LEAFY protein crystals with a honeycomb structure as platform for selective preparation of outstanding stable bio-hybrid materials

Lucile Chiari,[a] Philippe Carpentier,[a, b] Sylvie Kieffer-Jaquinod,[c] Alice Gogny,[a] Julien Perard,[a] Stéphane Ravanel,[d] David Cobessi,[e] Stéphane Ménage,[a] Renaud Dumas,*[d] Olivier Hamelin*[a]

[a] *Univ. Grenoble Alpes, CEA, CNRS, IRIG-LCBM, UMR5249, 38000, Grenoble, France. E-mail: olivier.hamelin@cea.fr.*
[b] *ESRF, the European Synchrotron, 71 Avenue des Martyrs, 38000 Grenoble*
[c] *Univ. Grenoble Alpes, CEA, INSERM, Biosanté 1292, 38000 Grenoble, France.*
[d] *Univ. Grenoble Alpes, CEA, CNRS, INRAE, IRIG-DBSCI-LPCV 38000, Grenoble, France.*
[e] *Univ. Grenoble Alpes, CEA, CNRS, IBS 38000, Grenoble, France.*

Supplementary materials

Experimental Section

Materials and Methods. $RuCl_3,3H_2O$ was purchased from Strem Chemicals. All chemicals were obtained from Sigma-Aldrich Merck. Solvents used in synthetic procedures were analytical grade and used without prior purification. Plasmid coding for cysteine mutant K84C was obtained by gene synthesis (ShineGene) from the initial GbLFY-SAM sequence cloned in pETM11.

Inorganic compounds
^{13}C and ^{1}H-NMR spectra were recorded on Bruker 300 MHz.

Elemental analyses were conducted at the Service Central d'Analyse - CNRS, Solaize, France using the ICP AES method for metal titration.

HRMS was performed at the Institut of Organic and Analytical Chemistry. UMR 7311 - Orléans University. Orléans.

Electrospray ionization mass spectrometry measurements were performed at LCIB/INAC-CEA-Grenoble on a Thermoquest Finnigan LCQ ion trap (Thermo Scientific, San Jose, CA, USA), equipped with an electrospray source.

Absorption spectra of the complexes were recorded with a Shimadzu UV-1800 spectrophotometer.

Protein
Mass spectra of proteins were recorded at EDyP/BGE-CEA-Grenoble with a nano-LC-MS/MS (NCS3500 UHPLC and QExactive HF, Thermo Fisher Scientific). The data are processed using Xcalibur software (Thermo Fisher Scientific). For MS/MS experiments, the Mascot Distiller software (version 2.5.1, Matrix Science) is used and then the peptides are identified by the Mascot software (version 2.6).

Hybrid material

Optical spectroscopy data of the hybrid material were obtained on the microspectrophotometer of the icOS laboratory at the ESRF Grenoble.[1]

The UV-visible spectra were measured using an Ocean Optics QE65 Pro spectrophotometer and an Ocean optics DH-2000-BAL UV-Visible white light source.

The resonance Raman spectra were measured using a Renishaw InVia Raman spectrometer with laser excitation at 785 nm 50 mW on the sample). The sample was maintained at 100 K using an Oxford cryostream 700 nitrogen gas cryogenic cooling system.

Resonance Raman spectroscopy. Resonant Raman spectra were collected at the Laboratory icOS (ESRF, Grenoble) on a dedicated microspectrophometer 5. The spectrograph is a Renishaw InVia system (www.renishaw.fr/), with a compact back-scattering Raman-probe remotely installed on a standard goniometer designed for biological crystals. Raman Resonance was obtained using a 50 mW near-infrared excitation source at 785 nm (RL785 diode laser) with a focal spot of 50 µm diameter size at the sample. Full spectra (200–2000 cm-1) were recorded on single crystals maintained at 100 K using an Oxford cryostream 700 nitrogen gas cryogenic cooling system and with 20 min total exposure time to attain good signal to noise ratios. Spectrum baseline correction, cosmic ray removal, and data smoothing, were processed with the software Wire2 developed by Renishaw.

Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) studies were performed on a Thermo Fisher Scientific iCAP RQ quadrupole mass instrument and data are processed using Qtegra software provided by Thermo Fisher Scientific.

1. Synthesis

Synthesis of $[Ru(tpy)Cl_3]$ [2]

To a solution of $RuCl_3$, $3H_2O$ (509.2 mg, 1.9 mmol, 1.3 eq) in absolute EtOH (200 mL), terpyridine (350.7 mg, 1.5 mmol) was added and the mixture was refluxed for 3h, cooled to room temperature. The product was filtered off, washed

successively with EtOH (3x60 mL) then with Et_2O (3x60 mL). After drying, the desired product was obtained as a brown powder (650.7 mg, 94%). Spectral data are identical to those reported in the literature.

Synthesis of [(tpy)(5-NH_2-phen)Ru-Cl][PF_6], ([**NH_2-Ru-Cl**][PF_6])

To a solution of Ru(tpy)Cl_3 (549.3 mg, 1.2 mmol) in a EtOH-H_2O mixture (75 : 25 mL) 5-amino-1,10-phenanthroline (235.3 mg, 1.2 mmol, 1,0 eq), triethylamine (243 µL, 1.8 mmol, 1.5 eq) and lithium chloride (287.1 mg, 6.8 mmol, 5.5 eq) were added. The resulting mixture was refluxed for 3h then cooled to room temperature. The solution was concentrated under vacuum to the fifth. Addition of an excess of KPF_6 results in the precipitation of the product which was further filtrated and subsequently washed with H_2O then Et_2O. After silica gel column chromatography (acetone-3% aqueous solution of KNO_3 (95 : 5)), anion metathesis was then achieved after concentration of the solution under vacuum, dissolution of the residue in a minimum of H_2O then precipitation of the product by adding a large excess of KPF_6. After filtration, subsequent washing with water and Et_2O, the desired compound was obtained as a a mixture of the two isomers (61:39 ratio) as a deep red powder (674 mg, 79%).

^{1}H-NMR (300 MHz, d_6-acetone) : Mixture of two isomers δ 10.56 (d, *J* = 5.2 Hz, 0.61H, phen), 10.18 (d, *J* = 6.3 Hz, 0.38H, phen), 9.14 (d, *J* = 8.3 Hz, 0.61H, phen), 8.77 (d, *J* = 7.9 Hz, 2.01H, tpy), 8.62 (d, *J* = 8.1 Hz, 2.04H, tpy), 8.55 (d, *J* = 8.2 Hz, 0.75H, phen), 8.41 (m, 0.61H, phen), 8.23 (m, 1.41H (1.05H tpy+0.35H phen), 7.96 (m, 2.56H (1.92H tpy + 0.64H phen), 7.90 (d, *J* = 5.5 Hz, 0.39H, phen), 7.72 (m, 2.03H, terpy), 7.48 (d, *J* = 5.2 Hz, 0.60H, phen), 7.42 (m, 0.29H, phen), 7.40 (s, 0.49H, phen), 7.3 (m, 2.07H, tpy), 7.20 (m, 0.61H, phen), 7.16 (s, 0.57H, phen), 6.41 (s, 1.2H, NH_2), 6.16 (s, 0.76H, NH_2); ^{13}C-NMR (75 MHz, d_6-acetone) : Mixture of two isomers δ 159.0; 158.9; 158.4; 158.4; 152.9; 152.4; 152.4; 152.3; 148.4; 148.1; 147.4; 144.9; 136.7; 133.3; 132.9; 132.6; 132.4; 132.3; 131.3; 130.1; 129.1; 127.1; 127.1; 125.5; 124.7; 124.5; 123.8; 123.6; 123.4; 123.3; 123.3 ;122.3; 122.2; 103.2; 102.4; MS (ESI) m/z (%): 565.0 [**NH_2-Ru-Cl**]$^+$ (100); UV-vis (acetone) : λ_{max} (ε)= 510 (10629), 422 (6451), 362 nm (9942 $M^{-1}.cm^{-1}$); HRMS (ESI+) : calcd for $C_{27}H_{20}ClN_6Ru$: 565.0476; found: 565.0472

