Volume 9
Number 1
7 January 2018
Pages 1-268

Chemical Science

rsc.li/chemical-science

ISSN 2041-6539

EDGE ARTICLE
Xinjing Tang et al.
Caged circular siRNAs for photomodulation of gene
expression in cells and mice

This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.

ROYAL SOCIETY
OF CHEMISTRY

rsc.li/chemical-science

ARTICLE

Unveiling Leaching-Oxidizing-Landing Paths of Pd Single-Atom Catalyzed Suzuki-Miyaura Reaction by Ambient Mass Spectrometry

Yiyan Yin[a], Xiaorong Wang[a], Xiyang Ge[a], Xiaotong Shen[a], Xiaomin Liu[a], Xiang Li[a,b], Jin Ouyang[c], Na Na[a,*]

The palladium-catalyzed Suzuki-Miyaura cross-coupling (SMCC) reaction is an essential technique for C-C bond formation. It is considered to occur through two distinct pathways involving homogeneous and heterogeneous mechanisms. However, there is still debate about these mechanisms given the lack of direct structural evidence in both spatial and temporal terms, especially regarding the conversion of active Pd species. In this study, the Pd single-atom catalyst (Pd SAC) SMCC reaction was monitored in real-time using ambient mass spectrometry (AMS) to study conversions of Pd species both on the catalyst surface and in the liquid phase. This revealed a leaching-oxidizing-landing paths as a contributor to the heterogeneous process which involves heterogeneous oxidative addition, Pd leaching along with transmetallation (rather than oxidative addition), and subsequent oxidation-landing back onto the catalyst surface. The leaching-oxidizing-landing mechanism of Pd active sites during Pd SAC-catalyzed SMCC reaction provides an explanation for the Pd migration on the catalyst surface. A crucial role of molecular oxygen during SMCC reaction was revealed and attributed to the re-deposition of active Pd species by coordinating with the catalyst support. Overall, the leaching-oxidizing-landing mechanism of the SMCC reaction has been revealed and it not only provides insights into mechanism studies and catalyst designs, but it enlarges AMS applications.

Introduction

The palladium-catalyzed Suzuki-Miyaura cross-coupling (SMCC) reaction is a notable discovery for the selective construction of carbon-carbon (C-C) bonds through the reaction between aryl halides and organoboron nucleophiles.[1] The traditional SMCC reaction is facilitated by homogeneous Pd complexes that are coordinated with different ligands, resulting in major disadvantages of toxicity and high cost of Pd complexes.[2-5] To address these limitations and improve the recovery and reutilization of solid Pd catalyst upon separation, one possible approach could be moving the reaction from homogeneous to heterogeneous systems.[6] Moreover, upon anchoring single metal atoms on different supports, Pd single-atom catalyst (Pd SAC) might be an ideal catalyst by maximizing the metal utilization while minimizing aggregation and biotoxicity.[7-9] Generally, the SMCC reaction catalyzed by Pd SAC could simultaneously involve both heterogeneous processes (occurring on the catalyst surface) and homogeneous processes (facilitated by Pd

species in solution), which remain under debate due to the lack of direct structural evidence in both the spatial and temporal terms.

Briefly, the main challenge for unveiling the homogeneous/heterogeneous paths of the SMCC reaction could be the examination of detailed changes of active Pd species throughout a series of conversions.[10] The transformation of Pd species may involve leaching from the solid phase, migration, and landing on supports for the subsequent catalytic cycles.[11] Due to the lack of sufficient evidence to directly demonstrate structural changes, the natural role of Pd catalytic species is still a debate currently.[12-14] Several studies have indicated that the soluble Pd species leaching from the solid support are responsible for the homogeneous SMCC reaction,[15-17] whereas others argue that the active sites for the heterogeneous reaction are located on the solid phase[18-20]. Nevertheless, there may exist a complex correlation between these two mechanisms, and both pathways could simultaneously occur during the SMCC reaction. In addition, the unique structural properties of Pd SAC would present additional difficulties in understanding the complex heterogeneous/homogeneous SMCC paths.[21] Although the roles of ligands on Pd have been studied in heterogeneous pathways[8, 22], the detailed Pd transformation during leaching, migration, and the recovery of Pd remains poorly understood. Furthermore, the environmental factors (such as oxygen) for the conversion of Pd active sites have not been adequately examined due to the challenge of directly identifying Pd species in real time. Hence, further efforts are necessary to provide structural information on Pd active sites during the leaching and

a. Key Laboratory of Radiopharmaceuticals, Ministry of Education, College of Chemistry, Beijing Normal University, Beijing, 100875, China.
b. State Key Laboratory for Quality Assurance and Sustainable Use of Dao-di Herbs, National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, 100700, China.
c. Department of Chemistry, Faculty of Arts and Sciences, Beijing Normal University, Zhuhai, 519085, China.
† Correspondence: nana@bnu.edu.cn
Supplementary Information available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

Chemical Science Accepted Manuscript

ARTICLE

Journal Name

migration of Pd active sites in homogeneous/heterogeneous SMCC paths.

Several strategies have been employed to distinguish, monitor, and examine homogeneous or heterogeneous SMCC paths in spatial and temporal terms, particularly through transmission electron microscopy, real-time fluorescence imaging, and sequential electrical signals.[10, 19, 23] However, the indirect structural information is still insufficient to clearly identify the structural changes of Pd species during homogeneous/heterogeneous paths. Ambient mass spectrometry (AMS) is a label-free technique that allows for the rapid

and direct acquisition of structural information, which has shown significant potential for the monitoring of multiple molecules in complex systems.[24-26] With the advantages of online analysis, AMS facilitates the observation and identification of transient intermediates, thereby revealing the roles and changes of active sites.[27-29] Therefore, the AMS-based online monitoring would substantially provide multiple pieces of structural information both on the SAC surface and in the liquid phase, facilitating the examinations of both leaching and migration of Pd active sites.[30]
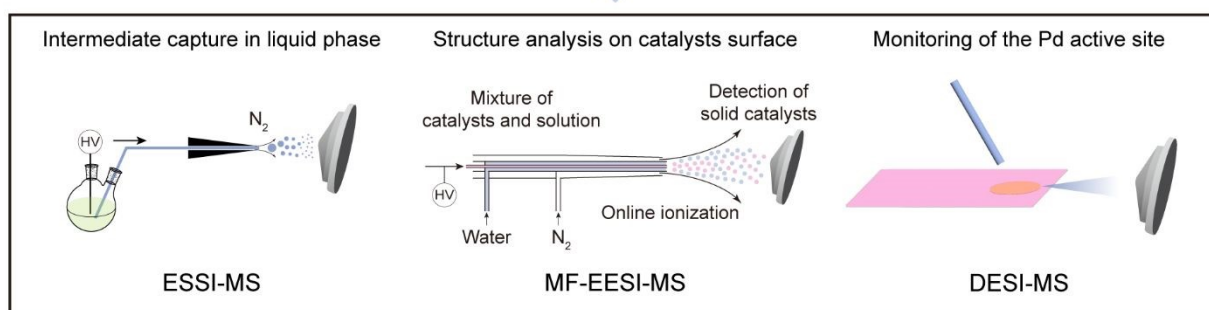
Scheme 1. Ambient mass spectrometry methods for the online monitoring and mechanistic study of the Pd SAC-catalyzed Suzuki-Miyaura cross-coupling reaction.

In this study, the Pd SAC-catalyzed Suzuki-Miyaura reaction was monitored by a series of AMS methods to investigate fundamental homogeneous/heterogeneous aspects. The series of AMS monitoring (Scheme 1) has been carried out during the SMCC reaction. The structural information of both the catalyst surface and

the liquid phase illustrates the leaching-oxidizing-landing process of the Pd active site. This study would not only enhance deeper comprehension of the Pd SAC-catalyzed SMCC reaction but also provide an effective AMS method to monitor the structural change of the single-atom active sites during the reaction.

Experimental procedure

Chemicals

2-bromopyridine, phenylboronic acid, 2-phenylpyridine, and phosphotungstic acid were obtained from Macklin reagent (Shanghai, China). KOH of analytical grade was purchased from Beijing Chemicals (Beijing, China). Palladium acetate was purchased from Aladdin (Shanghai, China). All other chemical reagents were of analytical grade and were used directly without further purification. Methanol of HPLC grade was purchased from Fisher Chemical (CA, USA). Ultrapure water (Mill-Q, Millipore, 18.2 MΩ) was used in all experiments.

