Biotechnology Journal
www.biotechnology-journal.com

Rapid Buffer and Ligand Screening for Affinity Chromatography by Multiplexed Surface Plasmon Resonance Imaging

Karin P. M. Geuijen, Daniëlle E. J. W. van Wijk-Basten, David F. Egging, Richard B. M. Schasfoort, and Michel H. Eppink*

Protein purifications are often based on the principle of affinity chromatography, where the protein of interest selectively binds to an immobilized ligand. The development of affinity purification requires selecting proper wash and elution conditions. In recent years, miniaturization of the purification process is applied to speed up the development (e.g., microtiterplates, robocolumns). The application of surface plasmon resonance imaging (SPRi) as a tool to simultaneously screen many buffer conditions for wash and elution steps in an affinity-based purification process is studied. Additionally, the protein A ligand stability after exposure to harsh cleaning conditions often limits the reuse of resins and is determined at lab scale. The SPRi technology to screen ligand life-time with respect to alkali stability is used. It is also demonstrated that SPRi can successfully be applied in screening experiments for process developments in a miniaturized approach. The amount of resin, protein and buffer in these studies is reduced 30–300-fold compared to 1 mL column scale, and approximately 10–1000-fold compared to filter plate experiments. The overall development time can be decreased from several months towards days. The multiplexed SPRi can be applied in screening affinity chromatography conditions in early stage development for ligand development and recombinant protein production.

1. Introduction

Affinity chromatography is a highly selective capture step in the purification of many recombinant proteins, where the interaction is based on reversible binding.[1] Within the biopharmaceutical industry, protein A chromatography is often applied as the first unit operation to remove the majority of impurities, such as host cell proteins (HCPs), fragments, DNA and media components, from a cell culture harvest to purify mAbs.[2–5]

Recombinant protein A has been engineered to obtain a more stable protein[3] that can withstand harsh conditions on a purification column, such as high flow rates, different wash buffers, and additives or cleaning conditions.[6] Many different engineered protein A-based resins are available nowadays for efficient IgG purification,[3,7] which have a longer resin lifetime compared to their native counterparts, mainly due to an improved alkali stability. Affinity columns are generally rigorously cleaned after each purification cycle to prevent cross-contamination, often by dilute NaOH solutions. The applied cleaning-in-place (CIP) solution depends on resin type and on ligand stability, and therefore ligand life-time has to be studied during the development of new affinity ligands.[8–11]

Process development of a protein A purification step for IgGs involves the selection of wash and elution conditions. HCP levels can vary significantly in protein A pools amongst different mAbs and removal thereof is dependent on the selected wash conditions.[12] In recent years, wash conditions have been investigated to remove a larger fraction of HCPs[13–15] and aggregates[16] while maintaining high overall yield. A rapid screening of the various steps in the purification process enables a fast transfer to pilot-scale experiments where only a selection of conditions can be tested.[17] Especially with the development of newly engineered protein A ligands, or any other affinity ligand, there is a broad interest in miniaturized screening approaches to speed up the development process.[18,19]

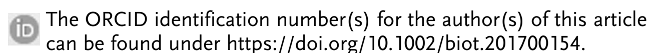
Here we describe an alternative screening technology, based on multiplexed SPRi, to miniaturize process development using protein A and IgG as a model system. We screened elution and

K. P. M. Geuijen, Dr. D. E. J. W. van Wijk-Basten, Prof. M. H. Eppink
Downstream Processing, Synthon Biopharmaceuticals BV, PO Box 7071, 6503 GN Nijmegen, The Netherlands
E-mail: karin.geuijen@synthon.com

K. P. M. Geuijen, Prof. M. H. Eppink
Bioprocess Engineering, Wageningen University, PO Box 16, 6700 AA Wageningen, The Netherlands

Dr. D. F. Egging
Preclinical Department
Synthon Biopharmaceuticals BV, PO Box 7071, 6503 GN Nijmegen, The Netherlands

Dr. R. B. M. Schasfoort
Medical Cell Biophysics Group, MIRA institute, Faculty of Science and Technology
University of Twente, PO Box 217, 7500 AE Enschede, The Netherlands


The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/biot.201700154.

DOI: 10.1002/biot.201700154

 © 2017 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

wash buffers in a simulated Protein A affinity chromatography step and we performed an alkali stability test of the protein A ligands at microgram scale. The use of a 48-channel continuous flow microspotter (CFM) to screen 48 different buffers for interaction analysis has been proven before[20] and we further optimized the experimental set-up to screen for protein A process development.

