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At-line quantification of bioactive antibody in bioreactor by surface plasmon resonance using epitope detection

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ABSTRACT

We report an innovative at-line method to monitor concentration of bioactive antibody (i.e., antibody with conserved antigen-binding activity) secreted during bioreactor culture by the use of surface plasmon resonance (SPR)-based biosensor technology. In a first series of experiments, conditions for SPR-based measurements were validated off-line by monitoring bioactive antibody concentration in conditioned medium from 500-ml baffled flask hybridoma cell cultures. A fully automated experimental setup in which the SPR-based biosensor was harnessed to a bioreactor was then used at-line to monitor the concentration of bioactive antibody produced in a 3.5-L bioreactor. Quantitative SPR measurements performed both at-line and off-line were in excellent agreement with quantitative Western blotting followed by densitometry analyses. Thus, our experimental study confirms that SPR biosensors can be applied to at-line quantification of correctly folded proteins that are secreted by cells cultured in a bioreactor. Our experimental approach represents a novel and robust analytical strategy to be applied to the control and optimization of the production of bioactive secreted proteins.

Recombinant proteins are becoming increasingly important, as indicated by their market share of more than $60 billion (U.S.) [1]. Of interest, 20% of the biopharmaceutical products in development are antibodies or derivatives [2]. To satisfy market needs, large-scale antibody production has been evaluated with various expression systems (i.e., hybridoma and other mammalian cell lines, transgenic plants and plant cells, or bacterial systems) [3,4]. In parallel, quantitative tools designed for the on-line monitoring of bioreactor-based cultures have also been mentioned as key to improving cell-specific productivity [5]. In that context, product concentration may be the most important parameter to be routinely measured [6].

Whereas off-line classical methodologies for the determination of antibody concentration (e.g., enzyme-linked immunosorbent assay [ELISA][2], quantitative Western blotting) are well documented, their implementation is long and labor-intensive [2,7]. Therefore, immunodetection methods cannot satisfy the need for at-line quantification of protein levels. Among the off-line techniques available for antibody characterization, Biacore surface plasmon resonance (SPR)-based biosensors are widely employed because measurements can be entirely automated, allowing unattended operation, good accuracy, and robustness [7,8]. Of interest, SPR biosensors are routinely used off-line for measuring bioactive concentrations of proteins within unpurified samples [9,10].

Recently, bioprocess at-line monitoring has been successfully applied to the production of chimeric proteins of interest fused to fluorescent reporters such as green fluorescent protein [5,11]. The main disadvantages of these strategies reside in the fact that protein levels can be overestimated due to cleavage of the reporter. Also, the tag must be cleaved from the protein after production and purification. Another strategy based on on-line mass spectrometry is available and provides qualitative information about protein integrity. However, protein levels are not quantified, and no information about protein bioactivity can be obtained [12]. Compared with these techniques, at-line SPR biosensing could rapidly monitor how changes in culture parameters affect product concentration, thereby providing key information for bioprocess improvement. Furthermore, the signal ultimately could be relayed to a control system aimed at optimizing cell-specific productivity.

In that effort, Vostiar and coworkers [13] demonstrated the usefulness of an on-line setup to monitor intracellular components of *Escherichia coli* fed batch cultures. In this approach, both bioreactor

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[2] *Abbreviations used:* ELISA, enzyme-linked immunosorbent assay; SPR, surface plasmon resonance; HEK, human embryonic kidney; PSMA, prostate-specific membrane antigen; mAb, monoclonal antibody; PFHM-II, protein-free hybridoma medium II; HBS, Hepes-buffered saline; EDTA, ethylenediaminetetraacetic acid; NHS, *N*-hydroxysuccinimide; EDC, *N*-ethyl-*N*-(3-diethylaminopropyl)carbodiimide hydrochloride; PDEA, 2-(2-pyridinyldithio)ethaneamine hydrochloride; PBS, phosphate-buffered saline; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; PVDF, polyvinylidene fluoride; TBS–Tween, Tris-buffered saline–Tween 20; IgG, immunoglobulin G; HRP, horseradish peroxidase; CCD, charge-coupled device.

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sampling and sample preparation were automatically performed on-line prior to sample manual transfer to an SPR biosensor for subsequent analysis. At-line SPR detection of cell-free protein synthesis was also reported by Lee and coworkers [14]. We recently demonstrated that an SPR biosensor can be used for the fully automated at-line detection of secreted protein in a bioreactor culture of transiently transfected human embryonic kidney (HEK) 293 cells [15]. In that study, semiquantitative measurements were achieved by taking advantage of a highly specific recombinant coil tag [16] present on the secreted protein of interest. However, our experimental approach did not discriminate between folded and denatured protein. In the current work, we clearly demonstrate that our SPR-based detection setup allows for a strict at-line quantification of bioactive monoclonal antibody produced by a hybridoma cell line cultured in a bioreactor. Furthermore, our improved fully automated methodology does not require the product to be tagged. Prior to at-line quantification of bioactive monoclonal antibody concentration produced in a bioreactor, preliminary off-line experiments were performed. In these experiments, the type of ligand, the ligand immobilization strategy, and the quality of the biosensor calibration and aging of the sensor surface were assessed. Off-line and at-line quantifications of standard batch cultures were then performed to validate the optimized quantification protocol.

Materials and methods

Cell line and secreted antibody

Truncated prostate-specific membrane antigen (PSMA) ectodomain and hybridoma cell line 17G1.1 corresponding to a derived subclone of the hybridoma cell line 17G1 (patent PCT/CA2004/000127), which secretes monoclonal antibody (mAb) binding to PSMA, were generous gifts from S. Moffett (ProScan Rx Pharma, Montreal, QC, Canada). Aliquots of purified mAb stock solution (1.5 g/L) were kept at −20 °C in 10% glycerol [17].

