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Luminescent Protein Staining with Re(I) Tetrazolato Complexes

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Abstract

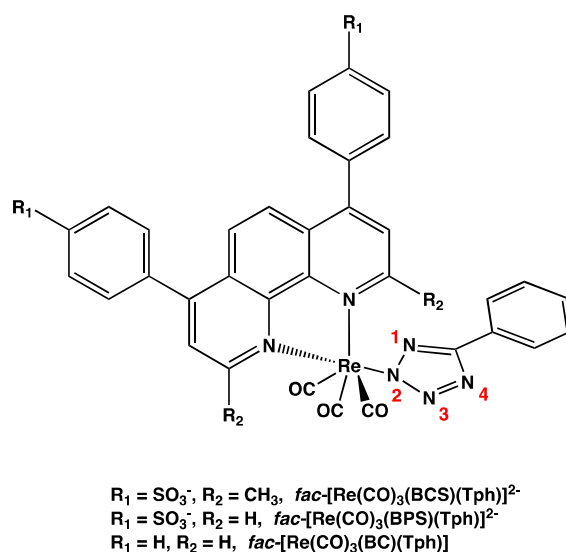
Within the general framework of our past and current studies dealing with the investigation of the photophysical properties and the biological behavior of the family of tetrazolato and tetrazole Re(I) complexes, we have endeavored to investigate their potential in the luminescent staining of proteins purified by acrylamide gel electrophoresis. With the aim to provide the first examples of luminescent Re(I) complexes to be exploited for this specific purpose, we have designed and prepared four new Re(I)-based species with the general formula $fac-[Re(CO)_3(N^{\wedge}N)(Tph)]^{2-/0}$, where **Tph** is the 5-(phenyl)tetrazolato anion and **N[∧]N** is in turn represented by bathophenanthroline disulfonate (**BPS**), bathocuproine disulfonate (**BCS**) or by the SO₃⁻ free bathocuproine (**BC**). In this latter case, the neutral complex $fac-[Re(CO)_3(BC)(Tph)]$ served as a model species for the characterization of the former disulfonate complexes. Its cationic analogue $fac-[Re(CO)_3(BC)(Tph-Me)]^+$ was also prepared by a straightforward methylation reaction. All complexes displayed bright phosphorescence in organic media and, relative to their water solubility, the dianionic species $fac-[Re(CO)_3(BPS)(Tph)]^{2-}$ and $fac-[Re(CO)_3(BCS)(Tph)]^{2-}$ were also highly emissive in aqueous solution. The sulfonate groups played a key role in promoting and significantly enhancing the luminescent staining performances of both the Re(I) complexes $fac-[Re(CO)_3(BPS)(Tph)]^{2-}$ and $fac-[Re(CO)_3(BCS)(Tph)]^{2-}$ for proteins. Highlighting a response superior to that of Coomassie Blue and comparable to the one obtained by the well-known silver staining method, these dianionic Re(I)-complexes could efficiently detect up to 50 ng of pure Bovine Serum Albumin (BSA), as well as all proteins found in a Standard Protein Marker mix and from a total protein extract. A lower but still good response for

luminescent protein staining was surprisingly obtained by employing the -SO_3^- free neutral and cationic complexes ***fac*-[Re(CO)₃(BC)(Tph)]** and ***fac*-[Re(CO)₃(BC)(Tph-Me)]⁺**, respectively. These preliminary results open up new possibilities for the further widening of the use of Re(I)-based complexes as luminescent protein staining agents.

Introduction

The development of luminescent sensors aimed at targeting biologically relevant molecules has been central to the research interests of many groups worldwide. Further to the well-established protocols that are centered on organic fluorophores, a great deal of attention has been dedicated to the use of the most popular classes of luminescent metal complexes, such as those based on Ru(II), Ir(III), Re(I) and Pt(II) for the extra and intracellular luminescent detection of bio-molecules.¹ Among the various targets, proteins are obviously one of the most important, and intense research efforts have been dedicated to their sensing by taking advantage of the different interactions (*i.e.* covalent, not covalent) that can occur between proteins and luminescent metal complexes. In addition to providing critical information into the involvement of key proteins in biological processes,² these studies have had a significant impact on proteomic analysis. For example a series of Ru(II)-based compounds, such as ruthenium(II) *tris*(bathophenanthroline disulfonate), abbreviated as RuBPS,³ and SYPRO Ruby[®] (whose structure is yet undisclosed),^{4a-b} have been indeed commercialized as luminescent agents for the staining of the discrete protein bands that are obtained from Sodium Dodecyl Sulphate - PolyAcrylamide Gel Electrophoresis (SDS-PAGE). The strategy underpinning the use of RuBPS relies upon the water solubility that is conferred by the sulfonate moieties and, importantly, by the occurrence of electrostatic interactions between the peripheral SO_3^- groups of the BPS ligands and the NH_2 groups of the proteins.^{3,4} The structure of RuBPS has inspired the successive development of BPS-based Ir(III) cyclometalated complexes with the general formula $[\text{Ir}(\text{C}^{\wedge}\text{N})_2(\text{BPS})]$, in which the peculiar and intense luminescent output of this class of metal complexes was combined with the “usual” protein recognition properties displayed by the sulfonate groups.⁵ In these regards, it is worth noting that a recent report by Zhou and coworkers showed that also cationic and neutrally charged Ir(III) cyclometalated complexes devoid of any SO_3^- moieties could also be used as protein staining agents, pointing to the importance of other factors aside of electrostatic interactions in determining the performances of protein stains.⁶ To the best of our knowledge, no examples of *fac*-triscarbonyl Re(I) diimines, one of the most extensively studied classes of luminescent d^6 metal complexes, have been considered for the specific purpose of protein staining. To this end, we have prepared a library of Re(I)-tetrazolato complexes with the general formula $\text{fac-}[\text{Re}(\text{CO})_3(\text{N}^{\wedge}\text{N})(\text{Tph})]^{0/+2-}$ where **Tph** denotes the 5-(phenyl)tetrazolato, to be exploited as new luminescent protein staining agents for SDS PAGE. In particular, our design strategy for the **N[^]N** ligand involves the use of disulfonate ligands; either bathophenanthroline

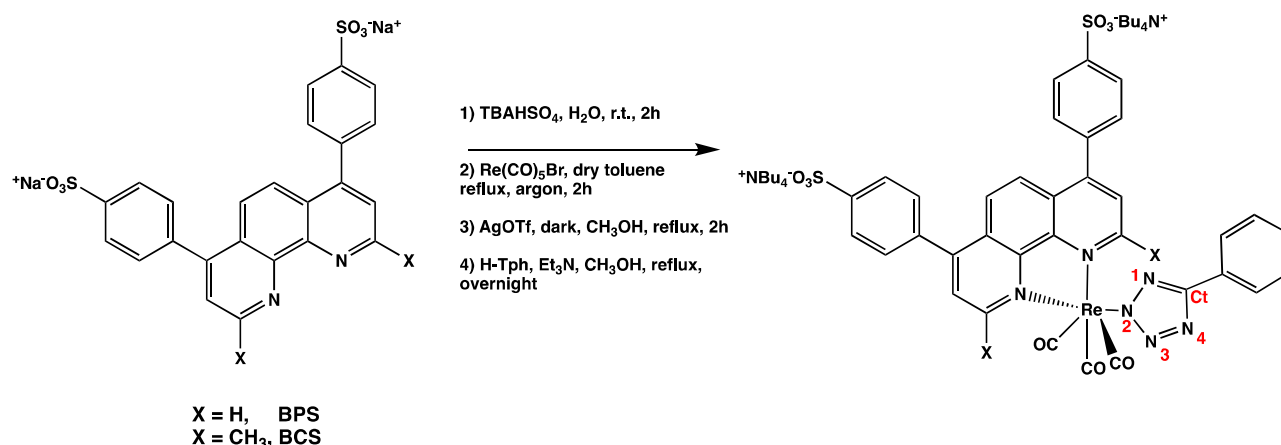
disulfonate (**BPS**), or bathocuproine disulfonate (**BCS**, see Scheme 1). We also prepared the neutral SO_3^- free bathocuproine (**BC**) complex *fac*-[**Re**(**CO**)₃(**BC**)(**Tph**)] and its cationic methylated analogue *fac*-[**Re**(**CO**)₃(**BC**)(**Tph-Me**)]⁺ (see Schemes 1 and 3) by taking advantage of the reactivity that the tetrazolate ligand displays toward electrophiles. Our results highlight how **Re**(I) disulfonate complexes can be regarded as very sensitive staining agents for proteins in SDS-PAGE while the non-sulfonate neutral and cationic derivatives, in addition to being useful model complexes, also display a promising potential for the detection of proteins.