2. Experimental Section

2.1. Elution Buffer Screening Using SPRi

A G-COOH sensor (SSens BV, Enschede, the Netherlands) was activated with EDC/NHS in 50 mM MES buffer according to manufacturer's instructions in the IBIS MX96 (IBIS Technologies BV, Enschede, the Netherlands). MabSelect SuRe (MSS) ligand (GE life sciences) was diluted to 1 μM in 50 mM sodium acetate pH 4.0/0.05% polysorbate 80 and immobilized on the entire surface of the sensor. The sensor was deactivated by 1M ethanolamine pH 8.5 during 10 min in the IBIS MX96.

Recombinant human IgG (Synthon Biopharmaceuticals BV, Nijmegen, the Netherlands) was diluted to 1 μM in PBS buffer pH 7.4/0.05% polysorbate 80 and injected over the MSS sensor with a total association time of 10 min until equilibrium was reached ($R_{eq}1$), after a baseline of 1 min. Subsequently, the sensor was transferred to the continuous flow microspotter (CFM, Wasatch, Salt Lake City, USA) where the surface was exposed to 48 different buffers simultaneously for 1 min in a 4×12 array. Elution buffers that were screened were prepared from a 50 mM sodium acetate stock solution at pHs of 6.0; 5.5; 5.25; 5.0; 4.8; 4.5; 4.0; 3.5; 3.25; 3.0; or 2.7 and 2 M NaCl stock solution. Buffers were mixed in different ratios and diluted with MQ water to obtain 25 mM sodium acetate buffers at the indicated pH, with salt concentrations of 0, 250, 500, or 1000 mM NaCl. PBS pH7.4 was included as a reference buffer for normalization of the results. The sensor surface was transferred back to the IBIS MX96 and a baseline of 1 min was followed by association of 1 μM IgG solution for 10 min again ($R_{eq}2$), followed by a complete regeneration of the surface with 25 mM phosphoric acid pH 3.0. This entire procedure, except immobilization, was repeated four times.

Sensorgrams were calibrated, referenced and zeroed and the binding levels at equilibrium were determined. First the recovery for each condition was calculated and normalized against PBS buffer to correct for dissociation of IgG from MSS ligand during buffer flushes in the system. Normalized recovery = $([1-(R_{eq}2/R_{eq}1)]_{\text{tested buffer}}/[1-(R_{eq}2/R_{eq}1)]_{\text{PBS}}) \times 100\%$. The normalized recovery was then translated into yield as follows: Yield = 100% − normalized recovery.

2.2. Elution Buffer Screening Using PreDictor Plates

PreDictor plates (GE life sciences) with 50 μL MabSelect SuRe media per well were prepared according to manufacturer's protocol. Equilibration and wash steps consisted of 3×200 μL PBS buffer pH 7.4. Purified IgG sample at 5 mg mL^{-1} was loaded to each well in 200 μL after equilibration. The wells were washed followed by IgG elution using buffers of 25 mM sodium acetate at pH 3.0–5.75 in 0.25 increments. IgG concentrations in the eluates were determined by OD_{280} measurements with Infinite M1000 reader (Tecan, Männedorf, Switzerland).

2.3. Wash Buffer Screening Using SPRi

A pre-activated Easy2Spot G-type sensor (SSens BV) was immobilized with 12 spots of MabSelect SuRe ligand (GE life sciences), 12 spots of MabSelect ligand (GE life sciences), and 12 spots of KanCapA ligand (Kaneka, Tokyo, Japan) at concentrations between 50 and 1.6 μg mL^{-1} in 50 mM sodium acetate pH 4.5/0.05% polysorbate 80. Immobilization was performed in the continuous flow microspotter (CFM) with a 5-min print time. The sensor was deactivated by 1M ethanolamine pH 8.5 during 10 min in the IBIS MX96.

