Using Artificial DNA Sequence to Suppress Non-specific Bindings in Crude Nuclear Extract During Surface Plasmon Resonance Assay

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Objective: Surface plasmon resonance (SPR) has been extensively used to characterize the interactions between molecules in terms of their binding specificity, affinity, and kinetics. Practical procedures, however, for measurement of the protein-DNA association in crude nuclear extract are yet to be defined.
Methods: Crude nuclear extract was obtained from MCF-7 cells or recombinant estrogen receptor alpha (ERα) was used for analysis. To suppress signal from non-specific bindings in SPR assay using Biacore, DNA fragments with minimal protein binding activity were identified in a database for transcription factors and included in the study.
Results: It is known that when analytes were purified transcription factors, the dissociation curves in Biacore sensorgrams exhibit exponential tendency. Based on statistical analysis, the dissociation phase between the ERα complex from crude nuclear extract and DNA oligonucleotides could be fitted exponentially. Following extrapolation of the dissociation phase, theoretical amount of bound antibodies could be estimated and compared for significant difference.
Conclusion: Our procedures made SPR technique such as Biacore a practical technique for measurement of protein-DNA associations in crude nuclear extract with reproducible and reliable results.

Key words: Surface plasmon resonance; Transcription factor; Crude nuclear extract; Protein-DNA binding; Estrogen receptor alpha

INTRODUCTION

The association of transcription factors to DNA is one of the crucial steps that initiate transcription of genes. Protein-DNA interactions are analyzed *in vitro* using such techniques as DNase footprinting [1] and/or electrophoretic mobility shift assay [2, 3]. When an antibody is used to identify a protein present in a protein-DNA complex during electrophoretic mobility shift assay (EMSA), the method is usually referred to as Supershift Assay. Ample knowledge concerning the specificity, affinity, and kinetics of protein-DNA association are brought to light with these techniques [4, 5]. Nowadays, chromatin immunoprecipitation (ChIP) has been employed [6] in large-scale genomic research of protein-DNA interaction [7]. Nevertheless, all these techniques are unable to reveal DNA-protein association and dissociation in real time such as the surface plasmon resonance (SPR) can exhibit [8].

Based on SPR mechanism, Biacore system allows measurement of changes in mass concentration on metal surface as molecules associate or dissociate [4]. The technique is well suited for determination of kinetic parameters, and both kinetic and affinity constants can be derived from the sensorgram data. An important chip made available by Biacore is the SA-Sensor Chip, which can be used to measure protein-DNA interactions. This chip was, for example, applied for measuring the kinetic constants of the interaction of the DNA-binding domain of hepatocyte nuclear factors (HNF)-3 alpha with its transferrin enhancer DNA specific target site [9]. In another study, it was used to analyze the difference in affinity for DNA between high mobility group (HMG) proteins 1 and 2 [10]. It is widely accepted that more information can be obtained by use of SPR assay than that of the corresponding conventional techniques.

Although other machines based on SPR have recently emerged in addition to the most recent Biacore model, only bindings between purified protein molecules and DNA oligonucleotides were analyzed in most of these studies [4]. Presently, there are only several reports including our own [11, 12] concerning the protein-DNA associations in crude nuclear extract using SPR technique [4, 13]. Due to the strong electrostatic nature of SPR, these authors have explored several new approaches in order to optimize experimental conditions for reproducible results. Despite of their efforts, it remains unclear how non-specific bindings could be identified. Furthermore, a statistical approach examining significance of the association in crude nuclear extracts is yet to be defined [14].

In the present study, DNA fragments with minimal protein binding activity were identified in a transcrip-

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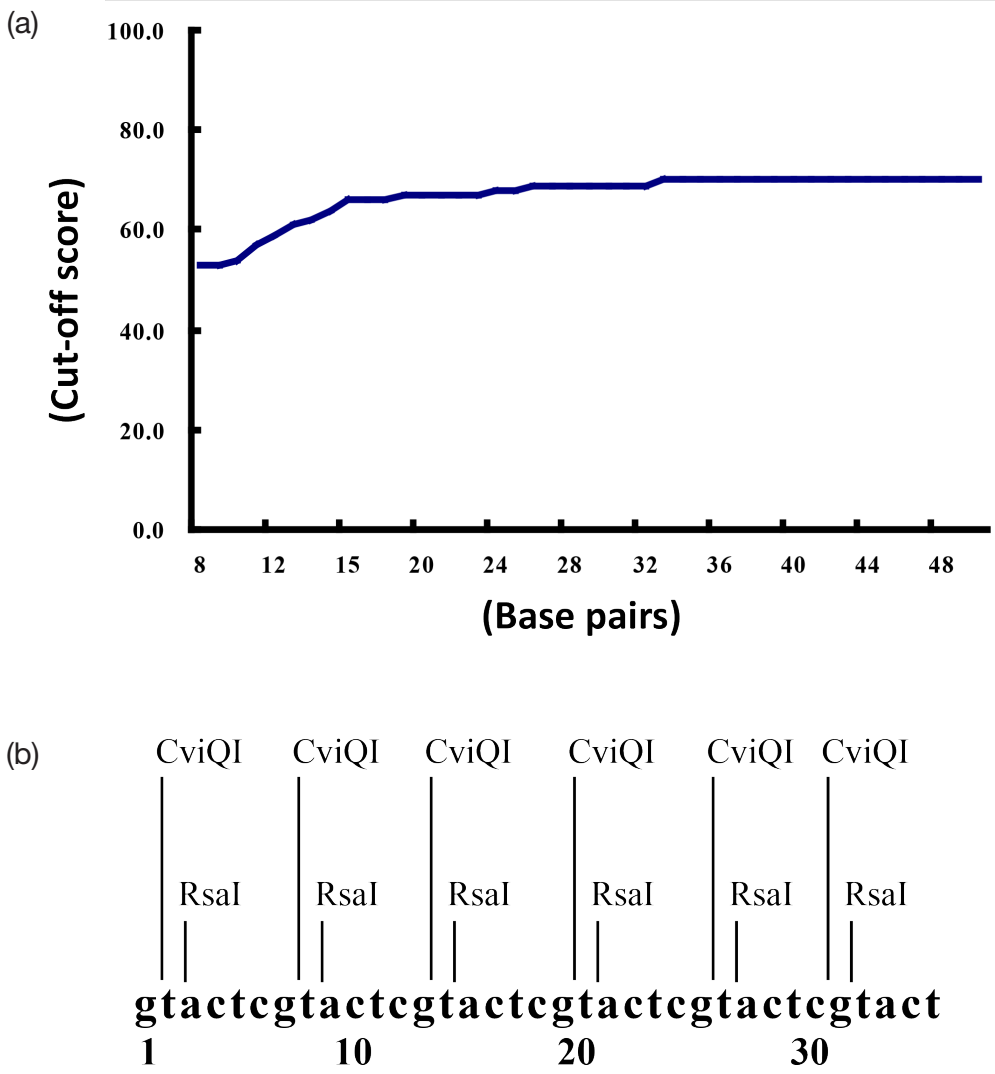