Synthesis of [(IA-phen)(tpy)Ru-Cl][PF_6] , ([**IA-Ru-Cl**] [PF_6])

To a solution of [**NH_2-Ru-Cl**][PF_6] (369.2 mg, 0.5 mmol) in dry CH_2Cl_2 (30 mL), iodoacetic anhydride (229.5 mg, 0.60 mmol, 1.2 eq) was added. The resulting mixture was then stirred at room temperature under inert atmosphere for 16h and subsequently washed with a 5% solution of $NaHCO_3$. The organic phase was dried over Na_2SO_4, concentrated under vacuum to the fifth. Addition of a large volume of ether results in the precipitation of the complex. After silica gel column chromatography (acetone-3% aqueous solution of KNO_3 (95 : 5)), anion metathesis was then achieved after concentration of the solution under vacuum, dissolution of the residue in a minimum of H_2O then precipitation of the product by adding a large excess of KPF_6. The resulting precipitate was washed with H_2O then Et_2O then dried. Pure complex was obtained as a mixture of two isomers (1:1 ratio) as a deep red powder (236.4 mg, 62%).

^{1}H-NMR (300 MHz, d_6-acetone) : Mixture of two isomers δ 10.51 (dd, *J* = 5.2, 1.0 Hz, 1H, phen), 10.40 (dd, *J* = 5.2, 1.2 Hz, 1H, phen), 10.09 (s, 1H, NH), 9.85 (s, 1H, NH), 9.06 (dd, *J* = 8.6, 1.1 Hz, 1H, phen), 8.85 (dd, *J* = 8.2, 1.0 Hz, 1H, phen), 8.67 (dd, *J* = 8.1, 1.8 Hz, 4H, tpy), 8.63 (s, 1H, phen), 8.52 (d, *J* = 8.3 Hz, 4H, tpy), 8.49 (s, 1H, phen), 8.45 (dd, *J* = 8.5 Hz, 1.1 Hz, 1H, phen), 8.42-8.30 (m, 2H, phen), 8.27 (dd, *J* = 8.2, 1.1 Hz, 1H, phen), 8.14 (dt, *J* = 8.1, 3.1 Hz, 2H, tpy), 7.90 (dd, *J* = 5.3, 1.0 Hz, 1H, phen), 7.85 (dt., *J* = 7.9, 1.5 Hz, 4H, tpy), 7.78 (dd, *J* = 5.3, 1.1Hz, 1H, phen), 7.61 (t, *J* = 5.4 Hz, 4H, tpy), 7.42-7.30 (m ,2H, phen), 7.19- 7.11 (m, 4H, tpy), 4.16 (s, 2H, CH_2I), 4.03 (s, 2H, CH_2I); ^{13}C-NMR (75 MHz, d_6-acetone) : Mixture of two isomers δ 153.5; 152.8; 152.6; 152.5; 151.8; 149.6; 136.9; 135.1; 133.9; 133.7; 133.6; 133.1; 130.4; 130.1; 129.3; 127.10; 127.1; 126.4; 126.3; 126.1; 126.0; 125.4; 125.4; 125.3; 125.2; 124.6; 124.5; 123.4; 122.9; -0.9; -1.1; MS (ESI) m/z (%): 733.1 [**IA-Ru-Cl**]$^+$ (100); 825.0 [**IA-Ru-Cl, -Cl$^-$, +I$^-$**]$^+$ (10; UV-vis (acetone) : λ_{max} (ε)= 508 (10801), 443 (6843 $M^{-1}.cm^{-1}$) ;HRMS (ESI+) : calcd for $C_{29}H_{21}ClN_6ORu$ 732.9548; found 732.9544.

Synthesis of [(ClA-phen)(tpy)Ru-Cl][NO_3], [**ClA-Ru-Cl**] [NO_3]

To a solution of Ru(tpy)Cl_3 (79.5 mg, 180.5 µmol) in EtOH (15 mL) 5-chloroacetamido-1,10-phenanthroline[3] (49.0 mg, 180.5 µmol, 1,0 eq) and lithium chloride (76.7 mg, 1.8 mmol, 10.0 eq) were added. The resulting mixture was refluxed for 7h, cooled to room temperature, filtrated and the solution concentrated *in vacuo*. The residue was dissolved in water and the complex precipitated in its PF_6 form by addition of a large excess of KPF_6. The product was then filtered of and

successively washed with water then Et_2O before being purified by silica gel chromatography (acetone-3% aqueous solution of KNO_3 (80 : 20). Pure complex was obtained as a mixture of two isomers (64:36 ratio) as a deep red powder (16.0 mg, 13%).

^{1}H-NMR (300 MHz, d_6-acetone) : Mixture of two isomers δ 10.77 (s, 0.36H, NH), 10.66 (s, 0.64H, NH), 10.57 (d, *J* = 4.4 Hz, 0.36H, phen), 10.49 (dd, *J* = 5.2, 1.2 Hz, 0.64H, phen), 9.23 (d, *J* = 7.6 Hz, 0.36H, phen), 8.75 (s, 0.86H, phen), 8.73-8.62 (m, 2.12H, phen), 8.52 (s, 0.46H, phen), 8.85 (dd, *J* = 8.2, 1.0 Hz, 1H, phen), 8.40 (d, *J* = 8.1 Hz, 2.48H, tpy), 8.35-8.29 (m, 0.82H, phen), 8.27 (d, *J* = 7.4 Hz, 2.63H, tpy), 8.23 (ds, 0.35H, phen), 7.85 (dt, *J* = 7.9, 2.7 Hz, 1.43H, tpy), 8.04 (ds, 0.27H, phen), 7.81 (dt, *J* = 7.8, 1.4 Hz, 2.39H, tpy), 7.58 (ds, 0.42H, phen), 7.55 (t, *J* = 5.9 Hz, 2.52H, tpy), 7.49 (dd, *J* = 5.3, 0.7 Hz, 0.49H, phen), 7.37-7.30 (m, 0.85H, phen), 7.28-7.22 (m, 0.57H, phen), 7.16 (t, *J* = 6.4 Hz, 2.27H, tpy), 4.58 (s, 0.62H, CH_2Cl), 4.48 (s, 1.38H, CH_2Cl); ^{13}C-NMR (75 MHz, d_6-acetone) : Mixture of two isomers δ 166.2; 166.1; 158.9; 158.8; 158.3; 153.4; 152.8; 152.7; 152.6; 152.6; 152.5; 151.8; 149.5; 145.5; 136.8; 135.1; 133.9; 133.6; 133.1; 130.4; 130.2; 130.1; 130.0; 127.10; 126.4; 126.3; 126.0; 125.3; 125.2; 124.4; 123.4; 122.4; 120.4; 119.9; 43.3; 43.1; MS (ESI) m/z (%): 641.2 [**ClA-Ru-Cl**]$^+$ (100); UV-vis (CD_2Cl_2) : λ_{max} (ε)= 507 (5332.3), 440 (3770.6 $M^{-1}.cm^{-1}$); HRMS (ESI+) : calcd for $C_{29}H_{21}Cl_2N_6ORu$ 641.0192; found 641.0187.

2- Overexpression in *E. coli*, purification, crystallization and quantification of proteins.