Association of 100 μL of an IgG CCF (Synthon Biopharmaceuticals BV) to the different ligands was performed for 3 min ($R_{eq}1$) after a baseline of 1 min. This was followed by injections of wash buffer for 3 min and then again an injection of the same IgG CCF for 3 min ($R_{eq}2$). The sensor was regenerated with 25 mM phosphoric acid pH3 for 30 s. This entire procedure was repeated three times for each wash buffer. A total of 48 different wash buffers were selected for this screening, and one per cycle was injected. The wash buffers consisted of 10, 25, or 100 mM Tris pH 8 or sodium acetate pH 5. The 25 mM buffers were used in combination with various additives at different concentrations. The tested additives in both buffers were: NaCl (0.5, 1, and 2 M), arginine (0.5, 1, and 2 M), $CaCl_2$ (0.5, 1, and 2 M), Na_2SO_4 (0.5, 1, and 1.5 M), isopropanol (5, 10, and 20%), ethanol (5, 10, and 20%), and urea (0.5, 1, and 2 M).

Sensorgrams were calibrated, referenced and zeroed and binding levels at equilibrium were determined. Recoveries were calculated and normalized against 10 mM Tris pH 8 buffer as follows: Normalized recovery = $([1-(R_{eq}2/R_{eq}1)]_{\text{tested buffer}}/[1-(R_{eq}2/R_{eq}1)]_{\text{10 mM Tris pH 8}}) \times 100\%$.

2.4. Wash Buffer Screening Using Filter Plates

A total of 96-well filter plates (Whatman) were filled with 40 μL resin (MabSelect SuRe or KanCapA) and equilibrated with 3×200 μL PBS buffer pH 7.4. Then 6×300 μL of IgG CCF was added to the wells and subsequently washed with 2×200 μL of the following buffers: PBS buffer pH 7.4; either 25 mM Tris pH 8 or 25 mM sodium acetate pH 5; each of the 48 selected wash buffers with additives (section 2.3) and at last 25 mM Tris pH 8 or 25 mM sodium acetate pH 5. The protein was eluted in 2×200 μL 25 mM acetate pH 3.0. Recovery of IgG for the wash and elution steps was determined by $OD_{280\,nm}$ with the Infinite M1000 reader (Tecan, Männedorf, Switzerland).

2.5. Alkali Resistance Testing Using SPRi

A pre-activated Easy2Spot G-type sensor was prepared as described in Section 2.3. Association of 100 μL of an IgG

© 2017 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

CCF (Synthon Biopharmaceuticals BV) at approximately 1–2 mg mL^{-1} IgG was performed for 15 min after a baseline of 1 min. The surface was regenerated with 25 mM sodium acetate pH 3 and then 10 cycles of 0.1 or 0.5 M NaOH were injected during 15 min. Each 10th cycle, the initial IgG CCF was injected again. This entire procedure was repeated 10 times to simulate a total of 100 NaOH cycles.

Sensorgrams were referenced and zeroed; responses at equilibrium were determined and expressed as binding capacity. Binding capacity of the first injection was set to 100% and binding capacity of subsequent cycles was calculated relative to the first cycle.

2.6. Alkali Resistance Testing Using Columns

Protein A columns of 1 mL with either MabSelect SuRe or KanCapA resin were used to determine the alkali resistance. First the dynamic binding capacity (DBC) was determined at 10% breakthrough using purified IgG, followed by a run with IgG CCF to determine product quality, followed by eight runs of sanitization with 0.5 M NaOH. This sequence of 10 runs was repeated 10 times to simulate 100 sanitization steps in total. The purification run with CCF consisted of following steps with 10 column volumes (CV) each: equilibration with PBS pH 7.4 at 1 mL min^{-1}; sample load at 0.5 mL min^{-1}; wash in four steps with different wash buffers; IgG elution with 25 mM sodium acetate pH 3.0; regeneration with 0.5 M acetic acid (MSS) or 1 M acetic acid (KCA); wash with purified water; sanitization with 0.5 M NaOH during 15 min and wash with purified water.

The DBC of the initial run was set to 100% and every 10th cycle the DBC was calculated, followed by normalization relative to the initial value.

2.7. Statistical Data Analysis

Statistical analysis of the data was performed in GraphPad Prism 6. Correlation analysis was used in the elution buffer and wash buffer screening. Linear regression analysis was performed on the alkali stability, and slopes of the regression curves were compared with each other.

