Electronic Supporting Information

Biohybrid catalysts for sequential tandem reactions based on an engineered transmembrane protein

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Table of Contents

General remarks

All manipulations were performed under argon atmosphere using standard Schlenk or glove box techniques. Prior to use, glassware was dried in an oven at 130 °C. Solvents were dried, distilled and degassed using standard methods. Imidazolium salt **3**[1], *N*-maleimido butanoyl chloride[2] and **GHC3**[1] were synthesized as reported elsewhere. All other chemicals are commercially available and were used as received. NMR measurements were performed on a Bruker DRX 400 at ambient temperature unless otherwise mentioned. The chemical shifts (δ ppm) in the ^{1}H and ^{13}C{^{1}H} NMR spectra were referenced to the residual proton signals of the deuterated solvents and reported relative to tetramethylsilane.[3] Abbreviations for NMR spectra: s (singlet), d (doublet), t (triplet), quint (quintet), br (broad). Circular dichroism (CD) spectra were recorded on a JASCO J1100 at ambient temperatures. MALDI–TOF MS spectra were measured on an Ultraflex III TOF/TOF mass spectrometer from Bruker using 2,5-dihydroxybenzoic acid (DHB) as matrix. High resolution ESI mass spectra were recorded on a LTQ-Orbitrap XL (ThermoFischer Scientific).

FhuA ΔCVFtev expression, extraction and analytics

Protein expression

E. coli BL21 (DE3) omp8 cells harboring the plasmid pPR-IBA1 FhuA ΔCVF^{tev} were used for production of the protein scaffold. Main cultures were started by inoculation with an overnight pre-culture to a final OD_{600} of 0.2. The main culture was incubated at 30°C, 250 rpm until an OD_{600} of 1 was reached. Gene expression was induced by addition of IPTG (1 mM final concentration). Cells were harvested after 16 h of induction at 30°C, 250 rpm by centrifugation (3220 g, 4 °C for 30 min) and stored at -20°C until further use.

FhuA ΔCVF^{tev} extraction

Solubilization of FhuA ΔCVF^{tev} using SDS was performed as previously described.[1]

Determination of protein concentration and coupling efficiency

Protein concentration was determined using Pierce™ BCA Protein Assay Kit (ThermoFisher Scientific). The fluorescence dye ThioGlo1® was used for determination of the accessibility of cysteine. FhuA ΔCVF^{tev} was used at a final concentration of 10 µM in 100 µL in a micro titer plate. 2 µL of a ThioGlo1® stock (1.5 mM) were added and incubated for 2 h in the dark. The fluorescence signal was detected applying an excitation wavelength of 379 nm and an emission wavelength of 513 nm using Infinite M1000 micro titer plate reader (TECAN). Cysteine occupations before and after coupling with the Grubbs-Hoveyda type and rhodium catalysts were compared to determine the coupling efficiency.

Synthesis of rhodium NHC catalysts

Synthesis of rhodium complex 4:

1,3-Dimesityl-4,5-dihydro-4-tetrahydropyranyl-1*H*-imidazol-3-ium-chloride (**3**) (500 mg, 1.09 mmol, 2.20 equiv.) was dissolved in THF (5 mL). A solution of KHMDS (235 mg, 1.17 mmol, 2.40 equiv.) in THF was added dropwise to the THF solution containing **3**. The resulting suspension was stirred for five minutes at room temperature until a pale orange solution was obtained. This solution was slowly added dropwise to solution of $[Rh(cod)Cl]_2$ (245 mg, 0.50 mmol, 1.00 equiv.) in THF (10 mL). The mixture was stirred for 16 h at 60 °C. The solvent was removed under vacuum and the remaining KHMDS was neutralized with water. The product was dissolved in DCM (20 mL), washed with water (3 x 15 mL) and the organic phase was dried over $MgSO_4$. DCM was removed under vacuum. Complex **4** was obtained as an orange solid (600 mg, 0.90 mmol, 89%).

^{1}H-NMR (400 MHz, CD_2Cl_2, 23 °C): δ = 7.10-6.90 (m, 4H, Aryl-*H*), 4.55-4.20 (m, 4H, *H*C=C*H*), 4.00-3.62 (m, 4H, N-CH_2, O-CH_2), 3.53-3.30 (m, 3.5H, *CH* and CH_2), 3.10-3.00 (m, 0.5H, C*H*), 2.72-2.20 (m, 18H, CH_3), 2.13-1.35 (m, 14H, CH_2 *and* CH(CH_2)) ppm.