Scheme 1: Structures, acronyms of the complexes and numbering for 5-(phenyl)tetrazolato (**Tph**) reported in this work.

Results and discussion

The 5-(phenyl)tetrazolato anion **Tph** was chosen due to its strong binding to the Re(I) centre which results in a substitution inert complex. This is important to ensure that any interactions between the complexes and proteins can be confidently ascribed to the disulfonate **N⁺N** ligands **BPS** or **BCS**. To this end, as depicted is Scheme 2, the preparation of the corresponding Re(I)-tetrazolato complexes *fac*-[Re(CO)₃(BPS)(Tph)]²⁻ and *fac*-[Re(CO)₃(BCS)(Tph)]²⁻ was pursued by a multistep procedure that required the preliminary counterion exchange of the sodium salts of the ligands **BPS** and **BCS**. This led to the corresponding tetrabutyl ammonium [Bu₄N]⁺ analogues which were found to be more soluble in organic solvents.⁷ Following this modification, the Re(I) complexes were obtained by our standard route^{8a-b} which involved the initial formation of the bromide precursors *fac*-[Re(CO)₃(BPS)(Br)]²⁻ and *fac*-[Re(CO)₃(BCS)(Br)]²⁻, followed by the Ag(I)-mediated bromide extraction and, finally, the coordination of the 5-(phenyl)tetrazolato anion to the Re(I) ion.



Scheme 2: Synthetic procedure used for the preparation of Re(I) disulfonate compounds described in this work (Ct means tetrazolic carbon).

After column chromatography, the target dianionic complexes *fac*-[Re(CO)₃(BPS)(Tph)]²⁻ and *fac*-[Re(CO)₃(BCS)(Tph)]²⁻ were characterized by performing Electro-Spray Ionisation Mass Spectrometry (ESI-MS) and Infrared (IR) spectroscopy experiments from dichloromethane solutions. These data supported their formation as tetrabutyl ammonium salts and with the facial arrangement of the three CO ligands (ESI Figures S1-4†, Table S1†). The analysis of the NMR (¹H and ¹³C) data for the disulfonate Re(I)-complexes required direct comparisons to the well resolved ¹H and ¹³C NMR spectra of the neutral sulfonate-free model complex *fac*-[Re(CO)₃(BC)(Tph)]. This

is due to the occurrence of the commercial **BPS** and **BCS** as mixtures of regioisomers,^{6, 9} which complicated the peak assignment (ESI Figures S5-8†). The pattern of signals of the neutral sulfonate-free model complex **fac-[Re(CO)₃(BC)(Tph)]** were consistent with the presence of one *cis*-chelate bathocuproine (**BC**) as the diimine (N^N) ligand and one axially coordinated 5-(phenyl)tetrazolato anion were clearly displayed (ESI Figure S9-11†). In addition, the direct comparison of the ¹³C-NMR spectra of **fac-[Re(CO)₃(BC)(Tph)]** with those relative to **fac-[Re(CO)₃(BPS)(Tph)]²⁻** and **fac-[Re(CO)₃(BPS)(Tph)]²⁻** revealed how, in all complexes, the tetrazolic carbon (Ct) was found to resonate at values of chemical shifts that, according to our previous work, is characteristic of the regioselective coordination of the tetrazolate ligand to the Re(I) ion through the N-2 atom (see Schemes 2 and 3 for atom numbering) of the pentatomic ring. This evidence was further corroborated by the analysis of the molecular structure obtained by X-ray diffraction of **fac-[Re(CO)₃(BC)(Tph)]**, which shows bonding parameters in excellent agreement with those reported earlier for related and isostructural Re(I) tetrazolato complexes.^{8a-b}

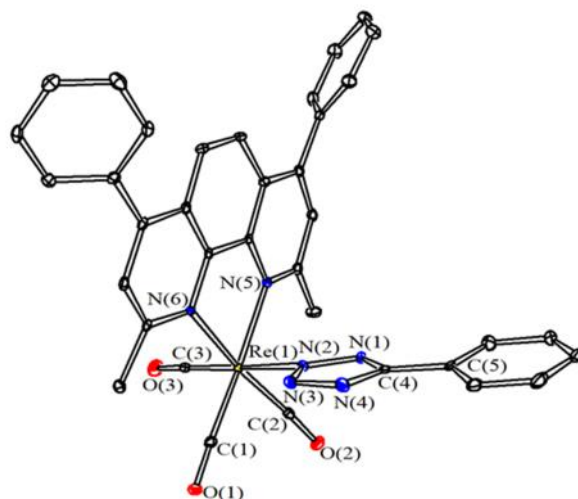
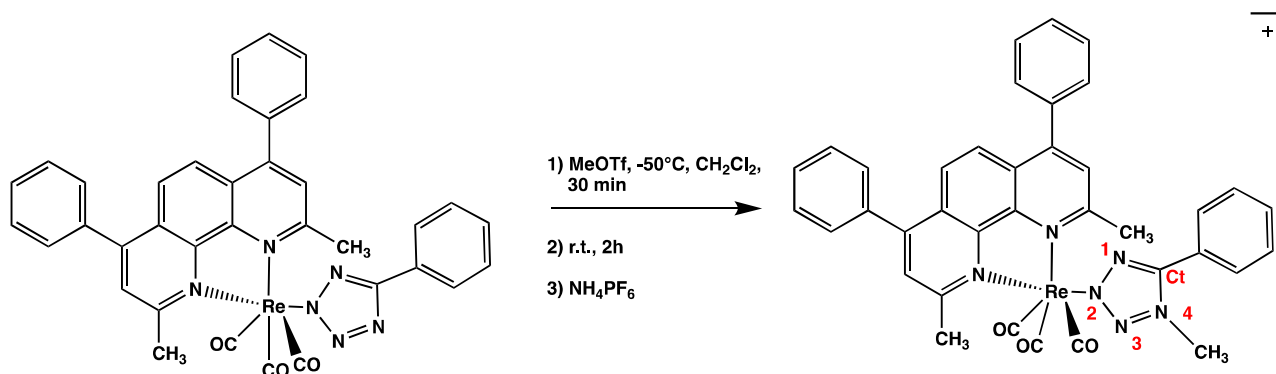


Figure 1: Molecular structure of **fac-[Re(CO)₃(BC)(Tph)]** with key atoms labelled. Displacement ellipsoids are at the 30% probability level. Hydrogen atoms have been omitted for clarity. Selected bond lengths (Å) and angles (°): Re(1)-C(1) 1.907(3), Re(1)-C(2) 1.918(4), Re(1)-C(3) 1.934(4), Re(1)-N(2) 2.172(3), Re(1)-N(5) 2.200(3), Re(1)-N(6) 2.199(3), N(3)-N(4) 1.328(4), N(2)-N(3) 1.323(4), N(1)-N(2) 1.352(4), N(1)-C(4) 1.340(5), C(4)-N(4) 1.343(5), N(5)-Re(1)-N(6) 75.65(11), C(1)-Re(1)-N(5) 176.93(14), C(2)-Re(1)-N(6) 170.90(13), C(3)-Re(1)-N(2) 178.59(14).

Over the past decade, we have extensively exploited the reactivity of luminescent Ru(II),^{10a-b} Ir(III)^{11a-b} and Re(I)-tetrazolato complexes toward electrophiles such as H⁺,¹² CH₃⁺ and divalent metal cations.^{8a-b} These studies highlight how such a relatively simple and straightforward chemical modulation of the tetrazolato ancillary ligand of the corresponding Ru(II) and, particularly, Ir(III) and Re(I)-tetrazolato complexes could be regarded as a viable strategy to modify

their luminescence outputs and moreover, to impart alternative biological behavior in terms of cellular incubation, localisation,^{13a-c} cytotoxicity,¹⁴ and antibacterial properties.¹⁵ On such basis, aiming to investigate the effect of the variation of the global charge of the SO_3^- free Re(I) tetrazolato complexes on their eventual luminescent protein staining properties, the neutral complex ***fac*-[Re(CO)₃(BC)(Tph)]** was converted into the corresponding cationic analogue ***fac*-[Re(CO)₃(BC)(Tph-Me)]⁺** by methylation reaction (Scheme 3).