3. Results and Discussion

3.1. Comparison of Elution Buffer Screening Using SPRi or Filter Plates

A screening of elution buffer conditions for the interaction between MabSelect SuRe (MSS) and IgG was performed on an SPRi sensor surface to prove the concept of the technology (Figure S1, Supporting Information). An array of 48 elution buffers was simultaneously flushed over the surface, to determine the influence of the buffer on the IgG yield, similar to the set-up in Geuijen et al.[20] The yields obtained in SPRi experiments match closely to the yields that were obtained in filter plate experiments and to values reported in literature (Application note 28-9277-92 AA, www.gelifesicences.com) (**Figure 1**). Correlation between the SPRi results and filter plate experiments was 0.94 with $P = 0.005$ for the buffers without NaCl addition. Slightly lower correlation values, of 0.67, 0.84, and 0.84 for 1 M NaCl, 0.5 M NaCl, and 0.25 M NaCl, respectively, were determined for the other elution buffer conditions.

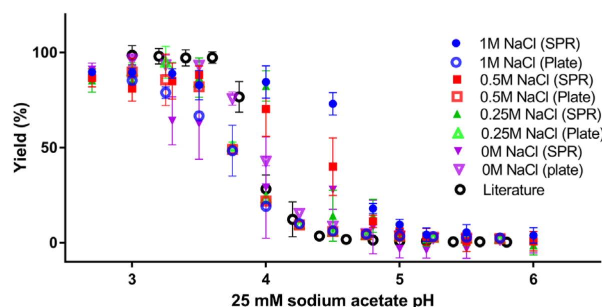


Figure 1. Elution pH profiles of IgG on MabSelect SuRe determined by multiplexed surface plasmon resonance, PreDictor plate experiments and the literature values of PreDictor plate experiments (Application note 28-9277-92 AA, www.gelifesicences.com). Each value of SPRi and filter plate experiments represents the IgG yield at indicated buffer pH of four replicates, with error bars indicating ± one standard deviation.

Minor differences between the different techniques were measured at pH 3.3 and 3, and between pH 4 and 5 upon NaCl addition. The infliction point of the binding equilibrium between IgG and MSS is around these pH values, and this may impact the results. The interactions between MSS and IgG are partly based on electrostatic interactions[21,22] and addition of NaCl to the buffer reduces these interactions. This directly impacts the binding equilibrium, resulting in reduced affinity around pH 4–5, which is translated to increased elution in the SPRi experiments upon NaCl addition. At pH 3.3 and 3, the slightly higher recovery in filter plate experiments may be explained by the presence of an agarose backbone on the resin, which may stabilize the interaction between MSS and IgG.[22] In SPRi experiments the MSS ligand is directly immobilized onto the sensor surface and this may have an impact on the stability of the interaction around the infliction point of the equilibrium. Furthermore, the intrinsic binding capacity of the filter plates and the SPRi sensor may be different, which can impact the results. Columns or filter plates are often loaded up to saturation, whereas the maximum binding capacity of MSS on the SPRi sensor is not reached.

3.2. Comparison of Wash Buffer Screening Using SPRi or Filter Plates

Wash buffers were screened on the SPRi platform with MabSelect (MS), MabSelect SuRe (MSS), and KanCapA (KCA) protein A ligand simultaneously on a single SPRi sensor (Figure S2, Supporting Information). No filter plate experiments with MS were performed, so focus will be on the results of MSS and KCA. A total of 48 wash buffers were selected (see Section 2) and IgG recovery was determined. IgG recoveries of the different wash buffers between filter plate experiments and SPRi experiments were compared. Results between SPRi and filter

© 2017 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

plates correlate well to each other as determined by statistical correlation analysis ($r = 0.57$ for pH 8 and $r = 0.83$ for pH 5; both with $P < 0.0001$).

Recoveries of the wash buffer screening at pH5 were comparable between SPRi and filter plates for four of the tested additives and in the sodium acetate buffer without additive (**Figure 2**B). Two additives (1 and 2 M arginine or $CaCl_2$) had reduced recoveries in the filter plate experiments and even lower recoveries in the SPRi experiments for both ligands. Additionally, a reduced recovery in SPRi experiments was detected with NaCl as additive, which was not observed in filter plate experiments. Protein interactions are based on complex formation between the two binding partners, and this is influenced by the experimental conditions. Especially at pH5, where the interaction between protein A and IgG is close to the infliction point of the equilibrium, the affinity is weaker compared to higher pH, and as a result dissociation will be faster. In SPRi experiments, the flow rate is relatively high compared to column chromatography ($\mu L\, s^{-1}$ vs $mL\, min^{-1}$). This translates to residence times of 0.24 s in SPRi experiments, compared to 3×1 min in filter plate experiments. As the interaction is based on equilibrium between association and dissociation, a higher flow rate or a shorter residence time may impact this equilibrium directly by preventing rebinding events and driving the equilibrium further towards dissociation, resulting in larger IgG loss (expressed as lower recovery).

