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COMMUNICATION

Recombinant Peptide Fusion Protein-templated Palladium Nanoparticles for Suzuki-Miyaura and Stille Coupling Reactions

Imann Mosleh,[a] Hamid R. Shahsavari,[b,c][‖] Robert Beitle,[a] and M. Hassan Beyzavi*[b]

Abstract: This study examined the use of nanoparticles created with recombinant 45-amino acid long peptides fused to green fluorescent protein (GFPuv) to catalyze twelve representative Suzuki-Miyaura and Stille coupling reactions. A method was developed to prepare freeze-dried powders (Pd@GFP) containing protein and synthesized nanoparticles. Next, coupling reactions were performed in a green solvent without nanoparticle purification. Pd@GFP had high turnover frequencies for the synthesis of model compounds including Tykerb® and could be recycled. This study establishes a potentially cost-effective approach to prepare heterogeneous catalysts containing well defined nanoparticles enabling key C–C bond formation leading to synthetically and pharmaceutically interesting compounds.

Palladium (Pd) is a fundamental element of many industrial processes including H_2 evolution, organic coupling reactions, and CO_2 reduction.[1] Due to its remarkable catalytic performance, operational simplicity, and high reaction selectivity, Pd has been widely used to construct many important molecules applicable in electronic device development, medicine, and biopharmaceutical improvement.[2] However, limited resources and high cost of Pd has encouraged scientists to design a cost-effective route for fabricating Pd-based catalyst.

One of the most exploited routes is to use Pd nanoparticles (Pd NPs). Unique chemical, physical, and mechanical properties of Pd NPs have raised from their high surface area to volume aspect ratio.[3] Various methods have been developed employing dendritic architectures and surface capping ligands.[4] On the other hand, bounding NPs to specific size and shape was achieved using hollow structures, metal-coordination complexes, and organic molecular cages.[5] Unfortunately, these methods were either expensive or unable to eliminate the challenge toward the size-controllable synthesis of NPs with narrow size distributions. Biomimetic 3-D ordered structures utilizing short peptides sequences have been used for NP synthesis to control size, morphology, and structure.[6] However, developing a cost-effective approach towards size-controllable NP synthesis remains unsolved.

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Any proposed method for the effective production of peptides for NP synthesis must recognize the cost-benefit analysis (CBA) of purification. On the one hand, the temptation to assume the requirements for highly purified peptide is common in literature.[3a] We recently demonstrated that even in a highly impure mixture that includes other biological materials, nanoparticle formation proceeds through the expected mechanism of nanoparticle nucleation and growth.[7] A logical next step for the work is to demonstrate that the NPs formed under these conditions could also be used without extensive purification.

In this work, we describe the successful synthesis of Pd NPs using three copies of peptide commonly referred to as Pd4 (TSNAVHPTLRHL) fused to green fluorescent protein (GFPuv). Inhomogeneous crude Escherichia coli lysate containing $(Pd4)_3$-GFPuv fusion protein can offer a remarkable scaffold which would be costly advantageous compared to not only chemically synthesized peptides, but also organic ligand, metal-coordination complexes, and organic cages. Furthermore, the successful synthesis of highly stable and well-dispersed Pd NPs with narrow size distribution using crude lysate containing $(Pd4)_3$-GFPuv fusion protein is described. It is also demonstrated that unpurified material is capable of controlling size, composition, and morphology of NPs. We have also investigated the catalytic activity of $(Pd4)_3$-GFPuv fusion protein-templated Pd NPs (Pd@GFP) in Suzuki-Miyaura and Stille coupling reactions. In addition, the ability of prepared fusion protein-templated Pd NPs towards anticancer drug (Tykerb®) precursor construction was studied. The synthesized NPs also showed high performance in environmentally friendly mixture and their catalytic activities did not suffer significant loss after cycling test.

$(Pd4)_3$-GFPuv fusion protein was constructed using splicing by overlap extension polymerase chain reaction (SOE PCR) technique. Using three steps PCR, $(Pd4)_3$-GFPuv gene was synthesized (Figure 1a). The PCR product of the third step and plasmid were double digested using *EcoRI*-HF® and *NcoI*-HF® restriction enzymes. The final plasmid containing $(Pd4)_3$-GFPuv fusion protein codon was obtained after ligation of digested PCR product and the digested plasmid (Figure 1b). The ligation product was transformed to into *E. coli* cells, and the protein mixture was obtained after the fermentation process.[7]

