Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

for

Immobilization of an Artificial Imine Reductase within Silica Nanoparticles Improves its Performance

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Experimental

General

Tetraethyl orthosilicate (TEOS, ≥ 99 %), (3-aminopropyl)-triethoxysilane (APTES, ≥ 98 %), ammonium hydroxide (ACS reagent, 28–30 %), ethanol (anhydrous), glutaraldehyde (Grade I, 25 % in water), 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline, 2-(N-morpholino)ethanesfonic acid, 3-(N-morpholino)propanesulfonic acid, sodium formate, sodium hydroxide, DCM, isopropanol, diethylamine, DMF, DMSO, *N*-Boc-ethylenediamine, 4-nitrophenylsulfonyl chloride, diethyl ether, *N*-methylmorpholine, 2- chloro-4,6-dimethoxy-1,3,5-triazine, Et_3N, acetonitrile, $[Cp^*IrCl_2]_2$ dimer and NAD^+ were purchased from commercial suppliers and used without further purification.

Mili-Q water (resistivity ≥ 18 MΩ cm^{-1}) was produced with a Millipore Synergy purification system. Streptavidin (Sav) mutants were produced, purified and characterized as previously described [1].

^1H and ^{13}C spectra were recorded on a Bruker 400 MHz. The chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane or a residual solvent peak, and the *J* values are given in Hz. Signals are quoted as s (singlet), d (doublet), t (triplet) and m (multiplet). Analyses of the catalytic runs were performed on an Agilent 1100 normal phase HPLC with an analytical Chiralpak IC column (250 · 4.6 mm, 5 µm). ESI-MS measurements were performed on a Bruker AmaZon™ X. The absorbance spectra were measured on a microplate reader Tecan, model Infinite M200.

Nanoparticles were imaged using a Zeiss SUPRA 40VP field emission scanning electron microscope, their size was measured using the acquired micrographs with the Olympus Analysis® software package.

ICP-MS analysis was performed on a 7500cx ICP-MS system (Agilent, Basel, Switzerland). ^{191}Ir was used for quantification, ^{193}Ir for verification of the results.

Stock solutions and buffers

MOPS/formate buffer: 3-(N-morpholino)propanesulfonic acid and sodium formate were dissolved in Milli-Q water to the concentrations of 6M (formate) and 1.2 M (MOPS). The pH was adjusted to 7 by addition of NaOH.

MES buffer: 2-(N-morpholino)ethanesulfonic acid was dissolved in Mili-Q water to a final concentration of 10 mM. The pH was adjusted to 6.2 by the addition of NaOH.

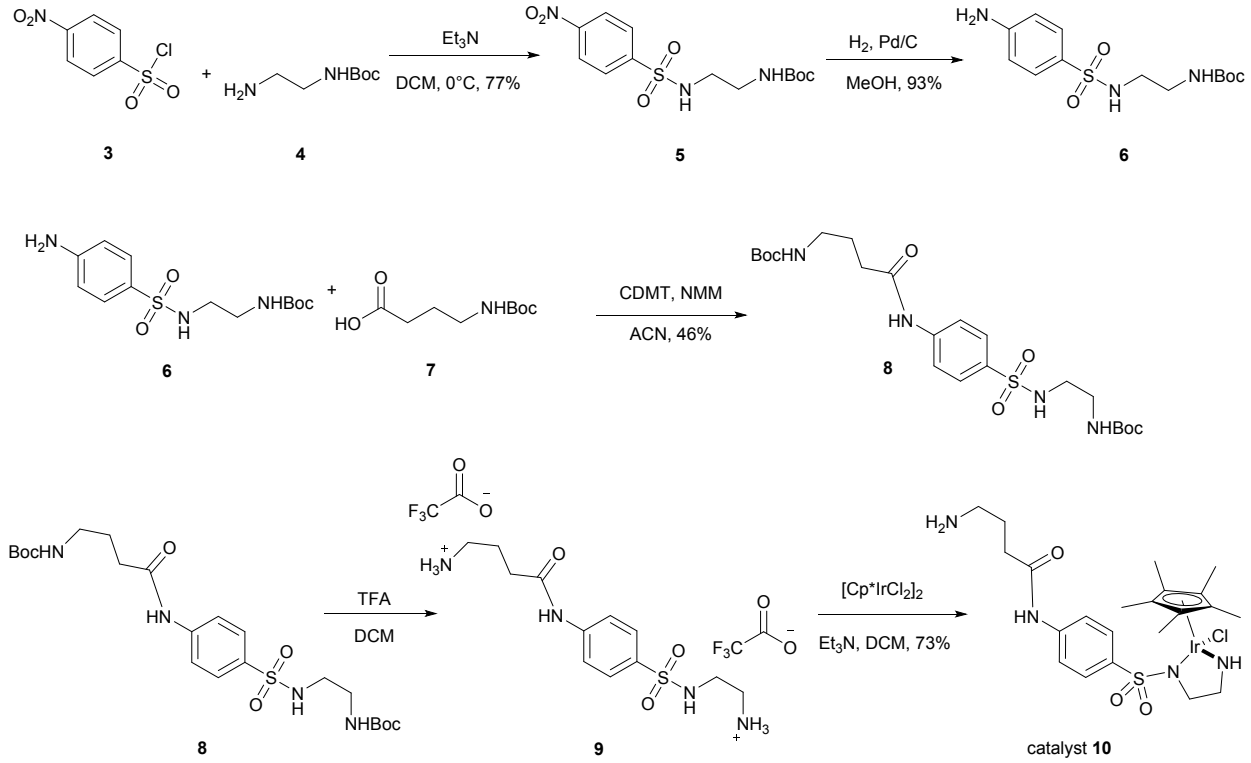
Activity assay buffer: Sodium formate and K_2HPO_4 were dissolved in Mili-Q water to a final concentration of 200 mM (formate) and 50 mM (phosphate). The pH was adjusted to 7.5 by addition of NaOH.

*Cp*Ir-complexes:* [Cp*Ir(biot-*p*-L)Cl)] was dissolved in DMF to a final concentration of 500 mM. For a detailed synthesis procedure, see reference [2]. [Cp*Ir(4C-L)Cl] was dissolved in DMF to a final concentration of 400 mM.

Substrate: 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline **1** was dissolved in Mili-Q water (2 ml) to a final concentration of 1 M.

Synthesis of the non-biotinylated catalyst 10

The synthesis was performed as summarized in following Scheme:



Scheme 1- Synthesis of catalyst **10**

N-Boc-*N'*-(4-nitrophenylsulfonyl)-ethylenediamine 5

To a solution of *N*-Boc-ethylenediamine **4** (1.74 g, 10.8 mmol, 1.0 eq.) and Et_3N (3 ml, 21.5 mmol, 2.0 eq.) in DCM (300 ml), a solution of 4-nitrophenylsulfonyl chloride **3** (2.51 g, 11.3 mmol, 1.05 eq.) in DCM (100 ml) was added dropwise at 5 °C and stirred overnight. The solution was concentrated to 100 ml, washed with water (3 x 20 ml), and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the crude product was purified by flash chromatography (silicagel, EtOAc : hexane (2 : 3)) The pure product **5** was obtained as a white powder (2.87 g, 8.32 mmol, 77 % yield). ^{1}H NMR (200 MHz, $CDCl_3$-CD_3OD, 22 °C): δ 1.40 (s, 9H; *t*-Bu), 3.03 (m, 2H; CH_2-CH_2- NH-S), 3.12 (m, 2H; CH_2-CH_2-NH-S), 4.47 (br, 2H; NH), 8.04 (d, J = 8.7 Hz, 2H; ArH), 8.37 (d, J = 8.7 Hz, 2H; ArH).