Instruments

Fourier transform infrared spectroscopy (FTIR) spectra were obtained on a Thermo Scientific Nicolet NEXUS 670. Ultraviolet-visible spectroscopy (UV-vis) measurements were recorded on a Shimadzu UV-3600 spectrophotometer. The yield of the SMCC reaction was analyzed by the Agilent 1260 liquid chromatography with mobile phase (acetonitrile/water v/v 6:4). The HRMS experiments for the liquid phase were detected by a G2-XS QTof mass spectrometer (Waters). The AMS experiments were performed on an LTQ XL linear ion trap mass spectrometer (Thermo Fisher Scientific).

Pd@PWO preparation

The catalyst of Pd@PWO was prepared following the reported procedures. Firstly, phosphotungstic acid (20.7 g, 6.29 mmol) and KCl (1.00 g, 13.4 mmol) were dissolved in 100 mL water under vigorous stirring. The solution was then adjusted to pH 5.5 by adding KHCO₃ (1 M). After removing the insoluble precipitate, the solution was dried to obtain lacunary PWO. Subsequently, PWO (50 mg, 0.02 mmol) and Pd(Ac)₂ (22.4 mg, 0.10 mmol) were added in 50 mL water. After a reaction for 10 h, excessive Pd(Ac)₂ was removed by filtering, and the solution was dried and washed with methanol to obtain Pd@PWO.

SMCC reaction

For the SMCC reaction examinations, 1 mg of Pd@PWO, 1.58 mg of 2-bromopyridine (1 mM), 1.83 mg of phenylboronic acid (1.5 mM), and 1.68 mg of KOH (3 equiv.) were added in 10 mL of methanol. The reaction mixture was stirred at room temperature for 2 h. The solid catalyst was separated by filtration, and the reaction mixture was analyzed by LC.

Online monitoring of the reaction

The electronsonic spray ionization (ESSI) source was constructed for the online monitoring of the SMCC reaction. A self-pumping effect from the reaction system (i.d. 250 μm, o.d. 365 μm) was generated by the nitrogen stream through an external capillary (i.d. 1000 μm, o.d. 1300 μm). The SMCC reaction was introduced in the MS inlet and the reactant and the product were monitored by MS analysis. The MS detection was in the full scan positive mode over an m/z range from 100 to 500. The capillary temperature was 275 °C, and the capillary voltage and tube lens voltage were set at 23.0 V and 75.0 V, respectively. The ion maximum injection time of the linear ion trap was 10 ms. The MS results were obtained and analyzed using Xcalibur software.

Online analysis of the solid catalyst

For the analysis of the solid Pd@PWO during the SMCC reaction, the multiphase flow of extractive electrospray ionization system (MF-EESI) was constructed for MS detection. Different from the MF-ESSI system for monitoring the reaction, an interlayer capillary (i.d. 530 μm, o.d. 690 μm) was added for introducing water, promoting the ionization of water-soluble solid catalysts in the spray. The MS detection was set in the negative mode over an m/z range from 800 to 1000. The capillary temperature was 275 °C, and the capillary voltage and tube lens voltage were set at -26.00 V and -85.35 V, respectively. The ion maximum injection time of the linear ion trap was 1000 ms. The MS results were obtained and analyzed using Xcalibur software.

DESI-MS analysis

The desorption electrospray ionization system (DESI) was used for the MS detection of the solid samples on the glass slides with water as the solvent. The MS detection was set in the negative mode over an m/z range from 800 to 1000. The capillary temperature was 275 °C, and the capillary voltage and tube lens voltage were set to -26.00 V and -85.35 V, respectively. The ion maximum injection time of the linear ion trap was 1000 ms. The MS results were obtained and analyzed using Xcalibur software.

Results

The feasibility of the SMCC reaction catalyzed by Pd@PWO

With the definite active sites and ionic structures, lacunary polyoxometalates-based SAC was chosen as the support for constructing Pd SAC for the SMCC reaction.[31, 32] As shown in Figure 1A, the SMCC reaction was catalyzed by the $PW_{11}O_{39}{}^{7-}$-derived Pd SAC (Pd@PWO).

Initially, the construction of Pd@PWO was identified by ESSI-MS during the synthesis. As shown in Figure 1B, the lacunary polyoxometalates were obtained by adjusting the pH of phosphotungstic acid to 5.5, leading to the significant ion of Keggin-derived lacunary PWO ($[PW_{11}O_{39} + 4H]^{3-}$ at m/z 893.72, calc. 893.76) upon removing a W=O group from $PW_{12}O_{40}{}^{3-}$. Notably, the anionic carrier of the lacunary polyoxometalates possesses a high ionization efficiency and facilitates the capture and identification of instantaneous intermediates using the ESSI system. The final catalyst of Pd@PWO was successfully recorded upon the coordination of Pd^{2+} with four O atoms in the PWO carrier, exhibiting the main ion peak at m/z 928.42 (calc. 928.39). This coordination between the Pd^{2+} and the Keggin-derived lacunary PWO was also confirmed by the UV-Visible absorption spectrum (Figure S1). Besides, the Pd-coordinated structure of Pd@PWO was further demonstrated by the peaks (P-Oa at ~1100 cm⁻¹, W-Ob-W at ~900 cm⁻¹ and W-Oc-W at ~750 cm⁻¹) in the infrared absorption spectrum after the coordination with Pd (Figure S2).[33]

Subsequently, the feasibility of the Pd@PWO-catalyzed SMCC reaction between 2-bromopyridine and phenylboronic acid (in the presence of KOH) was examined by liquid chromatography (LC) and HRMS analysis. As demonstrated in Figure 1C, the reactant 2-bromopyridine ($[C_5H_4NBr + H]^+$ at m/z 157.9608, calc. 157.9605) before the reaction and the product 2-phenylpyridine ($[C_5H_4NC_6H_5 +$

H]$^+$ at m/z 156.0822, calc. 156.0813) after the reaction were observed in the HRMS spectra. The relatively high signal intensity of the product indicated the efficient SMCC reaction catalyzed by Pd@PWO, whose yield of 2-phenylpyridine was 93% (1 h) at room temperature (determined by LC, Figure S3). Thereafter, the SMCC reaction was online monitored using ESSI-MS (Figure 1D). The SMCC reaction system was continuously provided for MS detection through the self-pumping of a nitrogen flow, without any sample pre-treatment or isolation. As shown in Figure 1E, the decreased reactant ions of 2-bromopyridine ([$C_5H_4NBr + K$]$^+$ at m/z 197.86, calc. 197.91) and phenylboronic acid ([$C_6H_5B(OH)_2 + H$]$^+$ at m/z 123.12, calc. 123.06), as well as the increased product of 2-phenylpyridine ([$C_5H_4NC_6H_5 + K$]$^+$ at m/z 194.06, calc. 194.04), were observed during the SMCC reaction for 2 h. Furthermore, the product yield was determined to be 97% (determined by LC) in 10 min at the relatively

