Design of Luminescent Biotinylation Reagents Derived from Cyclometalated Iridium(III) and Rhodium(III) Bis(pyridylbenzaldehyde) Complexes

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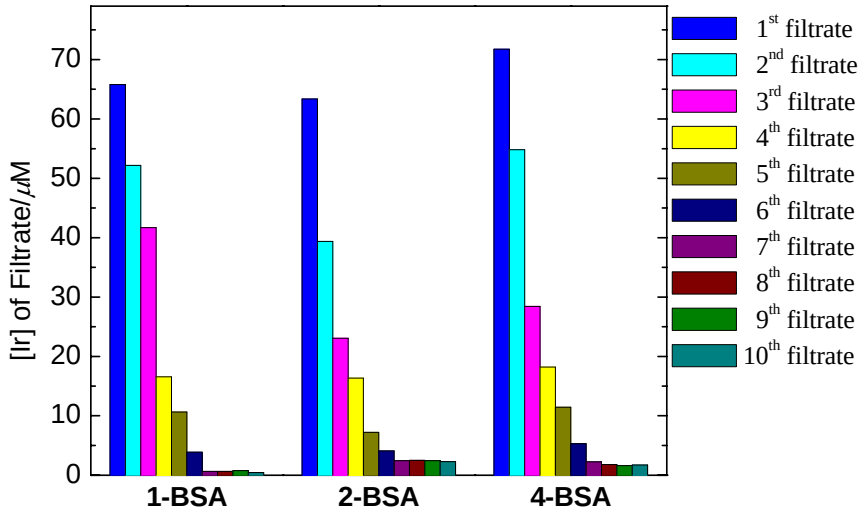
Supporting Information

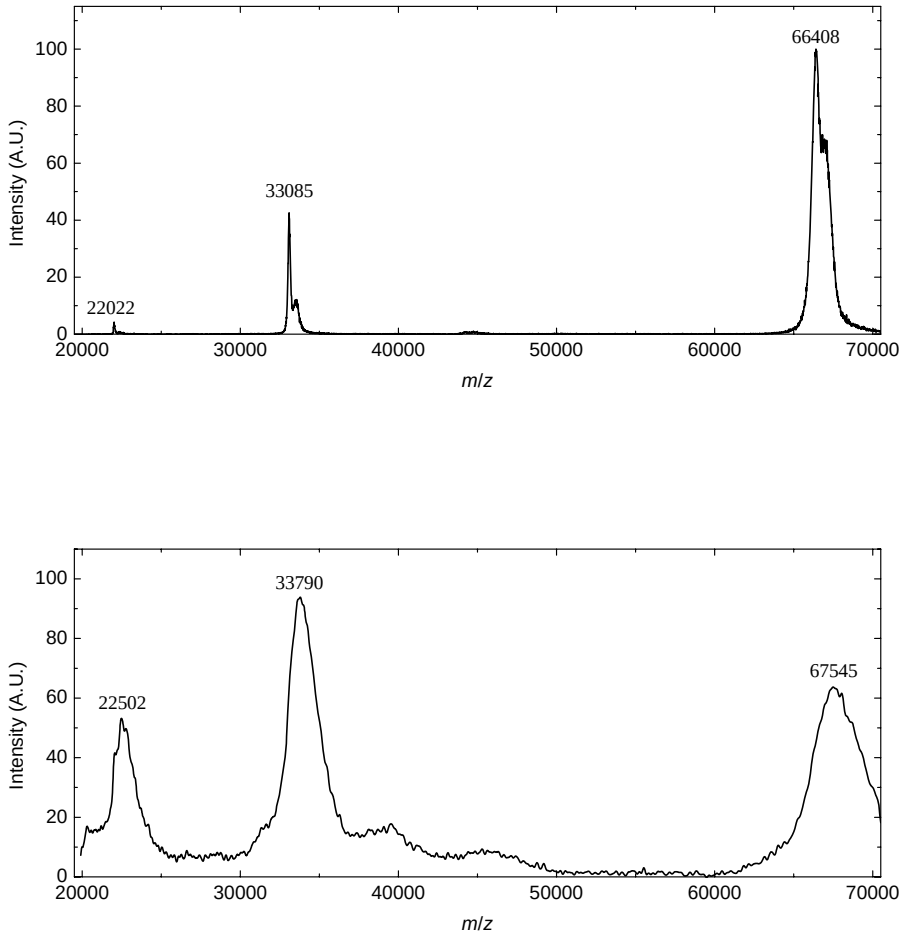
Evaluation of Purification of Bioconjugates. Upon typical labeling reactions and removal of the solid residue by centrifugation, solutions of the iridium(III) bioconjugates **1-BSA**, **2-BSA**, and **4-BSA** were concentrated with YM-30 centricons. The bioconjugate solution was washed successively with water (1 mL × 10). The washing after each step was collected and its iridium content analyzed by ICP-MS. We found that the iridium content in the washings decreased steadily, suggestive of successful removal of free iridium(III) complexes from the bioconjugate solutions (Figure S1). The iridium content in the filtrates became very low (0.5 – 2.2 μM) and steady after approximately seven rounds of washing, while highly concentrated iridium (230.4 – 488.1 μM) remained in the upper compartment of the centricon. We attributed the very small amount of iridium in the last few washings to the presence iridium(III) bioconjugates; the flow-through of a very small amount of the bioconjugate is an inevitable limitation of the spin centricon device.