^{13}C{^{1}H}-NMR (100 MHz, CD_2Cl_2, 23 °C): δ = 214.83-214.35 (d, $^1J_{Rh-C}$ = 47.8 Hz, N*C*N), 214.73-214.25 (d, $^1J_{Rh-C}$ = 47.8 Hz, N*C*N), 213.64-213.15 (d, $^1J_{Rh-C}$ = 47.8 Hz, N*C*N), 213.54-213.06 (d, $^1J_{Rh-C}$ = 47.8 Hz, N*C*N), 139.51, 139.36, 139.31, 139.26, 139.06, 138.94, 138.45, 138.36, 138.33, 138.29, 138.22, 138.14, 137.81, 137.56, 137.45, 137.33, 137.29, 136.70, 136.61, 136.46, 136.38, 136.18, 135.88, 135.57, 130.51, 130.47, , 130.35, 130.27, 130.08, 130.05, 129.34, 129.31, 129.20, 129.17, 129.07, 129.04, 129.00, 100.24-100.13 (m, *C*=*C*), 99.63, 98.25-98.10 (m, *C*=*C*), 97.41-97.07 (m, *C*=*C*), 71.15, 71.00, 69.14, 68.98, 68.83, 68.26, 67.62, 67.48, 67.44, 67.33, 67.30, 67.06, 66.90, 66.80, 66.76, 63.71, 63.49, 63.10, 62.78, 55.70, 55.32, 54.78, 54.18, 34.37, 33.31, 33.28, 32.94, 32.90, 31.95, 31.43, 31.02, 30.98, 30.91, 29.86, 29.80, 28.74, 28.36, 28.33, 27.52, 25.87, 25.83, 21.62, 21.37, 21.34, 21.32, 21.30, 21.24, 20.66, 20.60, 20.50, 20.46, 20.40, 20.28, 20.26, 20.13, 20.08, 20.02, 19.83, 19.38, 19.20, 19.11, 18.98, 18.87 ppm.

ESI-HR-MS positive mode (*m*/*z*)

Calculated $[M\text{-}Cl^-]^+$ ($C_{35}H_{48}N_2O_2Rh$): 631.2781

Found $[M\text{-}Cl^-]^+$: 631.2763

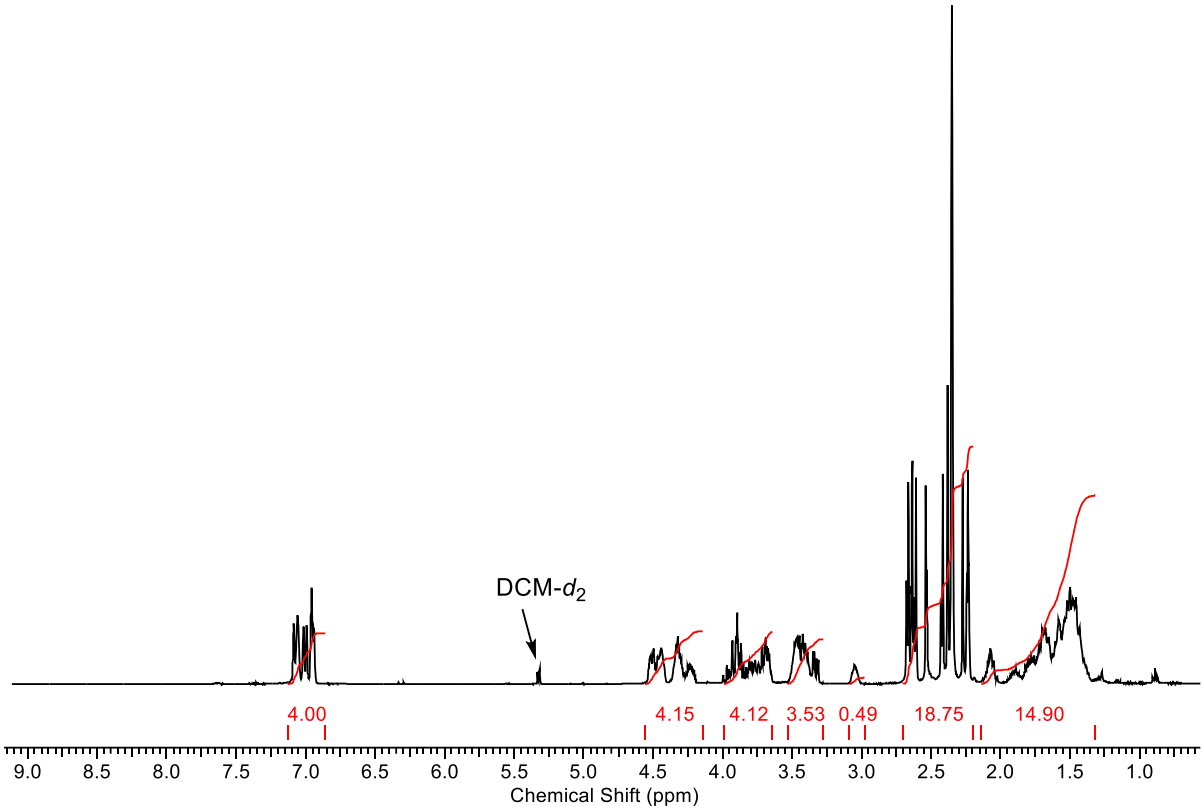


Figure 1. ^{1}H NMR spectrum of complex **4**.