higher temperature of 60 °C (Figure S4). The turnover number (TON) for the SMCC reaction was 31 and 33 at room temperature and 60 °C, and the turnover frequency (TOF) values were 31 and 198, respectively. The ESSI-MS detection demonstrated no SMCC reaction products or additional MS signals at 0 min (Figure S5A), and the real-time monitoring results of the reaction were basically consistent with the LC detection (Figure S5B). Consequently, any reaction acceleration in the microdroplets generated by ESSI on the SMCC reaction could be excluded. These results demonstrated that Pd@PWO was successfully constructed to efficiently catalyze the SMCC reaction at room temperature, arousing further investigations to reveal the reaction mechanism in homogeneous and heterogeneous pathways.
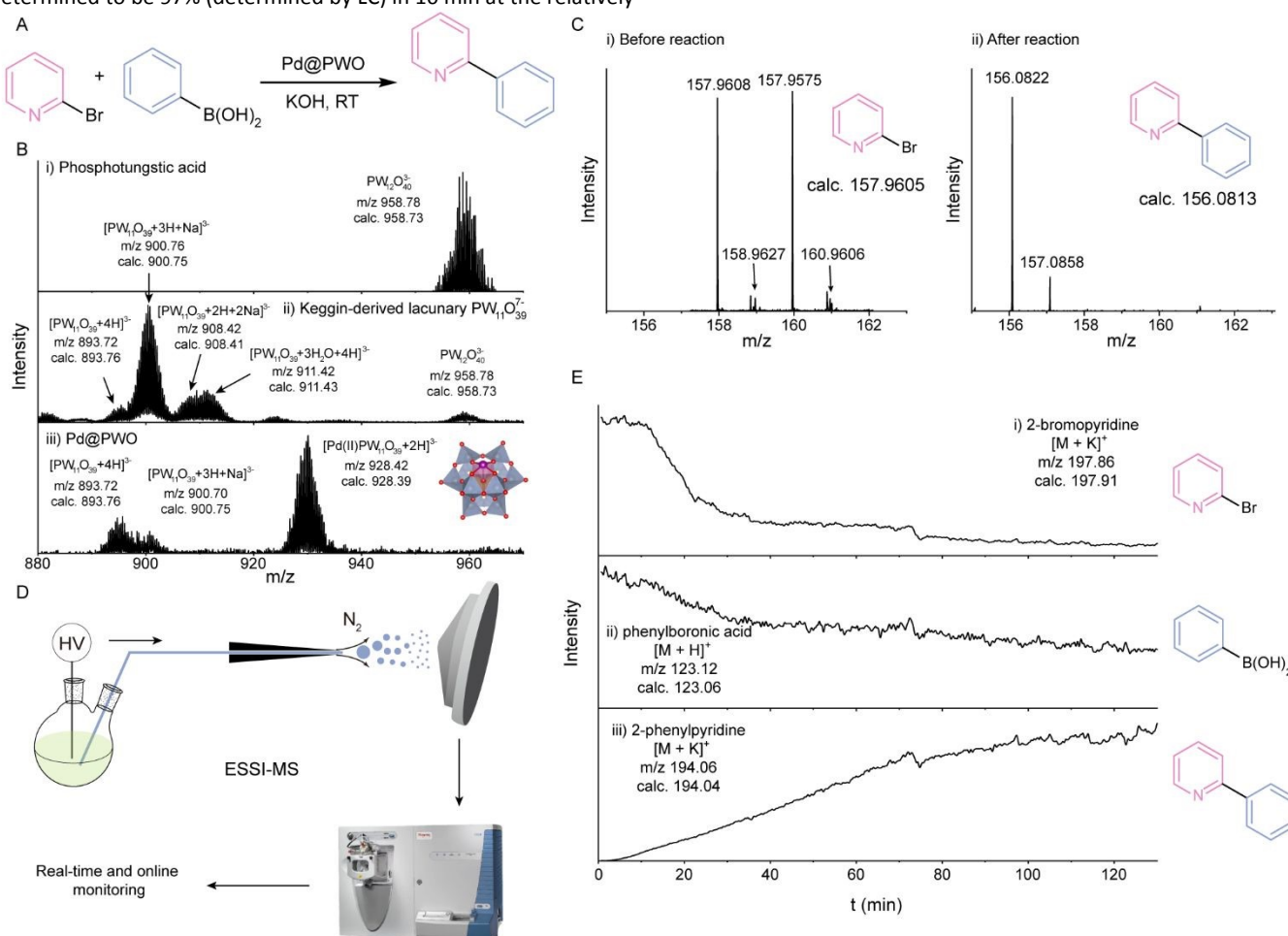
Figure 1. Confirming of Pd@PWO construction and the feasibility of catalyzing SMCC. A. SMCC reaction between 2-bromopyridine and phenylboronic acid to generate 2-phenylpyridine product, which was carried out at room temperature (RT) in methanol. B. Mass spectra of phosphotungstic acid (i), Keggin-derived lacunary PWO (ii), and Pd@PWO (iii). C. High-resolution mass spectrum of the reaction system before (i) and after SMCC reaction (ii) for 2 h. D. Diagram of the ESSI instrument for online monitoring of SMCC reaction. E. The extracted ion chromatograms (EICs) of 2-bromopyridine (m/z 197.86) (i), phenylboronic acid (m/z 123.12) (ii), and 2-phenylpyridine (m/z 194.06) (iii) during the SMCC reaction.

The heterogeneous mechanism of the SMCC reaction under air conditions

To pertinently examine the heterogeneous path or the homogeneous mechanism, the SMCC reaction was monitored for

100 min, with the Pd@PWO being removed at 20 min. Compared to the SMCC reaction catalyzed by Pd@PWO, no significant increase in the yield was recorded after removing Pd@PWO from the system at 20 min (Figure 2A). This suggests that the heterogeneous mechanism

ARTICLE

plays a significant role in the SMCC reaction, mostly occurring on the surface of Pd@PWO. Subsequently, to analyze the structural changes of the Pd active sites on the catalyst surface, the Pd@PWO during the SMCC reaction was analyzed. For direct and real-time obtaining of the intermediate structure, the multiphase flow of extractive electrospray ionization mass spectrometry (MF-EESI-MS) [28] was applied for the online ionization of Pd@PWO during the SMCC reaction. In brief, the MF-EESI was constructed by 3-layered concentric capillaries. The SMCC reaction system was self-pumping and introduced and electro-sprayed through the innermost capillary (connected to a high voltage) by nitrogen from an external capillary. Simultaneously, a stream of water in the middle layer was introduced (Figure S6). During MF-EESI, the reaction system within the

innermost capillary interacts with the water spray from the middle layer, facilitating the dissolution of heterogeneous catalysts to accomplish Pd@PWO ionization. As shown in Figure 2B, except for the Pd(II) species of Pd@PWO, an intermediate after the oxidative addition was observed on the surface of the catalyst ($[Pd(IV)(C_5H_4N)(OH)PW_{11}O_{39} + 2H]^{3-}$ at m/z 960.12, calc. 960.06). Therefore, it can be inferred that the heterogeneous pathway is employed by the Pd(II)-Pd(IV) catalysis. This could be attributed to the strong electron-donating capability of Keggin-derived lacunary PWO, which would activate Pd(II) to perform a similar role to classical Pd(0) in the SMCC reaction.
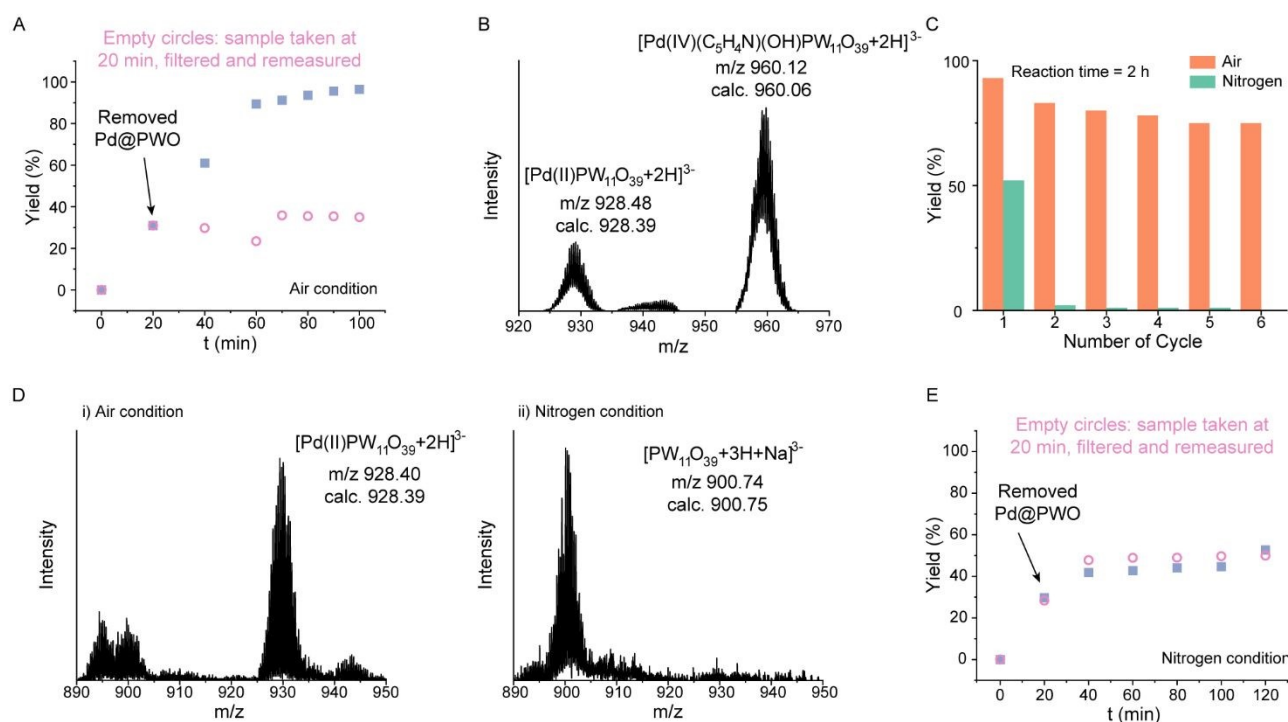