Figure S1. ICP-MS analysis of iridium concentration in the filtrate of ultrafiltration during the bioconjugate purification.



MALDI-TOF MS Analysis of Unmodified BSA and Bioconjugate 2-BSA. The protein samples were prepared in water containing 30% CH_3CN and 0.1% TFA. The sample solution (0.6 μL) was pipetted onto a MALDI plate and then air-dried at room temperature. Matrix solution of α-cyano-4-hydroxycinnamic acid in water (26 μM, 0.5 μL) containing 50% CH_3CN and 0.1% TFA was deposited on the sample and then air-dried. The sample was ionized by a 200-Hz pulsed Nd:YAG laser at 355 nm and subsequently analyzed using Applied Biosystems 4800 plus MALDI-TOF/TOF Analyzer in the positive-ion mode.

Figure S2. MALDI-TOF mass spectra of BSA (top) and bioconjugate **2-BSA** (bottom).



HABA Assays for Complexes 1 – 3 in the Absence and Presence of BSA. Complexes **1** – **3** (91 μM) were incubated, respectively, in 50 mM potassium phosphate buffer pH 7.4/DMSO (90:10, v/v) in the absence or presence of BSA (30 μM) at room temperature for 12 h. The solutions were then analyzed by HABA assays. It is noteworthy that, in contrast to bioconjugates **1-BSA** and **3-BSA**, complexes **1** – **3** bound to avidin in both the absence and presence of BSA. These observations indicate that the lack of avidin-binding of these two bioconjugates is due to steric hindrance between the conjugated BSA molecule and avidin. Interestingly, the biotin molecules of bioconjugate **2-BSA** are capable of binding to avidin as a consequence of the longer TEG spacer-arm.

Figure S3. Results of spectrophotometric titrations of avidin-$(HABA)_4$ with complexes **1** (solid triangles), **2** (solid circles), and **3** (solid squares), and unmodified biotin (open squares).

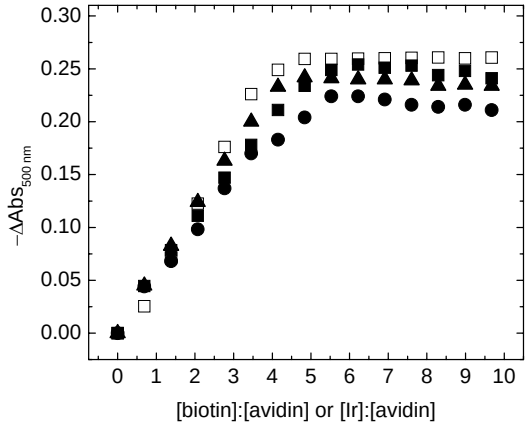
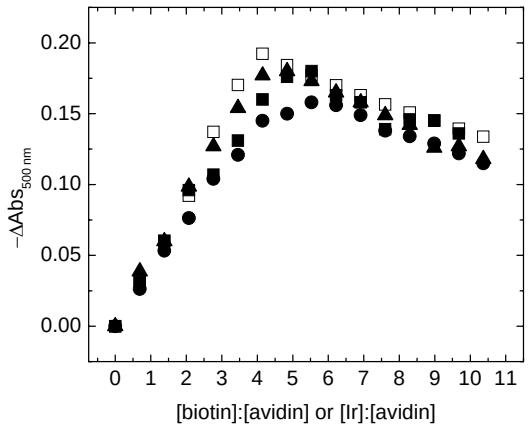


Figure S4. Results of spectrophotometric titrations of avidin-$(HABA)_4$ with complexes **1** (solid triangles), **2** (solid circles), and **3** (solid squares) and unmodified biotin (open squares) upon incubation with BSA (0.3 molar eqv) at room temperature for 12 h. The drop of the $-\Delta Abs_{500\ nm}$ after the equivalence points is due to the absorption changes of HABA in the presence of BSA.



Intracellular Co-localization of Bioconjugate 2-BSA with an Endocytic Marker. HeLa cells in growth medium were seeded on a sterilized coverslip in a 60 mm tissue culture dish and grown at 37°C under 5% CO_2 atmosphere for 48 h. The culture medium was then removed and replaced with a medium containing bioconjugate **2-BSA** and Alexa Fluor 633-labeled transferrin (50 μg mL^{-1}). After incubation for 1 h, the medium was removed and the cell layer was washed with PBS. The coverslip was mounted onto a sterilized glass slide and then imaged using a Leica TCS SPE confocal microscope.

Figure S5. Microscopy images of HeLa cells incubated with bioconjugate **2-BSA** (red) and Alexa Fluor 633-labeled transferrin (green) at 37°C for 1 h. The square illustrates the magnified region. The region that showed partial co-localization of these two bioconjugates (yellow) was indicated by an arrow.