The majority of wash buffers at pH8 had recoveries of >95% (Figure 2A), both in filter plates and SPRi experiments. Lower recoveries were determined in buffers with 1 or 2 M arginine (MSS and KCA) and 1 or 2 M $CaCl_2$ (MSS only) in filter plate experiments. In the SPRi experiments a similar trend in reduced recovery was observed with $CaCl_2$ as additive, although total recovery was higher as compared to filter plates. Both are strong basic additives, which may not be fully washed out upon start of elution. The lower recoveries are only observed using the buffers with higher molarities of either arginine or $CaCl_2$, which suggests that the pH during elution is possibly still too high to fully recover the IgG from the resin. As flow rates in SPRi experiments are higher compared to filter plate experiments, and therefore residence times of the buffer are shorter, as explained earlier, those basic additives are washed more rapidly in the SPRi

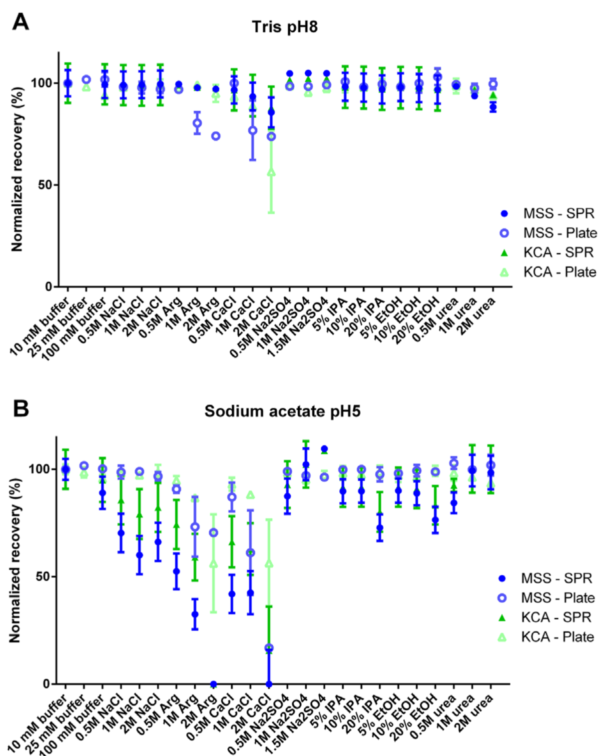


Figure 2. Wash buffer screening on MabSelect SuRe (MSS) and KanCap A (KCA) protein A ligands determined by multiplexed surface plasmon resonance and in filter plates in A) 25 mM tris buffer pH 8 and B) 25 mM sodium acetate pH 5. Various additives at different concentrations were tested in both buffer systems. Values indicate the IgG recovery in % after the wash step by elution at pH 3 (filter plates, $n = 2$), or by measurement of remaining IgG on the ligands (SPRi, $n = 3$). Error bars represent ± one standard deviation.