Figure 2. Examination of Pd@PWO-catalyzed SMCC reaction under air or nitrogen conditions. A. Yields of 2-phenylpyridine recorded during 100 min of the SMCC reaction in air. Blue squares: normal monitoring with the time interval injections. Pink circles: monitoring with catalyst removed at 20 min. B. Mass spectrum of Pd@PWO isolated from the SMCC reaction at 20 min. C. Yields of the SMCC reaction catalyzed by the recycled Pd@PWO in several cycles. Six catalytic cycles were employed in both nitrogen and air using recycled catalyst. The catalysts were washed with methanol and centrifuged after each reaction. D. Mass spectrum of the isolated Pd@PWO after SMCC reaction under nitrogen condition (i) and air condition (ii). E. Yields of 2-phenylpyridine were recorded during 100 min of the SMCC reaction under nitrogen conditions. Blue squares: normal monitoring with the time interval injections. Pink circles: monitoring with the catalyst removed at 20 min.

Normally, the oxidation of Pd(0) to Pd(II) in the presence of molecular oxygen can decrease the catalytic activity in the traditional Pd-catalyzed SMCC reaction.[34] Predictably, the present activation of Pd(II) in the Pd@PWO would effectively avoid poisoning of the Pd@PWO catalyst upon molecular oxygen oxidation. Excitingly, the yield of 2-phenylpyridine in air (93%) is significantly higher than that under nitrogen conditions (52%) (Figure S7). Consequently, the molecular oxygen did not reduce the catalytic activity of Pd@PWO but greatly enhanced the efficiency of the SMCC reaction. Meanwhile, a relatively reduced yield (67%) was recorded in the oxygen environments, due to the oxidation of phenylboronic acid by the

molecular oxygen. Furthermore, this product yield in oxygen conditions (67%) remained higher than that in nitrogen conditions (52%), suggesting that molecular oxygen had a significant improvement on the Pd@PWO-catalyzed SMCC reaction. Although this has also been reported in some heterogeneous catalysis,[19, 35] the detailed mechanism of molecular oxygen remains poorly understood. Hence, it is recommended to conduct more investigations on the functions of molecular oxygen, requiring the observation of structural changes of catalysts during the SMCC process.

The homogeneous mechanism under nitrogen conditions

The influence of molecular oxygen in the SMCC reaction was evaluated by examining the reusability of Pd@PWO in nitrogen and air environments. As shown in Figure 2C, relatively stable and high yields of the product were obtained across five cycles in air (75-95%). Lower yield was recorded under nitrogen conditions in the first cycle (52%) and declined substantially to less than 5% in the following cycles. The decrease in catalytic activity under nitrogen conditions could be attributed to the structural changes of the Pd@PWO catalyst after the SMCC reaction. To examine the changes in Pd@PWO structures after the SMCC reaction under nitrogen and air

conditions, the catalyst was isolated after the reaction for the ESSI-MS detections. Under air conditions (Figure 2D-i), the Pd species of $[Pd(II)PW_{11}O_{39} + 2H]^{3-}$ (at 928.40, calc. 928.39) were observed to be maintained after the reaction, indicating the good reusability of Pd@PWO under air conditions (Figure 2C). While in nitrogen (Figure 2D-ii), only the obvious lacunary $PW_{11}O_{39}^{7-}$ ($[PW_{11}O_{39} + 3H + Na]^{3-}$ at m/z 900.74, calc. 900.75) was recorded after the SMCC reaction. This suggests that Pd active sites were leached from the Pd@PWO catalyst, resulting in poor reusability under nitrogen conditions.
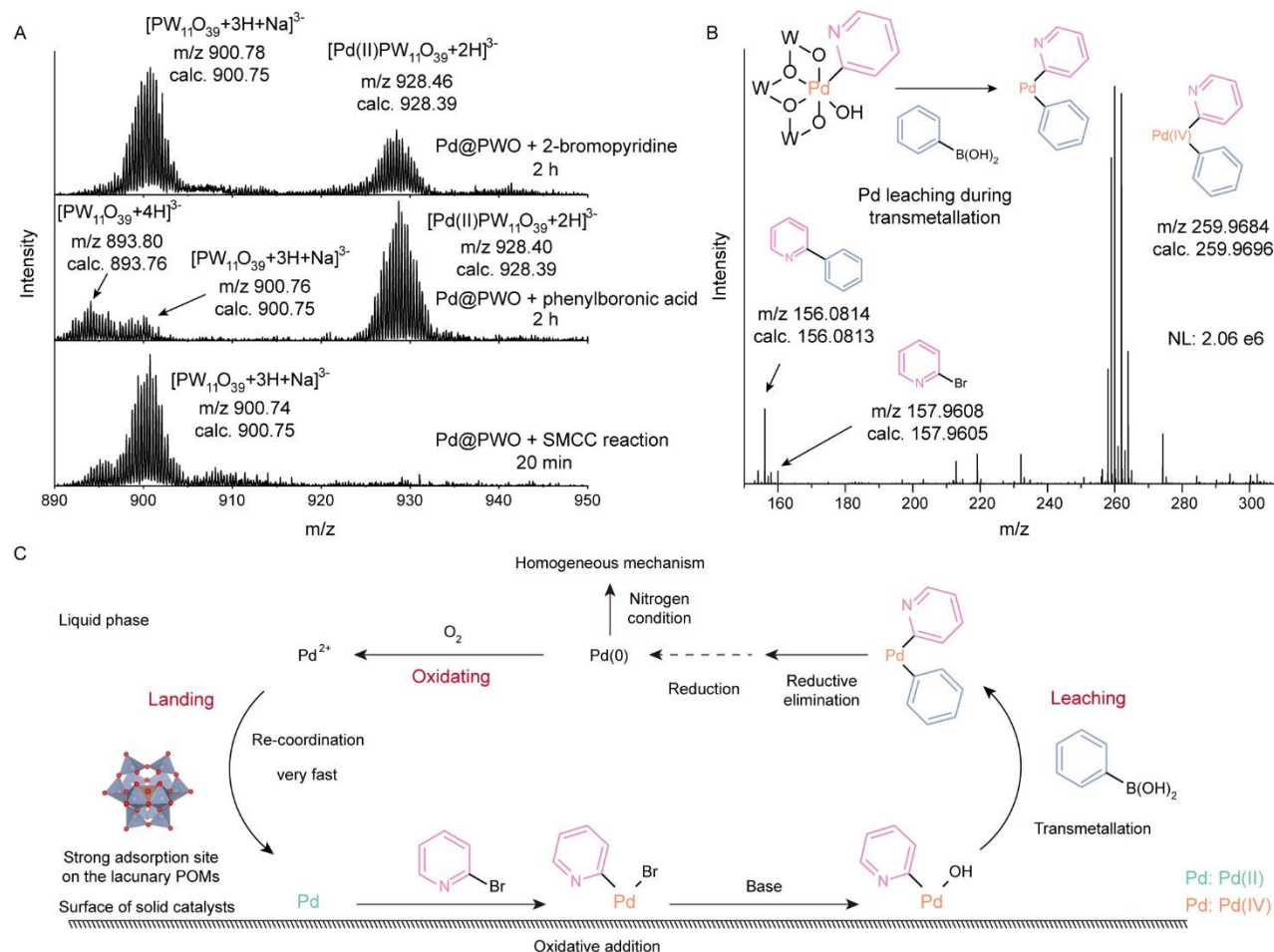

Figure 3. Investigation on leaching of Pd species from Pd@PWO. A. Mass spectra of the isolated Pd@PWO after adding 2-bromopyridine for 2h, phenylboronic acid for 2h, and employing SMCC reaction for 20 min in nitrogen. B. High-resolution mass spectrum of the liquid phase after SMCC reaction for 10 min in nitrogen. C. Illustration of the leaching-oxidizing-landing mechanism of the Pd@PWO catalyzed SMCC reaction involving the homogeneous and heterogeneous paths.

