Will We Witness Enzymatic or Pd-(Oligo)peptide Catalysis in Suzuki Cross-Coupling Reactions?

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ABSTRACT

Despite the development of numerous advanced ligands for Pd-catalyzed Suzuki cross-coupling reactions, the potential of (oligo)peptides serving as ligands has remained largely unexplored. This study demonstrates, via DFT modeling, that (oligo)peptide ligands can exhibit superior activity compared to classic phosphines in these reactions. The utilization of natural amino acids such as Met, SeMet, and His offers strong binding of the Pd center, thereby ensuring substantial stability of the system. The increasing sustainability and economic viability of (oligo)peptide synthesis open new prospects for applying Pd-(oligo)peptide systems as greener catalysts. The feasibility of *de novo* engineering an artificial Pd-based enzyme for Suzuki cross-coupling is discussed, laying the groundwork for future innovations in catalytic systems.

KEYWORDS

Cross-coupling, peptides, Suzuki coupling, DFT modeling, Pd catalysts, artificial enzymes

Main Text

The recent decades have seen remarkable success in Pd-catalyzed cross-coupling reactions. These reactions have become a cornerstone of academic research on organometallic catalysis and are widely used in pharmaceutical, agrochemical, and fine organic synthesis.[1–3] Additionally, they laid the groundwork for advanced 3d metal catalysis of cross-coupling reactions.[4] However,

despite these advancements, Pd-catalyzed cross-coupling reactions still exhibit several unwanted features, among which non-sustainability is the most discussed.

Although Pd itself is frequently blamed for the non-sustainability of Pd catalytic systems[5] (e.g., some experts put Pd among "unsustainable" metals[6]), this is not the sole issue. Pd loading of classic [(PPh_3)$PdCl_2$] in active systems can be as low as 25 ppm.[7] Non-negligible economic and sustainability challenges arise from using expensive ligands obtained through multistep non-atom-economic synthesis. The non-sustainability of standard phosphine and N-heterocyclic ligands is a critical aspect, as it impacts the sustainability of 3d metal catalysts that often depend on these advanced stabilizing ligands (see examples in Ref. [4]). Moreover, the use of non-green solvents[8] in these reactions is another concern.

Utilizing (oligo)peptide ligands in an aqueous medium may significantly enhance the sustainability of Pd catalytic systems for cross-coupling. This approach addresses the issue of ligand toxicity, as peptides readily degrade under environmental factors. Peptide synthesis is scalable, and its cost is decreasing due to advancements in synthetic methods.[9,10] Moreover, the development of greener methodologies for peptide synthesis is ongoing.[11] Finally, peptides offer vast possibilities for designing the coordination shell, providing a versatile tool to customize catalyst activity for a specific substrate.

De novo design of artificial organo-inorganic Pd-based enzymes will be an even more significant advancement. Conventional biotechnological methods for catalytic antibody engineering[12] are not applicable to cross-coupling reactions due to the inability to propose a transition-state analog molecule. Although metalloprotein design actively evolves,[13] and there is even an example of Ir incorporation in *E. coli* resulting in unnatural terpenoid synthesis,[14] currently known artificial metalloenzymes are based on cofactors containing biometals (see, e.g.,

[15,16]) rather than direct metal binding to amino acid side chains. Despite concerns about toxicity, some transition metal catalysts, like those used in CuAAC, have shown biocompatibility.[17] Regarding functional group tolerance, Suzuki coupling stands out, suggesting the potential compatibility of such cross-coupling with, albeit simple, biomolecular systems. All this clearly shows that finding peptide or protein ligand environments for cross-coupling reactions can be a genuine breakthrough.

This pursuit, however, presents a series of challenges, some of the most significant of which are addressed in this work. We explore the potential for stable coordination of Pd within a peptide environment that provides superior catalytic activity and discuss the impact of various reaction conditions.

Figure 1 illustrates the modeled Suzuki coupling mechanism, encompassing well-proven elementary steps: oxidative addition (OA, **1⟶2**, **TS1**), transmetalation (TM, **4⟶5**, **TS2**), and reductive elimination (RE, **5⟶1** (**P**), **TS3**). We examined N-acylated methylamides of naturally occurring amino acids (AA) such as histidine (His), glutamine (Gln), methionine (Met), and selenomethionine (SeMet) as ligands to model a concept active center where Pd is integrated into an oligopeptide/protein environment (Figure 1a, top). The activity of Pd centers with AA ligands was compared to those with traditional phosphine ligands, including L = PMe_3 and PPh_3. We evaluated two classic Ph-Ph and Ph-Vinyl(Vin) Suzuki-type couplings of PhBr with common boronic esters. We also estimated the thermodynamic effect of cis-trans isomerization of intermediates **2**, **4**, and **6** on activity and considered ligand dissociation from these intermediates to evaluate the stability of the proposed active center (see below).