Cell culture

17G1.1 cells were grown in suspension in protein-free hybridoma medium II (PFHM-II, Invitrogen Canada, Burlington, ON, Canada) supplemented with 1 ng/ml interleukin-6 (Cedarlane Laboratories, Burlington, ON, Canada), 0.1% Pluronic F-68 (Sigma–Aldrich, Oakville, ON, Canada), PenStrep (100 U/ml penicillin and 0.1 mg/ml streptomycin, Sigma–Aldrich), and amphotericin B (2.5 mg/L, Sigma–Aldrich) at 37 °C in batch mode. Cells were inoculated at 400,000 cells/ml for 500-ml baffled flask cultures and 275,000 cells/ml for bioreactor culture.

For off-line experiments, hybridoma cells were cultured for 10 days in 500-ml baffled flasks in a 37 °C humidified incubator maintaining 5% CO_2 and an agitation speed of 90 rpm. Samples were taken manually from cell cultures and centrifuged for 10 min at 1050 rpm. Samples destined for SPR analysis were filtered (0.22 μm), automatically diluted (1:20 dilution) by the biosensor, and injected 10 times over PSMA or PS0215 (sequence CGKSLYESWTKK, 95% purity, Bio-Synthesis, Lewisville, TX, USA) and related mock surfaces. In between injection cycles, surface regeneration was performed by injection of glycine buffer (pH 2.0). Samples analyzed by Western blot were kept at −20 °C.

Bioreactor culture was performed in a 3.5-L bioreactor (Chemap, Basel, Switzerland). Agitation speed was maintained at 90 rpm by a double helical ribbon impeller having the same geometrical ratios as in Ref. [18]. Dissolved oxygen was maintained at 45 ± 5% from air saturation by continuous feed of an air/oxygen/5% CO_2 mixture in the headspace. pH was maintained between 7.1 and 7.4 by the controlled addition of 7.5% sodium bicarbonate solution.

In addition to automated continuous sampling for at-line SPR monitoring (see below), manual sampling was performed. Samples were centrifuged for 10 min at 1050 rpm and kept at −20 °C for Western blot and off-line SPR analyses.

Bioreactor connection to SPR biosensor for at-line measurements

From 1 h postinoculation, the culture medium of the bioreactor was continuously pumped through a decantation column (20 cm height, 0.5 cm diameter, 50 μl/min), and remaining cells and cell debris were filtered with an in-line tangential filter (0.22 μm, Pall, Mississauga, ON, Canada) positioned at the inlet valve of a peristaltic pump (Ismatec, Cole Palmer Canada, Anjou, QC, Canada). Filtered, cell-free culture medium was then continuously delivered to a 7-mm plastic vial placed in a Biacore 3000 rack (Biacore, Piscataway, NJ, USA) that had been drilled at 5 mm from its top to insert tubing (Fig. 1).

SPR measurements

SPR measurements were performed using a Biacore 3000 system. For antibody detection, two ligands corresponding to PSMA and to PS0215 peptide were immobilized on different surfaces of CM4 sensor chips (Biacore).

Sensor chip surface preparation

PS0215 was chemically coupled to CM4 sensor chip surfaces using the standard thiol coupling procedure [16]. Coupling was performed in Hepes-buffered saline (HBS) running buffer (20 mM Hepes [pH 7.4], 150 mM NaCl, 3.4 mM ethylenediaminetetraacetic acid [EDTA], and 0.05% Tween 20) at a flow rate of 5 μl/min. Injections of 0.05 M *N*-hydroxysuccinimide (NHS) and 0.2 M *N*-ethyl-*N*-(3-diethylaminopropyl)carbodiimide hydrochloride (EDC) equimolar mixture (70 μl) were followed by a 70-μl injection of 4.5 mg 2-(2-pyridinyldithio)ethaneamine hydrochloride (PDEA) diluted in 250 μl of 10 mM sodium borate (pH 8.5) and by two 10-μl injections of PS0215 diluted in 100 μl of 10 mM sodium acetate (pH 4.0) to reach a maximal amount of coupled peptide (350–450 RU). A 70-μl injection of 1.5 mg cysteine and 14 mg sodium chloride diluted in 250 μl of 10 mM sodium acetate (pH 4.0) was then used to block the remaining activated groups on the sensor chip surface.

PSMA was coupled to CM4 sensor chips using the standard amine coupling procedure. That is, phosphate-buffered saline (PBS, Invitrogen Canada) with 0.05% Tween 20 was used as running buffer and the flow rate was set at 5 μl/min. Sequential injections consisted of an NHS/EDC mixture followed by two 10-μl injections of 17 mg/L PSMA dissolved in 10 mM sodium acetate (pH 5.5) to reach a maximal amount of coupled PSMA (2700–3300 RU). A 70-μl injection of 1.0 M ethanolamine–HCl (pH 8.5) was then used to block the remaining activated groups.

Mock surfaces related to each coupling strategy were generated by applying the same coupling procedure in which ligand injection was replaced by buffer injection.

Biacore sample injections

All injections were carried out at a flow rate of 20 μl/min, and all samples were diluted in PBS running buffer. Each sample was injected for 1 min over control and PS0215 surfaces (in a serpentine fashion) followed by a 1-min injection of running buffer. Regeneration of the sensor chip for subsequent injection cycles was performed by a 20-s pulse (100 μl/min) of 10 mM glycine (pH 2.0). In the case of PSMA surfaces, the antibody injection duration was 5 min.

