Supporting Information

Origin of Metal Cluster Tuning Enzyme Activity at the Bio-Nano Interface

Yufei Cao*, Yida Qiao*, Shitong Cui*, Jun Geab,c*

* Key Lab for Industrial Biocatalysis, Ministry of Education, Department of Chemical Engineering, Tsinghua University, Beijing 100084, China

b Institute of Biopharmaceutical and Health Engineering, Tsinghua Shenzhen International Graduate School, Shenzhen 518055, China

c Institute of Biomedical Health Technology and Engineering, Shenzhen Bay Laboratory, Shenzhen 518107, China

* Corresponding author. Email: junge@mail.tsinghua.edu.cn
Supplementary Materials and methods

Preparation of CALB-P Conjugates and Pd/CALB-P Nanohybrids

Palladium (II) acetate (Pd(OAc)$_2$, 99.9 wt.%), sodium borohydride (NaBH$_4$, 99 wt.%), sodium cyanoborohydride (NaCNBH$_3$, 95 wt.%) were purchased from Acros. *Candida Antarctic* lipase B (CALB, ≥5000 LU/G of liquid), Pluronic®F-127, p-nitrophenyl butyrate (p-NPB), were purchased from Sigma-Aldrich (St. Louis, MO, USA). The lipase was used after dialyzing against phosphate buffer (10 mM, pH 7.0) at 4 °C overnight.

The procedure of preparation of CALB-P conjugates and Pd/CALB-P Nanohybrids was the same as our previous work$^1$ (Fig. 1 and Supplementary Fig. 1). *Candida Antarctic* lipase B (CALB) (6 mg/mL) and aldehyde-functionalized Pluronic F-127 were dissolved in Na$_2$HPO$_4$/NaH$_2$PO$_4$ buffer (10 mM, pH 7.0). The molar ratio of the amine groups in lysine of CALB and the aldehyde groups in Pluronic was 1:1.1. After stirring vigorously for 2 h at room temperature, sodium cyanoborohydride (NaCNBH$_3$, 10% w/w of aldehyde-functionalized Pluronic) was dissolved in phosphate buffer (1 mL, 10 mM, pH 7.0) and then added into the reaction mixture dropwisely. The stirring was continued for 15 h. The solution was then dialyzed against phosphate buffer (10 mM, pH 7.0) at 4 °C overnight to remove unreacted reagents. Some detailed information about the CALB-P conjugates which we proved in the previous work were listed.

1. Each synthesized CALB-P conjugates contained one CALB molecule attached to one Pluronic F-127 (CALB-P), which has been determined by matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

2. The number of free amine groups on the surface of CALB and the CALB-P nanoconjugate was determined as 10 and 9, respectively. It indicates that a single polymer chain was attached to one enzyme molecule via one end of the polymer covalently bonding with an amine group from Lys residues of the protein. At pH 7, multipoint covalent attachment is hardly produced because of the low reactivity of Lys residues. Therefore, it is highly possible that the most reactive lysine residue on the protein surface was responsible for this conjugation. The pKa calculation of all Lys residues suggested that Lys 136 is the most possible bonding site of polymer.

After that, palladium acetate was dissolved at different concentrations in methanol (2 ml) and then added into the CALB-P aqueous solution (phosphate buffer, 8 ml, 50 mM, pH 7.0) under magnetic stirring at 25 °C. The Pd$^{2+}$ ions were reduced by methanol and Pd NPs formed. Through tuning the concentrations of Pd$^{2+}$ ions, we can synthesize Pd NPs of different sizes. The concentrations of Pd(OAc)$_2$ and CALB-P in the synthesis of the Pd/CALB-P nanohybrids with different NP sizes can be found in our previous work$^1$ and Table S1. The reaction solution was stirred for 20 h followed by dialysis against phosphate buffer (10 mM, pH 7.0) at 4 °C overnight. The powder of the nanohybrids was obtained by lyophilization and stored in a desiccator. Our previous results showed that the average size of the Pd NPs synthesized in CALB-P conjugates was readily tuned from 2.5 to 0.8nm with narrow size distributions (2.5±0.5, 2.2±0.8, 1.6±0.5 and 0.8±0.2nm) by decreasing the concentration of Pd(OAc)$_2$ (Table S1).

