Electronic Supporting Information

An artificial ruthenium-containing β-barrel protein for alkene-alkyne coupling reaction

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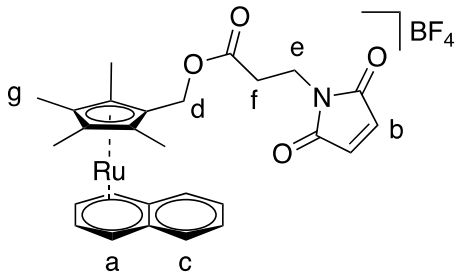
Table of contents

1. General Remarks

All operations were performed under an inert atmosphere of dry argon or nitrogen using standard Schlenk line or glovebox techniques. Acetonitrile (MeCN), D_3-Acetonitrile (D_3-MeCN), and CD_2Cl_2 were distilled under argon atmosphere from calcium hydride prior to use. Tetrahydrofuran (THF), diethyl ether (Et_2O), and dichloromethane (DCM) were purified using a MB SPS-800 solvent purification system. ^1H, $^{11}B\{^1H\}$, $^{13}C\{^1H\}$, and $^{19}F\{^1H\}$, NMR spectra were recorded on a *Bruker Avance II 400* or a *Bruker Avance III HD 400* spectrometer at 25 °C in J. Young-type NMR tubes. Chemical shifts for ^1H and $^{13}C\{^1H\}$ spectra were referenced internally using the residual solvent resonance.[1] The resonances in the ^1H and $^{13}C\{^1H\}$ NMR spectra were assigned on the basis of two-dimensional NMR experiments (COSY, HSQC, HMBC). High-resolution mass spectrometry (HRMS) was performed on an LTQ-Orbitrap XL ESI-MS spectrometer (ThermoFisher Scientific). MALDI–ToF MS spectra were recorded on an Ultraflex III ToF/ToF mass spectrometer from *Bruker* using 2,5-dihydroxybenzoic acid (DHB) as matrix. Komplex $[Ru(Me_4CH_2Cp^{OH})(C_{10}H_8)][BF_4]$ (**1**)[2], 3-(2,5-dioxo-2,5- dihydro-1H-pyrrol-1-yl)propanoyl chloride (**2**)[3] and the proteins (FhuA wildtype[4] and FhuA variant (FhuA Δ1-159_C545_V548_F501_tev or short FhuA ΔCVF^{tev})[5]) have been synthesized as previously reported.

2. Experimental Section

2.1. Synthesis of $[Ru(Me_4CH_2Cp^{Mal})(C_{10}H_8)][BF_4]$ (3)



Under argon atmosphere, $[Ru(Me_4CH_2Cp^{OH})(C_{10}H_8)][BF_4]$ (**1**) (100 mg, 0.21 mmol) and 3-(2,5-dioxo-2,5- dihydro-1H-pyrrol-1-yl)propanoyl chloride (**2**) (42.1 mg, 0.225 mmol) were placed in a Schlenk-flask and DCM (2 mL) was added. Subsequently, triethylamine *(74 µL, 0.54 mmol) was added dropwise*. After stirring 16 h at room temperature, the brown suspension was extracted with H_2O (3 x 2 mL) to afford a clear brown solution. The organic layer was dried over $MgSO_4$ and volatiles were removed *in vacuo.* Complex **3** was obtained as off-white solid (75 mg, 0.12 mmol, 57%).

^{1}H NMR (400 MHz, CD_2Cl_2): δ = 7.71 (dd, $^3J_{HH}$ = 6.8, 3.2 Hz, 2 H, naph-H_a), 7.55 (dd, $^3J_{HH}$ = 6.7, 3.3 Hz, 2 H, naph-H_a), 6.67 (s, 2 H, H_b), 6.60 (dd, $^3J_{HH}$ = 4.4, 2.4 Hz, 2 H, naph-H_c), 6.09 (dd, $^3J_{HH}$ = 4.4, 2.4 Hz, 2 H, naph-H_c), 4.63 (s, 2 H, H_d), 3.73 (t, $^3J_{HH}$ = 7.1 Hz, 2H, H_e), 2.58 (t, $^3J_{HH}$ = 7.1 Hz, 2H, H_f), 1.68 (s, 6H, H_g),1.65 (s, 6H, H_g) ppm.

^{13}C{^{1}H} NMR (101 MHz, CD_2Cl_2): δ = 170.86 (*C*=O), 170.52 (*C*=O, mal), 134.77 (H*C*=*C*H), 132.16 (*C*H), 128.20 (*C*H), 98.01 (C_q, naph), 95.90 (C_q, Cp), 95.33 (C_q, Cp), 89.60 (Cq, Cp), 89.05 (*C*H), 86.18 (*C*H), 57.75 (CH_2), 33.96 (CH_2), 33.12 (CH_2), 9.83 (CH_3), 9.73 (CH_3) ppm.

^{11}B{^{1}H} NMR (128 MHz, CD_2Cl_2): δ = -0.93 (s) ppm.

^{19}F{^{1}H} NMR (376 MHz, CD_2Cl_2): δ = -151.59 (s) ppm.

ESI MS (*m/z*): calc $[C_{27}H_{28}NO_4Ru]^+$: 532.1062; found: 532.1065.