The NP synthesis process was performed using the protein extract mixture contained approximately 10% $(Pd4)_3$-GFPuv fusion protein (Figure 2a).[7] The TEM image analysis of the Pd@GFP showed spherical and well-uniformed NPs with an average size of 2.4 ± 0.7 nm (Figure 2b and c). Prepared particles using lysate without $(Pd4)_3$-GFPuv fusion protein were neither discrete nor consistent in the absence of Pd4 peptide (Figure S1). It could be argued that Pd4 sequence is required to obtain uniform NPs as agglomeration, non-uniformity, and irregularity of particles occurred due to lack of nucleation site needed to anchor to the NPs in the absence of Pd4 sequence. It is expected that the histidine residues in the structure of Pd4 peptide provide suitable nucleation sites for Pd NPs formation due to the coordination of Pd^{+2} by the peptide. By adding the $NaBH_4$ as a reducing agent,

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Pd NPs were formed by means of coalescence, Ostwald ripening, and oriented attachment (OA).[7] The growth of Pd NPs was also inhibited by histidine residues present in $(Pd4)_3$-GFPuv fusion protein structure resulting in NPs with an average size of 2.4 ± 0.7 nm. $(Pd4)_3$-GFPuv fusion proteins would prevent Pd NPs from agglomeration through wrapping around the NPs and resulted in highly stable Pd NPs.

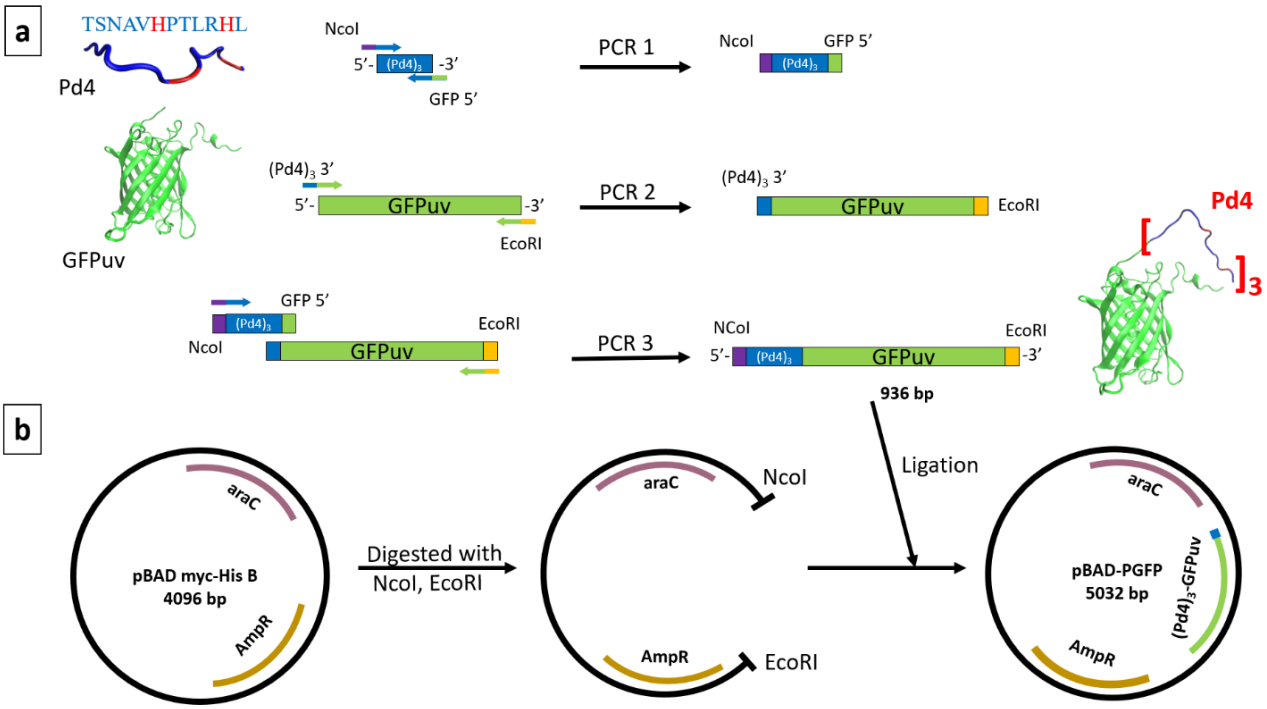

Figure 1. Illustration of cloning using PCR and overview of plasmid construction. A synthetic gene was constructed using SOE PCR.

The formation of Pd NPs was also confirmed by energy-dispersive X-ray spectroscopy (EDX) as shown in Figure 2d. Based on the representative EDX spectrum of the Pd@GFP, Pd peaks were detected at 3.3 and 21.2 keV.