The BSA-P conjugates and Pd/BSA-P nanohybrids were synthesized by a similar procedure and the same concentration of protein and Pd$^{2+}$ were used.
Enzyme Activity Assays of Pd/CALB-P

The hydrolytic activities of CALB, CALB-P and Pd/CALB-P were assayed by a standard method using p-NPB as the substrate. Triton X-100 (0.25g) was dissolved in \( \text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4 \) buffer (20 mL, 50 mM, pH 7.0) and then added to a 50 mL beaker with magnetic stirring. p-NPB was dissolved in acetone (1 mL) and then added to the above-mentioned Triton X-100 solution dropwisely. The mixture was stirred for 5 min at room temperature. The reaction was started by adding 50 µL of enzyme sample solution (50 µg/mL protein in 10 mM phosphate buffer, pH 7.0) to 950 µL of the substrate solution. The increase in absorbance was detected at 348 nm by a UV/Vis spectrophotometer SHIMADZU UV-2600.

Cascade reaction of 0.8Pd/CALB-P and 0.8Pd/BSA-P&CALB-P

0.8Pd/CALB-P (0.08 mg Pd, 6 U CALB) or 0.8 Pd/BSA-P (0.08 mg Pd)&CALB-P (6 U CALB), aryl halide (0.1 mmol), phenylboronic acid (0.4 mmol), potassium carbonate (41.5 mg, 0.3 mmol), deionized water (1 mL) were added to a 5-mL vial. Conversion of 1 µmol of p-NPB per minute in the assay at 25 °C was defined as one enzyme activity (U). The mixture was vigorously stirred at 50 °C for several minutes. The reaction mixture was then extracted with EtOAc (4 mL). The yield was determined by GC-MS analysis.
Supplementary Tables and Figures

Figure S1. The synthetic procedure of CALB- Pluronic F-127 conjugates.
Table S1. Concentrations of Pd(OAc)$_2$ and CALB-P in the synthesis of nanohybrids with different particle sizes.

| Pd NPs (d/nm) | CALB-P (mg/mL) | Pd(OAc)$_2$ (mg/mL) |
|---------------|----------------|-------------------|
| 0.8           | 16.7           | 0.6               |
| 1.6           | 16.7           | 1.3               |
| 2.2           | 16.7           | 2.7               |
| 2.5           | 16.7           | 3.3               |
Table S2. Formulas to calculate the number of atoms at different positions of truncated octahedron shaped Pd particles.

| Quantity                              | Formula                  |
|---------------------------------------|--------------------------|
| Total atom number of each particle (NT) | $16m^3 - 33m^2 + 24m - 6$ |
| Surface atom number of each particle (NS) | $30m^2 - 60m + 32$       |

The diameter of Pd atom ($d_{\text{Pd atom}}$) is 0.274 nm. Between the particle size ($d$) and the number of atoms involved in one truncated cuboctahedron shaped Pd particle follows the relationship of $d = 1.105 \times NT^{1/3} \times d_{\text{Pd}}$. $m$ is the number of atoms lying on an equivalent edge (corner atoms included).
Table S3. The total atom number of each particle (NT), surface atom number of each particle (NS), the ratio of NS and NT, and the diameter of four Pd NPs in our simulation.