Scheme 3: Synthetic procedure used for the preparation of ***fac*-[Re(CO)₃(BC)(Tph-Me)]⁺**.

The analysis of the NMR features of the methylated complex ***fac*-[Re(CO)₃(BC)(Tph-Me)]⁺** suggests its occurrence as a mixture of two structural isomers. In detail, the signals relative to ***fac*-[Re(CO)₃(BC)(Tph-Me)]⁺** appeared split into two distinct pattern of resonances, both in the case of the ¹H and ¹³C-NMR spectrum. While analysis of the ¹H-NMR spectrum suggests that the two isomers coexist in a 0.47:1 ratio, the ¹³C-NMR shows two different tetrazolic carbon resonances, both of which are centered below 160 ppm, highlighting the presence of two linkage isomers where methylation occurred regioselectively at the position N-4 of the tetrazole, while the Re(I) fragment was bound to either positions N-2 or N-1 of the same pentatopic ring (see Schemes 2 and 3 for atom numbering; ESI Figures S12-15[†]). This is consistent with our previous reports.^{8a}

Photophysical Properties

The relevant photophysical data of all the Re(I) complexes described herein are summarized in Table 1. While both the dianionic disulfonate complexes *fac*-[Re(CO)₃(BPS)(Tph)]²⁻ and *fac*-[Re(CO)₃(BCS)(Tph)]²⁻ were found to be water soluble as expected, they were also found to be soluble in organic media (dichloromethane for *fac*-[Re(CO)₃(BPS)(Tph)]²⁻ and methanol in the case of *fac*-[Re(CO)₃(BCS)(Tph)]²⁻). In contrast, the absorption and emission spectra of the -SO₃⁻ free neutral and cationic species *fac*-[Re(CO)₃(BC)(Tph)] and *fac*-[Re(CO)₃(BC)(Tph-Me)]⁺, respectively, could only be obtained from the corresponding dichloromethane solutions. However, as a general feature and irrespective of the nature of the solvent, the absorption profiles of the Re(I) complexes all display a UV region dominated by intense ligand-centered (LC) transitions (250-310 nm), followed by metal-to-ligand charge transfer (MLCT) processes (320-400 nm) tailing off in the visible region (Figure 2).

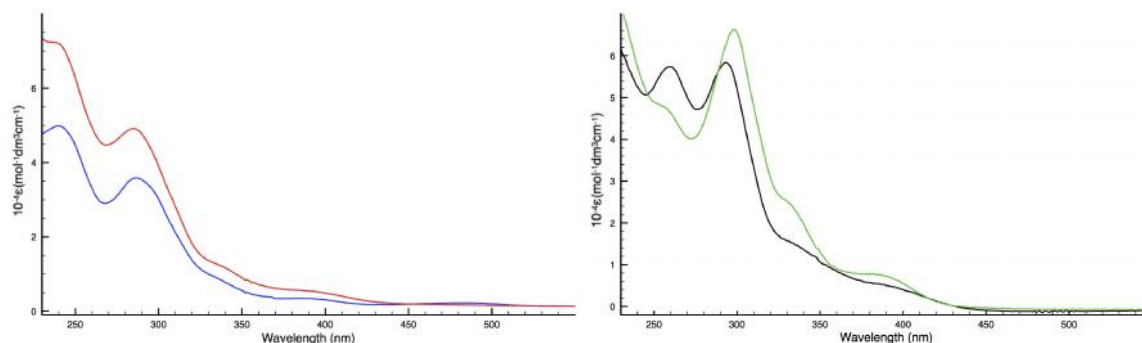


Figure 2: (left) absorption profile of *fac*-[Re(CO)₃(BCS)(Tph)]²⁻, in CH₃OH (red trace) and H₂O (blue trace); (right) absorption profile of *fac*-[Re(CO)₃(BC)(Tph)] (black trace), and *fac*-[Re(CO)₃(BC)(Tph-Me)]⁺ (green trace), CH₂Cl₂, 298K.

Table 1: Relevant photophysical data for the Re(I) complexes presented in this work.

| | | Absorption | Emission 298 K | | | | Emission 77 K | | |
|---|---------------------------------|--|-------------------------------|---|--|---|--|--|--|
| Complex | Solvent | λ_{abs} (nm) ($10^{-4}\epsilon$) ($\text{M}^{-1}\text{cm}^{-1}$) | λ_{em} (nm) | τ_{air} (μs) ^a | τ_{Ar} (μs) ^a | Φ_{air} (%) ^b | Φ_{Ar} (%) ^b | λ_{em} (nm) ^c | τ (μs) ^c |
| <i>fac</i> -[Re(CO) ₃ (BC)(Tph)] | CH ₂ Cl ₂ | 256(5.72), 293(5.84), 343(1.29), 392(0.49) | 592 | 0.33 | 0.87 | 1.97 | 6.35 | 534 | 2.41 |
| <i>fac</i> -[Re(CO) ₃ (BC)(Tph-Me)] ⁺ | CH ₂ Cl ₂ | 257(4.75), 298(6.62), 331(2.49), 390(0.73) | 546 | 1.36 | 12.1 | 2.96 | 15.01 | 514 | 39 |
| <i>fac</i> -[Re(CO) ₃ (BPS)(Tph)] ²⁻ | CH ₂ Cl ₂ | 288 (3.62), 392 (0.70) | 600 | 0.58 | 1.54 | 4.08 | 4.26 | 550 | 1.54 |
| <i>fac</i> -[Re(CO) ₃ (BCS)(Tph)] ²⁻ | CH ₃ OH | 285 (4.98), 383 (0.57) | 602 | 0.24 | 0.82 | 0.66 | 2.40 | 542 | 4.98 |
| <i>fac</i> -[Re(CO) ₃ (BCS)(Tph)] ²⁻ | H ₂ O | 240 (4.15), 286 (3.58), 389 (0.34) | 452 592 | 0.007 0.25 | 0.58 | 2.7 | 3.80 | /* | /* |
| <i>fac</i> -[Re(CO) ₃ (BPS)(Tph)] ²⁻ | H ₂ O | 289 (4.45), 387 (0.77) | 604 | 0.492 | 0.66 | 2.2 | 3.30 | /* | /* |

^a: "Air" means air equilibrated solutions, "Ar" means deoxygenated solutions under argon atmosphere; ^b: [Ru(bpy)₃]Cl₂/H₂O was used as reference for quantum yield determinations ($\Phi_r = 0.028$)²²; ^c: in frozen solvent matrix; *: not determined.

While the absorption profiles of the dianionic complexes did not significantly differ from each other, a subtle but evident *hypsochromic* shift of the MLCT features was observed when the neutral complex ***fac*-[Re(CO)₃(BC)(Tph)]** was converted into its cationic analogue ***fac*-[Re(CO)₃(BC)(Tph-Me)]⁺**. In agreement with our previous studies,^{8a, 12} this trend is consistent with the variation of the global net charge that occurs upon the addition of a CH₃⁺ moiety and the concomitant reduction of electron density on the tetrazole ring.

