Analyzing Protein:Nucleic Acid Interactions Using HaloLink™ Protein Arrays

ABSTRACT

Analysis of protein:nucleic acid interactions reveals information that can be important for understanding mRNA regulation, chromosomal remodeling and transcription. Techniques such as electrophoretic mobility shift assay and fluorescent anisotropy have been valuable in detecting protein:nucleic acid interactions; however, these techniques have disadvantages that make it difficult to study some interactions. In this paper we use the HaloLink™ Protein Array System to analyze protein:nucleic acid interactions using the DNA binding proteins p65 and p50. Our binding experiments show direct, specific interaction between DNA and p50 or p65. We also show that the NF-κB inhibitor, IκBα, decreases ability of the p50 protein to bind DNA. The HaloLink™ Protein Array System allows rapid generation of fusion protein using cell-free lysates coupled with covalent attachment of the proteins to the slide with HaloTag® protein. These features make the system an adaptable, medium-throughput assay.

Brad A. Hook, Nidhi Nath and Michael R. Slater

Promega Corporation

Publication Date: 2011

Introduction

Nucleic acid binding proteins are required for many processes in living organisms. Transcription factors play an important role in regulating transcription of DNA by binding to specific recognition sites on the chromosome. This regulation is required for cell viability, differentiation and growth(1) (2) . The ability to detect and confirm the interaction of such proteins with various nucleic acid targets provides valuable information about the cell signaling cascades that govern the ability of a cell to divide, migrate, interact with its neighbors, develop and maintain specialized functions, and terminate viability at the appropriate time.

Two common techniques used to detect the interaction of nucleic acid binding proteins with nucleic acids are the electrophoretic mobility shift assay (EMSA) and fluorescence anisotropy assay(3) (4) . EMSA involves binding protein to a radiolabeled DNA probe followed by resolution on a polyacrylamide gel. Due to the increase in mass, protein:probe complexes migrate slower than free probe, allowing comparison of free versus bound probe. The specificity of such complexes is determined using competition experiments with unlabeled specific and nonspecific oligos. This method works best with purified protein and can be quite labor intensive, particularly when numerous samples are being processed. In fluorescence anisotropy, a DNA binding protein is bound to a DNA substrate containing a fluorophore. Polarized light is shined onto the sample and then the emitted light is detected. Because a DNA:protein complex tumbles in solution more slowly than the free DNA, there is less deflected light. This method works best with purified protein and requires special equipment.

Protein chips have emerged as an approach for identification of DNA:protein interactions(5) (6) (7) . Functional protein microarrays are composed of arrays containing full-length functional proteins or protein domains. Fluorescently labeled DNA is used to probe the array and identify proteins that bind to that specific probe. Protein microarrays provide a method for high-throughput identification of DNA:protein interactions. However, microarrays are not easily adaptable. The HaloLink™ Protein Array System offers solutions to these challenges, as described in following paragraph, and certain issues have been specifically addressed. These include preventing false positives.

© Promega Corporation, Updated 2011.

The HaloLink™ Array System is an alternative approach for analysis of DNA:protein interactions. This system captures nucleic acid binding proteins on a slide surface (Figure 1) and previously has been shown to be a successful method for studying protein:protein interactions and enzymatic reactions(8) (9) . The system requires the generation of a fusion protein between the DNA-binding protein and the HaloTag® protein(8) (9) . The HaloTag® protein is a 34kDa modified hydrolase enzyme that can form a rapid, specific and covalent bond with its chloroalkane ligand(10) . Synthesizing the HaloTag® fusion-DNA-binding protein in cell-free systems adds to the speed and flexibility of the assay. The HaloLink™ Protein Array slides are hydrogel-coated glass slides containing immobilized HaloTag® ligand. Fifty individual wells are created by adding a silicon gasket to the slide. The expressed fusion protein can be bound to the surface covalently, allowing stringent washing and subsequent analysis of DNA binding by adding a DNA probe. This technique is rapid, requires no purified protein, provides a moderate data set (n = 50) and is adaptable to different throughputs.

