Supporting Information

An Unusual Ligand Coordination Gives Rise to a New Family of Rhodium Metalloinsertors with Improved Selectivity and Potency

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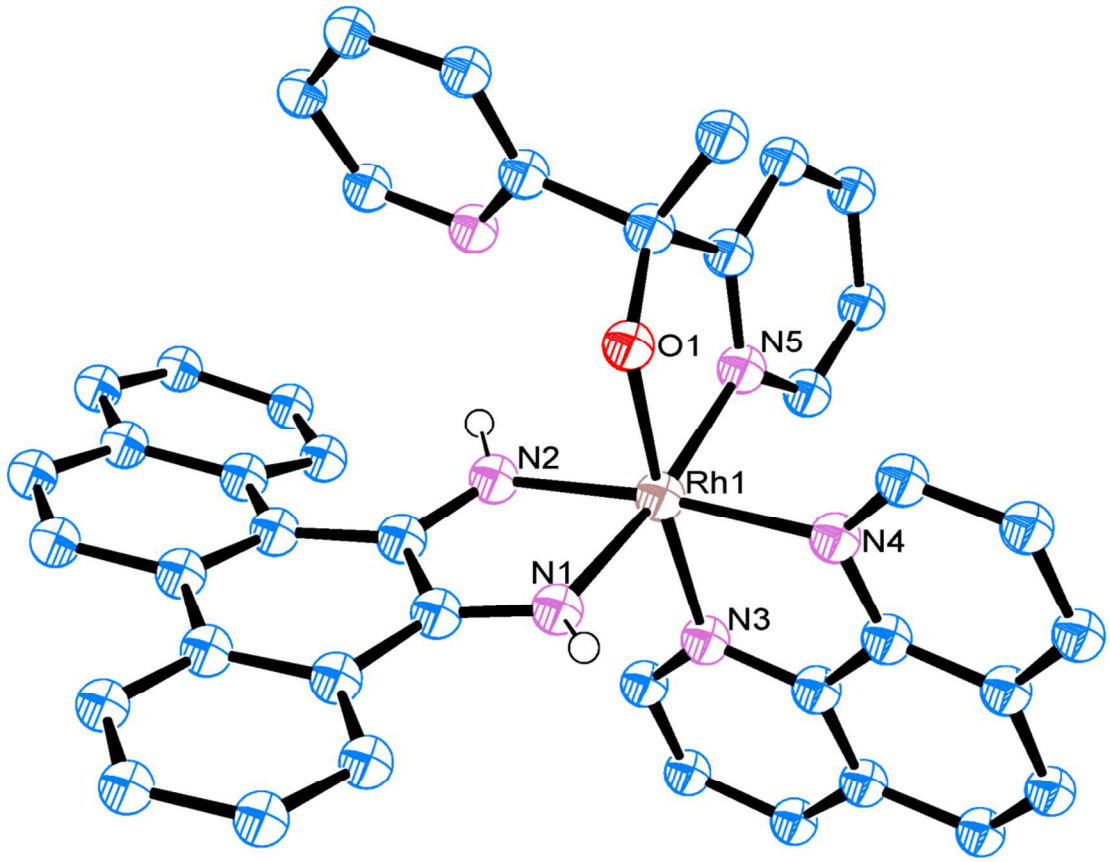

Figure S1 X-ray crystal structure of [Rh(chrysi)(phen)(DPE)]Cl_2. Displacement of ellipsoids are drawn at 50% probability. For clarity, chlorine atoms, solvent molecules, and hydrogen atoms (except immine protons) have been omitted. Selected bond lengths (Å): Rh-O 1.996, Rh-N1 2.006, Rh-N2 1.987, Rh-N3 2.046, Rh-N4 2.059, Rh-N5 2.031.

Table S1. Crystal data and structure refinement for [Rh(chrysi)(phen)(DPE)]Cl_2.

Identification code	ack007	
Empirical formula	C46 H47 Cl2 N6 O5 Rh	
Formula weight	937.70	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P -1	
Unit cell dimensions	a = 9.6145(8) Å	α= 64.434(4)°.

	b = 15.7645(13) Å β= 85.049(4)°.
	c = 16.8583(14) Å γ = 73.491(4)°.
Volume	2208.0(3) $Å^3$
Z	2
Density (calculated)	1.410 Mg/m^3
Absorption coefficient	0.560 mm^{-1}
F(000)	968
Crystal size	0.450 x 0.300 x 0.200 mm^3
Theta range for data collection	2.211 to 30.586°.
Index ranges	-13<=h<=13, -22<=k<=22, -24<=l<=24
Reflections collected	153083
Independent reflections	13536 [R(int) = 0.0310]
Completeness to theta = 25.242°	99.9 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7461 and 0.6678
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	13536 / 299 / 674
Goodness-of-fit on F^2	1.120
Final R indices [I>2sigma(I)]	R1 = 0.0418, wR2 = 0.1050
R indices (all data)	R1 = 0.0468, wR2 = 0.1110
Extinction coefficient	n/a
Largest diff. peak and hole	2.425 and -0.777 $e.Å^{-3}$

Low-temperature diffraction data (ϕ-and ω-scans) were collected on a Bruker Kappa diffractometer coupled to an Apex II CCD detector with graphite-monochromated Mo K_α radiation (λ = 0.71073 Å) for the structure of ack007. The structure was solved by direct methods using SHELXS[i] and refined against F^2 on all data by full-matrix least squares with SHELXL-2013[ii] using established refinement techniques.[iii] All non-hydrogen atoms were

refined anisotropically. Unless otherwise noted, all hydrogen atoms were included into the model at geometrically calculated positions and refined using a riding model. The isotropic displacement parameters of all hydrogen atoms were fixed to 1.2 times the *U* value of the atoms they are linked to (1.5 times for methyl groups).

ack007crystallizes in the triclinic space group $P\bar{1}$with one molecule in the asymmetric unit along with two chloride anions, two waters, and two molecules of ethanol. Each of the ethanol molecules were disordered over three positions. They were refined with the help of similarity restraints on the 1,2- and 1,3- distances and displacement parameters as well as rigid bond restraints for anisotropic displacement parameters. The occupancies of the two disordered ethanol molecules were freely refined to 0.509(3):0.261(3):0.229(3) and 0.453(3):0.295(3):0.252(3), respectively. Additional residual electron density is located near each of the disordered ethanol molecules. However, the refinement of additional ethanol positions was unsuccessful, and the current model represents the best model which led to a stable refinement. The coordinates for the hydrogen atoms bound to N1, N2, O1W and O2W were located in the difference Fourier synthesis and refined semi-freely with the help of a distance restraint. The N-H and O-H distances were restrained to be 0.88(4) Å and 0.84(4) Å, respectively. The hydrogen atoms bound to oxygen in the ethanol molecules could not be found and were included at geometrically calculated positions and refined using a riding model.