Expression and production of LEAFY WT and LEAFY$_{K84C}$

Expresssion and production of wild type and mutant GbLFY-SAM (**LEAFY**) were performed as described in Sayou et al.[4] *E. coli* Rosetta 2 (DE3) cells were transformed by heat shock with plasmids GbLFY-SAM (pETH195 for WT or pETH271 for the mutant K84C). After transformation, cells were grown at 37°C under agitation in 1 L of LB medium containing Kanamycin (50 mg/mL) and Chloramphenicol (34 mg/mL) up to an optical density of 0.6 at 600 nm. Then, protein expression was performed at 18°C overnight after induction by IPTG (0.4 mM). The pellet obtained after centrifugation (4000 rpm 30 min. 4°C) was frozen at -80°C and thawed until use.

LEAFY WT and LEAFY$_{K84C}$ proteins purification

Purification of wild type and mutant **LEAFY** were performed as described in Sayou et al.[3] Pellet were resuspended in buffer A (Tris-HCl 20 mM pH8.0. TCEP 1 mM) containing 1 tablet of Complete EDTA free protease inhibitor (Roche) and then sonicated. After centrifugation (12000 rpm. 30 min. 4°C), supernatant were loaded on columns containing 1.5 mL Ni-Sepharose High Performance resin (GE Healthcare) previously equilibrated with 20 mL of buffer A. After washing (20 mL of buffer containing 20 mM imidazole), proteins were eluted in buffer A containing 300 mM imidazole. Purified proteins are then dialyzed and cleaved at 4°C for 15 hours in the presence of protease 6His-TEV. After cleavage, **LEAFY** proteins were purified on Ni-Sepharose and on a 16/60 Prep Grade Superdex-200 column (GE Healthcare) equilibrated in buffer A. The purified fractions were frozen in liquid nitrogen and stored at -80°C. Expression yields are generally 30-70 mg of **LEAFY** for 1 L of LB.

Protein crystallization

LEAFY proteins were crystallized using the hanging drop method in EasyXtal 15-Well Tools plates at 20°C. The drops were prepared by mixing 2 µL of a 2.5 mg/mL solution of **LEAFY** protein with 2 µL of crystallization buffer. The wells contain 500 µL of crystallization buffer optimized for each protein (table below).

Protein	Crystallization buffer
LEAFY$_{K84C}$	12.5 mM Tris-HCl pH7.2; 550 mM ammonium sulfate ; 1 mM TCEP
LEAFY WT	25 mM Tris-HCl pH7.2; 320 mM ammonium sulfate

Protein quantification

The determination of **LEAFY$_{K84C}$** protein amount in crystals was measured after washing the crystals issued from one well of crystallization with water then subsequent solubilization in denaturating buffer (10 µL) at 90°C for 15min and comparison with a known range of **LEAFY WT** on SDS-PAGE.

3. Grafting procedures

Preparation of Ru-OH_2 ⊂ $LEAFY_{K84C}$ and Ru-OH_2 ⊂ LEAFY WT hybrids in solution
To 20 µL of a solution of the protein (2.5 mg/mL ; 195.5 µM), a solution of the complex **IA-Ru-Cl** in DMSO (1 µL of 32 mM; 8 equiv./monomer of protein) was added then left for 16h at 4°C. The resulting solution was then dialyzed (Tris-HCl (50 mL, 20 mM pH8), TCEP 1 mM, 5% DMSO) at 4°C for 5 days.

Preparation of Ru-OH_2 ⊂ $LEAFY_{K84C}$ and Ru-OH_2 ⊂ LEAFY WT crystals
To each crystallization drop (4 µL), a solution of **IA-Ru-Cl** in acetone (2 µL of a 0.8 mM solution; 14.2 equiv./cysteine) was added. After an overnight incubation, five crystallization drops were collected in an Eppendorf tube, centrifuged then washed several times with acetone then with crystallization buffer until complete disappearance of the ungrafted ruthenium complex.

Hybrids characterizations

1-Mass spectrometry

a-Bottom-up analysis

Sample preparation for ArgC digestion
Solutions of **Ru-OH_2 ⊂ $LEAFY_{K84C}$** and **Ru-OH_2 ⊂ LEAFY WT** were deposited on a 1D SDS page for « in gel digestion ». The corresponding bands were cutted and treated as previously described[5] except that ArgC was used as the protease.

LC/MS analysis
ArgC digested peptides were resuspended in 5 % acetonitrile and 0.1% trifluoroacetic acid solution. Peptides were then loaded on a PepMap C18 precolumn (300 µm × 5 mm) and separated on a C18 reversed-phase capillary column (75 µm i.d. × 15 cm ReproSil-Pur C18-AQ, 3 µm particles) using the UltiMate™ 3000 RSLCnano system (Thermo Fisher Scientific) coupled to a Q-Exactive + mass spectrometer (Thermo Fisher scientific). Mass spectrometry (MS) and MS/MS data were acquired using Xcalibur on a Data-Dependent mode, by alternating one full MS scan with Orbitrap detection acquired over the mass range 200 to 2000 m/z, at a target resolution of 70,000 and with an AGC of 10^6. The peptides were isolated for fragmentation by higher-energy collisional dissociation (HCD) with a normalized collision energy of 27 using an isolation window of 3 m/z, a target resolution of 17 500 and an AGC fixed to $3x10^3$.

Raw data have been digitized with Mascot Distiller (2.5.1 version of Matrix Science) then identified with Mascot (2.6 version) by adding sequence of Leafy K84C into the protein used database. Tolerance search on peptide masses has been enlarged to 4 Da in order to consider all isotopes of Ruthenium. Also different possibilities of modifications have been introduced : naked complex, ACN-complex and also ACN-complex considering a neutral loss of CH_3CN.

b-Top-down analysis
Sample have been retaken in a mixture of H_2O:CH_3CN: trifluoroacetic acid (94.9:5:0.1) in order to reach a concentration of 0.5 µM. Non digested protein have been analysed on a C4- LC/MS system using the same kind of mass spectrometer used for Bottom-up analysis. MS/MS analysis have been also generated to target the protein part containing the complex.

For both mode of analysis mMass software (5.5.0 version; Martin Strohalm) has been used in order to generate theoretical isotopic pattern of Ru containing peptide or LFY protein for comparison purposes.

2-Quantification of ruthenium contents by ICP-MS

A calibration range from 0.2 to 200 µg/L in ruthenium was performed using a commercial ruthenium solution at 1 g/L by dilution. To the crystals issued from a single crystallization well, 20 µL of a 65% HNO_3 solution (ICP grade) was added.

The resulting solution was allowed to mineralize for 10 h at 60°C. The solution was then diluted with milli-Q water to a final volume of 2 mL.

3- In cristallo Resonance Raman spectroscopy

In crystallo spectroscopy experiments were carried out on a micro-spectrophotometer dedicated for protein crystals in the laboratory icOS at the ESRF (Grenoble). Non-resonant Raman spectra were recorded at 100 K using an inVia Raman microspectrometer (Renishaw, Gloucestershire, UK) equipped with a near-IR 785 nm diode excitation laser, and a collection optics in back scattering geometry focused onto the crystal (ref). Each Raman spectrum were recorded on crystals over the spectral range 200-1000 cm^{-1} and during 30s of accumulation time. The final resulting spectrum is an average of 100 spectra sequentially recorded. All processing and spectral analysis were performed using Wire 2.0 (Remishaw) software.