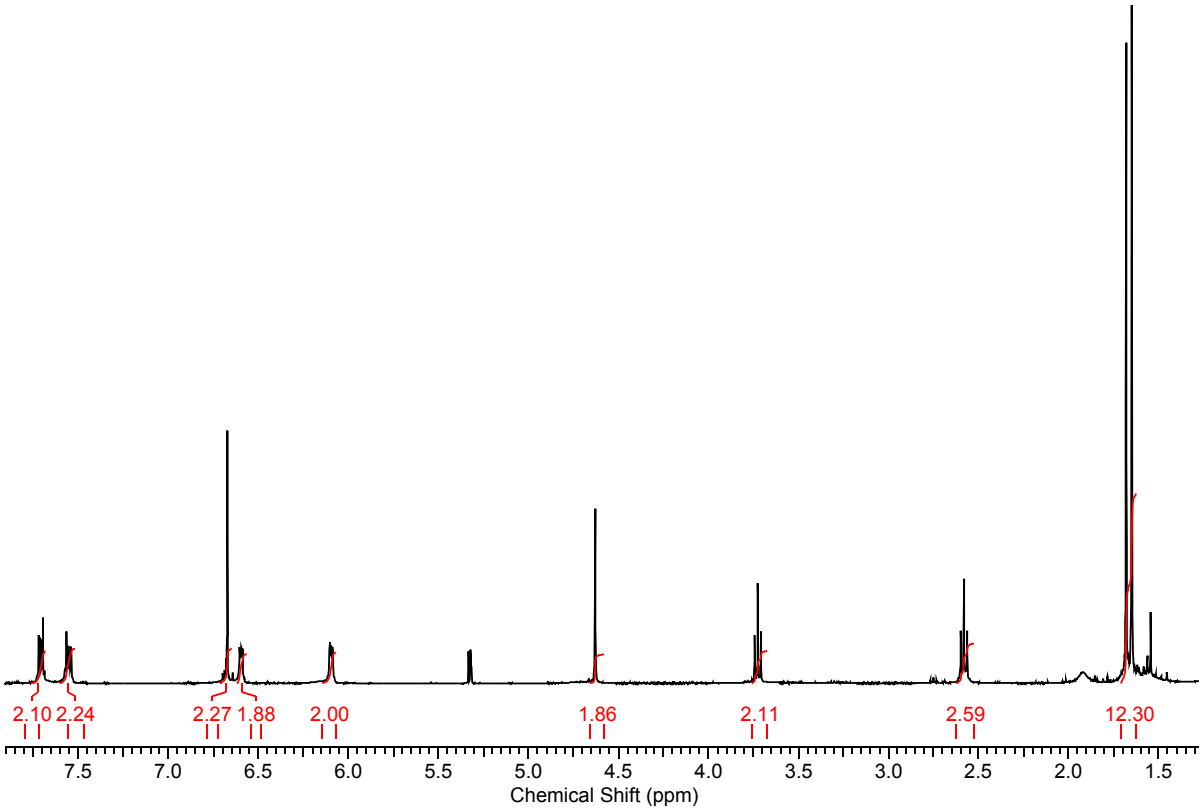


Figure S1: ^{1}H NMR spectrum (400 MHz, CD_2Cl_2, 27 °C) of $[Ru(Me_4CH_2Cp^{Mal})(C_{10}H_8)][BF_4]$ (**3**).

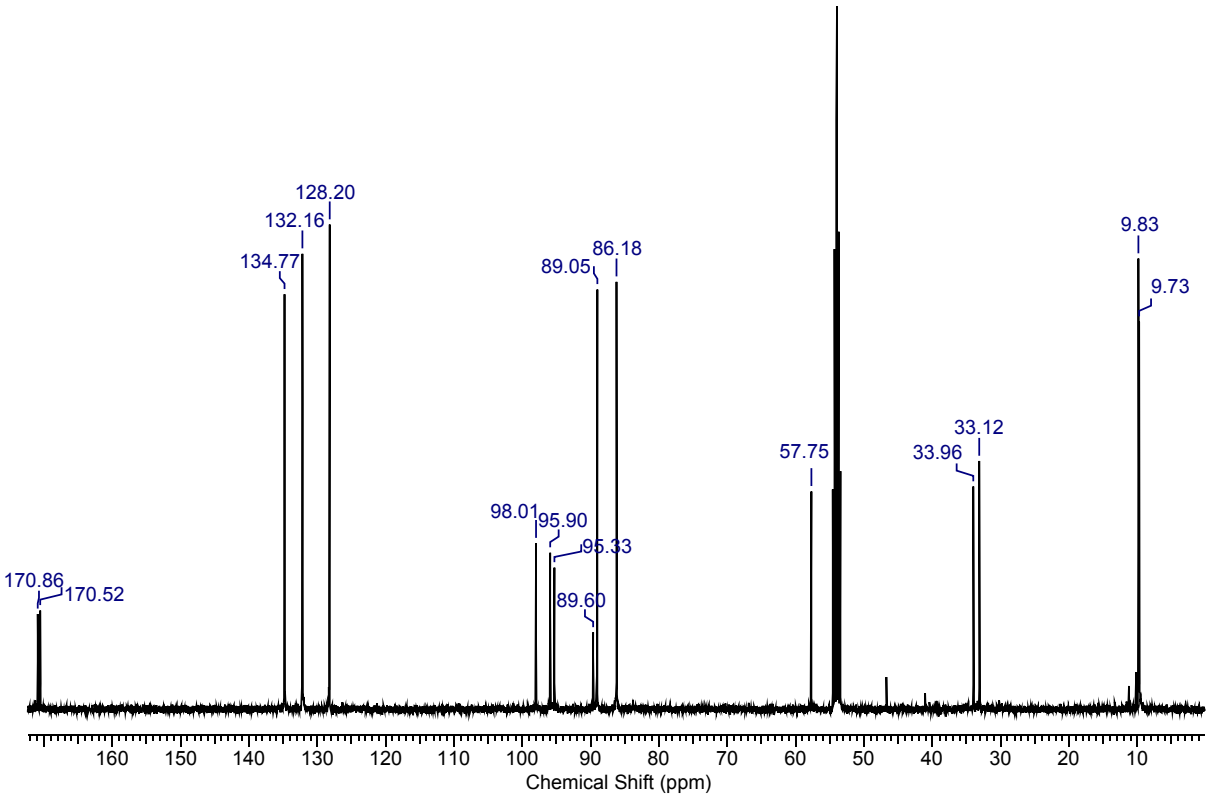


Figure S2: $^{13}C\{^{1}H\}$ NMR spectrum (101 MHz, CD_2Cl_2, 27 °C) of $[Ru(Me_4CH_2Cp^{Mal})(C_{10}H_8)][BF_4]$ (**3**).