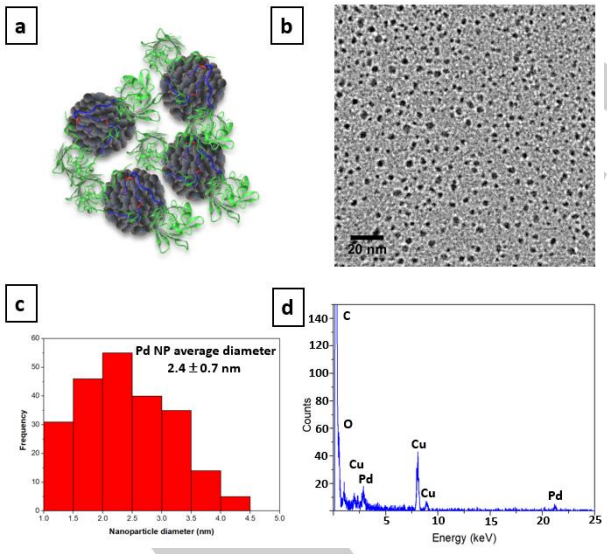

Figure 2. (a) Schematic of Pd@GFP, (b) TEM image of Pd@GFP, (c) size distribution of Pd@GFP, and (d) EDX analysis of Pd@GFP. The detection of the copper in the EDX is because of the carbon-coated copper (CCC) grid used for TEM analysis.

X-ray photoelectron spectroscopy (XPS) confirms the existence of Na, Cl, O, N, Pd, and C elements (Figure S2). Typically, NaCl concentration in LB is estimated to be within the range of 5-10 mg/g. Since neither sonication nor centrifugation would affect salt concentration in the soluble fraction of the lysate, the presence of NaCl is not surprising. Furthermore, the existence of O, N, and C elements is attributed to the organic materials and crude lysate in Pd@GFP structure. As shown in Figure 3, Pd 3d XPS spectrum shows the characteristic peaks of $3d_{3/2,5/2}$ doublets. The peaks at the binding energy (BE) around 341.0 eV (Pd $3d_{3/2}$) and 334.5 eV(Pd $3d_{5/2}$) are attributed to Pd^0 species, and the peaks around BE of 343.7 eV (Pd 3d3/2) and 337.8 eV(Pd $3d_{5/2}$) are related to Pd^{II} species, which probably result from the reoxidation of Pd^0.[8] Therefore, the chemical composition of Pd@GFP as catalyst was confirmed by XPS. The percentage of Pd was determined by inductively coupled plasma mass spectroscopy (ICP-MS) to be 1.2% (Figure S3). SEM analysis of Pd@GFP indicated that Na, Cl, O, and C were also present and accompanied the Pd NPs (Figure S4).

The C–C coupling reactions, Suzuki-Miyaura and Stille, provided information on the catalytic activity of Pd@GFP. Based on optimization (Tables S1-S4), coupling reactions were performed using 0.5 mmol% of Pd (5×10^{-5} eqiv.) and base (3 equiv.) in a green mixture of EtOH/H_2O (1:1, v/v) at 80 °C. Different aryl-iodides and -bromides containing electron-rich and electron-deficient groups reacted satisfactorily with various phenylboronic acids or phenyltin compounds (Table 1) furnished high to excellent yields. It was also found that different substituents such as $–CH_2OH$, –CHO, $–COCH_3$, $–OCF_3$, and –F remained intact during these transformations. Thus, Pd@GFP was determined to be an efficient, inexpensive, and green catalyst for the Suzuki-Miyaura coupling reaction.

In order to extend the potency of our Pd@GFP catalyst, the synthesis of Tykerb® precursor was performed.[9] Precursor synthesis of Tykerb® and an anticancer drug requires the Suzuki-Miyaura reaction to couple 5-formyl-2-furanylboronic acid with

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iodoquinazoline (Scheme 1).[9a] By employing Pd@GFP, the precursor of Tykerb® was obtained in a good yield (73%) which confirmed that the catalyst is a suitable candidate causing no conflict with the functional groups present in the drug precursor (Scheme 1). This achievement can be considered as a remarkable applicability of this protocol for the synthesis of pharmaceutically important complex molecules.

