CANCER GENOMICS & PROTEOMICS 7: 303-310 (2010)

Surface Plasmon Resonance (SPR) Spectrometry as a Tool to Analyze Nucleic Acid–Protein Interactions in Crude Cellular Extracts

FARID E. AHMED[1,+], JOHN E. WILEY[2], DOUGLAS A. WEIDNER[3], CHRIS BONNERUP[4] and HELVECIO MOTA[1,++]

Departments of [1]Radiation Oncology, Leo W. Jenkins Cancer Center, [3]Pediatrics-Genetics, [4]Microbiology and Immunology, The Brody School of Medicine, and [5]Department of Physics, East Carolina University (ECU), Greenville, NC 27858, U.S.A.

Abstract. *This study presents proof-of-principle application showing that label-free affinity enrichment surface plasmon resonance (SPR) biosensor binding is able to semiquantitatively detect molecular DNA–protein interactions in crude cellular extracts in a real-time ligand fishing analysis study. Crude cell extracts obtained from a confluent HT-28 human adenocarcinoma cell line, synchronized to the G_0/G_1 phase of the cell cycle, were extracted in a chaotropic medium and cryopreserved in liquid nitrogen. Various immunoprecipitation antibodies were used against defective human excision and mismatch repair genes, hDDB2 and hMSH2, respectively, which theoretically allow for protein binding to DNA ligands in their native conformation. A set of biotinylated DNA target sequence heteroduplexes were also utilized for binding hDDB2 and hMSH2, prepared by heating a biotinylated oligonucleotide strand with an equimolar amount of the complementary strand to form a DNA duplex for hMSH2; a UV-irradiated duplex was employed for hDDB2 instead of an irradiated single-strand DNA to enhance binding. SDS was used to regenerate heteroduplex-modified chips that were used in a BIAcore 2000 SPR instrument at 25˚C. Results showed that hMSH2 does not bind preferentially to the heteroduplex-complementary pair. In contrast, hDDB2 was found to bind preferentially to the UV-irradiated version of the heteroduplex-complementary pair. It is concluded that the choice of antibodies with appropriate epitopes is crucial to the success of these SPR binding studies because of enhanced specificity.*

[+]*Current address:* GEM Tox Consultants & Labs, Inc., 2905 South Memorial Drive, Greenville, NC 27834, U.S.A.
[++]*Current address:* Department of Radiation Oncology, Carolinas Medical Center, N.E., 920 Church Street, Concord, NC 20825, U.S.A.

Correspondence to: Dr. Farid E. Ahmed, Director, GEM Tox Consultants & Labs, Inc., 2905 South Memorial Drive, Greenville, NC 27834, U.S.A. Tel: +1 2522581808, Fax: +1 2523217261, e-mail: gemtoxconsultants@yahoo.com

Key Words: Affinity enrichment, binding, biosensor, kinetics, label-free, mass, probe, photons, nucleic acid-protein interactions, SPR spectroscopy.

Surface plasmons are electromagnetic waves formed by electrons propagating along the surface of a thin metal layer (*e.g.*, gold) giving rise to surface plasmon resonance (SPR). SPR is a unique, optoelectronic, label-free, noninvasive, direct-reading enrichment detection method that utilizes interaction of light photons with free electrons (surface plasmons) on gold surfaces to quantify the change in the concentration/amount of biomaterial on the surface (1).

In SPR biosensors, prism couplers are used to achieve optical excitation of metal electrons when a light beam undergoes total internal reflection at a prism-metal excitation interface (Figure 1A). Under these conditions, the electric field of the reflected photons penetrates beyond the reflecting surface to a distance of approximately a quarter of the photon wavelength of the photons and interacts with the surface electrons in the metal film. At a specific angle of the incident light, higher than the critical angle of reflection, the momentum of the incoming photon matches the momentum of the surface plasmon, resulting in energy transfer; therefore, the light is not reflected and SPR occurs. At that point, the excited surface plasmons generated at the site of total internal reflection are evanescent from the metal–solution interface (2) and tend to create an electric field that extends into the medium on either side of the metal surface to about 200 nm, decaying exponentially with the distance from the metal surface. The propagation of this evanescent electrical field is very sensitive to changes in the chemical composition of the solution, and such changes alter

the momentum of the surface plasmons. Therefore, if SPR is to occur under the new conditions imposed at the solution–surface interface, the incident angle of the optical wave must change. This angle is measured by the SPR instrument and is used to express changes occurring in the solution. The change in mass on the gold surface thus equals the change in the refractive index close to the surface of the sensor chip. This change in the refractive index of the solvent during complex association or dissociation reactions can be measured by the change in the resonance angle that is required to create SPR. The change in resonance angle is expressed in resonance units (RU), where 1 RU=1 pg/mm^2 of proteins (3).