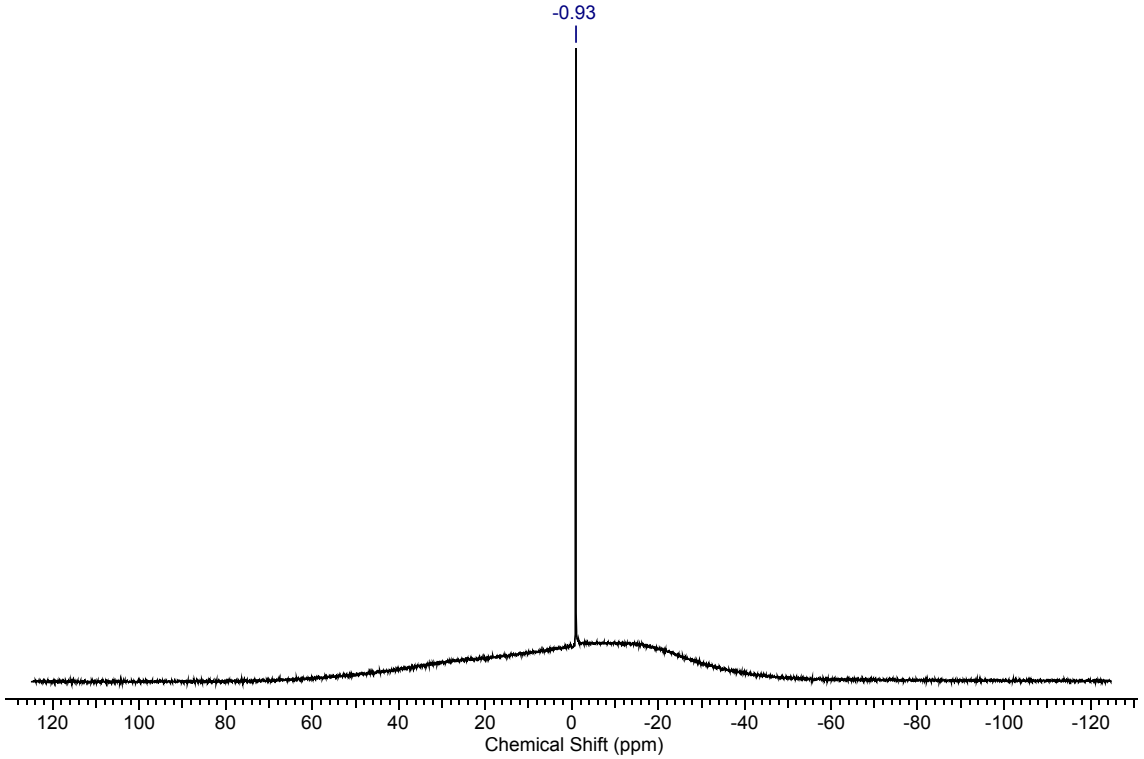


Figure S3: $^{11}B\{^1H\}$ NMR spectrum (128 MHz, $CD2Cl_2$, 27 °C) of $[Ru(Me_4CH_2Cp^{Mal})(C_{10}H_8)][BF_4]$ (**3**).

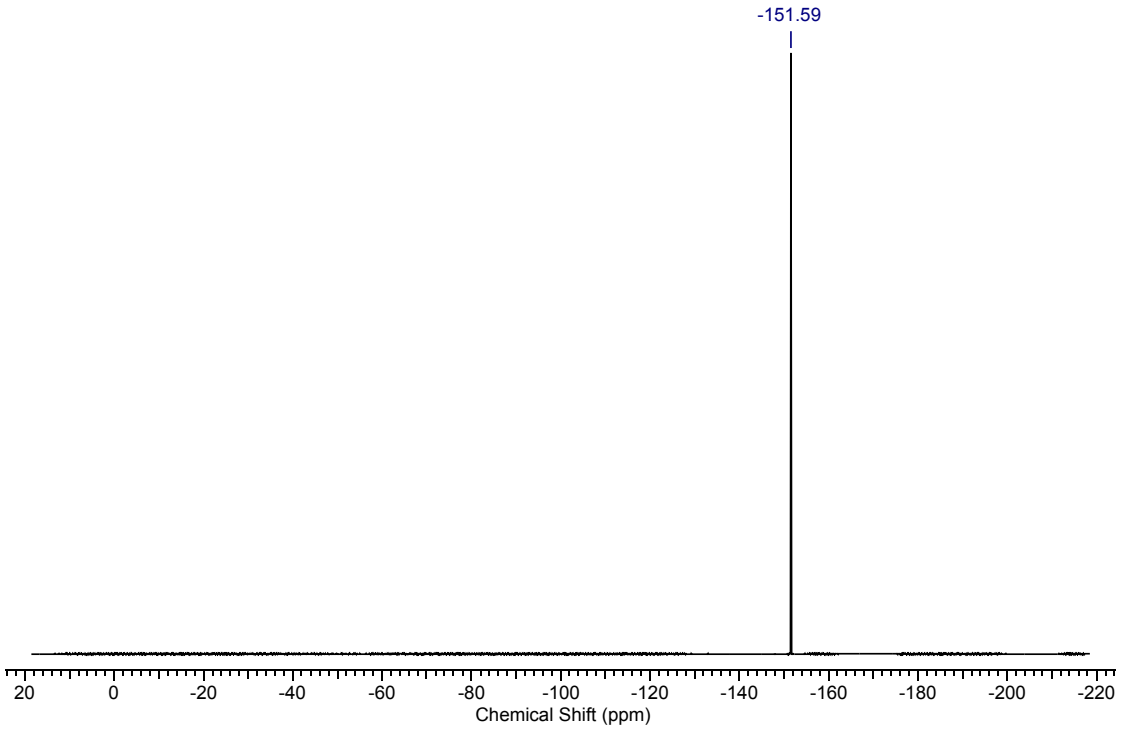
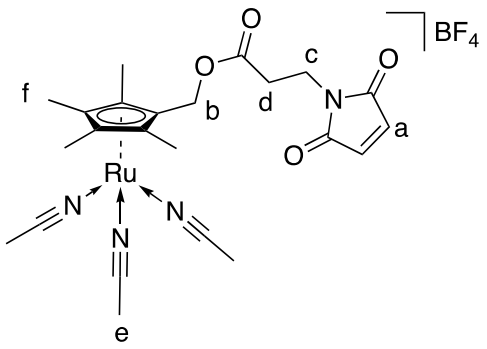


