Suzuki-Miyaura cross-coupling catalyzed by protein-stabilized palladium nanoparticles under aerobic conditions in water: application to a one-pot chemoenzymatic enantioselective synthesis of chiral biaryl alcohols†

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The preparation of palladium nanoparticles stabilized primarily within the protein cavity of a highly thermostable Dps protein (DNA binding protein from starved cells) and the use of this precatalyst system in Suzuki-Miyaura cross-coupling reactions under aerobic conditions in water is described, as well as a two-step one-pot Suzuki-Miyaura cross-coupling followed by an enantioselective enzyme-catalyzed reduction to form chiral biaryl alcohols.

In recent years, a large number of studies have been devoted by academic and industrial research groups to the development of environmentally friendly processes. In this context, the use of water as a reaction medium in transition metal-catalyzed processes has merited increasing attention and is currently one of the most important targets of sustainable chemistry.[1] Water, an inexpensive, readily available, non flammable, non toxic solvent, provides remarkable advantages over common organic solvents both from an economic and an environmental point of view.[2] Further, the hydrophobic effect[3] often plays a beneficial role when reactions involving water-insoluble substrates are carried out in water due to its high dielectric constant and density. Clearly, performing transition metal-catalyzed reactions in water requires that the key issue of catalyst solubility in this reaction medium be solved. Many studies—several applications of palladium catalysis in particular—have focused on transition metals coordinated to polar phosphine ligands so as to generate water-soluble catalysts,[4-6] on supported palladium precatalysts,[7-9] or on phase transfer conditions.[10] The use of precatalysts containing water molecules as ligands[11] or of hydrophilic CNC-pincer palladium complexes[12] has also been suggested.

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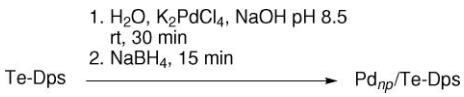
Proteins are very attractive building components for the construction of water-soluble transition metal precatalysts. They have been used to control the coordination structure of palladium complexes,[13] to encapsulate preformed nanomaterials[14] or to spatially limit nanoparticle growth. In this specific case shell-like oligomeric proteins are used that provide a cage able to control the size and shape of the formed nanostructures.[15] Nevertheless, there are only a few reports on the use of protein-nanoparticle precatalysts in organic synthesis. A palladium nanocluster formed in an apoferritin cage used in an elegant size-selective olefin hydrogenation[16] is the only example to the best of our knowledge.

Herein, we report the preparation of palladium nanoparticles stabilized primarily within the Dps protein (**D**NA binding **p**rotein from **s**tarved cells) from the thermophilic bacterium *Thermosynechoccus elongatus* (Te-Dps). Dps proteins belong to the ferritin superfamily and form a smaller cage structure than canonical ferritins (4.5 nm inner diameter as compared to 8 nm). The activity of this precatalyst system has been verified in the Suzuki-Miyaura cross-coupling reaction, one of the most powerful and convenient synthetic protocols for the formation of biaryls. We have also used this precatalyst system in a two-step one-pot Suzuki-Miyaura cross-coupling followed by an enantioselective enzyme-catalyzed reduction to form chiral biaryl alcohols.

Te-Dps stabilized palladium nanoparticles (Pd_{np}/Te-Dps) were prepared as a clear brown solution by exposure of K_2PdCl_4 to Te-Dps at room temperature and reduction with $NaBH_4$ (Scheme 1).



Scheme 1 Synthesis of Pd_{np}/Te-Dps.

The UV/Vis spectrum of the solution changed significantly after reduction, with the appearance of a broad intense absorption between 300-500 nm (Figure 1A). A similar spectroscopic change was observed previously for the reduction of Pd(II) species in the presence of apoferritin[16] and in dendrimers[17] due to the formation of zero-valent palladium clusters. Control experiments revealed that a black precipitate was formed when the reduction of K_2PdCl_4 was carried out under the same conditions in the absence of Te-Dps. Taken together, these results suggest that soluble protein-stabilized palladium species