Comprehensive characterization of how proteins interact with other molecules such as DNA or RNA should include information on parameters such as active concentration, binding, specificity, affinity (the strength of the interaction), ranking, reaction kinetics (the rates at which the interactions bind and dissociate), thermodynamics (when SPR analysis is applied at numerous temperatures to a particular molecular interaction), and residence time (time a chemical or drug spends on the protein under study). SPR detects mass concentration and conformational changes (association or dissociation) on a sensor surface based on wavelength modulation by label-free detection modes in real time, and data are presented graphically in an interaction profile known as sensogram, in which binding response is plotted over time (Figure 1B).

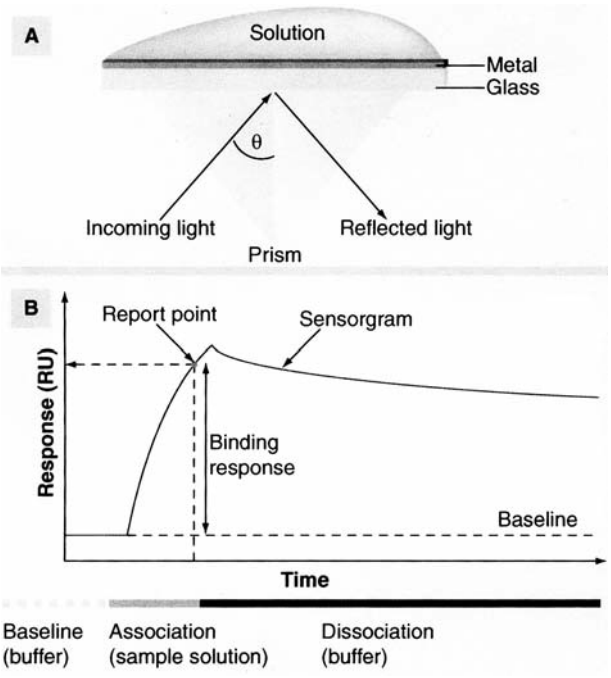

Figure 1. *A: SPR prism configuration: Total internal reflection of a monochromatic light at a prism–metal solution interface is a prerequisite for the SPR phenomenon. B: Sensogram for detecting mass concentration and dissociation changes on the sensor surface via label-free SPR detection.*

Besides the aforementioned capability of real-time quantitative analysis (4) and the lack of requirement for a label for detection, which increases versatility (5), there are several advantages of using SPR technology over other affinity-based methods. For example, SPR has a high sensitivity of up to 1 nM for a 20 kDa protein (6) and it can be performed in an automated fashion, thus increasing sample throughput (7). However, there are also disadvantages of SPR, as it cannot easily discriminate between specific and non-specific interactions with the sensor surface. Elaborate washing does not completely remove the non-specifically bound material; thus, reference material or control samples are needed to correct for the non-specific binding (8). Because SPR is mass sensitive, the sensitivity for high molecular weight molecules is good, but binding of low molecular weight compounds is more difficult to detect (6). In addition, a particular challenge in SPR application is the limited sensor area, leading to a diminished capacity; increasing the sensor surface, or accumulating several runs, might diminish this limitation (9).

Similar to other affinity-based methods, SPR enrichment consists of several sequential steps: (i) preparation of the surface *via* immobilization of a probe molecule, (ii) verification of the activity of the prepared sensor, (iii) incubation of the sensor with a target-containing sample to form a complex, (iv) dissociation of the complex to reuse the sensor, or to further analyze the target by employing stringent washing of several different pH solutions or different ionic strengths (10), and (v) elution of captured proteins for further analysis. However, quantitative elution can be challenging as the probe-target complexity usually has a rather high affinity (11).

To improve upon SPR measurements, and because SPR detection is non-destructive to the protein being analyzed, SPR can be combined with mass spectrometry (MS), either matrix-assisted laser desorbtion ionization (MALDI)-time-of-flight (TOF)-MS or electrospray ionization (ESI)-MS, in what has been termed as biomolecular interaction analysis (BIA)/MS. Hence, MS validates the protein by looking at its intact mass, which is an intrinsic property of each protein (1). Furthermore, MS analysis offers an additional insight into possible protein modifications that might exist as a result of point mutation or post-expression through *in vivo* processing (12).

The SPR phenomenon has been known for over a century, but only in the last two decades has it become accepted as a method for the detection of biomolecular interactions, and has been subsequently incorporated in a number of commercially available biosensors. Today, SPR-based biosensors are mostly used for characterizing protein

interactions under conditions where only one analyte of interest is targeted by an immobilized ligand on a single site on the sensor chip surface. This experimental design is a result of the fact that SPR does not discriminate between the types of molecules it detects, as it registers only the total amount of biomaterial retained on the surface (1, 11).

