\text{short}}) \) and a slow \( (A_{\text{long}}) \) decay component are resolved, normalized on the absolute number of reactive trajectories (SI Figure S16). For inert substrate \( 3_{\text{imm}} \), addition of Pd\(^0\) makes the short component disappear, leading to a monoeponential decay. This behavior is opposed to the reactive substrate \( 2_{\text{imm}} \) where the amplitude of the faster decay component of the allylated \( 2_{\text{imm}} \) absolutely increases (Figure 3d). Although the relative amplitude \( A_{\text{short}} \) of the biexponential fit increases only by ~ one third (from 0.28 to 0.38), the absolute raise corresponds to 7% of all trajectories of \( 2_{\text{imm}} \) during the presence of Pd\(^0\) (~120 trajectories of 487 reactive trajectories) and, hence, to roughly half of the increase of reactive trajectories upon addition of Pd\(^0\). Thus, this faster process is related to the synthetic reaction mechanism, although it competes here with photochemical conversion. An absolute increase of the slow component is noticed as well.

Secondly, the obtained rate constants are compared. Addition of Pd\(^0\) extends the dark state lifetime of inert substrate \( 3_{\text{imm}} \) to a single value \( t_{\text{dark}} \approx 30 \pm 5 \) s (Figure 3d). This time constant, however, may not necessarily reflect the binding time of Pd\(^0\) to the substrate. Considering the benzylated derivatives \( 3 \) are not prone to conversion by Pd\(^0\), this latter, slower process is not related to the catalytic cycle. This interpretation becomes important, as the same lifetime is found for the reactive substrate \( 2_{\text{imm}} \) as well. Here, Pd\(^0\) also elongates the long component of dark state lifetime (denoted by horizontal arrow in Figure 3d). At the same time, its short component lifetime slightly shortens upon addition of Pd\(^0\) from \( t_{\text{short,pd}} = 3.1 \pm 0.5 \) s to \( t_{\text{short,pd}} = 2.4 \pm 0.4 \) s opposed to its absence in the reaction of benzylated counterpart \( 3_{\text{imm}} \) (SI Figure S16b). Still, the observation of (at least) two lifetimes may also hint to more than one process being involved. The question therefore arises whether a distribution of rate constants is here a more appropriate description.

For analysis of kinetics with an intrinsic dispersion of rate constants, a Weibull analysis is employed\({}^{[53]}\). Here, a \( \beta \)-value results from a stretched exponential fit of the dataset with an effective rate constant \( k_{\text{eff}} \). The larger the discrepancy of \( \beta \) from 1 is, the more pronounced is the distribution of rates around \( k_{\text{eff}} \). Applied on the presented data, this analysis shows an increase of complexity of dark state kinetics of \( 2_{\text{imm}} \) through addition of Pd\(^0\), indicated by a decrease of the corresponding \( \beta \)-value. With the inert substrate \( 3_{\text{imm}} \), Pd\(^0\) exerts the opposite effect with a \( \beta \)-value close to one with the transition metal present (SI Figure S13d,e). One interpretation are additional contributions to reactive trajectories by e.g. the reaction of a mixture of various reactive Pd-ligand species\({}^{[25,54]}\) or the presence of different intermediate states like \( \eta^1 \) or \( \eta^2 \)-complexes\({}^{[33,34]}\). In summary, no more evidence for a distinct, sudden change than before results from the kinetics dispersion.

Nevertheless, having the previously discussed lack of a fast rising component in the cross-correlation (Figure 3a) and in the \( t_i \)-histogram (Figure 3c) as well as in direct color shifts of trajectories in mind, we therefore conclude that the vast majority of chemically relevant intermediate in the catalytic cycle has a lifetime of \( 2.4 \pm 0.4 \) s or longer (scheme 2). This interpretation is further supported from the kinetics obtained from FCS experiments, which disagrees with the expected rate constants of the fluorgenic approach, and excludes a \( \eta^2 \)-like shortcut from \( 2_{\text{imm}} \) to \( 1_{\text{imm}} \).