| Name | Pd19 | Pd55 | Pd201 | Pd459 |
|------|------|------|-------|-------|
| NT   | 19   | 55   | 201   | 459   |
| NS   | 18   | 44   | 122   | 227   |
| NS/NT| 0.95 | 0.79 | 0.61  | 0.49  |
| d (nm)| 0.8  | 1.2  | 1.8   | 2.3   |
Figure S2. The parameters of LJ potential of atom Pd and some common atoms in the protein of Amber ff14SB force field. The LJ potential $V_{LJ}(r) = 4\epsilon \left[ \left( \frac{\sigma}{r} \right)^{12} - \left( \frac{\sigma}{r} \right)^{6} \right]$ between the Pd atom and other protein atoms was plotted. The interatomic LJ parameters were calculated through the following formula: $\sigma_{ij} = \frac{1}{2}(\sigma_i + \sigma_j)$, $\epsilon_{ij} = \sqrt{\epsilon_i \epsilon_j}$. The LJ potential between Pd and different protein atoms does not have an obvious difference except for atom H. But atom H is located at the backbone of protein and it unlikely interacts with Pd NPs (side chain of protein provides the main distribution of interaction).
Figure S3. Docking results of CALB and Pd NPs with different sizes. The top 100 highest scored complex structures were aligned into one frame to observe the position distribution of Pd NPs. (a) CALB&Pd19. (b) CALB&Pd55. (c) CALB&Pd201. (d) CALB&Pd459.
Figure S4. The binding energy between protein and Pd NPs with different initial geometries. (a) Nine initial geometries of Pd19/CALB were selected. The binding energy with simulation time increasing was shown. The Pos-2 had the highest binding affinity and it was selected to do the following simulation. (b) Eight initial geometries of Pd55/CALB were selected. The binding energy with simulation time increasing was shown. The Pos-1 had the highest binding affinity and it was selected to do the following simulation. (c) Four initial geometries of Pd201/CALB were selected. The binding energy with simulation time increasing was shown. The Pos-3 had the highest binding affinity and it was selected to do the following simulation. (d) Five initial geometries of Pd459/CALB were selected. The binding energy with simulation time increasing was shown. The Pos-3 had the highest binding affinity and it was selected to do the following simulation.
Figure S5. Taking Pd19/CALB as an example to confirm that the Pd NPs binding is a shape matching behavior.  
(a) The number of contacts with time increasing during nine simulations of Pd19/CALB (nine different initial geometries). (b) Correlation between the number of contacts and binding energy of Pd19 in nine simulations with different initial geometries. Expect for the second initial geometry Pos-2, the binding energy has a good linear relationship with the number of contacts ($R^2=0.99$). The slope of the fitting line is $-34.5\pm1.9$ kJ/mol and it demonstrates that each contact contributes $-34.5$ kJ binding energy, which is similar to the results in Supplementary Fig. 10a. It further proves that Pd NPs binding is a shape matching behavior. The Pos-2 is not included in the above-mentioned linear relationship, because there exists a big glycosyl in this position and it contributes more binding energy than normal protein residues. The existence of glycosyl is the main reason that Pos-2 has the highest binding affinity. Hence, the position of glycosyl has the potential to optimally stabilize the metal NPs with small sizes (Pd19 and Pd55 in our work).
Figure S6. The root-mean-square deviation (RMSD) of CALB in the simulation of complex (a) Pd19/ CALB (b) Pd55/ CALB (c) Pd201/ CALB (d) Pd459/ CALB.
Finally obtained structures of Pd/CALB complexes (after 1 μs-long MD simulation, 2 μs for Pd19/CALB). Two drawing methods “NewCartoon” and “QuickSurf” were used to show the structure of CALB. Pd atoms were shown as yellow spheres. The residues interacting with Pd NPs were shown with blue color and the catalytic triad was also shown.
Figure S8. The binding energy between CALB and Pd NPs in the simulation. (a) Pd19/ CALB (b) Pd55/ CALB (c) Pd201/ CALB (d) Pd459/ CALB.
Figure S9. The number of contacts between CALB and Pd NPs in the simulation. (a) Pd19/ CALB (b) Pd55/ CALB (c) Pd201/ CALB (d) Pd459/ CALB. It has the same tendency as the binding energy in Supplementary Fig. 6. It also indicates that the binding energy of Pd NPs is determined by the contact area with the protein surface.
Figure S10. (a) The ratio between binding energy and number of contacts. The higher values of Pd19 and Pd55 come from the contribution of glycosyl. The binding energy distribution on the surface of (b) Pd19, (c) Pd55, (d) Pd201, (e) Pd459. We can observe that corner and edge atoms have more contribution of binding energy. It indicates that corner and edge atoms of metal NPs are easier to bind with protein surface.
Figure S11. The final obtained structures of Pd/CALB complexes were aligned onto the CALB equilibrium structure (1 μs-long MD simulation). The CALB in Pd/CALB complexes was shown as red color, while wild CALB was shown as blue color. The region binding with Pd NPs was shown as green. And the region in which obvious conformation change took place was marked by a black dotted circle. The root mean square deviation (RMSD) between the average structure of CALB in Pd NPs/CALB during equilibrium simulation and that of wild CALB is 1.12 Å (Pd19/CALB), 0.86 Å (Pd55/CALB), 1.65 Å (Pd201/CALB) and 1.68 Å (Pd459/CALB).
Figure S12. Root-mean-square fluctuation (RMSF) differences between the CALB in the CALB-Pd complex and wild CALB. The $\alpha_5$, $\alpha_{10}$ region and catalytic triad Ser105, Asp187 and His224 were marked.
Figure S13. Conformational drift measured by Cα root mean square deviation. (a) RMSD calculated as a function of the fraction of the total Cα atoms considered for structural alignment in CALB, Pd19/CALB, Pd55/CALB, Pd201/CALB and Pd459/CALB. The plots indicate that 90% of conformations in protein can be aligned to below 0.9 nm (CALB; black), 0.82 nm (Pd19/CALB; blue), 0.81 nm (Pd459/CALB; red), 0.76 nm (Pd55/CALB; blue), and 0.73 nm (Pd201/CALB; green). Fractional Cα RMSD calculated after identification of the core in (b) CALB, (c) Pd19/CALB, (d) Pd55/CALB, (e) Pd201/CALB and (f) Pd459/CALB. The bottom black line denotes the stable core and consists of 90% Cα atoms. The top red line is the remainder 10% Cα atoms. These constitute the non-core Cα atoms that display deviation in all simulations.