Figure S4: $^{19}F\{^1H\}$ NMR spectrum (376 MHz, $CD2Cl_2$, 27 °C) of $[Ru(Me_4CH_2Cp^{Mal})(C_{10}H_8)][BF_4]$ (**3**).

2.2. Synthesis of $[Ru(Me_4CH_2Cp^{Mal})(MeCN)_3][BF_4]$ (4)



$[Ru(Me_4CH_2Cp^{Mal})(C_{10}H_8)][BF_4]$ (**3**) (24 mg, 39 µmol) was dissolved in MeCN (1 mL) and stirred at ambient temperature for 24 h. The solution was concentrated to 0.5 mL and washed with Et_2O (3 x 2 mL) and cyclohexane (3 x 1 mL). The solution was evaporated affording compound **4** as forest-green solid (15 mg, 24 µmol, 63%).

^{1}H NMR (400 MHz, CD_3CN): δ = 6.71 (s, 2H, H_a), 4.69 (s, 2H, H_b), 3.69 (t, $^3J_{HH}$ = 7.0 Hz, 2H, *H*c), 2.57 (t, $^3J_{HH}$ = 7.0 Hz, 2H, H_d), 2.14 (s, 9H, H_e), 1.65 (s, 6H, H_f), 1.63 (s, 6H, *H*f) ppm.

^{11}B{^{1}H} NMR (128 MHz, CD_2Cl_2): δ = -1.19 (s) ppm.

^{19}F{^{1}H} NMR (376 MHz, CD_2Cl_2): δ = -153.369 (s) ppm.

ESI MS (m/z): $[C_{19}H_{23}N_2O_4Ru]^+$ calc: 445.0696; found: 445.0692. and $[C_{17}H_{20}NO_4Ru]^+$ calc: 404.0430; found: 404.0434.

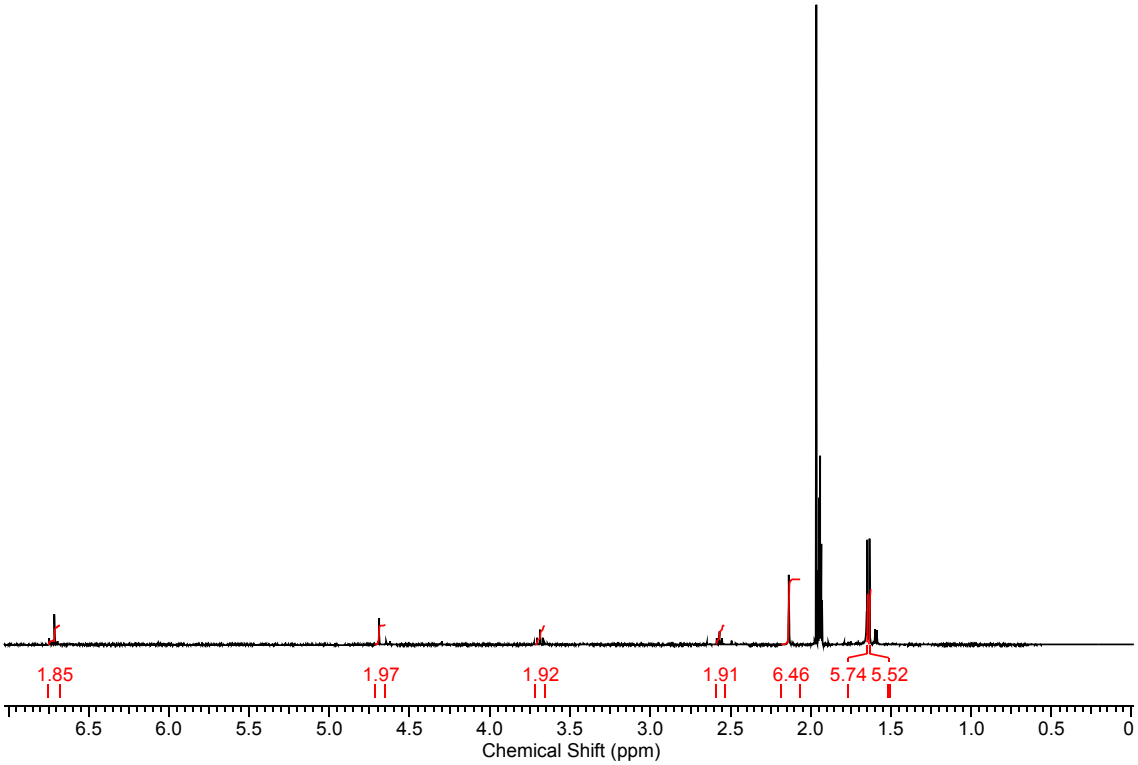


Figure S5: ^{1}H NMR spectrum (400 MHz, CD_3CN, 27 °C) of $[Ru(Me_4CH_2Cp^{Mal})(MeCN)_3][BF_4]$ (**4**).

