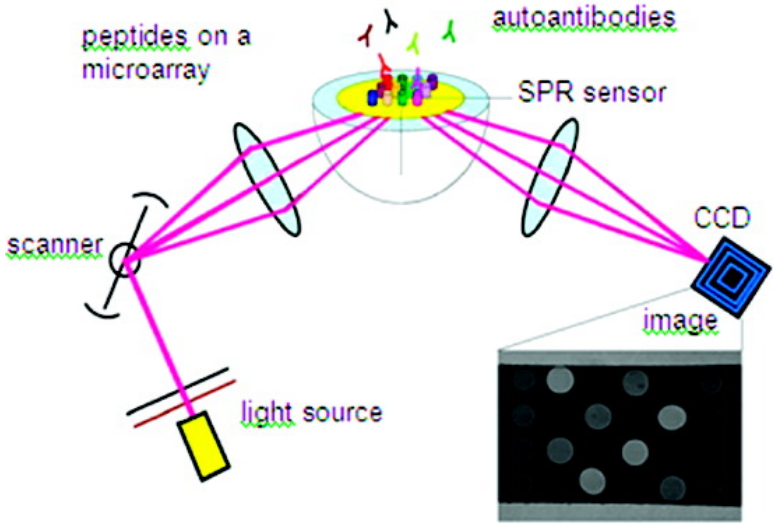
Article

Biomolecular Interaction Monitoring of Autoantibodies by Scanning Surface Plasmon Resonance Microarray Imaging

Angelique M. C. Lokate, J. Bianca Beusink, Geert A. J. Besselink, Ger J. M. Pruijn, and Richard B. M. Schasfoort

J. Am. Chem. Soc., **2007**, 129 (45), 14013-14018• DOI: 10.1021/ja075103x • Publication Date (Web): 17 October 2007



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 6 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
ARTICLES
Published on Web 10/17/2007

Biomolecular Interaction Monitoring of Autoantibodies by Scanning Surface Plasmon Resonance Microarray Imaging

Angelique M. C. Lokate,[‡] J. Bianca Beusink,[†] Geert A. J. Besselink,[†] Ger J. M. Pruijn,[‡] and Richard B. M. Schasfoort*[,†]

Contribution from the Biochip Group, MESA+ Research Institute for Nanotechnology, University of Twente, P. O. Box 217, NL-7500 AE Enschede, The Netherlands, and Department of Biomolecular Chemistry, Nijmegen Centre for Molecular Life Sciences, Institute for Molecules and Materials, Radboud University Nijmegen, P. O. Box 9101, NL-6500 HB Nijmegen, The Netherlands

Received July 10, 2007; E-mail: R.B.M.Schasfoort@tnw.utwente.nl

Abstract: A new commercial surface plasmon resonance (SPR) imaging analysis system with a novel SPR dip angle scanning principle allows the measurement, without the need for labeling, of the exact SPR dip angle. With this system hundreds of biomolecular interactions can be monitored on microarrays simultaneously and with great precision. The potency of this system is demonstrated by automatically monitoring the interactions between citrullinated peptides and serum autoantibodies of 50 rheumatoid arthritis (RA) patients and 29 controls in a single step. The smallest antibody concentration that could be measured in this experimental setup was 0.5 pM.

Introduction

Some of the outstanding questions in the fields of biology and medicine remain unsolved as a result of our limited understanding of the function, behavior, and concerted interaction of many significant biomolecules. To obtain information on complex biological systems, protein microarray technology facilitates sensing of multiple biomolecular interactions in parallel. However, labeling is required, which may cause additional problems for studying structure−function relationships of biomolecular interactions. A well-known approach to measure the binding of proteins and other biomolecules to sensor surfaces in a label-less and real-time manner is the surface plasmon resonance (SPR) imaging method, which has been described since 1988.[1−4] SPR is an optical method for measuring the refractive index of material absorbed on a thin metal layer, usually gold. Photons of p-polarized light can interact with the free electrons of the metal layer, inducing a wavelike oscillation of the free electrons and thereby reducing the reflected light intensity. The majority of systems used for SPR imaging represent fixed-angle instruments. These instruments are based on a relationship between the change in reflected light intensity and the mass of bound analyte; i.e., a fixed incident light angle is employed and mass changes are estimated from the intensity of the reflected light.[4] However, the applicable range of this linear relationship is limited and the optimal angles differ considerably when ligand or analyte panels having different molecular weights are to be monitored.[5] The use of only one fixed incident light angle is therefore not suitable for the monitoring of multiple biomolecular interactions in parallel on a microarray. Most commercially available instruments for SPR imaging measurements, such as instruments from GWC Technologies, Genoptics, and Kmac, although with different technologies, measure at fixed angles to monitor the interactions.