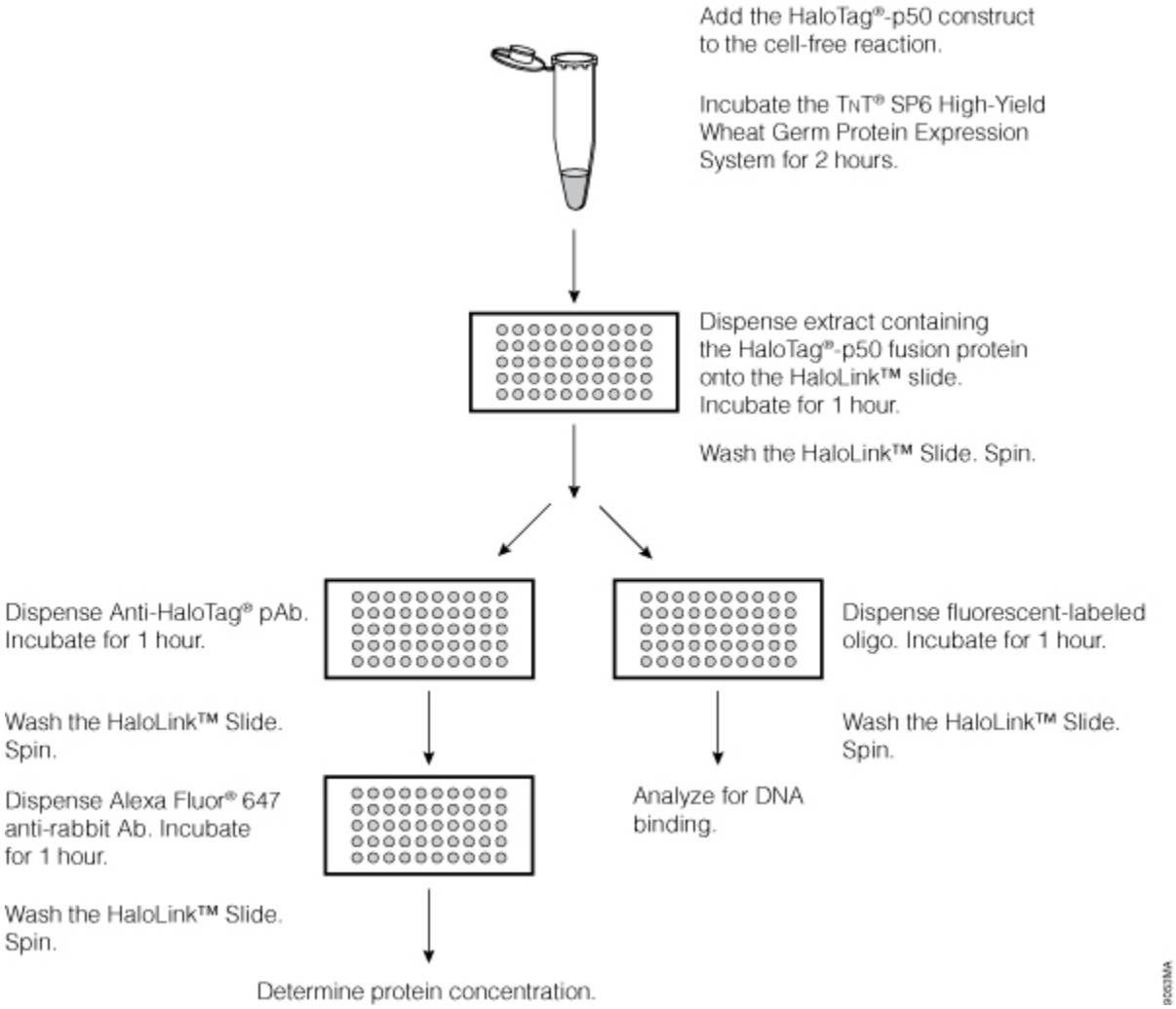

Figure 1. Schematic of HaloLink™ Protein Array System protocol. A HaloTag® fusion was expressed in a cell-free expression system and covalently attached to the surface. After washing and drying with centrifugation, the slide was processed to determine protein concentration and used to analyze the DNA: protein interaction. To detect the HaloTag® fusion protein attached to the slide, certain wells of the slide were probed with Anti-HT pAb to detect the HaloTag® fusion bound to the surface. After another wash and spin, the slide was probed with Alexa Fluor® 647 anti-rabbit antibody and analyzed to detect the HaloTag® fusion protein. Other wells had a fluorophore-labeled DNA probe added to them. After incubation, the slide was washed, dried and scanned.

We used the Rel/NF-κB transcription factor, p50(11) , as a model DNA binding protein to show that the HaloLink™ Protein Array slide provides a tool for analyzing DNA-binding protein:DNA interactions. Dimers of NF-κB proteins regulate transcription by directly binding enhancer sequences, referred to as κB DNA sequences, which are located in the regulatory regions of numerous genes.

HaloLink™ Protein Array Experimental Design

Figure 1 outlines the experimental design of the HaloLink™ Protein Array System. A HaloTag® fusion protein is expressed with a cell-free expression system and then covalently attached to the slide surface through the HaloTag® protein's interaction with the HaloTag® ligand (see supplementary information for details). After washing and drying, the slide is processed to determine protein concentration, and is then used to analyze the DNA: protein interaction. To detect the HaloTag® fusion protein, certain wells of the slide are probed with anti-HaloTag® antibody (Cat.# G9281). After a second wash followed with a drying step, the slide is probed with Alexa Fluor® 647 anti-rabbit antibody and analyzed on a Typhoon® 9410 slide scanner to detect the HaloTag® fusion protein. In addition, other wells have a fluorophore-labeled DNA probe added to them. After incubation, the slide is washed, dried, and scanned to detect the DNA:protein interaction.