Biacore (Uppsala, Sweden), now part of GE Healthcare Life Sciences, released its first commercial instrument in 1990 (13). Biacore technology is able to analyze interactions in the presence of the widely used solvent dimethylsulfoxide (DMSO). This technology, through the introduction of colloidal silica beads as a capturing and transporting agent, has made it possible, if desired, to recover proteins after affinity purification on the sensor surface for subsequent MS analysis (14).

Xeroderma pigmentosum (XP) is a rare autosomal recessive skin disorder characterized by sun-sensitivity, pigmentation abnormalities and a high incidence of malignant skin tumors, which is due to effective nucleotide excision repair (NER) of ultraviolet (UV)-induced damage to DNA (15). The NER deficiency in XP complementation group E (XPE) cells has been correlated with a damage-specific DNA binding protein (DDB). Human DDB is a heterodimer of 48 and 127 kDa (p48 and p127) subunits that recognizes a variety of UV-induced lesions, whose activity is absent from cell strains from a subset of XPE individuals (Ddb^-) (16, 17). Functional studies have also demonstrated a role for DDB in DNA repair *in vivo* (18). Human cDNAs for both DDB subunits have been sequenced (19, 20). The mouse DDB2 cDNA coding for DDB p48 subunit was cloned, and a search of databases revealed the amino acid sequence of mouse and *Drosophila* p127 subunit predicted homologs (21). The predicted human p127 amino-acid sequence has homology with monkey DDB 127 (22). P48, but not p127, has been shown to: (i) be inducible in response to UV damage in a p53-dependent manner (23), and (ii) stimulate E2F1 transcriptional activity (24). It has been proposed that a third domain may have evolved in higher eukaryotes in order to integrate p127-DDB functions with other cellular functions *via* its interactions with p48 (21).

Approximately 70% of hereditary non-polyposis colorectal cancer can be accounted for by mutations in one of several genes involved in DNA mismatch repair, and polymorphism in genes involved in carcinogen metabolism, as well as folate metabolism (25). In those cases, approximately 95% have shown alteration in the base sequence of the three mismatch repair genes (*hMSH2, hMLH1* and *hMSH6*), with a smaller proportion attributable to mutations in other mismatch repair genes (25). Early studies employed only conventional genomic DNA sequencing for mutation detection. However, later studies employing conversion analysis substantially increased the diagnostic yield of genetic testing for mismatch repair mutations in patients diagnosed with colorectal cancer (26).

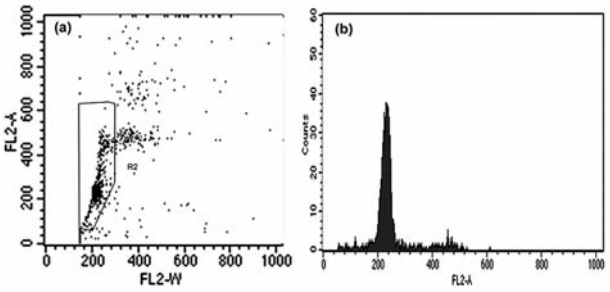

Figure 2. *Flow cytometry on HT-29 cells after cell trypsinization showing: (a) the population of cells selected for analysis, and (b) more than 90% of cells were synchronized in the G_0/G_1 phase of the cell cycle.*

Materials and Methods

Cell line and culture conditions. HT-29 human adenocarcinoma cells, passage 134 were obtained from the American Type Culture Collection (ATCC), Rockville, MD, USA. It is an established epithelial line, resembling colonic crypt cells (27). Cells were originally seeded in 75 cm^2 tissue culture flasks at ~1.5×10^5 cells/flask (2×10^3 cells/cm^2), then propagated in 150 cm^2 flasks that can accommodate ~70×10^6 cells/flask at full confluency. Isove's modified Dulbecco's medium (IMDM) containing 105 IU/l of penicillin and 0.1 g/l streptomycin was used for maintaining cells in an humidified atmosphere of 95% air and 5% CO_2 at 37˚C. Cells were subcultured at 2-day intervals (doubling time ~20 h) to maintain subconfluent growth conditions. The contact inhibited cells were grown for the last two days before removal from the flask to carry out affinity interactions in a serum-free IMDM containing antibiotic in order to synchronize them in the G_0/G_1 phase of the cell cycle (28).