![Scheme 2. Mechanism of deallylation reaction for 2a,b,c and 2imm with derived kinetic constants (I): allylic substitution through Pd0; (II): Pd-insertion into C–O-bond.](doi.org/10.1002/cctc.202000032)
in equation (2), rather pointing to the proposed mechanisms involving a Pd$^\text{II}$ insertion into the C–O-bond.\cite{33,34}

**Conclusion**

The interaction of Pd$^\text{II}$ with allyl-protected substrate molecules 2 and its chemically inert, benzyalted counterpart 3 has been studied by fluorescence spectroscopy and microscopy. Steady-state unselective fluorescence quenching provides evidence for formation of a 2-cation-moieties. The reduced $t_{\text{rel}}$ in FCS, only observed in the allylated compounds, is suggestive of formation of a chemically relevant intermediate. SM experiments with two-channel detection were performed to cover the time regime well above 0.1 s. Noise insensitive correlation analyses and noise affected analysis of fluorescence event histograms result in similar time-constants for the substrate decay and product formation, but only the latter method shows the kinetics of presumably photoinduced dark states and reveals the existence of a Pd$^\text{II}$ intermediate with a lifetime of 2–3 s. The present experiments are more in line with insertion of Pd$^\text{II}$ into the C–O-bond (in a S$_2$2-like mechanism we would expect an instantaneous release of the product 1$_\text{mm}$), but may reflect a selected mechanism for the Tsuji-Trost-deallylation. As our pyrene derivatives provide an excellent leaving group, the result may only be transferred to comparable substituents. Experiments with other allylated fluorophores\cite{15,16} are a convenient way to find out distinctions. It is yet unclear to which extent the investigated conversion, with simultaneous excitation of product and substrate formation agrees with the findings of our previous study based on a fluorogenic approach.\cite{18}

However, an intermediate, as found here with a lifetime $\ll 60$ s, would hardly be detected in a conventional fluorogenic approach or kinetic analyses in an ensemble and underpins the benefits of single-molecule studies.

To finally elucidate the mechanism, which takes place in synthesis, we therefore have to avoid any light-driven activation of the metal with its immediate ligand sphere, e.g. by using red labeled ligands.\cite{19,23,33} Labeled ligands, used for enantioselective chemistry, may be employed as well.\cite{23} With this development and some technical improvements, e.g. for accessing the ms-time regime,\cite{24} we head for resolving this widely applied metal-organic reaction on the SM-level.

**Acknowledgements**

This work was supported by the German Research Foundation, (DFG, project number JU650/10-1). The authors thank Reiner Wintringer (†) and Dr. Klaus Hollemeyer (Service Center Mass Spectrometry, Saarland University) for ESI-MS analysis. Also, the technical design of implemented measurement chamber and microfluidic cell by Rudolf Richter is gratefully acknowledged and Matthias Jourdain for carefully reading the manuscript.

**Conflict of Interest**

The authors declare no conflict of interest.

**Keywords:** fluorescence spectroscopy · single-molecule detection · palladium · Tsuji-Trost reaction · reaction mechanisms