In dilute dichloromethane (or as in the case of ***fac*-[Re(CO)₃(BCS)(Tph)]²⁻** as a methanol solution) at room temperature, upon excitation of the corresponding MLCT features (typically, $\lambda_{\text{exc}} = 350\text{--}370$ nm) all the Re(I) complexes are luminescent and, in line with the typical behavior of this family of *fac*-Re(I) triscarbonyl diimine complexes,¹⁶ display broad and structureless bands centered between *ca.* 540 and 600 nm (Table 1). In all cases, the emission can be confidently ascribed to phosphorescence originating from charge transfer states of triplet multiplicity, ³CT, similarly to the previously reported for neutral and ionic Re(I) tetrazolato complexes. In fact, the excited state lifetime τ and quantum yield Φ are sensitive to the presence of dissolved O₂ (Table 1). At 77 K, the emission profiles appear blue-shifted as a consequence of rigidochromism (ESI Fig. S7–9[†]) and the values of τ and Φ increase due to the lack of vibrational and collisional quenching.^{17a-b}

As we have previously documented for the methylation of similar Re(I) tetrazolato complexes,^{8a} the methylated and cationic complex ***fac*-[Re(CO)₃(BC)(Tph-Me)]⁺** displays phosphorescent emission which is significantly more intense, longer lived and blue shifted ($\Delta\lambda_{\text{max}} = 1433\text{ cm}^{-1}$) with respect to that exhibited by its neutral precursor ***fac*-[Re(CO)₃(BC)(Tph)]** (Figure 4). In particular, the photophysical properties of the N-3 and N-4 linkage isomers that represent the complex ***fac*-[Re(CO)₃(BC)(Tph-Me)]⁺** appear identical, as witnessed by their displaying one single band in the emission profile and by the satisfactorily monoexponential fitting of the excited state decay.

It is worth noting that the water-soluble sulfonated complexes ***fac*-[Re(CO)₃(BCS)(Tph)]²⁻** and ***fac*-[Re(CO)₃(BPS)(Tph)]²⁻** basically retained their phosphorescent properties on passing from organic media to aqueous solutions, as witnessed by their displaying almost unaltered broad and unstructured emission profiles centred at *ca.* 600 nm upon excitation of the corresponding MLCT manifold (Figure 3). The only exception was observed in the emission spectrum recorded from an air-equilibrated solution of the complex ***fac*-[Re(CO)₃(BCS)(Tph)]²⁻**, where an additional fluorescent feature ($\tau = 7\text{ ns}$, Table 1) centred at $\lambda = 452\text{ nm}$, whose intensity increased upon exciting the sample at wavelengths shorter than 370 nm (Figure S19[†] ESI), appeared further to the phosphorescent emission peaking at 592 nm. The different multiplicity of spin of the emissive

excited states was further suggested by their different response to the removal of dissolved O₂. Upon degassing, only the phosphorescent process centred at $\lambda_{\text{max}} = 592$ nm experienced both an elongation of the corresponding excited state lifetime and, in particular, a significant enhancement of the emission intensity, making the fluorescent emission peak at *ca.* 452 nm almost undetectable (Figure S18[†] ESI). However, we suspect that the occurrence of a similar behaviour might be explained by considering the possibility of the replacement of the tetrazolate anion [Tph⁻] with one water molecule in the coordination sphere of ***fac*-[Re(CO)₃(BCS)(Tph)]²⁻**. Indeed, the coexistence of fluorescent and phosphorescent emissions originating from ¹IL (intra-ligand) and ³MLCT (metal-to-ligand charge transfer) type excited states, respectively, has been previously described by Wolcan and co-workers for aqueous solutions of the parent Re(I) complex ***fac*-[Re(CO)₃(BCS)Cl]²⁻**,¹⁸ where the insurgence of a fluorescent emission centred at *ca.* 450 nm was rationalized as the result of the partial hydrolysis of the chloride complex ***fac*-[Re(CO)₃(BCS)Cl]²⁻**.

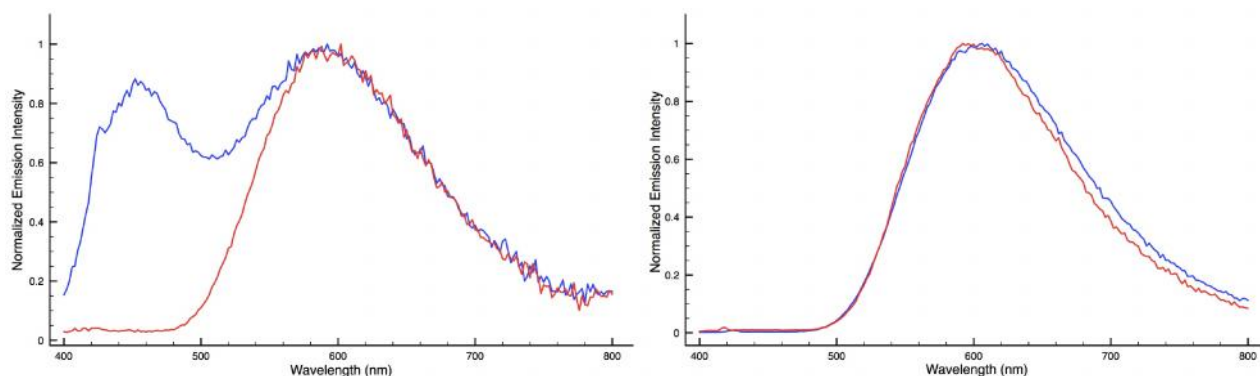


Figure 3: (left) Normalized emission profile of ***fac*-[Re(CO)₃(BCS)-(Tph)]²⁻**, in CH₃OH (red trace) and H₂O (blue trace); (right) Normalized emission profile of ***fac*-[Re(CO)₃(BPS)(Tph)]²⁻**, in CH₂Cl₂ (red trace) and H₂O (blue trace).

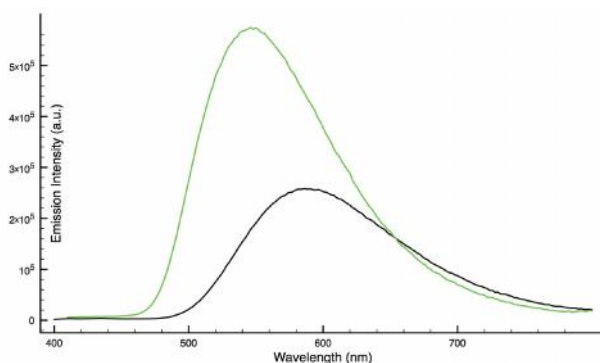


Figure 4: Emission profile of ***fac*-[Re(CO)₃(BC)(Tph)]** (black trace), and ***fac*-[Re(CO)₃(BC)(Tph-Me)]⁺** (green trace), CH₂Cl₂, 298K.

Luminescent staining of proteins

We then investigated the ability of the Re(I) tetrazolato complexes, either those containing disulfonated diimine ligands and SO₃-free bathocuproine ligands, to behave as luminescent probes for the visualization of proteins that are separated upon performing SDS-PAGE. In a preliminary assessment, the disulfonate Re(I)-based complexes *fac*-[Re(CO)₃(BCS)(Tph)]²⁻ and *fac*-[Re(CO)₃(BPS)(Tph)]²⁻, were tested as protein staining agents toward pure BSA (Bovine Serum Albumin, 66.2 kDa), which was selected as a target protein to be determined in polyacrylamide gels. Specifically, different dilutions of BSA (stock solution 1 mg/mL), ranging from 50 ng to 1 µg, were separated by SDS-PAGE. Gels were first fixed (30% ethanol, 10% acetic acid) then stained for a period of 15 hours with a 1 µM solution in 20% ethanol of *fac*-[Re(CO)₃(BCS)(Tph)]²⁻ and *fac*-[Re(CO)₃(BPS)(Tph)]²⁻, respectively. Thereafter, protein bands were readily visualised by using a UV trans-illuminator ($\lambda_{\text{exc}} = 302 \text{ nm}$). Both Re(I)-based stains (Figure 5) featured high sensitivity; concentrations of pure BSA down to 50 ng were detected. These charged dyes most likely interact with proteins in a non-covalent manner through a mechanism comparable to that of Coomassie staining, which is assumed to involve electrostatic interaction between sulfonate groups and basic amino acids.

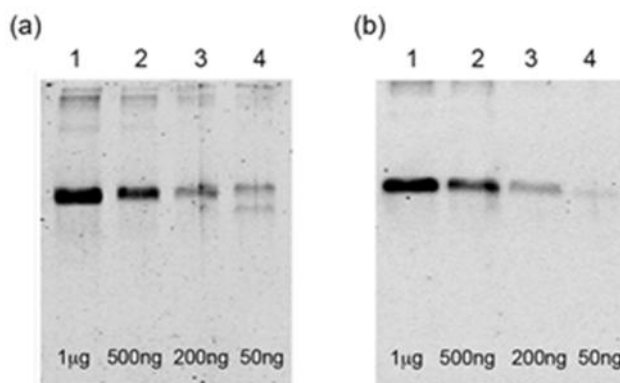


Figure 5: SDS-PAGE of different amounts of BSA. Lane 1 to 4: 1, 0.5, 0.2 and 0.05 µg BSA, respectively. (a) Staining with *fac*-[Re(CO)₃(BCS)(Tph)]²⁻ and (b) staining with *fac*-[Re(CO)₃(BPS)(Tph)]²⁻.