Fig. 1 Characteristics of DNA sequences with minimal cut-off scores
The minimal cut-off scores rise with the increase of DNA base pairs in length (a). Characteristics of one of the 62 sequences with the cut-off score ≤ 75 % and a core sequence sharing similar 35 base pairs in length (b). Some of the sites for restriction enzyme were indicated.

tion factor database and included in a SPR analysis using Biacore to suppress signals from non-specific bindings. Further experiments showed that the dissociation phase for the bound ERα complex from crude nuclear extract could be expressed exponentially, and the values of bound ERα complex could thus be extrapolated after the inflow of anti-ERα antibodies. Significant difference was defined by comparing the amount of bound antibodies between the wild-type and mutant DNA group. Our procedures made SPR analysis like Biacore a practical technique to measure protein-DNA complex in crude nuclear extracts with reproducible and reliable results.

MATERIALS AND METHODS

DNA sequences with minimal protein binding activity

To identify artificial DNA sequences that do not specifically bind to the known protein molecules, an online free program MATCH™ (pub ver 1.0) was used to search the TransFac Public database (matrixTFP60.lib) for all matrix groups including *Homo sapiens* (http://gene-regulation.com/). The default matrix similarity score was set at ≤ 70 %, which describes the quality of a match between a matrix and an arbitrary part of the input sequences. The default core similarity was set at ≤ 75 %, which denotes the quality of a match between the key sequence of a matrix and a part of the input sequence. The core similarity was used as a cut-off score, which in part reflects the binding specificity or affinity between a DNA sequence and one or a family of protein molecules [15]. For all the possible combination of four DNA bases up to 48 pairs in length (4^{48}), the sequences with the cut-off score ≤ 75 % were supposed that they did not contain a specific motif (NoMf) (Fig. 1a), and therefore pooled for further analysis. Hence, a core sequence sharing a similar 35 base pairs in length was found (Fig. 1b), and the protein binding activities in the 32 base pairs (NoMf-32) of which were tested in the following experiments (Table 1).

Preparation and immobilization of DNA oligonucleotides

Estrogen Response Element (ERE) can be grouped

Table 1 Oligonucleotides for protein-DNA binding assay

Oligonucleotides	Motif sequences	Cut-off score (%)
NoMf-32	5`-gta ctc gta ctc gta ctc gta ctc gta ctc gt	< 75 %
* EREm32	5`-ttg atc aag gcc ctg aca cca gac ttt ctc ga	< 75 %
EREw32	5`-ttg atc agg tca ctg tga cct gac ttt ctc ga	100 %

*ERE, Estrogen Responsive Element containing oligonucleotides.

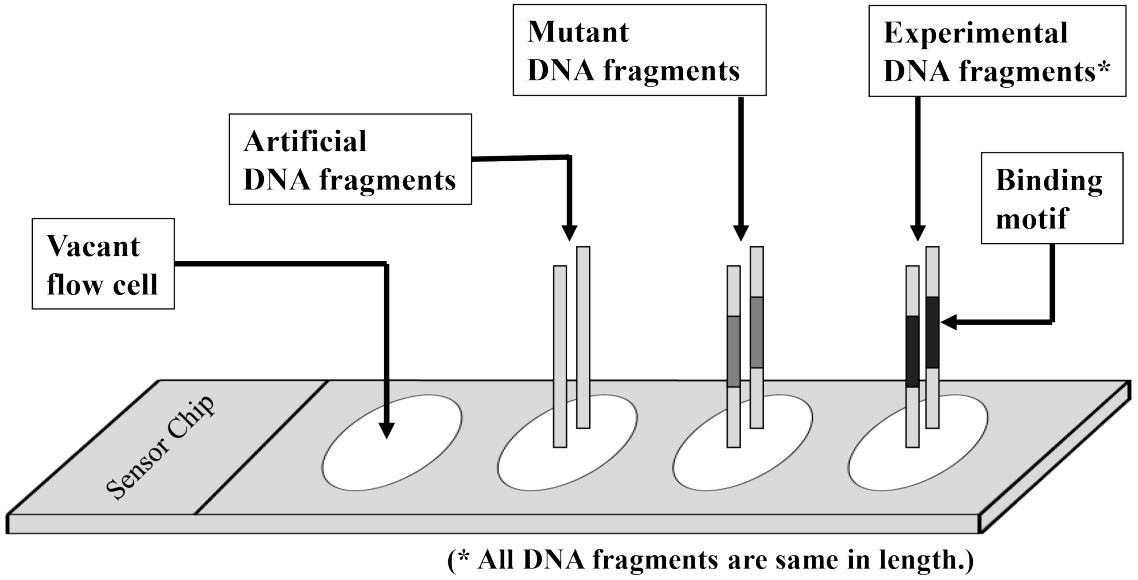

Fig. 2 Immobilization of DNA fragments in each of the flow cells on Biacore SA-sensor chip