4- In cristallo UV-visible spectroscopy

UV-visible light absorption microspectrophotometry were also carried out in the spectroscopy facility icOS, at the ESRF (Grenoble, France). The UV–vis absorption micro-spectrophotometer consists of two face-to-face objectives at 180 from each other, with the crystals positioned at the focused point in between them. The crystal is maintained at 100K using a liquid-nitrogen open-flow cooler (Cryostream Series 700, Oxford Cryosystems, Oxford, England). The reference white light was delivered by a halogen-deuterium source Mikropack DH2000-BAL (Ocean Optics) through the first objective. UV-visible light absorption spectra were collected through the second objective and recorded using QE65Pro a spectrophotometer (Ocean Optics) equipped with a CCD detector. The spectrometer was control and spectra were displayed by the commercial SpectraSuite software (Ocean Optics). Each absorption spectrum were recorded over the spectral range 200-1000nm and during typically 0.08 s of accumulation time, and the final spectrum is an average of 10 spectra.

5- Crystallization, data collection and structure resolution of Ru ⊂ $LEAFY_{K84C}$.

Diffraction data were collected on FIP-BM30A[6] at the ESRF, at 100 K, using an ADSC 315r detector. Diffraction data (Table 1) were processed and scaled using XDS.[7] Phasing was performed by molecular replacement using Phaser[8] from CCP4[9] using the structure of LEAFY (PDB code 4UDE[4]. After 10 cycles of refinement using REFMAC [10] from CCP4, no positive electron density for Cys84 and the complex was observed in the Fo-Fc electron density map.

Wavelength (Å)	0.976250
Resolution range (Å)	35.84 – 2.86 (3.04-2.86)
Space group	$P6_5$
Unit cell parameters (Å,°)	a= 80.62, c= 78.32
Number of total reflections	28,597 (4,701)
Unique reflections	6,663 (1,074)
Average multiplicity	4.29 (4.37)
Data completeness (%)	99.0 (99.2)
R_{sym} (%)	7.4 (83.7)
$<I/\sigma_{(I)}>$	13.5 (1.8)
CC(1/2)	99.8 (67.2)

Table S1. Statistics of data collection

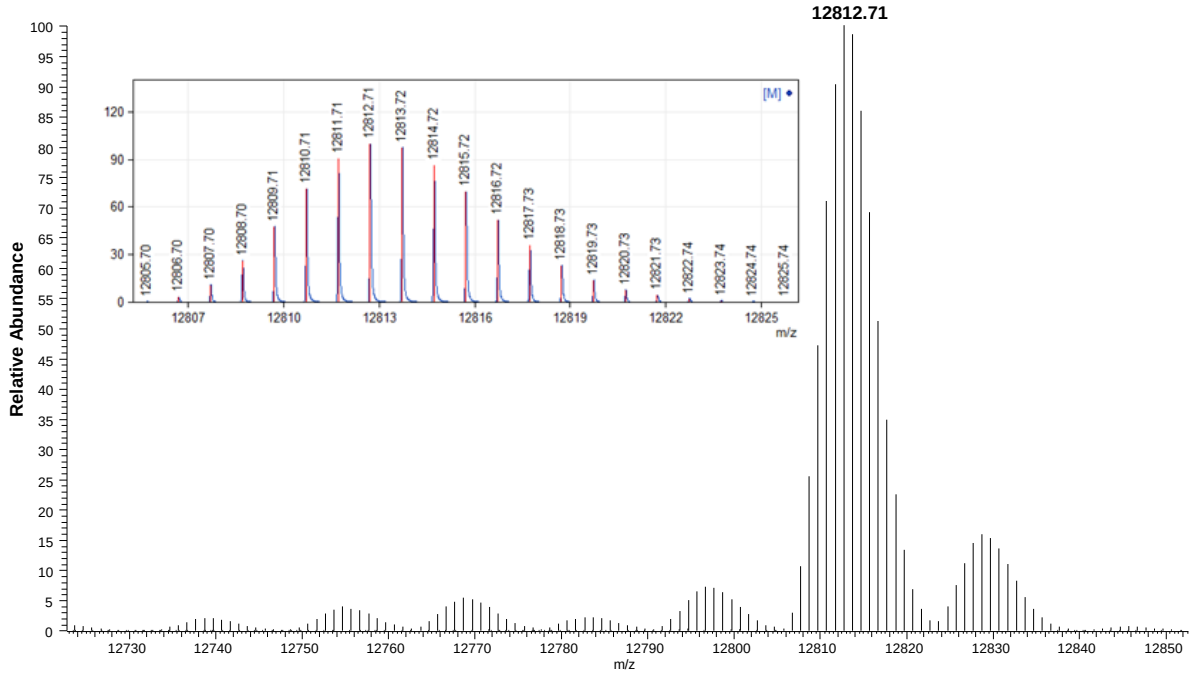


Fig. S1: Experimental and theoretical (inset) mass spectrum of **LEAFY WT**.