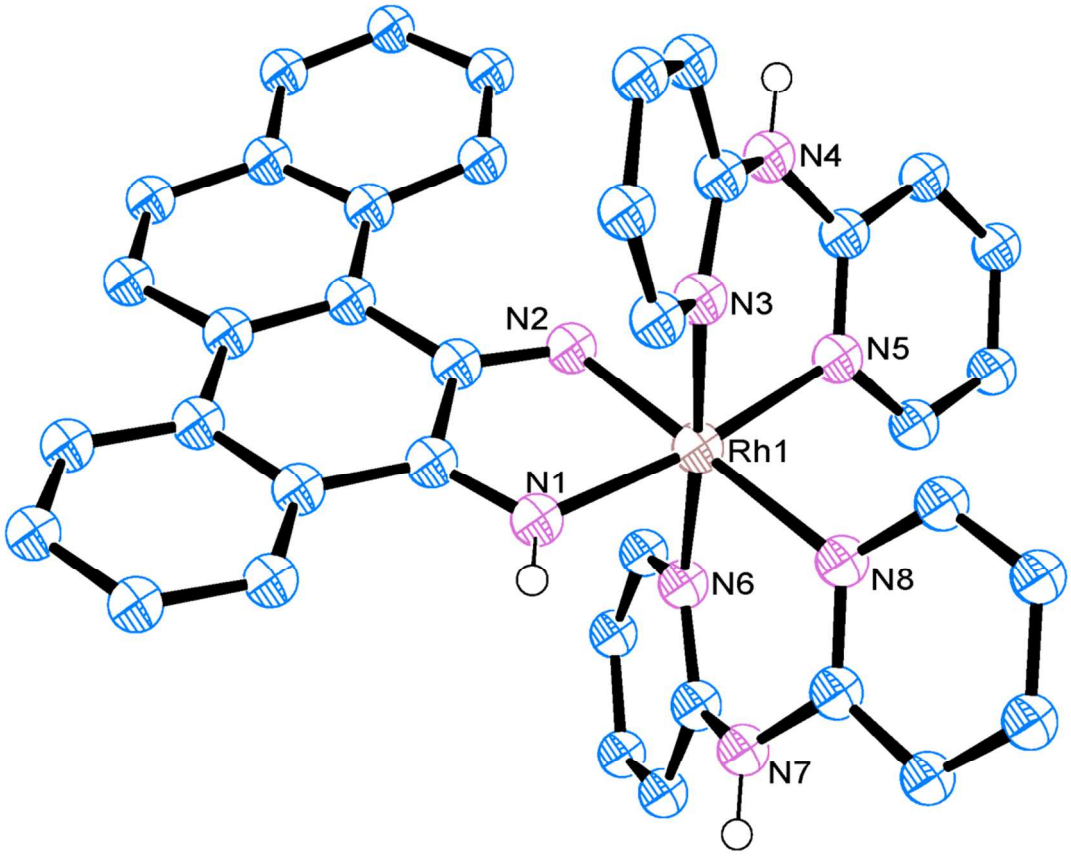

Figure S2 X-ray crystal structure of $[Rh(HDPA)_2(chrysi)]Cl_2$. Displacement of ellipsoids are drawn at 50% probability. For clarity, chlorine atoms, solvent molecules, and hydrogen atoms (except the immine proton) have been omitted. Selected bond lengths (Å): Rh-N1 2.002, Rh-N2 1.974, Rh-N3 2.042, Rh-N5 2.045, Rh-N6 2.041, Rh-N8 2.114.

Table S2. Crystal data and structure refinement for $[Rh(HDPA)_2(chrysi)]Cl_2$.

Identification code	ack002
Empirical formula	C42 H43 Cl2 N8 O3 Rh
Formula weight	881.65
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P 21/n
Unit cell dimensions	a = 9.4383(4) Å α= 90°.

	b = 25.4636(10) Å β= 96.746(2)°.
	c = 16.6094(6) Å γ = 90°.
Volume	3964.1(3) $Å^3$
Z	4
Density (calculated)	1.477 Mg/m^3
Absorption coefficient	0.617 mm^{-1}
F(000)	1816
Crystal size	0.500 x 0.400 x 0.150 mm^3
Theta range for data collection	2.315 to 30.547°.
Index ranges	-13<=h<=13, -35<=k<=36, -23<=l<=23
Reflections collected	178000
Independent reflections	12143 [R(int) = 0.0639]
Completeness to theta = 25.242°	99.9 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7461 and 0.6942
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	12143 / 13 / 538
Goodness-of-fit on F^2	1.035
Final R indices [I>2sigma(I)]	R1 = 0.0284, wR2 = 0.0641
R indices (all data)	R1 = 0.0371, wR2 = 0.0677
Extinction coefficient	n/a
Largest diff. peak and hole	0.745 and -0.767 $e.Å^{-3}$

Low-temperature diffraction data (ϕ-and ω-scans) were collected on a Bruker Kappa diffractometer coupled to a Apex II CCD detector with graphite monochromated Mo K_α radiation (λ = 0.71073 Å) for the structure of ack002. The structure was solved by direct methods using SHELXS[i] and refined against F^2 on all data by full-matrix least squares with SHELXL-2013[ii] using established refinement techniques.[iii] All non-hydrogen atoms were

refined anisotropically. All hydrogen atoms were included into the model at geometrically calculated positions and refined using a riding model. The isotropic displacement parameters of all hydrogen atoms were fixed to 1.2 times the *U* value of the atoms they are linked to (1.5 times for methyl groups).

ack002 crystallizes in the monoclinic space group $P2_1/n$ with one molecule in the asymmetric unit along with two molecules of methanol. One of the two chloride ions was disordered over two positions and refined with the help of similarity restraints on the displacement parameters. The occupancies of the two components refined to 0.930(4):0.070(4). The coordinates for the hydrogen atoms bound to N1, N4, N7, O1S, O1T, and O1W were located in the difference Fourier synthesis and refined semi-freely with the help of a distance restraint. The N-H distances were restrained to be 0.91(4) Å for N1, 0.88(4) Å for N4, N7 and 0.84(4) Å for all O-H bonds.

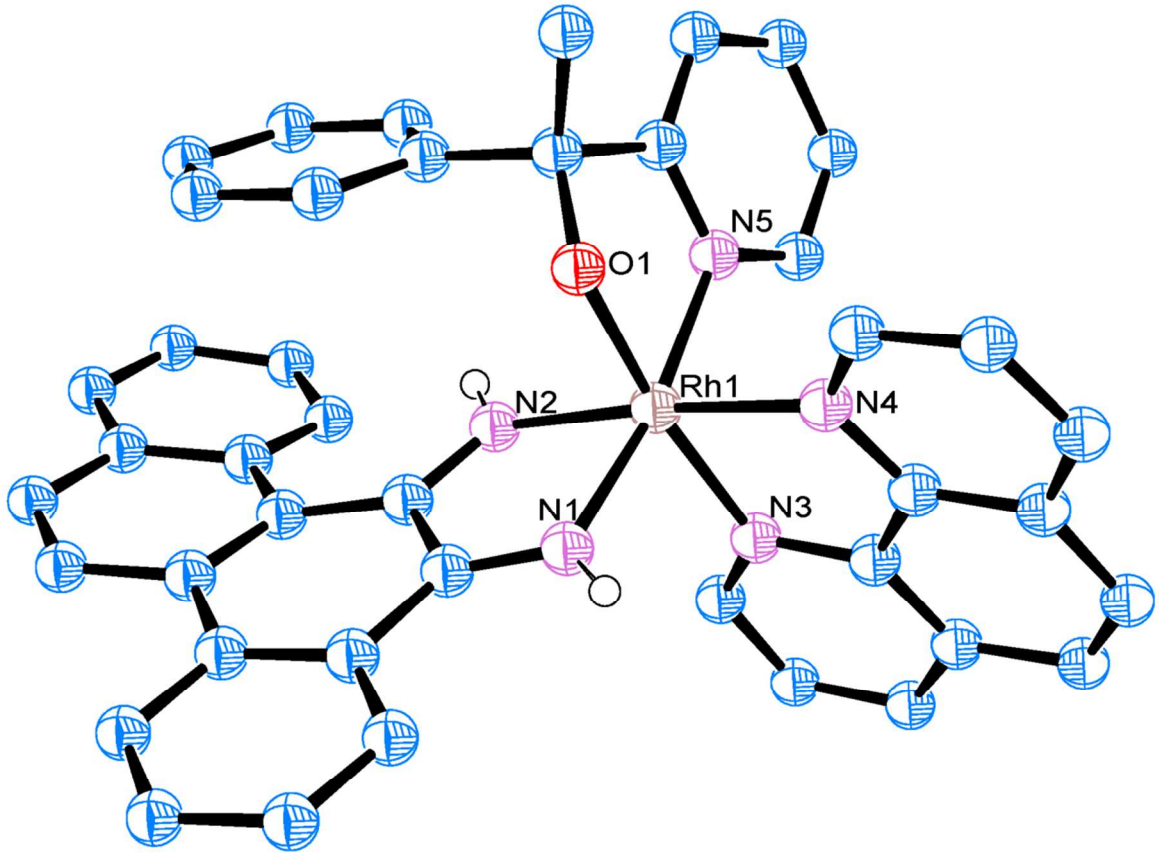

Figure S3 X-ray crystal structure of [Rh(chrysi)(phen)(PPE)]Cl_2. Displacement of ellipsoids are drawn at 50% probability. For clarity, chlorine atoms, solvent molecules, and hydrogen atoms (except immine protons) have been omitted. Selected bond lengths (Å): Rh-O 1.983, Rh-N1 1.991, Rh-N2 1.993, Rh-N3 2.053, Rh-N4 2.037, Rh-N5 2.034.

Table S3. Crystal data and structure refinement for [Rh(chrysi)(phen)(PPE)]Cl_2.

