Quantifying reversible nitrogenous ligand binding to Co(II) porphyrin receptors at the solution/solid interface and in solution†

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ABSTRACT: We present a quantitative study comparing the binding of 4-methoxypyridine, MeOPy, ligand to Co(II)octaethylporphyrin, CoOEP, at the phenyloctane/HOPG interface and in toluene solution. Scanning tunneling microscopy (STM) was used to study the ligand binding to the porphyrin receptors adsorbed on graphite. Electronic spectroscopy was employed for examining this process in fluid solution. The on surface coordination reaction was completely reversible and followed a simple Langmuir adsorption isotherm. Ligand affinities (or $\Delta G$) for the binding processes in the two different chemical environments were determined from the respective equilibrium constants. The free energy value of $-13.0\pm0.3$ kJ/mol for the ligation reaction of MeOPy to CoOEP at the solution/HOPG interface is less negative than the $\Delta G$ for cobalt porphyrin complexed to the ligand in solution, $-16.8\pm0.2$ kJ/mole. This result indicates that the MeOPy–CoOEP complex is more stable in solution than on the surface. Additional thermodynamic values for the formation of the surface ligated species ($\Delta H_c = -50$ kJ/mol and $\Delta S_c = -120$ J/mol) were extracted from temperature dependent STM measurements. Density functional computational methods were also employed to explore the energetics of both the solution and surface reactions. At high concentrations of MeOPy the monolayer was observed to be stripped from the surface. Computational results indicate that this is not because of a reduction in adsorption energy of the MeOPy–CoOEP complex. Nearest neighbor analysis of the MeOPy–CoOEP in the STM images revealed positive cooperative ligand binding behavior. Our studies bring new insights to the general principles of affinity and cooperativity in the ligand–receptor interactions at the solution/solid interface. Future applications of STM will pave the way for new strategies designing highly functional multisite receptor systems for sensing, catalysis, and pharmacological applications.

INTRODUCTION

Many biological systems contain metalloporphyrin binding sites that function as receptors that can regulate reversible ligand binding in order to perform tasks like respiration, photosynthesis, metal sequestration, transfer electrons, preform enzymatic catalysis and recognize target molecules.\textsuperscript{1-3} Duplication of this behavior in artificial porphyrin systems is desirable for the a wide range of application such as separating gas mixtures,\textsuperscript{4} energy storage and delivery,\textsuperscript{5,6} selective chemical sensing,\textsuperscript{7} cancer therapeutics,\textsuperscript{8,9} and catalysis.\textsuperscript{10,11} In both biology and technological applications, understanding how the ligands and receptors communicate requires that these interactions be quantified.\textsuperscript{12,13} This objective can be realized by carrying out investigations determining both ligand affinity and cooperativity through model binding experiments. Affinity denotes the strength of interaction between the ligand and the receptor. Cooperativity (synergism or allostery) occurs when the binding of one molecule to a receptor enhances (or weakens) the binding of other ligands to adjacent receptors.

Various techniques exist for characterizing ligand binding interactions and the resulting complexes at the ensemble level including electronic spectroscopy, electrochemistry, electron paramagnetic resonance and nuclear magnetic resonance spectroscopy and surface plasmon resonance. Direct molecular scale level chemical reaction monitoring methods (like
scanning tunneling microscopy, STM), however, offer the advantage of delivering more definitive mechanistic insights that are ordinarily hidden in ensemble level techniques.\textsuperscript{14-20} STM allows simultaneous access to spatial, temporal, and intra- and intermolecular reaction dynamics in different physical environments. Publications, albeit only a few at present, demonstrated that the solution/solid interface is an effective platform for probing reversible ligand binding to porphyrin receptor events to acquire both qualitative and quantitative information about binding affinity, reaction equilibrium kinetics, thermodynamics and cooperativity.\textsuperscript{21-31} These works include binding studies of biologically and chemically relevant species such as O\textsubscript{2} and nitrogen bases to porphyrin receptors substituted with first row transition metal elements supported on highly oriented pyrolytic graphite (HOPG), gold and silver substrates.\textsuperscript{22,24,26-29} (Electrochemical STM ligand binding studies are not considered here.)

Quantitative STM studies of molecular oxygen binding affinity to CoOEP at the phenyloctane/HOPG interface, demonstrated that while the O\textsubscript{2}–CoOEP complex is not detected in solution, it is stable at room temperature when supported on HOPG.\textsuperscript{22} Although studies of oxygen binding and oxidation reactions of manganese porphyrins on HOPG,\textsuperscript{21} Ag(111)\textsuperscript{29} and Au(111)\textsuperscript{30} in solution did not provide quantitative affinity data, they did report cooperative binding and reaction of adjacent porphyrins to oxygen.

ZnTDP, Zn(II)\textsubscript{5,10,15,20-meso-tetradecayl} porphyrin, adsorbed on graphite, was found to coordinate 3-nitropyridine more effectively than in fluid tetradecane solution.\textsuperscript{27} Imidazole, Im, reversibly coordinated to NiOEP supported on HOPG but not to the porphyrin receptors dissolved in solution.\textsuperscript{24}

Notable findings that emerged from the above reports are that (1) metalloporphyrins do not necessarily share the same ligand binding affinity on conducting surfaces and in solution and (2) enhanced binding of the axial ligands to surface supported porphyrin receptors is mediated by the substrate.

We are concerned with conducting STM experiments that quantitatively examine the binding affinity of ligands to metalloporphyrins at the solution/solid interface. Concurrently, we probe for existence of cooperativity or electronic communication between the substrate and the adsorbed porphyrins that may influence the receptors’ affinity toward ligands. In the report that follows we present a case study of 4-methoxy pyridine, MeOPy, ligation to CoOEP at the solution/solid interface and in solution. The binding of nitrogenous bases to Co(II) porphyrins in solution is well known\textsuperscript{32-35} and has been exploited in molecular recognition, chemical sensing and catalytic functions as well as for the synthesizing new types of porphyrin chelates and supramolecular structures.\textsuperscript{11,36-39} The binding of nitrogenous ligands to Co(II) porphyrins at the solution/solid interface has not been previously investigated. The MeOPy was chosen for this study because the ligand’s electron donating para-methoxy group is expected to increase the ligand–porphyrin complex stability and minimize steric effects.

Imaging is used to follow the dynamics of MeOPy binding to HOPG surface supported CoOEP in phenyloctane (Figure 1). Electronic spectroscopy was employed for binding reaction

Figure 1. Molecular models of cobalt(II)octaethyl porphyrin (CoOEP) and 4-methoxypyridine (MeOPy) molecules.
analysis in fluid toluene solution. In both experimental approaches, the respective ligand affinities (ΔG) were determined from the corresponding equilibrium constants. Temperature dependent STM studies yielded additional thermodynamic values. Associated theoretical calculations of the thermodynamic parameters provide comparison with the experimental values. Using the experimental distribution of MeOPy–CoOEP at the solution/solid interface and we demonstrate the existence of positive cooperativity in MeOPy binding to CoOEP.