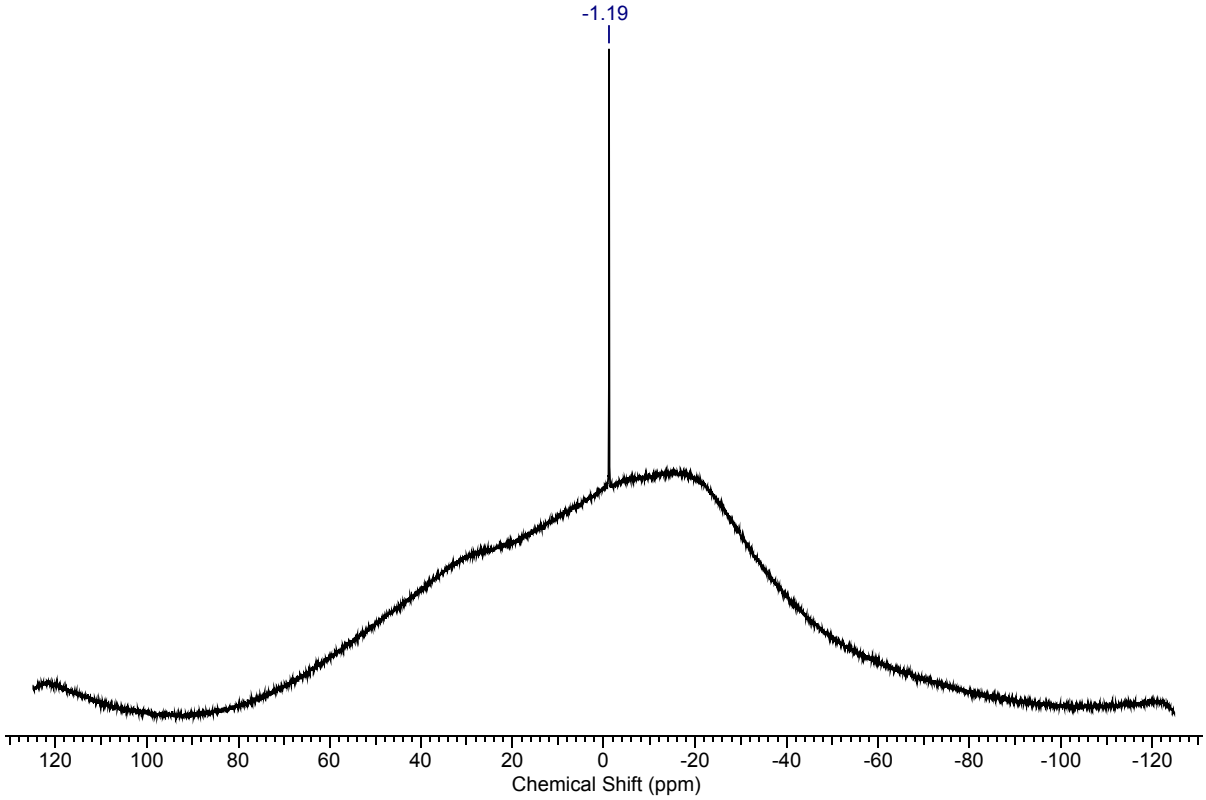


Figure S6: $^{11}B\{^{1}H\}$ NMR spectrum (128 MHz, CD_3CN, 27 °C) of $[Ru(Me_4CH_2Cp^{Mal})(MeCN)_3][BF_4]$ (**4**).