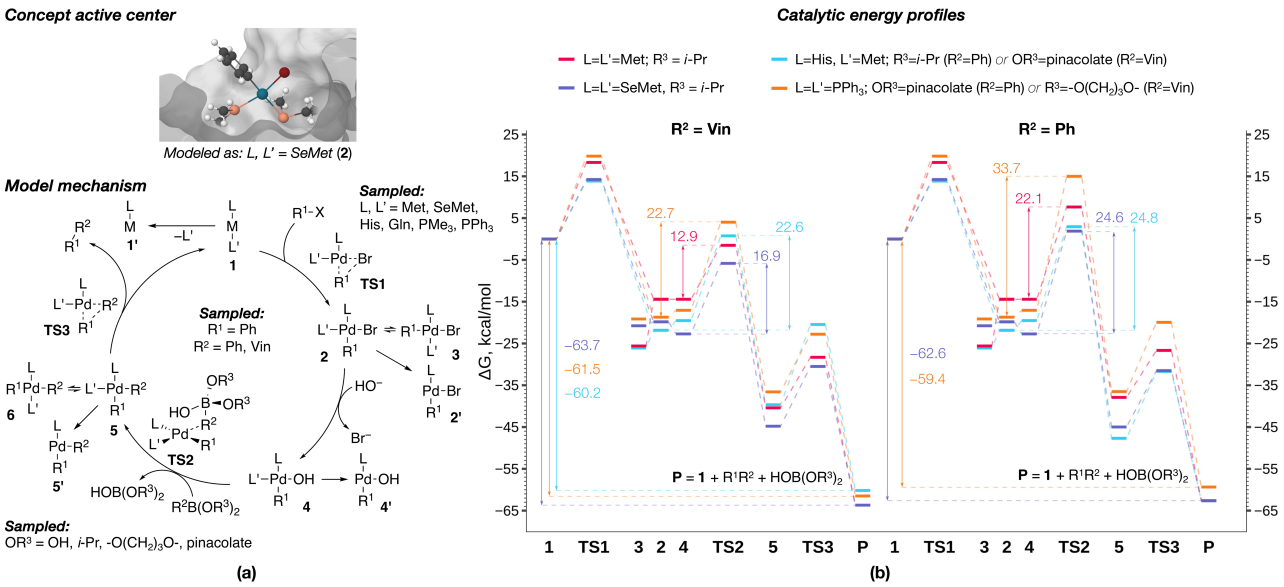

Figure 1. (a) The concept active center and the model catalytic cycle comprising both the reaction and catalyst degradation processes; (b) free energy profiles of selected Pd-ligand systems.

In all systems, a concerted transition state (**TS1**) was identified in OA. The initiation of TM by boronic esters required the formation of the hydroxo intermediate **4** that led to **TS2**. Optimized structures of **TS2** displayed a distinct migration of the R^2 group in the imaginary mode. The cycle is closed by concerted RE proceeding via **TS3** with the release of the coupling product.

The focal point of the study is the free energy profiles of the catalytic cycles of Ph-Ph and Ph-Vin couplings depicted in Figure 1b. Thermodynamic and kinetic parameters of elementary steps with all sampled ligands are given in the SI. The discussion here focuses on the AA ligands that exhibited the highest activity while strongly binding Pd and compares them to the commonly used PPh_3. A general downward trend in the thermodynamic effect is clear, with all steps within the cycle showing negative free energy, indicating that the overall reaction is markedly exergonic.

The barriers in the OA and RE steps did not exceed 19.8 and 19.2 kcal/mol, correspondingly. The TM step presented the highest barriers in the modeled cycle (Table 1), suggesting that the relative activity of Pd centers with varying ligands should primarily be evaluated in TM. We compared activity across phosphine and different AA ligand environments using the energetic span model.[18] The turnover frequency-determining transition state (TDTS) was identified as **TS2** in all cases, as shown in Figure 1b.