In systems that use a fixed wavelength the most reliable parameter that directly reflects mass changes on an SPR sensor chip is the SPR dip angle shift.[6,7] Thus, only when these shifts are monitored for all the spots on a microarray separately, the magnitude and affinity of biomolecular interactions can be reliably compared among all spots of any microarray.

The use of miniaturized and parallelized immunoassays in a microarray format in combination with scanning SPR imaging helps to improve autoimmunity research. Autoimmune diseases are characterized by the presence of high-affinity autoantibodies directed against self-proteins, such as rheumatoid factor for rheumatoid arthritis (RA),[8] Sm for systemic lupus erythematosus (SLE),[9] and Ro/SS-A and La/SS-B for Sjögren's syndrome.[9] Although at least some autoantibodies are known to be involved in cell and tissue damage, their mechanistic role

[†] University of Twente.
[‡] Radboud University Nijmegen.

(1) Rothenhäusler, B.; Knoll, W. *Nature* **1988**, *332*, 615−617.
(2) Jordan, C. E.; Frutos, A. G.; Thiel, A. J.; Corn, R. M. *Anal. Chem.* **1997**, *69*, 4939−4947.
(3) Thiel, A. M.; Frutos, A. G.; Jordan, C. E.; Corn, R. M.; Smith, L. M. *Anal. Chem.* **1997**, *69*, 4948−4956.
(4) Nelson, B. P.; Frutos, A. G.; Brockman, J. M.; Corn, R. M. *Anal. Chem.* **1999**, *71*, 3928−3934.
(5) Nelson, B. P.; Grimsrud, T. E.; Liles, M. R.; Goodman, R. M.; Corn, R. M. *Anal. Chem.* **2001**, *73*, 1−7.
(6) Liedberg, B.; Nylander, C.; Lundström, I. *Sens. Actuators* **1983**, *4*, 299−304.
(7) Liedberg, B.; Nylander, C.; Lundström, I. *Biosens. Bioelectron.* **1995**, *10*, i−ix.
(8) Mageed, R. A. In *Manual of Biological Markers of Disease*; Van Venrooij, W. J., Maini, R. N., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1994; pp 1−27.
(9) Routsias, J. G.; Tzioufas, A. G.; Moutsopoulos, H. M. *Clin. Chim. Acta* **2004**, *340*, 1−25.

in the pathogenesis of the disease is generally not known.[10] Nevertheless, the specificity of autoantibody responses highlights their potential as important tools for improved diagnosis, disease classification, and prognosis. Miniaturized multiplex assays can deliver a fingerprint of a patient's autoantibody repertoire requiring only a limited amount of patient material.

During the past decade various research groups made important contributions to the application of multiplex assays for autoantibody detection. In 2002, Robinson et al. employed protein and peptide ligand arrays, representing candidate autoantigens, to survey autoantibody binding.[11] Arrays of in situ synthesized peptides can also be generated with photolithography to perform antibody characterization.[12] Another approach is to apply "virtual arrays" in a homogeneous assay system with addressable beads.[13] However, in these systems at least one of the interactants must be labeled, which may disrupt the binding sites involved in the interaction, leading to false negatives. In addition, the label itself might interact with the immobilized proteins, leading to false positives.[14] A way to circumvent these problems is to use a labeled secondary binding molecule, which might result in improved assay sensitivity.

In this study scanning SPR microarray imaging (IBIS Technologies, Hengelo, The Netherlands) is used to measure the presence of anti-citrullinated protein antibodies (ACPA) in the sera of RA patients. Recently, it was shown that the so-called citrulline amino acid (which can be generated posttranslationally from arginine) is a critically important moiety of the antigenic determinants targeted by RA-specific autoantibodies.[15] Cyclic citrullinated peptides (CCPs) are widely used as antigenic targets in ELISA-based diagnostic tests for RA. The sensitivity and specificity are 71% and 99%, respectively.[16]

