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# **Critical parameters in surface plasmon resonance biosensor development: the interaction between estrogen receptor and estrogen response element as model**

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## Abstract

Estrogenic compounds are contaminants that may be active at low concentrations and are a major concern for environmental quality. They interact with organisms via Estrogen Receptors (ER). Some detection methods which have been developed use the ability of ER to interact with short consensus DNA sequences known as Estrogen Response Elements (ERE). Surface Plasmon Resonance (SPR) based techniques allow detection of interaction without labelled molecule use. Such optical transducers are widely used to convert the biological recognition signals into electric quantifiable signals. In this study, SPR is used to assess signal variation in the presence of estrogenic compounds. The combination of physical properties and biological recognition events (e.g. ER/ERE) permits the development of biosensors. These require several steps: activation of the surface, DNA sequence binding, ERE sequence evaluation, ER preparation, characterization of binding properties and regeneration of the surface. This article focuses on the mode of surface activation, protein-DNA binding conditions and the regeneration of ERE. After giving a summary of the literature concerning the usual conditions employed in these steps, an evaluation of some key parameters is given.

Keywords: biosensor, surface plasmon resonance, DNA, protein, binding, estrogen receptors

## 1. Introduction

In biosensor development for the detection of molecules and for monitoring molecular interactions several methodologies are used. This is due to variations in locally developed laboratory procedures and most of the time these procedures (e.g. the choice of the buffer and/or reactants) are not justified. This complicates the reproducibility of experiments and the possibility of comparing results from different studies. Indeed, discrepancy in the results is observed when the data has not been obtained with equivalent samples, or the same experimental conditions and quantification procedures. For instance, in estrogen receptor (ER) estradiol (E2) interaction studies, 9 different procedures were used in 11 articles [1]. These varied in terms of the ER concentration, the solvent used for E2 solubilisation and its final concentration in the reaction medium, composition of the buffer, the duration and temperature of ER and E2 incubation before analysis using an SPR instrument. The regularization of these parameters is nonetheless critical as they do not underline the same molecular effects, and can confuse ER dimerization [2] and ER denaturation [1] for example. These same parameters can also affect ER/ERE interaction, as previously shown [1]. Other factors, such as the protein thawing method or the ER/E2 mixing temperature are also essential [3]. Altogether, all protocol parameters must be detailed prior to the determination of biophysical parameters such as affinity constant or the quantification of molecules in a given sample.

To aid the establishment of parameters in future studies, these key factors should be studied and detailed in the literature. To this end, the present publication aims to detail the importance of the first steps in biosensor development for protein-DNA interaction experiments. Attention is therefore focused on the activation of the sensor surface, the buffers' effect on DNA binding, the regeneration of the surface, the evaluation of the efficiency of the regeneration step and finally the effect of time and temperature on ER binding levels after overnight incubation. We first summarize the existing methods described in the literature and then compare those most frequently employed. Experiments were performed on the optical biosensor based on the surface plasmon resonance included in BIAcore instruments, and output of the study can be generalized to other types of biosensors based on other label-free technologies.

## **2. Materials and methods**

### **2.1. Reagents**

All chemicals used were of analytical grade. The human recombinant ER $\alpha$ , 17 $\beta$ -estradiol (E2), and Estrogen Response Element (ERE) used were provided as has been previously described [1]. ERE sequences came from the promoter region of gene vitellogenin A2 (adapted from Cheskis *et al.* [4]).

### **2.2. Materials**

Experiments were performed using the BIAcore 1000 biosensor system. The sensor chips used were streptavidin-coated sensor chips (sensor chip SA, GE Healthcare Bio-Sciences AB, Uppsala, Sweden).

### **2.3. Methods**

#### **2.3.1. Surface activation**

Three solutions have been tested to activate the surface of SA sensor chips. Solution 1: 50 mM NaOH, solution 2: 50 mM NaOH and 1 M of NaCl and solution 3: 0.1% sodium dodecyl sulphate (SDS).

#### **2.3.2. Buffer choice for ERE binding**

In previous literature, different running buffers have been used to associate biotinylated ERE on streptavidin (SA) sensor chips (Table 1). In this study, two of these were compared, the HBS-EP buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA and 0.005% surfactant P20, pH 7.4, supplied by Biacore, GE healthcare) and TNMT buffer (50 mM Tris-HCl, 150 mM NaCl, 10mM MgCl<sub>2</sub>, 0.05 % Tween 20, pH 7.5). All buffers used in this study were of analytical grade, and were filtered (0.22  $\mu$ m) and degassed properly before use. ERE sequence was solubilized either in HBS-EP or TNMT buffers to a final concentration of 450 nM (10 mg/L), then 10  $\mu$ L of each solution was injected onto a SA sensor chip. The ERE binding level was determined 400 s after the beginning of the injection, the surface having been returned to the running buffer.

#### **2.3.3. Regeneration of ERE sequence**

Several methods for ERE regeneration have been used in the literature (Table 2). The two most frequent were tested here. The first consisted of the injection of 50 mM of NaOH. This solution aimed to dissociate both protein and the unbiotinylated DNA strand. This method then required the injection of the complementary single strand ERE to reconstitute the double strand DNA. The second method targeted only the bound ER protein on the ERE. The injection of 0.1% SDS solution (20  $\mu$ L/min) allowed the dissociation of ER from ERE.

#### **2.3.4. ER preparation**

ER (2600 nM stock solution) was diluted in TNMT buffer to a final concentration of 100 nM. ER solution was then equally diluted with estrogenic compounds (from  $2 \cdot 10^{-6}$  M to  $2 \cdot 10^{-9}$  M of E2 containing 0.2% methanol). The final concentration of ER was 50 nM containing estrogenic compound and 0.1% of methanol. The preparation was then incubated overnight at 4°C and kept in 4°C or ambient temperature until SPR analysis.