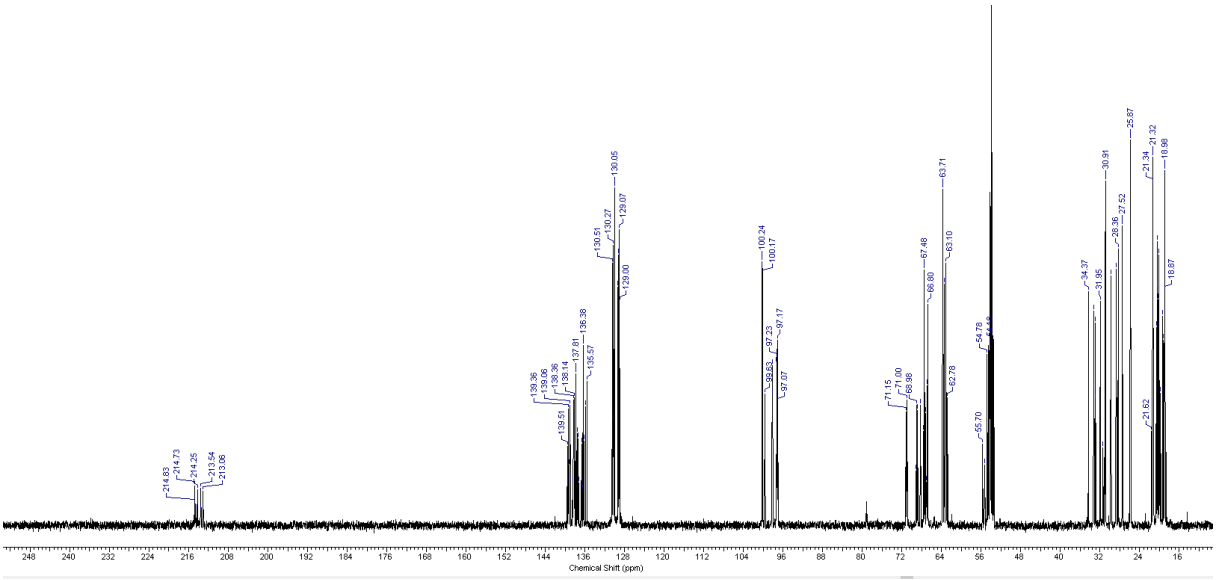


Figure 2. ^{13}C{^{1}H} NMR spectrum of complex **4**.

Synthesis of rhodium complex 5:

Complex **4** (600 mg, 0.90 mmol, 1.00 equiv.) was dissolved in ethanol (20 mL). An aqueous solution of HCl (1.0 M, 1 mL) was added and the resulting solution was sparged for 20 min. with argon. The solution was stirred under argon atmosphere for 72 h at 25 °C. Volatile compounds were removed under vacuum. The residue was dissolved in DCM (20 mL), washed with water (3 x 15 mL) and dried under $MgSO_4$. The orange residue was recrystallized from a THF/pentane solution. The solvent was removed under vacuum. Complex **5** was obtained as a yellow-orange solid (395 mg, 0.68 mmol, 76%).

^{1}H-NMR (400 MHz, CD_2Cl_2, 23 °C): δ = 7.13-6.90 (m, 4H, Aryl-*H*), 4.58-4.41 (m, 1 H, HC=C*H*), 4.37-4.25 (m, 1H, HC=C*H*), 4.17-4.08 (m, 1H, HC=C*H*), 3.99-3.72 (m, 3H, CH_2, HC=C*H*), 3.62-3.42 (m, 2H, CH_2), 3.39-3.33 (m, 0.25H, C*H*), 3.06-2.99 (m, 0.75H, C*H*), 2.82-2.19 (m, 18H, -CH_3), 1.80-1.22 (m, 9H, CH(CH_2)) ppm.

^{13}C-NMR (100 MHz, CD_2Cl_2, 23 °C): δ = 216.93-216.46 (d, $^1J_{Rh-C}$ = 46.8 Hz, N*C*N), 213.38-212.90 (d, $^1J_{Rh-C}$ = 48.5 Hz, N*C*N), 139.28, 139.18, 139.07, 138.54, 138.52, 138.42, 138.10, 137.88, 137.37, 137.27, 136.60, 136.41, 136.37, 136.29, 135.76, 130.91, 130.39, 130.29, 130.16, 129.69, 129.25, 129.18, 129.11, 98.30-98.23 (d, $^1J_{Rh-C}$ = 6.9 Hz, H*C*=*C*H), 97.82-97.74 (d, $^1J_{Rh-C}$ = 7.8 Hz, H*C*=*C*H), 97.36-97.29 (d, $^1J_{Rh-C}$ = 6.9 Hz, H*C*=*C*H), 96.59-96.52 (d, $^1J_{Rh-C}$ = 7.8 Hz, H*C*=*C*H), 71.27, 71.12, 68.29, 68.14, 68.00, 67.03, 66.89, 66.48, 65.34, 63.66, 62.52, 54.35, 34.37, 33.10, 32.85, 31.95, 29.82, 28.56, 28.42, 27.50, 21.59, 21.32, 21.29, 21.20, 20.62, 20.44, 20.26, 19.82, 19.34, 19.32, 18.64 ppm.

ESI-HR-MS positive mode (*m*/*z*)