Results

SPR Experimental Setup. When a spot meets the optimal SPR conditions, the reflected light at a certain region of interest (ROI) within this spot will reach a minimal value, resulting in a dark spot. Gray values are averaged from the pixels in predefined regions of interest, and in this way the reflected light intensity is determined. The intensity of the reflected light is plotted against the angle of the incident light. SPR measurements carried out in a fixed-angle mode often translate the changes in reflectivity signal to angle shifts of the SPR dip from the linear part of this "reflectivity versus angle plot". In this study the exact SPR dip angle was measured instead of estimated. This was done by fast incident angle scanning within a range of 4° around the SPR dip angle and imaging the corresponding reflected light via a CCD camera (Figure 1). Scanning is performed stepwise, e.g., an angle scan at steps of 50 mdeg of incident light, and at each angle an image is taken. By curve fitting the "reflectivity versus angle plots", the angle of the exact SPR dip can be calculated. In contrast to fixed-angle instruments,

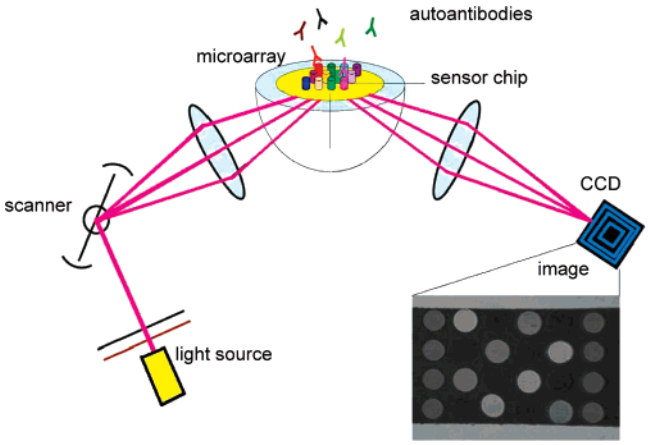


Figure 1. Schematic representation of the optical configuration of the scanning angle SPR imaging instrument. Citrullinated peptides and control peptides are chemically anchored on discrete spots of the microarray. Autoantibodies from diluted rheumatoid arthritis patient serum are flown over the sensor chip. The incident light is being reflected by the angle operated mirror before reaching the hemispheric prism and gold sensor, and the microarray is imaged on a CCD. The exact SPR minimum can be calculated from multiple reflectivity images and monitored for in principle hundreds of regions of interests.

which measure changes in reflectivity at one defined angle, the sensorgrams generated by the instrument used in this study represent SPR dip angles as a function of time (Figure 2). As a consequence, the *Y*-axis of the sensorgram does not represent an arbitrary reflectivity parameter, but contains the exact values for the SPR angle at maximal resonance. These exact SPR angles can be normalized for easy comparison of all the different sensorgrams of all the spots and are linearly correlated with the refractive index, corresponding to the mass of protein bound to the sensor surface.

Monitoring the Interaction of a Peptide Array with Serum Antibodies. In our proof-of-principle experiments, a 24-spot microarray containing human IgG as well as two different linear citrullinated peptides (CitA and CitB) and the corresponding control peptides (ArgA and ArgB, containing arginine instead of citrulline), were spotted (1 nL per spot) on an *N*-hydroxysuccinimide preactivated polycarboxylate-coated gold sensor surface using a noncontact spotting instrument.[17]

Images of the reflected light at four different scan angles after incubation of the 24-spot array with serum for 1 h are shown in Figure 2A−D. The SPR dip of each ROI can be visualized in a reflectivity-versus-angle-plot as shown in Figure 2E. The 0 mdeg setting is an arbitrary value, which can be set before each individual experiment by manual adjustment to obtain the best scan range. At an incident angle of −700 mdeg the background area near the array is in resonance (A in Figure 2); at −200 mdeg the spots coated with the arginine control peptides ArgA and ArgB are in resonance (B); at −100 mdeg the spots coated with citrullinated CitB are in resonance (C); at 100 mdeg the spots coated with citrullinated CitA are in resonance (D). Figure 2 demonstrates that the intensity profile images differ considerably between ROIs with complexes of varying masses attached to the surface.