[1] M. Orrit, Angew. Chem. Int. Ed. 2015, 54, 8004–8005; Angew. Chem. 2015, 127, 8116–8117.
[2] M. Orrit, T. Ha, V. Sandoghdar, Chem. Soc. Rev. 2014, 43, 973.
[3] N. G. Walter, C.-Y. Huang, A. J. Manzo, M. Sobhy, Nat. Methods 2008, 5, 475–489.
[4] T. Gordes, S. A. Blum, Nat. Chem. 2013, 5, 993.
[5] J. C. Scialiano, A. E. Lanterna, J. Org. Chem. 2017, 82, S011–S019.
[6] Q. T. Easter, S. A. Blum, Angew. Chem. Int. Ed. 2018, 57, 12027–12032; Angew. Chem. 2018, 130, 12203–12208.
[7] F. Zhang, P. Song, Q. Fu, M. Ruan, W. Xu, Nat. Commun. 2014, 5, 1–8.
[8] S. Dery, E. Amit, E. Gross, Top. Catal. 2018, 61, 923–939.
[9] A. Rybina, C. Lang, M. Wirtz, K. Grützmaier, A. Kurf, F. Maier, A. Schmitt, O. Trapp, G. Jung, D. P. Herten, Angew. Chem. Int. Ed. 2013, 52, 6322–6325; Angew. Chem. 2013, 125, 6445–6449.
[10] H. Shen, X. Zhou, N. Zou, P. Chen, J. Phys. Chem. C 2014, 118, 26902–26911.
[11] R. Ye, X. Mao, X. Sun, P. Chen, ACS Catal. 2019, 9, 1895–1992.
[12] R. Liebhuber, H. Gorris, Molecules 2014, 19, 14417–14445.
[13] K. P.-P. Janssen, G. De Cremer, R. K. Neely, A. V. Kubarev, J. Van Loon, J. A. Martens, D. E. De Vos, M. B. J. Roeffaers, J. Hofkens, Chem. Soc. Rev. 2014, 43, 990–1006.
[14] J. D. Ng, S. P. Upadhyay, A. N. Marquard, K. M. Lupio, D. A. Hinton, N. A. Padilla, D. M. Bates, R. H. Goldsmith, J. Am. Chem. Soc. 2016, 138, 3876–3883.
[15] A. Kiel, J. Kovacs, A. Mokhir, R. Krämer, D.-P. Herten, Angew. Chem. Int. Ed. 2007, 46, 3363–6; Angew. Chem. 2007, 119, 3427–3430.
[16] Q. T. Easter, S. A. Blum, Angew. Chem. Int. Ed. 2018, 57, 1572–1575; Angew. Chem. 2018, 130, 1588–1591.
[17] T. Chen, B. Dong, K. Chen, F. Zhao, X. Cheng, C. Ma, S. Li, P. Zhang, S. H. Kang, J. W. Ha, W. Xu, N. Fang, Chem. Rev. 2017, 117, 7510–7537.
[18] M. Wirtz, A. Grüter, F. Heib, V. Huch, J. Zapp, D.-P. Herten, M. Schmitt, G. Jung, Methods Appl. Fluoresc. 2015, 3, 4401.
[19] M. Wirtz, A. Grüter, P. Rebmann, T. Dier, D. A. Volmer, V. Huch, G. Jung, Chem. Commun. 2014, 50, 12694–12697.
[20] B. Finkler, I. Riemann, M. Vester, A. Grüter, F. Stracke, G. Jung, Photochem. Photobiol. Sci. 2016, 15, 1544–1557.
[21] G. Jung, A. Schmitt, M. Jacob, B. Hinkeldey, Ann. N. Y. Acad. Sci. 2008, 1130, 131–137.
[22] K. C. Nicolau, P. G. Bulger, D. Sarlah, Angew. Chem. Int. Ed. 2005, 44, 4442–4448; Angew. Chem. 2005, 117, 4516–4553.
[23] B. M. Trost, M. L. Crawley, Chem. Rev. 2003, 103, 2921–2943.
[24] D. C. Behenna, B. M. Stoltz, J. Am. Chem. Soc. 2004, 126, 15044–15045.
[25] M. S. G. Ahlquist, P. O. Norrby, Angew. Chem. Int. Ed. 2011, 50, 11794–11797; Angew. Chem. 2011, 123, 11998–12001.
[26] R. D. Hutchins, K. Lear, R. P. Fulton, Tetrahedron Lett. 1980, 21, 27–30.
[27] O. Dangles, F. Guibé, G. Balavoine, S. Lavielle, A. Marquet, J. Org. Chem. 1987, 52, 4984–4993.
[28] T. Kajimoto, H. Takahashi, J. Tsuji, J. Organomet. Chem. 1970, 23, 275–280.
[29] B. M. Trost, J. T. Fullerton, J. Am. Chem. Soc. 1973, 95, 292–294.
[30] K. Tsurugi, N. Nomura, K. Aoi, Tetrahedron Lett. 2002, 43, 469–472.
[31] B. M. Trost, D. L. Van Vranken, Chem. Rev. 1996, 96, 395–422.
[32] D. R. Vutukuri, P. Bharathi, Z. Yu, K. Rajasekaran, M. H. Tran, S. Thayumanavan, J. Org. Chem. 2003, 68, 1146–1149.
[33] N. H. Sherden, D. C. Behenna, S. C. Virgil, B. M. Stoltz, Angew. Chem. Int. Ed. 2009, 48, 6840–6843; Angew. Chem. 2009, 121, 6972–6975.
[34] M. Kollmar, B. Goldfuss, M. Reggeli, F. Rominger, G. Helimen, Chem. Eur. J. 2001, 7, 4913–4927.
[35] A. Miyake, G. Hata, K. Takahashi, A. Miyake, Chem. Commun. 1970, 2, 1392–1393.
[36] J. A. Menges, A. Clasen, M. Jourdain, J. Beckmann, C. Hoffmann, J. König, G. Jung, Langmuir 2019, 35, 2506–2516.
[37] B. Finkler, C. Spies, M. Vester, F. Walte, K. Omlor, J. Riemann, M. Zimmer, F. Stracke, M. Gerhards, G. Jung, Photochem. Photobiol. Sci. 2014, 13, 548–562.