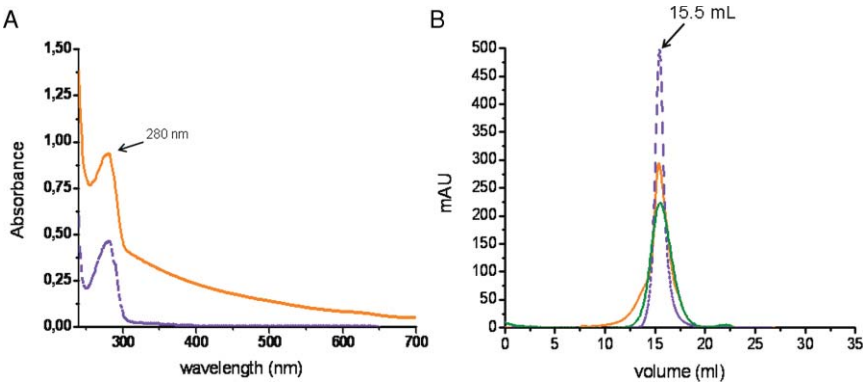


Fig. 1 (A) UV-Vis spectra of Te-Dps (dashed violet line) and Pd_{np}/Te-Dps (solid orange line). (B) Gel-filtration chromatogram of Te-Dps (280 nm, dashed violet line) and Pd_{np}/Te-Dps before and after heating at 100 °C for 24 hrs (405 nm, before heating, orange solid line: after heating, green solid line).

are formed by reducing K_2PdCl_4 in the presence of Te-Dps and presumably of other Dps proteins.

The protein-stabilized palladium species obtained according to Scheme 1 was analyzed by size-exclusion chromatography. The elution profile monitored at 280 nm (protein) and 405 nm (zero-valent palladium cluster) indicates clearly that the majority of the material has a composite nature since the largest part of the metal co-elutes with the protein. There are, however, small shoulders before and after the main peak indicative of composite material characterized by slightly smaller and bigger size (Figure 1B). Transmission electron microscopy (TEM, Figure 2A) images of the preparation indeed showed that Pd is mainly in the form of protein-enclosed, roughly spherical nanoparticles with a mean diameter of 3.5 ± 0.5 nm (Figure 2B) and that part of the metal is on the surface of the protein. Ueno *et al.*[16] obtained a similar pattern of Pd nanoclusters in the presence of apoferritin.

The catalytic properties of water-soluble Pd_{np}/Te-Dps were evaluated initially in the reaction of 4-iodobenzoic acid with 2-tolylboronic acid at 80 °C for 24 h using a precatalyst loading down to 0.05 mol% in the presence of a $NaHCO_3$/NaOH buffer at pH 10 in water. Under these conditions, the cross-coupling product **3a** was isolated in 56% yield. Thereafter we explored the influence of buffer and pH on the reaction outcome. Some results of these optimization studies are summarized in Table 1. CAPS (*N*-cyclohexyl-3-aminopropanesulfonic acid) at pH 10 (entry 7) and $NaHCO_3$/NaOH or CAPS at pH 11 (entries 9 and 10) gave similar results. **3a** was isolated in satisfactory yield with CAPS at pH 10.5 (entry 8). However, the highest yield was obtained when the reaction was carried out using Tris [tris(hydroxymethyl)aminomethane] at pH 8.5-9.0 (entries 4 and 5): the desired diaryl derivative **3a** was isolated in 90% yield (entries 4 and 5). Further experiments on the influence of pH employing Tris showed that the yield decreases with decreasing pH (entries 1-3). Importantly, the gel filtration profile of Pd_{np}/Te-Dps after exposure to 100 °C for 24 h is unaltered (Fig. 1B) due to the use of a highly thermostable protein.

The reuse of the aqueous solution containing the catalyst system was attempted. Compound **3a** was isolated in 81% yield in the second run but only in trace amounts in the third run. On this basis, the actual catalyst is likely a palladium species that is removed from the protein and is lost in the work-up (extraction with dichloromethane).

The reaction of 4-iodobenzoic acid with 2-tolylboronic acid was also performed with a palladium loading down to 0.001 mol% (Tris pH 8.9, 100 °C, 48 h). Under these conditions **3a** was isolated in 89% yield and the turn-over number (TON) was 22250.

Using the optimized conditions, we next explored the scope and generality of the process. The preparative results are

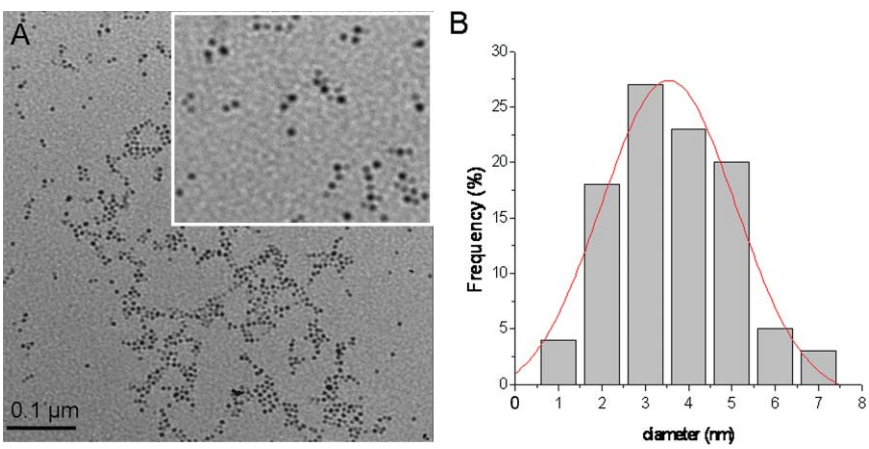
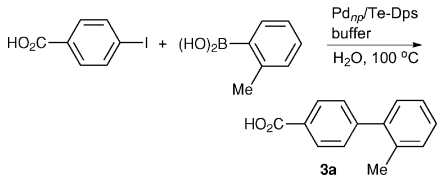