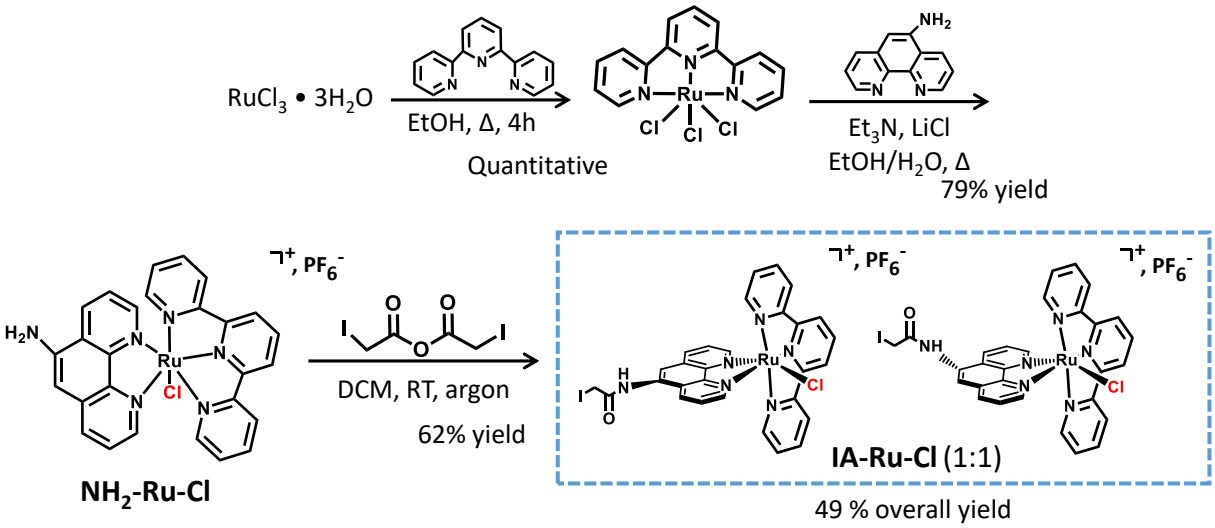


Fig.S2: Synthetic scheme of IA-Ru-Cl

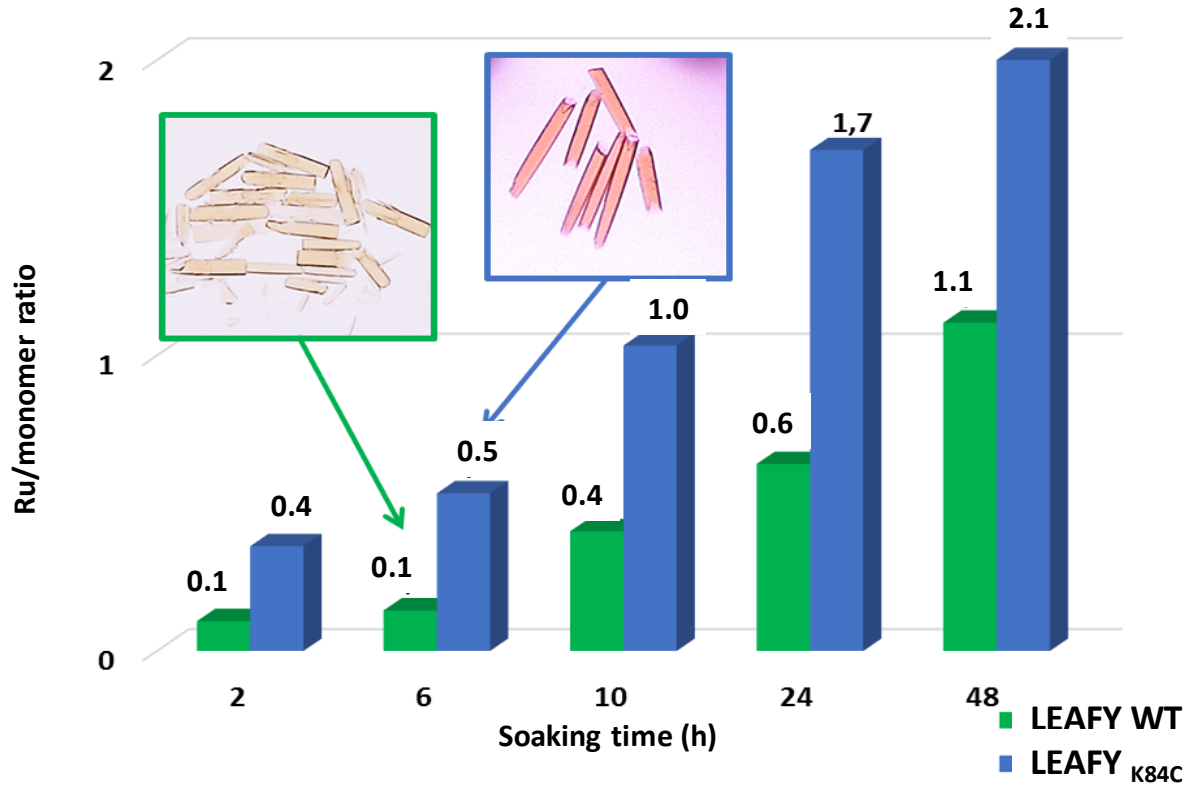


Fig.S3: Amount of grafted complex (determined by ICP-MS) per monomer of **LEAFY$_{K84C}$** and **LEAFY WT** unit according to the soaking time in the presence of **IA-Ru-Cl (**14 equiv. /monomer).

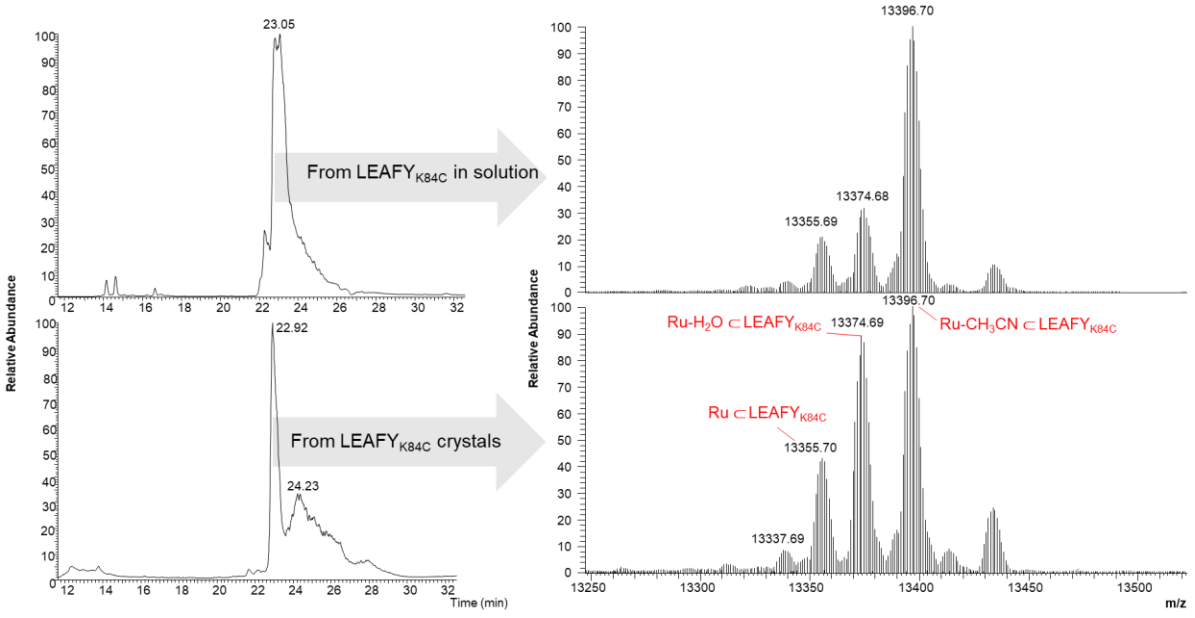


Fig. S4: LC chromatogram and corresponding deconvoluted mass spectra of the modified proteins issued from the binding of IA-Ru-Cl to LEAFY$_{K84C}$ in solution (up) and crystals (down).

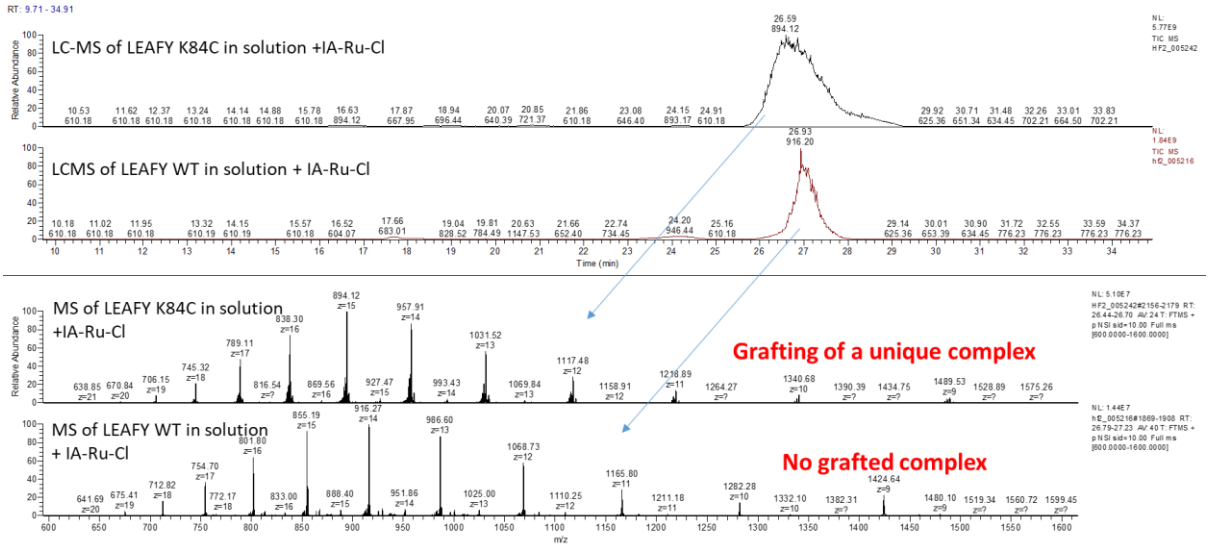


Fig. S5: LC-MS and MS spectra of LAFY WT and LEAFY$_{K84C}$ both in solution after reaction with IA-Ru-Cl showing the selectivity of the grafting in solution.

