Site-Specific Labeling of DNA-Protein Conjugates by Means of Expressed Protein Ligation

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Supporting Information
Materials and Methods

General. Cy3 monofunctional maleimide dye 5 was purchased from Molecular Probes. SERRS active dye 4 was synthesized as previously described.[1] All amino-modified oligonucleotides were obtained from Thermo Electron.

Site-specific labeling of MBP-DNA conjugate 3 with a SERRS active dye 4. The DNA-MBP conjugate 3 was synthesized using MBP-thioester 1 and cysteine-modified DNA 2 as previously described.[2, 3] After purification by anion exchange chromatography and ultrafiltration using a 10 kDa molecular cut-off filtration unit (Centricon 10, Milipore) 5 nmol of MBP-DNA conjugate 3 were diluted in TBSE buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 7.35) to a final concentration of 1.5 mg/ml. Subsequently, a 100 molar excess of 50 mM Tris-(2-carboxylethyl)phosphin (TCEP) solution in TBSE was added and the reaction mixture was incubated for 10 min at room temperature. After incubation, a 100 molar excess of maleimide dye 4, dissolved in 70 µl DMF, was mixed with the conjugate solution and the reaction mixture was incubated for 2 h in the dark at room temperature. The labeled conjugate 6 was concentrated to approx. 200 µl and the buffer was exchanged to elution buffer (20 mM Tris-HCl, pH 8.3) by ultrafiltration using a 10 kDa molecular cut-off filtration unit. The conjugate 5 was purified using anion-exchange chromatography on a MonoQ HR5/5 column (Pharmacia) by gradually increasing the NaCl concentration from 0 to 0.7 M. Peak fractions were pooled, concentrated and the buffer was exchanged to TE buffer (20 mM Tris-HCl, 5 mM EDTA, pH 7.5). Conjugate concentration was determined by absorbance measurement at 260 nm, 280 nm and 386 nm.

SERRS Measurements

SERRS spectra were recorded using Renishaw 2000 Raman spectrometer with 514.5 nm argon ion laser. Samples were analysed using a 20x objective to focus the laser beam into a microtiter plate containing the sample. Scan of 5 accumulations in 10 sec was used for all measurements.

Silver colloid was donated by Prof. Duncan Graham and was prepared by the citrate reduction of silver nitrate using a modified Lee and Meisel method.[4] Protein conjugate 6 was immobilized onto STV modified microtiter plates containing capture oligomer 8 (5′-biotin-CAA TTT CAC ACA GG-3′) through DNA directed immobilization and to each well 100 µl
silver colloid and 10 μl NaCl (1 M) to aid the aggregation were added. From wells containing controls (no capture oligomers and non complementary oligomer 9, 5’-biotin-TGA TAG GGT GCT TGC-3’) characteristic SERRS spectra were not obtained. As an additional control, silver colloid (100 μl) was mixed with conjugate 5 (10 μl, 70 nM) and 10 μl NaCl prior to immobilization and spectra obtained were similar to those of immobilized conjugate.

Site-specific labeling of a MBP-DNA conjugate with a Cy3-fluorescence dye 5.

The Cy3-labeled conjugate 7 was synthesized according to the same procedure used in the synthesis of 6. After concentration by ultrafiltration using a 10 kDa molecular cut-off filtration unit, the conjugate 7 was purified by size-exclusion chromatography (Figure S1) on a Superdex™ 200 column (Pharmacia) with TBS buffer (20 mM Tris-HCl, 150 mM NaCl, pH 7.35). Finally, conjugate concentration was determined by absorbance measurement at 260 nm, 280 nm and 550 nm.

![Figure S1: FPLC purification of the labeling mixture containing DNA-MBP conjugate 3 and Cy3 fluorescent dye 5. Shown are the absorbance at 280 nm (dashed) and 550 nm (black).](image)

Preparation of DNA microarrays.

For the attachment of the capture oligonucleotides 8 (5’-amino-CAA TTT CAC ACA GG-3’) and 9 (5’-amino-TGA TAG GGT GCT TGC-3’), a solution of the oligonucleotides in water
(10 µM) was spotted onto 3DProtein Slides (Chimera Biotec, Dortmund) using a piezo-driven spotting device (GeSiM). The slides were incubated overnight and stored at –20°C until use.

Microarray-fluorescence immunoassay.
To reduce non-specific binding of the reagents, all DNA microarrays were treated for 30 min with blocking solution (Chimera Biotec, Dortmund). After drying, adhesive hybridisation chambers (AB Gene) were fixed on top of the slides and the Cy3-labeled DNA-MBP conjugate 7 in TETBS buffer (20 mM Tris-Cl, pH 7.5, 150 mM NaCl, 5 mM EDTA, 0.01% Tween-20) in concentrations ranging from 100 nM to 1 nM was allowed to hybridize for 60 min at room temperature. The slides were washed twice with TETBS and serial dilutions of the antigen, mouse anti-MBP 10, were incubated for 60 min at room temperature. After antigen incubation, the slides were washed twice with TETBS, and 25 µl of the detection conjugate STV-Cy5-GAM 11 (100 nM in TETBS) were transferred into the hybridization chambers. Binding was carried out at room temperature for 45 min. Following, the slides were washed with TETBS and deionized H2O and dried by centrifugation in a microscope slide holder (Erie Scientific). The fluorescence intensity of the signals was measured with a microarray laser scanning system (Axon) by a photomultiplier of 500 and 100% laser power. Signals were analyzed and quantified using GenePix pro 4.1 software (Axon).