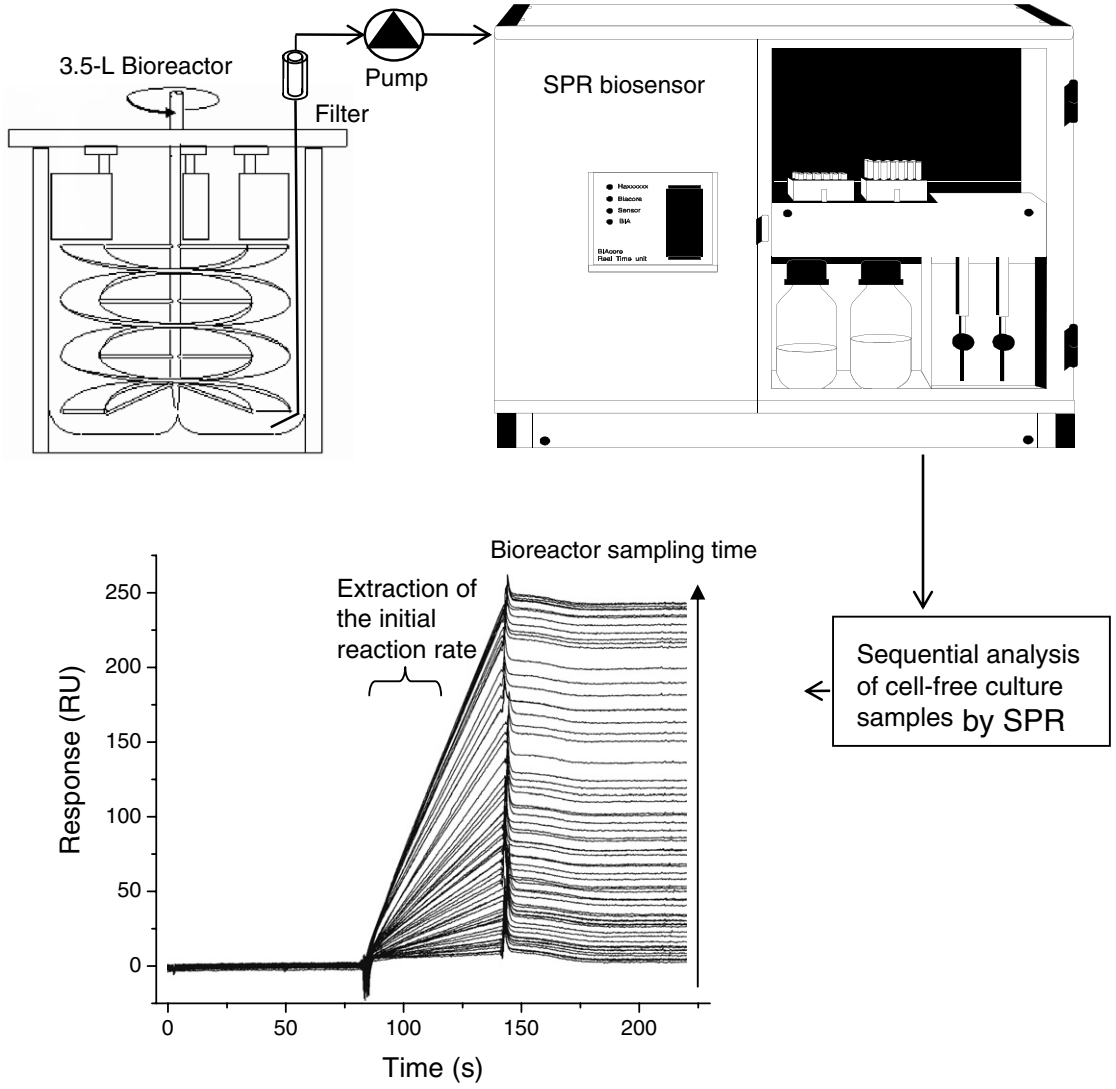


Fig. 1. Schematic representation of the experimental setup connecting the bioreactor to the biosensor for SPR at-line measurements.

For automated at-line monitoring of antibody concentration, the experimental setup consisted of a pump with an in-line filter that slowly and continuously delivered filtered culture medium to the modified vial placed inside the biosensor tube rack. At set intervals (i.e., when sufficient volume accumulated in the modified vial), the biosensor automatically prepared samples to be injected by transferring filtered culture medium to a separate empty vial and then diluting it with running buffer (1:20 dilution). Diluted samples were then automatically injected over the sensor surfaces, and surface regeneration was performed as described above. The biosensor was also programmed to totally empty the modified vial every 12 min (after sample injection); this filtered medium was kept in distinct 7-mm plastic vials for subsequent off-line analyses (600 μl). This cycle of events (1 h) was automatically repeated throughout the bioreactor cell culture with limited manual intervention (buffer tank and regeneration vial replenishment). Every 12 h, a freshly prepared antibody standard (0.45 g/L diluted 1:20) was automatically injected to readjust the calibration curve used for the determination of bioactive mAb concentration (see following section).

Reaction rate correlation with protein concentration

The accumulation of analyte (mAb) over the biosensor surface can be represented as a two-step process [19]:

$$\mathrm{mAb_{bulk}} \rightleftharpoons \mathrm{mAb_{surface}} \quad \text{and} \quad \mathrm{mAb_{surface}} + L \rightleftharpoons \mathrm{mAb} - L,$$

where $\mathrm{mAb_{bulk}}$ is mAb in solution, $\mathrm{mAb_{surface}}$ is mAb in the vicinity of the biosensor surface, L is the ligand that had been covalently immobilized at the surface (i.e., PSMA or PS0215), and mAb–L is the complex formed by the mAb and the ligand. The first step, which consists in the migration of the mAb from the bulk to the surface, is referred to as the diffusion step. The second step corresponds to the interactions between the mAb and its binding partner that had been covalently immobilized at the biosensor surface.