HaloTag®-p50 binds to the HaloLink™ Protein Array slide and has DNA binding activity.

Two human p50 proteins were expressed as N-terminal HaloTag® fusion proteins in a high-yield wheat germ cell-free protein expression system. One p50 protein contains the DNA binding domain, dimerization domain, and the nuclear localization sequence of the human p50 protein, while the second p50 protein (–DNA BD) has the DNA binding domain removed. Expression of the correctly sized fusion proteins was verified using the HaloTag® TMR Ligand (Cat.# G8251)(12) followed by SDS-PAGE (Figure 2, Panel A).

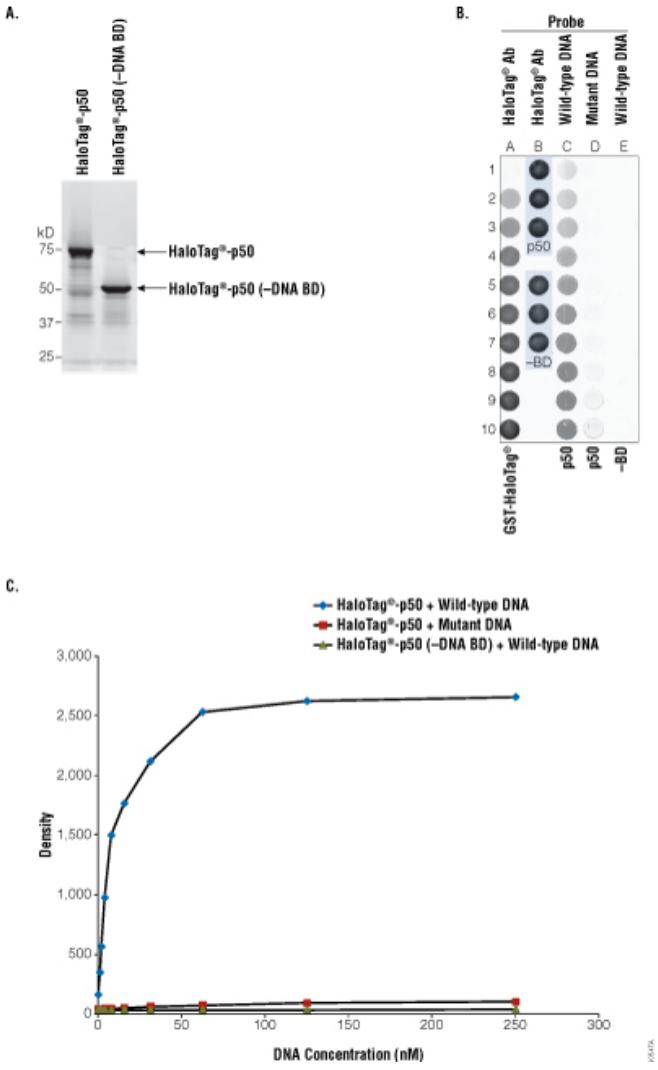

Figure 2. HaloTag®-p50 fusion protein attaches to the HaloLink™ Protein Array slide and shows DNA binding activity. Panel A. TMR-labeled SDS-PAGE of HaloTag® fusions. One microliter of both HaloTag®-p50 and HaloTag®-p50 (–DNA BD) fusion proteins expressed in a cell-free system was labeled with the HaloTag® TMR Ligand and separated using SDS-PAGE. A marker was used to assay for size. **Panel B.** HaloLink™ Protein Array slide. The numbers correspond to wells, and the letters correspond to columns. Column A: wells 1–10, 5µl of HaloTag®-GST Standard Protein at 0, 1.3, 2.6, 5.2, 10.5, 21, 42, 83, 166, and 332nM, respectively, was added to each well. Each well was then probed with Anti-HaloTag® pAb followed by Alexa Fluor® 647 anti-rabbit IgG antibody. Column B: wells 1–3, 5µl of lysate expressing HaloTag®-p50 fusion protein was added to each well; wells 5-7, 5µl of lysate expressing HaloTag®-p50 (–DNA BD) fusion protein was added to each well. These wells were then probed with Anti- HaloTag® pAb followed with the Alexa Fluor® 647 anti-rabbit IgG antibody. Column C: wells 1–10, 5µl of lysate expressing the HaloTag®-p50 fusion protein was added to each well. WT-DNA was added to the wells at 0, 1, 1.9, 3.9, 7.8, 15.6,

31.25, 62.5, 125, 250nM, respectively. Column D: wells 1–10, 5µl of lysate expressing the HaloTag®-p50 fusion protein was added to each well. Mut-DNA was added at the same concentrations as those in Column C. Column E: wells 1–10, 5µl of lysate expressing the HaloTag®-p50 fusion protein (–DNA BD) added to each well. WT-DNA was added at the same concentrations as those in Column C. **Panel C.** Graph showing binding of HaloTag®-p50 fusion proteins to DNA. Densities for each well were determined using GenePix® software. The concentration of the DNA was plotted versus the density of each well.