Considering the significant leaching of Pd species would facilitate the SMCC reaction in the homogeneous pathway, the SMCC reaction was evaluated by removing Pd@PWO at 20 min of the reaction under nitrogen conditions. This reaction path was simultaneously compared with the SMCC reaction in nitrogen without the catalyst removed. As shown in Figure 2E, there was no significant difference between the two reaction systems with or without removing the catalyst at 20 min. This result indicates that the SMCC reaction under nitrogen conditions was employed through the homogeneous path with the leached Pd species in the liquid phase. In general, the ligand-free Pd leached in the liquid phase would be

easily aggregated and lose catalytic activity during the homogeneous SMCC reaction, resulting in the low yield under nitrogen conditions (Figure S7, Figure 2C). Therefore, the SMCC reaction in nitrogen was carried out in the homogeneous path, which was dramatically distinct from the heterogeneous reaction in air.

Examination of the process of the leaching-oxidizing-landing Pd species

The leaching of Pd was further investigated due to its critical function in the Pd SAC-catalyzed SMCC reaction. In the examinations,

to ensure the satisfactory catching of leaching intermediates, the SMCC reaction was carried out under nitrogen conditions. It is generally believed that the oxidative addition (by 2-bromopyridine) is the prerequisite step of the leaching of Pd.[6] Therefore, the oxidative addition process was initially investigated by evaluating structural changes of Pd@PWO with the addition of 2-bromopyridine or phenylboronic acid to Pd@PWO, respectively. After interacting with the two reactants separately, the catalyst was isolated and analyzed by ESSI-MS to obtain the structural information of Pd@PWO. After interaction with 2-bromopyridine for 2 h, the peak of the Keggin-derived lacunary PWO ($[PW_{11}O_{39} + 3H + Na]^{3+}$ at m/z 900.78, calc. 900.75) was recorded, indicating the leaching of the Pd active site from Pd@PWO (Figure 3A). During the interaction between phenylboronic acid and Pd@PWO for 2 h, no obvious signal of lacunary PWO was recorded, indicating the inefficient leaching of Pd species from Pd@PWO. Nevertheless, the more efficient leaching of Pd species was exhibited after the SMCC reaction under nitrogen conditions for only 20 min, with only lacunary PWO observed and no other Pd-related ion recorded (Figure 3A). Consequently, the oxidative addition process facilitated the leaching of Pd species but was not the main force during the SMCC reaction.

Furthermore, the liquid phase of the SMCC reaction was analyzed using HRMS to investigate the intermediates generated during the leaching of Pd species from Pd@PWO. After 10 min of the reaction, the significant signal peak of $[Pd(IV)(C_5H_4N)(C_6H_5) - H]^+$ (at m/z 259.9684, calc. 259.9696) was observed in the liquid phase, which was identified as the transmetallation product after leaching from the Pd@PWO catalyst. Furthermore, other potential Pd-related substances (such as oxidative addition species $Pd(IV)(C_5H_4N)Br^{2+}$) were not detected in the liquid phase. This further demonstrates that the key step of Pd leaching in the SMCC reaction is likely to be the transmetallation process, rather than the oxidative addition process.

Significantly, the similar transmetallation product of $[Pd(IV)(C_5H_4N)(C_6H_5) - H]^+$ (at m/z 259.9684, calc. 259.9696) was also observed in the SMCC reaction under air conditions (Figure S8). This suggests that the presence of molecular oxygen did not hinder the leaching of Pd. Therefore, the mechanism of the SMCC reaction under air conditions is not a pure heterogeneous mechanism, which includes the leaching of Pd and the efficient recovery of the leached Pd back to the catalyst in the presence of molecular oxygen. Furthermore, the role of molecular oxygen was further demonstrated by observing the recovered Pd on Pd@PWO after the 2 h reaction in nitrogen and another 2 h exposure in air (Figure S9).

Under nitrogen conditions, the leached Pd species was unable to return to the catalyst, leading to homogeneous catalysis in the liquid phase. While under air conditions, the leached Pd species could be oxidized to Pd^{2+} by the molecular oxygen, which would then be rapidly captured by the lacunary PWO to form new Pd active sites upon their strong interactions. This rapid re-coordination of Pd^{2+} to the lacunary PWO ensured the excellent reusability of Pd@PWO, sustaining the efficient catalytic activity of the SMCC reaction. Therefore, molecular oxygen was demonstrated to play an important role in the landing of Pd species back to the catalyst and then maintaining the single-atom Pd active site during the SMCC reaction.

Besides, the intermediate of oxidative addition ($[Pd(IV)(C_5H_4N)(OH)PW_{11}O_{39} + 2H]^{3-}$) was observed on the catalyst during the SMCC reaction under air conditions (Figure 3B). However, the proposed transmetallation intermediate on the surface of the catalyst of $[Pd(IV)(C_5H_4N)(C_6H_5)PW_{11}O_{39} + 2H]^{3-}$ (calc. 980.08) was not observed (Figure S10). Based on the intermediate of the transmetallation product in the liquid phase (Figure S8), it can be further concluded that the leaching of Pd occurs during the transmetallation process rather than the oxidative addition process.