EXPERIMENTAL SECTION

Materials: 2,3,7,8,12,13,17,18-Octaethyl-21H,23H-porphine cobalt(II) [CoOEP] and 4-methoxypryidine (MeOPy) were purchased from Aldrich. Phenyl octane (99%) was acquired from TCI Organics. Toluene (99.7%) was obtained from JT Baker. All reagents were used without further purification. Highly ordered pyrolytic graphite (HOPG) substrates, 1 cm², ZYA grade were purchased from Tips Nano.

Scanning Tunneling Microscopy: In all STM experiments a freshly cleaved HOPG surface was affixed in a Teflon solution cell (maximum volume 100 μL). The substrate was in contact with either a variable temperature hot stage or a 1X Peltier heating/cooling stage outfitted with a Lakeshore 330 temperature controller with a range of −10 to 150 °C. A calibrated platinum resistance thermometer was used to monitor the temperature. The entire STM experiment was housed in an environmental chamber outfitted with gas inlets and outlets. All experiments were performed under argon. This set-up was described previously.28 Images were recorded using a Molecular Imaging (currently Keysight) Pico 5 STM equipped with a 1 μm² scanner. STM tips were made by mechanically cutting Pt₀.₈Ir₀.₂ wire (California Fine Wire Company Grover Beach, Ca.).

Electronic Spectroscopy: Solution phase ligand binding studies were carried out using a Thermo Scientific Evolution 260 Bio UV-Visible spectrophotometer with 1 cm path length quartz cuvettes. CoOEP and MeOPy solution samples were prepared with analytical grade toluene and phenyl octane and gave similar extinction coefficient values. Spectra were collected at 22 °C.

Computational Methods: Computations are performed with periodic density functional theory (DFT) using Vienna Ab-initio Simulation Package (VASP)⁴⁰,⁴¹, version 5.4.4. or with the program Gaussian 09.⁴² The DFT calculations were performed using the B3LYP functional and the 6-311G(d,p) basis. All Gaussian calculations were made on single molecules in the gas-phase or in toluene using the SCRF model with the SMD option. The VASP code uses the projector augmented wave (PAW) method⁴¹,⁴³ to describe the core electrons and valence–core interactions. We used optB88-vdW functional⁴⁴,⁴⁵ with PAW potentials optimized for the PBE functional⁴⁶ for all calculations. The electronic wavefunctions are sampled using a Gamma (Γ) point in the irreducible Brillouin zone (BZ) using the Monkhorst and Pack (MP)⁴⁷ method. A plane wave cut off energy of 550 eV was used for all simulations. Methfessel–Paxton smearing was used to set the partial occupancies for each wave function with a smearing width of 0.2 eV. All the geometries were fully optimized up to ~0.001 eV energy convergence. The choice of our DFT methodology, plane wave cutoff energies and k-point choice was based on previous periodic DFT simulations of similar systems of type²⁴,⁴⁸–⁵¹ and size.⁵² Additional computational details are presented in section 1 of the electronic supplementary information (ESI). VASP calculations were performed on species adsorbed to 2-layer graphite and on the same species in the gas phase.

RESULTS AND DISCUSSION
Binding of MeOPy to CoOEP in toluene solution.

Ligand affinity is quantitatively expressed by an experimentally measured equilibrium constant or, equivalently, free energy difference between the bound and free states of the system calculated from the equilibrium constant. Previous equilibrium binding studies of different nitrogenous bases to Co(II) porphyrins in solution have reported that these metal complexes tend to form five- and six-coordinate systems. Furthermore, the formation (or stability) constants, $K_s$, for the five-coordinate complexes were found to be significantly larger than $K_s$ for the cobalt porphyrins bound to two ligands. Pyridine based ligands are known to predominantly form penta-coordinate Co(II) porphyrin complexes.

The binding affinity studies of MeOPy to CoOEP in solution as a function of ligand concentration were carried in toluene under ambient conditions. Because five-coordinate amine cobalt porphyrin adducts do not bind oxygen in solution at room temperature, inert environment was not necessary. The titration experiments were followed by UV-visible absorption spectroscopy and the resulting spectral data are depicted in Figure 2. Increasing the solution concentration of MeOPy, produced a decrease in the intensity of the CoOEP Soret band at 394.5 nm, and Q bands at 553 nm, along with a concurrent appearance of a new peak at 422 nm. Clear isobestic points were observed at 403 and 542.5 nm (see Figure S2 in the ESI for more details) until the concentration of the ligand reached approximately 0.1 M confirming the formation of a stoichiometric, 1:1 MeOPy to CoOEP adduct. Above 0.1 M MeOPy solution concentration, at which the ligand to porphyrin ratio is greater than $10^4$:1, a nonisosbestic trend developed in the titration spectra. Similar solution equilibria trends were reported for the spectroscopic titration of tetra(p-methoxyphenyl)porphinaotocobalt(II), Co(p-OCH₃)TPP with different pyridine based ligands. Here, the nonisosbestic behavior (which also occurred near 0.1 M in amine) was attributed to the formation of molecular complexes between the aromatic amine and the π-system of the porphyrin.

For the complexation reaction:

$$\text{MeOPy} + \text{CoOEP} \rightleftharpoons \text{MeOPy–CoOEP} \quad (1)$$

the stability constant, $K_s$, defined as

$$K_s = \frac{[\text{MeOPy–CoOEP}]}{[\text{CoOEP}][\text{MeOPy}]} \quad (2)$$

was determined by fitting the change in the absorption maxima at 394.5 nm in Figure 2 as
function of the concentration of MeOPy added using a non-linear curve fitting algorithm. A similar fitting method was used to determine the equilibrium constant for the ligation of ZnTDP by 3-nitropyridine in n-tetradecane solution. As in the case of MeOPy-CoOEP system, a decrease in intensity and a nominal red shift of the zinc porphyrin Soret band was observed with increasing nitro pyridine ligand concentration.

The total absorbance measured at 394.5 nm in Figure 2 is equal to the sum of absorbance of the parent porphyrin and the absorbance of the ligated complex:

$$A_{\text{total}} = A_{\text{CoOEP}} + A_{\text{MeOPy-CoOEP}}$$ (3)

The observed change in absorbance is defined as:

$$\frac{\Delta A}{[\text{CoOEP}]_0} = \Delta \varepsilon \frac{K_{\text{MeOPy}}}{1+K_{\text{MeOPy}}}$$ (4)

where $\Delta \varepsilon$ is the difference in extinction coefficient between the porphyrin’s complex state and $[\text{CoOEP}]_0$, the initial concentration of CoOEP (additional details of the fitting process can be found in the ESI). By applying Equation 4 to the absorbance data in Figure 2 one obtains a very satisfactory fit for the formation of the 1:1 five coordinate porphyrin complex. This fit is represented by the solid line in the graph in Figure 3. The calculated stability constant for the MeOPy:CoOEP system is $890 \pm 65$ M$^{-1}$, a value comparable to $K_s$ for other cobalt porphyrins coordinated to a single pyridine based ligand.