Fig. 2 (A) TEM image of Pd_{np}/Te-Dps and 2× magnification in the inset. (B) Particle size distribution histogram of Pd_{np}/Te-Dps (particle size 3.5 ± 0.5 nm).

Table 1 The influence of buffers and pH on the Suzuki-Miyaura cross-coupling of 4-iodobenzoic acid and 2-tolylboronic acid catalyzed by Pd_{np}/Te-Dps[a]



Entry	Buffer	pH	Yield% of **3a**[b]
1	Tris	7.0	80
2	Tris	7.5	84
3	Tris	8.0	87
4	Tris	8.5	90
5	Tris	8.9	90
6	$NaHCO_3$/NaOH	10	50
7	CAPS	10	60
8	CAPS	10.5	78
9	$NaHCO_3$/NaOH	11	52
10	CAPS	11	47

[a] Reactions were carried out using 0.25 mmol of 4-iodobenzoic acid, 0.25 mmol of 2-tolylboronic acid, 2 mL of buffer (100 mM) at 100 °C in the presence of 0.05 mol% of Pd_{np}/Te-Dps for 24 h. [b] Yields are given for isolated products.

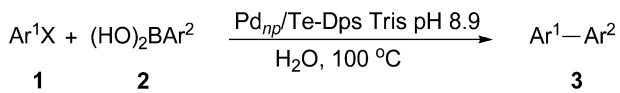
summarized in Table 2. Cross-coupling products were isolated in good to excellent yields with a variety of electron-rich and electron-poor aromatic rings both in the arylboronic acids and in the aryl iodides or bromides. Ortho substituents are also tolerated (entries 1, 3, 5, 13, 14). The reaction gave the desired product in moderate yield only with 4-bromoanisole (entry 15). Trace amounts of the coupling product were formed in the reaction of 3-(trifluoromethyl)phenyl chloride with 4-acetylphenylboronic acid (entry 20).

The positive results obtained with Pd_{np}/Te-Dps in the Suzuki-Miyaura cross-coupling reaction under aerobic conditions in water prompted us to investigate the combination of this palladium-catalyzed process with a biotransformation in a one-pot process.

A recent remarkable example of a palladium-catalyzed process combined with a biotransformation is due to Gröger *et al.*[18] who described a one-pot Suzuki-Miyaura cross-coupling followed by an enzyme-catalyzed reduction using water as the common reaction medium and 2 mol% of $PdCl_2(PPh)_3$ in the presence of 10 mol% of PPh_3 as the precatalyst system in the first step. Phosphines, however, are air-sensitive and utilization of phosphine-free conditions would be highly desirable.

Water-soluble Pd_{np}/Te-Dps appeared ideal for this type of transformation. In fact, its use proved to be successful in the palladium-catalyzed Suzuki-Miyaura cross-coupling followed by an asymmetric enzymatic reduction catalyzed by alcohol dehydrogenase from *Lactobacillus brevis*. Compounds **4a** and **4b** were isolated in high to excellent yields and in an enantiomeric excess higher than 99% (Scheme 2, see also ESI†). The two-step one-pot process was carried out under aerobic conditions in water by performing the first step (the Suzuki-Miyaura cross-coupling) under standard conditions. In the second step (the enzyme-catalyzed reduction), after cooling the reaction mixture to room temperature, *i*-PrOH, alcohol dehydrogenase [(*R*)-LB-ADH], and $NADP^+$ were added and the resulting reaction mixture was stirred at room temperature for 24 h. *In situ* conversion of $NADP^+$ to NADPH, the required reduced form, is catalyzed by alcohol dehydrogenase with *i*-PrOH acting as the hydrogen transfer agent. Excess *i*-PrOH makes the entire process irreversible by shifting the equilibrium towards the direction of the desired product.