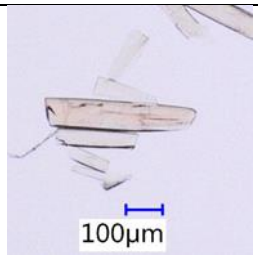
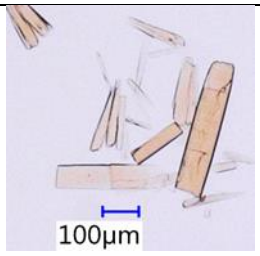
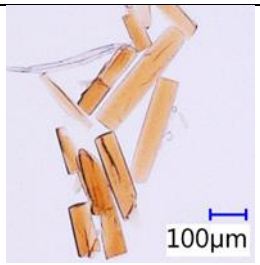
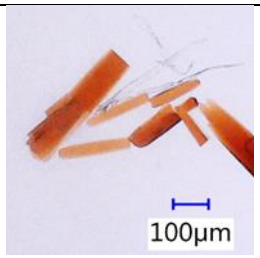
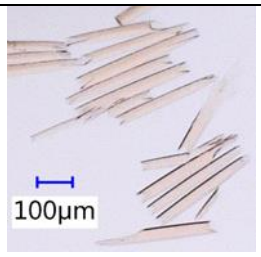
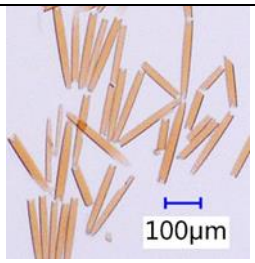
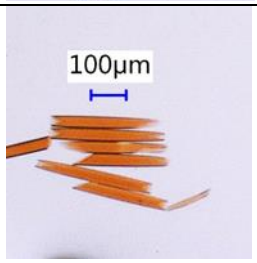
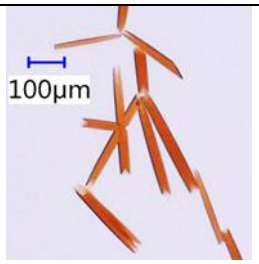
Complex	**ClA-Ru-Cl** (14 equiv./monomer)		**IA-Ru-Cl** (14 equiv./monomer)	
Soaking time	**16h**	**32h**	**16h**	**32h**
LEAFY WT				
LEAFY **K84C**				

Fig. S6: Visual comparison of the *in cristallo* grafting rate of the **ClA-Ru-Cl** and **IA-Ru-Cl** complexes within **LEAFY WT** and **LEAFY$_{K84C}$** crystals.

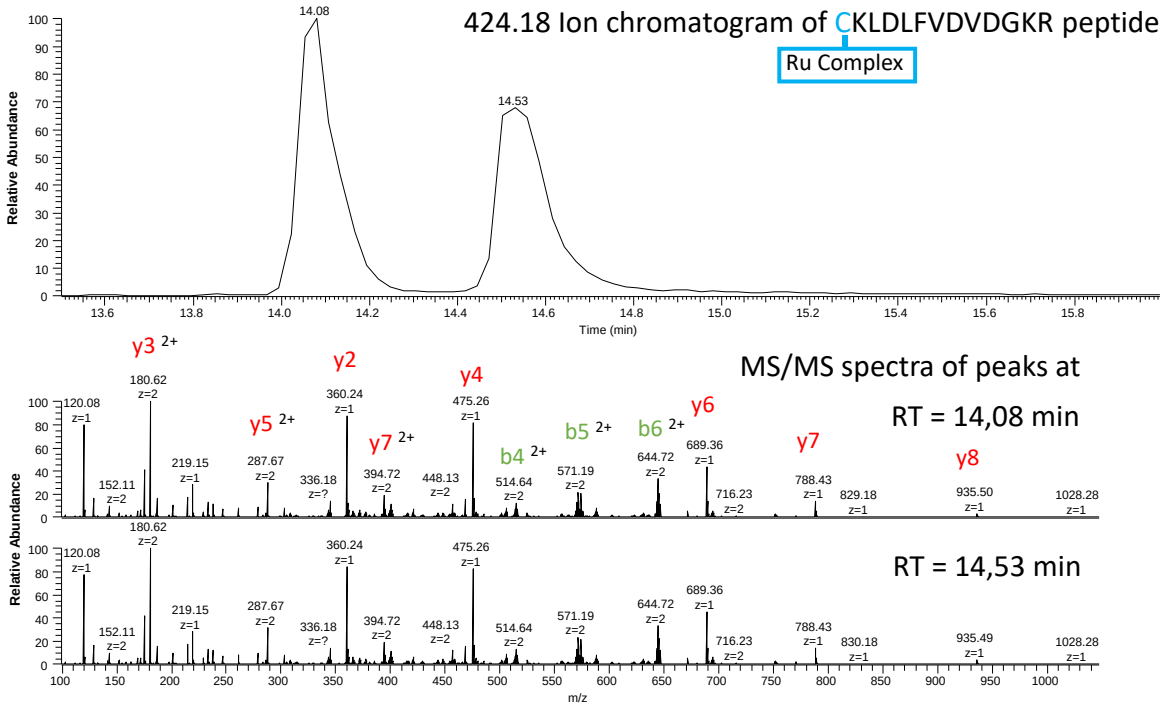


Fig. S7: Selective ion chromatogram of the modified peptide after ArgC digestion showing two distinctive peaks with the same m/z at 424.18 (z=5) (top) and their respective MS/MS fragmentation (bottom)

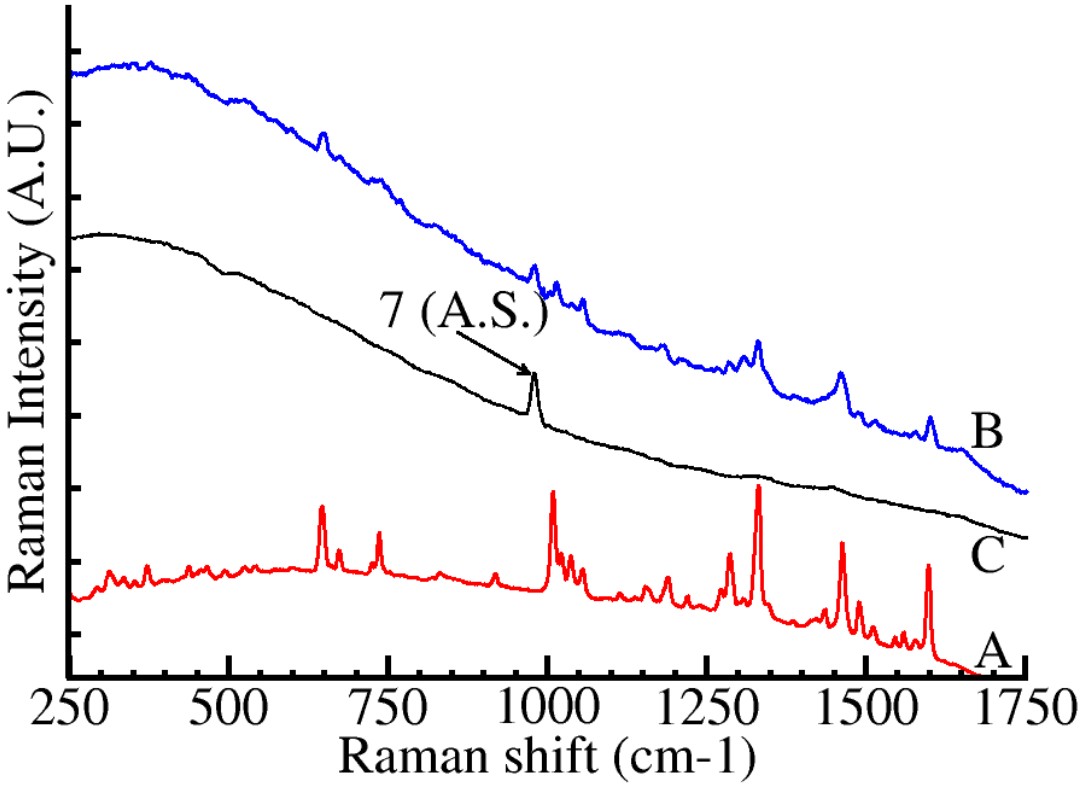


Fig. S8. Raw Raman data of pure the complex NH_2-Ru-Cl (A), the hybrid **Ru-$^{16}OH_2$ ⊂ LEAFY$_{K84C}$** crystals (B) the native protein LEAFY$_{K84C}$ crystal (C). Non-corrected backgrounds show that spectra are obscured by fluorescence. Prominent Peak 7 corresponds to the vibration of ammonium sulfate used as crystallization agent.