Figure S14. Core Cα root-mean-square deviation (RMSD) superimposition from CALB, CALB, Pd19/CALB, Pd55/CALB, Pd201/CALB and Pd459/CALB simulations. The structural alignment was calculated from the equilibrated section of all trajectories and rendered to illustrate 100 uniformly separated frames. The least mobile Cα atoms are colored blue and the most mobile atoms (red) provide the structural basis for the differential RMSDs.
Figure S15. Average positional Cα deviations of CALB, CALB, Pd19/CALB, Pd55/CALB, Pd201/CALB and Pd459/CALB. The averaged Cα positional deviations were mapped onto the averaged protein structures to visualize the largest relative displacements. The thickness of the cartoon corresponds to the Cα deviation.
**Figure S16.** Calculated dot product matrix between the eigenvectors identified by the PCA investigation on the wild CALB and those identified by PCA in the (a) Pd19, (b) Pd55, (c) Pd201 and (d) Pd459 bound form of the protein. Information of RMSD (Å) between the average structure of two different scenarios, subspace overlap and average maximum dot product were listed.
Figure S17. Dynamic cross-correlation maps (DCCMs) computed for CALB, Pd19/CALB, Pd55/CALB, Pd201/CALB, Pd459/CALB. The DCCMs for equilibrium trajectories were calculated. White regions indicate no correlation, blue indicates a negative correlation, while red regions indicate positive correlations.
Figure S18. Visualization of Jensen-Shannon (JS) divergence of the probability distribution of side-chain torsion-angle between Pd19/CALB and wild CALB. (a) The residues with JS divergence bigger than 0.5 (“responsive” residues) were displayed as “QuickSurf” drawing methods. From green to red, the JS divergence gradually increases. (b) According to the results of (a), we identified two pathways connecting the Pd19 binding site to the active site.
Figure S19. Probability density distributions of side-chain torsion angles from wild-type CALB (black) to Pd19/CALB (red) in pathway 1. Nine residues were included in pathway 1. In each figure of probability density distributions, the title of the figure shows the residue name, the name of side-chain torsion angles (chi1 and chi2), and the JS divergence.
Figure S20. Probability density distributions of side-chain torsion angles from wild-type CALB (black) to Pd19/CALB (red) in pathway 2. Eight residues were included in pathway 2. In each figure of probability density distributions, the title of the figure shows the residue name, the name of side-chain torsion angles (chi1 and chi2), and the JS divergence.
Figure S21. Visualization of Jensen-Shannon (JS) divergence of the probability distribution of side-chain torsion-angle between Pd55/CALB and wild CALB. (a) The residues with JS divergence bigger than 0.5 (“responsive” residues) were displayed as “QuickSurf” drawing methods. From green to red, the JS divergence gradually increases. (b) According to the results of (a), we identified two pathways connecting the Pd55 binding site to the active site.
Figure S22. Probability density distributions of side-chain torsion angles from wild-type CALB (black) to Pd55/CALB (red) in pathway 1. Nine residues were included in pathway 1. In each figure of probability density distributions, the title of the figure shows the residue name, the name of side-chain torsion angles (chi1 and chi2), and the JS divergence.
Figure S23. Probability density distributions of side-chain torsion angles from wild-type CALB (black) to Pd55/CALB (red) in pathway 2. Five residues were included in pathway 2. In each figure of probability density distributions, the title of the figure shows the residue name, the name of side-chain torsion angles (chi1 and chi2), and the JS divergence.
Figure S24. Visualization of Jensen-Shannon (JS) divergence of the probability distribution of side-chain torsion-angle between Pd201/CALB and wild CALB. (a) The residues with JS divergence bigger than 0.5 (“responsive” residues) were displayed as “QuickSurf” drawing methods. From green to red, the JS divergence gradually increases. (b) According to the results of (a), we identified a pathway connecting the Pd201 binding site to the active site.
Figure S25. Probability density distributions of side-chain torsion angles from wild-type CALB (black) to Pd201/CALB (red) in the pathway. Fifteen residues were included in the pathway. In each figure of probability density distributions, the title of the figure shows the residue name, the name of side-chain torsion angles (chi1 and chi2), and the JS divergence.
Figure S26. Visualization of Jensen-Shannon (JS) divergence of the probability distribution of side-chain torsion-angle between Pd459/CALB and wild CALB. (a) The residues with JS divergence bigger than 0.5 ("responsive” residues) were displayed as “QuickSurf” drawing methods. From green to red, the JS divergence gradually increases. (b) As the Pd459 binding site was close to the active site, no pathway was given. The residues surrounding the active site with high JS divergence were shown.
Figure S27. Probability density distributions of side-chain torsion angles from wild-type CALB (black) to Pd459/CALB (red) in the pathway. Ten residues surrounding the active site with high JS divergence were shown. In each figure of probability density distributions, the title of the figure shows the residue name, the name of side-chain torsion angles (chi1 and chi2), and the JS divergence.
**Figure S28.** The simulation procedure of CALB-P to find the equilibrium structure. Step-1: 20 ns NVT simulation under vacuum at 600 K. Step-2: 200 ns NPT simulation in water at 600 K. Step-3: 1 μs NPT simulation in water at 298.15 K.