Calculated $[M\text{-}Cl^-]^+$ ($C_{30}H_{40}N_2ORh$): 547.2207

Found $[M\text{-}Cl^-]^+$: 547.2186

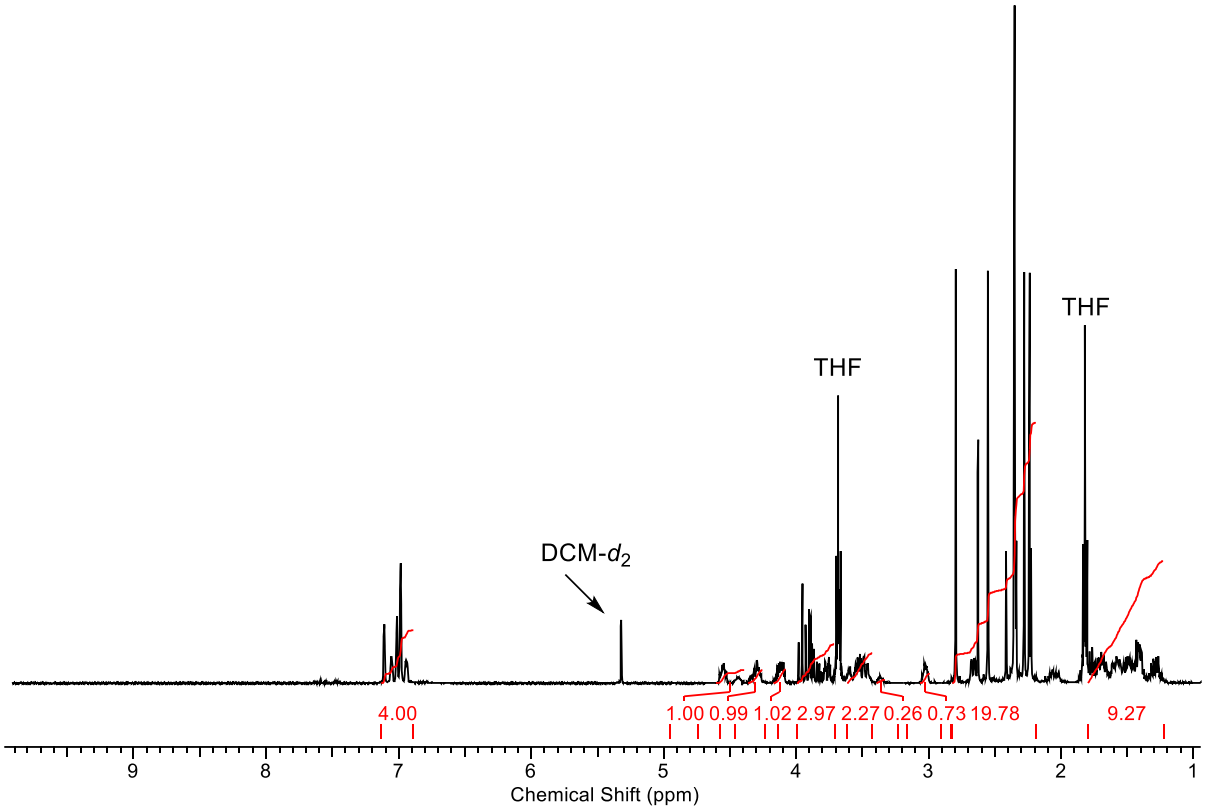


Figure 3. ^{1}H NMR spectrum of complex **5**.

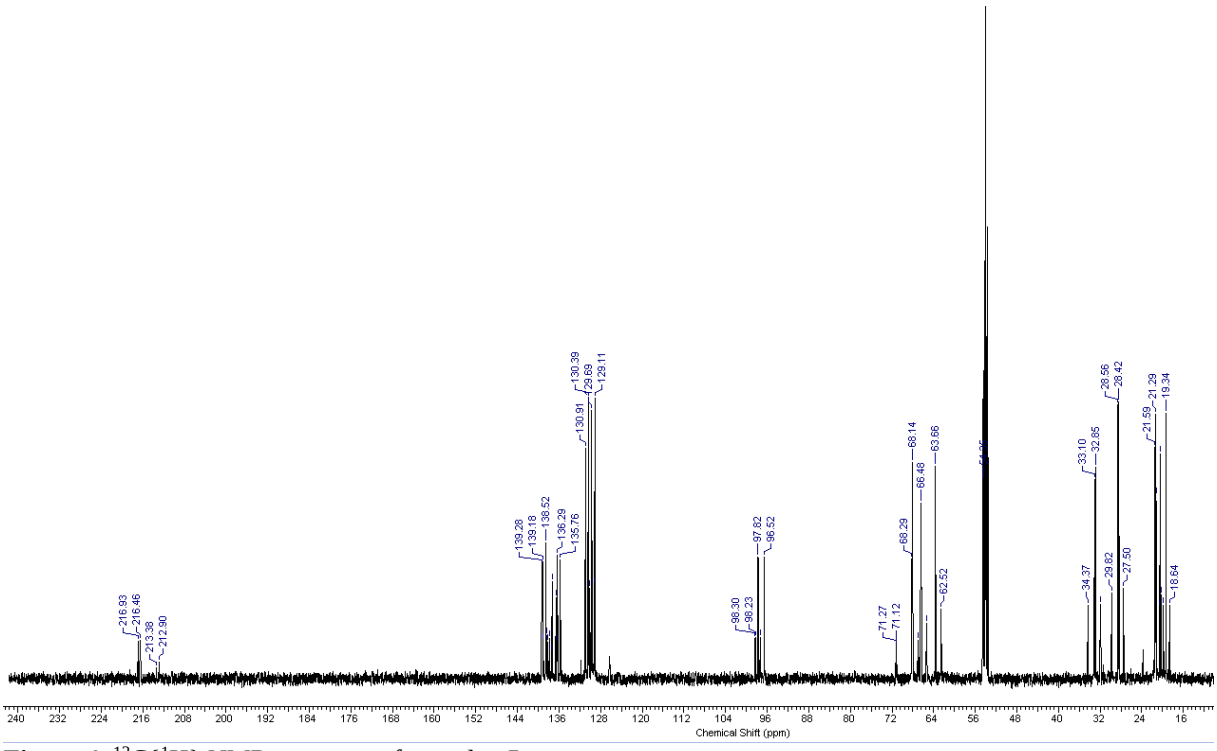


Figure 4. ^{13}C{^{1}H} NMR spectrum of complex **5**.