Identification code	ack010	
Empirical formula	C47 H48 Cl2 N5 O5 Rh	
Formula weight	936.71	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P -1	
Unit cell dimensions	a = 10.7782(6) Å	α= 72.345(3)°.

	b = 12.1739(6) Å β= 89.877(3)°.
	c = 17.5944(10) Å γ = 80.812(3)°.
Volume	2169.0(2) $Å^3$
Z	2
Density (calculated)	1.434 Mg/m^3
Absorption coefficient	0.570 mm^{-1}
F(000)	968
Crystal size	0.250 x 0.150 x 0.150 mm^3
Theta range for data collection	1.216 to 30.663°.
Index ranges	-15<=h<=15, -17<=k<=17, -25<=l<=25
Reflections collected	115348
Independent reflections	13351 [R(int) = 0.0555]
Completeness to theta = 25.242°	99.9 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7461 and 0.6896
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	13351 / 160 / 625
Goodness-of-fit on F^2	1.055
Final R indices [I>2sigma(I)]	R1 = 0.0372, wR2 = 0.0859
R indices (all data)	R1 = 0.0499, wR2 = 0.0919
Extinction coefficient	n/a
Largest diff. peak and hole	0.965 and -0.864 $e.Å^{-3}$

Low-temperature diffraction data (ϕ-and ω-scans) were collected on a Bruker Kappa diffractometer coupled to an Apex II CCD detector with graphite-monochromated Mo K_α radiation (λ = 0.71073 Å) for the structure of ack010. The structure was solved by direct methods using SHELXS[i] and refined against F^2 on all data by full-matrix least squares with SHELXL-2013[iii] using established refinement techniques.[iii] All non-hydrogen atoms were

refined anisotropically. Unless otherwise noted, all hydrogen atoms were included into the model at geometrically calculated positions and refined using a riding model. The isotropic displacement parameters of all hydrogen atoms were fixed to 1.2 times the *U* value of the atoms they are linked to (1.5 times for methyl groups). All disordered atoms were refined with the help of similarity restraints on the 1,2-distances and displacement parameters as well as rigid bond restraints for anisotropic displacement parameters.

ack010 crystallizes in the triclinic space group $P\bar{1}$ with one molecule in the asymmetric unit along with two chloride anions and four molecules of methanol. One of the methanol molecules hydrogen-bonds to the ruthenium molecule and was not disordered. The three other methanol molecules were modeled as disordered. For the second methanol, only the methyl group was disordered over two positions with the occupancy of the two components refined to 0.841(7):0.159(7). The third methanol was completely disordered over two positions with occupancies of 0.792(6):0.208(6). The fourth methanol was disordered over three positions with occupancies 0.0615(3):0.234(3):0.151(3). The coordinates for the hydrogen atoms bound to N1, N2, and O1M were located in the difference Fourier synthesis and refined semi-freely with the help of a distance restraint. The N-H and O-H distances were restrained to be 0.88(4) Å and 0.84(4) Å, respectively. The hydrogen atoms for the remaining methanol positions could not be located and were included at geometrically calculated positions and refined using a riding model.

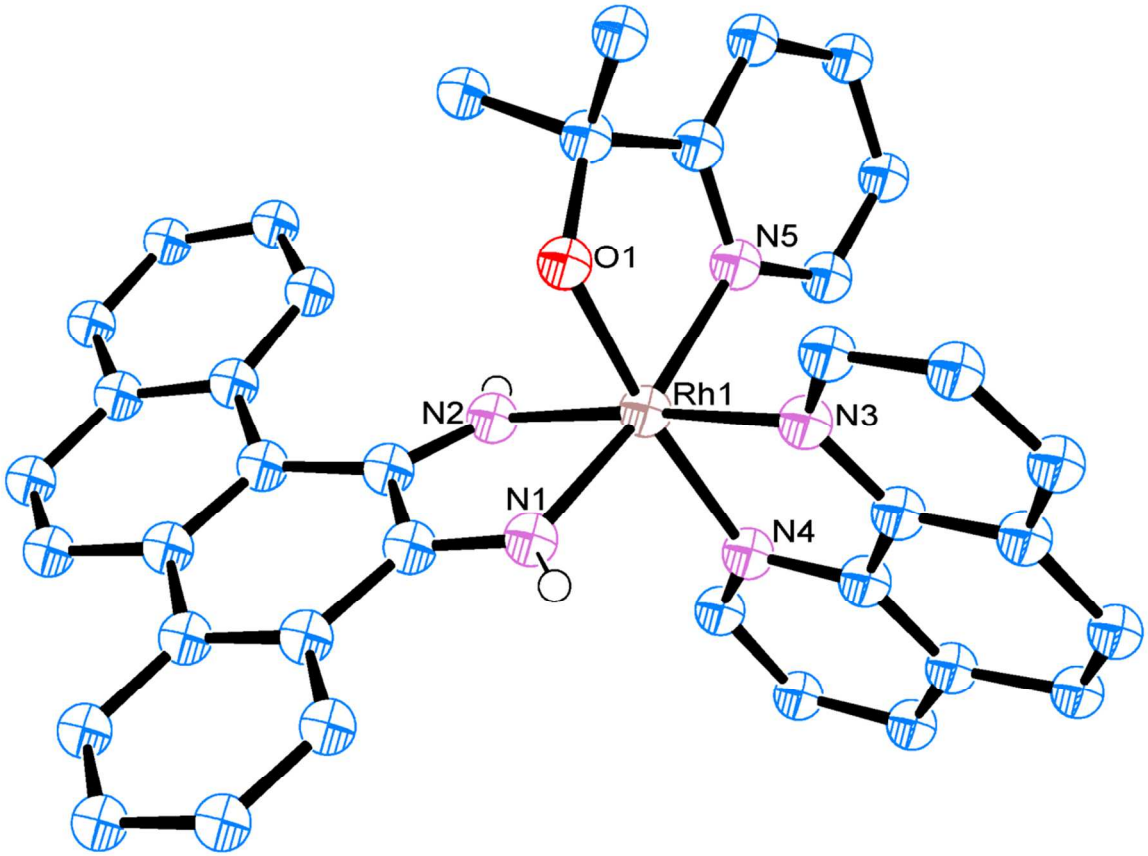

Figure S4 X-ray crystal structure of [Rh(chrysi)(phen)(PPO)]Cl_2. Displacement of ellipsoids are drawn at 50% probability. For clarity, chlorine atoms, solvent molecules, and hydrogen atoms (except immine protons) have been omitted. Selected bond lengths (Å): Rh-O 1.973, Rh-N1 1.980, Rh-N2 1.994, Rh-N3 2.039, Rh-N4 2.067, Rh-N5 2.041.

Table S4. Crystal data and structure refinement for [Rh(chrysi)(phen)(PPO)]Cl_2.

Identification code	ack013	
Empirical formula	C45 H48 Cl2 N5 O3 Rh	
Formula weight	880.69	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P -1	
Unit cell dimensions	a = 9.1633(11) Å	α= 110.881(2)°.

	b = 15.4046(19) Å	β= 94.610(2)°.
	c = 16.791(2) Å	γ = 105.705(2)°.
Volume	2090.2(4) Å^3	
Z	2	
Density (calculated)	1.399 Mg/m^3	
Absorption coefficient	0.583 mm^{-1}	
F(000)	912	
Crystal size	0.500 x 0.500 x 0.400 mm^3	
Theta range for data collection	1.324 to 30.043°.	
Index ranges	-12<=h<=12, -21<=k<=21, -23<=l<=22	
Reflections collected	61440	
Independent reflections	11087 [R(int) = 0.0277]	
Completeness to theta = 25.242°	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7460 and 0.6697	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	11087 / 3 / 521	
Goodness-of-fit on F^2	1.112	
Final R indices [I>2sigma(I)]	R1 = 0.0308, wR2 = 0.0703	
R indices (all data)	R1 = 0.0387, wR2 = 0.0750	
Extinction coefficient	n/a	
Largest diff. peak and hole	1.020 and -0.486 e.Å^{-3}	

Low-temperature diffraction data (ϕ-and ω-scans) were collected on a Bruker three-circle diffractometer coupled to a Bruker Smart 1000 CCD detector with graphite monochromated Mo K_α radiation (λ = 0.71073 Å) for the structure of compound ack013. The structure was solved by direct methods using SHELXS[i] and refined against F^2 on all data by full-matrix least squares with SHELXL-2013[ii] using established refinement techniques.[iii] All non-hydrogen atoms were

refined anisotropically. All hydrogen atoms were included into the model at geometrically calculated positions and refined using a riding model. The isotropic displacement parameters of all hydrogen atoms were fixed to 1.2 times the *U* value of the atoms they are linked to (1.5 times for methyl groups).

ack013 crystallizes in the triclinic space group $P\bar{1}$ with one molecule in the asymmetric unit along with two chloride anions, one molecule of diethyl ether, and one molecule of isopropyl alcohol. The coordinates for the hydrogen atoms bound to N1, N2, and O1T were located in the difference Fourier synthesis and refined semi-freely with the help of a distance restraint. The N-H and O-H distance were restrained to be 0.88(4) Å and 0.84(4) Å, respectively.