To determine the energetic span, δE, the turnover frequency-determining intermediate (TDI) was chosen as the cis-form of either intermediate **2** or **4**. The peptide/protein ligand environment in the concept active center (Figure 1a, top) may constrain the center's geometry to the *cis* form, potentially circumventing the *trans* intermediate, **3**, which exhibited lower free energy (Figure 1b). The higher stability of the *trans*-intermediate has been previously linked to reduced activity.[19] Consequently, only *cis* forms were considered for all ligand environments to emulate the proposed active center concept, including PPh_3, to ensure a consistent comparison. Since intermediate **2** is energetically lower for both Ph-Vin and Ph-Ph couplings in the bis-PPh_3 and His-Met systems, it was selected as the TDI. This results in δE values of 22.7 and 33.7 kcal/mol for Ph-Vin and Ph-Ph couplings with PPh_3, respectively. In the His-Met system, δE is 22.6 and 24.8 kcal/mol.

In the bis-Met and bis-SeMet systems, the *cis*-TDI was intermediate **4**. The energetic span, δE, in the bis-Met system was notably small, with values of 12.9 and 22.1 kcal/mol for the Ph-Vin and Ph-Ph couplings, respectively. For the SeMet system, δE exhibited moderate values of 16.9 and 24.6 kcal/mol for the Ph-Vin and Ph-Ph couplings.

Analyzing the δE values, we observe that AAs acting as ligands in the concept active center potentially offer activity levels far superior to the bis-PPh_3 system, given that TOF depends on

δE exponentially.[18] Using δE as a rough estimate of the activation energy for a thermocatalytic Suzuki coupling, values below 25.0 kcal/mol correspond to a kinetic constant of 1 per mol·hour at 60°C, which we can consider as well enough for catalytic activity for practical synthetic purposes. Although 60°C is an elevated temperature, enzymes stable at this temperature were developed,[20] and designing thermostable enzymes is a steadily growing field.[21] While monoligand phosphine systems sometimes show higher activity than bisligand counterparts,[22] this study focuses on bisligand phosphine and AA systems. This is due to the tendency of monoligand systems to form di- or oligopalladium clusters $L[ArPdX]_nL$[23,24] representing off-cycle resting states that reduce activity.[25] Additionally, strong ligand binding is crucial for Pd catalysts to prevent the release of Pd(0) and the formation of inactive metallic Pd(0) species.[24]

Table 1 illustrates ligand binding strength and corresponding free energies of activation ($\Delta G^{\ddagger}$) for the model coupling reactions depicted in Figure 1. Considering ligand dissociation as a barrierless process, energies $\Delta G_{\mathbf{1}\rightarrow\mathbf{1}'}$, $\Delta G_{\mathbf{4}\rightarrow\mathbf{4}'}$, and $\Delta G_{\mathbf{5}\rightarrow\mathbf{5}'}$, compared to the corresponding free energies of activation $\Delta G^{\ddagger}_{\mathbf{1}\rightarrow\mathbf{2}}$, $\Delta G^{\ddagger}_{\mathbf{4}\rightarrow\mathbf{5}}$, and $\Delta G^{\ddagger}_{\mathbf{5}\rightarrow\mathbf{1}(\boldsymbol{P})}$, serve as a measure of the stability of the catalytic system. In addition to $\Delta G_{\mathbf{1}\rightarrow\mathbf{1}'}$, $\Delta G_{\mathbf{2}\rightarrow\mathbf{2}'}$, $\Delta G_{\mathbf{4}\rightarrow\mathbf{4}'}$, and $\Delta G_{\mathbf{5}\rightarrow\mathbf{5}'}$ presented in Table 1, we computed energies of the detachment of the complete Pd-containing fragment from the concept active center and included these values in the SI. The values in Tables S2, S6, S11, S17, and S18 show that such a process is highly thermodynamically unfavorable.

If, for example, $\Delta G_{\mathbf{1}\rightarrow\mathbf{1}'}<\Delta G^{\ddagger}_{\mathbf{1}\rightarrow\mathbf{2}}$, ligand dissociation is more favorable than continuing the catalytic step **1⟶2**. Instances where $\Delta G_{\boldsymbol{n}\rightarrow\boldsymbol{n}'}<\Delta G^{\ddagger}_{\boldsymbol{n}\rightarrow\boldsymbol{k}}$ are highlighted in Table 1 in italics. Notably, for the His-Met system, we accounted for the dissociation of either ligand due to their significant differences in binding strength.

Table 1. Gibbs free energies of ligand dissociation vs. activation free energies of catalytic mechanism steps in kcal/mol.