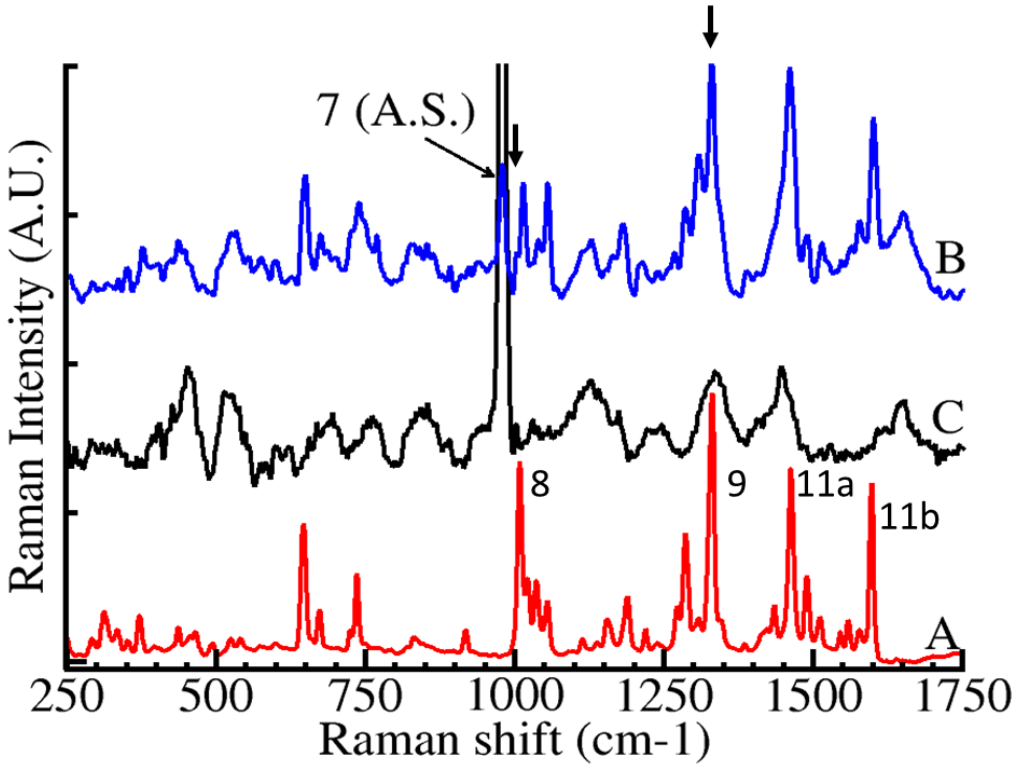


Fig. S9. Corrected Raman data (i.e. spikes removed, data smoothed and baseline subtracted) of pure the complex NH_2-Ru-Cl (A), the hybrid **Ru-$^{16}OH_2$ ⊂ LEAFY$_{K84C}$** crystals (B) the native protein LEAFY$_{K84C}$ crystal (C).

mode	Raman shift (cm^{-1})			Possible assignment [11-22]
	NH_2-Ru-Cl	**Ru-$^{16}OH_2 \subset$ LEAFY$_{K84C}$**	**Ru-$^{18}OH_2 \subset$ LEAFY$_{K84C}$**	
1	312	Absent	absent	ν (Ru-Cl) stretching vibration
2a	334	336	335	2a, 2b: ν(Ru-N) stretching vibration Pyridine, 2c: ν(Ru-N) stretching vibration Phenanthroline
2b	351	351	352	
2c	372	377	379	
3a	absent	410-430	390-410	ν (Ru-OH_2) stretching vibration, isotopic shift $\Delta\nu(^{16}O/^{18}O)$ ~ 20 cm^{-1}
3b	absent	450-470	430-450	
4	437	437	437	βC-C-C) pyridine Ring out of plane deformation ref[2,3]
5a	646	649	N.A.	α(C-C-C) pyridine Ring in plane deformation
5b	673	675	N.A	
5c	736	739	N.A.	
6	Absent	769	N.A.	ν(C-S) stretching vibration
7	Absent	980	N.A.	ν (SO_4) stretching vibration, ammonium sulfate, crystallization precipitant
8a	1008	1014	N.A.	ν(C-N, C-C) pyridine or phenantroline heterocyclic ring breathing
8b	1055	1056	N.A.	
9a	1287	1285	N.A.	ν(C=C, C=N) hybrid bonds stretching of pyridine or phenantroline heterocycle
9b	very weak	1308	N.A.	
9c	1331	1330	N.A.	
10	Absent	1340	N.A.	Leafy amide III region, α-helices
11a	1463	1461	N.A.	ν(C=N, C=C) double bong stretching of pyridine or phenantroline heterocycle
11b	1598	1602	N.A.	
12	Absent	1650	N.A.	Leafy amide I region, α-helices

Table S2. Attributions of the main Raman vibrational frequencies for the pure complex NH_2-Ru-Cl, and for the hybrid Ru-$^{16}OH_2 \subset$ LEAFY$_{K84C}$ and Ru-$^{18}OH_2 \subset$ LEAFY$_{K84C}$ crystals in the region 200-2000 cm^{-1} .

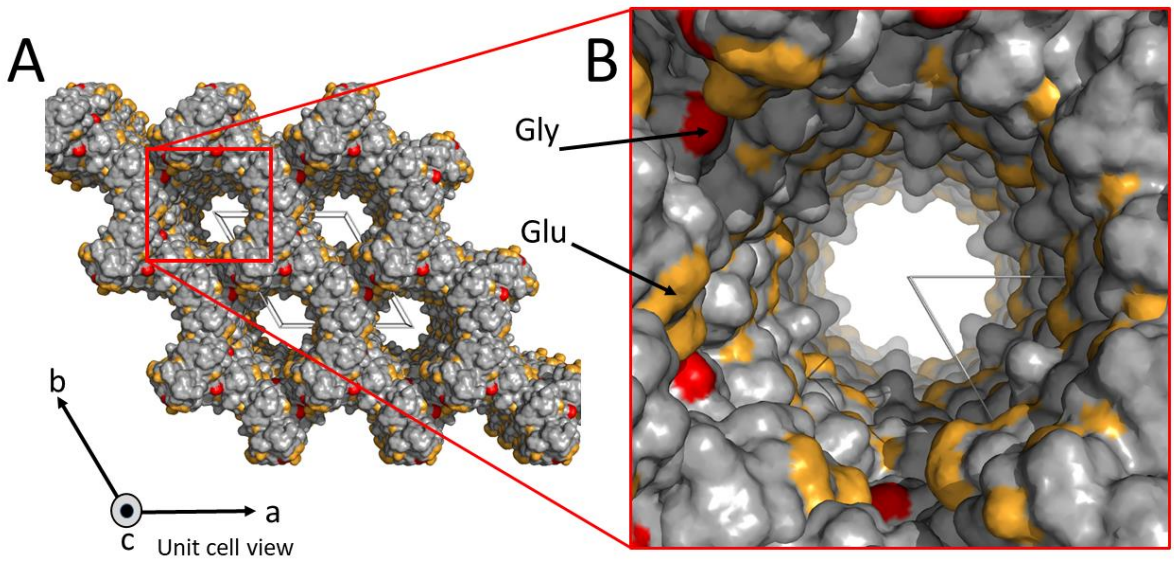


Fig. S10. View of solvent accessible crystal channels. **A,** general view the network of channels that cross entirely a LEAFY crystal along the C-axis. **B,** Close view of the details within a single channel. The surface represents areas of the proteins that are accessible to the solvent. The side chains of Glycine and Glutamate accessible to the solvent are coloured in red and orange respectively, while all the other ones are represented in grey.