Using the relationship, $\Delta G_{\text{soln}} = -RT \ln K_s$, the standard free energy of $-16.8 \pm 0.2$ kJ/mol was calculated for the formation of the MeOPy:CoOEP complex at 298 K. This free energy value is comparable to the $\Delta G_{\text{soln}}$ quantities of $-15.3 \pm 0.2$ kJ/mol and $-16.5 \pm 0.2$ kJ/mol reported for Co(p-OCH$_3$)TPP complexed to a single pyridine and 4-methylpyridine ligand, respectively. For pyridine ligated to Co(II)tetraphenylporphyrin, CoTPP, in toluene solution, $\Delta G_{\text{soln}}$ corresponded to $-15.3$ kJ/mol. In general, the magnitude of the free energy parallels ligand bond strength or ligand affinity. For nitrogenous bases, the ligand bond strength has been shown to increase with the increasing basicity (or $pK_a$) of the ligand. Thus, by comparing $pK_a$ values for pyridine (5.22), 4-methylpyridine (5.98) and MeOPy (6.58) it is clear that MeOPy is most basic in this series and is expected to have the highest binding affinity. This is supported by the trend in the $\Delta G_{\text{soln}}$ values for these ligands.

**Binding of MeOPy to CoOEP at phenyl octane/HOPG interface.**

1. **Ligand concentration dependence.**

For nitrogenous bases, the ligand bond strength has been shown to increase with the increasing basicity (or $pK_a$) of the ligand. Thus, by comparing $pK_a$ values for pyridine (5.22), 4-methylpyridine (5.98) and MeOPy (6.58) it is clear that MeOPy is most basic in this series and is expected to have the highest binding affinity. This is supported by the trend in the $\Delta G_{\text{soln}}$ values for these ligands.

**Figure 4.** STM image of equal volumes of $1 \times 10^{-5}$ M of CoOEP after addition of $5 \times 10^{-4}$ M acquired at the phenyl octane/HOPG interface. The constant current imaging was performed at room temperature under Ar with $+500$ mV bias and 20 pA setpoint. The white circles indicate ligated CoOEP/HOPG molecules. Cross-sectional profile is shown below the image.
All STM experiments investigating the binding of MeOPy to CoOEP were carried out in argon atmosphere to eliminate a possible interference of molecular oxygen which has been shown to readily bind to CoOEP at the octyl-benzene/HOPG interface.\textsuperscript{22} Initially, 10 μL of 20 μM cobalt porphyrin in phenyloctane was added to a STM solution cell holding the HOPG substrate. After verifying that the substrate surface was uniformly covered with a monolayer of the porphyrin, a varied concentration of MeOPy ligand in phenyloctane (10 μL volume) was introduced to the STM solution cell and mixed gently with a pipette tip. The sample was allowed to equilibrate for 20 minutes before imaging. Based on the binding of MeOPy to CoOEP in solution (Figure 2), the lowest concentration of the ligand added was 10 times greater than that of metal porphyrin in order to assure observation of surface MeOPY–CoOEP complexes.

Figure 4 represents a typical STM image for CoOEP at the phenyloctane/HOPG interface at room temperature after addition of a 5 x 10^{-4} M MeOPy solution. Here, two types of features are readily identified: bright and dim (circled white). The unligated CoOEP molecules are recognized as the bright features due to electron tunneling through the half-filled d_{z^2} orbitals of the cobalt ions.\textsuperscript{54} The dim spots are assigned to the MeOPY–CoOEP coordinated species. The cross-sectional profile in Figure 4, emphasizes the difference between the ligated and unbound molecules. The low conductivity of ligated cobalt cores is due to the MeOPy lack of electronic states near the Fermi level that attenuate the signal and allow differentiation between the ligated and unligated species in STM images. Similar tunneling contrast was observed in images CoOEP was bound to molecular oxygen at the phenyloctane/HOPG interface where the coordinated cobalt centers appeared dim.\textsuperscript{22}

To determine if the MeOPy ligation to CoOEP is a dynamic process, a series of consecutive scans were recorded for prolonged periods of time over the same sample area containing both bright and dark molecules (Figure S3 in the ESI). A ‘blinking’, i.e. vanishing and appearance of the dark molecules confirmed that the binding process was indeed reversible. Two sets of such consecutive image series where the concentration of the MeOPy ligand varied were analyzed for potential system equilibration. Here, the surface coverage (θ) is defined as the number of bright molecules in an image (MeOPY–CoOEP) divided by the total number of CoOEP surface molecules. The data in Figure 5 were collected after the system had been allowed to come to equilibrium for 2 hours at 22 °C. The STM images analyzed contained about 225 surface CoOEP molecules. Clearly, there is exchange of MeOPy occurring between the solution and the surface supported CoOEP and the ligation process has reached equilibrium.
For quantitative evaluation of the binding affinity of the MeOPy toward CoOEP/HOPG system, several of STM experiments were conducted where the concentration of the MeOPy ligand in solution varied from 0.1 mM to 0.8 mM. Using the previously defined $\theta$, as the surface coverage of the MeO Py–CoOEP complex, we plotted the quantity $\theta/(1-\theta)$ as a function of the solution concentration of the MeOPy ligand (Figure 6). The equilibrium data for MeOPy binding to CoOEP can be fit the Langmuir adsorption model which assumes a single binding event and a maximum binding capacity corresponding to monolayer surface coverage.

The Langmuir equilibrium constant for MeOPy binding to CoOEP/HOPG in solution can be written as

$$K_c = \frac{\theta}{(1-\theta)(c/c_0)}$$

where $c_0$ is taken the solution standard state of 1 M MeOPy and the standard state coverage is 0.5. The slope of the line in Figure 6 provides the value of $K_c$ which then can be used to calculate the change in the free energy. For the current system one arrives at $\Delta G_c(295 K)$ of $-13.0 \pm 0.3$ kJ/mol.

2. Temperature Dependence.

To obtain the remaining thermodynamic functions additional STM data was collected from ligand binding experiments at temperatures of 15° C, 30 °C and 45 °C (Figure 7). Thermodynamic values, $\Delta S_c$ and $\Delta H_c$ were determined from a linear curve fit of $\Delta G_c$ as a function of temperature, Figure 8. Using the definition for entropy as, $\Delta S_c = -\left(\frac{\partial G}{\partial T}\right)_c$ and the change in enthalpy as, $\Delta H_c = \Delta G_c + T\Delta S_c$ one obtains the values $\Delta S_c = -120 \pm 17$ J/mol·K and $\Delta H_c = -50 \pm 5$ kJ/mol for these state functions. The large

![Figure 6](image)

*Figure 6.* The quantity $\theta/(1-\theta)$ plotted as a function of MeOPy concentration in the solution at 22° C. Error bars indicate ±1 standard deviation.