Under mass transport limitation, concentration of bioactive mAb can be determined independently of its binding kinetics, with the initial reaction rate being proportional to mAb concentration [10] according to

$$\mathrm{d}R/\mathrm{d}t = \mathrm{MW}.k_\mathrm{m}.G.[A_\mathrm{bulk}],$$

where R is the response (RU) corresponding to the mAb accumulation at the biosensor surface, $\mathrm{d}R/\mathrm{d}t$ is the initial reaction rate ($\mathrm{RU.s^{-1}}$), MW is the molecular weight of the analyte, k_m is the mass transfer coefficient, and G is a conversion factor that takes into account that 1 RU of accumulated protein equals 1 $\mathrm{pg.mm^{-2}}$.

For every sample, the initial reaction rate was calculated by extracting the slope of the sensorgram for the first 30 s of the injection.

Western blotting and densitometry quantification

Here 5 μl of filtered samples was loaded onto nonreducing 7.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) mini-gels and transferred to polyvinylidene fluoride (PVDF) membrane (Bio-Rad, Hercules, CA, USA) using Mini Trans-Blot Cell (Bio-Rad) and according to the manufacturer's protocol. The membranes were then blocked with 5% (w/v) fat-free milk powder in Tris-buffered saline–Tween 20 (TBS–Tween, 50 mM Tris, 150 mM NaCl, and 0.1% [v/v] Tween 20). Membranes were rinsed and incubated for 1 h with an anti-mouse immunoglobulin G (IgG) secondary horseradish peroxidase (HRP)-coupled antibody (1:15,000 dilution, Bio-Rad) in TBS–Tween and 0.5% (w/v) fat-free milk powder and were rinsed three times for 10 min with TBS–Tween and once for 10 min with TBS only. The membranes were then submitted to chemiluminescent detection according to the manufacturer's protocol (Immun-Star HRP substrate, Bio-Rad) on X-ray film, followed by image acquisition with a charge-coupled device (CCD) camera and densitometry evaluation (Alpha Innotech, Mississauga, ON, Canada).

Results and discussion

Effect of ligand on performance of SPR measurement

Off-line preliminary experiments were conducted on the Biacore 3000 biosensor. Two ligands (i.e., PSMA and the linear peptide used for immunization [PS0215]) were compared. The covalent coupling strategy was optimized in each case to obtain the best performances for SPR measurement. Four criteria were taken into account to determine whether the PS0215 peptide or PSMA would be the most suitable sensor ligand: signal amplitude, signal linearity, detection sensitivity, and sensor chip lifetime. We estimated that the duration of our batch hybridoma culture would be 10 days according to previous cultures performed in baffled flasks. Because our goal was to obtain at least one measurement of bioactive mAb concentration per hour, the surface needed to give correct signal for at least 240 sample injections and regeneration cycles.

Efforts were made to immobilize a maximal amount of each ligand on different surfaces so as to favor mass transport limitation during subsequent mAb injections. The PSMA ligand was covalently coupled to the sensor chip by standard amine coupling, whereas the PS0215 synthetic peptide was engineered to contain an N-terminal cysteine to enable thiol coupling. We succeeded in immobilizing 400 RU of PS0215 and 3000 RU of PSMA. Considering that the molecular weights of PS0215, PSMA, and mAb are 1.5, 50, and 150 kDa, respectively, the theoretical maximal amounts of mAb that could then be captured on the biosensor surface (assuming a 1:1 binding stoichiometry) were 40,000 and 12,000 RU for PS0215 and PSMA, respectively, indicating a fourfold higher binding capacity for the peptide-coupled sensor surface relative to the protein-coupled sensor surface.