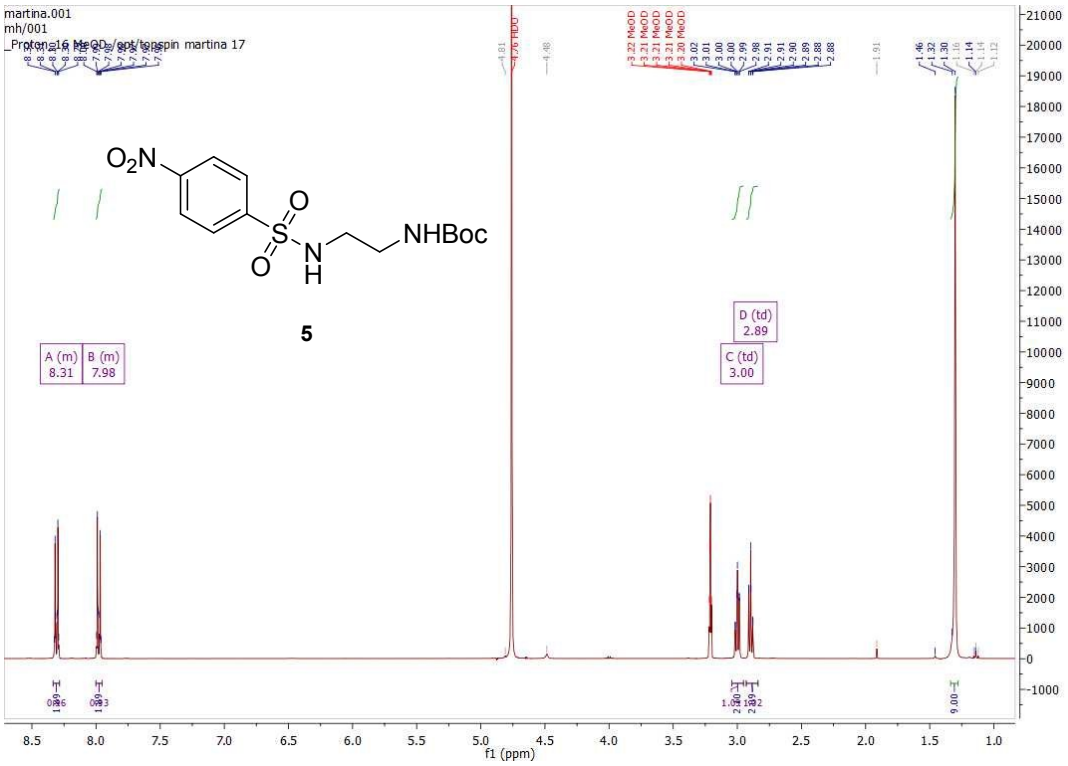


Figure 1- ^{1}H NMR of **5**

N-Boc-*N*'-(4-aminophenylsulfonyl)-ethylenediamine 6

An autoclave containing a suspension of Pd/C (5 %, 330 mg) in methanol (20 ml) was charged with *N*-Boc-*N*'-(4-nitrophenylsulfonyl)-ethylenediamine **5** (2.10 g, 6.08 mmol). The autoclave was purged three times with nitrogen and filled with hydrogen (3 bars). The reaction was stirred vigorously for 3 hours at RT. The hydrogen pressure was carefully released and the resulting suspension was removed by filtration through a celite plug. The solvent was evaporated under reduced pressure, which yielded product **6** as a white powder (1.78 g, 5.65 mmol, 93 %). ^{1}H NMR (400 MHz, $CDCl_3$-CD_3OD, 22 °C): δ 1.40 (s, 9H; *t*-Bu), 2.88 (m, 2H; CH2-CH2- NH-S), 3.12 (m, 2H; CH_2-CH_2-NH-S), 3.57 (br, 3H; NH; NH_2), 4.50 (br, 1H; NH), 6.62 (m, 2H; ArH), 7.47 (m, 2H; ArH).