![Figure 7](image)

*Figure 7.* Representative STM images collected in constant current imaging mode of MeOPy binding to CoOEP at the phenylloctane/HOPG interface at different temperatures (a) 15° C, (b) 30° C and (c) 45° C. Imaging conditions were (a) +400 mV bias and 15 pA setpoint, (b) -600 mV, 30 pA, (c) -600 mV, 35 pA.
negative entropy change is due to the loss of translational and rotational degrees of freedom by the ligand–porphyrin complex absorbed on the surface. For comparison, the change in the entropy was derived from statistical mechanics. The calculated value of $\Delta S_c$ is $-116 \text{ J/mol}$. The y-intercept of that line is $\Delta H_c$, with a value of $-47 \text{ kJ/mol}$.

The experimental free energy value for the ligation reaction of MeOPy to CoOEP in solution ($-16.8 \pm 0.2 \text{ kJ/mole}$) is more negative than the $\Delta G$ for cobalt porphyrin complexed at the solution/HOPG interface ($-13.0 \pm 0.3 \text{ kJ/mol}$). This is also the case for the computed values (Table 1). We infer that the ligand binding affinity of the metalloporphyrin is higher in a solvent than when CoOEP is bound to the HOPG substrate (Table 1).

The DFT calculated thermodynamic values (Table 1 and section 1 of the ESI) are in excellent agreement with experiment considering the relatively low level of theory used. Perhaps most importantly theory predicts that the surface bound MeOPy should be less stable than the same species in solution – as is observed.

### Table 1. Experimental and calculated thermodynamic values for the formation of a five coordinate MeOPy–CoOEP complex at 298 °C.

<table>
<thead>
<tr>
<th>System</th>
<th>$K_w(c)$ (M$^{-1}$)</th>
<th>$\Delta G$ (kJ/mol)</th>
<th>$\Delta H$ (kJ/mol)</th>
<th>$\Delta S$ (J/Kmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOPy–CoOEP (sol.)</td>
<td>890</td>
<td>-16.8±0.2</td>
<td>-23</td>
<td>-58</td>
</tr>
<tr>
<td>MeOPy–CoOEP (HOPG)</td>
<td>190</td>
<td>-13.0±0.3</td>
<td>-20</td>
<td>-55</td>
</tr>
</tbody>
</table>

**Figure 8.** $\Delta G_c$ plotted as a function of temperature. The solid line represents a best fit of the experimental data where the slope 120 J/mol is the measured $\Delta S_c$ relative to 1 M MeOPy standard state. The dashed line is shown with a slope equal to $\Delta S_c$ calculated through statistical mechanics, $-116 \text{ J/K mol}$.

**Figure 9.** Comparison of the fraction of ligated CoOEP plotted against the initial concentration of MeOPy in solution (blue line) and adsorbed onto HOPG (black line). The ligand affinity data in solution is based on electronic spectroscopy. The fraction of ligated CoOEP/HOPG molecules was determined from STM imaging experiments.
A different competition for ligand binding was reported when HOPG was exposed to premixed \( n \)-tetradecane solutions of 3-nitropyridine, \( \text{NO}_2 \text{Py} \) and \( \text{ZnTDP} \).\(^{27}\) Although the measured formation constant for \( \text{NO}_2 \text{Py} - \text{ZnTDP} \) in solution was very large (2.0±0.5 x 10^4 M\(^{-1}\)), STM images revealed that the percentage of bound surface molecules was greater than the percentage in solution.\(^{27}\) Thus, \( \text{NO}_2 \text{Py} \) binds more strongly to surface supported \( \text{ZnTDP} \) than it does to \( \text{ZnTDP} \) in solution.

### 3. High ligand concentration induced desorption of \( \text{CoOEP} \) from HOPG

Low affinity binding in solution implies that a relatively high concentration of the MeOPy ligand is required before the maximum binding to \( \text{CoOEP} \) on the surface is achieved. At ligand concentrations below about 0.8 mM, the number of bound porphyrin sites on HOPG scales roughly linearly with increasing concentration of MeOPy (Figure 6) with the CoOEP monolayer remaining intact. However, when the concentration of MeOPy is increased above 1 mM (i.e. 100 times the concentration of CoOEP), significant desorption of the molecular monolayer (about 20%) at the grain boundaries and within the monolayer itself is observed. Figure 10 shows STM images of an intact porphyrin monolayer and a partially disrupted monolayer. The depleted monolayer does not reconstruct or heal with scanning time. The same surface monolayer coverage trend was observed in STM experiments when premixed solutions containing similar ratios of the MeOPy and CoOEP as in sequential depositions were employed (Figure S4 in the ESI).

The above results are very surprising because previous STM studies of \( \text{CoOEP} \) at the phenyloctane/HOPG (or Au) interface showed that the porphyrin monolayer is quite stable and does not do not desorb from the substrate until about 70 °C.\(^{56-58}\) A possible explanation for the loss of monolayer from the HOPG surface, is that it forms a soluble six coordinate porphyrin complex at high MeOPy concentrations.

![Figure 10. Low resolution 40 x 80 nm STM images comparing two different ratios of MeOPy and CoOEP ratios at the phenyloctane/HOPG interface (a) 35:1 and (b) 100:1. Images were acquired under inert atmosphere at 22° C; -500 mV and (a) 25 pA, (b) 20 pA.](image)

Desorption from grain boundaries and defects is much faster than from within the monolayer and once the MeOPy–\( \text{CoOEP} \) or \( \text{CoOEP} \) complex desorbs it might be converted to the 6-coordinates species that does not form an ordered monolayer. This is less likely for the MeOPy–\( \text{CoOEP} \) complex for two reasons. First, pyridine based ligands mostly form penta-coordinate cobalt porphyrin complexes. Second, an MeOPy to CoOEP ratio greater than 10^4:1 is required for the formation of a six coordinate complex in solution.\(^{32}\) Under the conditions where loss of monolayer is first observed, the MeOPy ligand concentration is only 200 times that of the CoOEP.

An alternative explanation might be that the MeOPy-CoOEP complex desorbs more easily from HOPG than the parent CoOEP complex. In order to test this, we performed density functional calculations to determine the desorption energies both in UHV and in solution (details of the calculations are presented in the ESI).
The enthalpy change for the gas phase desorption reaction, Equation 6, at zero K is simply the electronic energy difference as computed by VASP. With the assumption that the internal energies of the HOPG and CoOEP do not change with desorption, the change in enthalpy is the change in zero point energy plus 3RT. This result and the related one for Equation 7 are given in Table 2.

\[
\text{CoOEP/HOPG} \rightleftharpoons \text{CoOEP} + \text{HOPG} \quad (6)
\]

\[
\text{MeOPy-CoOEP/HOPG} \rightleftharpoons \text{MeOPy-CoOEP} + \text{HOPG} \quad (7)
\]

The calculation in solution requires accounting for immersion of the reactants and products in solvent. The heat of immersion of CoOEP is a direct result of our Gaussian calculations (see ESI section 1). The heat of immersion of CoOEP/HOPG was determined using the solvent accessible area of the surface supported species and the computed heat of immersion of CoOEP (see Supplementary Information for details).\(^{59}\) A similar calculation was performed for MeOPy-CoOEP/HOPG. The heat of immersion of HOPG in toluene was also calculated using Gaussian in order to keep a consistent model throughout. To do this a 2-layer slab of HOPG was hydrogen terminated to make the super-molecule \(C_{164}H_{48}\) and the same SCRF(SMD) procedure was used to determine the heat of immersion. This heat of immersion (\(-256\) kJ/mole) was then multiplied by the ratio of the effective area per porphyrin divided by the area of carbon in the supermolecule in order to obtain the heat of immersion of HOPG per mole of porphyrin (\(-97\) kJ/mole – porphyrin).