into perfect and imperfect palindromic sequences with the imperfect sequences differing from the consensus sequence in one or more nucleotides. It is generally accepted that a perfect ERE does not exist in human DNA. Therefore, the bottom strand sequence information for the wild-type estrogen responsive element ERE (Table 1) in *Xenopus* vitellogenin A2 gene (No. X00205.1) was obtained from GenBank (NIH, Bethesda, MD, USA) and arbitrarily named (EREw32). Its mutant (EREm32) DNA sequence was generated using TransFac Public database for *Homo sapiens* with the cut-off score ≤ 75 [15], in order to confirm that the score system for the affinity of DNA-proteins in the TransFac works in the present experimental system. Biotinylated and purified DNA oligonucleotide fragments were obtained from Invitrogen (Carlsbad, CA, USA). Only the 3` ends of the bottom strand were biotinylated. To make double-stranded oligonucleotides, the non-biotinylated top strand and the biotinylated bottom strand were added at a molecular ratio of 3 : 1 to a standard PCR buffer containing 25 mM $MgCl_2$. Then the oligonucleotides were annealed by heating at 98 ˚C for 10 min, cooling to 50 ˚C at rate of about 2 ˚C/min, and then cooling on ice. Obtained double strands could be subcloned and sequenced (data not shown).

SPR assay using Biacore

Protein-DNA binding experiments were conducted as we and others previously reported [11, 16, 17] using Biacore 2000 system, Sensor Chip SA, and HBS buffer (pH 7.4) containing 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, and 0.005 % surfactant P20 (from Biacore AB, Uppsala, Sweden). The amount of molecules detected by Biacore is expressed as the resonance unit or response unit (RU). To immobilize the biotinylated DNA fragments to the sensor chip, 0.1 μM of the annealed oligonucleotides in HBS buffer containing 150 mM NaCl was injected at a rate of 5 μl/min over one of the four flow cell of SA Sensor Chip. The first flow cell was left vacant (Fig. 2); the second flow cell was immobilized with the DNA oligonucleotides without known protein-specific binding motifs. The remaining two were kept for oligonucleotides containing ERE motifs and their respective mutants. The average amount immobilized was 350 ± 5 RU for each type of oligonucleotides.

HBS buffer with 25 ng/μl poly [d (I-C)] was used to prepare the nuclear proteins for experiments. Human recombinant ERα (Promega Biosciences; Madison WI) was injected at a concentration of 8 footprinting units/ml, corresponding to 0.005 μg/μl of nuclear protein. A final concentration of 0.10, 0.15, or 0.25 μg/μl was used for crude nuclear extract from MCF-7 human cancer cells (Santa Cruz Biotechnology, Dallas, TX). The samples were incubated at 24 ˚C for 15 min. Crude nuclear extracts may contain other ERE binding transcription factors such as ER beta, and therefore, it is necessary to use anti-ER alpha antibodies to identify specific bindings. The anti-ERα antibody

Table 2 Results of fitting dissociation phases

Oligo	Para*	ERα Complex	MCF-7
EREw32	Y0	53.28 ± 0.16	56.46 ± 0.58
	A1	171.07 ± 24.88	662.47 ± 98.14
	Kd	123.29 ± 6.21	97.97 ± 13.75
EREm32	Y0	24.25 ± 0.21	35.68 ± 1.08
	A1	190.56 ± 30.91	101.92 ± 20.68
	Kd	113.03 ± 9.34	202.98 ± 27.23

*, parameters for exponentiation: $Y(T) = Y0 + A1 * e^{(-T/Kd)}$

(MC-20) was from Santa Cruz Biotechnology as reported previously [11], and the anti- ERα phosphorylated at serine 106 antibody (anti-pS106 ERα) was purchased from Abcam (330 Science Park, Cambridge CB4 0FL, UK). Each association/dissociation cycle was performed at a constant flow rate of 30 µl/min. For DNA-protein binding reactions, each sample was injected over the four flow cells for 5 min, followed by a constant flow of HBS buffer for 5 min to observe dissociation of bound proteins. Then 0.02 µg/µl of an antibody in HBS buffer was injected in the same manner. At the end of each cycle, bound proteins were removed by two-pulse injections of 1.5 M NaCl containing 0.05 % of surfactant P20 for 30 second (Sec) each to regenerate the chip. Sensorgrams were recorded automatically and adjusted to zero baseline level by subtracting the response recorded immediately before the injection of each sample. Each measurement was repeated three times with identical results. Sensorgrams were presented by subtracting the responses from the vacant flow cell or as indicated otherwise.

Analysis of sensorgrams

Exponential regression and exponential order

Although it is not known exactly how many molecules in crude nuclear extract bind to DNA oligos, the bound molecules could be considered as a complex of molecules in dissociation phase. Therefore, statistical method based on the exponential Langmuir Modes for purified analyte [18] could be tentatively applied to our data, and the implication of suitable functions might arise from trying to fit the bound proteins in dissociation phase using OriginPro 8.0 (OriginLab Corporation, Northampton, MA, USA). Presently, it is considered that there are no standard criteria for discrimination of the goodness-of-fit in terms of the exponential order [19]. Therefore, suitable exponential function and order approximate to the dissociation values between 380 and 680 sec were assumed to abide by three conditions in extrapolation (Fig. 5). First, the extrapolated values of bound nuclear proteins beyond 900 Sec should be lower than that of the antibodies. This is because the dissociation rate of antibodies is generally very slow. In addition, values of the bound antibodies in dissociation phase should always be higher than the extrapolated values for bound nuclear protein. And thirdly, these values at any time points should approximate zero gradually. Furthermore, Reduced Chi-squared (Chi-Sqr) test, Adjusted (Adj.) R-Square test, and Residual Plot Analysis were employed for evaluation of goodness-of-fit.