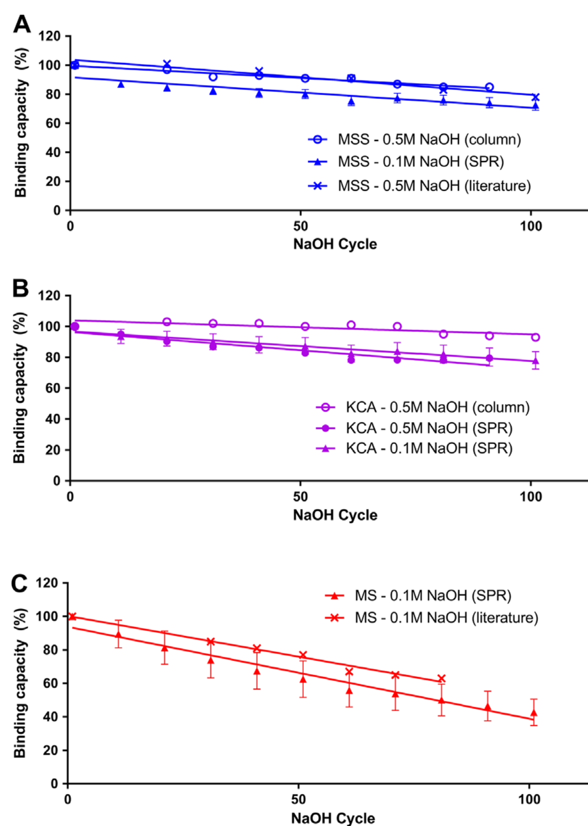


Figure 3. Alkali resistance testing of three protein A ligands: A) MabSelect SuRe [MSS], B) KanCapA [KCA] and C) MabSelect [MS]) determined by multiplexed surface plasmon resonance, on 1 mL chromatography columns and literature (MabSelect SuRe user instructions, 11-0026-01 AD, www.gelifesciences.com; KanCapA product sheet, USD3103, www.bioseparation.kaneka.com/kancap-a.html). Values represent the binding capacity of the ligand, determined by dynamic binding capacity at 10% breakthrough in column experiments ($n = 1$) and by IgG binding responses in SPRi ($n = 12$). Twelve independent spots on a single SPRi sensor for each of the ligands were evaluated and error bars indicate ± one standard deviation.

© 2017 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

experiments, resulting in higher recoveries because of a proper elution pH.

3.3. Comparison of Ligand Alkali Resistance Using SPRi or Small-Scale Columns

The resin life-time in chromatographic purifications is mainly affected by the cleaning-in-place procedure, which is often performed with 0.1 or 0.5 M NaOH solutions, especially for the engineered protein A ligands. CIP cycles were simulated on 1 mL columns and on SPRi up to 100 cycles. Every 10th cycle, the binding capacity expressed as the fraction of IgG that still bound was determined (sensorgrams shown in Figure S3, Supporting Information).

MS is not resistant to 0.5 M NaOH and therefore the SPRi experiments were performed with 0.1 M NaOH, since all three ligands were tested simultaneously. An initial experiment with only KCA ligand at the sensor surface was performed with 0.5 M NaOH. KCA and MSS have a binding capacity around 75–85% of the initial binding capacity after 100 cycles of NaOH, with an exposure time of 15 min per cycle (**Figure 3**). These results are comparable for the different methods that were applied. MS, which is less alkali-stable, had a binding capacity of only 50% after 80 cycles, which is in agreement with approximately 60% reported by the supplier (MabSelect SuRe user instructions, 11-0026-01 AD, www.gelifesciences.com). Resin life-time of all three ligands (MSS, KCA, and MS) is in agreement between column experiments, SPRi experiments, and the literature values (MabSelect SuRe user instructions, 11-0026-01 AD, www.gelifesciences.com; KanCapA product sheet, USD3103, www.bioseparation.kaneka.com/kancap-a.html). A linear regression analysis on the slopes of the binding-capacity curves shows no significant differences between techniques for each ligand individually ($P = 0.85$ for MSS; $P = 0.17$ for KCA; $P = 0.30$ for MS).

4. Conclusion

The SPR imaging technology provides an attractive alternative for screening of process parameters, such as wash and elution buffers or alkali stability for affinity-based purification strategies as demonstrated here. We have proven the concept of this screening based on the interaction between protein A and IgG with results comparable to filter plate and column experiments. The SPRi screening is economically attractive compared to filter plate screening or column screening (Table S1, Supporting Information), as consumption of all materials can be reduced nearly 20-fold up to >1000-fold. Especially reduction of IgG material needed for screening is an important benefit during early phase process development when often only limited material is available.

In a few experiments, we found differences between the tested techniques, which were mostly found around pH 4–5, where the interaction equilibrium changes.[3] The combination of wash buffer pH5 and certain additives led to a lower recovery in the SPRi experiments, and the elution profile changed around pH4 with the addition of NaCl. Differences in flow rate, chemical backbone and different spacer length in the SPRi technology compared to column resins may account for these deviations. For example, the higher flow rate in SPRi reduces rebinding effects, resulting in lower yields.[23] The agarose backbone of a resin may have a stabilizing effect on the interaction, as shown by molecular modeling of the protein A−IgG interaction by Salvalaglio et al.[22]

Overall column and filter plate experiments correlate well with the SPRi results. The major benefits of the SPRi technology are miniaturization and significant decrease in development time. Each of the described screenings was performed within 1 working day, whereas alkali stability on columns took several weeks. Buffer screening with filter plates is comparable in time, however much less material is needed in the SPRi screening strategy (Table S1, Supporting Information).