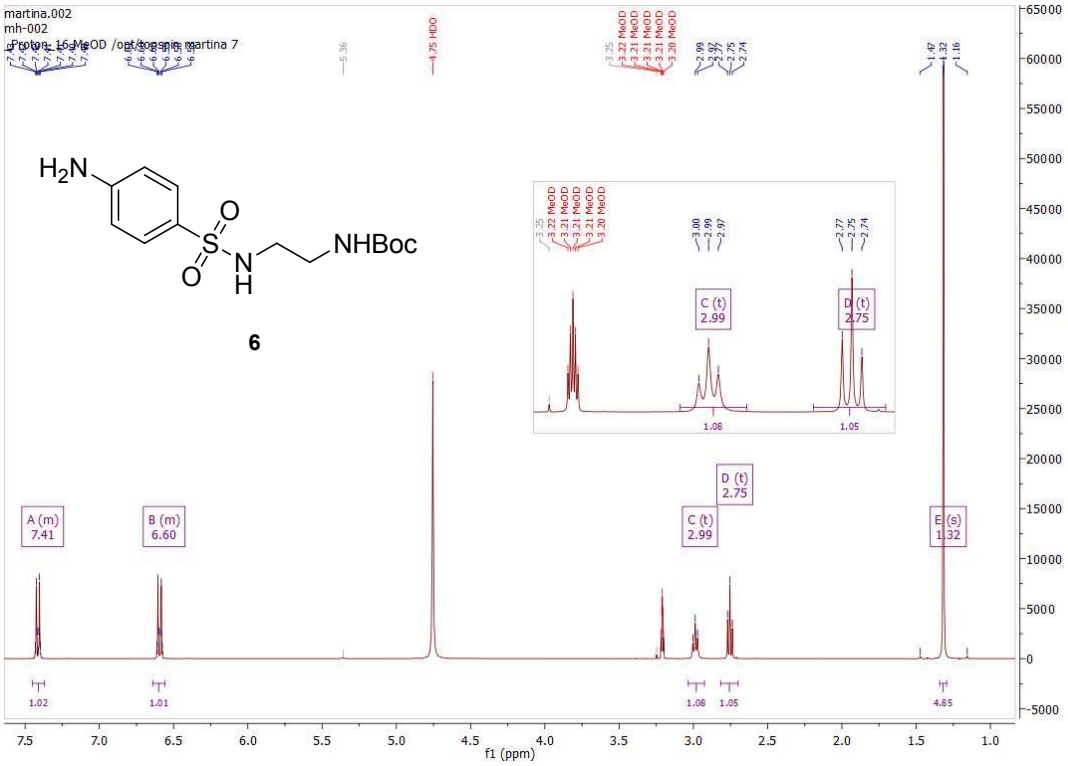


Figure 2- ^{1}H NMR of **6**

***N*-Boc-*N'*-(4-(*N'*-Boc-4-aminobutanamido)phenylsulfonyl)-ethylenediamine 8**

To a suspension of *N*-Boc-4-aminobutanoic acid **7** (13.2 mg, 0.065 mmol, 1.03 eq.), *N*-Boc-*N* '-(4-aminophenylsulfonyl)-ethylenediamine **6** (20 mg, 0.063 mmol, 1.0 eq.) and 2- chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) as a coupling agent (11.8 mg, 0.067 mmol, 1.06 eq.) in acetonitrile (10 ml), *N*-methylmorpholine (NMM) (9.6 µl, 0.095 mmol, 1.5 eq.) was added. The resulting reaction mixture was stirred at RT for 24 hours and heated to reflux for 6 hours. After cooling the reaction mixture to room temperature, water (3 ml) was added. The solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (silicagel using EtOAc : n-hexane (2:1)) to obtain the product **8** as a white powder (12.6 mg, 0.025 mmol, 40 % yield). ^{1}H NMR (400 MHz, Chloroform-d) δ 9.66 (s, 1H, NH), 7.78 (s, 4H, ArH), 5.40 (s, 1H, NH), 4.92 (s, , 2H, NH), 3.24 (m, , 4H, CH_2), 3.03 (q, *J* = 5.7 Hz, 2H, CH_2), 2.42 (m, 2H, CH_2), 1.88 (m, 2H, CH_2), 1.48 (s, 9H, *t*-Bu), 1.42 (s, 9H, t-Bu). ^{13}C NMR (101 MHz, Methanol-d_4) δ 174.69 (CO, *t*-Bu), 158.93 (CO, *t*-Bu), 144.45 (Ar-NH), 136.47 (Ar-S), 129.46 (2C, Ar-H) , 120.97 (2C, Ar-H), 80.66 (O-C-$(CH_3)_3$, *t*-Bu) , 80.43 (O-C-$(CH_3)_3$, *t*-Bu), 44.21 (CH_2-NH), 41.70 (CH_2-NH), 41.21 (CH_2-NH), 35.70 (CH_2-CO), 29.18 (3C, CH_3, *t*-Bu), 29.13 (3C, CH_3, *t*-Bu), 27.37 (CH_2- CH_2- CH_2).