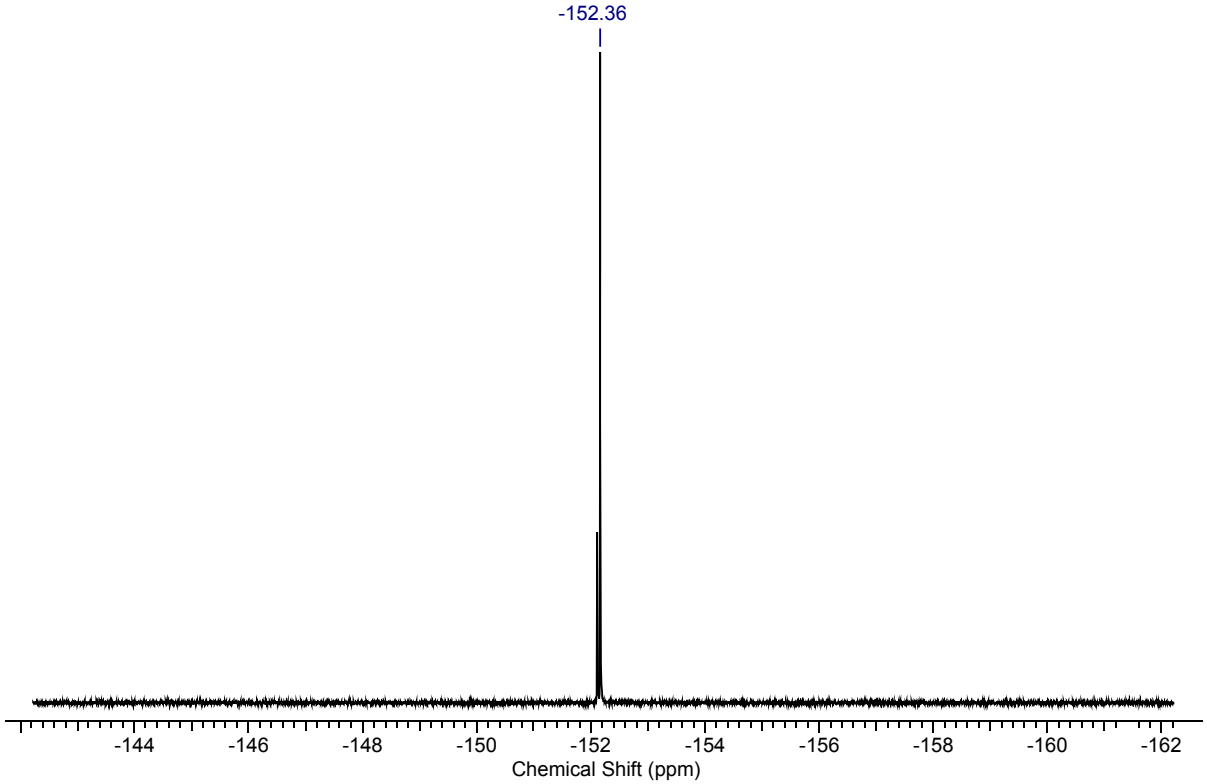


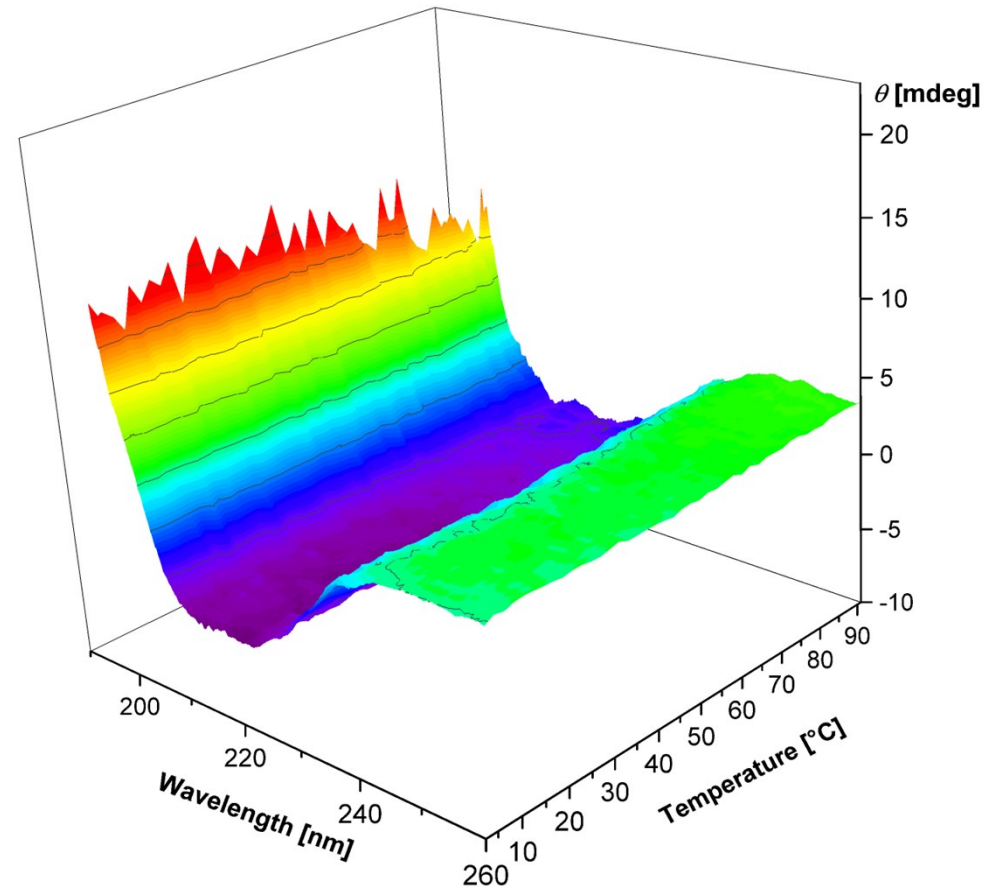
Figure S7: $^{19}F\{^{1}H\}$ NMR spectrum (376 MHz, CD_3CN, 27 °C) of $[Ru(Me_4CH_2Cp^{Mal})(MeCN)_3][BF_4]$ (**4**).

2.3. Coupling to FhuA

In a nitrogen filled glovebox, FhuA wildtype or FhuA ΔCVF^{tev} containing 1 (w/w)% SDS was dissolved in water (10 mL, 10 mg/mL), respectively. [Ru($Me_4CH_2Cp^{Mal}$)$(MeCN)_3$][BF_4] (**4**) (10 equiv.) in MeCN (10 (v/v)%) was added and stirred for 16 h. The solvent was removed *in vacuo* and the residue was washed with degassed MeCN (3 x 10 mL). The residue was dissolved in degassed water (10 mL) and transferred to a dialysis tube (MWCO = 12-14 kDa). Dialysis proceeded against a sodium phosphate buffer with 100-fold volume compared to the protein solution. The buffered solution contained sodium phosphate (NaP_i, 100 mM, pH = 8), EDTA (1 mM), 2-Methyl-2,4-pentanediol (MPD, 50 mM) and water. The dialysis solution was changed after 24 h and dialysis was performed for 48-96 hours.

2.4. CD-Spectroscopy

Circular dichroism (CD) spectra were recorded on a *J-1100* spectrometer (JASCO) equipped with a single position Peltier element. The temperature was set to 20 °C. The pathlength of the cuvette was 0.5 mm. Protein concentrations were adjusted to 20 µM. For variable temperature CD (VTCD) spectroscopy, temperatures from 4 °C to 92 °C were measured in 2 °C steps with a heating-ramp of 1 °C/min. Measurements were started when the target temperature was stable for more than 10 seconds. The temperature was measured inside the cuvette. Spectra were recorded with four accumulations in a range from 260 to 190 nm.