The resulting solution phase desorption energies,

\[
\Delta H_{\text{sol}} = \Delta H_g + \Delta H_i(\text{HOPG}) + \Delta H(\text{porphyrin}) - \Delta H(\text{porphyrin/HOPG}) \quad (8)
\]

are given in Table 2.

Although the VASP computed desorption energies are probably an overestimate and they are significantly reduced by the effects of solvation, they are clearly very similar in size. The values in Table 2 are probably too high since it is known from previous experiments,\(^{56,57}\) that the activation energy for desorption is \(125\) kJ/mole for CoOEP/HOPG in phenylloctane.\(^{58,59}\) We note, however, that thermodynamic desorption energies should be smaller than the desorption activation energy.\(^{60}\) The important result here is that the desorption energy for MeOPy-CoOEP is very close to that of CoOEP and is quite large. Thus, it seems unlikely that a preferential desorption of MeOPy–CoOEP is responsible for the loss of the monolayer.

A third origin for the monolayer dissolution may lay in the proposal offered by Walker for nonisosbestic behavior of the absorption spectra of related complexes.\(^{32}\) Aggregation of ligand molecules about the porphyrin ring may somehow inhibit the formation of an ordered monolayer (once a complex has desorbed from a grain boundary or defect). Such aggregation onto the adsorbed complex might also significantly lower the desorption energy. While this is occurring at about \(1/10^{th}\) the concentration where it is clearly seen in the absorbance spectrum, there may be more than one possible aggregate structure. It is also possible that the residence time of MeOPy on HOPG is long enough that at high concentrations it effectively blocks return of the porphyrin to the HOPG surface. Interestingly, the dissolution effect is reduced with increasing temperature.

Experiments are currently underway to better understand the driving cause(s) for the desorption of MeOPy–CoOEP from the graphite surface.

<table>
<thead>
<tr>
<th>System</th>
<th>(\Delta H_{\text{vap}}) kJ/mole</th>
<th>(\Delta H_{\text{sol}}) kJ/mole</th>
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<tbody>
<tr>
<td>CoOEP/HOPG</td>
<td>373</td>
<td>240</td>
</tr>
<tr>
<td>MeOPy–CoOEP/HOPG</td>
<td>383</td>
<td>237</td>
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</table>
4. Cooperativity

Electronic communication between the substrate and the adsorbed porphyrin can influence the receptor’s affinity toward an exogenous ligand. Possible cooperativity operating between adjacent CoOEP receptors on HOPG and the MeOPy ligands was examined by inspecting the distribution of dark molecules (ligated molecules) in the cobalt porphyrin monolayer and comparing the result with calculated random distribution. The details of the nearest neighbor analysis are provided in the Supplementary Information along with a representative STM image (Figure S5) that identifies the different grouping of the MeOPy−CoOEP surface species. In Figure 11, the experimental distribution (orange bars) was determined by counting the number of adjacent molecules that are ligated. Images that showed 15% surface ligated species were used in this analysis for a total of nearly 4000 molecules counted. The random distribution was modeled by a binomial distribution, green bars in Figure 11, for the case when \( \theta = 15\% \). The random distribution simulates the case where there is no preference for MeOPy binding to adjacent CoOEP molecules. If the distribution of dark nearest neighbors was random, we would expect the calculated fractions to equal to the experimentally determined fraction of MeOPy−CoOEP. This, however, is not what we observe. Comparing the experimental and random distributions we see an increase in the numbers of pairs and larger groupings of three and four of molecules for the experimental set. This result suggests that the binding of MeOPy to a given CoOEP molecule on HOPG substrate increases the chance that another MeOPy will bind to a neighboring molecule in the monolayer – an indication of surface mediated positive cooperativity.

It is important to note that the coverage represented by Figure 11 is at the extreme end of the data used to derive the Langmuir equilibrium constant. Cooperativity, therefore, is playing only a small role in the data used in Figure 8 and the corresponding analysis. The concentration region where cooperativity would play a significant role is the same region where the monolayer begins to dissolve.

CONCLUSION

In this work we have shown that MeOPy binds to CoOEP adsorbed on HOPG and when dissolved in fluid solution. The coordination of the ligand to CoOEP adsorbed on HOPG at low MeOPy concentration followed a simple Langmuir adsorption isotherm. Contributions of free energy, enthalpy and entropy to the binding process on the surface and in solution were experimentally determined and computationally estimated. Both methods are in satisfactory agreement. The free energy value for the ligation reaction of MeOPy to CoOEP/HOPG at the solution interface, \(-13.0\pm0.3\) kJ/mol, is less negative than the \( \Delta G \) for cobalt porphyrin complexed in solution, \(-16.8\pm0.2\) kJ/mol, leading us to conclude that the ligand binding affinity of the metalloporphyrin is higher in solution than when CoOEP is bound to the HOPG substrate.

Increasing the concentration of MeOPy to 100
times that of the CoOEP receptor initiates a significant desorption of the molecular monolayer at the grain boundaries and within the monolayer itself. The depleted monolayer does not reconstruct or heal with scanning time, although there are obvious changes in shape of the bare areas. The same surface monolayer coverage trend was observed in STM experiments where premixed solutions containing similar ratios of the MeOPy and CoOEP as in cases where sequential depositions were employed. At this time, we speculate that dissolution of the molecular monolayer may be due to solvation of the five-coordinate pyridinate complex and/or the CoOEP complex by excess pyridine.

At higher concentrations of MeOPy, the distribution of ligated porphyrins in the CoOEP/HOPG monolayer shows a preference for MeOPy binding in groups of two or more indicating that the CoOEP receptors’ reactivity is moderated by the HOPG substrate in a way that leads to positive cooperativity.

Computational studies based on density functional methods were used to determine $\Delta S$ and $DH$ values for the solution and surface reactions that were in very good agreement with experiment. The agreement between measured and computed changes in free energy are good, but the computed values are too large. They do show the appropriate trend – The $DG$ for reaction at the surface is not as negative as that for the reaction in solution.

ACKNOWLEDGMENT
This material is based upon work supported by the National Science Foundation under grant CHE-1800070. We gratefully acknowledge their support.

FOOTNOTE
†Electronic Supplementary Information (ESI) is available. Included are the details of the DFT calculations for the equilibrium thermodynamic data for the binding of MeOPy–CoOEP and for the desorption energies; additional STM images of the MeOPy binding process; nearest neighbor analysis of cooperative ligand binding. See DOI:

REFERENCES


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CONFLICTS OF INTEREST
The authors declare no competing financial interest.