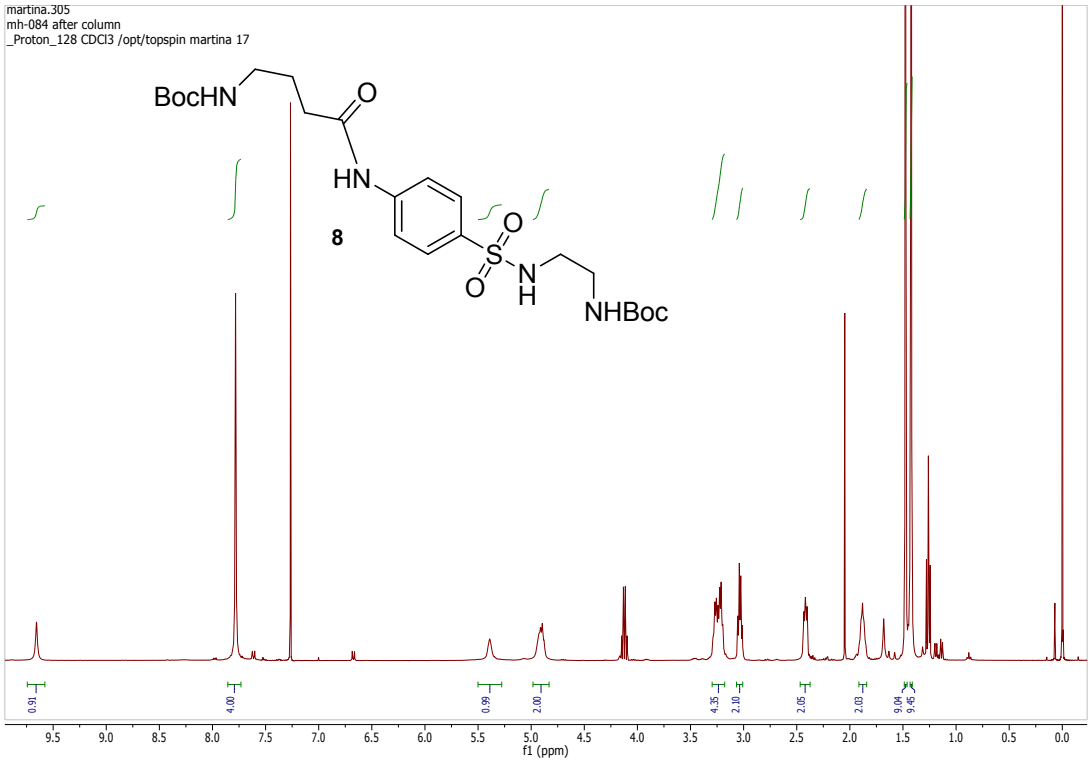


Figure 3- ^{1}H NMR of **8**

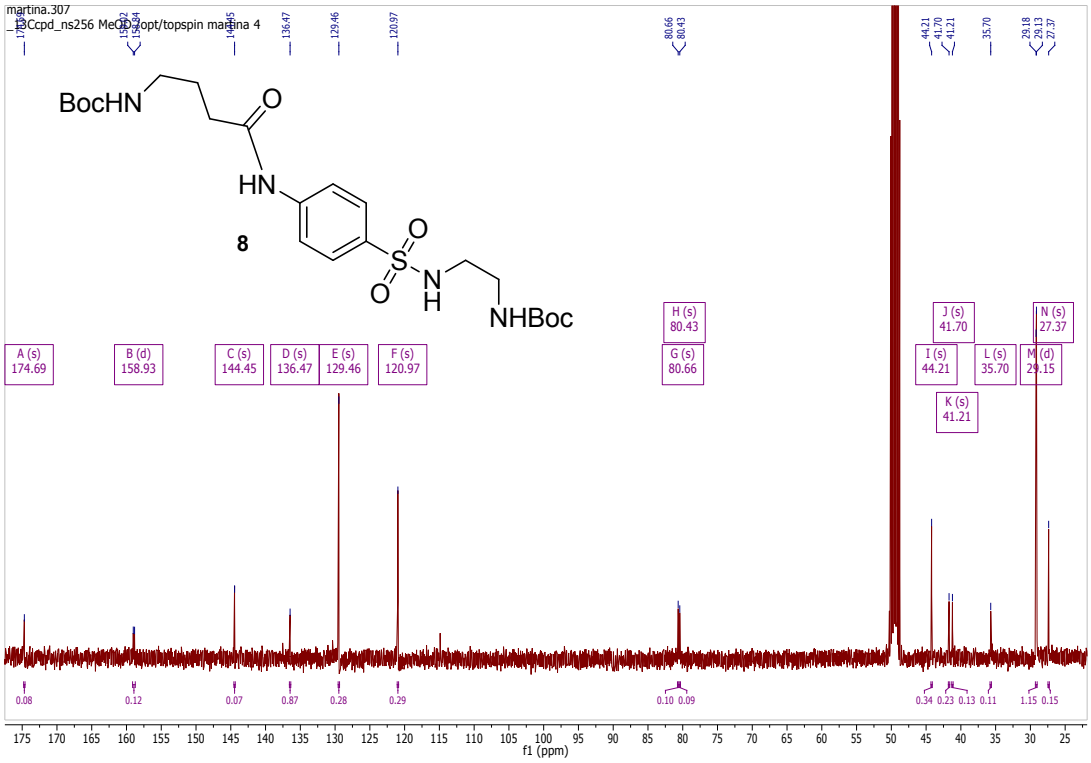


Figure 4- ^{13}C NMR of **8**

***N*-(4-(4-aminobutanamido)phenylsulfonyl)-ethylenediamine 9**

Trifluoroacetic acid (300 µl, 3 mmol, 30 eq.) was added dropwise to a suspension of *N*-Boc-*N'*-(4-(*N'*-Boc-4-aminobutanamido)phenylsulfonyl)-ethylenediamine **8** (50 mg, 0.1 mmol) in dry DCM (5 ml) at 0 °C. The resulting solution was stirred at RT for 6 hours. The solvent was evaporated under reduced pressure. The crude product dissolved in MeOH and diethyl ether was added until a white precipitate formed. The precipitated product was collected by filtration and dried under reduced pressure. Pure product **9** was obtained as white powder (39 mg, 0.074 mmol, 75 %). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.82 (s, 4H, ArH), 3.11 – 3.00 (m, 6H, CH_2-CH_2- CH_2), 2.59 (t, *J* = 7.0 Hz, 2H, CH_2), 2.02 (p, *J* = 7.1 Hz, 2H, CH_2). ^{13}C NMR (101 MHz, Methanol-d_4) δ 173.77 (CO), 144.91 (Ar-NH) , 135.82 (Ar-S), 129.90 (2C, ArH), 121.29 (2C, ArH), 41.97 (CH_2-NH_2), 41.29 (CH_2-NH_2), 40.94 (CH_2-NH_2), 35.00(CH_2-NH_2), 24.61 (CH_2-CH_2-CH_2).