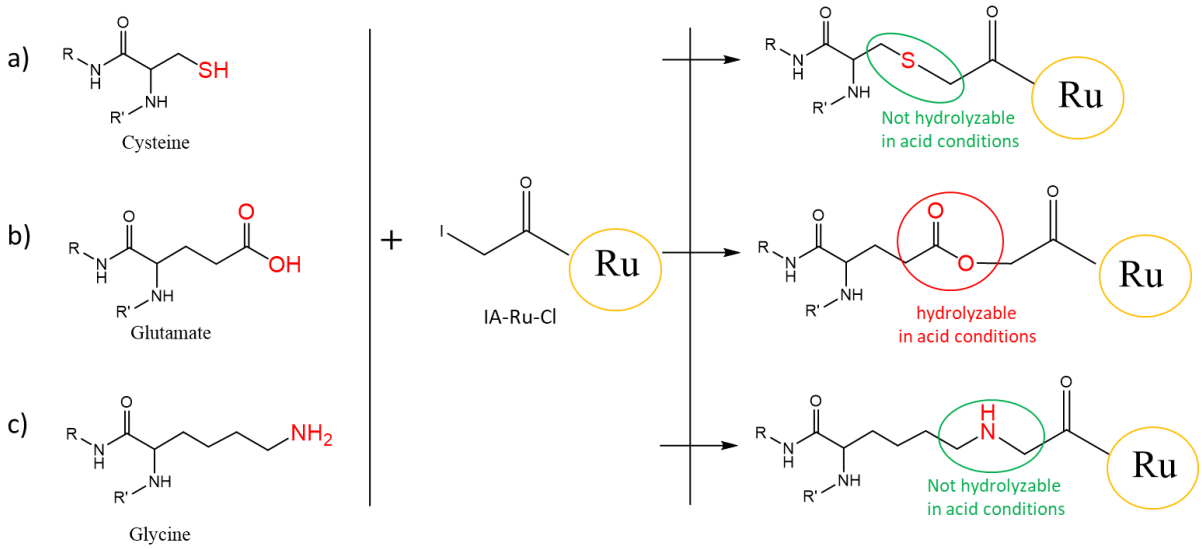
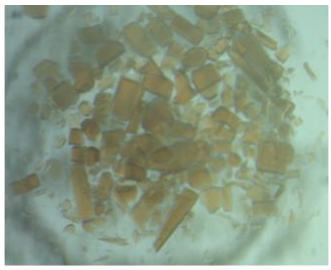
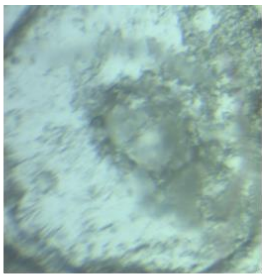


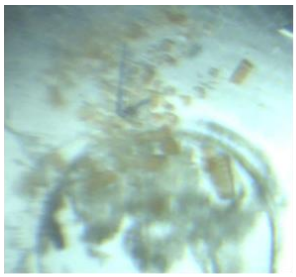
Fig. S11. Reactivity of cysteine a), glutamate b) and Glycine c) toward IA-Ru-Cl and stability of the resulting group in acid conditions



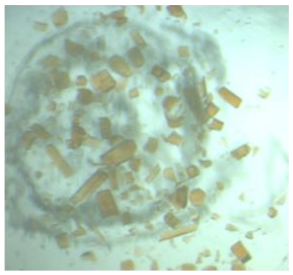
Ru ⊂ LEAFY WT



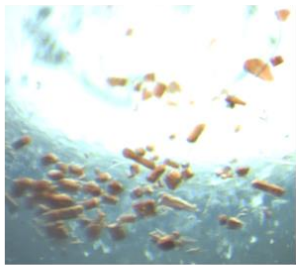
Ru ⊂ LEAFY WT
after 15 min in 0,5% formic acid



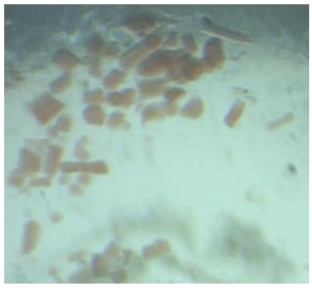
Ru ⊂ LEAFY WT
after 20h in 0,05% formic acid



Ru ⊂ LEAFY WT
in a CH_3CN-DMSO 1:1
mixture before irradiation



Ru ⊂ LEAFY WT
in a CH_3CN-DMSO 1:1
mixture after 4h irradiation
(blue LED 455 nm- 15
$mW.cm^{-2}$)



Ru ⊂ LEAFY WT
in a CH_3CN-DMSO 1:1
mixture after 48h irradiation
(blue LED 455 nm- 15
$mW.cm^{-2}$)

Fig. S12. Study of **Ru ⊂ LEAFY WT** crystals stability under acidic conditions and in the presence of organic solvents under light irradiation.

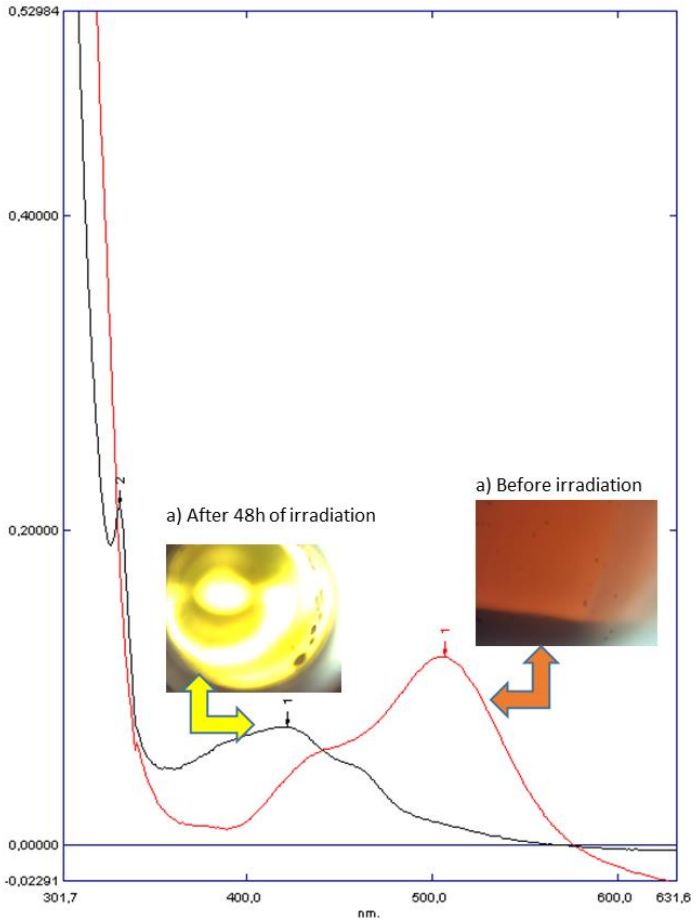


Fig. S13. Changes in the absorption spectrum of IA-Ru-Cl in a 1:1 mixture of CH_3CN-DMSO upon blue light irradiation; before irradiation a) and after 48h of light irradiation b).

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