Synthesis of rhodium complex 6:

Complex **5** (50.0 mg, 0.086 mmol, 1.00 equiv.) was dissolved in THF (5 mL). A solution of *N*-maleimido butanoyl chloride (20.0 mg, 0.095 mmol, 1.10 equiv.) in THF (2 mL) was added dropwise followed by the addition of $NaHCO_3$ (50 mg, 0.58 mmol, 6.8 equiv.). The mixture was stirred for 24 h at 25 °C. The mixture was filtered to remove solid $NaHCO_3$. DCM (15 mL) was added and the solution was washed with water (3 x 15 mL) and dried over $MgSO_4$. The solvent DCM was removed under vacuum. Complex **6** was obtained as a yellow-orange solid (52.6 mg, 0.068 mmol, 79% yield).

^{1}H-NMR (400 MHz, CD_2Cl_2, 23 °C): δ = 7.15-6.91 (m, 4H, Aryl-*H*), 6.73-6.62 (br s, 2H, *H*C=C*H* (maleimide)), 4.60-4.40 (m, 1H, *H*C=C*H*), 4.40-4.25 (m, 1H, N-CH_2), 4.16-4.07 (m, 1H, CH_2,), 4.00-3.73 (m, 3H, CH_2, C*H*), 3.62-3.33 (m, 4.5H, CH_2CH$_2$, CH_2, C*H*), 3.05-2.97 (m, 0.5H, C*H*) 2.80-2.21 (m, 20H, CH$_2$CH_2, CH_3), 1.97-1.87 (quint, 2H, J = 7.03 Hz, CH$_2$CH_2CH$_2$), 1.86-1.35 (m, 9H, CH_2) ppm.

^{13}C{^{1}H}-NMR (100 MHz, CD_2Cl_2, 23 °C): δ = 216.99-216.52 (d, $^1J_{Rh-C}$ = 46.7 Hz, N*C*N), 213.44-212.96 (d, $^1J_{Rh-C}$ = 48.6 Hz, N*C*N), 171.36, 168.95, 139.29, 139.19, 138.54, 138.51, 138.43, 138.11, 137.88, 137.27, 136.61, 136.36, 136.29, 135.75, 134.70, 134.64, 130.92, 130.40, 130.30, 130.16, 129.69, 129.25, 129.18, 129.11, 98.29-98.23 (d, $^1J_{Rh-C}$ = 6.6 Hz, *C*=*C*), 97.81-97.74 (d, $^1J_{Rh-C}$ = 6.6 Hz, *C*=*C*), 97.36-97.30 (d, $^1J_{Rh-C}$ = 6.6 Hz, *C*=*C*), 96.59-96.52 (d, $^1J_{Rh-C}$ = 6.6 Hz, *C*=*C*), 71.23, 71.09, 68.27, 68.13, 67.99, 67.02, 66.88, 66.48, 65.35, 64.45, 64.22, 63.67, 62.54, 62.38, 62.26, 54.35, 37.13, 34.37, 33.09, 32.92, 32.85, 31.95, 30.25, 29.82, 28.56, 28.42, 27.51, 23.73, 21.59, 21.29, 21.18, 20.63, 20.44, 20.26, 19.81, 19.34, 19.32, 18.63 ppm.

ESI-HR-MS positive mode (*m*/*z*)