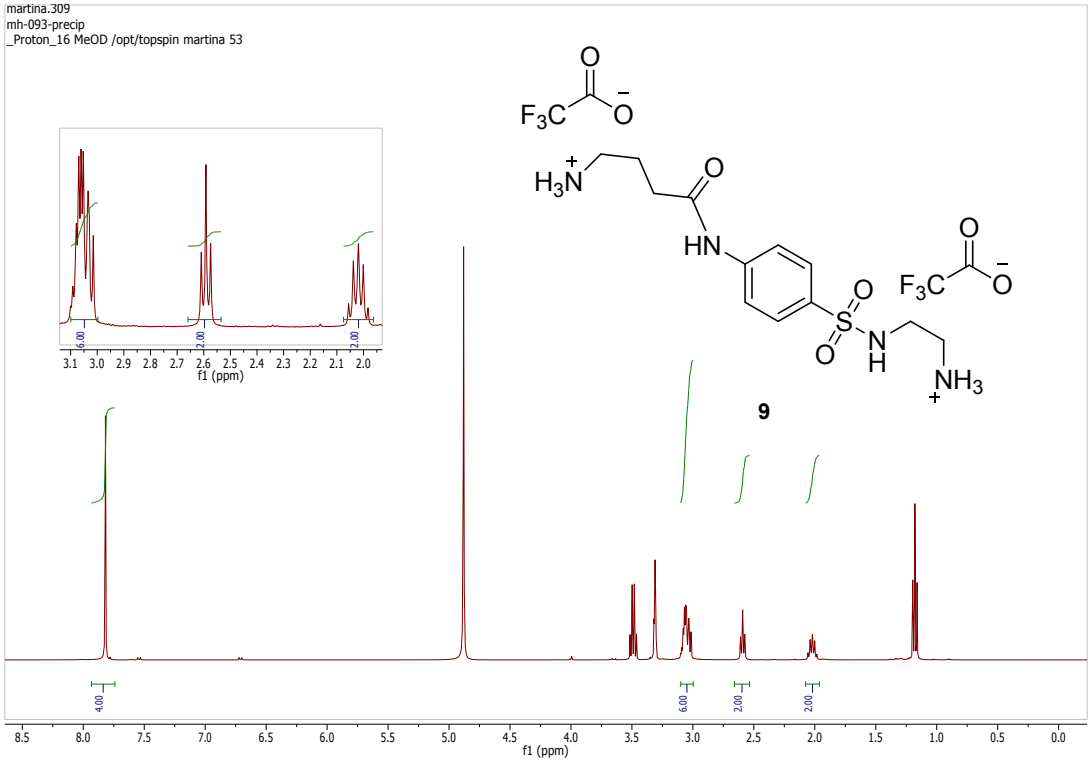


Figure 5- ^{1}H NMR of **9**

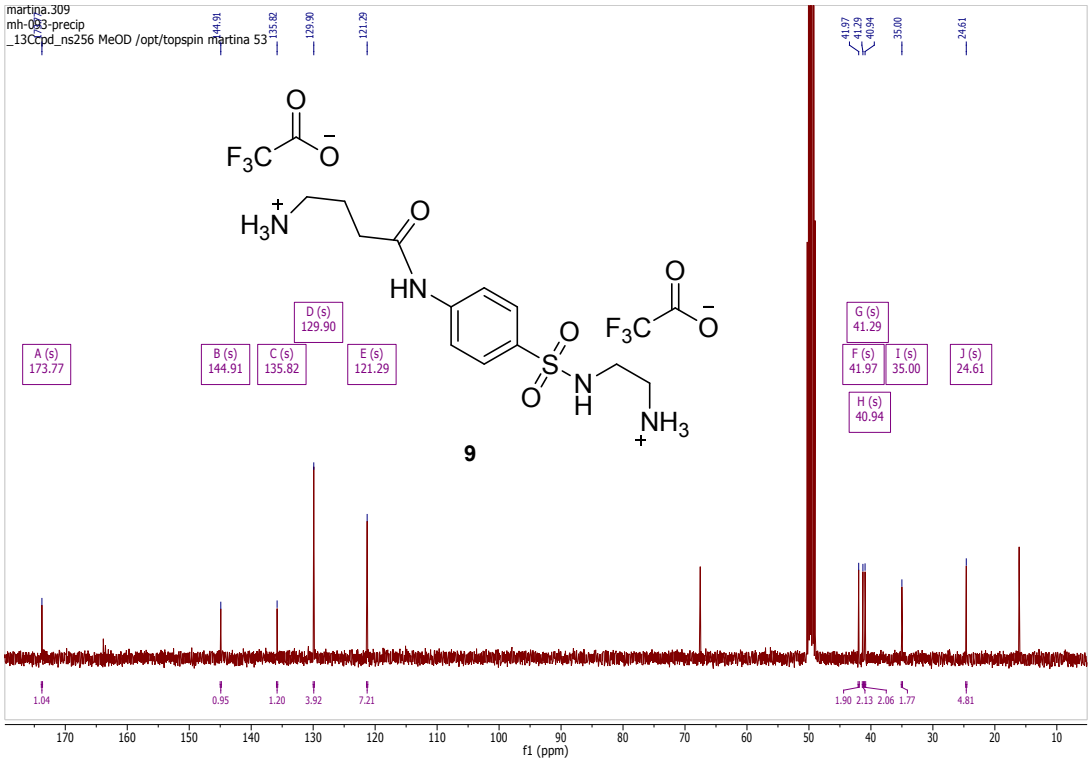


Figure 6- ^{13}C NMR of **9**

Synthesis of catalyst [Cp*Ir(4C-L)Cl] 10

The $[Cp^*IrCl_2]_2$ dimer (30 mg, 0.038 mmol, 0.475 eq.), the sulfonamide ligand **9** (40 mg, 0.076 mmol, 1 eq.) and Et_3N (26 µl, 0.19 mmol, 5 eq.) were dissolved in dry DCM (5 ml) under inert atmosphere. After stirring for 24 hours at room temperature, the solution was heated to reflux for 3 hours. The precipitate was filtrated, dissolved in MeOH (1 ml) and precipitated with diethyl ether. The pure catalyst **10** was obtained as a dark orange powder (15 mg, 0.022 mmol, 60 %). ^1H NMR (400 MHz, Methanol-d_4) δ 7.90 (d, *J* = 8.9, 2.7 Hz, 2H, ArH), 7.62 (d, *J* = 8.8, 2.0 Hz, 2H, ArH), 3.02 (t, 2H, CH_2), 2.63 – 2.51 (m, 4H, CH_2-CH_2), 2.48 – 2.42 (m, 2H, CH_2), 2.04 – 1.96 (m, 2H CH_2), 1.73 (s, 15H, Cp*- CH_3). ^{13}C NMR (101 MHz, Methanol-d_4) δ 172.95 (CO) , 142.07(Ar-NH) , 139.41 (Ar-S), 130.09 (2C, ArH) , 119.98(2C, ArH) , 86.76 (5C, Cp*-C) , 40.42 (2C, CH_2-NH_2) , 34.50 (2C, CH_2) , 24.11 (CH_2), 9.45 (5C, Cp*-CH_3). ESI-MS for $C_{22}H_{34}ClIrN_4O_3S$: [Cp*Ir(4C–L)Cl] : 625,2 [M-Cl].