**Figure S29.** 1 μs-long NPT simulation of CALB-P. (a) RMSD of CALB-P, CALB, and polymer. The large RMSD of polymer shows the flexibility of the polymer. The RMSD results indicate that the simulations are reaching equilibrium. (b) The structure at 0 μs (blue) and 1 μs (red) of simulation. Although the conformation of the polymer has a big change, the relative position of the protein and polymer does not change.
Figure S30. Similar equilibrium structures of protein-polymer complexes with different initial structures. We employed three different initial structures and similar equilibrium structures of complexes through the same simulation procedure (Supplementary Fig. 27) were obtained. It indicates that the approximate equilibrium state was obtained.

Figure S31. The final obtained structure of CALB-P was aligned onto the CALB equilibrium structure (1 μs-long MD simulation). The CALB in CALB-P complex was shown as red color, while the wild CALB was shown as blue color. The root mean square deviation (RMSD) between the average structure of CALB in CALB-P during equilibrium simulation and that of wild CALB is 0.53 Å.
Figure S32. Root-mean-square fluctuation (RMSF) and the difference between the CALB in the CALB-P complex and wild CALB. The $\alpha_5$, $\alpha_{10}$ region and catalytic triad Ser105, Asp187 and His224 were marked.
Figure S33. (a) RMSD calculated as a function of the fraction of the total Cα atoms considered for structural alignment in CALB and CALB-P. The plots indicate that 90% of conformations in protein can be aligned to below 0.9 nm (CALB; black) and 0.67 nm (CALB-P; blue). Fractional Cα RMSD calculated after identification of the core in (b) CALB, (c) CALB-P. The bottom black line denotes the stable core and consists of 90% Cα atoms. The top red line is the remainder 10% Cα atoms. These constitute the non-core Cα atoms that display deviation in all simulations. (d) Core Cα root-mean-square deviation (RMSD) superimposition from CALB-P simulations. The structural alignment was calculated from the equilibrated section of all trajectories and rendered to illustrate 100 uniformly separated frames. The least mobile Cα atoms are colored blue and the most mobile atoms (red) provide the structural basis for the differential RMSDs. (e) Average positional Cα deviations of CALB-P. The averaged Cα positional deviations were mapped onto the averaged protein structures to visualize the largest relative displacements. The thickness of the cartoon corresponds to the Cα deviation.
Figure S34. (a) Dynamic cross-correlation maps (DCCMs) computed for CALB-P. We can observe that it does not have an obvious difference with DCCMs of CALB. (b) Visualization of Jensen-Shannon (JS) divergence of the probability distribution of side-chain torsion-angle between CALB-P and wild CALB (left). The residues with JS divergence bigger than 0.5 (“responsive” residues) were displayed as “QuickSurf” drawing methods (right). From green to red, the JS divergence gradually increases. The polymer has no obvious influence on the probability distribution of side-chain torsion-angle.