Accepted manuscript

### 3. Results

#### 3.1. Activation of sensor chip surface

Several conditioning solutions have previously been used to activate the sensor chips SA as described in the literature dealing with ER/ERE interaction experiments (Table 1). The use of a conditioning solution is an essential first step to remove protein contaminants and dextran loosely bound on the surface. This then allows the binding of the biotinylated ERE on the surface.

Among the methodologies detailed in Table 1, two were chosen for testing. Both required NaOH injection either alone (Nilsson *et al.* (1995)) or in association with 1 M NaCl. However, when the latter solution (50 mM NaOH, 1 M NaCl) was used, NaCl crystals on the sensor chip were visible to the naked eye upon the chips' removal from the apparatus (data not shown). This solution was consequently excluded from further tests. The first solution (50 mM NaOH) was preferred. In routine experiments, two injections of 20  $\mu$ L of NaOH solution (50 mM) at a flow rate 20  $\mu$ L/min are performed on the SA commercial surface. The first injection reduces the baseline level by 400 - 600 RU and the second one by 50 - 200 RU (Figure 1).

We routinely added a second step in the activation process which consisted of an injection of 0.1% SDS. The injection of SDS was found to decrease SPR signal by 100 - 400 RU (Figure 1). This SDS washing step during the surface activation procedure was found to be essential for improving the stability of the baseline after ER/ERE binding experiments. For the activation of the surface the two injections of 50 mM NaOH, followed by one injection of 0.1% SDS solution was determined to be the best for obtaining a stable baseline before DNA association. This last injection was added in order to ensure that no baseline modification is observed if this solution is used for surface regeneration

**Table 1: Condition parameters of sensor chip SA before ERE binding.**

Reference	Conditioning solution	Concentration (mM)	Number of injections	Flowrate ( $\mu$ L/min)	Injected volume ( $\mu$ L)
GE healthcare	NaOH	50	3	nd	nd
	NaCl	1000			
Nilsson <i>et al.</i>	NaOH	50	5-6	nd	nd

[5], 1995

Pearson <i>et al.</i> [6], 2001	NaOH	50	nd	5	nd
	NaCl	1000			
Murata <i>et al.</i> [7], 2004	NaOH	100	nd	20	5
	HCl	50			5
Asano <i>et al.</i> [8], 2004	NaOH	100	nd	nd	nd
	HCl	50			

nd: not described; in grey: condition not tested in this study

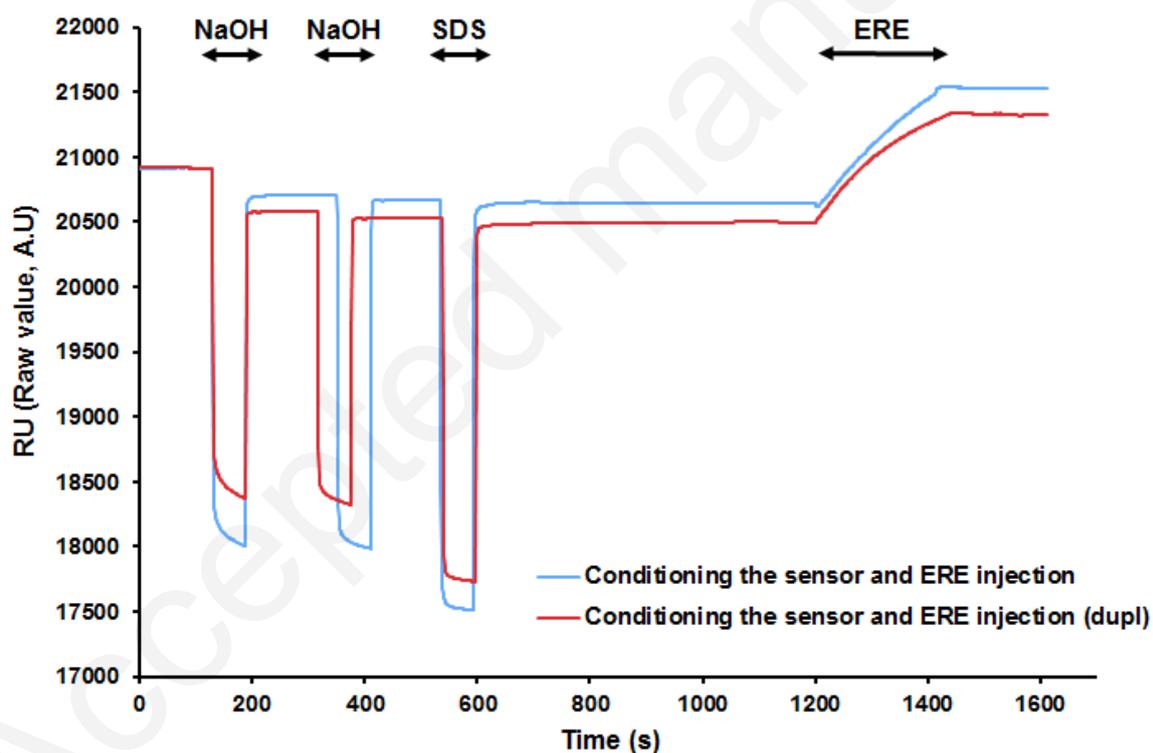


Figure 1: Sensorgrams obtained during the conditioning of sensor chip SA.

### 3.2. ERE binding and Buffer used

For ERE binding on the SA surface, two main strategies have been developed. Both are based on the streptavidin-biotin interaction. The first uses the ability of single strand DNA containing ERE sequences to form hairpin structures. The palindromic sequence found on a long single biotinylated strand forms a hairpin in order to reconstitute a perfect double strand ERE sequence through base complementarity [4, 6, 9, 10]. The second strategy uses a shorter single biotinylated strand. In order to reconstitute the double strand ERE sequence, the complementary strand is injected on the surface, leading to the formation of the palindromic sequence [1, 2, 7, 8, 11, 12].