Figure 3 shows the sensorgrams obtained during incubation of the microarray with an RA serum. ROIs in a background

(10) van Gaalen, F. A.; Linn-Rasker, S. P.; Van Venrooij, W. J.; De Jong, B. A.; Breedveld, F. C.; Verweij, C. L.; Toes, R. E.; Huizinga, T. W. *Arthritis Rheum.* **2004**, *50*, 709−715.
(11) Robinson, W. H.; et al. *Nat. Med.* **2002**, *8*, 295−301.
(12) Fodor, S. P.; Read, J. L.; Pirrung, M. C.; Stryer, L.; Lu, A. T.; Solas, D. *Science* **1991**, *251*, 767−773.
(13) Fulton, R. J.; McDade, R. L.; Smith, P. L.; Kienker, L. J.; Kettman, J. R., Jr. *Clin. Chem.* **1997**, *43*, 1749−1756.
(14) Cooper, M. A. *Anal. Bioanal. Chem.* **2003**, *377*, 834−842.
(15) Schellekens, G. A.; de Jong, B. A.; Van den Hoogen, F. H.; Van de Putte, L. B.; Van Venrooij, W. J. *J. Clin. Invest.* **1998**, *101*, 273−281.
(16) van Venrooij, W. J.; Zendman, A. J.; Pruijn, G. J. M. *Autoimmun. Rev.* **2006**, *6*, 37−41.
(17) Gutmann, O.; Niekrawietz, R.; Kuehlewein, R.; Steinert, C. P.; Reinbold, S.; De Heij, B.; Daub, M.; Zengerle, R. *Analyst* **2004**, *129*, 835−840.

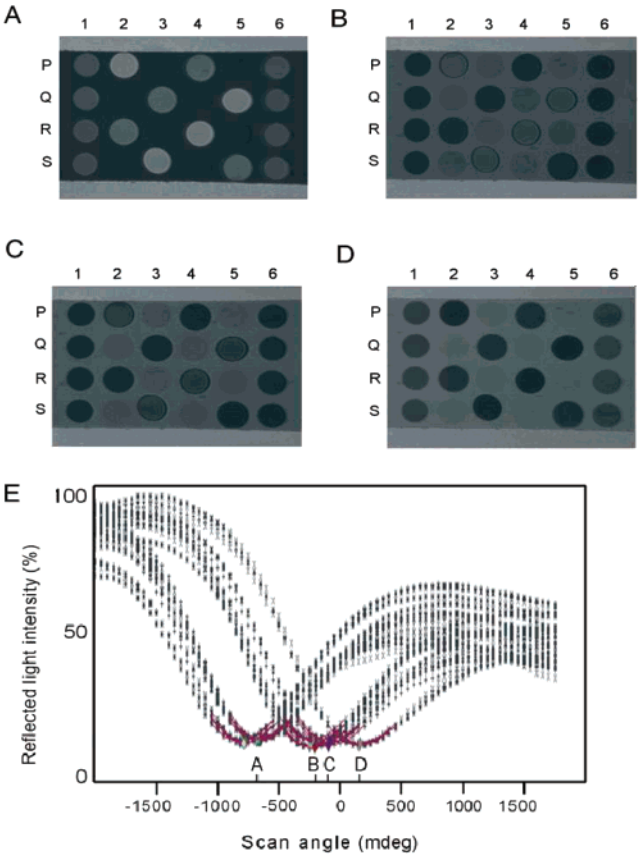


Figure 2. Reflectivity images at four different incident angles and the corresponding reflectivity-versus-angle plot. Spotted array contains in columns 1 and 6, human IgG; spot 4P,3Q,2R,1S, CitA; 2Q,3R,1S,4S, arginine control of CitA, ArgA; spot 2P,5Q,4R,3S, CitB; spot 3P,5P,4Q, 5R, arginine control of CitB, ArgB. Every 50 mdeg a reflectivity image is taken from which the intensity of the reflected light is measured. This reflected light intensity is plotted against the angle at which the image is taken, which results in a reflectivity-versus-angle plot (E). Incident angle is (A) −700 mdeg and the areas surrounding the spots are in resonance; (B) −200 mdeg, arginine control peptides of both citrullinated peptides are in resonance; (C) −100 mdeg, citrullinated CitB is in resonance; and (D) 100 mdeg, citrullinated CitA is in resonance. Note from (E) that, if reflectivity values are computed only instead of angle shifts, which is the case in current SPR imaging instruments, the results will be highly inaccurate.

region near the array and ROIs within the spots of immobilized human IgG and of two arginine-containing peptide controls showed relatively small angle shifts, namely 10, 100, 18, and 30 mdeg, respectively. Binding to the two corresponding citrullinated peptides, however, resulted in angle shifts of 250 and 400 mdeg, respectively. The noise, i.e., the baseline difference in resonance angle between the highest value and the lowest value, measured over 100 time points (1000 s) in one individual ROI, was 1.35 mdeg, making angle shifts of 5 mdeg significant. In Figure 4 the repeatability is shown for various injections of positive RA serum and control normal sheep serum.