To better investigate their staining capability, both *fac*-[Re(CO)₃(BCS)(Tph)]²⁻ and *fac*-[Re(CO)₃(BPS)(Tph)]²⁻ were then tested against a standard Protein Molecular Weight Marker (Thermo Scientific™ Pierce), a mixture of seven known proteins such as Beta-galactosidase (116 kDa), Bovine serum albumin (66.2 kDa), Ovalbumin (45.0 kDa), Lactate dehydrogenase (35.0 kDa), REase Bsp98I (25.0 kDa), Beta-lactoglobulin (18.4 kDa) and Lysozyme (14.4 kDa). The Protein Marker was diluted sequentially from the stock solution (0.1-0.2 mg/mL of each protein), and run

in a gel using decreasing concentrations from lane 1 to 4 (Figure 6 a and b). Both ***fac***-[Re(CO)₃(BCS)(Tph)]²⁻ (Figure 6, a) and ***fac***-[Re(CO)₃(BPS)(Tph)]²⁻ (Figure 6, b) produced very clear and sharp bands, being able to detect, with a similar intensity, each loaded protein (down to 0.5 μL, corresponding to about 50-100 ng of protein/band). Interestingly both ***fac***-[Re(CO)₃(BCS)(Tph)]²⁻ and ***fac***-[Re(CO)₃(BPS)(Tph)]²⁻ had a similar response to protein, suggesting that the sulfonate residues in the **BCS** and **BPS** diimine ligands plays a key role in the Re(I) complex-protein interaction.

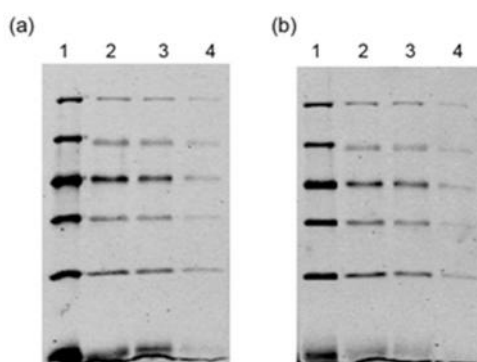


Figure 6: SDS-PAGE of different dilutions of the Protein Molecular Weight Marker. Lane 1 to 4: 6, 3, 1.5 and 0.5 μL, respectively; (a) staining with ***fac***-[Re(CO)₃(BCS)(Tph)]²⁻ and, (b) staining with ***fac***-[Re(CO)₃(BPS)(Tph)]²⁻.

To ensure that the Re(I)-based staining agents can also be used for the recognition of proteins from more complex mixtures, total protein extracts from *Escherichia coli* cells were loaded on polyacrylamide gels and then stained as previously indicated. As depicted in Figure 7, a total protein profile was identified independently of the characteristics of the protein mixture. Both ***fac***-[Re(CO)₃(BCS)(Tph)]²⁻ and ***fac***-[Re(CO)₃(BPS)(Tph)]²⁻ featured comparable efficacy to recognize unknown proteins, being able to detect down to a few micrograms/lane.

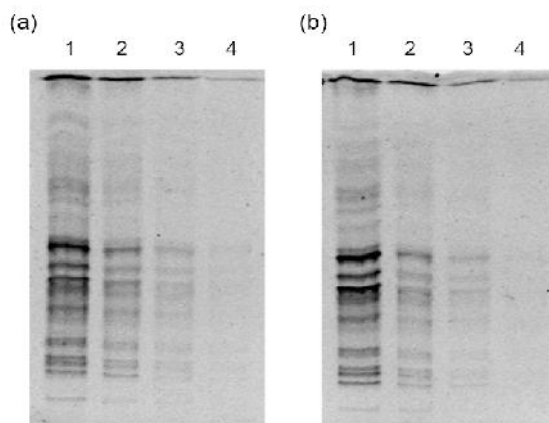


Figure 7: SDS-PAGE of total protein extracts. Lane 1 to 4: 5, 2, 1 and 0.5 μg, respectively; (a) staining with ***fac***-[Re(CO)₃(BCS)(Tph)]²⁻ and (b) staining with ***fac***-[Re(CO)₃(BPS)(Tph)]²⁻.

Comparison with colorimetric staining

Once the efficacy of $fac-[Re(CO)_3(BCS)(Tph)]^{2-}$ and $fac-[Re(CO)_3(BPS)(Tph)]^{2-}$ as staining agents to detect proteins in gels was verified, a comparison with two classical colorimetric stains, Coomassie Blue R250 and Silver staining, was carried out. To achieve this, three identical gels containing an equal amount of each standard protein were prepared (same dilutions as those shown in Figure 6). Gels were treated separately with a different staining protocols,¹⁹ then the intensity of the bands were compared, revealing how the fluorescent method based on $fac-[Re(CO)_3(BCS)(Tph)]^{2-}$ and $fac-[Re(CO)_3(BPS)(Tph)]^{2-}$ features comparable sensitivity, if not superior, to the traditional Silver staining (Figure 8). In particular, both Re(I) based stains were easily able to detect up to the last dilution of the protein marker (gels a and c, lane 1-4, Figure 8). In contrast, Coomassie Blue staining (gel b, Figure 8) exhibited a lower level of sensitivity with lanes containing less than 3 µg/L being visually difficult to distinguish from background. Beyond the high quality of detection, a conventional ethanol-acetic acid destaining, required for the Coomassie procedure, is not necessary when the luminescent Re(I)-compounds are used as stains instead a simple washing with water can efficiently reduce background staining. Interestingly, gels stained with Re(I)-based dyes can be re-stained with Coomassie Brilliant Blue without altering its sensitivity, and viceversa.

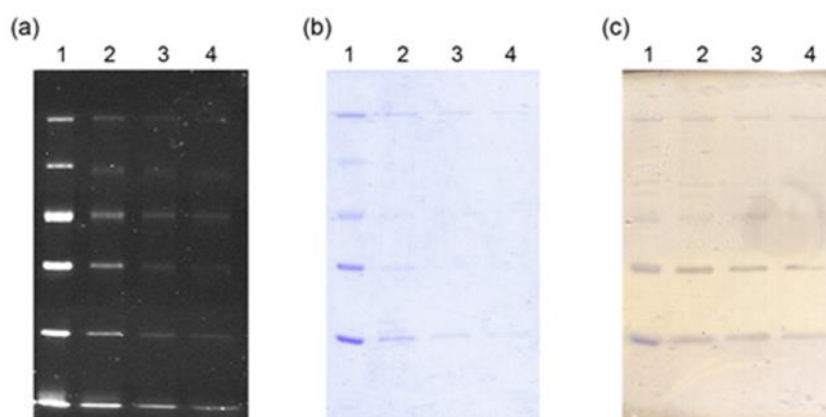


Figure 8: Comparison of three staining methods for the detection of different dilutions of the Protein Molecular Weight Marker in SDS-polyacrylamide gels. Lane 1 to 4: 6, 3, 1.5 and 0.5 µL of protein marker, respectively. (a) $fac-[Re(CO)_3(BCS)(Tph)]^{2-}$ staining, (b) Coomassie Blue staining and (c) Silver staining.