Extrapolation of bound nuclear proteins and antibodies

Using the exponential functions in Table 2, it was possible to extrapolate and plot the values after the injection of antibodies. Furthermore, the area-under-curve (AUC) from 1000 to 1200 Sec was presented as the sum of bound antibodies and nuclear proteins (AUCan) in the dissociation phase of antibodies. All calculation were carried out using OriginLab 7.5. Theoretical AUC of bound nuclear proteins (AUCnp) was calculated in the same way. The amount of bound antibodies (AUCab) in its dissociation phase was obtained by subtraction of AUCnp from AUCan, i.e., AUCan = AUCab + AUCnp.

RESULTS

Artificial DNA sequence with minimal protein binding activity

Searching all possible combination of four DNA bases up to 48 pairs in length (4^{48}) against TransFac Public database for all matrix groups (http://gene-regulation.com/), 62 sequences with the cut-off score ≤ 75 % (Fig. 1a) were identified (S. 1). These sequences exhibit a core sequence of 35 base pairs in length, and can be recognized by such restriction enzymes as CviQI, RsaI (Fig. 1b), MSPJI, and SgelI. The cut-off score was as low as 52 for a sequence of 8 base pairs in length. Then the cut-off score reaches a plateau of 75 % for sequences between 33 and 48 base pairs. Further searching the core sequence against the databases of NBCI Genomes and human and mouse genomic plus transcript [20] with BLASTN 2.6.0 + [21] showed that no significant similarity was found, which indicates that the presented sequences are artificial. For the core sequence of 35 base pairs in length, the protein binding activities in the 32 base pairs (NoMf-32) were tested in the following experiments (Table 1).

Following association of either recombinant ERα or nuclear extract from MCF-7 cells, while responses from NoMf-32 oligos with the minimal protein binding activity could always be detected (Fig 3a, c; Fig 4a, c), they were always lower than that of mutant (EREm32) and wide type ERE (EREw32). For recombinant ERα, while the highest value of 67.45 RU was detected on EREw32, 40.23 RU was shown on EREm32, but only 15.82 RU was exhibited on NoMf-32. For crude nuclear protein, while the highest value of 216.67 RU was detected on EREw32, 200.32 RU was shown on EREm32, but only 166.35 RU was exhibited on NoMf-32. It should be noted that the bound molecules from crude nuclear extract dissociate faster from NoMf-32 and EREm32 than that from EREw32. The results indicate low affinity of the DNA oligos with the minimal