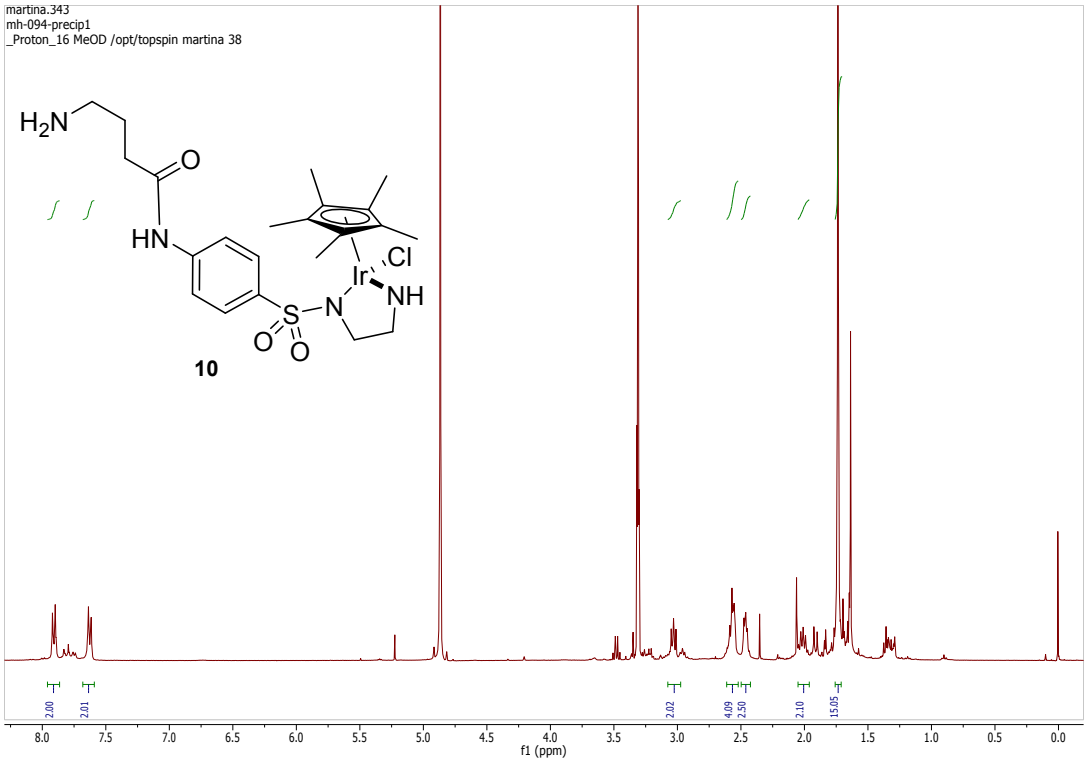


Figure 7- ^{1}H NMR of **[Cp*Ir(4C–L)Cl]**

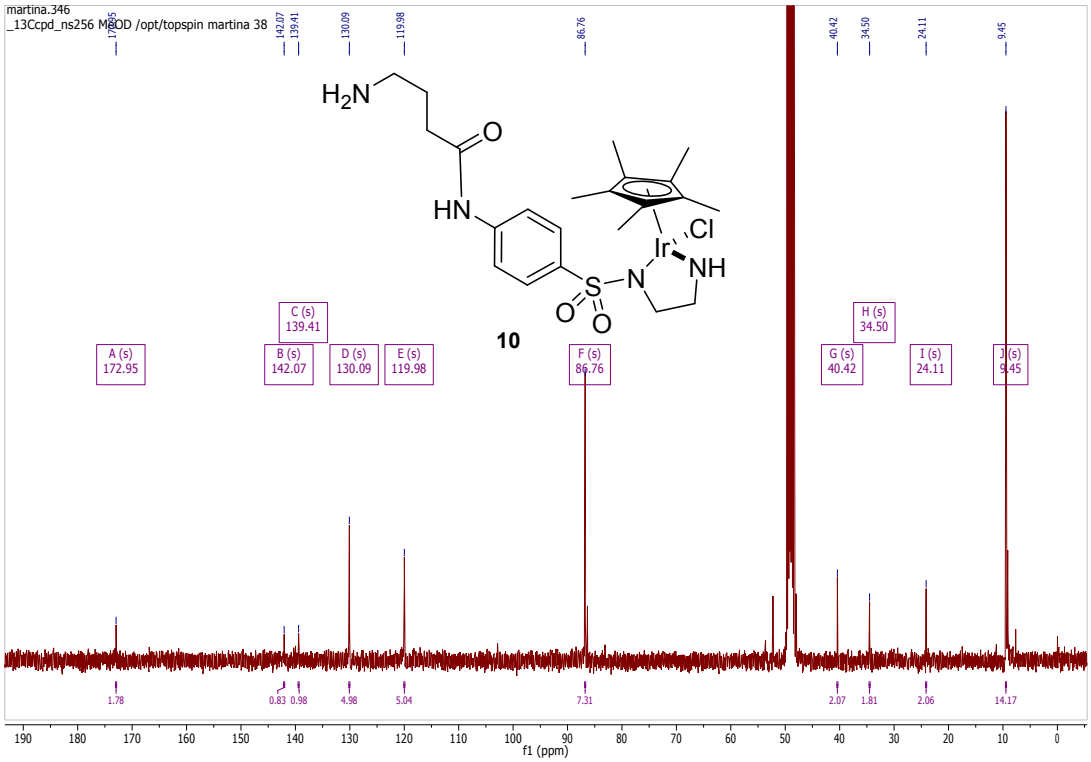


Figure 8- ^{13}C NMR of **[Cp*Ir(4C–L)Cl]**

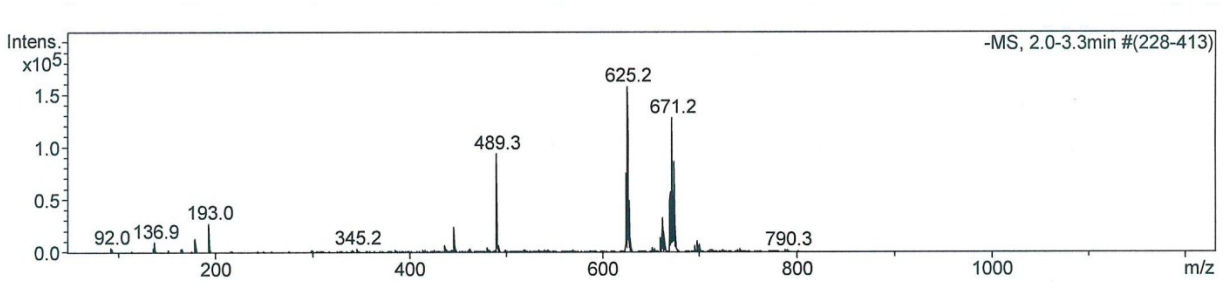


Figure 9- ESI-MS of **[Cp*Ir(4C–L)Cl]**

SNPs synthesis

The SNPs were prepared by adapting the procedure described elsewhere [3] as follows. All chemicals and solvents were equilibrated in a water bath (20 °C, 1 h) before use. Ammonium hydroxide (40 ml, 28–30 %) and ethanol (345 ml) were mixed in a round bottom flask (1 l), under stirring (600 rpm). TEOS (15 ml) was added and the solution kept under stirring for 20 h. The resulting milky suspension was centrifuged (3'220 g, 10 min) and the white pellet resuspended in ethanol. This operation (hereafter called 'washing cycle') was repeated once with ethanol and three times with water to yield the SNPs (4 g). Into the stock solution of SNPs in water (18 ml of 3.2 mg·ml^{-1}), APTES was added (11 µl , 0.047 mmol) and incubated in a water bath (30 min, 20 °C, 400 rpm). After two washing cycles, the resulting nanoparticles were stored at 4 °C.

All washing steps were performed by centrifugation (3'220 g, 5 min) and the pellets were resuspended by ultrasonic treatment using an ultrasonic bath (2 min).

Sav preincubation and lyophilisation

In a typical experiment, purified Sav mutant (25 mg , approx. 3,8 x 10^{-7} mol) was dissolved in water. The average number of free binding sites per purified Sav tetramer was determined with a biotin-4-fluorescein assay, as described elsewhere [4]. The corresponding amount of [Cp*Ir(biot-*p*-L)Cl] ligand stock solution was added to produce protein with binding sites fully filled with the iridium cofactor (ratio of Ir : free binding sites 1 : 1) , or half fulled respectively (ratio of Ir : free binding sites 1 : 2). Samples were incubated (37 °C, 2 h), frozen (-80°C) and lyophilised to dryness. A stock solution (3 mg / ml) was used for protein immobilization.

SNPs synthesis and protection

The aminomodified nanoparticles (15 ml, 3 mg / ml) were incubated (30 min) with aqueous glutaraldehyde solution (60 µl of 25% (v/v)). After two washing cycles with Mili-Q water, the particles were spinned down (3'220 g for 10 min), resuspended in MES buffer (14 ml, 10 mM, pH 6.2) and incubated with the corresponding Sav mutant or iridium catalyst while stirring (1 h, 400 rpm). Subsequently, TEOS was added to the reaction mixture (72 µl, 0.324 mmol) and allowed to react for 1 h. To produce the protected SNPs, the nanoparticles were incubated with APTES (18 µl, 4 , 20°C). Protected nanoparticles were centrifuged (4000 rpm, 5 min) and, after two washing cycles, resuspended in Mili-Q water. The concentration of each SNPs stock solution was determined by freeze-drying of each stock solution (100 µl) and weighted using a microbalance.

Activity assay

In a typical experiment, SNPs (3.2 mg) were resuspended in MES buffer (250 µl, 10 mM, pH 6.2). The solution was mixed with the activity assay buffer (1 : 1 ratio, total V : 400 µl) and NAD^+ (20 µl, 2 mM stock solution) was added.

The reaction medium was then incubated and shaken (30 °C, 1200 rpm). After the desired reaction time (1h), the suspension was centrifuged (16'100 g, 1 min) and the supernatant (70 µl) was collected for UV-Vis analysis on a TECAN plate reader (absorbance spectrum 240 – 360 nm).

Bradford assay

A bovine serum albumin standard curve was prepared in buffer (see reference [5]). 5X Bradford blue reagent (50 µl) was added to a sample or albumin solution (200 µl), mixed and incubated for 5 min at room temperature. The sample was pipetted into a 96-well plate and the absorbance was determined at 595 nm by a spectrophotometer (Synergy™ H1 Hybride reader from BioTek).

Scanning electron microscopy and particle size measurement

Each sample (2 µl) was spread on freshly cleaved mica sheets, dried at room temperature and sputter-coated with a gold–platinum alloy (15 s, 10 mA, SC7620 Sputter coater). Micrographs were acquired using the InLens mode with an accelerating voltage of 20 kV. Particle sizes were measured using the acquired micrographs with the Olympus Analysis® software package. About 100 measurements were recorded per type of nanoparticles. The protective layer thickness was determined by comparing their diameter with the diameter of unprotected native Stöber SNPs.