Figure S35. Eight starting positions of Pd NPs in the simulation of Pd/CALB-P. Taking Pd19 as an example, we selected eight starting geometries to find the optimal conformation of Pd/CALB-P.
Figure S36. The binding energy between protein and Pd NPs with different initial geometries. Eight initial geometries of (a) Pd19/CALB, (b) Pd55/CALB, (c) Pd201/CALB, (d) Pd459/CALB were selected. The structure had the highest binding affinity and it was selected to do the following 1 μs-long simulation.
Figure S37. 1 μs-long NPT simulation of Pd19/CALB-P. (a) RMSD of CALB-P, CALB, and polymer. The RMSD results indicate that the simulation reached equilibrium. (b) The structure at 0 μs (blue) and 1 μs (red) of simulation.

Figure S38. 1 μs-long NPT simulation of Pd55/CALB-P. (a) RMSD of CALB-P, CALB, and polymer. The RMSD results indicate that the simulation reached equilibrium. (b) The structure at 0 μs (blue) and 1 μs (red) of simulation.
Figure S39. 1 μs-long NPT simulation of Pd201/CALB-P. (a) RMSD of CALB-P, CALB, and polymer. The RMSD results indicate that the simulation reached equilibrium. (b) The structure at 0 μs (blue) and 1 μs (red) of simulation.