Flow cytometric analysis. After trypsinization, cells were harvested by adding 4 ml of culture medium to the flask, detaching the cells onto a sterile 12 ml polyallomer tube, centrifugation to remove the medium and staining with Trypan blue vital dye to obtain ~10^6 viable cells/ml. Cells were suspended in phosphate-buffered saline (PBS) buffer and fixed by adding 3 ml of cold 100% ethanol (final ethanol concentration of 70%), and the fixed cells then stored at –20˚C until further processing (29). When ready to proceed, cells were resuspended in 0.5 ml of PBS buffer, to which 500 µl of a buffer containing 200 mM sodium phosphate (dibasic, pH 7.8) and 100 mM citric acid was added. Cells were stained by resuspension for 30 min in 1 ml solution containing 50 µg/ml propidium iodate and 3.8 mM sodium citrate, to which 50 µl of 10 mg/ml Ribonuclease A were added. Analysis of fluorescence, for subdiploid population detection of various phases of the cell cycle (30), was performed by fluorescence-activated cell sorting in a Becton Dickinson, Franklin Lake, NJ, USA) FACScan single-cell-based flow cytometer (31).

Cell irradiation by short-wave UV light. Confluent cells in flasks were trypsinized, washed twice with cold PBS, placed in a 2-cm Petri dish, precooled for 15 min at 4˚C and exposed to UV light (wavelength: 245 nm; Strategene Cross Linker, San Francisco, CA, USA) from above, with a total energy yield of 27.2 J/m^2 (32).

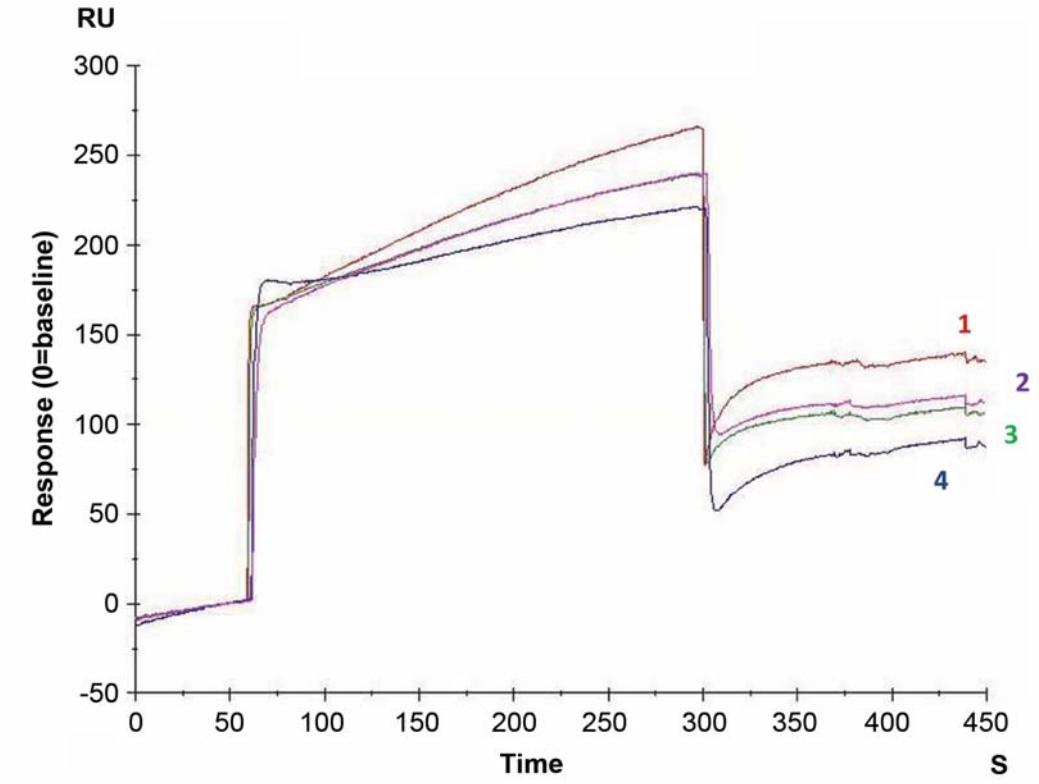

Figure 3. *Sensogram showing that DDB2 fragment (10 μg/ml) was bound to: (1) UV-irradiated DNA heteroduple on the surface of the SA chip (red line, 1) more stringently than to (2) UV-irradiated DNA homoduplex (purple line, 2) on the chip surface, or (3) to unirradiated DNA heteroduplex on the chip surface (green line, 3), and least to (4) no DNA on the chip surface (blue line, 4).*

Cell extract preparations. H-29 cells were trypsinized, counted and ~2×10^6 cells were placed in cryovials, to which 1 ml of the chaotropic TRI reagent TR-118 (TRI Research Institute, Cincinnati, OH, USA), containing the chaotropic reagent guanidinium hydrochloride, was added per 10^6 cells (*i.e.*, total 2 ml) to minimize nucleic acids degradation. Protein content in the crude cellular extract was measured by the Bradford assay (33). Stabilized cells were then flash frozen by dipping in liquid nitrogen. Cryovials containing the crude cellular extracts were kept in a –70˚C freezer until ready for SPR analysis.