Staining of gels with non-sulfonate complexes

In order to further investigate the importance of the SO_3^- groups for the luminescent staining of proteins, the two non-sulfonated neutral and cationic Re(I) tetrazolate complexes, namely ***fac*-[Re(CO)₃(BC)(Tph)]** and ***fac*-[Re(CO)₃(BC)(Tph-Me)]⁺**, were tested as well. As described before, different dilutions of the protein molecular marker were run on SDS-PAGE, then gels were stained with a solution 1 μM solution of either neutral ***fac*-[Re(CO)₃(BC)(Tph)]** or the cationic ***fac*-[Re(CO)₃(BC)(Tph-Me)]⁺** in 20% ethanol. Interestingly, protein staining was easily observed for both non-sulfonated complexes (Figure 9, a and b), albeit the sensitivity was somewhat reduced compared with the disulfonated complexes (Figure 6). Recently, an analogous behavior has been reported relative to neutral and cationic brightly emissive Ir(III) complexes containing non-sulfonated ligands.⁶ In these cases, the performance of the protein stains was attributed to the extended π -conjugation across either the cyclometalated or the ancillary ligand. Therefore, we suspect that a similar effect arising from the **BC** ligand may account for the luminescent protein staining performances displayed by the neutral and cationic SO_3^- free Re(I)-complexes ***fac*-[Re(CO)₃(BC)(Tph)]** and ***fac*-[Re(CO)₃(BC)(Tph-Me)]⁺**, respectively. Interestingly, when the same gels were subjected to Coomassie staining, a comparable intensity of each protein band was obtained (Figure 9, c and d). These results highlight the relevance of the design of the ligands beyond to their “simple” decoration with $-\text{SO}_3^-$ groups in the preparation of luminescent complexes as protein staining agents. In addition, it is worth mentioning that the time-saving synthesis that is required for the non-sulfonated complexes can open promising perspectives for the use of SO_3^- - free Re(I) complexes in protein staining.

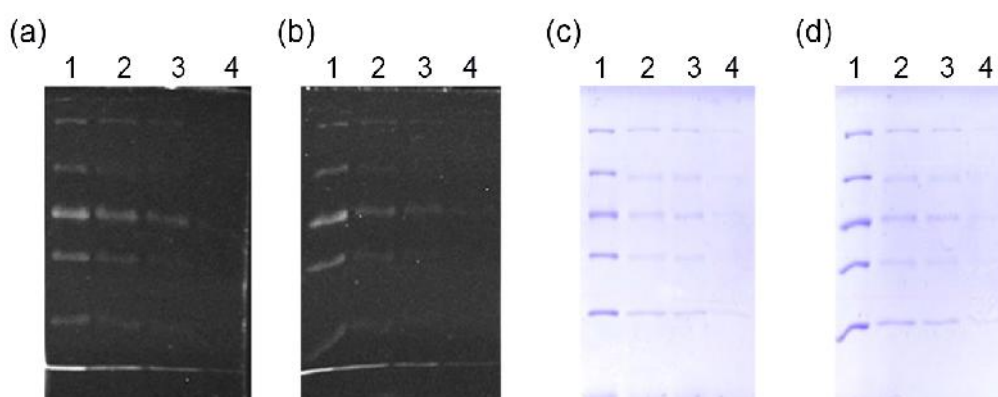


Figure 9: SDS-PAGE of different dilutions of the Protein Molecular Marker (lane 1, 2, 3, 4): staining with ***fac*-[Re(CO)₃(BC)(Tph)]** (a) and ***fac*-[Re(CO)₃(BC)(Tph-Me)]⁺** (b); (c) and (d): subsequent Coomassie staining of gels (a) and (b).

Conclusions

In summary, we have demonstrated how Re(I) tetrazolato complexes with the general formula ***fac*-[Re(CO)₃(N[^]N)(Tph)]^{0/+2-}**, where Tph means the 5-phenyl tetrazolato anion, do interact with proteins and, particularly, display a promising potential for the luminescent staining of SDS gels. These results pave the way for the exploitation of this general class of luminescent Re(I) complexes as protein staining agents. In this specific context, we could observe that the decoration of the diimine ligand (N[^]N) with sulfonate (-SO₃⁻) end groups appears as a favorable requisite for the enhancement of their protein staining performances. This is in line with the design strategy that has been pursued for the isoelectronic Ru(II)-polypyridyls and Ir(III)-cyclometalates and also for this family of substitution inert ***fac*-[Re(CO)₃(N[^]N)L]** type complexes. Our findings suggest that the sulfonated and dianionic Re(I) complexes herein described, namely ***fac*-[Re(CO)₃(BCS)(Tph)]²⁻** and ***fac*-[Re(CO)₃(BPS)(Tph)]²⁻**, where BPS and BCS denote bathophenanthroline disulfonate and bathocuproine disulfonate respectively, can detect a variety of protein targets that span from pure BSA to a standard mixture of proteins of known molecular weights and, interestingly, to curde protein extracts. This is achieved with very good sensitivity and by displaying a response superior to the traditional colorimetric method based on Coomassie Blue R250 and was comparable to the well-established Silver staining method.

Interestingly, both -SO₃⁻ free derivatives that are represented by the neutral model complex ***fac*-[Re(CO)₃(BC)(Tph)]** and the corresponding cationic methylated analogue ***fac*-[Re(CO)₃(BC)(Tph-Me)]⁺** were found to detect proteins subjected to SDS-PAGE in a response that was comparable to that of Coomassie Blue under the same experimental conditions. This is in contrast to what may be expected on the basis of a structure – activity relationship, which from the literature would have suggested that the -SO₃⁻ free derivatives would possess only limited staining capability. In addition to report for the first time that tricarbonyl Re(I) diimine complexes can be used for this application, these latter findings are very encouraging since they may open up new possibilities for furthering the studies dealing with the use of the synthetically more easily accessible Re(I) complexes as luminescent protein staining agents. Nevertheless, future experiments will be focused on testing if our staining protocol could be shortened (e.g. by reducing the incubation time), and if it is compatible with mass spectrometry (MS) for proteomic analysis.

Experimental Section

General considerations. All the reagents and solvents were obtained commercially (Sigma Aldrich/Merck, Alfa Aesar, Strem Chemicals) and used as received without any further purification, unless otherwise specified. All the reactions were carried out under an argon atmosphere following Schlenk protocols. Where required, the purification of the Re(I) complexes was performed via column chromatography with the use of SiO₂ as the stationary phase. ESI-mass spectra were recorded using a Waters ZQ-4000 instrument (ESI-MS, acetonitrile as the solvent). Nuclear magnetic resonance spectra (consisting of ¹H and ¹³C) were always recorded using a Varian Mercury Plus 400 (¹H, 399.9; ¹³C, 101.0 MHz). ¹H and ¹³C chemical shifts were referenced to residual solvent resonances.

Photophysics. Absorption spectra were recorded at room temperature using a Perkin Elmer Lambda 35 UV/vis spectrometer. Uncorrected steady-state emission and excitation spectra were recorded on an Edinburgh FLSP920 spectrometer equipped with a 450 W xenon arc lamp, double excitation and single emission monochromators, and a Peltier-cooled Hamamatsu R928P photomultiplier tube (185–850 nm). Emission and excitation spectra were acquired with a 10 nm slit (excitation filter (395 nm) and corrected for source intensity (lamp and grating) and emission spectral response (detector and grating) by a calibration curve supplied with the instrument. The wavelengths for the emission and excitation spectra were determined using the absorption maxima of the MLCT transition bands (emission spectra) and at the maxima of the emission bands (excitation spectra). Quantum yields (Φ) were determined using the optically dilute method by Crosby and Demas²⁰ at excitation wavelength obtained from absorption spectra on a wavelength scale [nm] and compared to the reference emitter by the following equation:²¹

$$\phi_s = \phi_r \left[\frac{A_r(\lambda_r)}{A_s(\lambda_s)} \right] \left[\frac{I_r(\lambda_r)}{I_s(\lambda_s)} \right] \left[\frac{n_s^2}{n_r^2} \right] \left[\frac{D_s}{D_r} \right]$$

where A is the absorbance at the excitation wavelength (λ), I is the intensity of the excitation light at the excitation wavelength (λ), n is the refractive index of the solvent, D is the integrated intensity of the luminescence, and Φ is the quantum yield. The subscripts r and s refer to the reference and the sample, respectively. A stock solution with an absorbance > 0.1 was prepared, then two dilutions were obtained with dilution factors of 20 and 10, resulting in absorbances of about 0.02 and 0.08 respectively. The Lambert-Beer law was assumed to remain linear at the concentrations of the solutions. The degassed measurements were obtained after the solutions

were bubbled for 10 minutes under Ar atmosphere, using a septa-sealed quartz cell. Air-equilibrated $[\text{Ru}(\text{bpy})_3]\text{Cl}_2/\text{H}_2\text{O}$ solution ($\Phi = 0.028$)²² was used as reference. The quantum yield determinations were performed at identical excitation wavelengths for the sample and the reference, therefore deleting the $I(\lambda_r)/I(\lambda_s)$ term in the equation. Emission lifetimes (τ) were determined with the single photon counting technique (TCSPC) with the same Edinburgh FLSP920 spectrometer using pulsed picosecond LED (ELED 360, FWHM < 800ps) as the excitation source, with repetition rates between 1 kHz and 1 MHz, and the above-mentioned R928P PMT as detector. The goodness of fit was assessed by minimizing the reduced χ^2 function and by visual inspection of the weighted residuals. To record the 77 K luminescence spectra, the samples were put in quartz tubes (2 mm diameter) and inserted in a special quartz Dewar filled with liquid nitrogen. The solvent used in the preparation of the solutions for the photophysical investigations was of spectrometric grade. Experimental uncertainties are estimated to be $\pm 8\%$ for lifetime determinations, $\pm 20\%$ for quantum yields, and ± 2 nm and ± 5 nm for absorption and emission peaks, respectively.