To test DNA binding activity of p50, we used the HaloLink™ Protein Array System. Extract expressing HaloTag®-p50 and HaloTag®-p50 (–DNA BD) were added to the slide (Figure 2, Panel B). Three wells for each protein were probed with anti-HaloTag® antibody followed by Alexa Fluor® 647 anti-rabbit IgG antibody to verify that each protein was bound to the slide (Figure 2, Column B; wells 1–3 for HaloTag®-p50 and wells 5–7 for HaloTag®-p50 [–DNA BD]). A dilution series of the HaloTag®-GST Standard Protein at known concentrations was added to the slide to make a titration curve for bound HaloTag® protein (Figure 2, Panel B; Column A). A titration series of wild-type-DNA (WT-DNA) and mutated-DNA (Mut-DNA) both containing Alexa Fluor® 647 was added to the slide. Following scanning, the fluorescence intensity of each well was determined and plotted (Figure 2, Panel C). The HaloTag®-p50 protein bound the WT-DNA to near saturation levels, and did not significantly bind the Mut-DNA. The HaloTag®-p50 (–DNA BD) protein had no detectable DNA binding activity. Probing with a ^{32}P-labeled nucleotide gave similar results (data not shown). This experiment shows that the HaloLink™ Protein Array slide is able to bind HaloTag®-fusion proteins and is capable of analyzing DNA:protein interactions.

To test further the specificity of the HaloTag®-p50/WT-DNA interaction, we performed a competition assay using unlabeled WT-DNA. Replicates of the HaloTag®-p50 protein expressed in a wheat germ cell-free protein expression system and the HaloTag®-GST Standard Protein (20µg/ml) were bound to a HaloLink™ Protein Array slide (Figure 3, Panel A). The slide was then probed with Alexa Fluor® 647-labeled WT-DNA and mixed with increasing amounts of unlabeled WT-DNA. The binding signal decreased as the amount of unlabeled competitor increased. A plot of the fluorescent intensity of each HaloTag®-p50 well versus the percent of competitor DNA shows a linear decrease in signal (Figure 3, Panel B). The HaloTag®-GST Standard Protein did not bind the WT-DNA. This competition assay shows that the HaloTag®-p50 interaction is specific for the WT-DNA and that competition assays can be performed on the HaloLink™ Protein Array slides.

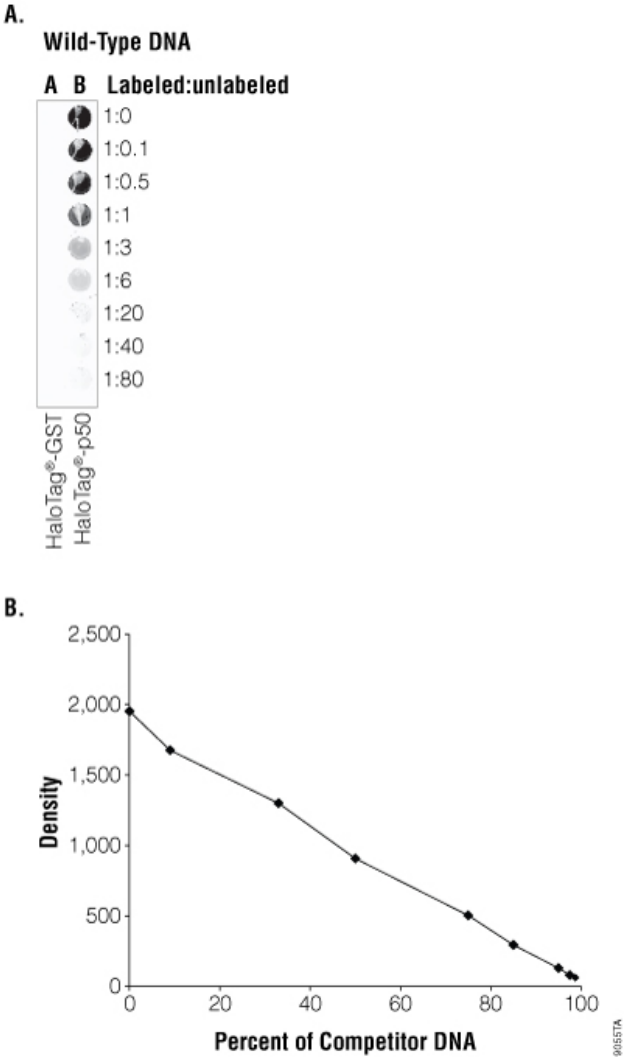

Figure 3. Specificity of DNA binding using a competition assay with the HaloLink™ Protein Array slide. Panel A. Column A: Five microliters of HaloTag® Standard Protein (332nM) was added to each of the nine wells. Column B: Five microliters of lysate expressing HaloTag®-p50 was added to each of the nine wells. WT-DNA was added to Columns A and B. The DNA contained a ratio of labeled (Alexa Fluor® 647) to unlabeled WT-DNA. The ratios are indicated next to each row. The slide was scanned with a GenePix® slide scanner at a wavelength of 635nm. **Panel B.** The densities for each well were determined using GenePix® software. The percent of competitor DNA versus the density of each well was plotted.