Therefore, based on the monitoring of both catalyst and liquid phase systems, the leaching-oxidizing-landing process of Pd species during the Pd@PWO-catalyzed SMCC reaction was determined. As shown in Figure 3C, the oxidative addition process was employed on the surface of Pd@PWO. Subsequently, in the presence of phenylboronic acid, the Pd species were leached from the catalyst surface through the transmetallation process. Thereafter, reductive elimination in the liquid phase is carried out, resulting in the generation of leached Pd(0) through reduction by ligands or phenylboronic acid. Due to the difficulty in ionizing neutral leached Pd for MS detections, future research should focus on the mechanism of Pd(0) generation. Under nitrogen conditions, leached Pd(0) will undergo a homogeneous pathway in the liquid phase for the SMCC reaction. In the presence of molecular oxygen, leached Pd will be oxidized to Pd^{2+}, and then quickly coordinated with the lacunary PWO to land back on the catalyst surface. This process ensures single-atom Pd active sites for the subsequent catalytic cycles. In fact, the leaching-oxidizing-landing process not only points out the role of molecular oxygen in the heterogeneous mechanism but also offers a comprehensive explanation for the migration of Pd active sites.
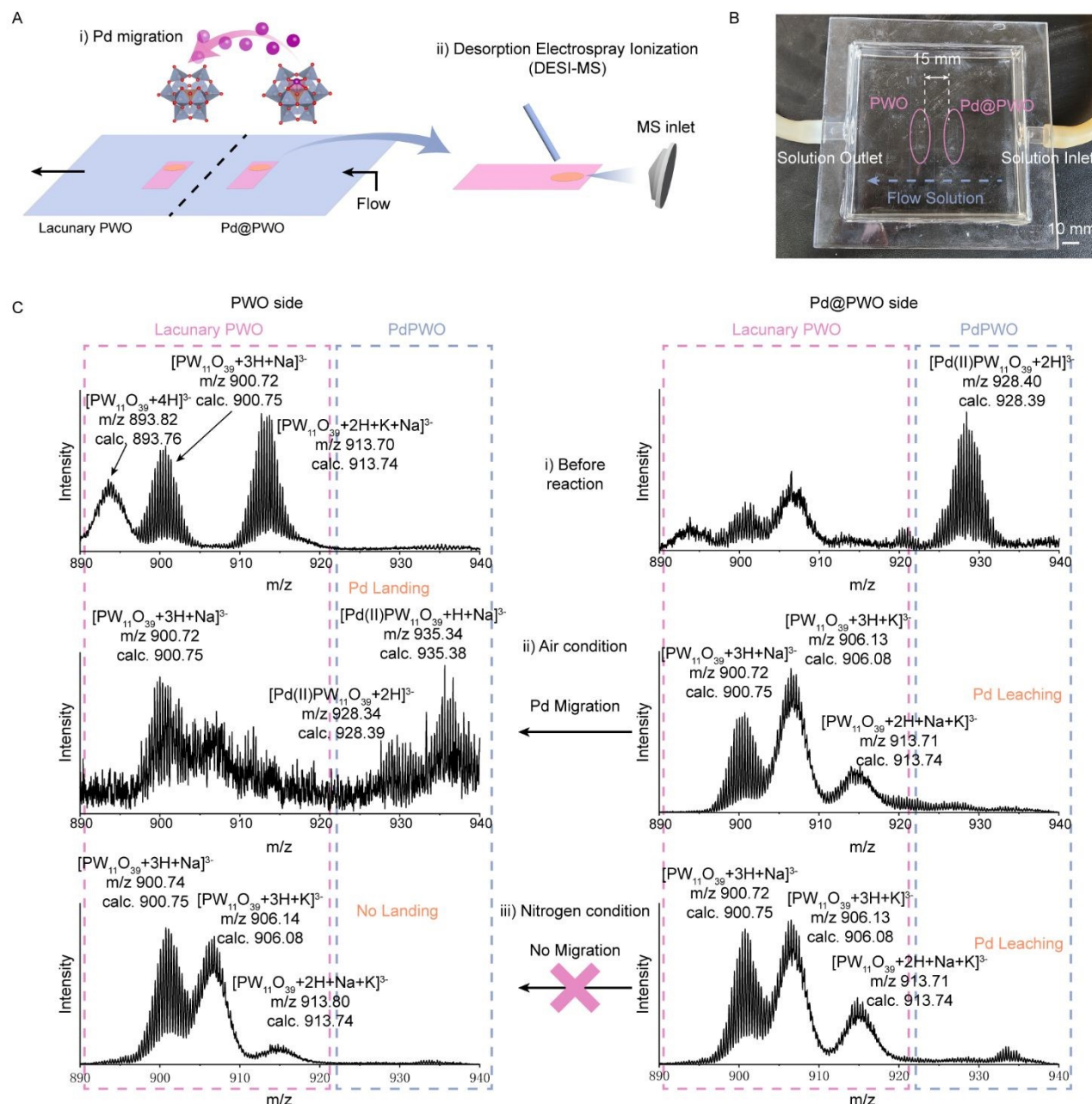
ARTICLE

Figure 4. Evaluation of migration of Pd species from Pd@PWO to lacunary PWO. A. (i) Schematic illustration of the quartz reaction cell with reactants flow through. Two glass slides coated with the catalyst of Pd-free lacunary PWO and Pd@PWO were deposited on the bottom of the cell. (ii) DESI instruments for detecting Pd-related species on two glass slides. B. Picture of the quartz reaction cell with two sheets located on the cell bottom 15 mm apart. C. MS spectra of the species on the glass slides with Pd@PWO and PWO before (i) and after SMCC reaction in air (ii) and nitrogen (iii) for 2 h, respectively. Pink dashed frame: the characteristic peaks attributed to the lacunary PWO. Blue dashed frame: the characteristic peaks of Pd@PWO.

The migration of the active catalytic site of Pd species

The migration of Pd during the SMCC reaction is a significant phenomenon in heterogeneous catalysis, which has been examined

by several characterizations.[10, 19] Actually, the proposed landing-oxidizing-landing pathway effectively accounts for the generation of new Pd active sites during the SMCC reaction. Nevertheless, the detailed structural mechanism of migration remains inadequately explained. To confirm the migration of Pd active sites during the SMCC reaction, the AMS detection was conducted under air and nitrogen conditions. The direct AMS detection of migration was conducted by a previously reported device with slight modifications.[19] Briefly, a quartz reaction cell with reactants continuously circulated was constructed to magnify the migration tendency (Figure 4A). To evaluate the migration across a relatively long distance, two catalysts, Pd@PWO and lacunary PWO, were respectively coated on two glass slides and positioned on the cell bottom about 15 mm apart. Subsequently, the reactants of 2-bromopyridine and phenylboronic acid were added in methanol and flowed from the Pd@PWO side to the PWO side for the SMCC reaction (Figure 4B). To verify the proposed migration of Pd active sites during the SMCC reaction, the catalyst on the surface of two glass slides was directly detected by another AMS technique, desorption electrospray ionization mass spectrometry (DESI-MS), without any pretreatments.[25, 36] The solvent (water) was electrosprayed and hit onto the glass slides, where the deposited catalysts (Pd@PWO or lacunary PWO) were transported to the mass spectrometer for analysis.

Initially, we assessed the viability of detecting catalysts on the glass slide using DESI-MS. As shown in Figure 4C, before the SMCC reaction, the main peak of Pd-related species of $[Pd(II)PW_{11}O_{39}+2H]^{3-}$ (at m/z 928.40, calc. 928.39) was detected on the Pd@PWO parts. While on the glass slide with the lacunary PWO coating, only the ions of lacunary PWO ($[PW_{11}O_{39} + 4H]^{3+}$ at m/z 893.82, calc. 893.76, $[PW_{11}O_{39} + 3H + Na]^{3-}$ at m/z 900.72, calc. 900.75, $[PW_{11}O_{39} + 2H + K + Na]^{3-}$ at m/z 913.70, calc. 913.74) were observed, and no Pd species was identified. This confirms the successful construction of DESI-MS evaluation systems.

To evaluate the oxygen-initiated landing of Pd species in the migration process, the catalytic SMCC reaction was conducted in nitrogen and oxygen for comparison. The reactants flowed from the Pd@PWO side to the PWO side during 2 h of the SMCC reaction in air, and then the two glass slides were detected by DESI-MS. As displayed in Figure 4C, no significant ion of Pd species was observed on the glass slide with Pd@PWO originally coated, which demonstrated the leaching of Pd active sites. While the corresponding ions of Pd species were detected on the lacunary PWO side ($[Pd(II)PW_{11}O_{39} + 2H]^{3-}$ at m/z 928.34, calc. 928.39, $[Pd(II)PW_{11}O_{39} + H + Na]^{3-}$ at m/z 935.34, calc. 935.38). This experiment validated the migration of Pd active sites, leaching from the Pd@PWO part and subsequently landing on the PWO side. Significantly, the intensity of $[Pd(II)PW_{11}O_{39}+2H]^{3-}$ (at m/z 928.42) on the Pd@PWO side dramatically decreased after the SMCC reaction under nitrogen conditions, indicating the leaching of Pd was also employed in the absence of molecular oxygen. However, no obvious Pd signal was found on the PWO side after the SMCC reaction under nitrogen conditions (Figure 4C), which indicated no significant landing of Pd species under nitrogen conditions. Consequently, the oxidizing process by molecular oxygen is crucial for the landing of Pd

and further plays a significant role in the migration of Pd active sites. In fact, molecular oxygen facilitated the landing of Pd species back to the lacunary PWO upon Pd^{2+} coordination, leading to the migration of Pd active sites and thereby maintaining efficient catalytic activity and the reusability of Pd@PWO for the SMCC reaction.

Discussion

The homogeneous/heterogeneous paths in the SMCC reaction catalyzed by a Pd SAC (Pd@PWO), supported by an anionic ligand (Keggin-derived lacunary $PW_{11}O_{39}^{7-}$), were investigated by AMS monitoring. Although both the homogeneous and heterogeneous mechanisms were studied previously,[8, 19, 23, 30] the dynamic changes and conversions of Pd-species between the catalyst surface and liquid phase remain ambiguous. Upon monitoring by AMS, the intermediates both on catalyst surfaces and in the liquid phase were captured and analyzed. This study provides valuable structural information on conversions of Pd-related species between homogeneous and heterogeneous paths, revealing the leaching-oxidizing-landing process during the SMCC reaction.