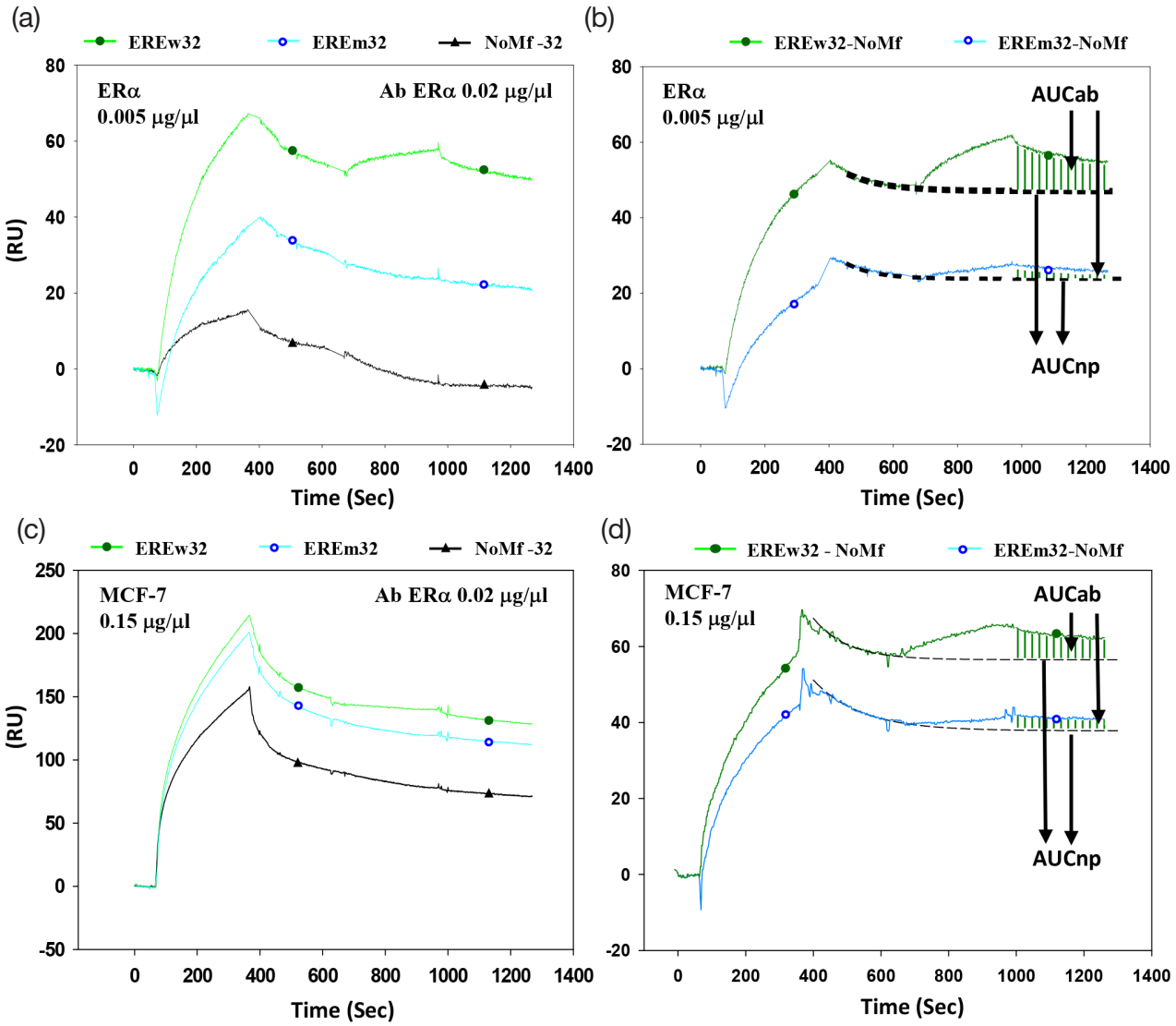

Fig. 3 Sensorgrams showing binding of recombinant ERα or ERα in crude nuclear extract
After association of 0.005 μg/μl of recombinant ERα (a, b) or 0.15 μg/μl of nuclear extract (c, d) anti-ERα (MC-20: sc-542) was injected. Sensorgrams were plotted after subtraction of responses from vacant flow cell (a, c) or that immobilized with NoMf-32 (b, d). The dashed lines were extrapolated and plotted based on regression of dissociation phases for bound nuclear proteins (Table 2). Area Under Curve for antibodies (AUCab) or nuclear proteins (AUCnp) was indicated based on Table 4. The amount of molecules detected by Biacore is expressed as the resonance unit or response unit (RU).

protein binding activity.

The responses differ markedly following inflow of antibodies. After injection of anti-ERα antibody, no obvious visual responses could be observed in NoMF-32 or EREm32 (Fig. 3a). However, a slightly uprising curve for EREm32 was exhibited after subtraction of NoMf-32 (Fig. 3b). Similar responses were also shown in the dissociation phase of crude nuclear extract (Fig. 3c, d), indicating the highest rise in sensorgrams for EREw32 was always observed for recombinant ERα and nuclear extract (Fig. 3, 4). It should be noted that there was an acute and sharp rise in sensorgram when inflow of antibody against phosphorylated (S106) ERα (Fig. 4) was initiated, and then a drastic decrease was exhibited in dissociation phase. For recombinant ERα (Fig. 4a), while the highest value of 269.67 RU was exhibited on EREw32, 204.58 RU was shown on EREm32, but even 183.33 RU was detected on NoMf-32. For crude nuclear protein (Fig. 4c), while the highest value of 296.37 RU was recorded on EREw32, 252.52 RU was reflected on EREm32, but 233.35 RU was still detected on NoMf-32. The results showed that the anti-pS106 ERα recognize phosphorylated ERα that are mostly not bound by the anti-ERα antibody (MC-20). One of the reasons might be that a large amount of phosphorylated ERα and its co-factors could be loosely associated either specifically or non-specifically. All these results indicate that using vacant flow cell as a control might lead to deviation in evaluation of DNA motif-specific bindings.

Exponential order and goodness-of-fit

To extrapolate the dissociation values after injection of antibody, 0.25 μg/μl of MCF-7 nuclear extract was injected for 5 min, followed by inflow of running buffer for 15 min as an actual dissociation phase (Fig.