SPR Assay Reproducibility. After placing the spotted sensor chip on top of the hemisphere prism in the flow cell based instrument, self-defined liquid handling procedures (LHPs) were used to increase the interexperiment reproducibility. Serum incubation, washing, and regeneration were done in an automated manner.

Because none of the peptides contained lysine residues, coupling is expected to occur exclusively via the N-terminal primary amino groups, thereby ensuring oriented, end-on immobilization of the peptides. One set of peptides was synthesized with a C-terminal biotin tag. These peptides were used to investigate possible differences in immobilization efficiency by assessing the SPR angle shift that resulted from incubation of the array with an anti-biotin antibody. The corresponding SPR angle shifts were 211 ± 10 and 212 ± 12 mdeg for the citrullinated peptide and arginine-containing peptide, respectively. From these data it can be concluded that there was no difference in immobilization efficiency between both peptides.

One advantage of multiplexing the monitoring of protein−protein interactions is the ability to test several interactions under the exact same conditions. The intra-array variation in the iSPR system was very low, as is illustrated by the sensorgrams of the quadruplicate interactions in Figure 3. The interaction of the 24-spot array with an RA serum showed an average angle dip shift of 202.5 mdeg with a standard deviation of 1.3 mdeg.

The sensor chips could be used for up to 50 interaction/regeneration cycles by treatment of the sensor surface with two repetitive incubations with 10 mM glycine·HCl, pH 1.5, for 30 s after each serum incubation step. In Figure 4, the repeatability of 12 injections for testing the chip performance is shown. Sequential measurements of patient sera on a single spot showed variations of less than 5% in binding to the citrullinated peptide, even when six interaction/regeneration cycles were performed between the two sequential measurements.

Reactivity of Patient Sera in SPR Assay. The reactivity of sera was quantified by calculating the ratio between the angle shifts observed for the citrullinated peptide A and the corresponding arginine control peptide (hereafter designated C/R ratio) upon binding of serum antibodies. The results for 50 RA sera and 29 control sera (9 SLE patient sera; 10 osteoarthritis (OA) patient sera; 10 normal healthy control sera) are shown in Figure 5. The mean C/R ratio for the RA sera that tested positive in a CCP ELISA (RA CCP+) is 8.6. The C/R ratio for the RA sera that tested negative in the CCP ELISA (RA CCP−), normal controls, SLE patients, and OA patients are 0.96, 1.01, 1.15, and 1.09, respectively. Although some RA CCP+ patients showed a C/R around 1, most of these patients were only weakly positive in the anti-CCP ELISA. Most likely the use of a linear peptide in the SPR assay leads to this slightly reduced sensitivity as the cyclic peptides used in the anti-CCP ELISA are known to be more sensitive.

Discussion

Surface plasmon microscopy and SPR imaging were first described in 1988.[1−4] Without exceptions, the systems used for SPR imaging represent fixed-angle instruments; i.e., the mass change is estimated from the intensity of the reflected light.[4] However, the only reliable and underived parameter that directly reflects the concomitant mass change on an SPR-sensor chip is the SPR dip shift.[6,7] Thus, only when these shifts are monitored separately for all the spots on a microarray, the magnitude and affinity of biomolecular interactions can be reliably compared among all spots of any microarray. The innovative nature of the approach in this study is the accurateness of SPR dip determination in combination with the unique features of imaging the sensor surface and real-time microarray spot