L, L'	$\Delta G_{1\rightarrow 1'}$	$\Delta G_{2\rightarrow 2'}$	$\Delta G_{4\rightarrow 4'}$	$\Delta G_{5\rightarrow 5'}$	
				Vin-Ph	Ph-Ph
L=L'=PPh_3	23.8	34.8	*15.1*	25.4	25.1
L=L'=Met	19.5	17.1	21.7	25.1	27.9
L=His, L'=Met (−His)	18.5	20.2	*22.4*	30.6	26.3
L=His, L'=Met (−Met)	22.4	17.8	33.0	28.5	23.0
L=L'=SeMet	18.1	23.0	30.8	31.9	31.8
L, L'	$\Delta G^{\ddagger}_{1\rightarrow 2}$	$\Delta G^{\ddagger}_{4\rightarrow 5}$		$\Delta G^{\ddagger}_{5\rightarrow 1(P)}$	
		Vin-Ph	Ph-Ph	Vin-Ph	Ph-Ph
L=L'=PPh_3	19.8	32.1	21.1	16.6	13.8
L=L'=Met	18.3	22.1	12.9	11.2	12.1
L=His, L'=Met	13.9	22.5	20.3	16.0	19.2
L=L'=SeMet	14.2	24.6	16.9	13.5	14.3

In the few instances where $\Delta G_{n\rightarrow n'}<\Delta G^{\ddagger}_{n\rightarrow k}$, the His-Met system exhibits this condition, with $\Delta G_{4\rightarrow 4'}$ of the dissociation of His residue being smaller than $\Delta G^{\ddagger}_{4\rightarrow 5}$ in the Vin-Ph coupling by 0.1 kcal/mol. This can be ascribed to the harder nature of the ligand. However, Gibbs free energy of the dissociation of the second ligand with the formation of free [PhPdOH] is relatively thermodynamically unfavorable, +12.9 kcal/mol (Table S11), which prevents easy liberation of Pd from the concept active center. Consequently, the His-Met type active center is expected to be stable, although its stability under catalytic conditions needs further investigation. Importantly, the high stability of AA systems is attributed to the chelating nature of the concept active center. Dissociated AA ligands were considered as remaining bound to the peptide chain, thereby not gaining translational entropy upon dissociation (see the SI for details). Surprisingly, $\Delta G_{4\rightarrow 4}<\Delta G^{\ddagger}_{4\rightarrow 5}$ is also observed in the bis-PPh_3 system, suggesting a need for a better understanding of dynamic changes in ligand state within this classic catalytic system, although this investigation falls outside the scope of the current study.

The stability of the peptide system under the basic conditions typical of Suzuki coupling is worth discussion. Designing an oligopeptide ligand system for this purpose may be more straightforward than a long-chain peptide or protein alternative due to the simpler structure of the former and since natural peptides and proteins may be prone to denaturation and structural modifications of AAs in basic environments.[26,27] Extremophile organisms can tolerate pH higher than 9 and, in some instances, above 12-13 as external environment,[28] so only a minor portion of their proteins is subjected to basic environments. Some engineered proteins are resistant to basic environments such as 0.5 M NaOH solutions (and 1 M for some domains).[29] Therefore, a necessary requirement for Pd-based enzymatic catalysis in Suzuki cross-coupling reactions is either a rational design of a suitable protein/peptide or a fundamental search for new biocompatible transmetalating agents.

In summary, according to the presented computational analysis, we see that (oligo)peptides can serve as greener ligands for Pd in Suzuki couplings and offer superior activity compared to the classic PPh_3. The engineering of an artificial peptide or protein capable of incorporating a Pd atom is a complex task, but the concept active centers presented here already can serve as a blueprint for designing short oligopeptide ligands. The chelating nature of such ligands, the ability to tailor their structure for strong Pd binding, and the increasing economic viability and sustainability of oligopeptide synthesis indicate that we indeed can witness Pd-oligopeptide catalysis of Suzuki couplings soon.

COMPUTATIONAL DETAILS

DFT modeling was conducted using the ORCA program[30] at the ωB97X-V/def2-TZVP//B97-3c level[31–33] within the RIJCOSX approximation[34,35]. SMD[36] was employed to simulate the

solvent environment. Additional computational details are provided in the SI. ChatGPT v.4 was used for initial text proofreading.

SUPPORTING INFORMATION

The supporting PDF file includes computational details and all reaction Gibbs free energies and free energies of activation.

CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

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