Figure S40. 1 μs-long NPT simulation of Pd459/CALB-P. (a) RMSD of CALB-P, CALB, and polymer. The RMSD results indicate that the simulation reached equilibrium. (b) The structure at 0 μs (blue) and 1 μs (red) of simulation.
Figure S41. The pattern of polymer absorbed on the surface of (a) Pd201 (b) Pd459 in Pd/CALB-P complex.
Figure S42. The binding energy between CALB-P and Pd NPs in the simulation. (a) Pd19/ CALB-P (b) Pd55/ CALB-P (c) Pd201/ CALB-P (d) Pd459/ CALB-P.
Figure S43. The number of contacts between CALB-P and Pd NPs in the simulation. (a) Pd19/ CALB-P (b) Pd55/ CALB-P (c) Pd201/ CALB-P (d) Pd459/ CALB-P. It has the same tendency as the binding energy in Supplementary Fig. 42.
Figure S44. (a) Binding energy and the ratio between binding energy and the number of surface atoms. (b) The ratio between binding energy and number of contacts. The binding energy distribution on the surface of (c) Pd19, (d) Pd55, (e) Pd201, (f) Pd459.
Figure S45. The final obtained structures of Pd/CALB-P complexes were aligned onto the CALB equilibrium structure (1 μs-long MD simulation). The CALB in Pd/CALB-P complexes was shown as red color, while wild CALB was shown as blue color. The region binding with Pd NPs was shown as green. The RMSD between the average structure of CALB in Pd NPs/CALB-P during equilibrium simulation and that of wild CALB is 0.71 Å (Pd19/CALB-P), 0.82 Å (Pd55/ CALB-P), 1.01 Å (Pd201/ CALB-P) and 1.24 Å (Pd459/ CALB-P).
Figure S46. Conformational drift measured by Cα root mean square deviation. (a) RMSD calculated as a function of the fraction of the total Cα atoms considered for structural alignment in CALB, Pd19/CALB-P, Pd55/CALB-P, Pd201/CALB-P and Pd459/CALB-P. The plots indicate that 90% of conformations in protein can be aligned to below 0.9 nm (CALB; black), 0.75 nm (Pd19/CALB-P; blue), 0.74 nm (Pd459/CALB-P; red), 0.73 nm (Pd55/CALB-P; blue), and 0.68 nm (Pd201/CALB-P; green). Fractional Cα RMSD calculated after identification of the core in (b) CALB, (c) Pd19/ CALB-P, (d) Pd55/ CALB-P, (e) Pd201/ CALB-P and (f) Pd459/ CALB-P. The bottom black line denotes the stable core and consists of 90% Cα atoms. The top red line is the remainder 10% Cα atoms. These constitute the non-core Cα atoms that display deviation in all simulations.
Figure S47. Core Ca root-mean-square deviation (RMSD) superimposition from CALB, Pd19/CALB-P, Pd55/CALB-P, Pd201/CALB-P and Pd459/CALB-P simulations. The structural alignment was calculated from the equilibrated section of all trajectories and rendered to illustrate 100 uniformly separated frames. The least mobile Ca atoms are colored blue and the most mobile atoms (red) provide the structural basis for the differential RMSDs.
**Figure S48.** Average positional Cα deviations of CALB, Pd19/CALB-P, Pd55/CALB-P, Pd201/CALB-P and Pd459/CALB-P. The averaged Cα positional deviations were mapped onto the averaged protein structures to visualize the largest relative displacements. The thickness of the cartoon corresponds to the Cα deviation.
Figure S49. Calculated dot product matrix between the eigenvectors identified by the PCA investigation on the wild CALB and those identified by PCA in the (a) Pd19, (b) Pd55, (c) Pd201 and (d) Pd459 bound form of the CALB-P. Information of RMSD (Å) between the average structure of two different scenarios, subspace overlap and average maximum dot product were listed.
Figure S50. Dynamic cross-correlation maps (DCCMs) computed for CALB, Pd19/CALB-P, Pd55/CALB-P, Pd201/CALB-P, Pd459/CALB-P. The DCCMs for equilibrium trajectories were calculated. White regions indicate no correlation, blue indicates a negative correlation, while red regions indicate positive correlations.
