Supporting Information

Peptide Beacons: A New Design for Polypeptide-Based Optical Biosensors.

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Materials and Methods

Synthesis of the Peptide Beacon.

Synthesis of N-methyl-N'-(2)bromoethyl-4,4'-bipyridinium. To produce N-methyl-4,4'-bipyridinium, 10 g of 4,4'-bipyridine was dissolved in 500 mL of chloroform. Twenty-five mL of iodomethane was added to the reaction, and was allowed to react at room temperature for 48 hours. The product was separated from the starting materials by performing phase extractions with H_2O, three times at 25 mL each. The starting material remained in the chloroform layer. The product was then further purified via HPLC using a gradient of 5% to 25% acetonitrile in H_2O and 0.1% trifluoroacetic acid, and the product eluted at 14 minutes. The product in solution was then lyophilized to a dry powder, and stored at –20˚ C. The product was verified on an Applied Biosystems PE Sciex QSTAR-Pulsar quadrupole orthogonal time-of-flight mass spectrometer fitted with an electrospray source (ESI-MS), m/z = 171. Five g of the product was dissolved in 100 mL acetonitrile and 10 mL of THF to which 15 mL of 1,2-dibromoethane was added. The solution was then allowed to reflux for 8 hours. The solvent mixture was then removed by rotary evaporation. The product was purified from the starting material by reverse-phase HPLC on a Waters Xterra MSC18 column, using gradient of 5% to 25% acetonitrile in H_2O and 0.1% trifluoroacetic acid, and the product eluted at 12 minutes. The reverse-phase mobile phase solvent was then removed by lyophilization, and the dry powder was stored at –20˚ C. The product was verified by ESI-MS, m/z = 278.

Synthesis of Ru(bipy)$_2$phen-5-iodoacetamide (adapted from *11*). Five mg of Ru(bipy)$_2$phen-5-amine (Fluka; diastereomers not determined), was dissolved in 100 mL of acetonitrile followed by the addition

of 100 mg of iodoacetic anhydride (Sigma-Aldrich). The solution was allowed to react for 8 hours at 4˚ C. The solvent was removed by rotary evaporation. The product was separated by reverse-phase HPLC on a 10% to 50% acetonitrile gradient over 60 minutes, the product elutes at 36 minutes. The product was then verified by ESI-MS, m/z = 777.

Conjugation of p17 peptide epitope to Ru(bipy)$_2$phen-5-iodoacetamide. Twenty mg (17 μmol) of the p17 peptide (sequence CEKIRLRC; from Biopeptide, San Diego) was treated with a 10x excess TCEP to reduce any disulfide cross linkages in 100mM TRIS-HCl buffer, pH = 7.5. The peptide was then purified via reverse-phase HPLC, lyophilized, and subsequently resuspended in a 200 mM of argon-degassed TRIS-HCl buffer, pH 7.8. Thirteen mg of Ru(bipy)$_2$phen-5-iodoacetamide was dissolved in the above buffer. The two solutions were mixed and allowed to react for 24 hours at room temperature under argon. The solution was then lyophilized, and purified via reverse-phase HPLC on a 10% to 50% acetonitrile gradient in H_2O and 0.1% trifluoroacetic acid over 60 minutes. The product elutes at 34 minutes. The product was verified using electrospray mass spectrometry, m/z = 1711. Ten mg of the p17 peptide-Ru(bipy)$_2$phen conjugate in 10 mL of 100 mM TRIS-HCl buffer, pH 7.5 was then treated with a 10x excess of TECP to reduce any disulfide cross-links and purified via reverse-phase HPLC to separate it from any unreacted TECP. The reduced p17 peptide-Ru(bipy)$_2$phen conjugate was then lyophilized to a powder, resuspended in 10 mL of 200 mM TRIS-HCl buffer, pH 7.5, degassed with argon. Twenty mg of N-methyl-N'-2-bromoethyl-4,4'-bipyridinium, dissolved in 4 mL of the same buffer, was added and the resulting solution and was allowed to react for 48 hours at room temperature under argon. The solution was then lyophilized, and purified via reverse-phase HPLC on a 10% to 50% acetonitrile gradient in H_2O and 0.1% TFA over 60 minutes. The product elutes at 28 minutes. The product was verified by ESI-MS, m/z = 1910. The resultant product was stored at –20˚ C and exhibits stability over long periods of time as a lyophilized powder. Of note, our PBs are a mixture of constructs in which the fluorophore is on either the amino- or carboxy-end of the

polypeptide, thus simplifying synthesis. Because electron-transfer is highly distance dependent, the orientation of the fluorophore and quencher does not affect the PB signaling.

Spectral Interrogation

Fluorescence Measurements were performed on a Varian Cary Eclipse Fluorimeter. Samples were excited at 450 nm. Titrations were performed with a fixed concentration of PB in a 1 mL quartz cuvette, in 100 mM TRIS buffer, pH 7.8. The anti-p17 antibody (Zeptometrix, Buffalo, New York) clone 32/1.24.89 was aliquoted for the three different titrations of buffer, saliva, and blood serum in increments of 0.25, 0.25 and 0.25 normal equivalents, respectively. After each addition, the solution was allowed to stand for two minutes at 28˚C, and then a measurement was acquired. The reported spectra represent the mean of multiple measurements. All of the reported experiments were conducted in the presence of dissolved oxygen. Experiments with oxygen-free buffers (obtained via purging with argon for 40 minutes) produce only slightly increased fluorescence intensity (data not shown)