IκBα Interferes with the DNA Binding Activity of HaloTag®-p50

IκBα is an ankyrin repeat protein that inhibits NF-κB transcriptional activity by sequestering NF-κB outside of the nucleus in resting cells. IκBα forms a very stable complex with NF-κB by binding tightly to the nuclear localization sequence (NLS) and weakly to the DNA-binding domain(13) (14) (15) (16) . In the absence of IκBα, we have shown that the p50 protein can bind the WT-DNA using the HaloLink™ Protein Array slide. We hypothesized that there will be a decrease in the binding of the p50 protein to the WT-DNA when IκBα is bound to the p50 protein.

To detect IκBα, a T7 epitope tag was added to the N-terminus. We expressed both the p50 protein and p50 co-expressed with IκBα in a wheat germ cell-free protein expression system. A Western blot using the T7 antibody verified the expression of IκBα (Figure 4, Panel A). A dilution series of the HaloTag®-GST Standard Protein, the HaloTag®-p50 lysate, and the HaloTag®-p50 + IκBα lysate were added to three HaloLink™ Protein Array slides (Figure 4, Panel C). One slide was probed for HaloTag® fusion protein binding using the Anti-HaloTag® pAb. Another slide was probed for IκBα using the anti-T7 epitope antibody. The third slide was probed with WT-DNA at a concentration of 100nM. After scanning each slide, fluorescent intensities for each well were determined. The concentrations of HaloTag®-p50 in columns B and C were calculated from a titration curve determined using the HaloTag®-GST Standard Protein. These concentrations were plotted versus fluorescent intensities of each spot on slide 3. DNA binding was normalized to compensate for a small decrease in HaloTag®-p50 in column 3 versus column 2 on slide 1. Probing with anti-T7 antibody shows the presence of IκBα in column 3. DNA binding of p50 decreased about threefold when IκBα was present, which is consistent with the reported values(13) .

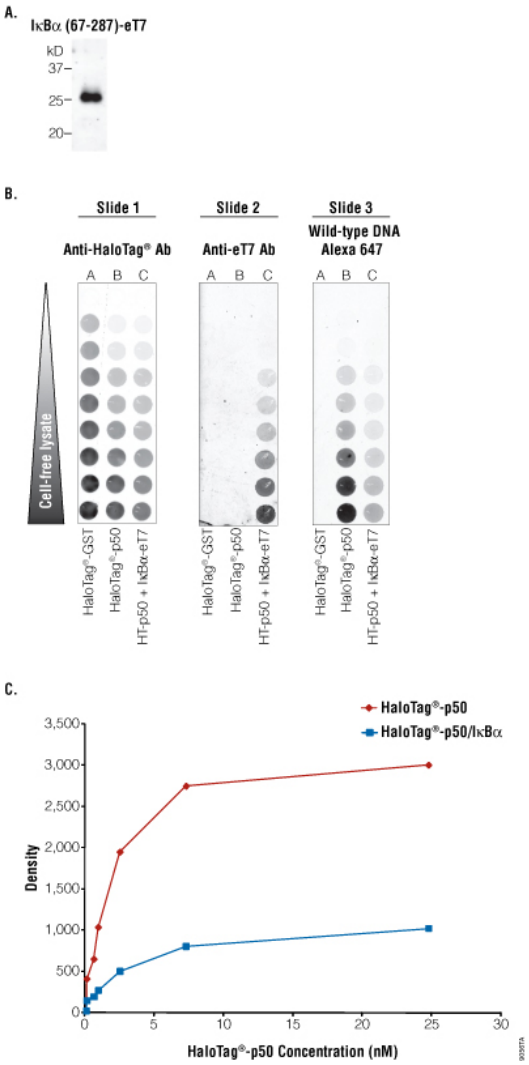

Figure 4. IkappaBalpha inhibits the binding of p50 to DNA. Panel A. Western Blot analysis of lysate expressing both HaloTag®-p50 fusion protein and IκBα. IκBα contains a T7 epitope tag, which was used for analysis. **Panel B.** HaloLink™ Protein Array slides. Three slides were imprinted identically with HaloTag®-GST Standard Protein and lysate expressing either HaloTag®-p50 or HaloTag®-p50 + IκBα proteins. Column A: wells 1–9, 5µl of HaloTag®-GST Standard Protein at 0, 2.6, 5.2, 10.5, 21, 42, 83, 166 or 332nM, respectively, was added to the wells. Column B: wells 1–9, 0, 0.039, 0.078, 0.156, 0.3125, 0.625, 1.25, 2.5 or 5µl of lysate expressing HaloTag®-p50, respectively, was added to the wells. Column C: rows 1–9 had 0, 0.039, 0.078, 0.156,