Calculated $[M\text{-}Cl^-]^+$ ($C_{38}H_{47}N_3O_4Rh$): 712.2633

Found $[M\text{-}Cl^-]^+$: 712.2608

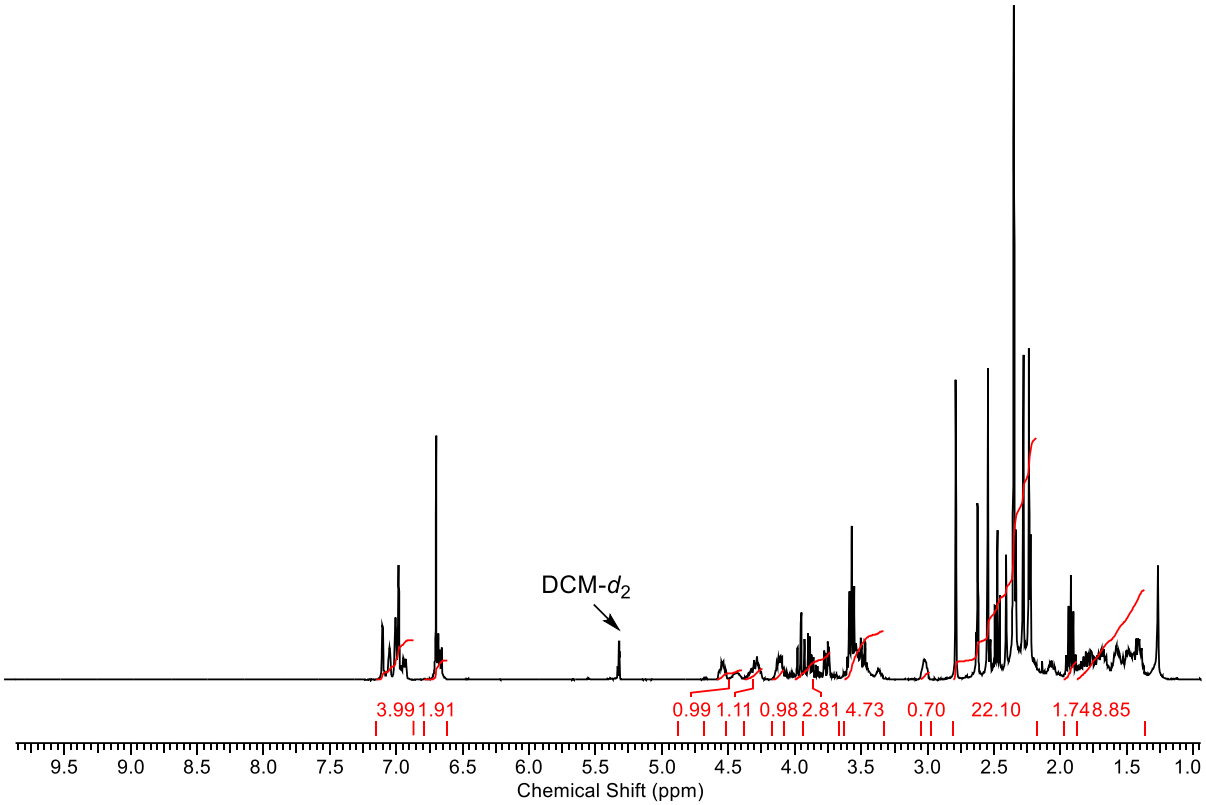


Figure 5. ^{1}H NMR spectrum of complex **6**.

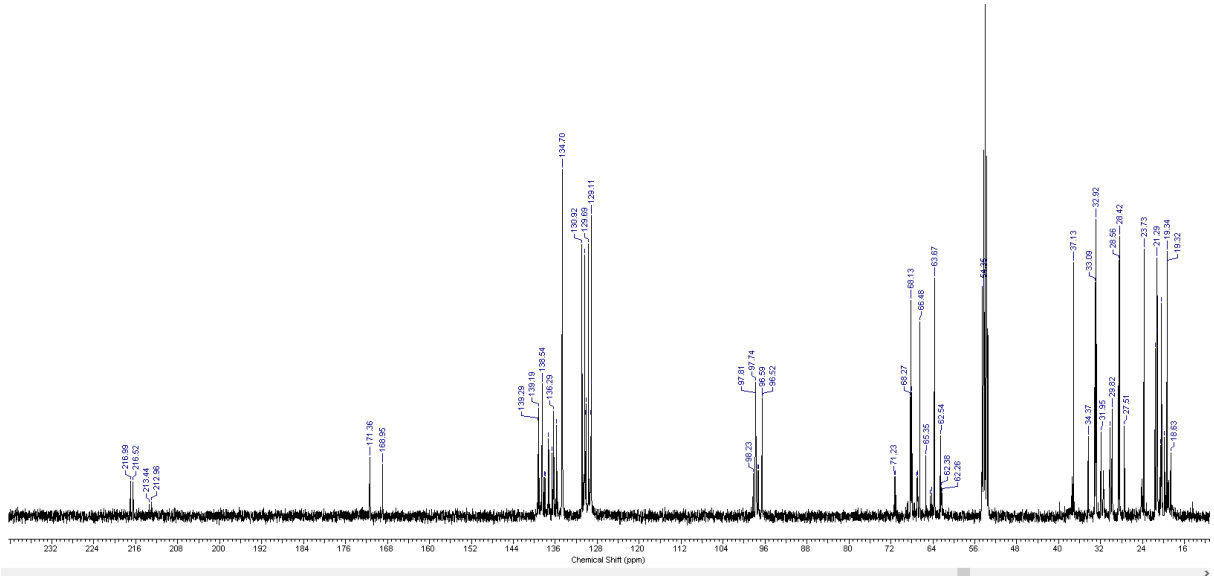
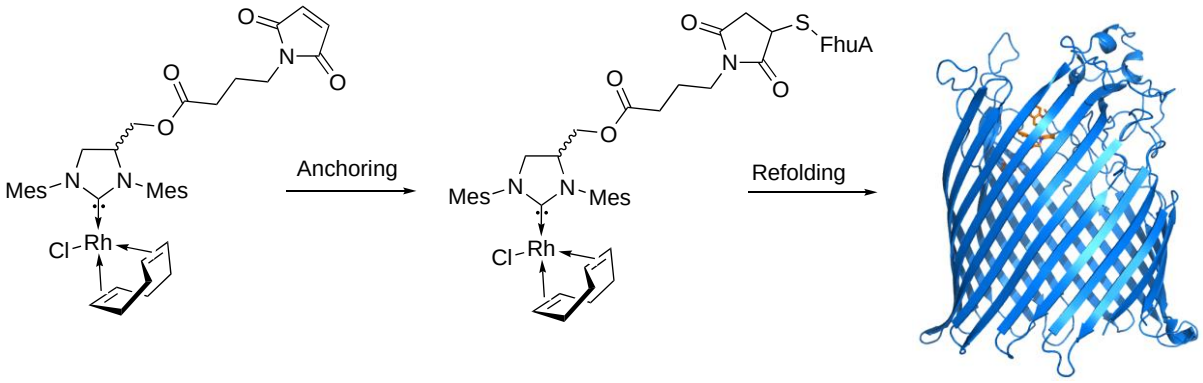


Figure 6. ^{13}C{^{1}H} NMR spectrum of complex **6**.