Four different buffers have been described in the literature for binding DNA on the surface of the sensor chip (Table 2). However, until now no comparison had been done concerning the influence of the buffer chosen on the level and stability of DNA binding. Among the 4 buffers, only HBS-EP and TNMT buffers were compared in this study as tricine and water buffers are not commonly used. We also decided to use only the double strand ERE, which was prepared extemporaneously before its injection on the surface. The sensorchips SA were conditioned as detailed above. Then, ten microliters of double strand Biotin BERE/EREC (ERE) solution (450 nM) were injected on the surface over the course of 1 minute (10  $\mu\text{L}/\text{min}$ ). Four independent ERE injections were performed for each buffer. Figure 2 shows sensorgrams of the mean of the four independent ERE injections in each buffer. The variability and stability of the four injections are illustrated in table 3.

**Table 2: ERE injection parameters on SA sensors chips documented in the literature**

Publication	Running buffer	Injected volume ( $\mu\text{L}$ )	Concentration	Flowrate ( $\mu\text{L}/\text{min}$ )	Time of injection	Binding level (RU)
Nilsson <i>et al.</i> [5]	HBS	30	ERE, 2 $\mu\text{mol}/\text{L}$	2	nd	nd
Kostelac <i>et al.</i> [10]	Water	20	ERE, 1 $\mu\text{mol}/\text{L}$	5	nd	nd
Pearson <i>et al.</i> [6]	HBS	nd	ERE, 5 $\text{mg}/\text{L}$	nd	nd	900

Murata <i>et al.</i> [7]	Tricine	5	<b>B</b> ERE nd	5	nd	200
		10	ERE <b>C</b> nd			
Asano <i>et al.</i> [8]	Water	nd	<b>B</b> ERE 10 mg/l	nd	2 min	60
			ERE <b>C</b> 1 $\mu$ g/ml			
Jisa <i>et al.</i> [11]	TNMT	nd	ERE, 1 $\mu$ mol/L	5	4 min	nd
Cheskis <i>et al.</i> [4]	TNMT	50	ERE, 33 mg/L	nd	nd	nd

nd: not described; **B**ERE: ERE single strand biotinylated; ERE**C**: ERE complementary single strand

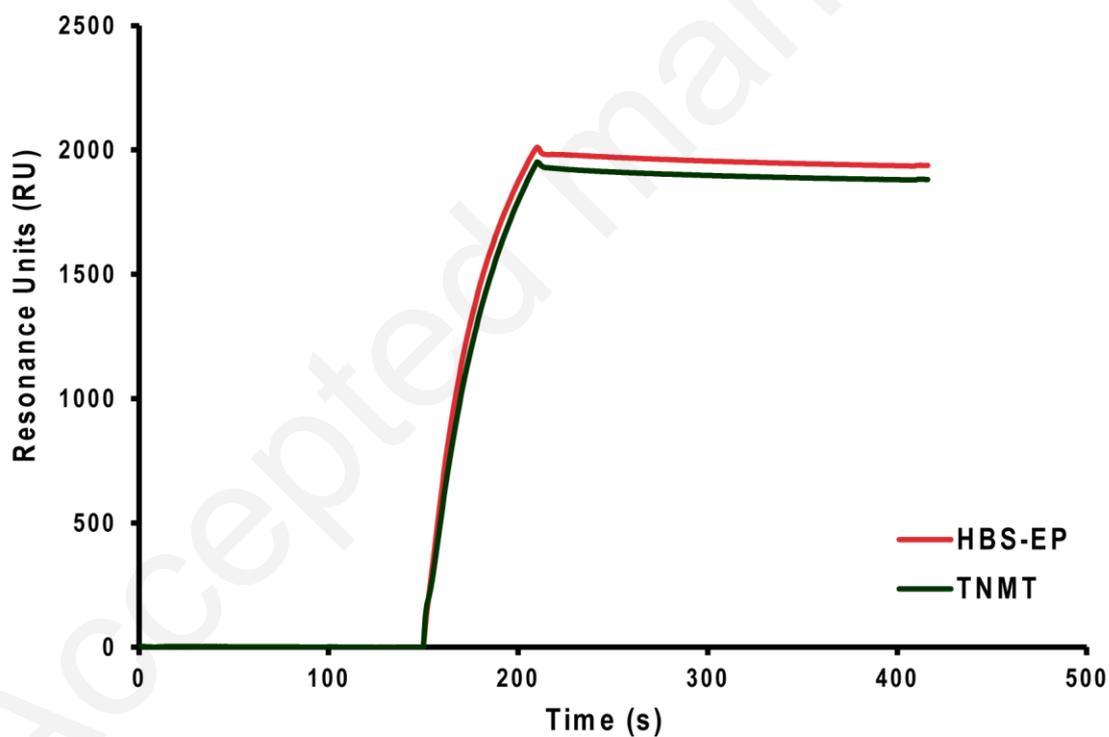


Figure 2: Means of 4 sensorgrams of ERE fixation on SA sensor chips in the presence of HBS-EP or TNMT running buffer