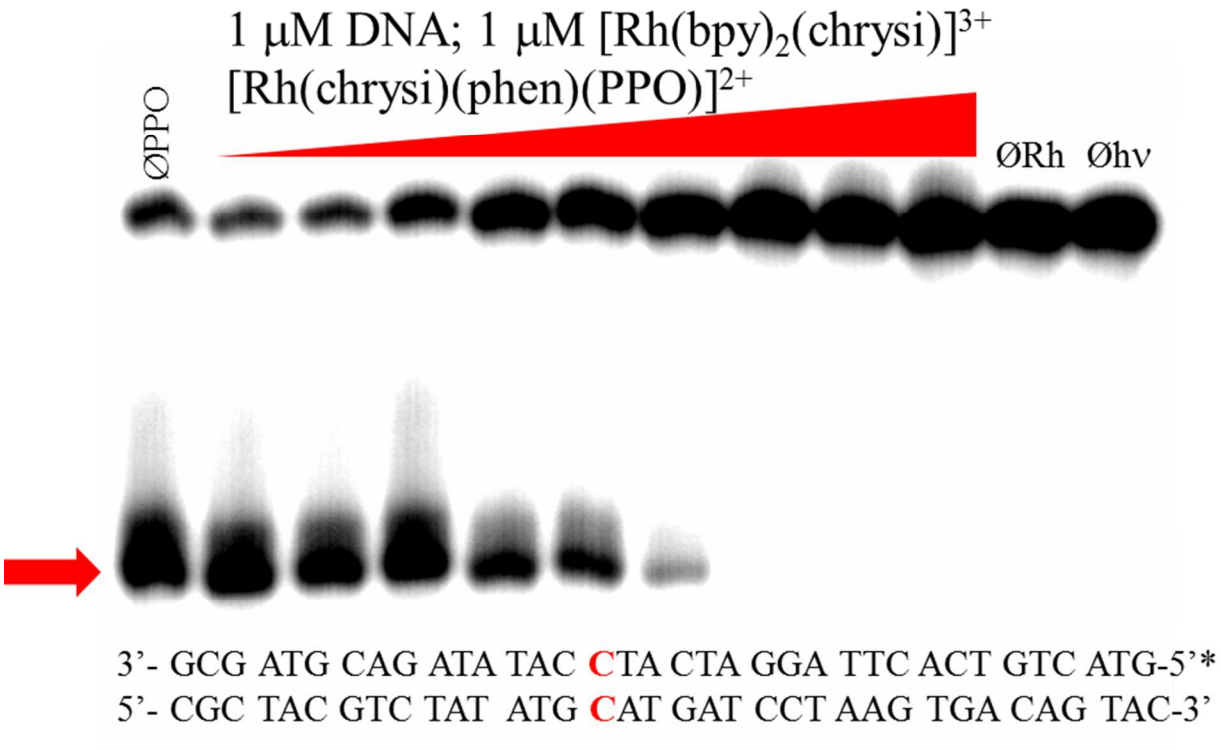

Figure S5 Binding affinities determined through DNA photocleavage. The DNA sequence is as shown (red denotes the mismatch, asterisk denotes the radiolabel). Samples were irradiated and electrophoresed through a 20% denaturing PAGE gel. A light control (ØRh, without rhodium) and dark control (Øhν, without irradiation) were included. A representative autoradiogram of a photocleavage competition titration between 1 μM *rac*-$[Rh(bpy)_2(chrysi)]^{3+}$ and 0-100 μM $[Rh(chrysi)(phen)(PPO)]^{2+}$ is shown. Arrow indicates photocleavage by *rac*-$[Rh(bpy)_2(chrysi)]^{3+}$ at the mismatch.

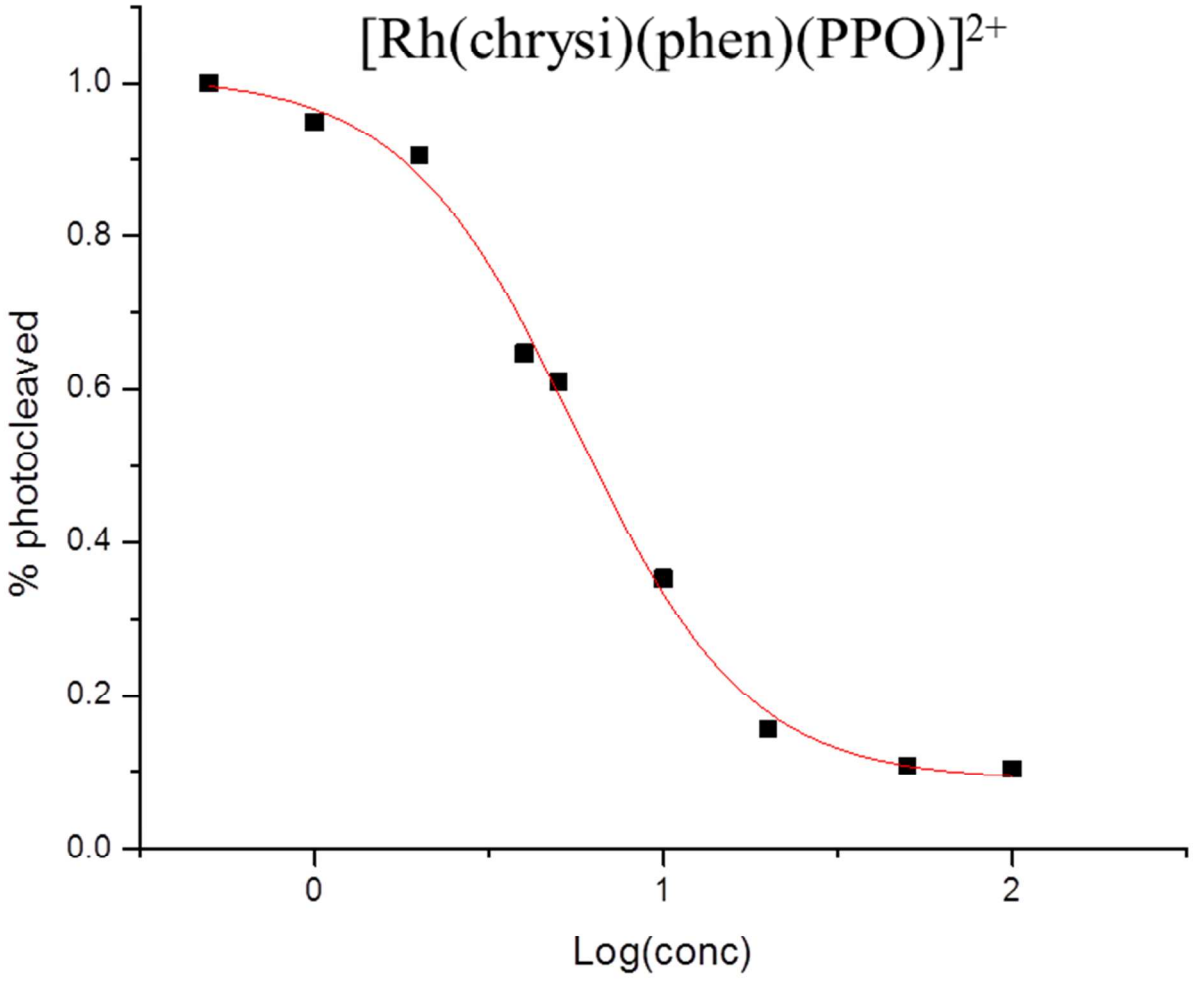

Figure S6 Representative sigmoidal curve for binding affinity determination. Shown is a plot of data from the photocleavage competition titration between 1 μM *rac*-$[Rh(bpy)_2(chrysi)]^{3+}$ and 0-100 μM $[Rh(chrysi)(phen)(PPO)]^{2+}$ shown in **Figure S5** for binding constant determination.