0.3125, 0.625, 1.25, 2.5, or 5µl lysate expressing HaloTag®-p50 + IκBα, respectively, was added to the wells. Slide 1 was then probed Anti-HaloTag® pAb followed by Alexa Fluor® 647 anti-rabbit IgG antibody. Slide 2 was probed with anti-T7 antibody followed by Alexa Fluor® 647 anti-mouse IgG antibody. Slide 3 was probed with WT-DNA (100nM). **Panel C.** DNA binding of HaloTag®-p50 fusion protein with and without IκBα was plotted. Densities for each well were determined using GenePix® software. The concentration of the HaloTag®-p50 fusion protein in each well was determined using a calibration curve of HaloTag®-GST Standard Protein. The concentrations were plotted versus the density of each well. The DNA binding signal of HaloTag®-p50 + IκBα proteins was normalized to that of HaloTag®-p50 fusion protein alone to compensate for the small decrease in HaloTag®®-p50 in Column C.

HaloTag®-p50 binds WT-DNA with a greater affinity than HaloTag®-p65

To compare the binding of a similar DNA binding protein, p65, to p50, we expressed p65 (amino acids 19–325) and p50 as HaloTag® fusion proteins in a cell-free protein expression system. Expression of the correctly sized fusion proteins was verified using the HaloTag® TMR Ligand followed by SDS-PAGE (Figure 5, Panel A). To test the ability of these proteins to bind DNA while bound to the HaloLink™ Protein Array slide, we first added lysates expressing HaloTag®-p50 or HaloTag®-65 to the slide (Figure 5, Panel B). Three wells for each protein were probed with Anti-HaloTag® pAb followed by Alexa Fluor® 647 anti-rabbit IgG antibody to verify that each protein was properly bound to the slide (Figure 5, Column B, wells 1–3 for HaloTag®-50 and wells 5 and 6 for HaloTag®-p65). The concentrations of the HaloTag®-p50 and HaloTag®-p65 proteins were calculated to be nearly identical on the slide. Varying concentrations of WT-DNA were added to the slide as shown in Figure 5. After scanning the slide, the densities of each well were determined and fit to a graph. Both proteins bound the WT-DNA, with HaloTag®-p50 binding about twofold better than HaloTag®-p65. These results agree with those reported previously (using fluorescence anisotropy and EMSA)(13) .