**Table 3: Binding level of ERE at 400 s (the mean of 4 independent injections)**

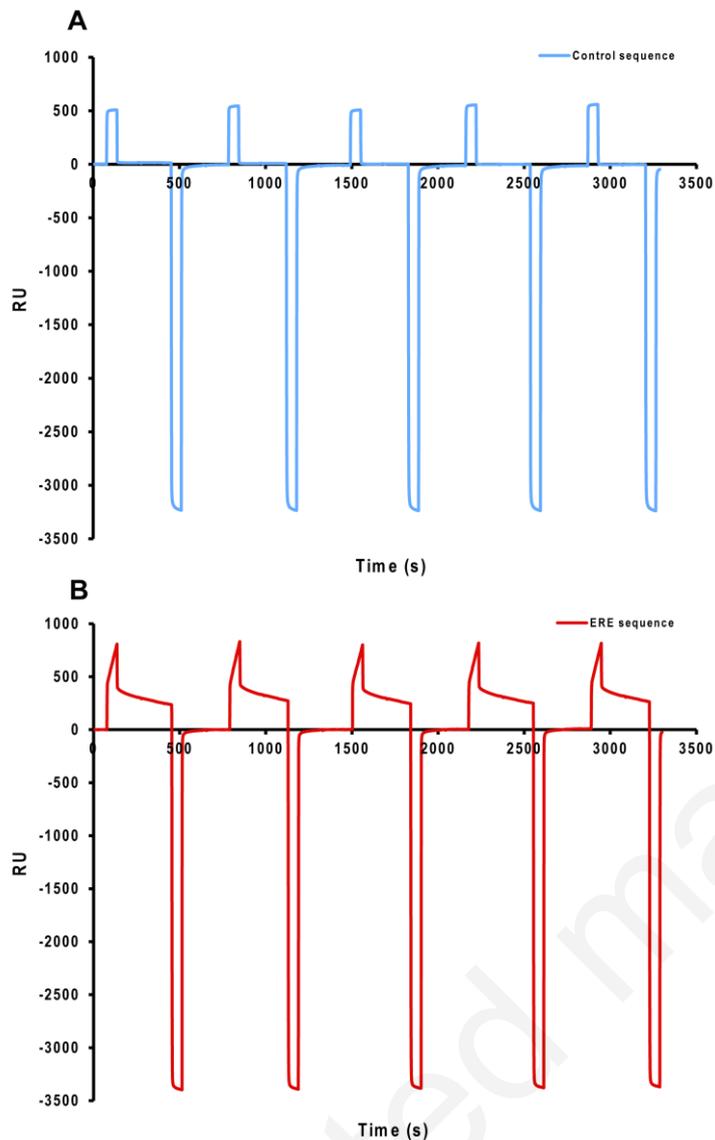
	HBS-EP	TNMT
Binding level (RU)	1936	1880
Standard deviation	30.23	14.27
CV (%)	1.56	0.76

As shown in figure 2, the composition of the running buffer does not affect the ERE binding ability on the chip. However, ERE fixation is more stable when using TNMT than when using HBS-EP buffer (as seen in table 3). In the presence of TNMT buffer, the variation of the signal is 0.75% as opposed to 1.5% for HBS-EP buffer. Therefore it is slightly preferable to use TNMT buffer to associate ERE and SA surface. Based on these results, it was decided that TNMT buffer would be used for all subsequent experiments in the laboratory.

### **3.3. ER binding specificity and ERE regeneration**

#### **3.3.1. Verification of ER binding specificity**

In the absence of ERE sequence coated on the sensor chip, the injection of 50 nM of ER shows low association signals with an average of  $12 \pm 0.7$  RU. This low level illustrates that ER does not interact in a specific manner on SA surface (Figure 3A). In contrast, the injection of 50 nM of ER on the ERE coated surface induced a clear and reproducible interaction signal illustrated by an increase the response level by  $392 \pm 18$  RU (Figure 3B).



**Figure 3: Injection of 40  $\mu\text{L}$  of ER (50 nM) onto (A) a bare SA sensor chip, or (B) a chip coated with 1200 RU of ERE**

### 3.3.2 ERE regeneration methods

The strong binding of biotinylated ERE to SA ( $K_D \sim 10^{-14}$  M [12]) permits regeneration of ERE after protein binding without altering the level of ERE coated on the SA sensor chip. The regeneration step enables the reuse and recycling of surfaces. Several strategies have been documented in the literature and no clear consensus is observed (Table 4). These can be grouped into two main approaches, one targeting the protein, and the other targeting the DNA. The first requires the use of 0.1% of SDS to partially denature the protein ER, which then no longer interacts with the ERE DNA

sequence [4]. The second approach dissociates the complementary unbiotinylated DNA strand and/or temporarily dissociates the DNA hairpin, removing ER at the same time [8].

In order to test the first approach (0.1% SDS), 5 successive injections of ER were performed, each followed by the 0.1% SDS injection. The ERE baseline level was monitored in order to observe the efficiency of regeneration (Figure 3B). The 0.1% SDS solution allows the dissociation of all bound ER molecules without changing the baseline corresponding to the ERE bound sequence.

The second general approach dissociates the double strand DNA (Figure 4) and all bound protein molecules. Among the different methods described in table 4, it was decided to test only the injection of a NaOH solution (50 mM) alone as the adjunction of HCl did not modify in NaOH efficiency (data not shown). Tests were conducted on double strand DNA. The double strand DNA approach requires, after NaOH regeneration, an additional step that consists of the hybridization of a new complementary non-biotinylated (ssDNAC) strand on the biotinylated ERE (Figure 5). Consequently, the baseline level was monitored during the regeneration step to evaluate the efficiency of this methodology.

The initial double strand ERE binding level on the surface reached 825 RU. After a first wash with 0.1% SDS (Figure 4, step 1), which eliminates any double strand ERE not attached to the surface of the sensor chip SA, the level dropped to 820 RU. The decrease due to the first SDS wash was very low.