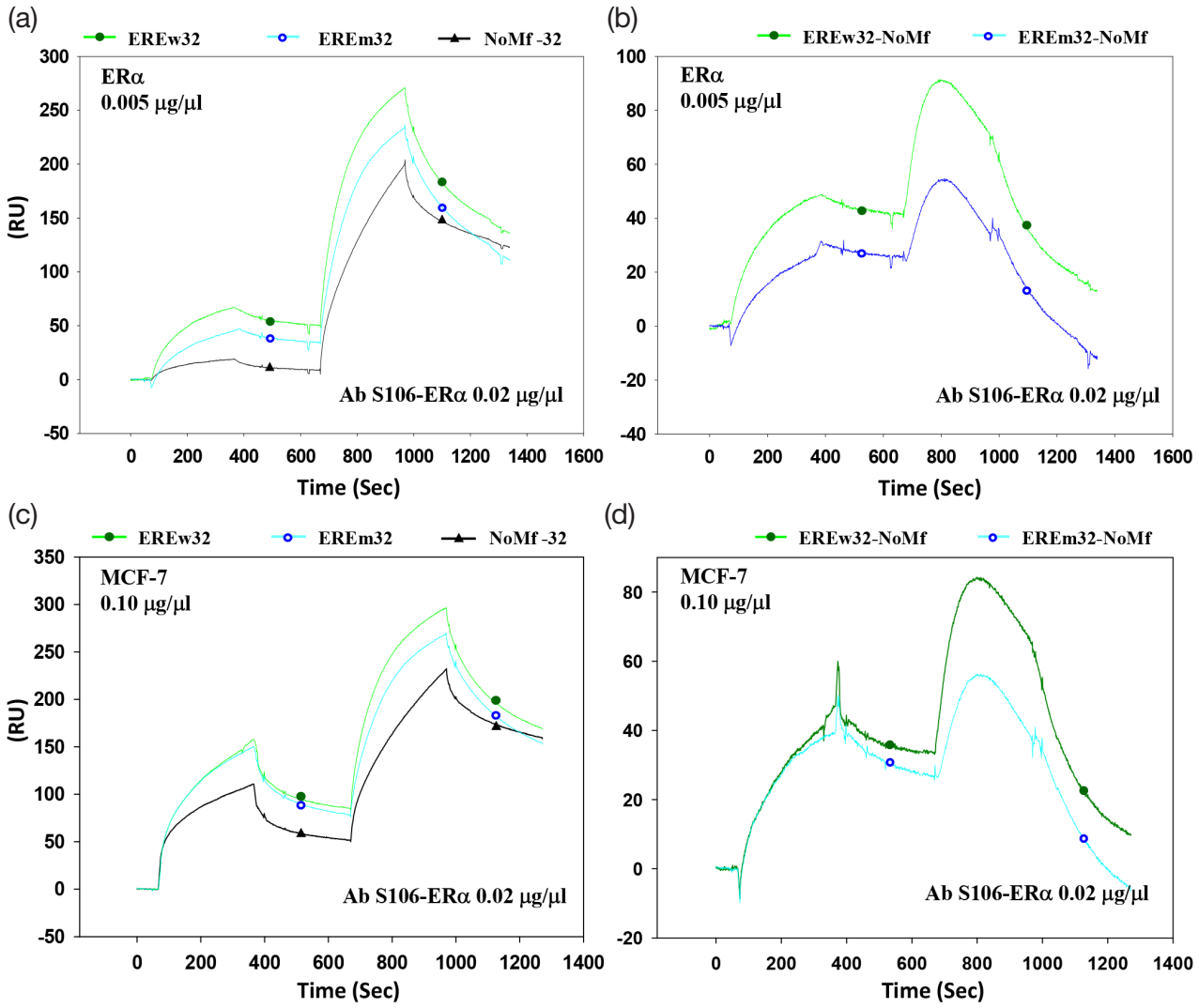

Fig. 4 Binding activities of ERα phosphorylated at serine 106 (pS106 ERα)
After association of 0.005 μg/μl of recombinant ERα (a, b) or 0.15 μg/μl of nuclear extract (c, d) anti-pS106-ERα (ab75753) was injected. Sensorgrams were plotted after subtraction of responses from vacant flow cell (a, c) or that immobilized with NoMf-32 (b, d). The amount of molecules detected by Biacore is expressed as the resonance unit or response unit (RU).

5a). Based on the values from the first 5 min of dissociation phase (Fig. 5b), a bi-exponential equation was established. The values were extrapolated and plotted for 10 min (Fig. 5c). A nearly perfect overlapping was shown when merging the dissociation phase (Fig. 5d) in running buffer (Fig. 4a) and extrapolation (Fig. 5c). The results indicate the feasibility to analyze the dissociation phase.

After subtraction of the NoMf-32 values from EREw2 (Fig. 3b) or EREm32 (Fig. 3d), the dissociation phase for ERα or ERα complex from crude nuclear extract could be fitted mono-exponentially (Table 2). Statistical analysis of goodness-of-fit (Table 3) showed that Degrees of Freedom from ANOVA linear fitting was 98 for EREw32 and 99 for EREm32 with bound ERα, being better fitted than that with bound ERα complex. Similar tendency could also be identified in results from Reduced Chi-squared (Chi-Sqr) and Adjusted (Adj.) R-Square test. The Residual vs. Independent plot from Residual Plot Analysis (Fig. 6) showed that most of the residuals are distributed within ± 0.5 of range along the time axis.

Extrapolation of bound nuclear proteins and antibodies

Beginning from the inflow of anti-ERα antibodies in the present study, each actual data point represents both the bound nuclear proteins and antibodies, including the non-specific signals. Based on the exponential function and parameters of regression (Table 2), the values of dissociation were extrapolated after inflow of the antibody and plotted for the bound ERα (Fig. 3b) or ERα complex from crude nuclear extract (Fig. 3d). Calculation of AUC showed that the bound antibodies (Table 4) against recombinant ER on EREw32 was 9.51 times more than that on EREm32 (AUCab: EREm/w32). On the other hand, the bound antibodies on EREw32 against ERα from crude nuclear extract were 3.78 times more than that on EREm32 (AUCab: EREm/w32).

Table 3 Goodness-of-fit for dissociation phases

Statistics	ERα Complex		MCF-7	
	EREw32	EREm32	EREw32	EREm32
Number of Points	101	102	86	96
Degrees of Freedom	98	99	96	97
Reduced Chi-Sqr	0.02	0.03	0.42	0.35
Adj. R-Square	0.99	0.97	0.95	0.94