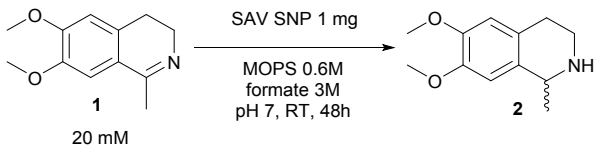
Table 1 Particle size measurement analysis

			st dev	st error	
	size (nm)	diameter increase (nm)	±		layer thickness
SNP native	241	-	9.12	0.91	nm
Cp*Ir(biot-*p*-L)Cl]·S112A@prot-SNP	264	23	10.08	1.00	12
Cp*Ir(biot-*p*-L)Cl]·S112K@prot-SNP	266	25	11.05	1.10	13
Cp*Ir(biot-*p*-L)Cl]·S112A-K121A@prot-SNP	264	23	9.47	0.94	13

ICP-MS determination of the total Ir content

Each type of lyophilized nanoparticles (1 mg) was incubated with NaOH solution (0.5 M, 95°C, 5 min) until complete dissolution [6]. Samples were neutralized using HNO_3 solution (65 %, semiconductor grade, Sigma Aldrich) and diluted using Milli-Q water (final HNO_3 concentration 3 %). Analysis was performed on a 7500cx ICP-MS system (Agilent, Basel, Switzerland) using standard operational settings as described elsewhere [7]. Quantification was perfomed via multi-element standards (Sigma-Aldrich) in matrix-matched calibration solutions containing the same amount of NaOH / HNO_3 as the samples. Rh was used as internal standard to account for residual matrix effects. The octopole was pressurized (4.5 $mL \cdot min^{-1}$ helium) to remove polyatomic interferences.

Typical catalysis procedure in buffer

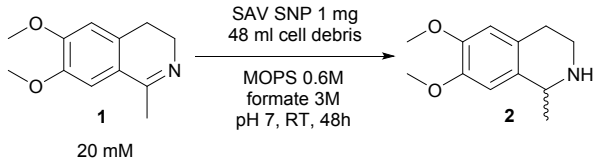


Scheme 2- Typical catalysis reaction

Protected nanoparticles (1 mg) were resuspended in MOPS / formate buffer (50 µl, 1.2 M MOPS, 3 M sodium formate, pH 7) and Mili-Q water (48 µl) in an HPLC vial, equipped with a stirring bar. The reaction was initiated by addition of the substrate stock solution (2 µl, 1 M stock solution) and the resulting mixture was shaken for 48 hours or 7 days at room temperature (final volume: 100 µl).

The reaction was quenched by centrifugation of the reaction mixture (30 s, 3'220 g). The supernatant was collected and the nanoparticle pellet was washed with Mili-Q water (3 x). The joined water phases were basified by addition of NaOH solution (50 µl, 20 % stock solution), extracted with DCM (2 x 0.5 ml), dried over Na_2SO_4 and analyzed by the means of normal phase HPLC.

Typical catalysis procedure in cellular debris



Scheme 3- Catalysis in the presence of cellular debris

Protected nanoparticles (1 mg) were resuspended in MOPS / formate buffer (50 µl, 1.2 M MOPS, 3 M sodium formate, pH 7) and cellular debris containing solution (48 µl) in an HPLC vial, equipped with a stirring bar. The reaction was initiated by addition of the substrate stock solution (2.5 µl, 400 mM stock solution) and the resulting mixture was shaken for 48 hours at room temperature (final volume: 100 µl).

The reaction was quenched by centrifugation of the reaction mixture (30 s, 3'220 g). The supernatant was collected and the nanoparticle pellet was washed with Mili-Q water (5 x). The joined water phases were basified by addition of NaOH solution (50 µl, 20 % stock solution), extracted with DCM (2 x 0.5 ml), dried over Na_2SO_4 and analyzed by the means of normal phase HPLC.

Table 2 Asymmetric imine reduction yielding salsolidine **2** using Cp*Ir(biot-*p*-L)Cl]·Sav@prot-SNP in the presence of E. coli cell lysate.

entry	SNP	ee (%)[a,b]	Conv. (%)[b]	TON
1	[Cp*Ir(biot-*p*-L)Cl]·S112A Sav@prot-SNP	25	1	78
2	[Cp*Ir(biot-*p*-L)Cl]·S112K Sav@prot-SNP	-20	1	171
3	[Cp*Ir(biot-*p*-L)Cl]·S112A-K121A Sav@prot-SNP	31	1	131
4	[Cp*Ir(biot-*p*-L)Cl]·S112A Sav free enzyme	0	0	0
5	[Cp*Ir(biot-*p*-L)Cl]·S112K Sav free enzyme	0	0	0
6	[Cp*Ir(biot-*p*-L)Cl]·S112A-K121A Sav free enzyme	0	0	0
7	[Cp*Ir(biot-*p*-L)Cl]	0	0	0

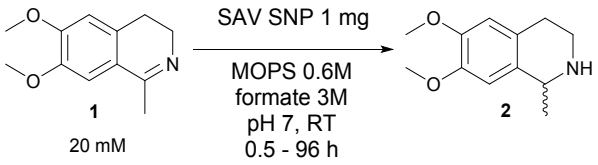
Reactions performed in a reaction buffer using 1 mg of SNPs of free enzyme with the corresponding Ir concentration in 100 µl reaction mixture with 20 mM substrate concentration at RT for 48 h; [a] Enantiomeric excess and conversion was determined by HPLC analysis on a Chiracel-IC column. [b] Positive ee values correspond to (*R*)-salsolidine; negative ee values correspond to (*S*)-salsolidine.

HPLC measurements

Each reaction mixture was analyzed by HPLC using a Chiralpak IC column (5 µm, 4.6 mm · 25 mm) using dichloromethane containing diethylamine (0.06 %) and isopropanol (0.5 %) as an eluent , flow of 1 ml/min; detection at λ=280 nm, 25 °C.

T_R 8.5 min ((S)-6,7-dimethoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline); 9.8 min (6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline); 14.6 min ((R)- 6,7-dimethoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline; referred to as salsolidine). Yields were calculated using a response factor of 1.95, as determined elsewhere [8].

Evolution of enantioselectivity over time



Scheme 4- Reaction set up for the time-point assay

Protected nanoparticles (1 mg) were resuspended in MOPS / formate buffer (50 µl, 1.2 M MOPS, 3 M sodium formate, pH 7) and Mili-Q water (48 µl) in an HPLC vial, equipped with a stirring bar. The reaction was initiated by addition of the substrate stock solution (2 µl, 1 M stock solution) and the resulting mixture was shaken for 0.5 to 96 hours at room temperature (final volume: 100 µl).

The reaction was quenched by centrifugation of the reaction mixture. The supernatant was collected and the nanoparticle pellet was washed with Mili-Q water (3 x). The joined water phases were basified by addition of NaOH solution (50 µl, 20 % stock solution), extracted with DCM (2 x 0.5 ml), dried over Na_2SO_4 and analyzed by the means of normal phase HPLC.