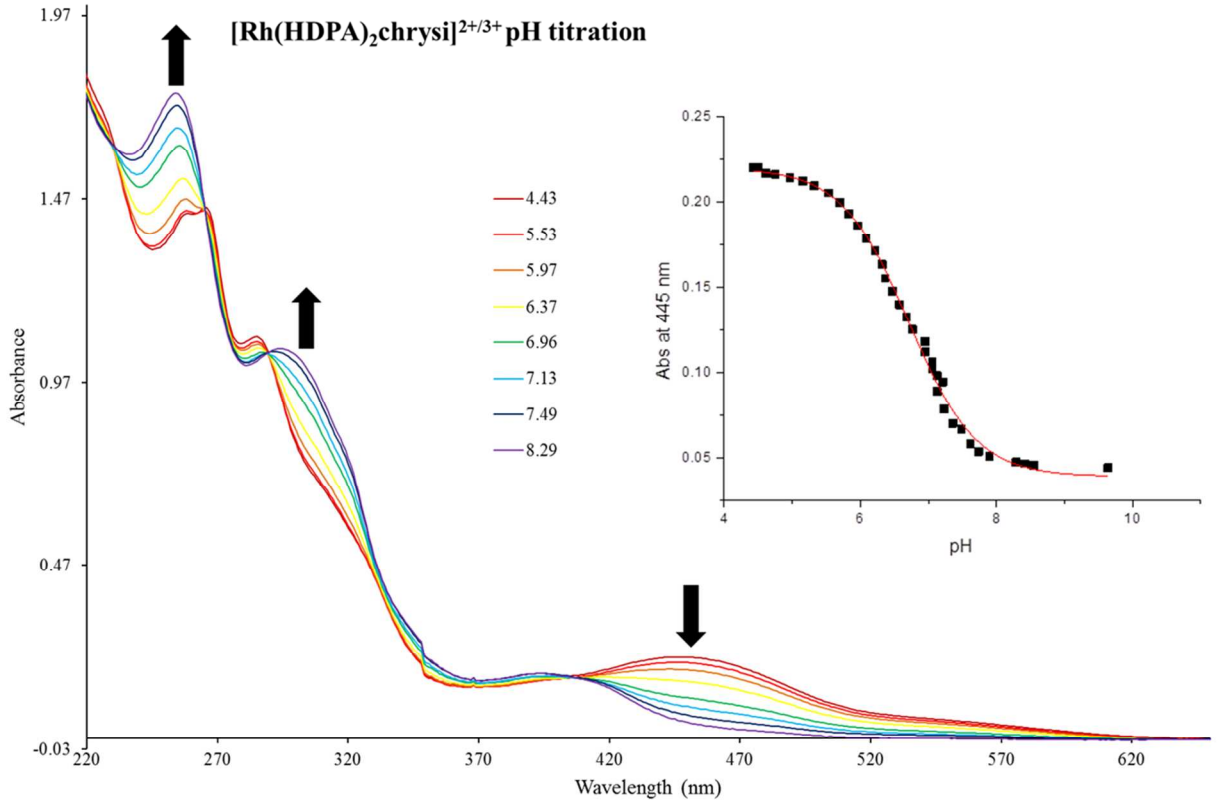

Figure S7 pH titration of $[Rh(HDPA)_2(chrysi)]^{3+}$. Shown are absorption spectra of a 25 μM solution of $[Rh(HDPA)_2(chrysi)]^{3+}$ as the pH changes from 4.5 (red) to 8.5 (purple). The black arrows exhibit the direction in which the various bands change as the pH increases. (Inset) The absorbance at 445 nm was plotted as a function of pH and fit to a sigmoidal curve. The pK_a was determined from the inflection point of this curve.

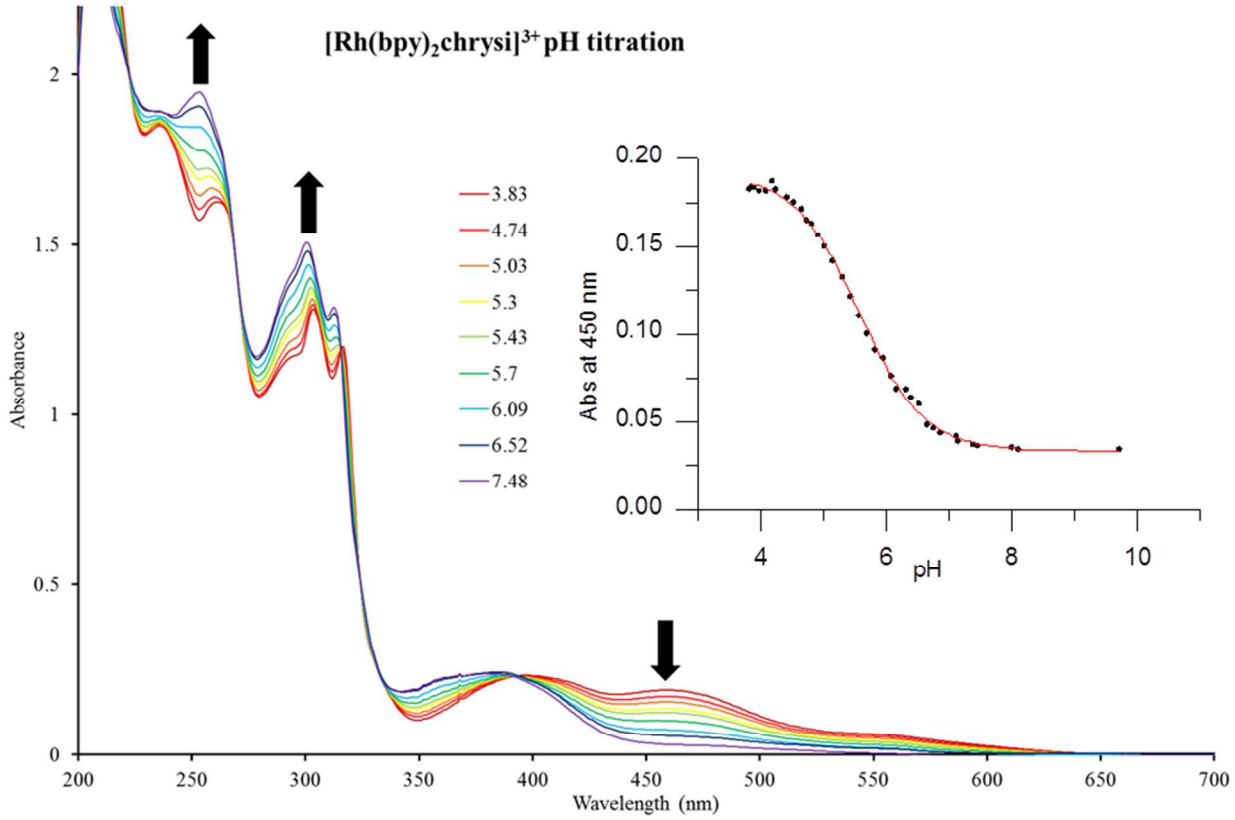

Figure S8 pH titration of $[Rh(bpy)_2(chrysi)]^{3+}$. Shown are absorption spectra of a 25 μM solution of $[Rh(bpy)_2(chrysi)]^{3+}$ as the pH changes from 3.8 (red) to 7.5 (purple). The black arrows exhibit the direction in which the various bands change as the pH increases. (Inset) The absorbance at 450 nm was plotted as a function of pH and fit to a sigmoidal curve. The pK_a was determined from the inflection point of this curve.