**Table 4: Methodology for surface regeneration after ER interaction.**

Publications	DNA	Regeneration	Hybridation
Asano <i>et al.</i> [8]	Double strand	100 mM NaOH, 50 mM HCl	yes
Zhang <i>et al.</i> [13]	Double strand	5 mM NaOH	no
Berthier <i>et al.</i> [14]	Double strand	0.1% SDS, 100 mM EDTA	no
Li <i>et al.</i> [15]	Double strand	1.5 M NaCl, 0.05 % of surfactant P20, two pulses	no
Berthier <i>et al.</i> [16]	Double strand	0.5 M imidazole, two pulses	no
Su <i>et al.</i> , [17]	Double strand	0.1% SDS	no
Habauzit <i>et al.</i> [1, 2]		0.1% SDS	
Bayle <i>et al.</i> [3]		0.1% SDS	
Jisa <i>et al.</i> [11]		0.1% SDS, two pulses	
Murata <i>et al.</i> [7]	Double strand	100 mM NaOH, 50 mM HCl	yes
Cheskis <i>et al.</i> [4]	Single strand,	0.1 SDS	no
Pearson <i>et al.</i> [6]	Hairpin DNA		

Kostelac <i>et al.</i> [10]	Single strand, Hairpin DNA	50 mM NaCl, 0.5 M NaOH	no
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First we evaluated the NaOH/hybridization step which was completed twice. The regeneration of the surface was achieved through two successive injections of NaOH, followed by the EREC hybridization step. After the first NaOH regeneration cycle, the baseline decreased to 41% of the initial value (774 RU to 320 RU, Figure 4, step 1). This level is less than the theoretical value estimated at 387 RU (half of 774). After the second NaOH regeneration step (Figure 4, step 2) the baseline level decreased to 32% of the level of double strand DNA measured at the beginning of the second step (618.7 RU to 198 RU).

The hybridization of the complementary strand leads to the reconstitution of the double strand DNA (Figure 5). The baseline level increased to 723 RU (93% of the initial level, beginning of step 2) and to 587 RU (95% of the baseline level, beginning of step 3). The regeneration of the surface is incomplete. Indeed, the overall baseline level between the initial double strand interaction and the final state of the surface decreased by 30% (From 820 RU to 587 RU). This is mainly due to the NaOH washing step that induces the surface alteration and also increases the surface sensitivity to SDS. After each NaOH step, the surface sensitivity to SDS treatment is increased. The average rate of baseline level decreases from  $0.9 \pm 0.7\%$  after step 1, to  $1.6 \pm 0.6\%$  after step 2, and finally to  $2.0 \pm 0.6\%$  (Step 3).

Taken together, these results demonstrate that the NaOH washing step alters the integrity of the surface, evidenced by a decrease in the amount of ERE bound to it. Secondly, the surface is more sensitive to the different treatments during washing steps as a result of NaOH treatment. This decrease appears to be due to an alteration of both streptavidin and double strand DNA. Thus, this regeneration procedure was not retained in our experiment plan. However, this regeneration procedure may be adapted to use with the hairpin strategy. Indeed, no additional hybridization step is required when using this strategy. In conclusion, when using the double strand DNA strategy, the 0.1% SDS washing step should be favored.

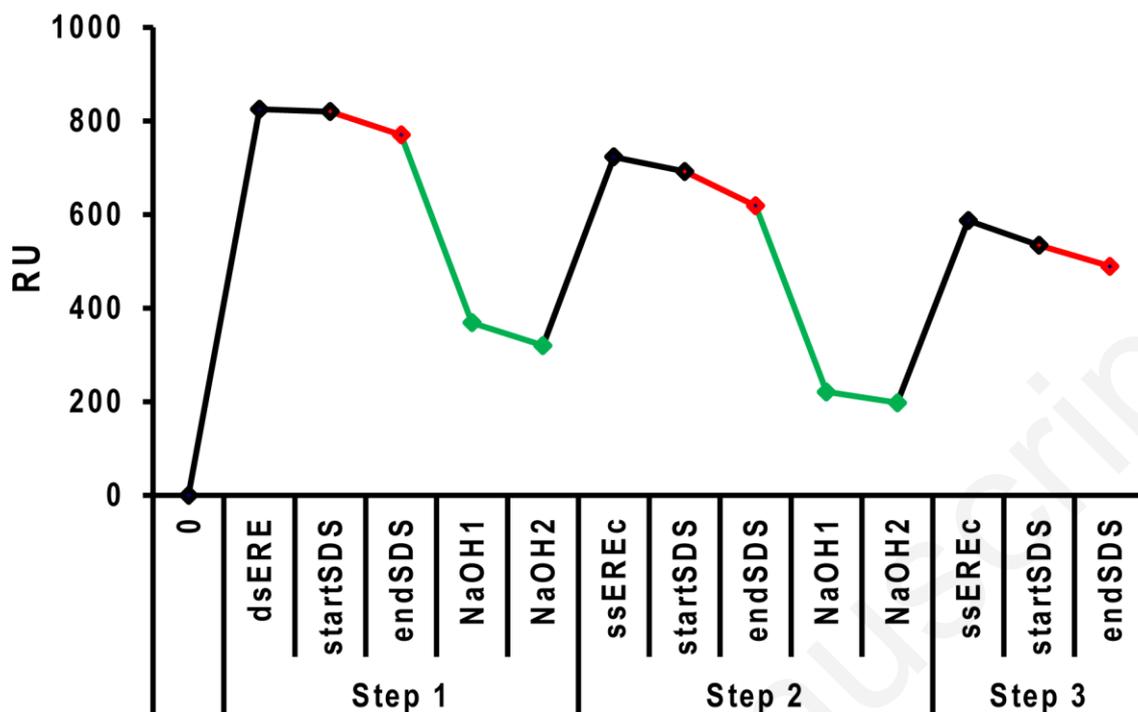


Figure 4: Baseline level obtained on BIAcore 1000 after ERE fixation and subsequent steps of washing and surface regeneration