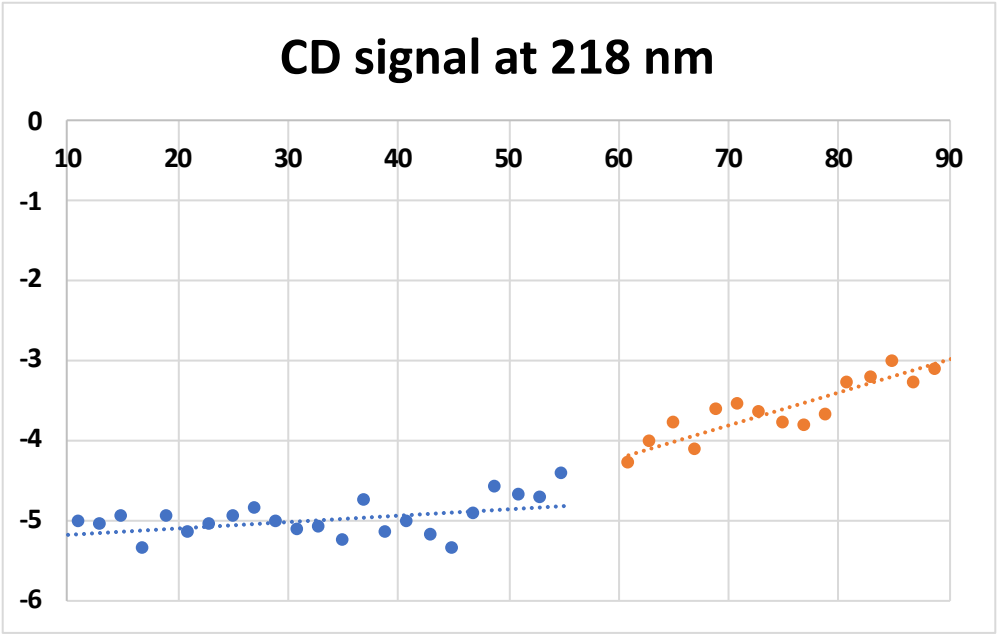


Figure S8: Variable temperature CD spectroscopy. The CD spectrum of FhuA shows characteristic minimum at 218 nm and maximum at 196 nm, indicating a β-sheet structure. Structural integrity is confirmed for temperatures up to 60 °C. Above ca. 60 °C, minimal changes in the CD spectra are observed, however, the protein is not fully denatured up the 90°C.

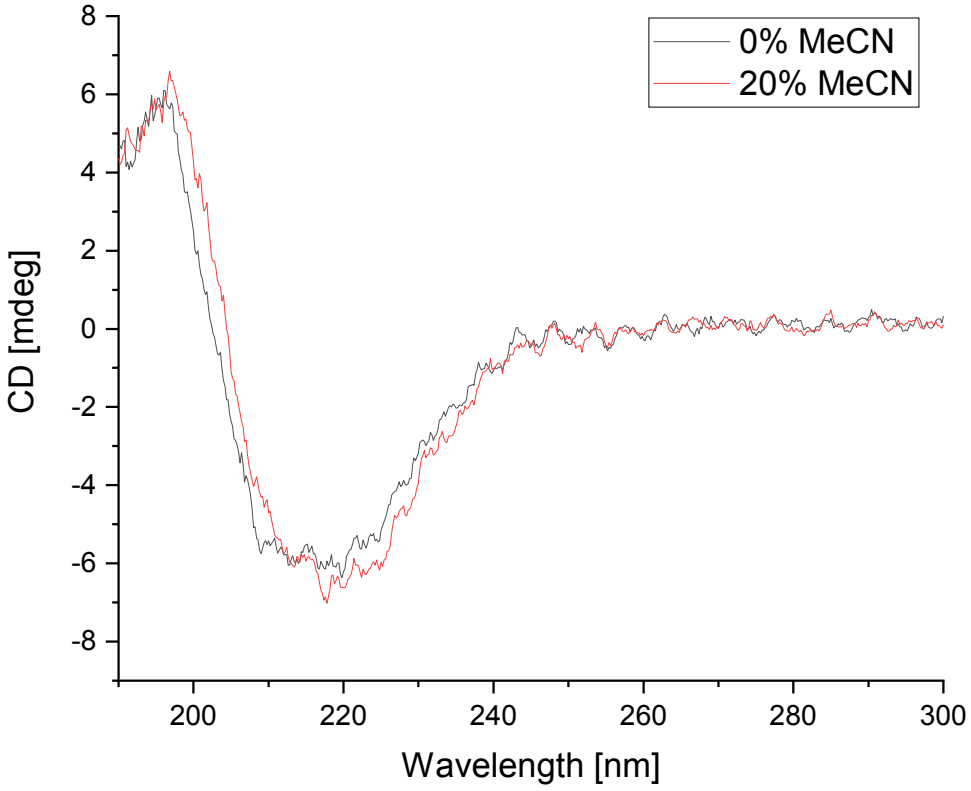


Figure S9: Comparison of CD spectra of **6** with (red) and without (black) 20% MeCN.