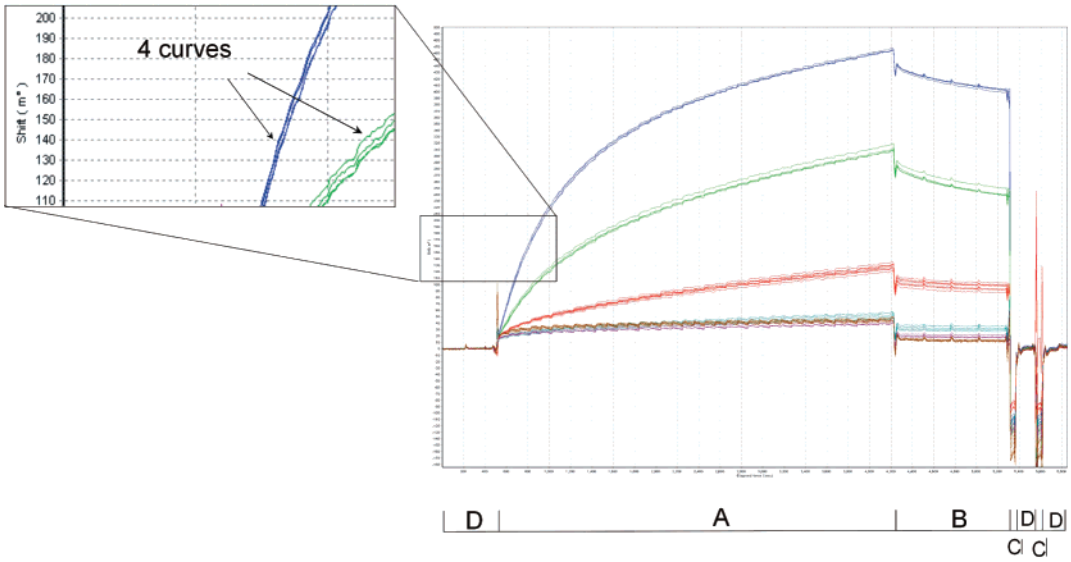


Figure 3. Typical interaction of RA patient antibodies with peptides immobilized on the SPR sensor chip. Sensorgram showing the 24 SPR dip angle shifts as a function of time. Baselines of the 24 regions of interests are zeroed. After the association phase (A), the array was washed with PBS, 0.03% Tween-20 (B), and regenerated with two sequential steps of 10 mM glycine·HCl, pH 1.5, for 30 s (C). In between the sequential glycine·HCl steps the array was rinsed with PBS, 0.03% Tween-20 (D). Blue (upper) and dark green lines (lowest) are the quadruplicates of CitA and the corresponding arginine control, respectively; light green (second upper) and purple lines are the quadruplicates of citrullinated CitB and the corresponding arginine control, respectively. Red lines are from human IgG ($n = 8$), and the brown lines are from regions close to the array (blank controls) ($n = 8$). The enlarged section shows the four individual curves from the quadruplicates of CitA and CitB.