Table 3 Salsolidine precursor reduction using [Cp*Ir(biot-*p*-L)Cl]·S112A Sav SNPs

entry	Reaction time (h)[a]	ee (%)[b,c]	Conv. (%)[c]	TON
1	0,5	87	1.2	91
2	1	86	4.6	357
3	3	87	6.1	477
4	5	86	9.8	764
5	24	84	22.2	1723
6	48	83	30.7	2384
7	72	81	34.3	2662
8	96	78	41.5	3222

[a]Reactions performed in a reaction buffer using 1 mg of SNPs in 100 µl reaction mixture with 20 mM substrate concentration, [b] Enantiomeric excess and conversion was determined by HPLC analysis on a Chiracel-IC column.[c] Positive ee values correspond to (*R*)-salsolidine; negative ee values correspond to (*S*)-salsolidine.

NAD⁺ reduction measurements

SNPs (1 mg) of free enzymes with the corresponding iridium concentration were resuspended in MES buffer (120 µl, 10 mM, pH 6.2). The solution was mixed with the activity assay buffer (1 : 1 ratio, total V : 245 µl) and NAD^+ (5 µl, 10 mM stock solution) was added. The reaction medium was then incubated at 30 °C and shaken (1200 rpm). After the desired reaction time, the suspension was centrifuged (16'100 g, 1 min) and the supernatant (200 µl) was collected for UV-Vis analysis on a TECAN plate reader (absorbance at λ= 340 nm). The NADH quantification was calculated using the molar absorption coefficient of 6220 $M^{-1}cm^{-1}$.

Table 4 NADH regeneration catalyzed by protected [Cp*Ir(biot-p-L)Cl]·Sav@prot-SNP or [Cp*Ir(biot-p-L)Cl]·Sav as purified proteins.

	NADH Concentration (uM)						TON					
	nanoparticles			free enzymes			nanoparticles			free enzymes		
time (h)	Cp*Ir(biot-*p*-L)Cl]·S112A @prot-SNP	Cp*Ir(biot-*p*-L)Cl]·S112K @prot-SNP	Cp*Ir(biot-*p*-L)Cl]·S112A-K121A@prot-SNP	Cp*Ir(biot-p-L)Cl]·S112A	Cp*Ir(biot-p-L)Cl]·S112K	Cp*Ir(biot-p-L)Cl]·S112A-K121A	Cp*Ir(biot-*p*-L)Cl]·S112A @prot-SNP	Cp*Ir(biot-*p*-L)Cl]·S112K @prot-SNP	Cp*Ir(biot-*p*-L)Cl]·S112A-K121A@prot-SNP	Cp*Ir(biot-p-L)Cl]·S112A	Cp*Ir(biot-p-L)Cl]·S112K	Cp*Ir(biot-p-L)Cl]·S112A-K121A
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0	0	0	0
0.1	9.3	9.3	12.7	3.6	2.8	4.1	714	1557	1687	278	459	544
0.2	14.8	9.0	13.1	4.6	5.1	10.7	1137	1498	1749	354	856	1430
0.3	15.3	12.8	17.3	5.3	6.7	11.6	1176	2127	2306	410	1120	1551
0.5	15.3	14.7	22.5	8.6	7.8	16.3	1177	2448	2994	659	1301	2170
0.8	16.0	16.1	25.4	13.9	12.4	22.7	1235	2681	3392	1068	2067	3025
1.0	16.8	17.8	26.4	16.7	15.9	29.7	1292	2971	3520	1288	2644	3961
2.0	18.6	18.5	32.4	29.0	29.1	48.7	1431	3076	4322	2231	4853	6489
3.0	37.4	27.8	36.5	38.4	39.0	65.0	2878	4628	4867	2951	6495	8662
4.0	38.9	28.1	39.1	48.4	44.9	76.9	2993	4683	5218	3723	7488	10247
5.0	45.4	29.3	42.8	59.8	52.2	84.4	3495	4882	5707	4601	8707	11252
6.0	49.9	35.8	49.3	68.1	58.2	91.2	3836	5965	6569	5235	9697	12162
7.0	59.0	40.6	65.0	74.4	61.1	96.9	4539	6764	8672	5725	10184	12915
8.0	68.0	47.2	65.5	88.2	70.0	99.3	5232	7874	8734	6783	11671	13236
9.0	77.0	50.2	74.3	93.4	70.6	106.0	5926	8361	9908	7182	11764	14129
10.0	83.5	55.3	79.3	100.8	73.8	106.7	6426	9221	10576	7752	12294	14233
11.0	92.0	57.7	92.3	103.4	74.6	109.0	7080	9619	12309	7958	12427	14528
12.0	127.6	112.5	115.3	114.2	76.2	112.2	9818	18750	15374	8787	12695	14956
24.0	128.8	119.2	127.3	122.1	101.1	115.7	9910	19860	16975	9390	16845	15431
36.0	134.8	123.3	135.1	130.3	105.7	118.0	10367	20554	18016	10023	17623	15739
48.0	137.6	146.2	138.8	131.0	106.1	120.8	10583	24366	18508	10073	17682	16109

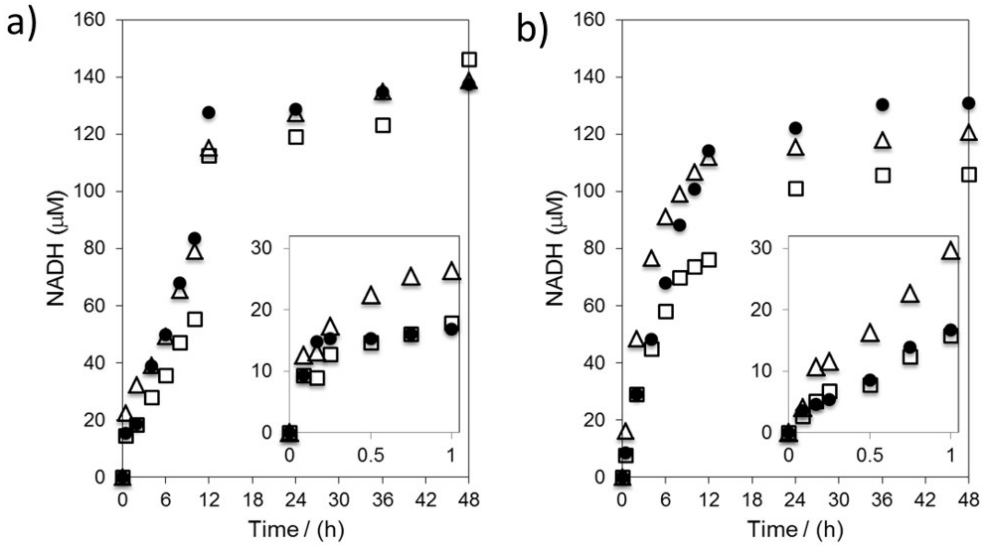


Figure 10- NADH regeneration catalyzed by a) protected [Cp*Ir(biot-*p*-L)Cl]·Sav@prot-SNP and b) [Cp*Ir(biot-*p*-L)Cl]·Sav as purified proteins over time as determined by absorption at λ= 340 nm. Mutant S112A is shown as full circles, S112K as empty squares and S112A-K121A as triangles.

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