Supporting Information

Dynamics of on surface ligation of cobalt(II)octaethyl porphyrin by 4-methoxypyridine: a scanning tunneling microscopy study

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STM time dependent MeOPy and CoOEP reaction imaging series  4
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DFT Computational Details

Gaussian (ub3lyp/6311g for the cobalt complexes and b3lyp/6-311G for all singlet state species) were performed on all species reported here. VASP calculations were performed with the optB88-vdW functional with PAW potentials optimized for the PBE functional for all calculations.

In both the free molecule and adsorbed state calculations, the low energy configuration of the MeOPy-CoOEP was the one shown in Figure S1 with the Pyridine ring bisecting the N-Co-N angle. Also shown in S1 is the supercell used in the VASP calculations but expanded by two in the a and b directions to aid understanding of the system calculated. The graphite layer is colored yellow for clarity. The 2 layer graphite was first optimized and then the bottom layer fixed in all subsequent optimizations. The top layer and all atoms of adsorbates were optimized to an energy of 1 mV or better. VASP calculations were performed for MeOPy, MeOPy-CoOEP, and CoOEP in 2x2x1 (otherwise empty) supercells in order to more correctly calculate free molecule energies.

Table S1 presents the computed energies of the various systems with “Big Box” indicating that a

<table>
<thead>
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<th>Species</th>
<th>eV</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOPG</td>
<td>-1150.6446</td>
</tr>
<tr>
<td>CoOEP M</td>
<td>-472.9750</td>
</tr>
<tr>
<td>CoOEP BigBox</td>
<td>-472.6469</td>
</tr>
<tr>
<td>CoOEP hopg</td>
<td>-1627.0814</td>
</tr>
<tr>
<td>MPyCoOEP M</td>
<td>-556.6455</td>
</tr>
<tr>
<td>MPyCoOEP Big Box</td>
<td>-556.4028</td>
</tr>
<tr>
<td>MPyCoOEP hopg</td>
<td>-1710.9419</td>
</tr>
<tr>
<td>MPy big box</td>
<td>-82.7643</td>
</tr>
<tr>
<td>CoOEP/G = G + CoOEP</td>
<td>3.79</td>
</tr>
<tr>
<td>MPyCoOEP/G = G + MPyCoOEP</td>
<td>3.89</td>
</tr>
</tbody>
</table>
2x21 supercell was used to minimize communication between molecules in cells. The M notation indicates that the same monolayer structure as for the HOPG supported system was used, but the HOPG was not included. For example, the condensation of the CoOEP into the monolayer structure in the absence of HOPG is predicted to be 328 mV more stable. The last two entries in the table are the electronic desorption energies into the gas phase. It is useful to note that the gas phase electronic energy difference predicted for the reactions (where s designates adsorbed in the HOPG surface):

\[
\text{MeOPy(g) + CoOEP(s) = MeOPy-CoOEP(s) \quad \text{rxn 1}}
\]
\[
\text{MeOPy(g) + CoOEP(g) = MeOPy-CoOEP(g) \quad \text{rxn 2}}
\]

are \(\Delta E_1 = -106 \text{ kJ/mole for rxn 1}\) and \(-96 \text{ kJ/mole for rxn 2}\).

This is a small difference and indicates that the HOPG surface is not significantly affecting the reaction energetics.

Table S2. Gaussian calculations for molecules in toluene. * indicates entropy correction for free volume of solution

<table>
<thead>
<tr>
<th></th>
<th>H (eV)</th>
<th>G(eV)</th>
<th>(E_{\text{elect}}) (eV)</th>
<th>St+r</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOPy-CoOEP conf 1 vacuum G09</td>
<td>6-311G</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MeOPy-CoOEP conf 2 vacuum G09</td>
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<td>-3362.087827</td>
<td>-3362.226061</td>
<td>-3362.9895</td>
<td></td>
</tr>
<tr>
<td>MeOPy-CoOEP conf 2 toluene G09</td>
<td></td>
<td>-3362.128864</td>
<td>-3362.262154</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delta of immersion in kJ/mole</td>
<td></td>
<td>-107.7</td>
<td>-106.9*</td>
<td>353.13</td>
<td>1173.61</td>
</tr>
</tbody>
</table>

|                     |       |       |                         |      |         |
| MeOPy vacuum       |        | -362.65915 | -362.697921 | -362.78817 |         |
| MeOPy in toluene   |        | -362.668469 | -362.707349 |             |         |
| Delta of immersion in kJ/mole |        | -24.5 | -36.9* | 284.05 | 342.38 |

|                      |        |       |                         |      |         |
| CoOEP vacuum G09    |        | -2999.408699 | -2999.523341 | -3000.1787 |         |
| CoOEP toluene G09   |        | -2999.442608 | -2999.555535 |             |         |
| Delta of immersion in kJ/mole |        | -89.0 | -96.7* | 348.40 | 994.54 |
| \(\Delta(\text{RXN})\) Gas |        | -52.45 | -12.60 | -59.47 | -163.3 |
| \(\Delta(\text{RXN})\) Solution |        | -46.70 | -9.37* |             | -125.4* |

Table S2 collects the results of the Gaussian calculations. Note that the predicted electronic energy change for rxn 2 is of the same order but about 30% less than that from the VASP calculation.

An additional pair of Gaussian calculations were performed in order to obtain a heat of immersion for HOPG that would be internally consistent with the other heats of immersion. We created an H terminated slab of graphite from the 2 layers used in the calculation but containing a total of 164 carbons (C\(_{164}H_{48}\)) and used the SCRF(SMD) model to calculate the heat of immersion. The result was -249.086 kJ/mole of the flake, The convert this to per mole of CoOEP (and therefore MeOPy-
CoOEP) we used carbon area of the flake (4.25 nm²) and that of the CoOEP (1.61 nm²) to get a heat of immersion of HOPG of -94.55 kJ/mole of CoOEP area.

One final thermodynamic issue must be addressed. The Gaussian calculation of entropy and free energy in solution treats the system as if it has the full volume of a mole of gas at 298K. Obviously, this is not appropriate for a solution. One way to correct for this is to simply compress that gas from 24.45 L to the 1 mole/L standard state. This is often done. Clearly, it leaves too much free volume since the space occupied by the solvent is not considered. In this work we chose to compute the free volume in one liter of toluene by first computing the effective volume of a toluene molecule (0.132 nm²) and the volume available per molecule based on the density (0.178 nm²) and taking the difference to get 0.0455 nm²/molecule of toluene or 250 cm³ per L of toluene. The entropy correction for going from STP as a gas to 1 mole/L is therefore Rln(24.45/.25) = 38.10 J/mole K. reduction in entropy and 298*Rln(24.45/.25) (=11.35 kJ/mole) increase in G.

With these computed values, the following analysis was applied.

Consider the reaction \( A + B = C \), where each component is treated as ideal in the gas and solution phase and the total energy is assumed to be a sum of electronic, vibrational, rotational and translational parts.