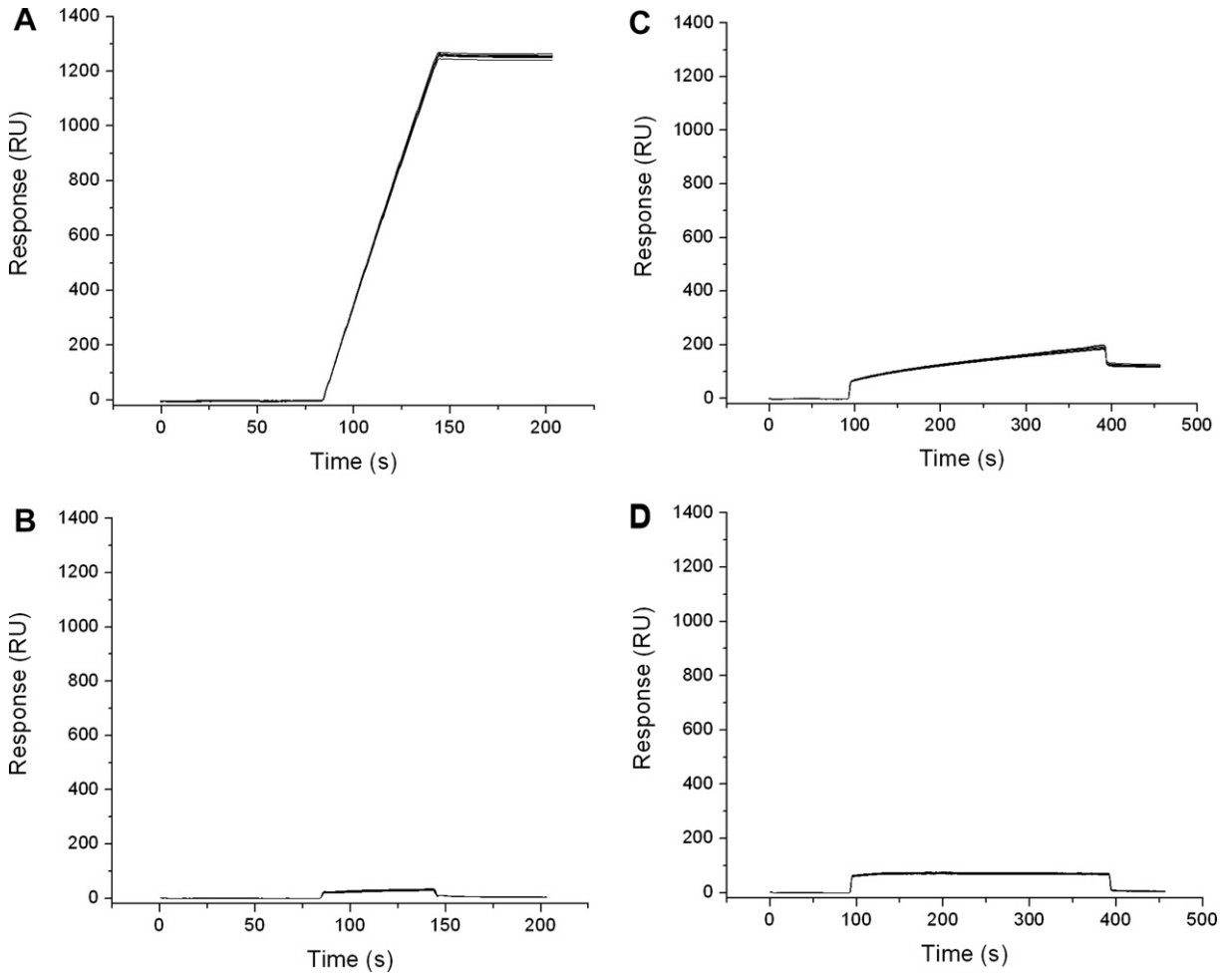


Fig. 2. Sensorgrams corresponding to 10 injections of diluted filtered hybridoma culture medium over PS0215 (A), PSMA (C), and respective mock surfaces (B,D). The reference sample corresponded to filtered medium collected after 10 days of a 17G1.1 culture in baffled flasks. The sample was automatically diluted 1:20 in running buffer prior to injection.

PSMA and PS0215 surfaces were first tested with culture medium obtained at day 10 from a standard batch culture of hybridoma cells performed in baffled flasks (400,000 cells/ml at inoculation). Raw data collected using the different surfaces are shown in Fig. 2. mAb binding levels at the end of the injections, as well as initial reaction rates that were determined after control correction of the sensorgrams, are given in Table 1. A comparison of the sensorgrams that were recorded when injecting diluted medium over PS0215 and PSMA surfaces with those recorded over their respective mock surfaces indicated that, as expected, interactions between the carboxymethylated dextran matrix of our biosensor surfaces and the proteins within the conditioned medium are minimal even when medium contained nonnegligible concentrations of intracellular proteins (cell viability was < 20% after 10 days of culture in baffled flasks).

Based on the excellent reproducibility achieved with PS0215-immobilized surfaces (< 0.5% variation for both initial reaction rate and antibody accumulation [Table 1]), in addition to the linearity of the sensorgrams during the injection phase that eased initial reaction rate determination (Fig. 2A), PS0215 peptide surfaces were then further employed to develop our at-line assay for bioactive antibody concentration.

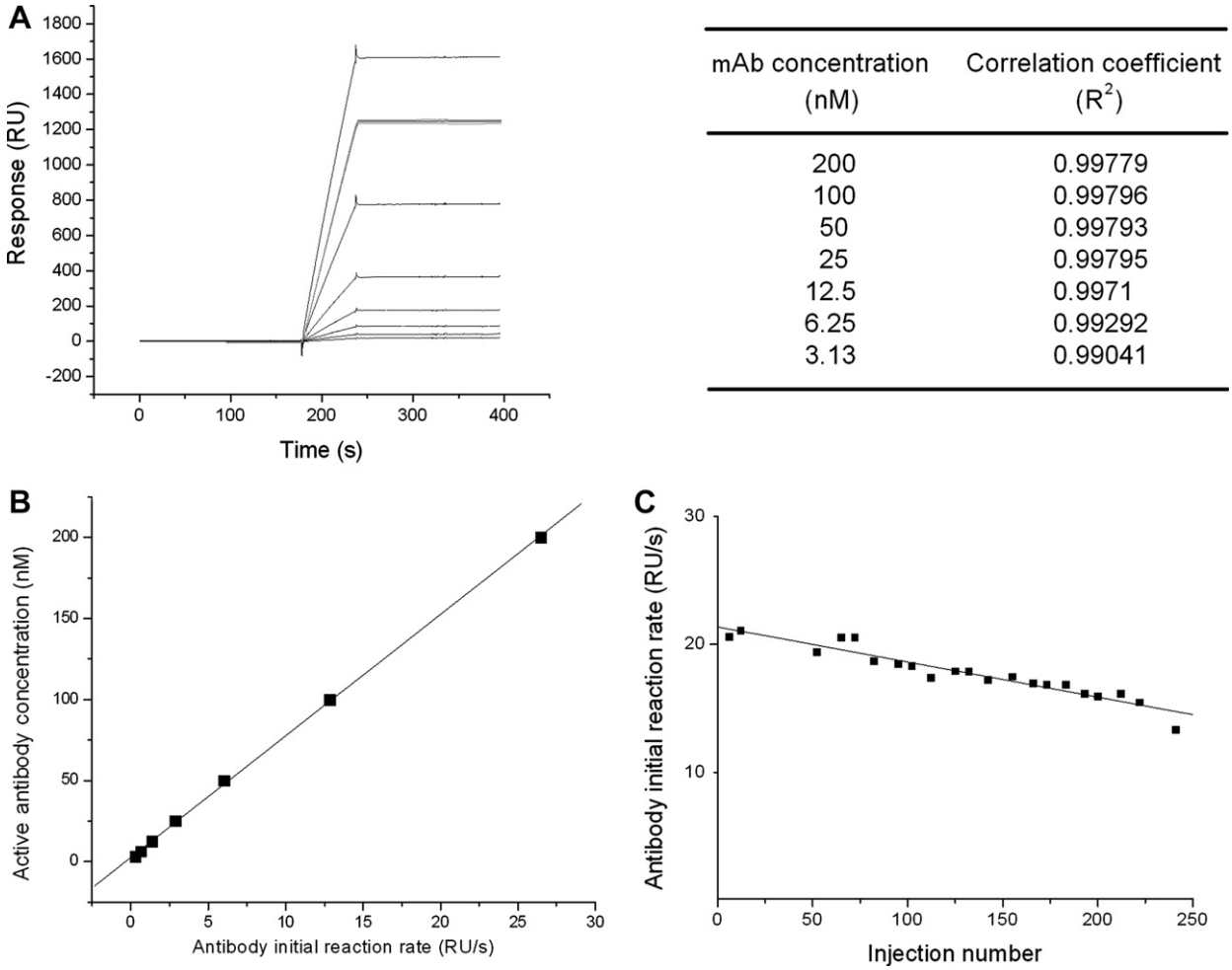
Table 1
Comparison of mAb binding to PSMA and PS0215 surfaces