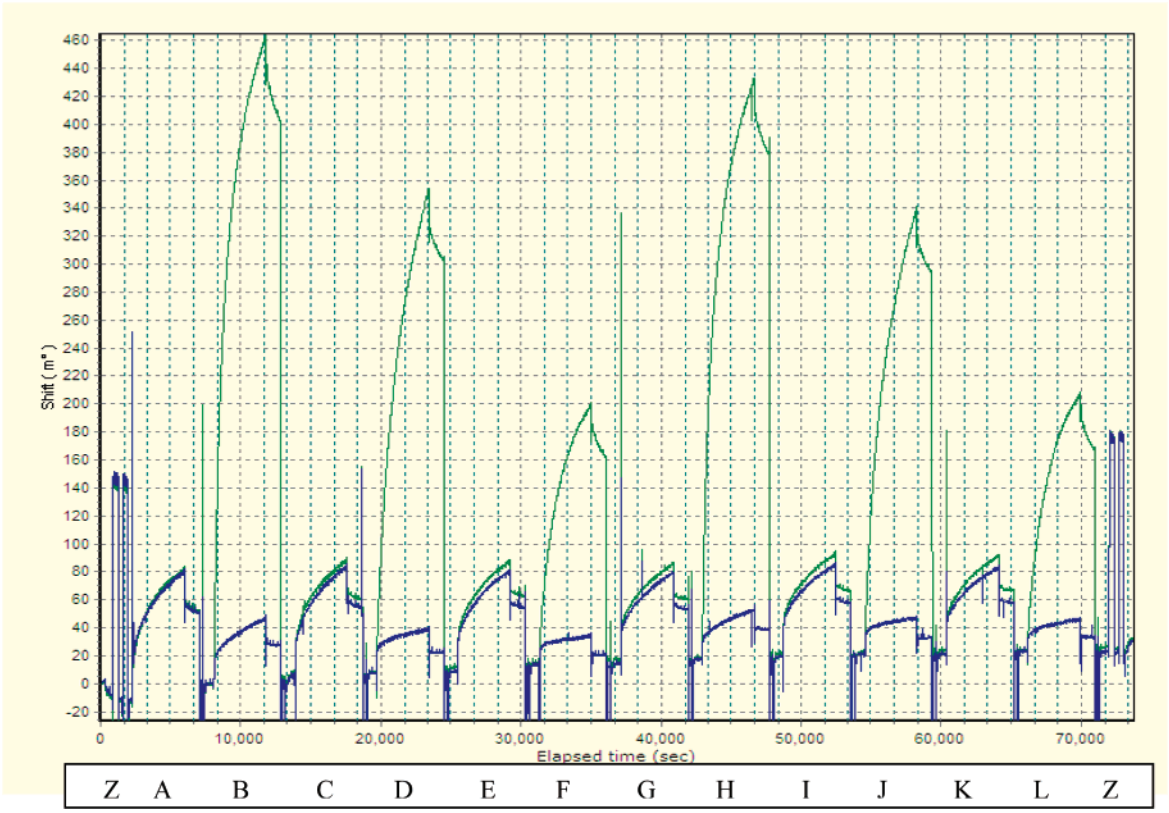


Figure 4. Reproducibility of sequential interactions. Sensorgram (raw data) of a citrulline peptide containing spot and its arginine control spot from the same array as shown in Figure 2. The array was probed two times with three different RA sera: (B, H) serum 1, (D, J) serum 2, and (F, L) serum 3. Between each incubation with RA serum the array was incubated with normal sheep serum (A, C, E, G, I, K) to increase the number of regenerations. After every serum (RA serum or NSS) the sensor was regenerated with 10 mM glycine·HCl. Residual serum components after regeneration will form a layer that blocks the aspecific binding sites, which hardly increase after application of more sera. At the beginning and end of the sensorgram a calibration cycle (Z) is performed for sensor calibration purposes by injection two times of a 1% glycerol solution.

monitoring. In this way the SPR dip of each spot is monitored real-time by fast scanning of the angle of incidence and imaging the corresponding reflected light beam via a CCD camera. Currently, SPR dip angles of microarrays of up to 500 ROIs can be determined and this number is limited by the current computing power, since collecting and processing the images

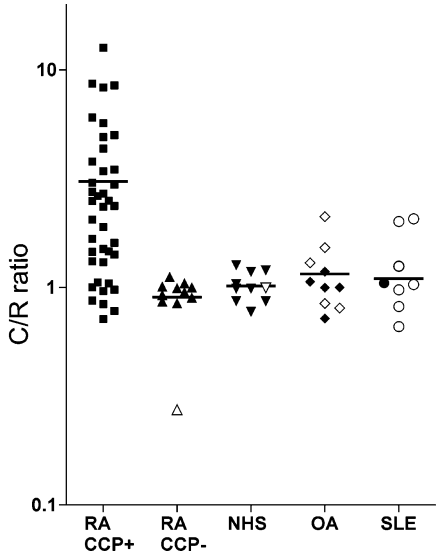


Figure 5. Autoantibody reactivity to a citrullinated peptide monitored by SPR imaging. Fifty RA sera (39 tested positive (RA CCP+) in the CCP ELISA and 11 tested negative (RA CCP−)), 10 normal control sera, 10 OA sera, and 9 SLE sera were tested. Sera for which the citrulline immobilized spots showed an angle shift of less than 10 mdeg are depicted with open symbols.

including the fast curve-fit calculations for all individual ROIs require a huge data transport.

Autoimmune diseases are characterized by the prevalence of autoantibodies recognizing self-proteins. Though many autoantigens have been identified and characterized, to date most of the assays that have been developed to detect autoantibodies to these antigens are ELISA-based, thus allowing only separate analyses for each type of autoantibodies, which is laborious, time-consuming, and not suitable for the development of multiplex systems. Recent observations that the specificity of the detection of anti-CCP antibodies in RA patient sera can be increased by monitoring reactivities with both citrulline- and arginine-containing peptides in parallel emphasize the need for multiplex analysis systems.[18] In most of the studies that monitor multianalyte protein−protein interactions, a secondary antibody conjugate was necessary to visualize bound antibodies. Here, we showed that SPR imaging of protein/peptide microarrays provides a method that allows a one-step multianalyte detection of autoantibodies in patient sera, which does not require additional reagents to visualize antibody binding. The ability to measure in a fully automated fashion with liquid handling procedures increases reproducibility, and once an experiment is started it can continue unattended. The ligand-containing sensor chips can be efficiently regenerated and reused, increasing the interassay reproducibility as well.

The smallest antibody concentration that could be measured when a peptide concentration of 1 ng/nL was used during generation of the array was 0.5 pM. Although the sensitivity of detecting RA-specific autoantibodies by the scanning SPR microarray imaging system under the conditions applied in this study is slightly lower than that of the ELISA systems, we expect that further optimization will lead to similar sensitivities. Moreover, for the low-titered sera a signal amplification step, e.g., by using a (gold-labeled) secondary antibody, may raise the sensitivity to levels that allow positive signals for all anti-CCP autoantibody containing sera.