Suppose \( F^p_i = F(int)_i^p + F(t, R)_i^p \) where \( F \) is a function of state and \( t,R \) are translational and rotational contributions referenced in the \( p \) phase. Because these are ideal systems, we have \( H(t, R)_i^g = 3RT \) and also \( S_i^l = S(int)_i^l + S(t, R)_i^g - Rln\left(\frac{24.45}{V_f}\right) \) where \( V_f \) is the free volume available for solute in 1 L of solution.

For a reaction:

\[
\Delta S^l = \sum (\Delta S(int)_i^l + \Delta S(t, R)_i^g - Rln\left(\frac{24.45}{V_f}\right))
\]

Since the correction term is the same for all components, one product and two reactants results in:

\[
\Delta S^l = \sum (\Delta S(int)_i^l + \Delta S(t, R)_i^g) + Rln\left(\frac{24.45}{V_f}\right)
\]

The value in () is the calculated value of \( S \) for the solution phase provided by the Gaussian program. Thus

\[
\Delta S^l = \Delta S^l_{G09} + Rln\left(\frac{24.45}{V_f}\right)
\]  

a1

Since \( \Delta H^l = \Delta H^l_{G09} \) we have

\[
\Delta G^l = \Delta G^l_{G09} - RTln\left(\frac{24.45}{V_f}\right)
\]  

a2

Or more generally for a reaction with a net loss of \( n \) moles:
\[ \Delta G^l = \Delta G^l_{G09} - nRT \ln \left( \frac{24.45}{V_f} \right) \]

And

\[ \Delta H^l = \Delta H^l_{G09} \quad \text{a3} \]

Now consider the reaction with mixed phases:

\[ A(g) + B(g) = C(g) \quad \text{a4} \]

\[ A(g) + B(s,g) = C(s,g) \quad \text{a5} \]

\[ A(l) + B(l) = C(l) \quad \text{a6} \]

\[ A(l) + B(s,l) = C(s,l) \quad \text{a7} \]

Where (s,g) designates a surface supported monolayer in contact with gas and (s, l) is the same monolayer in contact with liquid.

\[ \Delta E_4\text{(electronic)} and \Delta E_5\text{(electronic)} were computed from VASP while all the thermodynamic variables were computed for eqn a4 and a6 using Gaussian. We will now combine this information to derive \( \Delta H_7 \) and \( \Delta S_7 \), from which \( \Delta G_7 = \Delta H_7 - T \Delta S_7 \). Three approximations will be used:

1) The entropy and enthalpy of the adsorbed species B and C associated with the 6 frustrated translations and rotations cancel. This is a good approximation for the case where the adsorbates have similar physisorption forces and similar masses (as is the case here).

2) The heats of wetting of the adsorbed species are proportional to the solvent accessible surface area (SAS). 3

3) The internal motions of the adsorbed molecules are little changed by adsorption to the thermodynamic functions associated with internal motions are the same in solution as on the surface.

Thus:

\[ \Delta H_5 = -\varepsilon + \Delta H_4 - H_{T,R}(C; g) + H_{T,R}(B; g) \] where \( H_{T,R}(B; g) \) is the translational and rotational enthalpy of species B in the gas phase. The later 2 terms cancel at 298 K.

\[ \Delta H_7 = \Delta H_5 + \Delta H_t(C; s) - \Delta H_t(B; s) - \Delta H_t(A) \]

Where \( \Delta H_t(C) \) is the enthalpy associated with transporting a mole of C in the gas phase at 1 atm to a monolayer of C on an HOPG surface. \( \Delta H_t(A) \) is the enthalpy of immersion for A. That is, the enthalpy associated with transferring 1 mole of gas A at 1 atm to a 1 M solution.

\[ \Delta S_7 = \Delta S_6 - S_{T,R}(C; l) + S_{T,R}(B; l) + S_{\text{config}} \]
The last term, $S_{\text{config}}$, is the configurational entropy of a half filled monolayer that is the standard state for the Langmuir adsorption and is equal to $R \ln(2)$.

$$\Delta G_7 = \Delta H_7 - T \Delta S_7$$

Similarly

$$G(C;s) = -\varepsilon + G(C;g) - G(C;t,R)$$

$$G(B;s) = G(B;g) - G(B;t,R)$$

So $\Delta H_1 = -\varepsilon + \Delta H_0$

Where $\Delta H_0$ refers to:

$$A(g) + B(g) = C(g) \quad \text{rxn 1}$$

$$\Delta S_1 = \Delta S_0 + S(C;t,R) - S(B;t,R)$$

$$\Delta G_1 = -\varepsilon + \Delta G_0 - T(S(C;t,R) - S(B;t,R))$$

Finally, consider the reaction:

$$A(l) + B(s,l) = C(s,l) \quad \text{rxn 2}$$

In order to relate rxn 2 to rxn 1, we need to immerse all three components into the solvent. The heat and free energy of immersion of A is straightforward. They are simply given by

$$\Delta F(A, \text{imm}) = F(A, l) - F(A, g) \quad \text{a4}$$

Where the appropriate values of $F(A,l)$, the thermodynamic function of species A in the liquid phase are given by eqns a1 to a3, and $F(A,g)$ are the gas phase values read directly from Gaussian.

The wetting energies of the two surfaces are more problematic. We have adopted the following strategy. We start by assuming that the enthalpy of wetting is proportional to the solvent available surface area (SAS) of the species. With the SAS for species B and C were calculated using gaussian and the enthalpy of immersion determined as in a4 were scaled by the SAS to determine their value per unit area. For example, the enthalpy of wetting of surface C would be:

$$\Delta H(C, \text{imm}) = \text{SAS}(C) \times (H_{G09}(C, l) - H_{G09}(C, g)) \quad \text{a5}$$

We further assumed that the SAS for a CoOEP monolayer was half the total area, and the SAS for MPyCoOEP was that of the free molecule – half the area of CoOEP. These areas were then used to compute functions of wetting.
One additional term is required for adjusting the entropy change associated with rxn 2. The configurational entropy of the half-covered surface that is the standard state for adsorption. This is 

\[ S = R \ln(2) \]

With these assumptions and definitions

\[ \Delta H_2 = \Delta H_1 + \Delta H(C,\text{imm}) - \Delta B(B,\text{imm}) - \Delta H(A,\text{imm}) \]

\[ \Delta S_2 = \Delta S_1 - TR\ln(2) \]

Desorption energy in solution

\[ \text{A/HOPG} (s,g) = \text{A} (g) + \text{HOPG} (s,g) \quad \text{rxn 5} \]

\[ \text{A/HOPG} (s,l) = \text{A} (l) + \text{HOPG} (s,l) \quad \text{rxn 6} \]

The difference between rxn 6 and 5 is the difference in wetting of the two solid surfaces and the immersion of A into solution. Thus

\[ \Delta F_6 = \Delta F_5 + \Delta F(\text{HOPG,imm}) - \Delta F(\text{A/HOPG,imm}) + \Delta F(A,\text{imm}) \]

Using the same argument used above:

\[ \Delta H_6 = +\Delta H_5 + \Delta H(\text{HOPG,imm}) - \Delta H(\text{A/HOPG,imm}) + \Delta H(A,\text{imm}) \]

\[ \Delta H_5 = \Delta E_5 + RT \]