[38] W. P. Ambrose, P. M. Goodwin, J. P. Nolan, Cytometry 1999, 36, 224–31.

[39] I. Sgouropoulos, S. Presé, Biophys. J. 2017, 112, 2021–2029.

[40] E. Lerner, T. Cordes, A. Ingargiola, Y. Alhadid, S. Chung, X. Michalet, S. Weiss, Science 2018, 359, 1–12.

[41] R. M. Dickson, A. B. Cubitt, R. Y. Tsien, W. E. Moerner, Nature 1997, 388, 355–358.

[42] O. Stern, M. Volmer, Phys. Z. 1919, 20, 183–188.

[43] J. Panchompoo, L. Aldous, M. Baker, M. I. Wallace, R. G. Compton, Analyst 2012, 137, 2054–2062.

[44] J. Widengren, U. Mets, R. Rigler, J. Phys. Chem. 1995, 99, 13368–13379.

[45] P. Karagiannidis, S. K. Hadjikakou, P. Aslanidis, A. Hountas, Inorg. Chim. Acta 1990, 178, 27–34.

[46] B. Hinkeldey, A. Schmitt, G. Jung, ChemPhysChem 2008, 9, 2019–2027.

[47] A. M. Huynh, J. Menges, M. Vester, T. Dier, V. Huch, D. A. Volmer, G. Jung, ChemPhysChem 2016, 17, 433–442.

[48] A. Hoffmann, M. T. Woodside, Angew. Chem. Int. Ed. 2011, 50, 12643–12646; Angew. Chem. 2011, 123, 12854–12857.

[49] K. Bacia, Z. Petrášek, P. Schwille, ChemPhysChem 2012, 13, 1221–1231.

[50] H. Yuan, M. Orrit, ChemPhysChem 2012, 13, 681–683.

[51] J. P. Fouassier, F. Morlet-Savary, J. Lalevée, X. Allonas, C. Ley, Materials 2010, 3, 5130–5142.

[52] T. Cordes, J. Vogelsang, P. Tinnefeld, J. Am. Chem. Soc. 2009, 131, 5018–5019.

[53] K. Kitagawa, S. A. Blum, ACS Catal. 2017, 7, 3786–3791.

[54] A. Biffis, P. Centomo, A. Del Zotto, M. Zecca, Chem. Rev. 2018, 118, 2249–2295.

[55] P. Wang, J. Xia, Y. Gu, Tetrahedron Lett. 2015, 56, 6491–6494.

[56] J. Yan, X. Wang, L. Zhou, L. Zhang, RSC Adv. 2017, 7, 20369–20372.

[57] M. Navarro, S. Wang, H. Müller-Bunz, G. Redmond, P. Farris, M. Albrecht, Organometallics 2017, 36, 1469–1478.

[58] S. Sehayek, Y. Gidi, V. Glembockyte, H. B. Brandaõ, P. François, G. Cosa, P. W. Wiseman, ACS Nano 2019, 13, 11955–11966.

Manuscript received: January 7, 2020
Revised manuscript received: February 15, 2020
Accepted manuscript online: April 1, 2020