	Antibody reaction rate (RU/s)[a]	Antibody accumulation at the end of the injection (RU)[a]	Surface lifetime (number of cycles)
PS0215	21.4 ± 0.1 (n = 10)	1250 ± 6 (n = 10, 1-min injections)	⩾250
PSMA	0.50 ± 0.016 (n = 10)	109 ± 4 (n = 10, 5-min injections)	⩾100

[a] Note that sensorgrams shown in Fig. 2 were double referenced prior to antibody reaction rate and antibody accumulation measurements.

Robustness of antibody quantification method

In a series of subsequent experiments, injections of purified mAb were then performed at concentrations ranging from 3 to 200 nM (0.00135–0.03 g/L) over both mock and PS0215 surfaces. Injections were performed at a low flow rate (20 μl/min) so as to favor mass transport limitation (Fig. 3A). For every concentration tested, the initial reaction rate was calculated by extracting the initial slope of the control-corrected sensorgram (30 s of the injection phase, from 180 to 210 s in Fig. 3A). For every antibody concentration, regression coefficients were found to be excellent (R^2 > 0.99 [table in Fig. 3]). Furthermore, a plot of these initial reaction rates as a function of the mAb concentrations was found to be linear (R^2 = 0.9996 [Fig. 3B]). This indicated unambiguously that our ap-



mAb concentration (nM)	Correlation coefficient (R^2)
200	0.99779
100	0.99796
50	0.99793
25	0.99795
12.5	0.9971
6.25	0.99292
3.13	0.99041

Fig. 3. Design of the quantification method. (A) Control-corrected sensorgrams corresponding to the injection of purified mAb samples (3–200 nM, black solid line) and sensorgrams from Fig. 2A (gray solid line). (B) Calibration curve obtained by extracting initial reaction rates from the sensorgrams shown in panel A. (C) Antibody initial reaction rate variations when injecting spent medium at the same dilution over the PS0215 and control surfaces. The table shows correlation coefficients related to the linear regression performed for initial reaction rate measurements of the various sensorgrams shown in panel A.

proach combining PS0215 surface and mAb initial reaction rate measurement was able to measure bioactive mAb concentrations spanning from 0.00135 to 0.03 g/L.

Of interest, both pure mAb injections (Fig. 3A) and injections of diluted cell culture medium containing mAb (Fig. 2A) gave sensorgrams with similar profiles. This observation strongly suggested that the interaction between mAb and immobilized PS0215 was highly specific and that culture medium components did not interfere significantly with detection. That is, if analytes other than mAb, present in the medium at the end of the culture, had bound to PS0125, one would have expected the corresponding sensorgram profile to be different from the ones related to pure mAb injections, for example, sensorgrams displaying different dissociation profiles. These results further supported our decision to use surface-immobilized PS0215 in our quantitative assay. Using this sensor chip set of surfaces, a 1:20 dilution of a 10-day-old cell culture medium sample (Fig. 2) gave an initial reaction rate value (Table 1) within the linear range of our calibration curve (Fig. 3A). These data demonstrated that bioactive mAb concentration at the end of our first baffled flask culture was 0.48 g/L, a standard value for batch mAb production (S. Moffet, personal communication).

Aging of PS0215 surfaces was then investigated by injecting diluted medium over both PS0215 and control surfaces for 250 cycles. The initial reaction rate corresponding to mAb binding to immobilized PS0215 was observed to decrease with injection number (Fig. 3C). This drift would need to be taken into account for at-line monitoring. We anticipated that injections of a standard of known concentration at set intervals (every 12 h) would permit our calibration curve to be readjusted and, thus, to remain precise and accurate throughout the culture period.