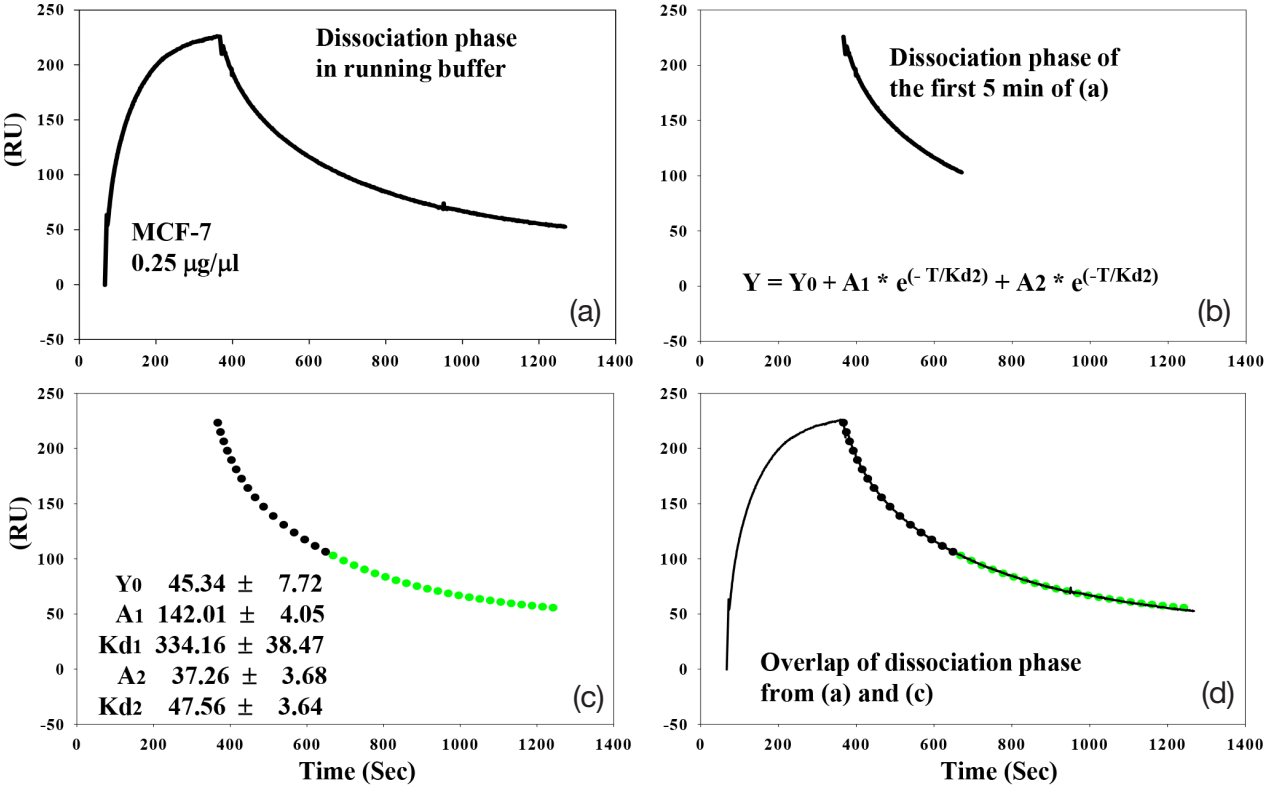

Fig. 5 Fitting dissociation phase of the bound ERα complex on EREw32
Crude nuclear extract was injected for 5 min, followed by a dissociation phase in running buffer for 15 min (a). The first 5 min of dissociation was fitted bi-exponentially (c) and the extrapolated curve was merged with the actual dissociation phase (d). Sensorgrams were presented by subtracting the responses from the flow cell immobilized with NoMf-32. The amount of molecules detected by Biacore is expressed as the resonance unit or response unit (RU).

DISCUSSION

Although analysis based upon the surface plasmon resonance (SPR) like Biacore has been extensively used in measurement of the association and dissociation between molecules, there are only a few reports concerning the protein-DNA complex in crude nuclear extracts [11−14]. In the present study, the DNA sequences with the minimal protein binding activity were identified and included in Biacore assay. After subtraction of the non-specific bindings to these sequences, it was found that the dissociation for bound ERα complex in crude nuclear extract could be expressed exponentially, which made it possible to extrapolate the values of bound ERα complex after the inflow of ERα antibodies. Then significant association could be defined by comparing the amount of bound antibodies between the wild-type and mutant DNA group.

Artificial DNA sequences with minimal protein binding activity

Searching all possible combination of four DNA bases up to 48 pairs in length against TransFac Public database for *Homo sapiens*, 62 sequences with a core sequence of 35 base pairs in length (Fig. 1) were identified with a cut-off score set at ≤ 75 %. Further searching the core sequence against the databases of NBCI Genomes and human and mouse genomic plus transcript [20] with BLASTN 2.6.0 + [21] showed that no significant similarity was found, which indicates that the presented sequences are artificial. For both purified crude recombinant ERα and MCF-7 crude nuclear protein, the results from Biacore assay showed that the core artificial sequence carried the lowest protein binding activity (Fig 3, 4). By subtraction of the binding values from the core sequence, an indistinctive associations between the wild and mutant ERE oligos became visually evident especially for the crude nucle-