Based on identifying intermediates on the catalyst surface and in liquid phases, the leaching of Pd was confirmed under nitrogen conditions. The leaching comparison between the SMCC reaction and adding only the oxidative reactant of 2-bromopyridine demonstrates that the oxidative addition was not the crucial step for Pd leaching. The oxidative addition products were observed on the catalyst surface, and the transmetallation products were observed in the liquid phase rather than on the catalyst surface. Therefore, the transmetallation process may play a crucial role in Pd leaching. Under nitrogen conditions, the leached Pd species will undergo a homogeneous path in the liquid phase, resulting in the poor reusability of Pd@PWO for the SMCC reaction. Nevertheless, due to the technical limitations of the AMS technique, it is not possible to determine the specific mechanism of the reductive elimination process in the liquid phase, since the neutral leached Pd is difficult to be ionized for the AMS monitoring. Consequently, further studies on the generation of Pd(0) species by reductive elimination are encouraged.

Molecular oxygen does not inhibit the leaching of Pd species in the SMCC reaction under air conditions, but rather, it occurs concurrently with the transmetallation process. Furthermore, the molecular oxygen facilitates the rapid oxidation and landing of the leached Pd species. In brief, molecular oxygen would further trigger the oxidation of leached Pd(0) to Pd^{2+}, facilitating the landing of the Pd active site by coordinating with lacunary PWO. Due to the strong coordination ability of PWO, Pd is able to quickly land back onto the catalyst surface after leaching, resulting in the excellent reusability and catalytic activity of Pd@PWO. Given the requirement of anaerobic conditions in the traditional reaction system, understanding this mechanism will significantly advance the development of the SMCC reaction under air conditions.

This leaching-oxidizing-landing mechanism effectively explains the Pd migration during the SMCC reaction, which was verified by DESI-MS detection. As demonstrated, only the leaching process but no landing behavior of Pd was observed under nitrogen conditions. While under air conditions, the long-distance migration (~15 mm) of Pd active sites was observed upon the leaching-oxidizing-landing

process. Therefore, molecular oxygen was demonstrated to facilitate the landing of leached Pd species back to the catalyst surface,

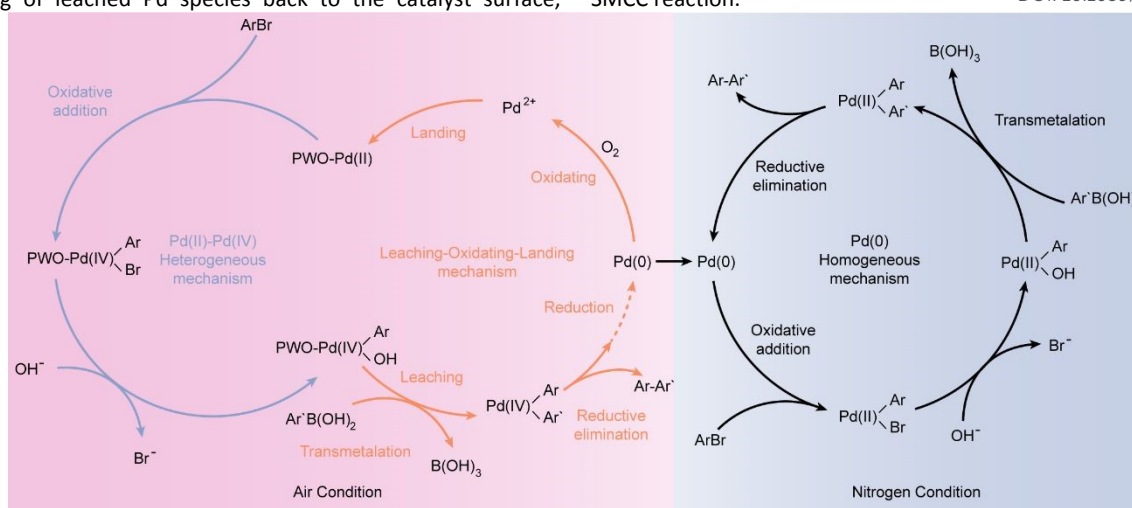thereby supporting the leaching-oxidizing-landing mechanism in the SMCC reaction.

Figure 5. Catalytic cycles of the Pd(II)-Pd(IV) heterogeneous mechanism (blue), the homogeneous mechanism (black), and the leaching-oxidizing-landing mechanism of Pd species (orange).

Conclusions

In conclusion, the AMS monitoring of the SMCC reaction catalyzed by Pd@PWO suggested the leaching-oxidizing-landing process of Pd to reveal the fundamental aspects of homogeneous/heterogeneous paths. The heterogeneous oxidative addition between Pd(II) and Pd(IV) was performed on the surface of the Pd@PWO catalyst (Figure 5). Subsequently, Pd leaching occurred concurrently with the transmetallation process, and the reductive elimination was employed in the liquid phase. Under nitrogen conditions, the homogeneous path was employed by the leached Pd species. This process exhibited the low catalytic efficiency and reusability of Pd@PWO without Pd landing back on the catalyst surface (Figure 5). While this was efficiently overcome in the presence of molecular oxygen. In this way, the subsequent oxidation-landing process was initiated under air conditions (Figure 5). Significantly, the molecular oxygen facilitated the generation of Pd^{2+} in the liquid phase, which could efficiently land back onto the catalyst surface. Furthermore, this leaching-oxidizing-landing mechanism effectively explains the migration of Pd active sites on the Pd@PWO. The unveiling of leaching-oxidizing-landing paths not only provides new insights into the SMCC mechanism and catalyst designs but also expands the application of AMS in mechanism examinations.

Author contributions

Y. Yin, X. Ge, and N. Na conceived the idea and designed the experiments; X. Shen and J. Ouyang supported the characterizations of the catalyst; N. Na supervised the project and acquired the funding; Y. Yin, X. Wang, X. Liu and X. Ge conducted the experiments with the assistance from N. Na; X. Wang and X. Liu carried out the catalyst synthesis; X. Li carried out the HRMS analysis; Y. Yin, X. Ge, and N. Na analyzed the data and interpreted the results. All authors read, discussed, and commented on the manuscript.

Conflicts of interest

The authors declare no competing interests.

Data availability

The data that support the findings of this study are available on request from the corresponding author.

Acknowledgements

Notes and references

1   Miyaura, N., Suzuki, A. Palladium-Catalyzed Cross-Coupling Reactions of Organoboron Compounds. *Chem. Rev.* 1995, **95**, 2457.
2   Han, F.-S. Transition-metal-catalyzed Suzuki–Miyaura cross-coupling reactions: a remarkable advance from palladium to nickel catalysts. *Chem. Soc. Rev.* 2013, **42**, 5270.
3   I. Beletskaya, P., Alonso, F., Tyurin, V. The Suzuki-Miyaura reaction after the Nobel prize. *Coordin. Chem. Rev.* 2019, **385**, 137.
4   Handa, S., Wang, Y., Gallou, F., Lipshutz, B. H. Sustainable Fe–ppm Pd nanoparticle catalysis of Suzuki-Miyaura cross-couplings in water. *Science* 2015, **349**, 1087.
5   Torborg, C., Beller, M. Recent Applications of Palladium-Catalyzed Coupling Reactions in the Pharmaceutical, Agrochemical, and Fine Chemical Industries. *Adv. Synth. Catal.* 2009, **351**, 3027.
6   Srivastava, A., Kaur, H., Pahuja, H., Rangarajan, T. M., Varma, R. S., Pasricha, S. Optimal exploitation of supported

heterogenized Pd nanoparticles for C-C cross-coupling reactions. *Coordin. Chem. Rev.* 2024, **507**, 215763.

7   Chen, Z., Vorobyeva, E., Mitchell, S., Fake, E., Ortuño, M. A., López, N., Collins, S. M., Midgley, P. A., Richard, S., Vilé, G., Ramírez, J. P. A heterogeneous single-atom palladium catalyst surpassing homogeneous systems for Suzuki coupling. *Nat. Nanotechnol.* 2018, **13**, 702.

8   Tao, X., Long, R., Wu, D., Hu, Y., Qiu, G., Qi, Z., Li, B., Jiang R., Xiong, Y. Anchoring Positively Charged Pd Single Atoms in Ordered Porous Ceria to Boost Catalytic Activity and Stability in Suzuki Coupling Reactions. *Small* 2020, **16**, 2001782.

