Protein-induced fluorescence enhancement detects protein-nucleic acid interactions in microplates

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- Detect structure and sequence specificities of protein-DNA interactions
- Well-scanning function of BMG LABTECH’s multi-mode reader used to increase measurement surface and stability

Introduction

Various biological processes depend on the interaction of proteins with nucleic acids. These comprise packaging of DNA, transcriptional regulation, DNA replication and DNA repair. The binding of proteins to nucleic acids are hitherto detected by electrophoretic mobility shift assays (EMSAs), fluorescence polarization (FP) assays or chromatin immunoprecipitations (ChIP). However, these methods are either laborious (EMSA, ChIP) or limited to specific targets (EMSA, FP, ChIP). A novel fluorescence assay detects interactions of nucleic acids with proteins to identify binding, sequence and structure specificities or dissociation constants. The microplate assay is a quick and cost-effective alternative and can be read on a fluorescence reader with the capability to scan wells such as the FLUOstar® Omega.

Assay Principle

Materials & Methods

- Black 96 well microplate (Greiner, FluoTracTM 600, high binding, #655077) coated with 100 µl NeutrAvidin (Pierce, binding capacity ~15pmol D-biotin/well) and blocked with SuperBlock blocking buffer (ThermoScientific, #15117)
- Proteins*: BamHI (ThermoScientific), XPF/ERCC1 (expressed in Escherichia coli strain BL21 (DE3)), KU70/KU80 (expressed in HI5 insect cells)
- Cy3-labeled oligonucleotides (IDT)*
- FLUOstar Omega microplate reader (BMG LABTECH)

The Cy3-labeled oligonucleotides (100 µl, 25nM) were incubated on the plate for 2 h to bind and subsequently washed three times to remove residual nucleic acids. A first scan determined baseline fluorescence, a second scan detected binding of the protein to the oligonucleotide after incubation with 100 µl of the protein solution.

Results & Discussion

Using protein-induced fluorescence enhancement in microplates, the binding of the endonuclease BamHI to its recognition site was verified by a PIFE-value of 30 % (Fig. 2B). Addition of non-labelled oligonucleotides containing the recognition site, but sequence changes outside the recognition motif decreased the PIFE value.

Keywords: Binding studies, Cy3, Nucleic acids, Protein-DNA interaction, Well Scanning
Having shown that mwPIFE detects sequence preferences of protein-DNA binding, preferential binding of proteins to specific DNA structures was investigated. The XPF/ERCC1 heterodimer incises damaged DNA for repair and specifically binds structures of damaged DNA. A truncated XPF/ERCC1 heterodimer retaining the DNA-binding site was used to determine structure specific interaction. The dimer minimally bound ssDNA or short, blunt ended DNA if competing with the probe displayed in Figure 3B. Reduction of the PIFE-value due to binding of the competing DNA-structure was observed for 10 nt 3’ overhang, a hairpin and a splayed DNA-structures (Fig. 3).

The KU70/KU80 heterodimer is responsible for binding broken dsDNA ends, thereby protecting them from degradation and keeping both ends in proximity to allow for ligation. Increasing the concentration of KU-heterodimer resulted in higher PIFE-values and allowed for the determination of the equilibrium constant KD (Fig. 4).

**Conclusion**

The mwPIFE detects interaction of nucleic acids with proteins and reports on sequence and structure specificities as well as on binding constants. The change in fluorescence intensity needs to be captured on a huge surface of the well to provide stable measurements. This has been enabled by the well-scan function of the FLUOstar Omega combined with its high sensitivity. The simple and cost-effective assay broadens the application to detect structure-specific bindings. Its plate format accelerates the determination of protein-nucleic acid interactions.*

**References**


*For further information and when publishing data using the mwPIFE please refer to Valuchova et al.*

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**Fig. 2:** Sequence specificity of BamHI binding. [A] Sequences of Cy3-labelled DNA probe and competitors (B) dsDNA competitors were added in 1–10x excess over labeled and immobilized DNA probe. Error bars indicate standard deviations from three wells. Data published in Valuchova et al.

**Fig. 3:** Structure specific binding of XPF/ERCC1. Competitors were added to DNA-probe-protein complex in 1x, 10x, and 100x excess and PIFE values were calculated. Data published in Valuchova et al.

**Fig. 4:** Analysis of Ku-DNA interaction by mwPIFE. The indicated probe was titrated with Ku dimer. The binding curve was reconstituted from 3 independent replicas with standard deviations indicated. The calculated KD value was 10 ± 5 nM. Data published in Valuchova et al.

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**Fig. 3:** Structure specific binding of XPF/ERCC1. Structure of competitor oligonucleotides. Number indicate lengths of oligonucleotides.