SPR measurements. SPR measurements were performed on a BIAcore 2000 instrument (Uppsala, Sweden, now part of GE Healthcare, Piscataway, New Jersey, USA) at 25˚C. In such 'ligand fishing binding experiments' involving protein–nucleic acid interaction, the targeted HT-29 cell preparations, containing nucleic acid DNA sequence for gene *hDDB2* (50-100 μg) that was irradiated with 27.2 J/m^2 of UV light in order to cause complete inhibition of UV-DDB activity (34), was placed onto a flow cell, and a 50-100 μg of control cell preparations were placed onto a second flow cell. Nucleic acid DNA preparations were made by synthesizing a biotinylated oligonucleotide DNA heteroduplex sequence and capturing it using a streptavidin-coated sensor chip. Antibodies (Abs) against *hDDB2* (p48) and the human mismatch repair protein Mut S homology 2 colon cancer nonpolyposis type 1 (*hMSH2*) that are suitable for immunoprecipitation, rather than those used for Western blotting, which are believed to recognize the target protein (35), were obtained from several companies, namely Santa Cruz Biotechnology, Santa Cruz, CA, USA), Novus Biologicals (Gilbert, AZ, USA), Promega Corporation (Madison, WI, USA) and Proteintech Group, Inc. (Chicago, IL, USA). Once the nucleic acids were captured, the protein was injected over both treated and control crude cell extracts and the reference-subtracted data were observed.

For nucleic acid binding studies, a set of DNA heteroduplexes was synthesized, each of which contained one of the eight possible single base pair mismatches located at the same position within the heteroduplex molecules, as detailed by Su *et al*. (34). Heteroduplex preparations were made as described by Allen *et al*. (36), since without a heteroduplex formation there would be no mismatch. The streptavidin sensor chip (SA chip; Pharmacia, which is now part of Pfizer, Groton, CT, USA) was derivatized with a 31 bp biotinylated (biotin) G-T heteroduplex prepared from biotin oligonucleotides (oligo) 5'-biotin-GCCGAATTTCTAGAATCGAGAGCTTGCTAGC and 5'-GCTAGCAAGCTTTCGATTCTA GAAATTCGGC (25 nmoles), by heating an equimolar amount of the complementary strand to form a duplex to 80˚C and cooling to room temperature over a 2 h period in order to form a DNA duplex. An otherwise identical A-T homoduplex was prepared by hybridizing 5'-biotin-GCCGAATTTCTAGAATCGAAAGC TTGCTAGC to the second oligo. A complementary DNA strand was also made, which had the following oligonucleotide sequence: 5'-GCTAGCAAGCTTTCG ATTCTAGAAATTCGGC. A fraction of the homoduplex/

complement pair was then treated with the UV light, as described above, in order to make a ligand for *hDDB2* binding. The heteroduplex DNA strand that paired with an equimolar amount of the complementary strand served as a control for *hDDB2*. Binding of *Escherichia coli* MutS to the heteroduplex or homoduplex modified SA chip was performed at a flow rate of 20 µl/min in a solution containing 0.01 M HEPES-KOH, pH 7.4, 0.1 M NaCl, 3.4 mM EDTA, 8.4 mM $MgCl_2$, 0.005% (v/v) Surfactant P20. The heteroduplex-modified SA chip was regenerated following MutS binding by a 20 µl injection of 0.5% sodium dodecyl sulfate (DDS) (36). The chip layout for the SPR studies that measured DNA–protein interaction was as follows: (a) heteroduplex experimental, (b) UV-irradiated homoduplex, (c) non-irradiated DNA heteroduplex control, and (d) streptavidin only (no DNA) for *hDDB2* binding or *hMSH2* homoduplex.

Results

Figure 2 represents an output of a FACScan flow cytometer showing that more than 90% of serum-deprived HT-29 cells were synchronized such that they were in the G_0/G_1 phase of the cell cycle.

Results of measuring nucleic acid DNA–protein interactions are given in the SPR sensogram showing that 10 µg/ml of *hDDB2* fragment is bound most tightly to UV-irradiated heteroduplex (curve 1, red line in Figure 3) when using the Ab from Proteintech Group, Inc. Progressively less tight binding is observed to UV-irradiated DNA homoduplex (curve 2, purple line), non-irradiated DNA heteroduplex (curve 3, green line), and no DNA (curve 4, Blue line).

In contrast, the sensogram for *hMSH2* revealed no binding of the mismatch repair gene fragment to DNA heteroduplex using any of the commercial Abs.

Discussion

This study employed a prolific epithelial cell line in order to obtain an extract that contained enough nucleic acids and proteins from fast growing cells to enable the assays for several experimental parameters without having to repeat extraction preparations for affinity SPR analysis, which may have led to variations in the results due to biological and/or technical errors. Each strand of the heteroduplex was estimated to have a molecular weight of approximately 10 nM (10^4 g/mole × 10^{-8} mole=10^{-4} g).