*) If the reaction energy in solution is -1 ev and on the surface it is -1.2 eV then \( \varepsilon = 0.2. \)
Figure S1. UV-Vis spectra of MOPy-CoOEP in toluene. The initial concentration of CoOEP was 5.2 μM and the concentration of MOPy was consecutively larger in each spectrum from 8.2 μM to 0.066 M. Upper chart shows the full spectrum while lower charts show zoomed in regions of the spectrum that include isosbestic points at 403 and 542.5 nm.
**Absorbance curve fitting derivation for titration of MeOPy and CoOEP in toluene**

Definitions: $P = \text{CoOEP}; L = \text{MOPy}; PL = \text{MOPy-CoOEP}; \varepsilon_P = \text{extinction coefficient of } P; \varepsilon_{PL} = \text{extinction coefficient of } PL; A = \text{absorbance at } 395 \text{ nm.}$ The electronic spectra above shows the solution phase complexation of CoOEP and MOPy following the reaction:

$$
P + L \leftrightarrow PL
$$

At equilibrium:

$$
[P] = [P]_0 - [PL]
$$

$$
[L] = [L]_0 - [PL]
$$

where $[P]_0$ is the initial concentration of the porphyrin and $[L]_0$ is the initial concentration of the ligand which is much greater than the concentration of the complex and we will use the approximation $[PL] \ll [L]_0$. Combining the above equations gives:

$$
[PL] = \frac{K[L]_0[P]_0}{1 + K[L]_0}
$$

The complex and the unbound porphyrin have differing extinction coefficients, and each follows beer’s law. Here, the path length is 1 cm and the ligand alone does not absorb light at the wavelength of interest. Therefore, the total absorbance of the system in equilibrium is given by:

$$
A_{tot} = A_p + A_{PL} = \varepsilon_P[P] + \varepsilon_{PL}[PL]
$$

Initially, absorbance is only due the porphyrin:

$$
A_0 = \varepsilon_P[P]_0
$$

$$
\Delta A = A_{tot} - A_0 = \varepsilon_P[P] + \varepsilon_{PL}[PL] - \varepsilon_P[P]_0 = \varepsilon_P([P]_0 - [PL]) + \varepsilon_{PL}[PL] - \varepsilon_0[P]_0 = \Delta \varepsilon[PL]
$$

$$
\Delta A = \Delta \varepsilon \frac{K[L][P]_0}{1 + K[L]}
$$

The titration shown in Figure SA1 provides a series of absorbance values at 395 nm. Subtracting these absorbance values from that of the same amount of CoOEP in toluene gives data points of absorbance difference versus the MeOPy concentration are shown in Figure SA2. Curve fitting the plot with the equation shown above we get $K=890 \pm 65$. Taking $\Delta G = -RT \ln K$, gives $\Delta G(295 \text{ K}) = -16.6 \text{ kJ/mol}$. Derivation was adapted from 4
Figure S2. Representative STM images collected sequentially with [CoOEP] = 10 μM and [MeOPy] = 500 μM. These images are a part of the sequence of STM images that were used to create Figure 6. Images are 15x15 nm in size, were collected at +500 mV 20 pA and took 42 sec.
STM Images of premixed CoOEP and MeOPy

Figure S3. Large scale (200 x 200 nm) STM images illustrate the difference between two sample preparation methods. The top row shows samples that were prepared first adding 10 μL of 20 μM CoOEP solution to the HOPG substrate and then adding 10 μL methoxypyridine solution with the desired concentration. All images shown in the main paper were created in this way. The lower row shows the samples that were prepared by first mixing the CoOEP and MeOPY solutions and adding 20 μL of the mixed solution to the HOPG substrate. We observe a difference in the amount of HOPG that is covered by organized adsorbed porphyrin molecules depending on the concentration of MeOPy and method of sample preparation. Numbers shown on each image show the average percentage of HOPG surface that contains organized porphyrins, organized porphyrins may occur in either bound or unbound state.

MeOPy–CoOEP nearest neighbor distribution analysis
Figure S4. Representative STM image used for nearest neighbor distribution analysis, bias is 500 mV and setpoint is 20 pA. Image collected at 22°C and 500 μM MOPy concentration. Total porphyrin molecules in image is 610, total ligated porphyrin molecules (dark spots) in image is 95; therefore, θ is 15.6%. Circles denote all ligated porphyrins in the imaging area, and they are color coded based on number of adjacent ligated porphyrins. Each CoOEP has a total of 6 neighbors when incorporated into the monolayer. The molecules near the edges of the image that do not have 6 total visible neighbors are colored grey they are used in determining number of neighbors for any adjacent porphyrins that do have 6 visible neighbors, but are not, themselves, included in the analysis.
Table S1. Completed Nearest Neighbor Analysis for Representative STM Image

<table>
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<tr>
<th>Counting type</th>
<th>Number of Ligated Neighbors</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
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<tr>
<td>Experimental count</td>
<td></td>
<td>21</td>
<td>19</td>
<td>21</td>
<td>16</td>
<td>9</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Experimental fraction</td>
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<td>0.22</td>
<td>0.24</td>
<td>0.18</td>
<td>0.10</td>
<td>0.01</td>
<td>0.00</td>
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<tr>
<td>Random Fraction (θ=15.6%)</td>
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<td>0.18</td>
<td>0.05</td>
<td>0.01</td>
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</tr>
</tbody>
</table>

Nearest Neighbor Statistics

To investigate the correlation of the ligation of porphyrin molecules and their distribution within the monolayer statistical analysis in the style of Hulsken et al. was undertaken. In the case where all ligated porphyrins are randomly distributed throughout the monolayer, each porphyrin molecule has equal probability of complexing with MOPy, and ligation events are independent of neighboring porphyrin molecules. Then at any given time the ligated porphyrins in the monolayer follow a binomial distribution. In general, the binomial distribution goes as:

\[ f(n, k, p) = \binom{n}{k} p^k (1-p)^{n-k} \]

where \( n \) is the number of independent trials, \( k \) is the number of successes, and \( p \) is the probability of success. The CoOEP monolayer has a pseudo-hexagonal lattice in which each porphyrin is surrounded by 6 nearest neighbors, so in this case \( n=6 \). The probability of success will be related to the fraction of ligated molecules, \( \theta \), and the number of successes, \( k \), will be the number of ligated nearest neighbors each porphyrin in the imaging area has. So, the chance of having \( i \) ligated direct neighbors goes as:

\[ f_i = \binom{6}{i} \theta^i (1-\theta)^{6-i} \]

In Figure SB1 and Table S1 analysis of one representative image is shown. To get Figure 4 in the main text a total of 12 individual STM images were used for a total of 3990 porphyrin molecules of which 630 were ligated to give an overall \( \theta = 15.7\% \), this \( \theta \) was used in determining the bars labeled random in Figure S3. The individual images had local \( \theta \)'s ranging from 14.1\% to 16.8\%. 

13
References