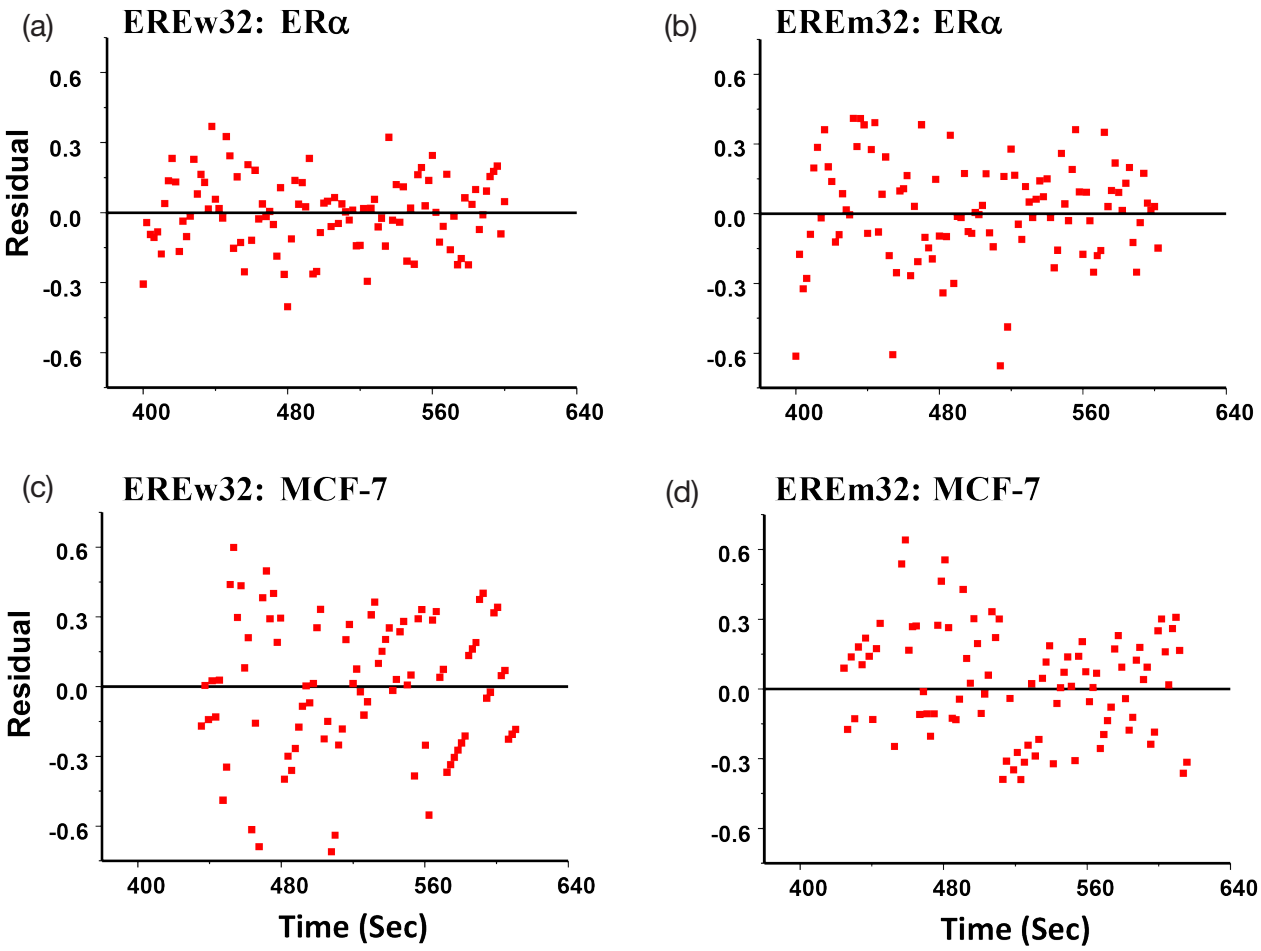

Fig. 6 Residuals showing goodness-of-fit
Residuals of exponential dissociation for recombinant ERα bound to EREw32 (a) or EREm32 (b), and for ERα complex in crude nuclear extract bound to EREw32 (a) or EREm32 (b).

Table 4 Amount of bound anti-ERα antibody

Area Under Curve (RU·Sec)	ERα *		MCF-7 #	
	EREw32	EREm32	EREw32	EREm32
AUCan	12652.95	8264.85	12262.77	5354.14
AUCnp	6159.21	7581.72	10661.72	4930.61
AUCab	6493.74	683.13	1601.72	423.53

*, AUCab: EREw/m32 = 9.51; #, AUCab: EREw/m32 = 3.78

ar extract (Fig. 4).

Due to the strong electrostatic nature and hyper sensitivity of SPR, such binding controls as the vacant flow cell, fragmented genomic DNA of E. Coli, poly [d (I-C)], or poly [d (A-T)] in some studies might make it hard to obtain reproducible results for a protein-DNA complex in crude nuclear extracts. A DNA oligo with equal length to that of the experiment without specific binding motifs is essential to detect non-specific bindings (Fig. 4) and obtain reproducible, comparable, and reliable results. As the DNA sequences identified in the present study are 48 base pairs in length, they could cover most of the DNA sequences that specifically bind proteins. In addition, because both to recombinant ERα or ERα complex in nuclear protein, the binding value is always the lowest for our artificial DNA sequence, such sequences could be used for other sequences in experiments *in vitro* just like the present study.

Exponential order and goodness-of-fit

It is considered [19] that the criteria for discrimination of the exponential order are still a controversial issue, and a higher score for goodness-of-fit does not necessarily indicate a better regression. Between purified molecules, mono-exponential Langmuir Model [8] is usually employed for dissociation phase of a 1:1 interaction. In the present study, implication of the exponential tendency in dissociation phase was derived from linear regression after various transformations of the binding values and time, and our data could be fitted to mono- or bi-exponential function, with the average scores being higher than 95 % at default setting.

Although the actual mechanism is not known, it is

possible that for numerous exponential dissociations that occur simultaneously, measured data could be fitted to a sum of exponentials. Theoretically, the exponential order depends upon the difference in their initial values and dissociation constants [19]. Accordingly, if bound proteins are close in their dissociation constants and/or initial values, the data from crude nuclear extracts could still be fitted mono-exponentially. Therefore, Langmuir Model is still applicable in simultaneous events of numerous exponential dissociations.

Extrapolation of bound nuclear proteins and antibodies

Although it has been empirically determined that a significant binding reaction could be expected when the ratio of the amount of antibodies to antigens is more than two fold, no clear definition for significant association has been reported for a protein-DNA complex from crude nuclear extracts [13, 14]. Beginning from the inflow of anti-ERα antibodies in the present study, each actual data point represents both the bound nuclear proteins and antibodies, including the non-specific signals. Although a core sequence and then the mutant ERE always exhibits lower binding value than that of wide type ERE even in crude nuclear extract, using antibody is still a reliable procedure to further confirm that a target molecule exists in bound ERα complex. Our results indicate that because exponential fitting of the dissociation data allows extrapolating the amount of bound nuclear proteins and corresponding antibodies in dissociation phase, it is feasible to compare the amount of bound antibodies between the experimental and mutant statistically so as to define significant association.

CONCLUSIONS

Our results showed that both statistically and experimentally the existence of artificial DNA sequences with minimal protein binding activity. These sequences could be used in SPR analysis to suppress non-specific bindings for protein-DNA complex especially in crude nuclear extracts. Based on the characteristics of these sequences, mutant oligos with minimal protein binding activity could be designed for analysis of protein-DNA binding specificity and binding site mapping. In the dissociation phase of SPR assay, extrapolation of bound molecule complex in crude nuclear extract made it possible to determine significant binding of the corresponding antibodies for a target molecule. By following these procedures, SPR assay like Biacore could be used to measure protein-DNA complex in crude nuclear extract with consistent and reliable results.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

SUPPLEMENTARY MATERIALS

S. 1 All 62 artificial DNA sequences identified in the present study. These 62 sequences share a similar 35 bases of sequence gtactcgtactcgtactcgtactcgtactcgtac

actatagtactcgtactcgtactcgtactcgtactcgtactatactat
actatagtactcgtactcgtactcgtactcgtactcgtactatactac
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