Coupling, anchoring and refolding of rhodium catalyst 6 to FhuA and characterization of the biohybrid conjugate



Scheme S1: Coupling and refolding of rhodium catalyst 6 to FhuA.

In a glovebox, rhodium catalyst **6** was dissolved in THF (1 mg catalyst per mL ***FhuA ΔCVF^{tev}** solution, 20 (v/v)%) and was added dropwise to the ***FhuA ΔCVF^{tev}** solution in degassed water (1 (w/w)% SDS, c(*FhuA ΔCVF^{tev}) = 5 mg/mL). This mixture was stirred for 24 h at 25 °C. The solvent was removed under vacuum and the residue was washed with THF (6 x 10 mL).

ThioGlo fluorescence titration

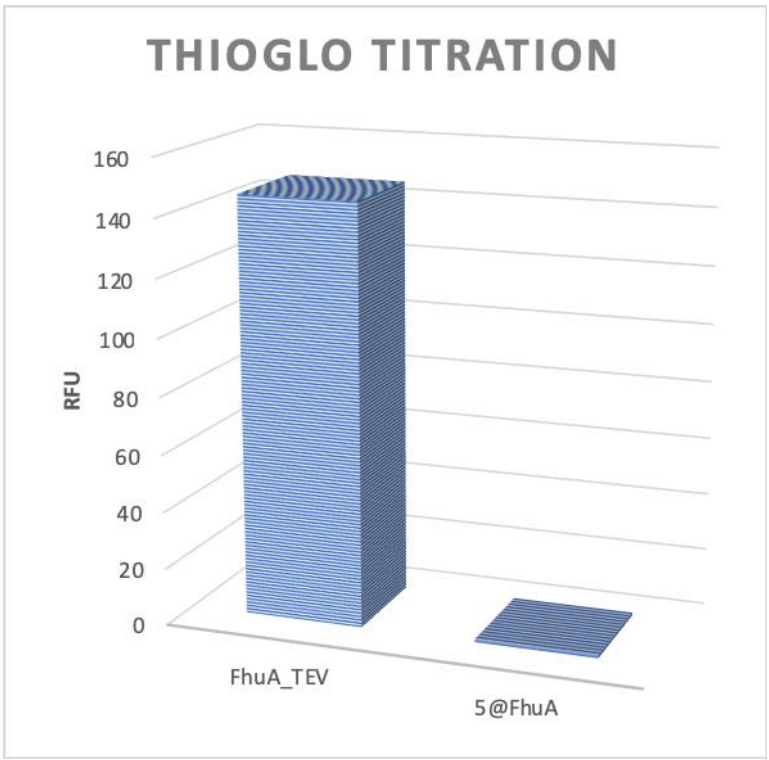


Figure S7. ThioGlo® fluorescence titration.

CD spectroscopy

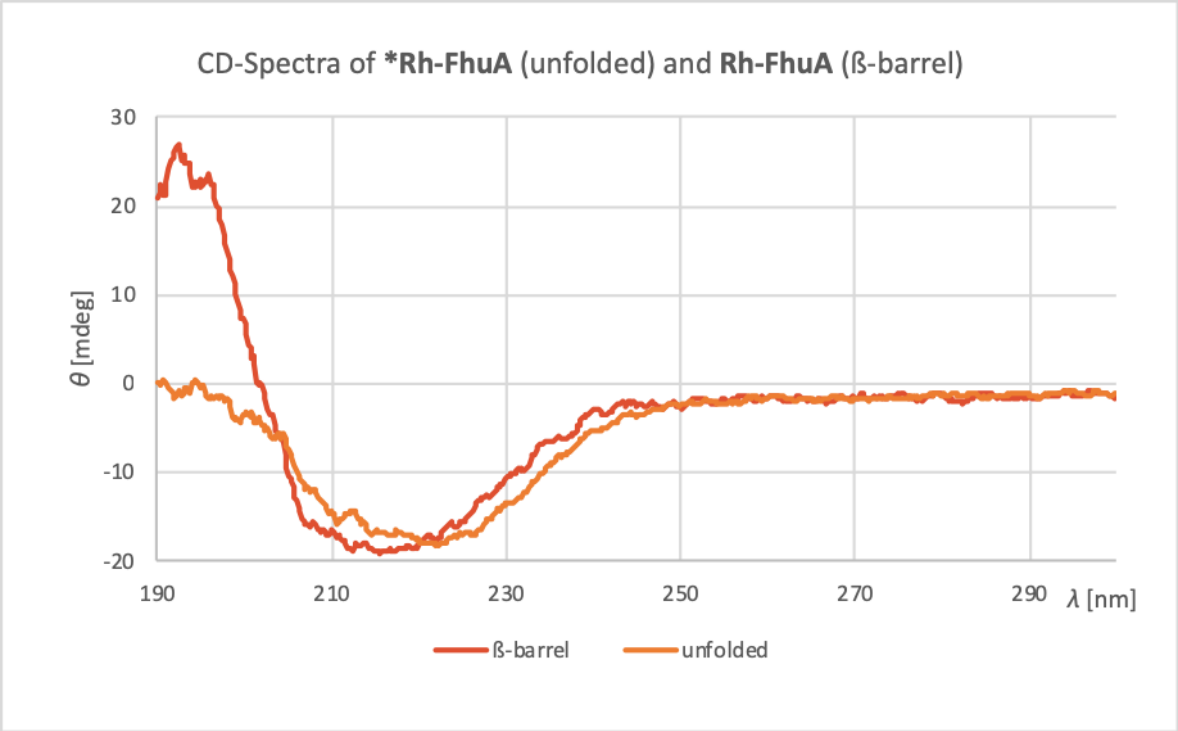


Figure S8: CD spectroscopy of partially unfolded and refolded **5**@FhuA.