Figure S51. **Visualization of Jensen-Shannon (JS) divergence of the probability distribution of side-chain torsion-angle between Pd19/CALB-P and wild CALB.** The residues with JS divergence bigger than 0.5 ("responsive" residues) were displayed as “QuickSurf” drawing methods. From green to red, the JS divergence gradually increases.
Figure S52. Visualization of Jensen-Shannon (JS) divergence of the probability distribution of side-chain torsion-angle between Pd55/CALB-P and wild CALB. (a) The residues with JS divergence bigger than 0.5 (“responsive” residues) were displayed as “QuickSurf” drawing methods. From green to red, the JS divergence gradually increases. (b) According to the results of (a), we identified a pathway connecting the Pd55 binding site to the active site.
Figure S53. Probability density distributions of side-chain torsion angles from wild-type CALB (black) to Pd55/CALB-P (red) in the pathway. Seven residues were included in the pathway. In each figure of probability density distributions, the title of the figure shows the residue name, the name of side-chain torsion angles (chi1 and chi2), and the JS divergence.
Figure S54. Visualization of Jensen-Shannon (JS) divergence of the probability distribution of side-chain torsion-angle between Pd201/CALB-P and wild CALB. (a) The residues with JS divergence bigger than 0.5 (“responsive” residues) were displayed as “QuickSurf” drawing methods. From green to red, the JS divergence gradually increases. (b) According to the results of (a), we identified a pathway connecting the Pd201 binding site to the active site.
Figure S55. Probability density distributions of side-chain torsion angles from wild-type CALB (black) to Pd201/CALB-P (red) in the pathway. Eleven residues were included in the pathway. In each figure of probability density distributions, the title of the figure shows the residue name, the name of side-chain torsion angles (chi1 and chi2), and the JS divergence.
Figure S56. Visualization of Jensen-Shannon (JS) divergence of the probability distribution of side-chain torsion-angle between Pd2459/CALB-P and wild CALB. (a) The residues with JS divergence bigger than 0.5 ("responsive" residues) were displayed as "QuickSurf" drawing methods. From green to red, the JS divergence gradually increases. (b) According to the results of (a), we identified a pathway connecting the Pd459 binding site to the active site.
Figure S57. Probability density distributions of side-chain torsion angles from wild-type CALB (black) to Pd459/CALB-P (red) in the pathway. Sixteen residues were included in the pathway. In each figure of probability density distributions, the title of the figure shows the residue name, the name of side-chain torsion angles (chi1 and chi2), and the JS divergence.
Figure S58. (a) TEM images of Pd/BSA-P. (b) TEM images of Pd/CALB-P. (c) Activity test of Pd NPs in 0.8Pd/BSA-P and 0.8Pd/CALB-P. 0.8Pd/CALB-P (0.08 mg Pd) or 0.8 Pd/BSA-P (0.08 mg Pd), aryl halide (0.1 mmol), phenylboronic acid (0.4 mmol), potassium carbonate (41.5 mg, 0.3 mmol), deionized water (1 mL) were added to a 5-mL vial. The mixture was vigorously stirred at 50 °C for 30 min. The reaction mixture was then extracted with EtOAc (4 mL). The yield was determined by GC-MS analysis. The figure showed the yield of the Suzuki reaction.
Supplementary reference

1. Li, X.Y., Y.F. Cao, K. Luo, Y.Z. Sun, J.R. Xiong, L.C. Wang, Z. Liu, J. Li, J.Y. Ma, J. Ge, H. Xiao, and R.N. Zare, Highly active enzyme-metal nanohybrids synthesized in protein-polymer conjugates. *Nat Catal.,* **2019**, 2, 718-725.

2. Mateo, C., O. Abian, R. Fernandez–Lafuente, and J.M. Guisan, Increase in conformational stability of enzymes immobilized on epoxy-activated supports by favoring additional multipoint covalent attachment. *Enzyme and Microbial Technology,* **2000**, 26, 509-515.