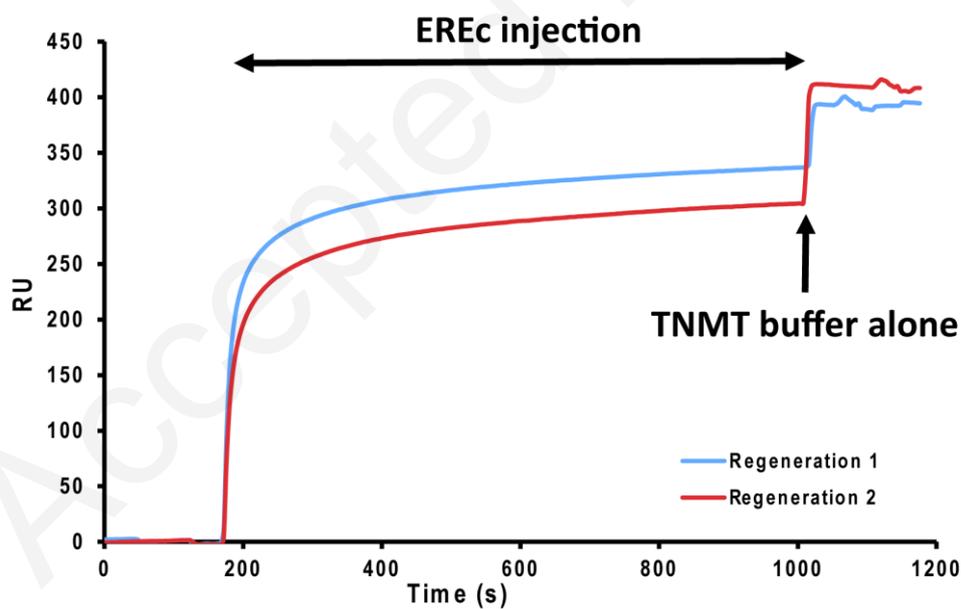


Figure 5: Regeneration of double strand DNA by injection of complementary ssDNA on remaining biotinylated ssDNA.

### 3.3.3 Efficiency of the SDS washing step

In order to validate the efficiency of the SDS washing step, solutions of bovine serum albumin (BSA) with or without E2 were injected on the sensor surface before and after ER binding step and SDS washing steps, as described in the literature [6, 18]. After the washing step, the remaining amount of ER on the surface could interact with BSA-E2. In these experiments, ERE sequence (1250 RU) was immobilized on the sensor chip surface. The solutions were injected in the following order: BSA, BSA-E2, ER, BSA and finally BSA-E2.

As can be seen in figure 6, the first injections of BSA, and BSA-E2 did not bind to the surface. After the first injections, the binding levels were respectively 0.1 and 3.5 RU. The third injection of ER interacted specifically with the surface with binding levels of 436 RU (Figure 6). After the SDS washing step, the second injection of BSA and of BSA-E2 did not bind on residual ER. Therefore the 0.1% SDS step is efficient enough to eliminate all ER bound on the ERE coated surface.