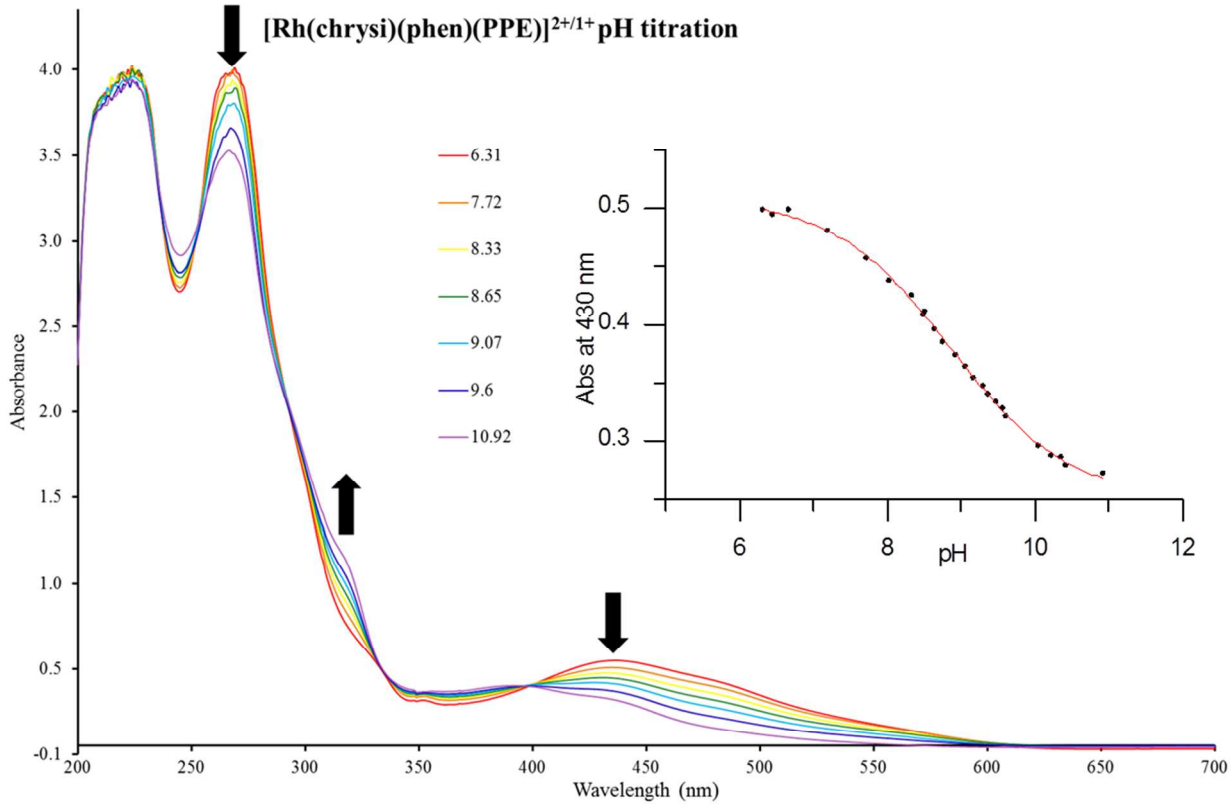

Figure S9 pH titration of $[Rh(chrysi)(phen)(PPE)]^{2+}$. Shown are absorption spectra of a 25 μM solution of $[Rh(chrysi)(phen)(PPE)]^{2+}$as the pH changes from 6.3 (red) to 10.9 (purple). The black arrows exhibit the direction in which the various bands change as the pH increases. (Inset) The absorbance at 430 nm was plotted as a function of pH and fit to a sigmoidal curve. The pK_a was determined from the inflection point of this curve.

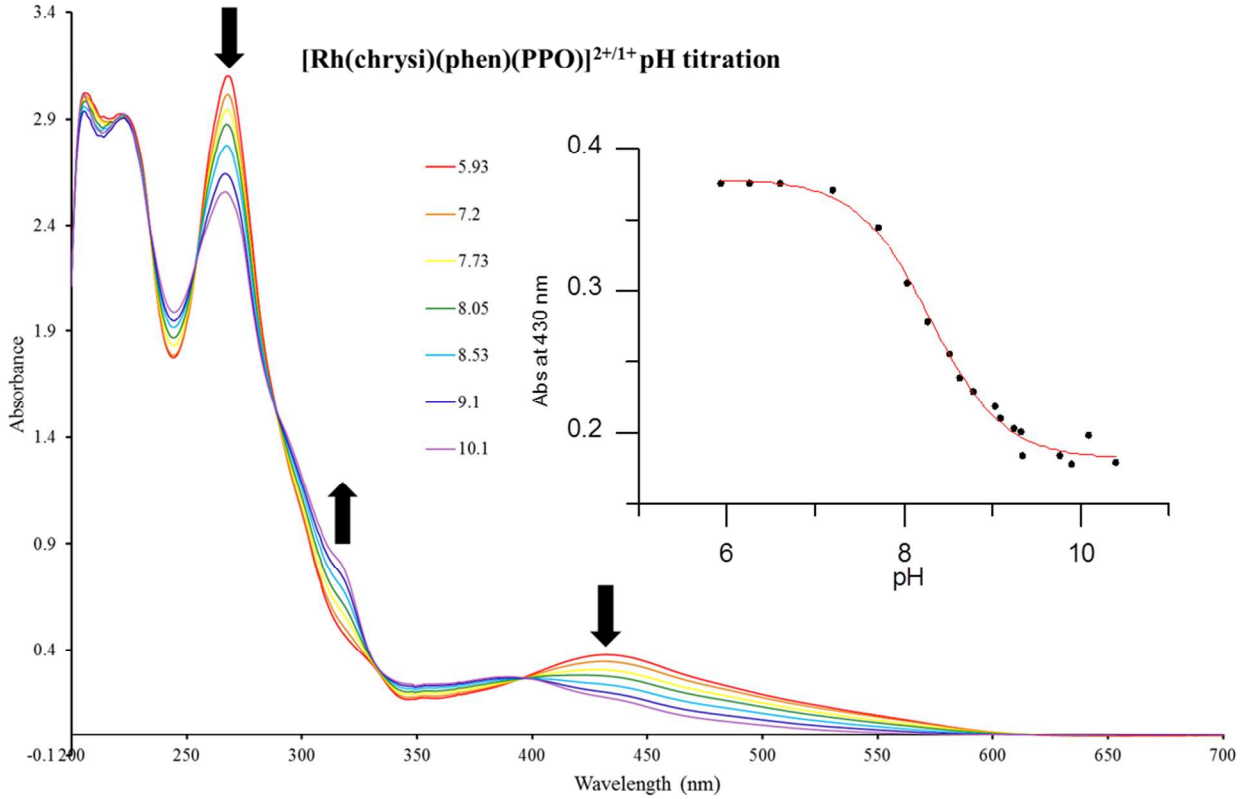

Figure S10 pH titration of $[Rh(chrysi)(phen)(PPO)]^{2+}$. Shown are absorption spectra of a 25 μM solution of $[Rh(chrysi)(phen)(PPO)]^{2+}$as the pH changes from 5.9 (red) to 10.1 (purple). The black arrows exhibit the direction in which the various bands change as the pH increases. (Inset) The absorbance at 430 nm was plotted as a function of pH and fit to a sigmoidal curve. The pK_a was determined from the inflection point of this curve.