The currently most widely applied target for the detection of anti-citrullinated protein antibodies, cyclic citrullinated peptides, are only recognized by about 70% of RA patients.[16] Due to the heterogeneity of the anti-citrullinated protein response in RA,[15] the use of additional citrullinated peptides may allow the detection of such antibodies in patients that are not reactive with the peptides used in the CCP2 ELISA. The use of microarrays monitored by SPR imaging will facilitate the simultaneous detection of the various anti-citrullinated protein antibodies.

The applicability of this novel scanning SPR technology goes beyond monitoring the presence of autoantibodies in sera of autoimmune patients. Real-time monitoring of the binding allows the user to study the association and dissociation rate constants for determining the affinity constants of the biomolecular interaction. Besides kinetic studies, the accurate measurement of the exact SPR dip angle allows the accurate comparison of each curve, including subtraction of, e.g., common mode effect (i.e., bulk shift jumps). This new scanning SPR technique will be of great use in any field that requires high-detection power and high-throughput analyses.

Experimental Procedures

Serum Samples. The sera were obtained from the Department of Rheumatology, University Hospital Nijmegen. Sera were collected from patients visiting the outpatient clinic who had been diagnosed as having RA according to the revised criteria of the American College of Rheumatology. To further assess specificity, we analyzed a group of serum samples from healthy individuals and groups of sera from patients with osteoarthritis and SLE, obtained from various clinics and hospitals. Sera were stored at −80 °C until used.

Preparation of Arrays. SPR detects changes in refractive index in the hydrogel (200 nm) which is linked to the gold surface. Due to the small molecular weight of the synthetic peptides used (∼1500 Da), the contrast of the immobilized array to the background is not high. To visualize the array, human IgG was spotted as well. The peptides and human IgG were spotted using a noncontact spotter[17] (1 nL drops with spotting concentration of 1 ng/nL in 50 mM Mes, pH 5.4) on EDC/NHS-activated Xantec HC 200 nm sensor chips and placed in a humidity chamber at room temperature for 1 h. Unreacted active groups were blocked with 1 M ethanolamine, pH 8.0, for 10 min. The sensor chip was rinsed with PBS and placed on a hemisphere with index-matching oil and inserted in the SPR microarray imaging instrument.

SPR Microarray Interaction Studies. Incubation, washing, and regeneration were performed in an automated way using liquid handling procedures (LHPs) in the instrument for biomolecular interaction sensing (IBIS-iSPR, IBIS Technologies BV, Hengelo, The Netherlands). A serum sample plug of 400 μL (diluted 1:50 in PBS, 0.03% Tween-20) was guided backward and forward over the array in a flow cell with a speed of 1 μL/s. The serum sample plug was surrounded by two air plugs to prevent the diffusion of serum components into the buffer. Between all steps the flow cell was rinsed with PBS, 0.03% Tween-20. The array was regenerated by injection of 400 μL of 10 mM glycine·HCl, pH 1.5, twice for 30 s. Two incubation/regeneration cycles were completed before applying the sera in order to block aspecific binding sites and create an optimal reactive sensor surface. Analysis of the data was done using the supplied software.

Peptide ELISA. Anti-CCP2 ELISA was performed by IMMUNOSCAN RA (Euro-Diagnostica, Arnhem, The Netherlands), in accordance with the manufacturer's instructions with the recommended 25 U/mL cutoff.

(18) Vannini, A.; Cheung, K.; Fusconi, M.; Stammen-Vogelzangs, J.; Drenth, J. P.; Dall'Aglio, A. C.; Bianchi, F. B.; Bakker-Jonges, L. E.; Van Venrooij, W. J.; Pruijn, G. J. M.; Zendman, A. J. W. *Ann. Rheum. Dis.* **2007**, *66*, 511−516.

Acknowledgment. We thank Dr. Jan Wouter Drijfhout (Leiden University Medical Centre, The Netherlands) for providing the synthetic peptides used in this study. This research is supported by the Dutch Technology Foundation STW, applied science division of NWO, and the Technology Program of the Ministry of Economic Affairs.

Supporting Information Available: Movie of the scanning SPR imaging principle (.avi). Every point of a sensorgram is obtained from the scanning angle process by calculating the minimum of reflectance of a region of interest from a spot of the microarray. The minimum of reflectance of various spots are at different SPR angles and accurately determined using high-speed imaging processing techniques. Complete ref 11. This material is available free of charge via the Internet at http://pubs.acs.org.

JA075103X