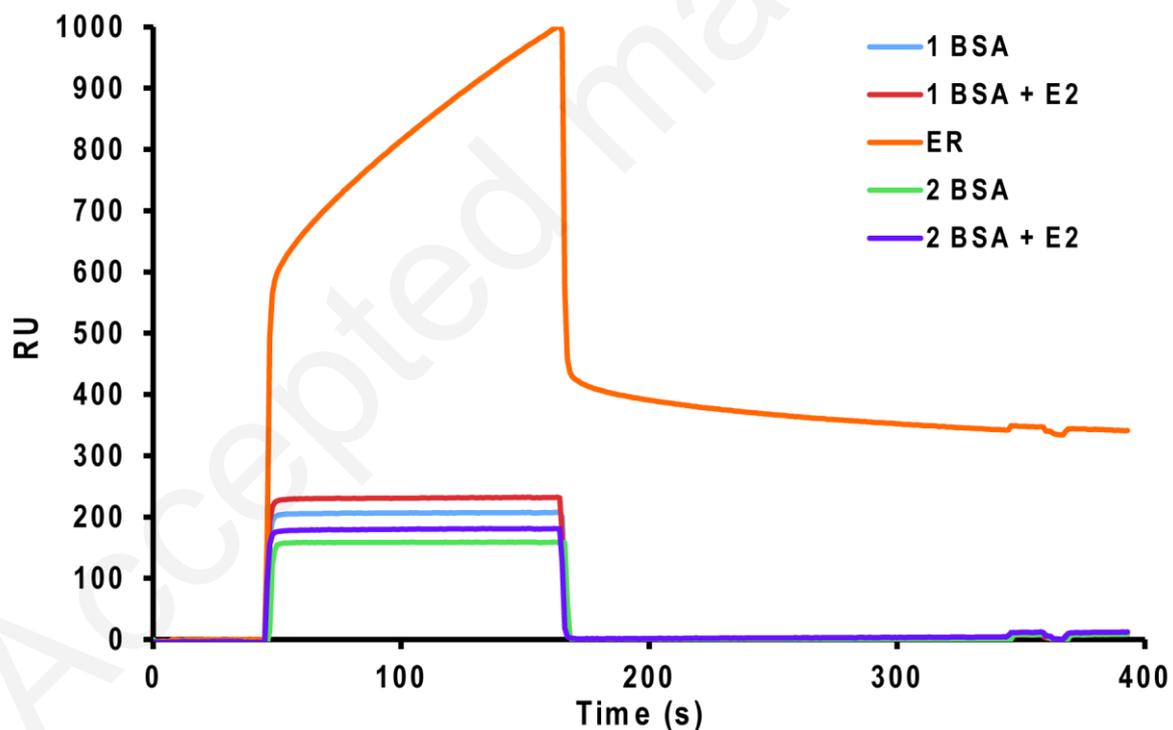


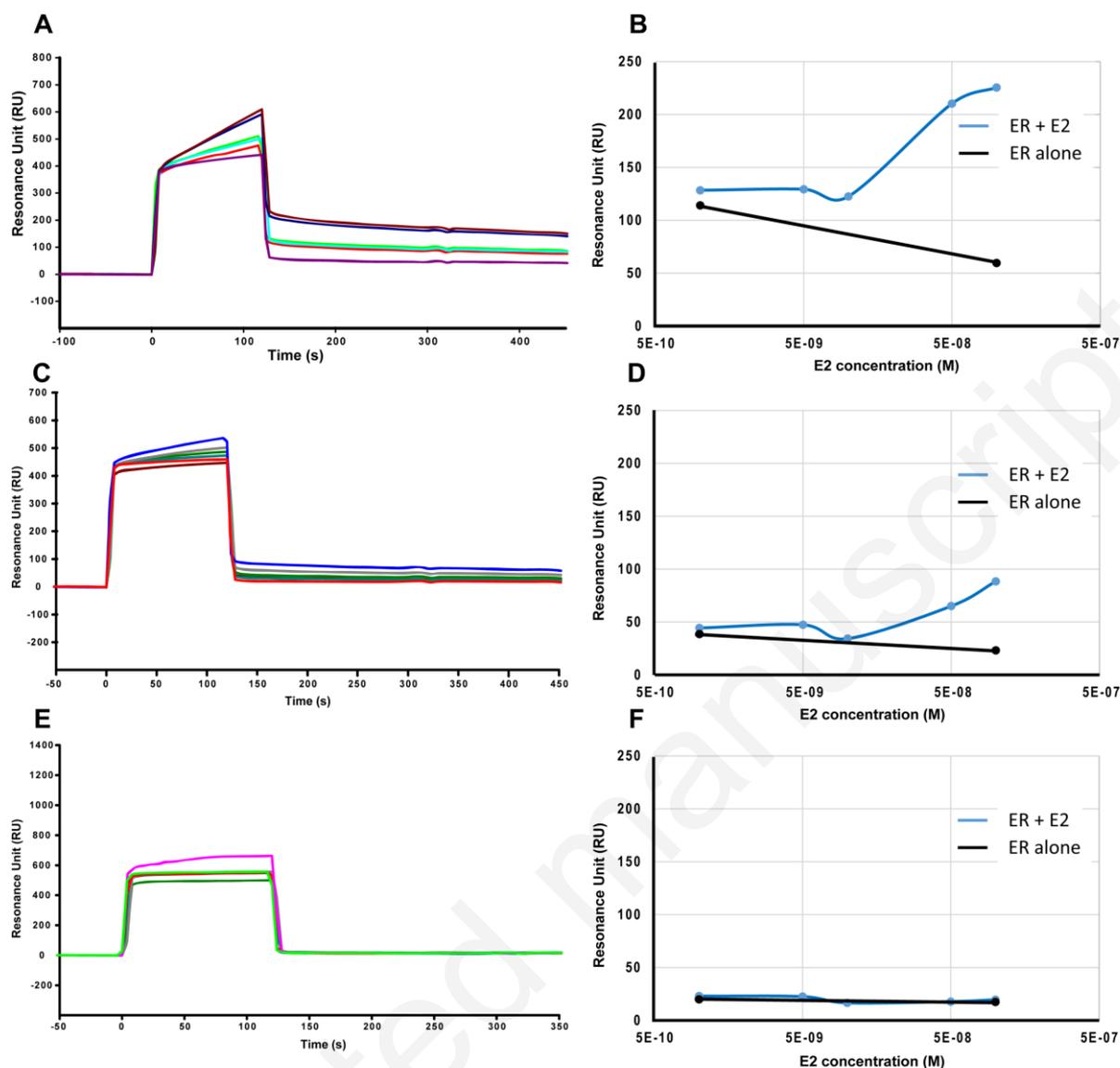
Figure 5: Sensorgrams obtained upon injection of 50 nM of BSA or BSA-E2 before and after injection of 50 nM ER.

#### 3.4. Effect of time and ambient temperature on the ER binding level

The aim of this part is to evaluate the impact of sample conservation at ambient temperature after an incubation of ER and E2 overnight at 4°C, before their final injection into the SPR instrument (Figures 7A, 7C, 7E). Three sets of samples of 50 nM of ER were incubated with a range of E2 from 1 nM to 100 nM. Each set was separated by one injection of ER onto the sensor chip surface. The evaluation of the binding level is indicated by the black line in Figure 7 (B, D, F). The use of the external injection of ER was used in the literature [8] to calibrate the binding level according to this external injection. At ambient temperature, the ER binding level decreases.

As found in previous studies [1, 3, 8], the temperature and time of sample conservation induce a modification of the ER binding level. Indeed, the ER binding level decreased by between 114 and 59 RU (Figure 7A-B) in 1h30 at ambient temperature. This decrease corresponds with the findings of Asano *et al.* [8], in whose study the signal decreases by a factor of 2. After 1h30, the signal decreases from 38 to 23 RU in the following 1h30 (Figure 7C-D) and finally after 3h the signal decreases to 20 to 17 RU (Figure 7E-F) in the last 1h30. While Asano *et al.* used the ER binding level to correct the dose curve, this is no longer possible after the second cycle of analysis.

For the first set of injections (Figure 7A) the E2 dose effect on the ER binding is observed to produce nearly a two-fold increase in the SPR signal from 128 RU to 225 RU (Figure 7B). The difference between the loss of binding of ER (59 RU) and the last point of the dose curve is due to the presence of E2, which permits greater stability of ER in ambient temperature [1, 3]. During the second set of injections (Figure 7C-D), the overall ER binding level decreases even in the presence of E2. The SPR signal increases from 42 RU (E2 = 0.5 nM) to 88 RU (E2 = 100 nM). The protective effect of E2 begins to decrease, however the two-fold increase of the SPR signal remains the same. In the third cycle (Figure 7F), the low amount of ER binds onto ERE. The overall binding level is between 16 (low dose of E2) and 22 RU (high dose of E2). The two-fold increase of ER binding level is no longer observed. The lack of E2 effect may suggest that all ER proteins have been rendered inactive. As the three ranges of experiments were prepared simultaneously, these results are evidence that temperature has an effect on ER binding.