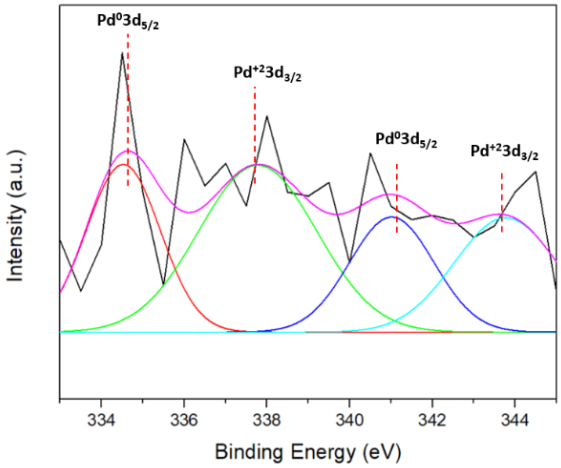
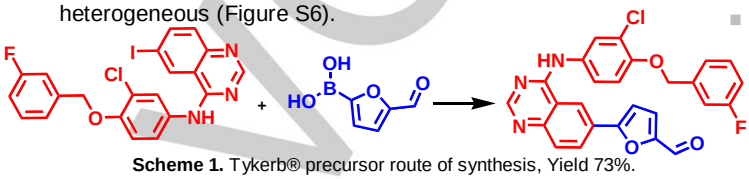


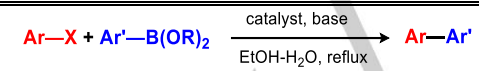
Figure 3. Pd3d XPS spectrum of Pd@GFP catalyst.

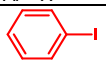
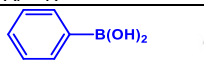
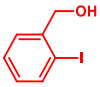
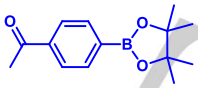
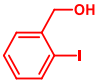
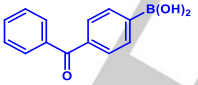
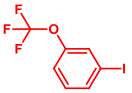
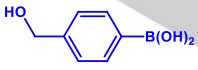
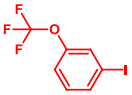
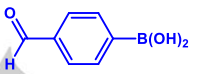
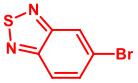
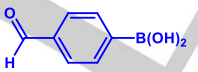
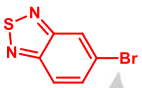
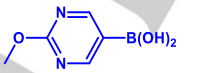
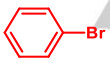
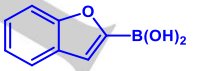
Environmental protection concerns as well as cost-effective approaches for appropriate catalyst used in chemical reactions are of high importance. The reusability of Pd@GFP catalyst for Suzuki-Miyaura and Stille couplings was investigated (Figure S5). The catalyst was reused for five times and the catalytic activity decrease of about 13% and 18% after five cycles was achieved in Suzuki-Miyaura and Stille coupling reactions, respectively (Figure S5). Also, no aggregation or morphological changes were observed in used catalyst after five cycles and the Pd NPs remained spherical and uniformed without any aggregation. The average size of nanoparticles remained almost unchanged (2.2 ± 0.7 nm in Suzuki-Miyaura coupling and 2.1 ± 0.6 nm in Stille coupling) confirming an excellent recyclability (Figure 4). To confirm the nature of the catalyst, the filtrate of the reaction mixture showed no progress indicating that the catalyst is indeed heterogeneous (Figure S6).

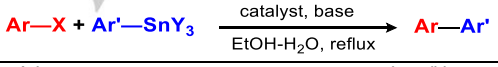


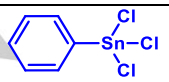
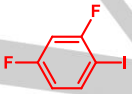
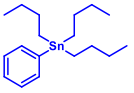
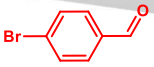
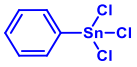
Scheme 1. Tykerb® precursor route of synthesis, Yield 73%.

Table 1. Substrate scope for Suzuki-Miyaura and Stille coupling reactions.[a]



Entry	Ar—X	Ar'—R	Time (h)	Yield [b] (%)	TOF(h^{-1})
1			1.5	97	12,933
2			2	86	8,600
3			6	83	2,766
4			2	88	8,800
5			2.5	94	7,520
6			15	82	1,093
7			16	69	920
8			4	95	4,750



Entry	Ar—X	Ar'—R	Time (h)	Yield [b] (%)	TOF (h^{-1})
9			5.75	96	3,324
10			18	72	800
11			12	86	1,433

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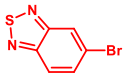
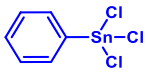
12			40	93	465

[a] Reaction conditions: Aryl halide (0.1 mmol), arylboronic acid (1.2 mmol), K_2CO_3 (0.3 mmol) (entries 1-8), and Aryl halide (0.1 mmol), Phenyltin compound (1.2 mmol), K_3PO_4 (0.3 mmol) (entries 9-12) and Pd NPs (0.005 µmol) were mixed in 1 mL H_2O:EtOH (1:1, v/v) and refluxed under N_2 under 80 °C. [b] Yields were determined by HPLC.