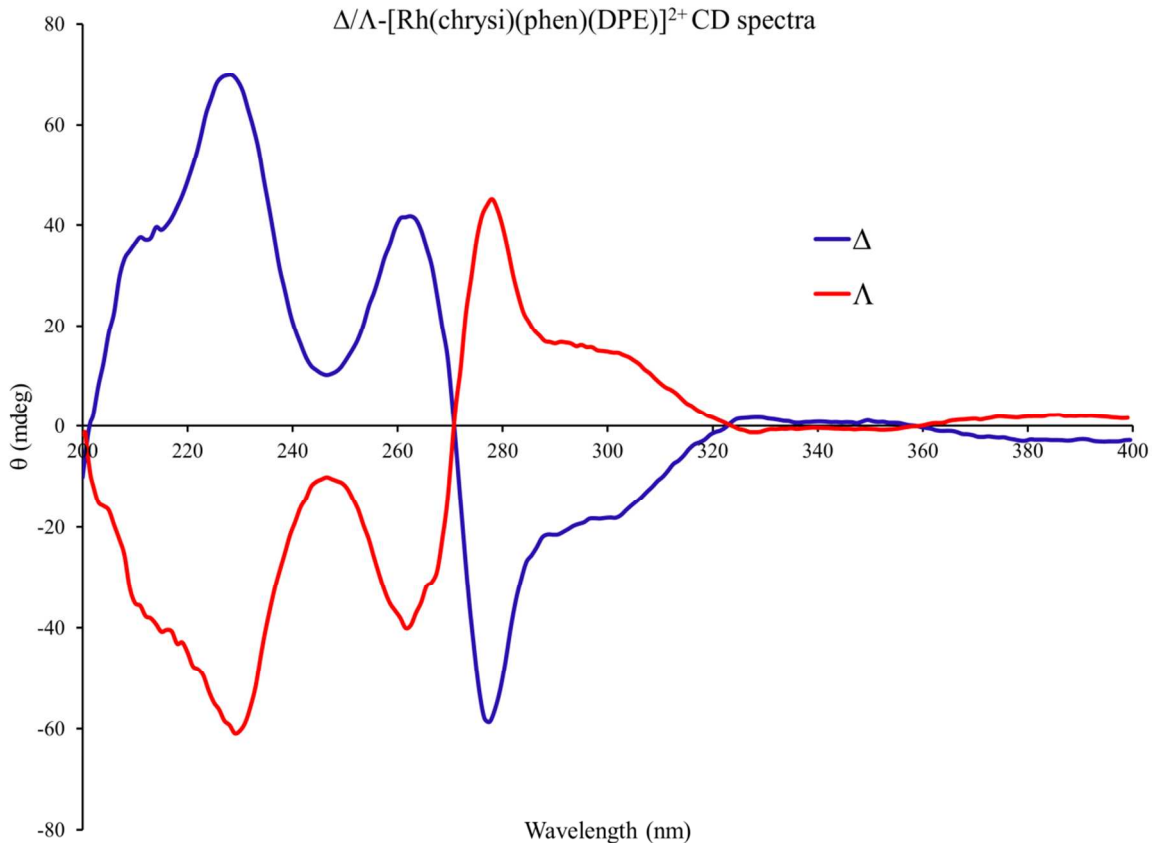

Figure S11 Circular dichroism (CD) spectra of Δ- and Λ- $[Rh(chrysi)(phen)(DPE)]^{2+}$ in water (blue and red, respectively). Δε values for Δ- $[Rh(chrysi)(phen)(DPE)]^{2+}$: 229 nm (77 M^{-1} cm^{-1}), 262 nm (46 M^{-1} cm^{-1}), 277 nm (-64 M^{-1} cm^{-1}), 295 nm (-21 M^{-1} cm^{-1}). Δε values for Λ-$[Rh(chrysi)(phen)(DPE)]^{2+}$: 229 nm (-82 M^{-1} cm^{-1}), 262 nm (-53 M^{-1} cm^{-1}), 277 nm (61 M^{-1} cm^{-1}), 295 nm (22 M^{-1} cm^{-1}).

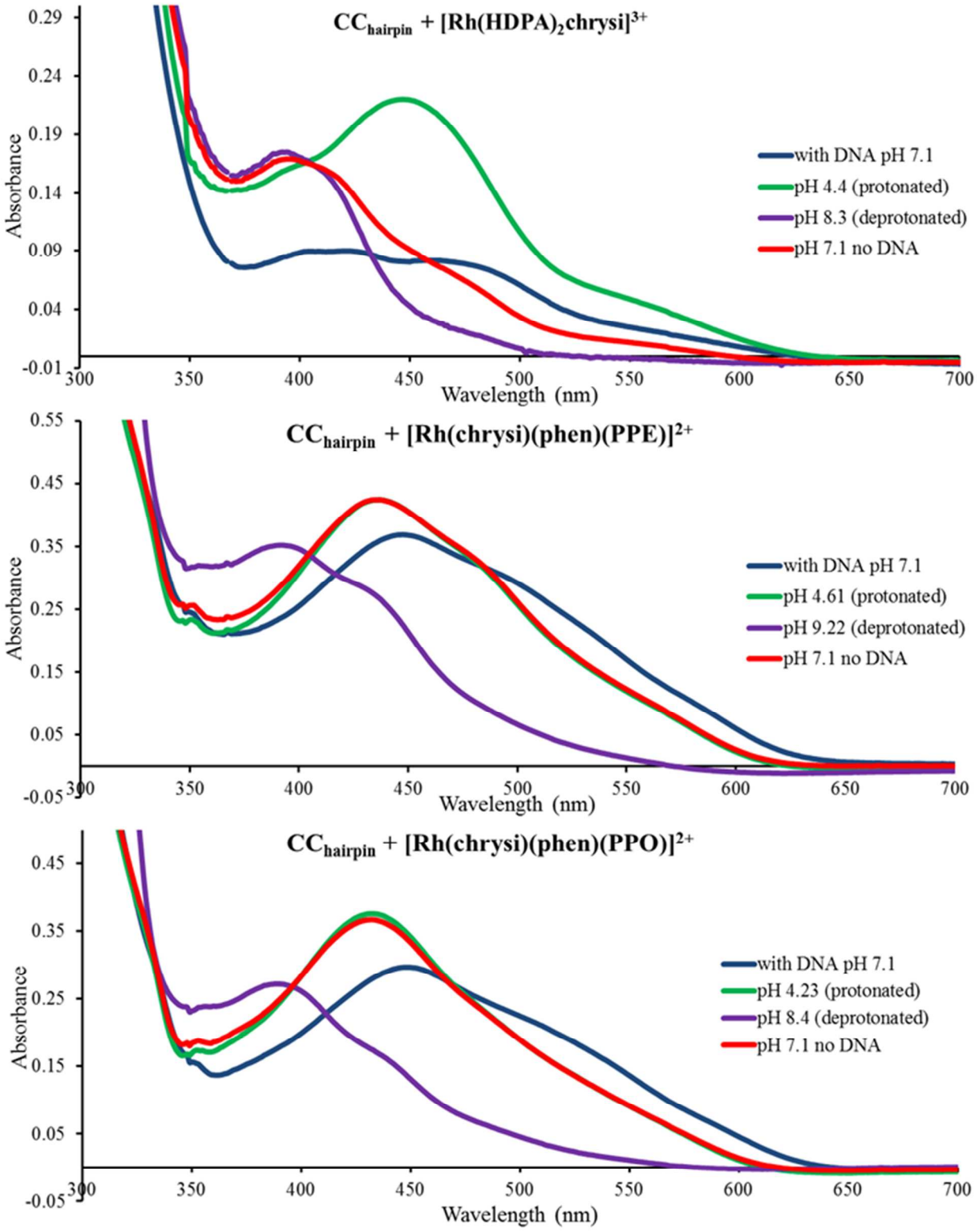

Figure S12 Absorption spectra of [Rh(HDPA)$_2$(chrysi)]$^{3+}$(top), [Rh(chrysi)(phen)(PPE)]$^{2+}$ (middle), and [Rh(chrysi)(phen)(PPO)]$^{2+}$ (bottom) bound to mismatched DNA. Shown in blue

are absorption spectra of 25 μM solutions of the various metal complexes with 25 μM of the DNA hairpin 5′-GGCAGGCATGGCTTTTTGCCATCCCTGCC-3′ (underline denotes the CC mismatch). The absorption spectra of the fully protonated species are shown in green, those of the fully deprotonated species are in purple, and those of the compounds at pH 7.1 with no DNA are shown in red.