Ligand synthesis

Warning! Tetrazole derivatives are used as components for explosive mixtures.²³ In this lab, the reactions described here were only run on a few grams scale and no problems were encountered. However, great caution should be exercised when handling or heating compounds of this type. Following the general method reported by Koguro and co-workers,²⁴ tetrazole ligand [H-Tph] was obtained in quantitative yield. [H-Tph] ¹H-NMR (DMSO d_6 , 400 MHz) δ (ppm) = 8.06 - 8.03 (m, 2H), 7.62 - 7.60 (m, 3H).

General Procedure for the Preparation of *fac*-[Re(CO)₃(N[^]N)-(Tph)]²⁻-type complexes

To an aqueous solution (5 mL) of TBAHSO₄ (tetrabutylammonium hydrogensulphate) (0.140 g, 0.42 mmol) was added the desired diimine ligand BCS or BPS as sodium salt (0.102 g, 0.18 mmol). The resulting solution was stirred for 2h at r.t. and then extracted with CH₂Cl₂ (3 x 3 mL). The organic phase was then dried with MgSO₄ and the removal of the solvent afforded the targeted [Bu₄N] salt of BCS or BPS in almost quantitative yield. To a suspension of Re(CO)₅Br (46 mg, 0.11 mmol) in dry toluene (5 mL) was added either [Bu₄N][BCS] or [Bu₄N][BPS] (0.11 mmol). The mixture was heated for 2h at reflux under argon atmosphere to afford an orange gel. The solvent was decanted, removed and the gel formed was washed several times with hexane until it became solid, then

dried to give *fac*-[Re(CO)₃(N[^]N)Br]²⁻. To a solution of *fac*-[Re(CO)₃(N[^]N)Br]²⁻ in CH₃OH (0.11 mmol, 10 mL) repaired from light was added AgOTf (0.11 mmol). The mixture was heated at reflux for 2h. The resulting solution was filtered over a celite pad to remove AgBr and reduced in volume (to about 10 mL). To the latter one was added dropwise a CH₃OH + Et₃N mixture of [H-Tph] (2 mL + 0.23 μL) and heated at reflux, overnight. The resulting yellow solution was evaporated to dryness and purified over SiO₂ column chromatography (CH₂Cl₂/CH₃OH 3:1) to give *fac*-[Re(CO)₃(N[^]N)-(Tph)]²⁻ as second fraction (N[^]N = BCS: 0.055 g, 0.038 mmol, Y = 35%; N[^]N = BPS: 0.076 g, 0.055 mmol, 50%).

***fac*-[Re(CO)₃(BPS)(Tph)]²⁻** ESI-MS [M]²⁻ = 452 *m/z* (CH₃OH); IR **ν** (cm⁻¹) = 2026.4 (CO), 1918.7 (CO), (CH₂Cl₂); Given the uncertainty about the effective position of the SO₃⁻ groups on the commercial BPS ligand (*i.e* the presence of structural isomers in the starting material),^{5, 8} it was not possible to confidently attribute the various signals in both ¹H and ¹³C NMR of ***fac*-[Re(CO)₃(BPS)(Tph)]²⁻**. ¹H-NMR (CD₃OD, 400 MHz) δ (ppm) = 7.34-7.25 (m, 2H), 7.46-7.39 (m, 1H), 7.63-7.55 (m, 1H), 7.82-7.67 (m, 5H), 8.21-8.00 (m, 9H), 9.68-9.62 (m, 1H). ¹³C-NMR (CD₃OD, 100 MHz) δ (ppm) = 197.72, 194.43, 166.26, 155.25, 155.12, 152.34, 150.12, 149.31, 149.22, 148.05, 147.76, 147.70, 141.64, 138.66, 137.09, 132.58, 131.02, 130.39, 129.93, 128.34, 128.27, 128.21, 127.87, 127.77, 127.62, 126.80, 123.39, 120.22, 59.51, 59.48, 59.46, 58.85, 51.29, 49.63, 49.42, 49.21, 49.00, 48.78, 48.57, 48.36, 47.92, 24.93, 24.76, 24.60, 20.68, 20.67, 20.66, 20.49, 13.92, 13.73. Anal. Calcd. For C₆₆H₉₁N₈O₉Re₁S₂ (1390.84) C 57.00, H 6.59, N 8.06. Found: C 57.08, H 6.66, N 8.00.

***fac*-[Re(CO)₃(BCS)-(Tph)]²⁻** ESI-MS [M]²⁻ = 467 *m/z*, [M+Na]²⁻ = 957 *m/z*, [M]⁻ = 1176 *m/z* (CH₃OH); IR **ν** (cm⁻¹) = 2029.0 (CO), 2015.5 (CO), 1914.3 (CO) (CH₂Cl₂); Given the uncertainty about the effective position of the SO₃⁻ groups on the commercial BCS ligand (*i.e* the presence of structural isomers in the starting material),^{5, 8} it was not possible to confidently attribute the various signals in both ¹H and ¹³C NMR of ***fac*-[Re(CO)₃(BCS)(Tph)]²⁻**; ¹H-NMR (CD₃OD, 400 MHz) δ (ppm) = 3.50 (s, 6H), 7.26-7.33 (m, 3H), 7.35-7.41 (m, 2H), 7.43-7.49 (m, 3H), 7.52-7.57 (m, 2H), 7.61-7.69 (m, 6H), 7.79-7.85 (m, 1H), 8.01-8.04 (m, 9H). ¹³C-NMR (CD₃OD, 100MHz) δ (ppm) = 164.77, 162.72, 160.93, 158.78, 150.53, 148.65, 148.38, 145.81, 145.42, 145.04, 137.86, 135.94, 131.17, 129.41, 129.28, 128.88, 128.68, 128.59, 128.55, 128.34, 128.28, 128.23, 126.72, 126.33, 126.26, 125.81, 125.73, 125.70, 124.41, 124.14, 124.00, 122.73, 58.10, 30.15, 23.35, 19.20, 12.51. Anal. Calcd. For C₆₈H₉₅N₈O₉Re₁S₂ (1418.89) C 57.75, H 6.75, N 7.90. Found: C 57.85, H 6.64, N 7.98.