Off-line validation of SPR measurements

We performed 10-day-long 500-ml baffled flask hybridoma cell cultures (in triplicate) for which mAb concentration was followed off-line by SPR. As shown in Fig. 4A, cells grew exponentially up to 57 h after inoculation at a specific rate of $0.033 \pm 0.0012\ h^{-1}$. Meanwhile, bioactive mAb concentration also increased at an exponential rate with a specific rate of $0.035 \pm 0.0017\ h^{-1}$ (see Fig. 4C), indicating that antibody production was growth associated. After the cell growth phase (60 h), the cells started to die and the net bioactive antibody production rate decreased but was still positive and exponential ($0.0062 \pm 0.0005\ h^{-1}$).

To validate SPR off-line measurements, antibody present in filtered conditioned medium obtained from samples taken for cell counts was analyzed by Western blot densitometry (Figs. 4C and 5). At the end of the culture, minor degradation products appeared (Fig. 5); these were considered as nonbioactive mAb fragments and, therefore, were not taken into account for densitometry quantification because only bioactive antibody was quantified by SPR.

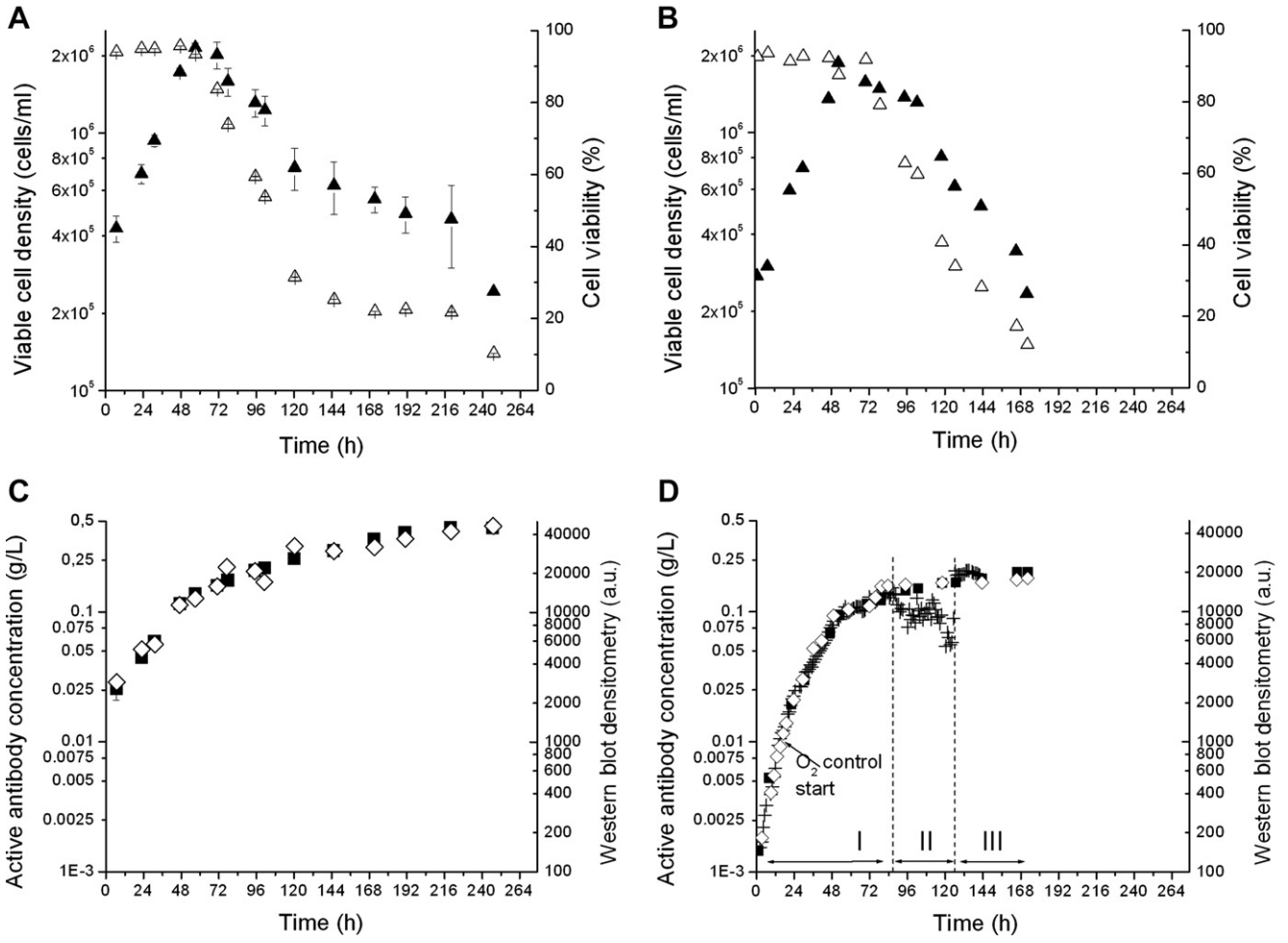


Fig. 4. Shake flask and bioreactor hybridoma cell culture monitoring. (A,B) Cell density (▲) and viability (Δ) evaluated off-line in flask cultures and bioreactor culture, respectively. (C,D) Off-line (■) and at-line (+) quantification of bioactive antibody by SPR biosensing and off-line quantification by Western blot densitometry (◇) for flask cultures (C) and bioreactor culture (D). In panel D, areas I and III correspond to periods of time during which the in-line filter was functional, whereas area II corresponds to the period of time during which the in-line filter was damaged.

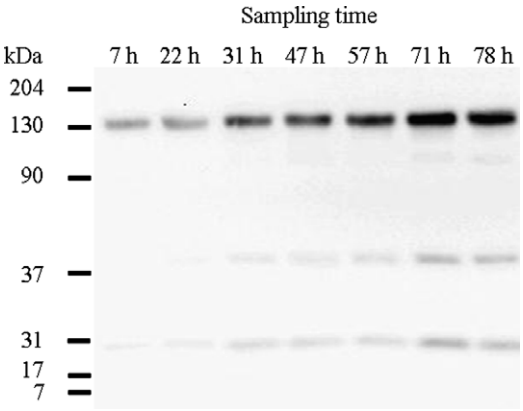


Fig. 5. Example of Western blot used for densitometry off-line analysis of centrifuged samples taken manually during the flask cultures. The estimated size of antibody was 155 kDa.