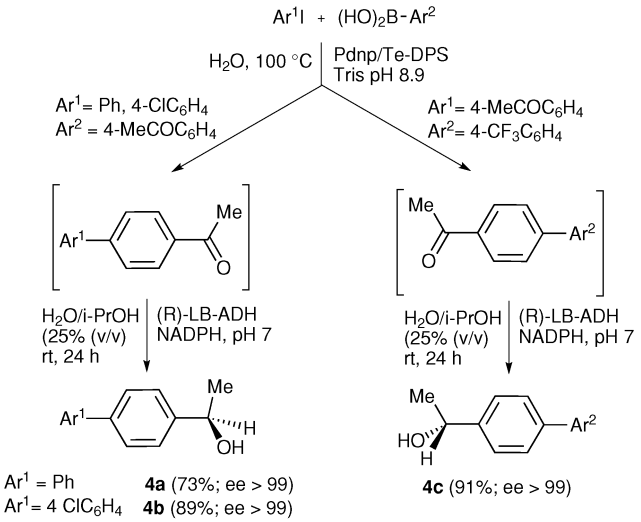
Table 2 Reaction of aryl halides with arylboronic acids catalyzed by Pd_{np}/Te-Dps[a]



1 **2** **3**

Entry	Ar^1X **1**	$Ar^2B(OH)_2$ **2**	Time (h)	Yield% of **3**[b]	
1	$4\text{-}HO_2CC_6H_4I$	$2\text{-}MeC_6H_4B(OH)_2$	24	90	**3a**
2	$4\text{-}MeOC_6H_4I$	$4\text{-}CF_3C_6H_4B(OH)_2$	48	87	**3b**
3	$4\text{-}CNC_6H_4I$	$2\text{-}MeC_6H_4B(OH)_2$	24	77	**3c**
4	$3\text{-}CF_3C_6H_4I$	$4\text{-}MeC_6H_4B(OH)_2$	24	80	**3d**
5	$3\text{-}CF_3C_6H_4Br$	$4\text{-}MeC_6H_4B(OH)_2$	48	61	**3d**
6	$4\text{-}NO_2C_6H_4I$	$2\text{-}MeC_6H_4B(OH)_2$	24	70	**3e**
7	$2\text{-}NO_2,4\text{-}MeC_6H_4I$	$4\text{-}MeCOC_6H_4B(OH)_2$	24	72	**3f**
8	$4\text{-}HO_2CC_6H_4I$	$4\text{-}MeOC_6H_4B(OH)_2$	24	92	**3g**
9	$4\text{-}ClC_6H_4B(OH)_2$		24	83	**3h**
10	$4\text{-}HO_2CC_6H_4Br$	$4\text{-}MeOC_6H_4B(OH)_2$	48	71	**3g**
11	$4\text{-}HO_2CC_6H_4Br$	$C_6H_5B(OH)_2$	48	83	**3i**
12	$4\text{-}ClC_6H_4I$	$4\text{-}MeCOC_6H_4B(OH)_2$	24	89	**3l**
13	$4\text{-}MeCOC_6H_4Br$	$4\text{-}CF_3C_6H_4B(OH)_2$	48	63	**3m**
14	$4\text{-}MeCOC_6H_4Br$	$4\text{-}MeOC_6H_4B(OH)_2$	48	55	**3n**
15	$4\text{-}MeOC_6H_4Br$	$4\text{-}CF_3C_6H_4B(OH)_2$	48	45	**3b**
16	$4\text{-}NO_2C_6H_4I$	$4\text{-}MeCOC_6H_4B(OH)_2$	5	90	**3o**
17	$2\text{-}HO_2CC_6H_4I$	$4\text{-}MeCOC_6H_4B(OH)_2$	24	87	**3p**
18	$4\text{-}MeOC_6H_4I$	$2\text{-}MeC_6H_4B(OH)_2$	24	65	**3q**
19	PhI	$4\text{-}MeCOC_6H_4B(OH)_2$	24	91	**3r**
20	$3\text{-}CF_3C_6H_4Cl$	$4\text{-}MeCOC_6H_4B(OH)_2$	48	traces	**3r**

[a] Reactions were carried out using 0.25 mmol of aryl halide, 0.25 mmol of arylboronic acid, 2 mL of Tris (pH 8.9, 100 mM) at 100 °C in the presence of 0.05 mol% of Pd_{np}/Te-Dps. [b] Yields are given for isolated products.



Scheme 2 One-pot chemoenzymatic synthesis of chiral biaryl alcohols.

In conclusion, we have shown that palladium nanoparticles stabilized within the cage of a Dps protein from a thermophilic protein (Pd_{np}/Te-Dps) can be used successfully in the Suzuki-Miyaura cross-coupling reaction under aerobic phosphine-free conditions in water. In addition, Pd_{np}/Te-Dps and enzyme catalysis can be efficiently coupled in a two-step one-pot chemoenzymatic approach to form chiral biaryl alcohols using water as the common reaction medium. The process is simple and fast, affords the desired products in high to excellent isolated yields with excellent enantiomeric excess.

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