General Procedure for the Preparation of *fac*-[Re(CO)₃(BC)(Tph)] and *fac*-[Re(CO)₃(BC)(Tph-Me)]⁺ complexes

***fac*-[Re(CO)₃(BC)(Tph)]⁺.** A 0.100 g aliquot of *fac*-[Re(BC)(CO)₃Br] (0.140 mmol) was dissolved in 20 mL of an ethanol/water mixture (3:1 v/v) under an Ar atmosphere. A 5.0 mL portion of an ethanol/water (3:1 v/v) solution containing 0.033 g (0.22 mmol) of Tph⁻ was added drop wise. Once the addition was completed, the resulting suspension was stirred at the reflux temperature for 24 h. After this time, the mixture was cooled to r.t. and filtered through a glass frit, affording the desired complex as a yellow microcrystalline powder, requiring any further purification process (Y = 0.056 g, 0.072 mmol, 51%). **ESI-MS** [M+H]⁺ = 777 *m/z*; **IR** ν (cm⁻¹) = 2022.71 (CO), 1918.04 (CO), 1898.22 (CO) (CH₂Cl₂); **¹H NMR** (Acetone-d₆, 400 MHz) δ (ppm) = 3.57 (s, 6H, CH₃ BC), 7.23-7.29 (m, 3H), 7.60-7.65 (m, 10H), 7.72-7.95 (m, 2H), 7.97 (m, 2H), 8.09 (m, 2H). **¹³C-NMR** (Acetone-d₆, 100 MHz) δ (ppm) = 197.72(CO), 194.38(CO), 164.91(Ct), 163.65, 152.16, 149.74, 136.88, 131.31, 130.57, 130.48, 129.91, 129.18, 128.98, 128.05, 127.76, 126.73, 125.24, 31.56 (CH₃-BC). Anal. Calcd. For C₃₆H₂₅N₆O₃Re₁ (775.84) C 55.73, H 3.25, N 10.83. Found: C 55.65, H 3.18, N 10.91.

***fac*-[Re(CO)₃(BC)(Tph-Me)]⁺.** 0.100 g of *fac*-[Re(CO)₃(BC)-(Tph)] was dissolved in 20 mL of CH₂Cl₂ and the mixture was allowed to cool down by immersion into an ethanol/liquid nitrogen cold bath. Then, methyl trifluoromethanesulfonate (1.2 equiv., solution in dichloromethane 0.179 M) was added. The reaction was stirred under nitrogen for 30 minutes while being kept in the cold bath, and then allowed to warm up to room temperature and stirred for 3 hours. Anion exchange was carried out by adding an excess of NH₄PF₆ in water to the solution and stirring for 20 minutes. The product was then extracted using dichloromethane (3×10 mL) and the organic components were combined and dried over anhydrous MgSO₄. Subsequent purification by column chromatography on alumina (gradient: CH₂Cl₂/acetone 8:2, second fraction) yielded *fac*-[Re(CO)₃(BC)(Tph-Me)]⁺[PF₆]⁻ (Y = 0.059 g, 0.063 mmol, 49%). **ESI-MS** [M]⁺ = 791 *m/z*; [M]⁻ = 145 *m/z* (PF₆); **IR** ν (cm⁻¹) = 2037 (CO), 1934 (CO, br), (CH₂Cl₂); The ratio of system *a* : *b* is 1 : 0.47. **¹H NMR** (Acetone-d₆, 400 MHz) δ (ppm) = 3.23 (s, 6H, -CH₃ BC, *a*), 3.54 (s, 3H, -CH₃ BC, *b*), 3.73 (s, 3 H, -CH₃ Tph, *a*), 4.18 (s, 1.71 H, -CH₃ Tph, *b*), 7.34-7.27 (m, 2.60 H), 7.59-7.54 (m, 3.84 H), 7.75-7.63 (m, 15 H, aromatic system BC *a*, *b*), 8.05-8.03 (m, 3.96 H, Tph, *a*), 8.08 (m, 1.19 H, Tph, *b*), 8.19 (m, 1.31 H, Tph, *b*). **¹³C-NMR** (Acetone-d₆, 100 MHz) δ (ppm) = 164.62, 164.44, 157.23, 156.59, 151.96,

151.81, 148.75, 148.21, 135.61, 135.57, 132.52, 132.27, 129.86, 129.83, 129.29, 129.26, 129.16, 129.11, 128.79, 128.68, 127.55, 127.36, 127.33, 127.19, 124.80, 124.71, 121.69, 120.71, 36.20, 34.89, 30.78, 30.58. Anal. Calcd. For $C_{37}H_{28}N_6O_3Re_1P_1F_6$ (935.84) C 47.49, H 3.02, N 8.98. Found: C 47.53, H 3.11, N 9.10.

Gel electrophoresis

12.5 % SDS-PAGE gels were used for the proteins separation in a Mini-PROTEAN® Bio-Rad electrophoresis system. The unstained Protein Molecular Marker (Thermo Scientific™ Pierce) contained a mixture of seven proteins (0.1 to 0.2 mg/mL of each) with a known molecular weight: beta-galactosidase (116 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), lactate dehydrogenase (35.0 kDa), REase Bsp98I (25.0 kDa), beta-lactoglobulin (18.4 kDa), lysozyme (14.4 kDa). Before loading onto the gel, the marker solution was boiled for 5 minutes. A stock solution 1 mg/mL of bovine serum albumin (BSA) in water was used as protein standard.

All protein samples (in a 20 µL volume) were prepared by adding 5 µL of sample buffer 5x (250 mM Tris·HCl pH 6.8, 10% SDS, 40% (v/v) glycerol, 5% (v/v) β-mercaptoethanol, 0.1% (w/v) Bromophenol Blue). Samples were boiled for 5 minutes then loaded on the gel. Electrophoresis was carried out at 120 V for 2 h in Tris/glycine running buffer (25 mM Tris pH 8.3, 192 mM glycine, 0.1 % SDS).

Total protein extracts

Escherichia coli TOP10 cells (Invitrogen, Carlsbad, CA, USA) were grown in 50 mL of liquid medium LB (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl) at 37 °C for 15 h under shaking (180 rpm). Cells were harvested by centrifugation (4500g for 20 minutes at 4 °C) then lysed by sonication in 50 mM TrisHCl pH 8, 150 mM NaCl, 1 mM EDTA (3 cycles on ice, 15 sec on/off at 15 w). Protein quantification was performed according to the Bradford assay.²⁵

Gel staining and imaging

Later after electrophoresis, polyacrylamide gels were stained. When the Coomassie Brilliant Blue (CBB) protein staining was used, gels were briefly rinsed in distilled water, then incubated with gentle shaking at room temperature for 2-3 h in 50 mL of CBB solution (30% v/v ethanol, 10% v/v acetic acid, 0.1% w/v Coomassie brilliant blue R-250). Gels were destained in a solution of 30% ethanol and 10% acetic acid until the protein bands appeared. Alternatively, when the silver nitrate staining was used, gels were washed with distilled water for 5 minutes and fixed for 30

minutes in 30% ethanol, 10% acetic acid solution. Gels were rinsed twice in 10% ethanol for 5 min, then in distilled water (2 x 5 minutes). The following Sensitizer, Stain and Developer working solutions were prepared and used according to the Pierce Silver Stain Kit (Thermo Scientific™ Pierce). When the protein bands were visible, 20 mL of Stop solution (5% acetic acid) were added. For the fluorescent staining, gels were firstly fixed in a solution of 30% ethanol, 10% acetic acid for 2 h at room temperature. After three rinsing in 20% ethanol (10 minutes each), gels were incubated with 30 mL of the stain solution (1 μM of each Re-complex in 20% ethanol) on small plastic box covered with an aluminium foil to protect the dye from light. The staining was carried out overnight with gentle shaking at room temperature. Gels were destained in water (2 x 10 minutes) before the imaging using the Gel Doc™ XR System (BioRad) equipped with UV light illuminators (excitation 302 nm).

X-ray crystallography

Crystal data and collection details for ***fac*-[Re(CO)₃(BC)(Tph)]** are reported in ESI (Table S2†). The diffraction experiments were carried out on a Bruker APEX II diffractometer equipped with a PHOTON100 detector and using Mo-Kα radiation. Data were corrected for Lorentz polarization and absorption effects (empirical absorption correction SADABS).²⁶ Structures were solved by direct methods and refined by full-matrix least-squares based on all data using F^2 .²⁷ H-atoms were placed in calculated positions and refined isotropically using a riding model. All non-hydrogen atoms were refined with anisotropic displacement parameters. CCDC 1843521 for ***fac*-[Re(CO)₃(BC)(Tph)]** contain the supplementary crystallographic data for this paper.

Electronic Supporting Information (ESI †) available: IR, ESI-MS and NMR (¹H, ¹³C, NOESY, COSY) spectra of all the Re(I) based species. UV-vis absorption, emission and excitation spectra recorded at r.t and 77K. Crystallographic data for ***fac*-[Re(CO)₃(BC)(Tph)]**.

Conflicts of Interests. There are no conflicts to declare.

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Graphics



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