Using an SPR system, one interaction partner (*e.g.*, nucleic acid) is immobilized on a sensor chip surface, while the other (*e.g.*, protein) flows over the surface *via* a microfluidic flow bath. A general problem in chip-based affinity separation systems is the large surface-to-volume ratio of the fluidic system. Extreme care must be exercised in order to avoid non-specific adsorption, which might result in the loss of target protein and the carry-over during the affinity purification process, leading to reduction in sensitivity, even if MS analysis is also employed (37). MS analysis was not employed in this proof-of-principle study as this analytical step was not necessary; if MS analysis had been used, then there would have been investigation and experimentation to find the optimal regulatory conditions and materials compatible with MS, such as 0.1% trifloroacetic acid (38, 39).

It should be stated that the *hDDB2* protein should not bind to its own DNA sequence, unless that sequence has been UV-irradiated. To avoid any effect of cell cycle variation due to UV radiations, which may induce cell cycle delay and may also be dose dependent, synchronized cells were used, mostly in the G_0/G_1 phase of the cell cycle, where cells are known to be sensitive to repair of irradiation-induced damage to DNA (40). Although it may not be possible to observe events that are specific to other phases of the cell cycle, this approach is justified for an initial proof-of-principle study. It is possible that if other UV-irradiation doses had been employed, different results may have been obtained. Moreover, different Abs might have resulted in different SPR sensograms, as the epitopes necessary for the attachment of the Ab to its target protein may vary from one Ab to another. Proteintech Group, Inc Abs showed that label-free binding between UV-irradiated *hDDB2* heteroduplex fragment and protein in a crude cellular extract can be measured by SPR technology. In contrast, Abs to damage repair protein DDB2 from three other companies (Santa Cruz Biotechnology, Novus Biological and Promega) did not show such binding. It should be noted that Abs that only work in Western blot tend to recognize only denatured proteins (41), and thus are not useful for such affinity studies.

When studying the binding of *hMSH2* protein by SPR technology in crude cell extracts, none of the Abs obtained from any of the companies exhibited affinity interaction. This is a problem that is often encountered when employing Abs, probably due to lack of proper epitopes. The Human Proteome Organization project aims to overcome this problem by making high quality human Abs with complete epitopes commercially available (42, 43).

SPR experiments typically give great detail about a specific interaction, which has important applications in the pharmaceutical and biotechnology industries for studying drug discovery, biopharmaceutical developments and clinical immunogenicity assays, as well as in manufacturing and quality control methods. By contrast, 2D gel electrophoresis gives a protein profile for a given state of a cell line employed. Protein arrays can identify interactions for a given protein, but do not characterize a given interaction. The design of a protein array may also lead to the inability to observe low affinity or transient interactions of interest as weak interactants may be washed off before detection. Chromatin immunoprecipitation will identify a DNA-binding sequence for a given protein, but will not give detailed information on the interaction between the protein and the target DNA (1, 3).

The combination of detecting binding properties of hundreds of therapeutic proteins with SPR microarrays from phage display libraries with automated protein purification methods promises to accelerate the discovery of high-affinity leads significantly (1, 44). Miniaturization, cost reduction and automation of SPR sensors will have wide applications not only in the laboratory, but also in the field for real-time process control (45).

SPR is expected to move into the domain of high throughput analysis through the development of SPR protein arrays. Several companies have developed prototypes of SPR instruments capable of analyzing tens to hundreds of ligand spots or active sites (*e.g.*, Biacore; HTS Biosystems, http:// www.htsbiosystems.com; GWC Instruments, http:// www.gwcinstruments.com; Graffinity, http:// www.graffinity.com). On-chip MS analysis of such SPR arrays will be the next logical step, as these arrays will be used for the screening of interacting patterns from biological samples, where it is crucial to gain insight on the identity and structure of interacting biomolecules (1).

Acknowledgements

We express our deep gratitude to: Eric Rouch for advice on sample preparation for SPR analysis and for the time he spent running the initial SPR and producing initial sensograms; Paul Modrich for discussion on the SPR technology and methods of sample analysis; Hiram Gay and Ron R. Allison for insightful clinical discussions; Claudio Sibbata for dosimetry advice; and Peter Kragel for chosing a sensitive cell line to employ during this initial proof-of-principle work.

The Authors declare no conflict of interest in carrying out this research, or in preparing this manuscript.

References

1 Ahmed FE: Mining the oncoproteome and studying molecular interactions for biomarker development by 2DE, ChIP and SPR technologies. Exp Rev Proteomics *5*: 469-496, 2008.