2.5. ThioGlo

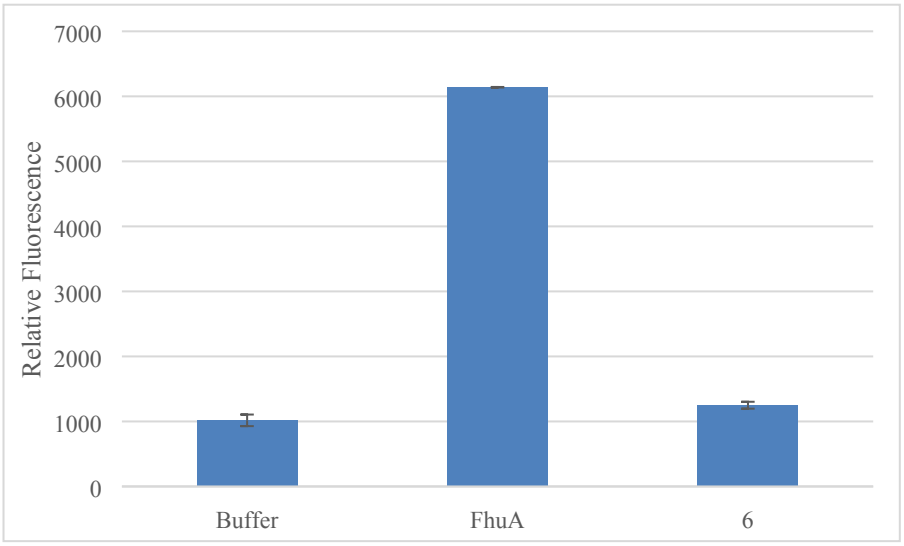


Figure S10: Fluorescence measurement of FhuA and biohybrid catalyst **6** treated with ThioGlo. Low fluoresence indicate a high coupling efficiency, because ThioGlo becomes fluoresent upon conjugation to a thiol. Sample preparation was performed as previously reported.[5] In the given example, the coupling efficiency was 95% (1-((1249.5-1017)/(6140-1017))*100 = 95%). As reference, untreated FhuA of the same biological batch was used.

2.6. MALDI-ToF MS

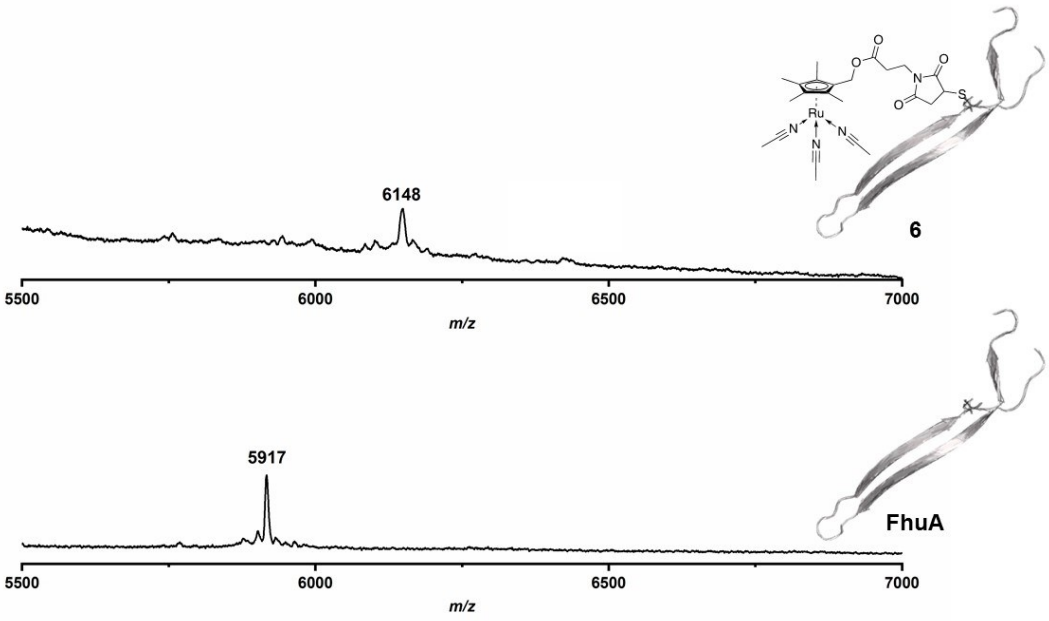


Figure S11. MALDI-ToF MS spectrum after TEV cleavage[5] of **6** (top) and FhuA without a catalyst attached (bottom). The calculated mass for the cleaved FhuA fragment (5902 Da) is in good agreement with the observed mass (5917 Da). The observed signal at *m/z* = 6148 was assigned to the ligand having the ester moiety cleaved and the maleimide moiety hydrolyzed (calc. $[C_7H_{13}NNaO_6]^+$: 6147 Da).

2.7. Catalysis

In a typical catalysis run, protein-free catalyst **4** (5 mol%) or biohybrid catalyst **6** (0.5 mol%) were dissolved in sodium phosphate buffer (NaPi, 100 mM, 1 mM EDTA) containing 2-Methyl-2,4-pentanediol (MPD, 50 mM) and 20 (v/v)% MeCN inside a nitrogen-filled glovebox. The coupling efficiency of the biohybrid catalyst **6** was determined with the ThioGlo cysteine titration prior to catalysis (see section "2.5 ThioGlo"), as previously established.[4] The concentration is given with respect to the coupled catalyst. That is, the metal loading was constantly 0.5 mol% in all catalytic runs. For example, if the coupling efficiency was determined to be 96%, the amount of protein corresponds to 0.52 mol% (0.5 $mol\%_{metal\ loading}$ / 0.96 = 0.52 $mol\%_{protein\ loading}$). 4-butenol (c(**7**) = 32 mM) and 5-hexynenitrile (c(**8**) = 32 mM) were added (Total reaction volume: 1 mL). The reaction vial was placed in an oil bath at 60 °C. After the reaction time indicated, the reaction mixture was extracted with DCM containing 1 mM mesitylene as internal standard, dried over $MgSO_4$ and analyzed *via* GCMS. The TOF value was calculated by dividing the TON by the reaction time indicated.

3. References

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