9   Qiao, B., Wang, A., Yang, X., Allard, L. F., Jiang, Z., Cui, Y., Liu, J., Li, J., Zhang, T. Single-atom catalysis of CO oxidation using Pt1/FeOx. *Nat. Chem.* 2011, **3**, 634.

10 Galushko, A. S., Boiko, D. A., Pentsak, E. O., Eremin, D. B., Ananikov, V. P. Time-Resolved Formation and Operation Maps of Pd Catalysts Suggest a Key Role of Single Atom Centers in Cross-Coupling. *J. Am. Chem. Soc.* 2023, **145**, 9092.

11 Eremin, D. B., Ananikov, V. P., Understanding active species in catalytic transformations: From molecular catalysis to nanoparticles, leaching, "Cocktails" of catalysts and dynamic systems. *Coordin. Chem. Rev.* 2017, **346**, 2.

12 Gaikwad, A. V., Holuigue, A., Thathagar, M. B., ten Elshof, J. E., Rothenberg, G. Ion- and Atom-Leaching Mechanisms from Palladium Nanoparticles in Cross-Coupling Reactions. *Chem. Eur. J.* 2007, **13**, 6908.

13 Schmidt, A. F., Kurokhtina, A. A., Distinguishing between the homogeneous and heterogeneous mechanisms of catalysis in the Mizoroki-Heck and Suzuki-Miyaura reactions: Problems and prospects. *Kinet. and Catal.* 2012, **53**, 714.

14 Sigeev, A. S., Peregudov, A. S., Cheprakov, A. V., Beletskaya, I. P. The Palladium Slow-Release Pre-Catalysts and Nanoparticles in the "Phosphine-Free" Mizoroki–Heck and Suzuki–Miyaura Reactions. *Adv. Synth. Catal.* 2015, **357**, 417.

15 Sun, B., Ning, L., Zeng, H. C. Confirmation of Suzuki–Miyaura Cross-Coupling Reaction Mechanism through Synthetic Architecture of Nanocatalysts. *J. Am. Chem. Soc.* 2020, **142**, 13823.

16 Li, B., Zeng, H. C. Minimalization of Metallic Pd Formation in Suzuki Reaction with a Solid-State Organometallic Catalyst. *ACS Appl. Mater.* 2020, **12**, 33827.

17 Shi, W., Niu, Y., Li, S., Zhang, L., Zhang, Y., Botton, G. A., Wan, Y., Zhang, B., Revealing the Structure Evolution of Heterogeneous Pd Catalyst in Suzuki Reaction via the Identical Location Transmission Electron Microscopy. *ACS Nano* 2021, **15**, 8621.

18 Ellis, P. J., Fairlamb, I. J. S., Hackett, S. F. J., Wilson, K., Lee, A. F. Evidence for the Surface-Catalyzed Suzuki–Miyaura Reaction over Palladium Nanoparticles: An Operando XAS Study. *Angew. Chem. Int. Ed.* 2010, **49**, 1820.

19 Costa, P., Sandrin, D., Scaiano, J. C. Real-time fluorescence imaging of a heterogeneously catalysed Suzuki–Miyaura reaction. *Nat. Catal.* 2020, **3**, 427.

20 Cai, X., Liu, Y., Li, G., Hu, W., Liu, X., Chen, M., Ding, W., Zhu, Y. A Functionalized Heterogeneous Catalyst from Atomically Precise Pd1Au8 Clusters Facilitates Carbon–Carbon Bond Construction. *Adv. Mater.* 2023, **35**, 2301466.

21 Lang, R., Du, X., Huang, Y., Jiang, X., Zhang, Q., Guo, Y., Liu, K., Qiao, B., Wang, A., Zhang, T. Single-Atom Catalysts Based on the Metal–Oxide Interaction. *Chem. Rev.* 2020, **120**, 11986.

22 Usteri, M. E., Giannakakis, G., Bugaev, A., Pérez-Ramírez, J., Mitchell, S. Understanding and Controlling Reactivity Patterns of Pd1@C3N4-Catalyzed Suzuki–Miyaura Couplings. *ACS Catal.* 2024, **14**, 12635.

23 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.

24 Zhang, X., Su, R., Li, J., Huang, L., Yang, W., Chingin, K., Balabin, R., Wang, J., Zhang, X., Zhu, W., Huang, K., Feng, S., Chen, H. Efficient catalyst-free N2 fixation by water radical cations under ambient conditions. *Nat. Commun.* 2024, **15**, 1535.

25 Takáts, Z., Wiseman, J. M., Gologan, B., Cooks, R. G. Mass Spectrometry Sampling Under Ambient Conditions with Desorption Electrospray Ionization. *Science* 2004, **306**, 471.

26 Sun, J., Yin, Y., Li, W., Ouyang, J., Na, N. CHEMICAL REACTION MONITORING BY AMBIENT MASS SPECTROMETRY. *Mass Spectrom. Rev.* 2022, **41**, 70.

27 Liu, X., Chen, J., Wei, Z., Yi, H., Lei, A. Deciphering reactive intermediates in electrooxidative coupling of indoles through real-time mass spectrometry. *Chem* 2024, **10**, 2131.

28 Wang, Y., Sun, M., Qiao, J., Ouyang, J., Na, N. FAD roles in glucose catalytic oxidation studied by multiphase flow of extractive electrospray ionization (MF-EESI) mass spectrometry. *Chem. Sci.* 2018, **9**, 594.

29 Lu, H., Yin, Y., Sun, J., Shen, X., Feng, X., Ouyang, J., Na, N. Accelerated plasma degradation of organic pollutants in milliseconds and examinations by mass spectrometry. *Chin. Chem. Lett.* 2021, **32**, 3457.

30 Cheng, H., Yang, T., Edwards, M., Tang, S., Xu, S., Yan, X. Picomole-Scale Transition Metal Electrocatalysis Screening Platform for Discovery of Mild C–C Coupling and C–H Arylation through in Situ Anodically Generated Cationic Pd. *J. Am. Chem. Soc.* 2022, **144**, 1306.

31 Hülsey, M. J., Sun, G., Sautet, P., Yan, N., Observing Single-Atom Catalytic Sites During Reactions with Electrospray Ionization Mass Spectrometry. *Angew. Chem. Int. Ed.* 2021, **60**, 4764.

32 Ge, X., Yin, Y., Wang, X., Gao, Y., Guan, X., Sun, J., Ouyang, J., Na, N. Multienzyme-Like Polyoxometalate-Based Single-Atom Enzymes for Cancer-Specific Therapy Through Acid-Triggered Nontoxicity-to-Toxicity Transition. *Small* 2024, **20**, 2401073.

33 Kuznetsova, L. I., Detusheva, L. G., Fedotov, M. A., Likholobov, V. A. Catalytic properties of heteropoly complexes containing Fe(III) ions in benzene oxidation by hydrogen peroxide. *J. Mol. Catal. A Chem.* 1996, **111**, 81.

34 Liu, C., Li, X., Oxygen-Promoted Suzuki–Miyaura Reaction for Efficient Construction of Biaryls. *Chem. Rec.* 2016, **16**, 84.

35 Liu, C., Liu, C., Li, X. -M., Gao, Z. -M., Jin, Z. -L. Oxygen-promoted Pd/C-catalyzed Suzuki–Miyaura reaction of potassium aryltrifluoroborates. *Chin. Chem. Lett.* 2016, **27**, 631.

36 Ghosh, J., Mendoza, J., Cooks, R. G., Accelerated and Concerted Aza-Michael Addition and SuFEx Reaction in Microdroplets in Unitary and High-Throughput Formats. *Angew. Chem. Int. Ed.* 2022, **61**, e202214090.

37 Miura, Y.-k., Kamiya, Y. Highly Selective Sorption of Small Polar Molecules by a Nonporous Ionic Crystal of a Lacunary Keggin-type Heteropoly Anion and Alkali Metal Cations. *Chem. Lett.* 2012, **41**, 331.

View Article Online
DOI: 10.1039/D5SC04760D

Chemical Science Accepted Manuscript

Data availability

The data that support the findings of this study are available on request from the corresponding author.

Chemical Science Accepted Manuscript