The SPRi screening as demonstrated here can be very useful in early-phase discovery. The proof of concept was based on protein A−IgG interaction, but any type of interaction for affinity chromatography can be analyzed in this set-up. The technology is especially useful for screening many different ligands simultaneously, for example, in selecting new ligands for affinity chromatography.[24]

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

We would like to thank Sanne Wilmsen, Lonnie Joosten-Stoffels, Eefje Verhofstad, and Thomas de Beijer for executing part of the experimental work. We thank Kaneka Corporation for providing their protein A ligand free of charge.

Conflict of Interest

The authors declare no commercial or financial conflict of interest.

Keywords

continuous flow micro elution screening, ligand selection, process miniaturization, protein A affinity chromatography, surface plasmon resonance

Received: February 23, 2017
Revised: June 9, 2017
Published online: August 10, 2017

[1] P. Cuatrecasas, *Annu Rev. Biochem*. **1971**, *40*, 259.
[2] K. Huse, H. J. Bohme, G. H. Scholz, *J. Biochem. Biophys. Methods* **2002**, *3*, 217.
[3] S. Hober, K. Nord, M. Linhult, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*. **2007**, *1*, 40.
[4] H. F. Liu, J. Ma, C. Winter, R. Bayer, *MAbs* **2010**, *5*, 480.
[5] S. Chollangi, R. Parker, N. Singh, Y. Li, M. Borys, Z. Li, *Biotechnol. Bioeng*. **2015**, *11*, 2292.

© 2017 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

[6] T. M. Pabst, R. Palmgren, A. Forss, J. Vasic, M. Fonseca, C. Thompson, W. K. Wang, X. Wang, A. K. Hunter, *J. Chromatogr. A* **2014**, 180.
[7] R. Hahn, R. Schlegel, A. Jungbauer, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*. **2003**, *1–2*, 35.
[8] R. Hahn, K. Shimahara, F. Steindl, A. Jungbauer, *J. Chromatogr. A* **2006**, *1-2*, 224.
[9] C. Jiang, J. Liu, M. Rubacha, A. A. Shukla, *J. Chromatogr. A* **2009**, *31*, 5849.
[10] A. Gronberg, M. Eriksson, M. Ersoy, H. J. Johansson, *MAbs* **2011**, *2*, 192.
[11] A. Rathore, M. Pathak, G. Ma, D. Bracewell, *BioPharm. Int.* **2015**, *3*, 28.
[12] B. Nogal, K. Chhiba, J. C. Emery, *Biotechnol. Prog*. **2012**, *2*, 454.
[13] T. Ishihara, M. Hosono, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*. **2015**, 107.
[14] A. A. Shukla, P. Hinckley, *Biotechnol. Prog*. **2008**, *5*, 1115.
[15] G. R. Bolton, K. R. Selvitelli, I. Iliescu, D. J. Cecchini, *Biotechnol. Prog*. **2015**, *2*, 406.
[16] T. Yada, K. Nonaka, M. Yabuta, N. Yoshimoto, S. Yamamoto, *Biotechnol. J*. **2016**, 1.
[17] G. Lye, J. Hubbuch, T. Schroeder, E. Williman, *BioProcess Int*. **2009**, *7*, 18.
[18] P. Fernandes, F. Carvalho, M. P. Marques, *Recent Pat. Biotechnol*. **2011**, *3*, 160.
[19] T. Bergander, K. Nilsson-Valimaa, K. Oberg, K. M. Lacki, *Biotechnol. Prog*. **2008**, *3*, 632.
[20] K. P. Geuijen, R. B. Schasfoort, R. H. Wijffels, M. H. Eppink, *Anal. Biochem*. **2014**, 38.
[21] E. T. Gedig, in *Handbook of Surface Plasmon Resonance* (Eds: R. B. M. Schasfoort, A. J. Tudos) The Royal Society of Chemistry, Cambridge, UK **2008**, p. 173.
[22] M. Salvalaglio, L. Zamolo, V. Busini, D. Moscatelli, C. Cavallotti, *J. Chromatogr. A* **2009**, *50*, 8678.
[23] P. Schuck, H. Zhao, *Methods Mol. Biol*. **2010**, *627*, 15.
[24] T. M. Pabst, M. Wendeler, X. Wang, S. Bezemer, P. Hermans, A. K. Hunter, *Biotechnol. J*. **2016**, *12*, 1.

© 2017 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim