Colorimetric Assays

DOI: 10.1002/anie.201100378

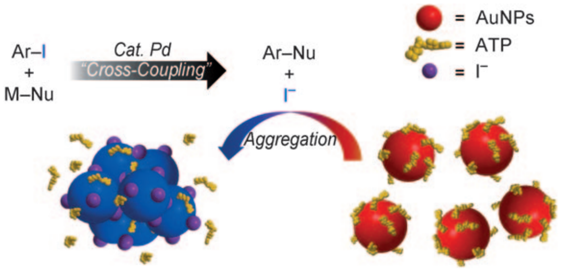
A Colorimetric High-Throughput Screening Method for Palladium-Catalyzed Coupling Reactions of Aryl Iodides Using a Gold Nanoparticle-Based Iodide-Selective Probe**

Eunhye Jung, Sudeok Kim, Yong Kim, Seong Hyeok Seo, Soo Suk Lee, Min Su Han, and Sunwoo Lee**

Palladium-catalyzed cross-coupling reactions are among the most important methods in organic synthesis, the pharmaceutical industry, and materials science.[1] Recently, newly developed catalytic systems have been reported for use in coupling reactions, such as Suzuki, Stille, Heck, Sonogashira, Hiyama, α arylation and Buchwald–Hartwig amination.[2] Developing an efficient catalytic system requires costly and time-consuming trial and error and consideration of numerous variables, including catalyst, ligand, base, additive, solvent, stoichiometry, and temperature. To address this problem, we employed high-throughput screening (HTS) assays, which were originally developed to accelerate the discovery process in the pharmaceutical industry.[3] The major advantage of HTS is its more rapid monitoring of chemical transformations. Gas chromatography (GC),[4] high-performance liquid chromatography (HPLC),[5] mass spectrometry (MS),[6] infrared spectroscopy (IR),[7] colorimetry,[8] and fluorescence spectroscopy[9] are currently employed as analytical methods. Recently, Hartwig, Taran, and Rozhkov independently reported fluorescent screening methods for palladium-catalyzed C–C bond formation.[10] However, these methods have the disadvantage of allowing HTS of variable parameters within the target reaction only, so that the target materials have to be synthesized for the screening of the coupling reactions to be viable. Therefore, the development of more general and simple methods to apply to all types of coupling reactions is desired.

Gold nanoparticles (AuNPs) have been widely used as chromogenic components in colorimetric sensing systems owing to their high extinction coefficients and distance-dependent optical properties. As a result, many AuNP-based colorimetric sensors have been developed to detect metal ions, proteins, DNA, small molecules, and biocatalysts.[11] However, to the best of our knowledge, colorimetric systems using AuNPs have not been developed for high-throughput screening of chemical reactions, such as palladium-catalyzed cross-coupling reactions.

Recently, colorimetric sensing systems employing adenosine triphosphate (ATP)-stabilized AuNPs (sAuNPs) have been developed to detect biocatalysts, metal ions, and anions.[12] Unlike citric acid capped AuNPs, the sAuNPs are stable over a wide pH range, even in high salt concentrations, but quickly aggregate when exposed to soft metal ligands, such as thiol and iodide.[12,13] In palladium-catalyzed cross-coupling reactions, aryl iodide is the most reactive substrate, and its iodide ion is released after completion of the coupling. With this information, we speculated that sAuNPs could be used to detect released iodide by means of a colorimetric sensing system. This method would allow a more general and faster screening in palladium-catalyzed coupling reactions (Scheme 1).



Scheme 1. Sensing of iodide in palladium-catalyzed cross-coupling reactions by using sAuNPs. ATP = adenosine triphosphate.

First, we evaluated whether sAuNPs would selectively detect iodide ions and if we could accurately assess the concentration of iodide by measuring UV absorbance. A stock solution of iodide ions was added to an assay solution containing sAuNPs (3 nM) in a pH 7.0 buffer solution (10 mM

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[*] E. Jung,[+] Y. Kim, Prof. Dr. S. Lee
Department of Chemistry and Institute of Basic Science
Chonnam National University
Gwangju, 500-757 (Republic of Korea)
Fax: (+82) 62-530-3389
E-mail: sunwoo@chonnam.ac.kr

S. Kim,[+] S. H. Seo, Prof. Dr. M. S. Han
Department of Chemistry, Chung-Ang University
Seoul 156-756 (Republic of Korea)
Fax: (+82) 2-825-4736
E-mail: mshan@cau.ac.kr

Dr. S. S. Lee
Bio & Health Group, Emerging Center
Samsung Advanced Institute of Technology
Yongin-si, Gyeonggi-do, 446-712 (Republic of Korea)

[+] These authors contributed equally to this work.

[**] This work was supported by a National Research Foundation of Korea Grant funded by the Korean Government (2010-0002350 & 2009-0072357), POSCO, and the Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093817).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201100378.

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sodium phosphate buffer (SPB) + 0.1 M NaCl), so that the final concentration was between 0 and 5 μM. The changes in the absorbance of the assay mixtures were recorded at 520 nm after adding the iodide ions. The observed absorbance intensity at 520 nm decreased almost in proportion to the decreasing iodide concentrations (See Figure S2 in the Supporting Information). Furthermore, the sAuNPs showed high selectivity for iodide over various other anions (Figure 1 a), and the changes in absorbance caused by the addition of iodide ions were almost unaffected by the presence of other anions (Figure 1 b). This means that the sAuNPs can selectively detect iodide produced in a palladium-catalyzed cross-coupling reaction despite the presence of various anions from additive materials, such as NaOAc, K_2CO_3, CsF, and K_3PO_4. Moreover, we found that sAuNPs afforded the blue solution in the presence of iodide (Figure 1 c). Therefore, the palladium-catalyzed cross-coupling reaction can be evaluated simply by detecting the concentration of iodide ions.

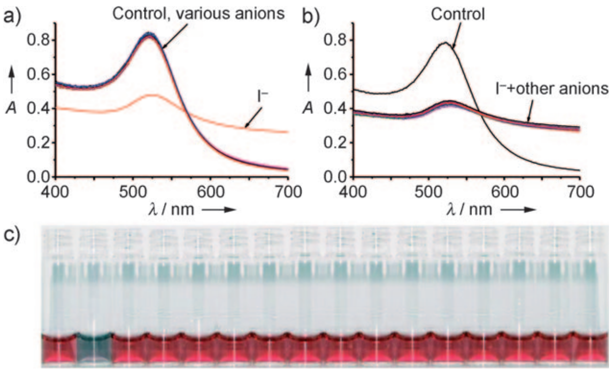
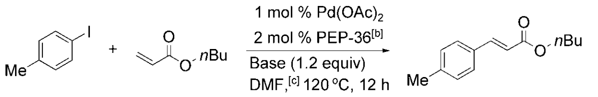


Figure 1. a) UV/Vis spectra obtained after the addition of various anions (5 μM) to a buffer solution (pH 7.0, 10 mM SPB + 0.1 M NaCl) containing sAuNPs (3 nM). b) UV/Vis spectra obtained by the addition of I^- (5 μM) to a buffer solution (pH 7.0, 10 mM SPB + 0.1 M NaCl) containing sAuNPs (3 nM) and other anions (25 μM). c) The color of the solution in the absence and presence of anions (5 μM, from left to right): no anion, I^-, F^-, Cl^-, Br^-, CH_3COO^-, ClO_4^-, NO_3^-, CN^-, N_3^-, SO_4^{2-}, HSO_3^-, HSO_4^-, $P_2O_7^{4-}$, HPO_4^{2-}, and HCO_3^-.

With the above data, we investigated the correlation of absorbance with the conversion extent of aryl iodide in the palladium-catalyzed coupling reaction. To do this, the Mizoroki–Heck coupling reaction of iodobenzene and *n*-butyl acrylate was chosen as a model reaction and the reactions were carried out with a variety of bases.[14] All of the samples were taken from the reaction mixture and then diluted to 5 μM. After treatment with sAuNPs, the absorbance was recorded at 10 min.[15] The absorbance was then used to calculate the conversion extent by means of a linear plot correlating absorbance to mole fraction of iodide.[16] As shown in Table 1, the absorbance decreased as the conversion extent increased. All conversion extents obtained from the absorbance showed a high correlation with those from GC with an internal standard material.

We also found that the color of the solution changed from red to blue as the conversion extent increased, as shown in Figure 2. This allowed us to apply this method in the colorimetric assay of the cross-coupling reactions.

Table 1: The correlation of conversion extents by GC and UV in the Heck reactions.[a]



Entry	Base	A[g]	Conv (UV) [%][h]	Conv (GC) [%][i]	Yield [%][g]
1	–	0.789413	1	0	0
2	DBN[d]	0.758287	8	5	4
3	NaOAc[e]	0.693869	21	21	18
4	Pyridine	0.653862	30	37	37
5	NaOAc[f]	0.548959	52	50	56
6	NaOAc	0.465500	69	70	65
7	K_2CO_3	0.317895	100	100	98

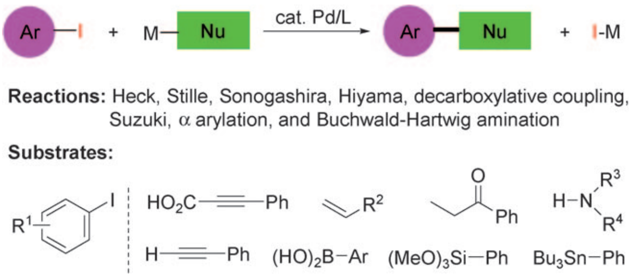
[a] Reaction conditions: 4-iodotoluene (0.3 mmol) and *n*-butyl acrylate (0.36 mmol). [b] PEP-36 = bis(2,6-di-*tert*-butyl-4-methylphenyl)pentaerythritol diphosphite. [c] DMF = dimethylformamide. [d] DBN = 1,5-diazabicyclo[4.3.0]non-5-ene. [e] Reaction time = 1 h. [f] Reaction time = 7 h. [g] Absorbance intensity. [h] Determined by UV absorbance. [i] Determined by GC with an internal standard material.

Figure 2. The color of the solutions corresponding to entries 1–7 of Table 1 (from left to right).

The success of the Mizoroki–Heck reaction allowed us to expand this assay for HTS of various palladium-catalyzed cross-coupling reactions without the need to synthesize specific target materials. We evaluated this assay in the coupling reaction of aryl iodide with eight kinds of palladium-catalyzed reactions: Heck,[14] Stille,[17] Sonogashira,[18] Hiyama,[19] Suzuki,[20] α-arylation,[21] Buchwald–Hartwig amination,[22] and decarboxylative couplings.[23] The scope is shown in Scheme 2.

After complete reaction, each sample was diluted to 5 μM using H_2O and transferred to a 96-well plate, and using a multichannel pipette, a sAuNP solution was added.[25] The resulting plate was left to stand for 2 min and then analyzed on a microplate reader at 520 nm absorption. The correlation equation obtained from the six standard samples was used to convert the absorbance values to conversion extents (Supporting Information, Figure S3, red line[26]). All reaction results are shown in Figure 3 and Figure 4.

Several features were readily apparent from our results. First, all of the conversion extents were obtained in a few minutes using a microplate reader, and they compared favorably with those from GC analysis for 90 representative samples from the crude reaction mixtures. The response time



Scheme 2. Scope of the palladium-catalyzed cross-coupling reactions of aryl iodides for high-throughput screening.[24]

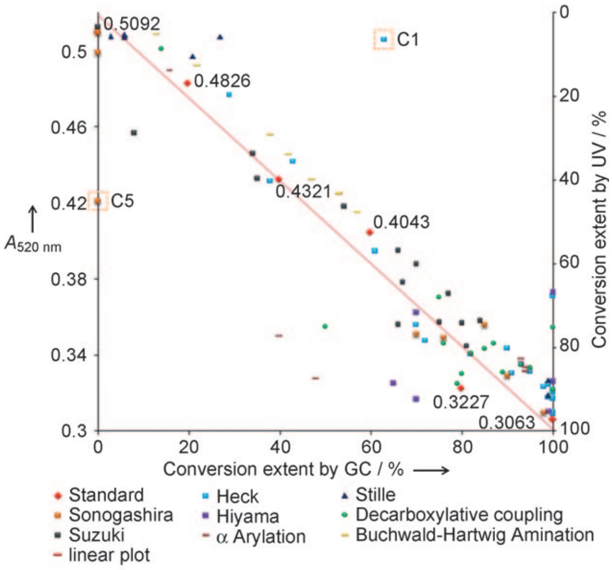


Figure 3. The correlation between GC and UV spectroscopy for the extent of conversion. Values for the standard (absorbance, red diamonds) are shown.

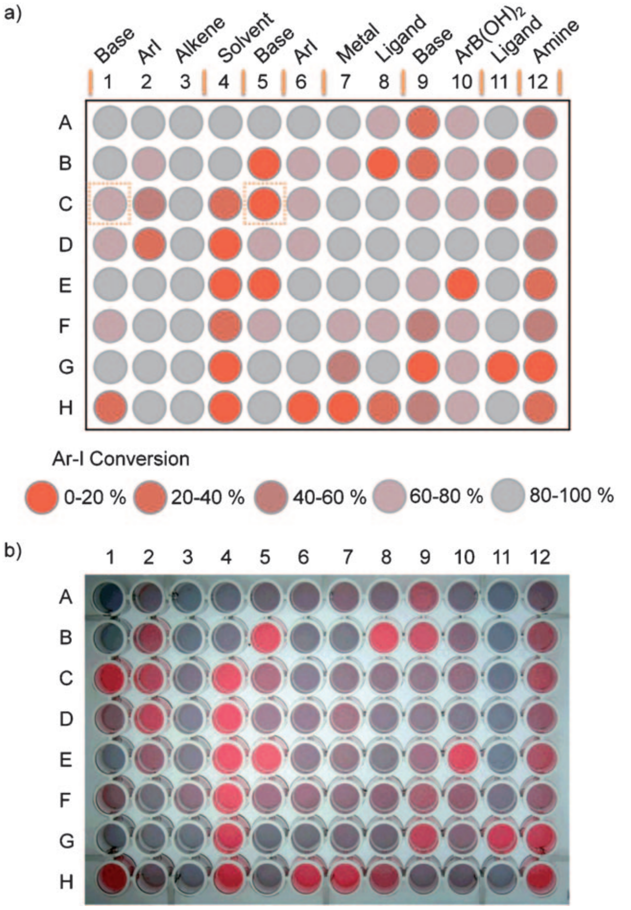


Figure 4. Colorimetric assay for palladium-catalyzed coupling reactions. a) The expected colors drawn from the conversion extents of the GC analysis. b) The detected colors. 1–3 Heck, 4 Stille, 5 Sonogashira, 6 Hiyama, 7,8 decarboxylative coupling, 9,10 Suzuki, 11 α arylation of ketones, 12 Buchwald–Hartwig amination.

for the change in absorbance and color decreased from 10 min to 2 min owing to the small scale of the assay.

As shown in Figure 3, most of conversion extents in this assay showed high correlation with those from GC, with errors in the range of ±10%. Furthermore, the color change gives information about the range of conversion extents (Figure 4). We compared the detected colors of this assay with the expected colors obtained from the conversion extents of the GC analysis (given as five colors corresponding to five conversion ranges).

A variety of aryl iodides and nucleophiles were screened, and all showed reliable conversion extents, regardless of whether they had an electron-donating or electron-withdrawing group. Notably, this assay does not suffer interference from stoichimetric amounts of metals present in nucleophiles, such as tin (Stille), silane (Hiyama), and boron (Suzuki), even though they could produce complexes with iodide ions in the reaction mixture (Figure 4, columns 4, 6, 9, and 10). Moreover, this assay showed reliable conversion extents in the presence of 5 mol% CuI (Sonogashira, column 5). It is well known that finding a suitable palladium source and ligand is the most important factor in cross-coupling reactions. This assay could be readily applied to screening palladium sources and ligands (columns 7, 8, and 11). Most coupling reactions require a base or an activator, so we screened a variety of bases, including organic, inorganic, strong, and weak bases. Most bases showed a high correlation between the assay data and GC data even though competitive anions were present in the reaction mixtures. However, correlations were not found when either Ag_2CO_3 or tetrabutylammonium iodide (TBAI) was employed as a base or an additive (row C, columns 1 and 5,: see orange dotted boxes in Figures 3 and 4a). In the case of Ag_2CO_3 (C1), the absorbance data showed lower conversion than the GC conversion because the silver cation reacts with the iodide ion rather than the carbonate ion to form a precipitate. In the case of TBAI (C5),[27] the iodide ion reacted with sAuNPs to decrease the absorbance. All of the organic solvents tested showed high correlation regardless of the solubility of the iodide ion in the various solvents. Reliable data were obtained when this assay was conducted two further times.[28]

In conclusion, we have developed a gold nanoparticle based assay for sensing iodide ions and demonstrated the usefulness of this method as a general and simple way for

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screening palladium-catalyzed cross-coupling of aryl iodides. One of the most important features of this assay is that it does not require the synthesis of a specific sensing substrate to screen each target coupling reaction. Furthermore, this assay showed a high tolerance of the functional groups of substrates, metals in nucleophiles, bases, solvents, and even anions of salts. We obtained information on conversion extents from the absorbance and the change in color under very dilute conditions. Moreover, this assay allows the conversion extents of 96 samples to be recorded in a few minutes and at concentrations of less than 5 μM. Although the assay presented herein is restricted to obtaining the extent of conversion, we believe that its efficiency and versatility will be of great help in finding optimal conditions for any substituted reactions of organic iodides.

Received: January 16, 2011
Published online: April 11, 2011

Keywords: cross-coupling · gold · high-throughput screening · palladium · sensors

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[15] The recorded sample was a 1.0 mL solution.

[16] Conversion extent [%] = 21.8 × [(absorbance intensity−0.84321)/−0.10417]−9.9.

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[24] For further information regarding palladium species, ligands L, bases, solvents, and reaction conditions, see the Supporting Information.

[25] The recorded samples were 0.2 mL solutions.

[26] Linear plot: Absorbance = -0.00217 (conversion extent [%]) + 0.51825.

[27] The absorbance before the reaction is 0.4278 and after the reaction is 0.4210.

[28] Data from two additional experimental runs are shown in the Supporting Information, Tables S15 and S16.

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