It has been reported that NPs prepared using dendritic architectures and surface capping ligands may show better stability, but their catalytic activity is low.[10] This is attributed to their low surface accessibility while most of the surface is covered by organic supports. Also, employing Pd@GFP preparation, the particle size was dictated by the structure of Pd4 due to the structure of Pd4 peptide fused to GFPuv protein. Regarding to the catalytic activity, Pd@GFP showed slightly higher catalytic activity (TOF = 3.3 × 10^3 h^{-1}) comparing to the Pd NPs prepared using chemically synthesized Pd4 peptides.[3a, 11] This shows that the surface accessibility and catalytic activity of Pd NPs prepared through recombinant approach were not compromised. In other words, a recombinant approach to provide Pd4 or a similar construct appears to be attractive from both a scientific and economic standpoint.

To conclude, a facile aqueous solution phase NP synthesis method was exploited in a heterogeneous biological protein environment. Pd@GFP catalyst was utilized for Suzuki-Miyaura and Stille coupling reactions in green solvents. The catalyst was more active compared to chemically synthesized peptide-templated Pd NPs as exhibits higher catalytic activity with very good stability (5 cycles). Also, Pd@GFP was successfully used for the preparation of an anti-cancer drug (Tykerb®) precursor. Pd@GFP not only extends the application of peptide-templated NPs, but also provides a cost-effective approach to design heterogeneous catalysts. This catalyst resulted in a three order of magnitude decrease in cost due to elimination of purification steps.

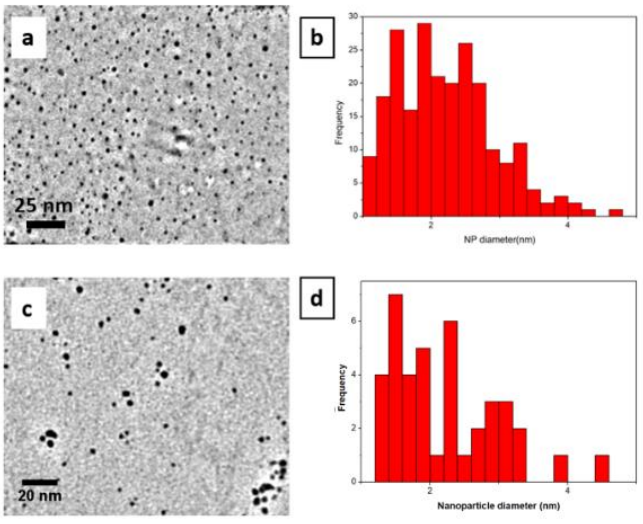


Figure 4. (a) TEM image, and (b) particle size distribution of Pd@GFP catalyst after five cycles for Suzuki-Miyaura coupling. (c) TEM image, and (d) particle size distribution of Pd@GFP catalyst after five cycles for Stille coupling.

Supporting Information (see footnote on the first page of this article):

Full experimental details and characterization data (NMR and Mass spectra) for compounds.

Acknowledgements

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Keywords: Palladium nanoparticles • Peptides • Bio-templated synthesis • Heterogenous catalysis • Coupling reactions.

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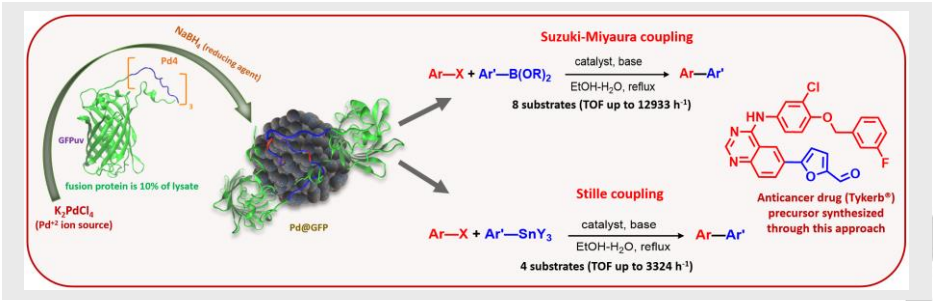

A peptide-templated Palladium nanoparticles are synthesized and utilized for Suzuki-Miyaura and Stille coupling reactions.

*Imann Mosleh, Hamid R. Shahsavari, Robert Beitle, and M. Hassan Beyzavi**

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Recombinant Peptide Fusion Protein-templated Palladium Nanoparticles for Suzuki-Miyaura and Stille Coupling Reactions