2 Homola J: Present and future of surface plasmon resonance biosensors. Anal Bioanl Chem *377*: 528-539, 2003.

3 Ahmed FE: Expression microarray proteomics and the search for cancer biomarkers. Curr Genom *7*: 399-426, 2006.

4 Homola J, Yee SS and Gauglitz G: Surface plasmon resonance sensors: review. Sens Actuators B Chem *54*: 3-15, 1999.

5 Mullett WM, Li EP and Yeung JM: Surface plasmon resonance-based immunoassays. Methods *22*: 77-91, 2000.

6 Englebienne P, Van Hoonacker A and Verhas M: Surface plasmon resonance: principles, methods and applications in biomedical sciences. Spectroscopy *17*: 255-273, 2003.

7 Grote J, Dankbar N, Gedig E and Koenig S: Surface plasmon resonance/mass spectrometry interface. Anal Chem *77*: 1157-1162, 2005.

8 Rich RL and Myszka DG: Why you should be using more SPR biosensor technology. Drug Discov Today Technol *5*: 1-8, 2004.

9 Catimel B, Rothacker J and Nice E: Use of biosensors for microaffinity purification: an integrated approach to proteomics. J Biochem Biophys Methods *49*: 289-312, 2001.

10 Dutra RF, Mendes RK, Lins da Silva V and Kubota LT: Surface plasmon resonance immunosensors for human cardiac troponin T based on self-assembled monolayer. J Pharm Biomed Anal *43*: 1744-1750, 2007.

11 Visser NFC and Heck AJR: Surface plasmon resonance mass spectrometry in proteomics. Exp Rev Proteomics *5*: 425-433, 2008.

12 Krone JR, Nelson RW, Dogruel D, Williams P and Granzow R: BIA/MS: Interfacing biomolecular interaction analysis with mass spectrometry. Anal Biochem *244*: 124-132, 1997.

13 Jönsson U, Fägerstam L and Ivarsson B: Real-time biospecific interaction analysis using surface plasmon resonance and a sensor chip technology. BioTechniques *11*: 620-627, 1991.

14 Rich RL and Myszka DG: Advances in surface plasmon resonance biosensor analysis. J Mol Recog *13*: 388-407, 2000.

15 Cleaver JE: Defective repair replication of DNA in xeroderma pigmentosum. Nature *218*: 652-656, 1968.

16 Chu G and Chang E: Xeroderma pigmentosum group E cells lack a nuclear factor that binds to damaged DNA. Science *242*: 564-567, 1988.

17 Keeney S, Wein H and Linn S: Biochemical heterogeneity in xeroderma pigmentosum complementation group E. Mutat Res *273*: 49-56, 1992.

18 Keeney S, Eker APM, Brody T, Vermeulen W, Bootsma D, Hoeljmakers JHJ and Linn S: Correction of the DNA repair defect in xeroderma pigmentosum group E by injection of a DNA damage-binding protein. Proc Natl Acad Sci USA *91*: 4053-4056, 1994.

19 Dualan R, Keeney S, Nichols AF, Admon A and Linn S: Chromosomal localization and cDNA cloning of the genes (*DDB1* and *DDB2*) for the p127 and p48 subunits of a human damage specific DNA binding protein. Genomics *29*: 62-69, 1995.

20 Hwang BJ, Liao JC and Chu G: Isolation of a cDNA encoding a UV-damaged DNA binding factor defective in xeroderma pigmentosum group E cells. Mutat Res *362*: 105-117, 1996.

21 Zolezzi F and Linn S: Studies of the murine *DDB1* and *DDB2* genes. Gene *245*: 151-159, 2000.

22 Takao M, Abramic M, Moors M Jr, Ortin VR, Wootton JC, McLenigan M, Levine AS and Protic M: A 127 kDa component of a UV-damaged DNA-binding complex which is defective in some xeroderma pigmentosum group E patients is homologous to a slime mold protein. Nucl Acids Res *21*: 4111-4118, 1993.

23 Hwang BJ, Ford JM, Hanawalt PC and Chu G: Expression of the p48 xeroderma pigmentosum gene is p53-dependent and is involved in global genomic repair. Proc Natl Acad Sci USA *96*: 424-428, 1999.

24 Hayes S, Shiyanov P, Chen X and Raychaudhuri P: DDB a putative DNA repair protein can function as a transcriptional partner of E2F1. Mol Cell Biol *18*: 240-249, 1998.

25 Ahmed FE: Gene–gene, gene–environment & multiple interactions in colorectal cancer. J Env Sci Health C *24*: 1-101, 2006.

26 Casey G, Lindor NM, Papadopoulos N, Thibodeau SN, Moskow J, Steelman S, Buzin CH, Sommer SS, Collins CE, Butz M, Aronson M, Gallinger S, Barker MA, Young JP, Jass JR, Hopper JL, Diep A, Bapat B, Salem M, Seminara D and Haile R: Conversion analysis for mutation detection in *MLH1* and *MSH2* in patients with colorectal cancer. J Amer Med Assoc *293*: 799-809, 2005.