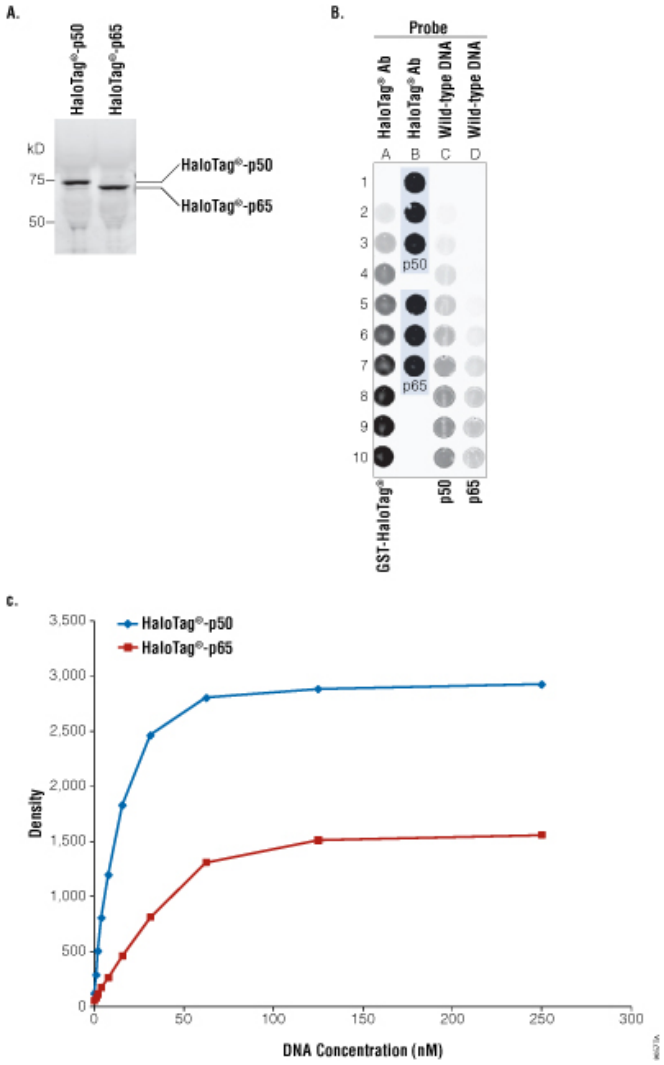

Figure 5. Binding affinity of p50 and p65 proteins for wildtype DNA. Panel A. TMR-labeled SDS-PAGE of HaloTag® fusion proteins. One microliter of both HaloTag®-p50 and HaloTag®-p65 fusion proteins expressed in a cell-free expression system was labeled with the HaloTag® TMR Ligand and separated using SDS-PAGE. A marker was used to assay for size. **Panel B.** HaloLink™ Protein Array slide. Column A: wells 1–10, 5µl of HaloTag®-GST Standard Protein at 0, 1.3, 2.6, 5.2, 10.5, 21, 42, 83, 166 or 332nM, respectively, was added to each well. Each well was then probed with Anti-HaloTag® pAb followed by Alexa Fluor® 647 anti-rabbit IgG antibody. Column B: wells 1–3, 5µl of lysate expressing HaloTag®-p50 was added to each. Wells 5–7, 5µl of lysate expressing HaloTag®-65 was added to each well. Wells 1–3 and 5–7 were then probed with Anti-HaloTag® pAb followed by Alexa Fluor® 647 anti-rabbit IgG antibody. Column C: wells 1–10, 5µl of lysate expressing HaloTag®-p50 was added to each well. WT-DNA was added at to the rows as 0, 1, 1.9, 3.9, 7.8, 15.6, 31.25, 62.5, 125 or 250nM, respectively. Column D: wells 1–10, 5µl of lysate expressing HaloTag®-p65 added to each. WT-DNA was added at the same concentrations as Column C. The slide was scanned with a GenePix® slide scanner at a wavelength of 635nm. **Panel C.** The densities for each well were determined using GenePix® software. The concentration of the DNA was plotted versus the density of each well.

Discussion

The three main components of the HaloLink™ Protein Array system are cell-free expression, the HaloTag® protein and the HaloLink™ Slides. All three contribute to the efficiency, robustness and overall speed of the assay. This system can also be used for functional characterization of purified recombinant protein. In a previous study, the Halolink Protein array system was used to study protein: protein interactions found in the NF-kB complex(17) . We have shown that the HaloLink™ Protein Array

System can be used to analyze Protein: DNA interactions using p50 and p65 transcription factors as model DNA-binding proteins.

First, cell-free translation systems, such as the TNT® SP6 High-Yield Wheat Germ Protein Expression System or the TNT® Rabbit Reticulocyte System, allow for rapid protein production directly from DNA. In addition, protein can be produced in less than two hours using cell-free systems, versus days for production from *E. coli*-based systems. Cell-free systems also eliminate the expression difficulties some transcription factors have in *E. coli*-based systems. This adds the flexibility of quickly expressing protein truncations, mutations, or fusions and testing them for activity.

Second, the HaloTag® protein enables covalent and oriented capture of proteins on solid surfaces directly from the cell-free expression system without any prior purification step. The HaloTag® protein forms a covalent bond with its HaloTag® ligand. A HaloTag® ligand with reactive end group is used to activate hydrogel-coated glass slides and, subsequently, to capture proteins of interest expressed as HaloTag®-fusion proteins in cell-free protein expression systems. This approach eliminates the need for protein purification, and allows the fused protein of interest to be oriented for maximum biological activity.

Third, polyethylene glycol (PEG)-coated glass slides are known to resist nonspecific adsorption of unwanted protein and to prevent surface-induced denaturation of specific proteins. The low-background binding of the HaloLink™ Slides allows the detection of low abundant binding proteins. A silicone gasket creates 50 wells on the glass slide, so that multiple assays can be performed manually on the same slide without any specialized equipment. This offers an improvement over EMSA, which generally allows approximately 15 reactions per gel.

Using the HaloLink™ Protein Array System, we found that p50 bound WT-DNA specifically, and p50 that is lacking its DNA binding domain had no DNA binding activity. This highlights one advantage of using cell-free lysates, truncation and expression is quick, and many different mutations can be tested. The DNA probes used in the experiments were generated synthetically, adding to the speed of the assay. Multiple DNA probes can be tested quickly for binding to one protein, or multiple proteins can be tested for binding to one probe. The DNA can be detected using a fluorophore (as shown in these experiments) or a radiolabeled DNA probe. Both detection methods have similar sensitivity. Using a mutated DNA in a competition assay with wild-type, unlabeled DNA, we showed that the interaction is specific and the HaloLink Protein Array slides have little background binding to the DNA probes.

Furthermore, we compared the binding of two different DNA binding proteins, p50 and p65 and showed that the HaloLink™ Protein Array slide has the sensitivity to show binding differences between different nucleic acid binding proteins and target binding sites, which agrees with previous studies(11) .