**Figure 6: Time and temperature effect on the ER binding level.**

### 3.5. Measurement of ER interaction properties.

Several processes have previously been used to measure the difference and/or the modification of the interaction between ER and the estrogen response element. Most relied on a comparison between ER on its own, and ER with the studied molecule [2, 8, 10, 14–17, 19, 19]. The binding level is the most commonly used parameter for comparisons, however another indicator that can be used is the fold change in binding level [2, 3, 10]. Some publications have also calculated affinity constants between ER and ERE depending upon the treatment. But most have only used one ER concentration and few molecules have been screened (Table 5). The difference in the  $K_D$  varies by nearly two orders of magnitude between manipulations. These variations may be due to the difficulties occurring with

the conservation of ER depending upon the ER preparation and experiment conditions [1, 3, 20]. This makes it difficult to calculate reliable affinity constants for ER/ERE interaction.

**Table 5: Rate constant of association ( $k_a$ ) and dissociation ( $k_d$ ) and the dissociation constant ( $K_D$ ) between ER and ERE depending upon estrogenic stimulation.**

Compounds	References	$k_a$ ( $10^3 \text{ M}^{-1} \cdot \text{s}^{-1}$ )	$k_d$ ( $10^{-5} \text{ s}^{-1}$ )	$K_D$ ( $10^{-10} \text{ M}$ )
No ligand	[4]	99	6.8	7.4
	[11]	8.17	50.6	619
	[13]	137.7	118.2	86
	[17]	8.77	63.3	70
	Dns	191	102	53.1
17 $\alpha$ -ethinyl estradiol	[4]	105	194	184
17 $\beta$ - $\alpha$ estradiol	[4]	78	15	18
	[11]	96.2	186	193
	Dns	252	180	71.3
Raloxifen	[4]	74	2.9	3.8
	[11]	14.5	17.1	117

Dns: Data not shown

#### 4. Discussion

Biosensors have long been developed to monitor several kinds of molecular interactions and to determine the overall properties of these interactions. Among biosensors, surface plasmon resonance-based technologies are increasingly popular due to their ability to easily determine the kinetic and equilibrium affinity constants through direct monitoring of interactions. As a result, SPR technology has been widely used for the characterization of interaction properties of several

biological partners, e.g. molecule/protein, protein/protein, protein/DNA. In the present study, the described biosensor has been used to study and characterize the properties of the Estrogen Receptor (protein) / Estrogen response element (consensus DNA sequence) interaction. This biosensor was developed to mimic the natural interaction of ER and ERE occurring in cells that induce gene expression. Several kinds of molecules have been found to induce some modification of gene expression [20, 21]. However, cellular approaches do not inform on the direct modification of ER interactions with ERE. Several estrogenic compounds could therefore modify this interaction. Biosensors are therefore essential for the characterization of molecules' effect on ER binding properties.

In ER/ERE interactions measured by SPR, previous literature showed that each research group had developed their own system even though the strategies adopted were similar. Methodologies diverged at each step (activation of the surface, DNA binding, shape of the DNA, washing step, protein preparation). The goal of this study was to compare the most commonly used parameters in order to determine which ones were the most reliable. We demonstrate that surface activation with NaOH, followed by one injection of SDS permits activation of the streptavidin coated surface. This activation permits the stable binding of double strand DNA when it is diluted in TNMT buffer. This double strand DNA allows ER interaction. After the injection, all ER protein is removed with a single injection of 0.1% SDS solution. ER binding properties are significantly modified by the time and temperature of the ER's conservation, and conservation of ER at 4°C until its analysis by SPR is a crucial parameter for reliable interaction measurements.

The differences in biosensor strategies have several consequences, the first being how results are expressed. In most publications only comparisons of binding levels are mentioned [2, 8, 10, 14–17, 19], while a few take into account unwanted factors such as protein denaturation or precipitation [1–3, 8]. These unwanted factors are difficult to explain as they may be due to a modification of the dimerization properties of ER, a true modification of the ER affinity, or a precipitation of ER resulting from uncontrolled experimental conditions. Results of different publications are accordingly difficult to compare. These same unwanted factors may also influence the calculation of the affinity constant and could explain the discrepancies in their values [4, 11, 13, 19]. In order to resolve these difficulties, we have established a reliable protocol for the design of interaction experiments. The resulting test facilitates the evaluation of endocrine disrupting chemicals. It can also be applied in environmental monitoring as this method reached a detection limit of 5 nM of E2 [1], which is consistent with the detection limit of other biosensors involving SPR [18, 22, 23] and is below the detection limit of biosensors based on other technologies [24, 25].

## 5. Conclusion

Several publications have been dedicated to the development of ER/ERE interaction studies and many different methodologies were developed, without being compared with one another. The aim of this publication was to sum up the different methodologies used for surface activation, ERE binding, ER injection, and binding conditions and surface regeneration by comparing these methodologies. These steps are essential for the development of protein-DNA experiments and should be made clear before developing any further biosensors. They should moreover be adapted depending upon the aim of the study and experimental conditions. Until now, parameter comparisons have not been sufficiently described in the literature, even though they are crucial for the development of better and more reliable biosensors. The overall objective of the present study, together with our earlier publications [1–3], is the unification of methodologies in ER/ERE interaction studies in order to favor the comparison of results from different laboratories and improve overall understanding of endocrine disrupting chemical disturbances.

### Conflict of interest

The authors declare no conflict of interest.

### Highlights

- Biosensor development requires preliminary steps not previously detailed
- Preliminary steps are essential to ensure the reliability of biosensors
- Parameter comparison serves to understand their influence on ER interactions
- Time and temperature modify the interaction properties of proteins

### Author contributions

Conception of the work: DH and SB; collection of data: DH, SB and DB; analysis of data: DH, SB, JC and BR; writing of manuscript DH, SB, BR and JC. All authors contributed to the discussion and approved the manuscript.

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