27 Tom BH, Rutzky LP, Jakstys MM, Oyasu R, Kaye CI and Kalhan BD: Human colonic adenocarcinoma cells. I. Establishment and description of a new line. In Vitro *12*: 180-191, 1976.
28 Cooper S: Reanalysis of the protocol for *in vitro* synchronization of mammalian astrocytic cultures by serum deprivation. Brain Res Protocols *15*: 115-118, 2005.
29 Davis PK, Ho A and Dowdy SF: Biological methods for cell–cell synchronization of mammalian cells. BioTechniques *30*: 1322-1331, 2001.
30 Frantz DJ, Hughes BG, Nelson DR, Murray BK and Christensen MJ: Cell cycle arrest and differential gene expression in HT-29 cells exposed to an aqueous garlic extract. Nutr Cancer *38*: 255-264, 2000.
31 Orfao A and Ruiz-Argüelles A: General concepts about cell sorting techniques. Clin Biochem *29*: 5-9, 1996.
32 Ortin VR, McLenigan M, Takao M, Levine AS and Miroslava P: Translocation of a UV-damaged DNA binding protein into a tight association with chromatin after treatment of mammalian cells with UV light. J Cell Science *110*: 1159-1168, 1997.
33 Bradford MM: A rapid and sensitive method of the quantitation of microgram quantities of proteins utilizing principles of protein-dye binding. Anal Biochem *72*: 249-254, 1976.
34 Su SS, Lahue RS, Au KG and Modrich P: Mispair specificity of methyl-directed DNA mismatch correction *in vitro*. J Biol Chem *263*: 6829-6835, 1988.
35 Scopes RK: Protein Purification: Principles and Practice. New York: Springer, 1994.
36 Allen DJ, Makhov A, Grilley M, Taylor J, Thresher R, Modrich P and Griffith JD: MutS mediates heteroduplex loop formation by a translocation mechanism. EMBO J *16*: 4467-4476, 1997.
37 Larsericsdotter H, Jansson O, Zhukov A, Areskoug D, Oscarrson S and Buijs J: Optimizing the surface plasmon resonance/mass spectrometry interface for functional proteomics applications: How to avoid and utilize nonspecific adsorption. Proteomics *6*: 2355-2364, 2006.
38 Ahmed FE: Application of MALDI/SELDI mass spectrometry to cancer biomarker discovery and validation. Current Proteomics *5*: 415-438, 2008.
39 Ahmed FE: Liquid chromatography-mass spectrometry: A tool for proteome analysis & biomarker discovery and validation. Exp Opin. Mol Diag *3*: 429-444, 2009.
40 Davis PK, Ho A and Dowdy SF: Biological methods for cell–cell synchromization of mammalian cells. BioTechniques *30*: 1322-1331, 2001.
41 Ahmed FE: Differences in mRNA and microRNA microarray expression profiles in human colon adenocarcinoma HT-29 cells treated with either intensity-modulated radiation therapy (IMRT), or conventional radiation therapy (RT). Cancer Genom Proteom *6*: 109-128, 2009.
42 Haab B: Application of antibody array platforms. Curr Opin Biotechnol *17*: 415-421, 2006.
43 Omenn GS, States DJ, Adamski M, Blackwell TW, Menon R, Hemjakob H, Apweiler R, Haab BB, Simpson RJ, Eddes JS, Kapp EA, Mortiz RL, Chan DW, Rai AJ, Admon A, Aebersold R, Eng J, Hancock WS, Hefta SA, Meyer H, Paik YK, Yoo JS, Ping P, ounds J, Adkins J, Qian X, Wang R, Wasinger V, Wu CY, Zhao X, Zeng R, Archakov A, Tsugita A, Beer I, Pandey A, Pisano M, Andrews P, Tammen H, Speicher DW and Hanash SM: Overview of the HUPO plasma proteome project: results from the pilot phase with 35 collaborating laboratories and multiple analytical groups, generating a core database of 3020 proteins as a publicly available database. Proteomics *5*: 3226-3245, 2005.
44 Wassaf D, Kuang G, Kopacz K, Wu QL, Nguyen Q, Toews M, Cosic J, Jacques J, Witshire S, Lambert J, Pazmany CC, Hogan S, Lander RC, Nixon AE and Saxton DJ: High throughput affinity ranking of antibodies using surface plasmon resonance microarrays. Anal Biochem *351*: 241-253, 2006.
45 Chinowsky TM, Soelberg SD, Baker P, Swanson NR, Kaufman P, Mactutis A, Grow MS, Atmar R, Yee SS and Furlong CE: Portable 24-analyte surface plasmon resonance instruments for rapid, versatile biodetection. Biosensors Bioelectronics *22*: 2268-2275, 2007.

Received August 29, 2010
Revised September 22, 2010
Accepted September 27, 2010