The HaloLink™ Protein Array slides also provide a platform to study protein:protein:nucleic acid binding. Using the p50 protein and its inhibitor IκBα, we were able to show a decrease in p50 binding to WT-DNA when the inhibitor was present and showed that the HaloLink™ Protein Array slide has the sensitivity to show binding differences between different nucleic acid binding proteins and target binding sites. This decrease in binding was previously observed(13) (14) . The HaloLink™ Protein Array slide system can be used to analyze the effect that one protein has on the DNA binding activity of another protein.

HaloLink™ Protein Array slides provide a medium-throughput assay for analyzing protein:protein, protein:nucleic acid, and protein:small molecule interactions. Unlike conventional protein arrays, HaloLink™ Protein Array slides allow flexibility of content printing. The user can easily create protein mutations and truncations to assay important binding regions on a protein. Using these slides, a user can screen quickly a number of different potential targets, determine important binding regions and determine the even amino acids required for the interaction.

REFERENCES

1. Mankan, M.A. *et al.* (2009) NF-kappaB regulation: the nuclear response. *J. Cell. Mol. Med.* **13**, 631–43.
2. Gosh, S. *et al.* (1998) NFκB and Rel proteins: Evolutionarily conserved mediators of immune responses. *Annu. Rev. Immunol.* **16**, 225–60.
3. Lane, D., Prentki, P. and Chandler, M. (1992) Use of gel retardation to analyze protein-nucleic acid interactions. *Microbiol. Rev.* **56**, 509–28.
4. LiCata, V.J. and Wowor, A.J. (2008) Applications of fluorescence anisotropy to the study of protein-DNA interactions. *Methods Cell Biol.* **84**, 243–62.
5. Hall, D.A., Ptacek, J. and Snyder, M. (2007) Protein microarray technology. *Mech. Ageing Dev.* **128**, 161–7.
6. Hall, D.A. *et al.* (2004) Regulation of gene expression by a metabolic enzyme. *Science* **306**, 482–4.
7. Hudson, M.E. and Snyder, M. (2006) High-throughput methods of regulatory element discovery. *Biotechniques* **41**, 673, 675, 677 passim..
8. Hurst, R. *et al.* (2009) Protein-protein interaction studies on protein arrays: Effect of detection strategies on signal-to-background ratios. *Anal. Biochem.* **392**, 45–53.
9. Nath, N. *et al.* (2008) Improving protein array performance: Focus on washing and storage conditions. *J. Proteome Sci.* **7**, 4475–82.
10. Urh, M. *et al.* (2008) Methods for detection of protein-protein and protein-DNA interactions using HaloTag. *Methods Mol. Biol.* **421**, 191–209.
11. Phelps, C.B. *et al.* (2000) Mechanism of kappa B DNA binding by Rel/NF-kappa B dimers. *J. Biol. Chem.* **275**, 24392–9.
12. *HaloTag® Technology: Focus on Imaging* Technical Manual TM260
13. Malek, S. Huxford, T. and Ghosh, G. (1998) Ikappa Balpha functions through direct contacts with the nuclear localization signals and the DNA binding sequences of NF-kappaB. *J. Biol. Chem.* **273**, 25427–35.
14. Bergqvist, S., *et al.* (2006) Thermodynamics reveal that Helix Four in the NLS of NT-κB p65 anchors IκBα, forming a very stable complex. *J. Mol. Biol.* **360**, 421.
15. Baeuerle, P.A. and Balitmore, D. (1988) I kappa B: a specific inhibitor of the NF-kappa B transcription factor. *Science* **242**, 540–6.
16. Truhlar, S.M. *et al.* (2008) Pre-folding IkappaBalpha alters control of NF-kappaB signaling. *J. Mol. Biol.* **380**, 67–82.
17. Hurst, R., Ohana, R. and Nath, N. (2011) Interactions Analysis Using Cell-Free Protein Expression Systems and HaloLink™ Protein Arrays Promega Corporation Web Site. Accessed June 30 2011..

HOW TO CITE THIS ARTICLE

Scientific Style and Format, 7th edition, 2006

Hook, B.A., Nath, N. and Slater, M.R. Analyzing Protein:Nucleic Acid Interactions Using HaloLink™ Protein Arrays. [Internet] 2011. [cited: year, month, date]. Available from: http://www.promega.com/resources/pubhub/analyzing-protein-nucleic-acid-interactions-using-halolink-protein-arrays/

American Medical Association, Manual of Style, 10th edition, 2007

Hook, B.A., Nath, N. and Slater, M.R. Analyzing Protein:Nucleic Acid Interactions Using HaloLink™ Protein Arrays. Promega Corporation Web site. http://www.promega.com/resources/pubhub/analyzing-protein-nucleic-acid-interactions-using-halolink-protein-arrays/ Updated 2011. Accessed Month Day, Year.