Antibody levels quantified by Western blotting were in excellent agreement with antibody concentration obtained by off-line SPR-based measurements (R^2 = 0.95 [Fig. 4C]).

At-line measurement of bioactive mAb concentration along a bioreactor culture

We then performed hybridoma cell culture in a 3.5-L bioreactor to which our SPR biosensor was harnessed. Prior to initiating the culture, two sets of PS0215 and control surfaces were freshly prepared and calibrated (data not shown). Continuous delivery of filtered culture medium to one biosensor vial was synchronized with an automated biosensor protocol allowing for measurements of bioactive mAb concentration every hour throughout the duration of the entire culture (172 h). In addition, cell culture samples were taken manually through the bioreactor sampling port so as to evaluate cell growth and viability and to prepare cell-free samples for subsequent off-line SPR measurements and quantification by Western blotting and densitometry.

In the case of bioreactor batch culture, cells grew exponentially from 7 to 52 h at a specific rate of $0.039 \pm 0.0019\ h^{-1}$, which was slightly higher than the rate for flask cultures ($0.035 \pm 0.0017\ h^{-1}$). This could be attributed to a better control of culture parameters in the bioreactor. Thus, the growth phase was shorter in the bioreactor than in the flasks, but the maximal cell densities were similar in both cases (2×10^6 cells/ml [Fig. 4A and B]), suggesting that the yields in cells per medium nutrient content were similar in both cultures. After 4 days, cell viability decreased more rapidly in the bioreactor batch culture than in the shake flask cultures, thereby limiting bioreactor culture to 172 h (vs. 247 h in shake flasks). This is more likely to be attributed to increased shear stress during agitation.

Fig. 4D shows results for at-line quantification of bioactive mAb concentration. During the first 86 h (phase I in Fig. 4D), mAb concentration increased as expected. However, after 86 h (phase II), at-line quantification showed a sudden decrease in bioactive mAb concentration as well as an increased variability in the calculated values. Previous results from baffled flask cultures indicated that such a decrease in bioactive mAb concentration could not be attributed to mAb degradation. A visual inspection of the in-line filter revealed that the latter was clogged and possibly damaged. Thus, we postulated that cells and cell debris had been injected over the biosensor surface; this would explain our sporadic phase II results. Therefore, the in-line filter was replaced, the biosensor microfluidic parts were unclogged, and at-line monitoring was continued using the remaining fresh PS0215 and control surfaces we had prepared and calibrated previously (phase III). As expected, at-line concentration measurements for the end of the culture were observed to be consistent with those recorded during the early stages of the culture (phases I and III).

After at-line monitoring and extensive cleanup of the biosensor, a new set of PS0215 and control surfaces was prepared. Centrifuged samples collected for cell counts were then manually filtered prior to dilution and injected on both surfaces after calibration. In parallel, mAb concentrations were also evaluated by quantitative Western blotting. As can be see in Fig. 4D, our hypothesis of in-line filter failure was confirmed by both off-line SPR (closed squares) and Western blot (open diamonds) quantification.

Conclusions

In this study, we have demonstrated that an experimental setup harnessing an SPR-based biosensor to a bioreactor is suitable for at-line monitoring of native bioactive monoclonal antibody concentration, obviating the need for a recombinant tag. To monitor production from the very beginning to the end of the culture, we optimized our SPR measurements by selecting a high-affinity antibody binder as ligand to be immobilized at the surface of our biosensor. Off-line SPR and Western blotting experiments were in excellent agreement with at-line measurements except for the few hours during which the in-line filter was damaged, a problem encountered previously by others [20]. In striving toward the development of a fully automated system, data suggest that more optimal monitoring could be achieved by more infrequent sampling or by aseptically changing the in-line filter daily (a routine manipulation in bioprocesses). Alternatively, bioreactor sampling could be achieved with commercially available sampling probes capable of withdrawing sterile cell-free samples from a bioreactor (e.g., probes commercialized by Flownamics, Madison, WI, USA, and Groton Biosystems, Boxborough, MA, USA).

We were able to demonstrate the robustness of our novel SPR at-line quantification method that enabled concentration measurement every hour. Such a sampling rate enables fine monitoring and precise determination of production behavior and kinetics. If needed (e.g., for transient transfection processes), higher frequencies for concentration determination could be attained without drastic modifications of the experimental protocol.

A further step in at-line culture characterization would be to quantify both total and bioactive antibody concentrations. We believe that our experimental setup is versatile enough to achieve this goal. On a similar note, Canziani and coworkers [7] developed a multiple injection SPR assay in which an Fc-specific antibody was immobilized at the sensor chip surface to determine total antibody concentration, although subsequent injection of antigen over captured mAb would be necessary to permit bioactivity assessment. An alternative strategy would be to perform both total and bioactive mAb quantifications in parallel. That is, two of the four available surfaces on a sensor chip could be dedicated to total mAb concentration measurements via anti-Fc antibody interactions, whereas bioactive mAb concentration could be measured as described in this article using the two remaining surfaces of the biosensor chip.

Our experimental approach has the great advantage of being adaptable to any protein for which a specific high-affinity binding partner is available. Therefore, the coupling of an SPR biosensor to a bioreactor constitutes a powerful tool for at-line monitoring, control, and optimization of bioreactor protein production.

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