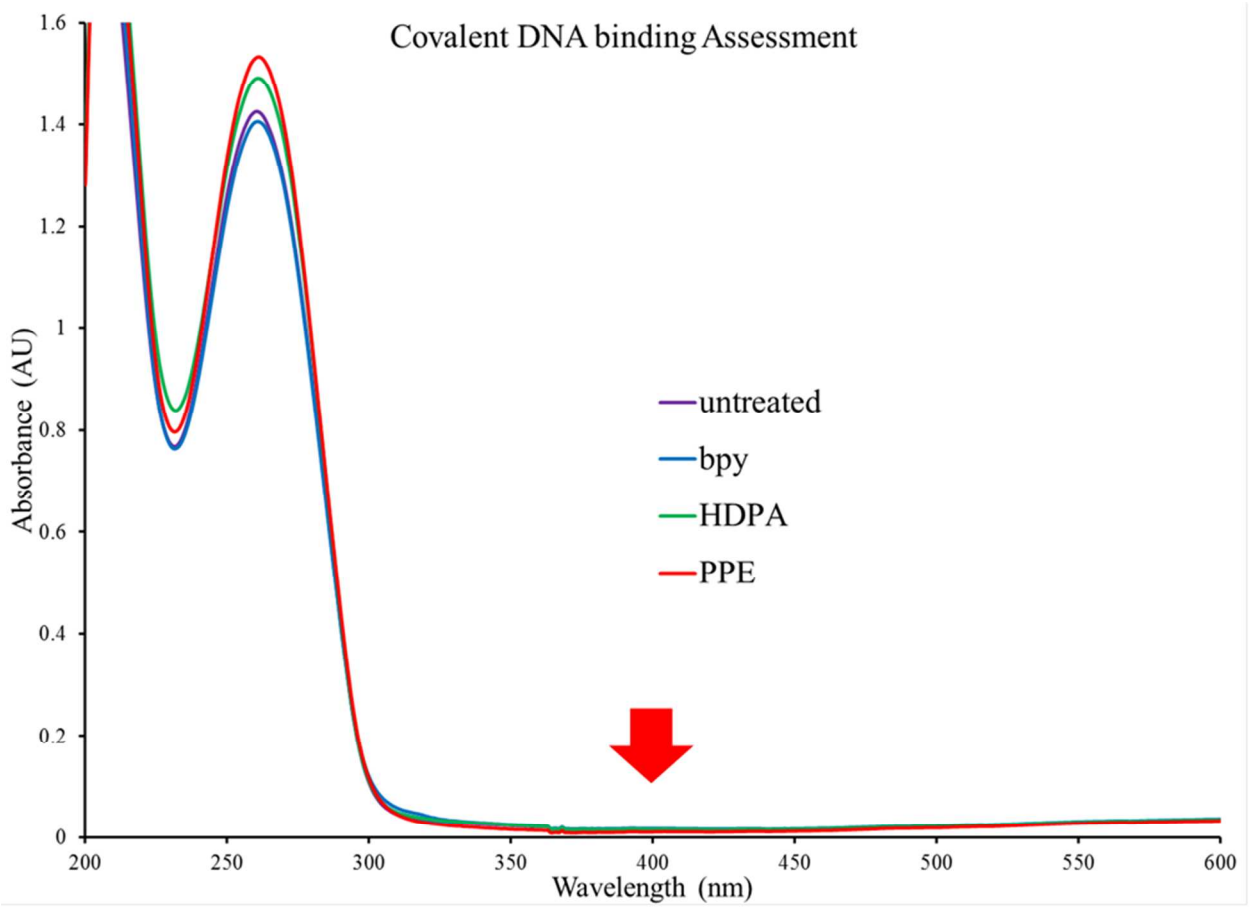

Figure S13 Assessment of the ability of $[Rh(chrysi)(phen)(PPE)]^{2+}$ to bind covalently to mismatched DNA. A 29-mer hairpin with a CC mismatch was incubated with water, $[Rh(chrysi)(phen)(PPE)]^{2+}$, $[Rh(bpy)_2(chrysi)]^{3+}$, or $[Rh(HDPA)_2(chrysi)]^{3+}$ (latter two compounds were included as control compounds that do not covalently bind to DNA). Following DNA melting and precipitation, a UV-Vis spectra was taken of the various samples. The absence

of a band around 400 nm (indicated with red arrow) indicates that no Rh complex was still bound (this MLCT band is a characteristic band in all Rh-chrysi compounds).

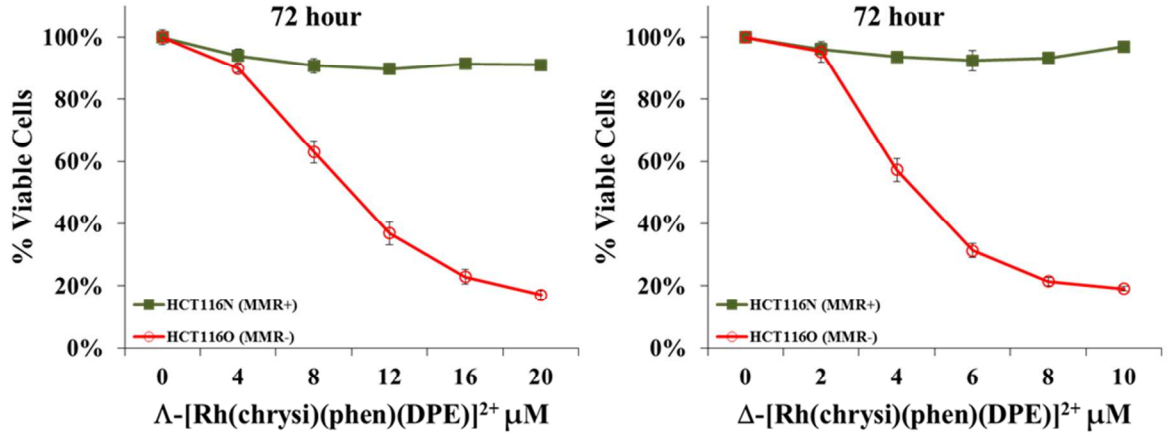

Figure S14 Differential cytotoxicities of Δ-and Λ-$[Rh(chrysi)(phen)(DPE)]^{2+}$. HCT116N (green) and HCT116O (red) cells were plated in 96-well format at densities of 5 x 10^4 cells/well and treated with the concentrations of rhodium metalloinsertors indicated. After 72 hours, the cells were labeled with MTT for 4 hours.

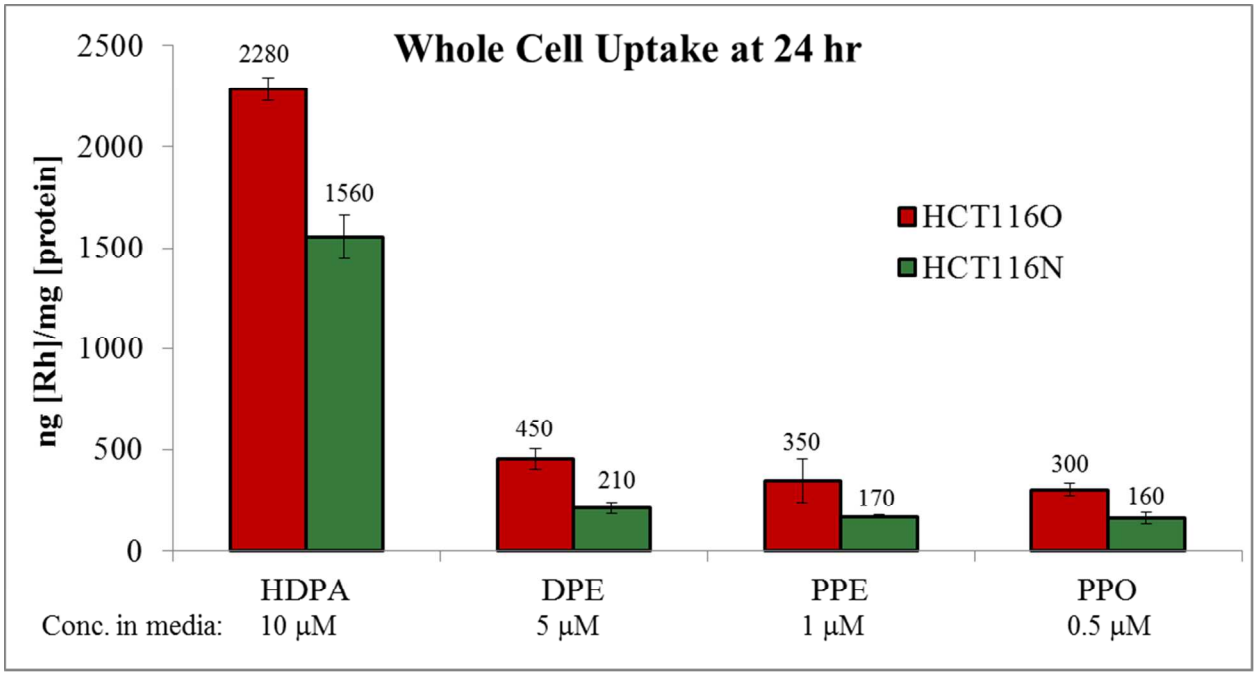

Figure S15 ICP-MS assay for whole-cell rhodium accumulation. HCT116O and HCT116N cells were treated with $[Rh(HDPA)_2(chrysi)]^{3+}$, $[Rh(chrysi)(phen)(DPE)]^{2+}$, $[Rh(chrysi)(phen)(PPE)]^{2+}$, or $[Rh(chrysi)(phen)(PPO)]^{2+}$ at the concentrations shown for 24 h. The cells were analyzed for rhodium content by ICP-MS. The rhodium counts were normalized to protein content, which was determined by a BCA assay. See experimental. It should be noted that because the rhodium concentrations are normalized to protein content, rather than number of cells, the concentrations from the two cell lines cannot be directly compared to one another.

i. Sheldrick, G. M. *Acta Cryst.* **1990**, *A46,* 467-473.

ii. Sheldrick, G. M. *Acta Cryst.* **2008**, *A64,* 112-122.

iii. Müller, P. *Crystallography Reviews* **2009**, *15*, 57-83.