MALDI ToF MS

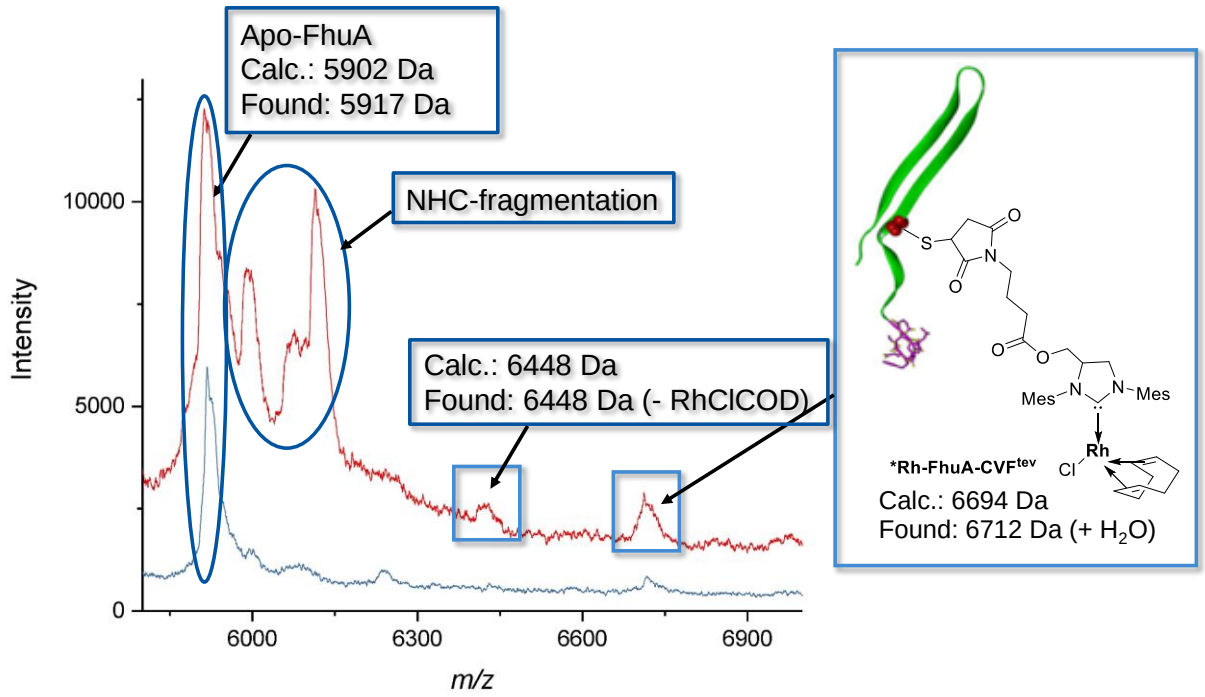


Figure S9. MALDI ToF MS analysis of protein without metal catalyst (blue) and with metal catalyst **6** (red).

General protocols for catalysis

All reactions were performed in a glass autoclave. Reaction vessel was placed in a preheated oil bath.

Metathesis reaction: Reaction conditions: c(catalyst) = 0.2 mM, c(substrate) = 20 mM, water, pH = 6.0 (100 mM NaP_i), c(NaCl) = 50 mM, 1 (w/w)% SDS, 20 (v/v)% THF, final volume: 2 mL. After reaction time indicated, the reaction was quenched by addition of ethyl vinyl ether (50 equiv.). The mixture was extracted with DCM and analyzed via GC-MS.

Hydrogenation reaction: Reaction conditions: c(catalyst) = 0.2 mM, c(substrate) = 20 mM, water, pH = 6.0 (100 mM NaP_i), c(NaCl) = 50 mM, 1 (w/w)% SDS, 20 (v/v)% THF, final volume: 2 mL. Hydrogen gas (1 bar) was introduced after three cycles of freeze-pump-thaw. After the reaction time indicated, the reaction mixture was extracted with DCM and analyzed via GC-MS.

Cascade reaction: Reaction condition: c(respective catalyst) = 0.1 mM, c(substrate) = 10 mM, water, pH = 6.0 (100 mM NaP_i), c(NaCl) = 50 mM, 1 (w/w)% SDS, 20 (v/v)% THF, final volume: 4 mL. 48 h, hydrogen gas (1 bar) was introduced after three cycles of freeze-pump-thaw. After the reaction time indicated, the reaction mixture was extracted with DCM and analyzed via GC-MS.

References

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S2. R. M. de Figueiredo, P. Oczipka, R. Fröhlich and M. Christmann, *Synthesis*, 2008, DOI: 10.1055/s-2008-1032016, 1316-1318.

S3. G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw and K. I. Goldberg, *Organometallics*, 2010, **29**, 2176-2179.