Specific capture and detection of *Staphylococcus aureus* with high-affinity modified aptamers to cell surface components

A. Baumstummler¹, D. Lehmann¹, N. Janjic² and U.A. Ochsner²

¹ Merck Millipore, Lab Solutions, BioMonitoring, Molsheim, France
² SomaLogic, Inc., Boulder, CO, USA

**Significance and Impact of the Study:** Monitoring for microbial contamination of food, water, nonsterile products or the environment is typically based on culture, PCR or antibodies. Aptamers that bind with high specificity and affinity to well-conserved cell surface epitopes represent a promising novel type of reagents to detect bacterial cells without the need for culture or cell lysis, including for the capture and enrichment of bacteria present at low cell densities and for the direct detection via qPCR or fluorescent staining.

**Keywords**
aptamer, cell capture, cell surface, protein A, staining, *Staphylococcus aureus*.

**Abstract**
Slow off-rate modified aptamer (SOMAmer) reagents were generated to several *Staphylococcus aureus* cell surface-associated proteins via SELEX with multiple modified DNA libraries using purified recombinant or native proteins. High-affinity binding agents with sub-nanomolar $K_d$’s were obtained for *staphylococcal* protein A (SpA), clumping factors (ClfA, ClfB), fibronectin-binding proteins (FnB,A, FnB,B) and iron-regulated surface determinants (Isd). Further screening revealed several SOMAmers that specifically bound to *Staph. aureus* cells from all strains that were tested, but not to other staphylococci or other bacteria. SpA and ClfA SOMAmers proved useful for the selective capture and enrichment of *Staph. aureus* cells, as shown by culture and PCR, leading to improved limits of detection and efficient removal of PCR inhibitors. Detection of *Staph. aureus* cells was enhanced by several orders of magnitude when the bacterial cell surface was coated with SOMAmers followed by qPCR of the SOMAmers. Furthermore, fluorescence-labelled SpA SOMAmers demonstrated their utility as direct detection agents in flow cytometry.

**Introduction**
SOMAmer (slow off-rate modified aptamer) reagents are made from single-stranded DNA (ssDNA) that contain pyrimidine residues modified at their 5-position with mimics of amino acid side-chains and have quite long (>30 min) dissociation rates (Gold et al. 2010). These features lead to better affinity and better kinetic properties of SOMAmers compared with standard RNA or DNA aptamers. Virtually any protein can be used for SELEX (systematic evolution of ligands by exponential enrichment) to generate specific, high-affinity SOMAmers in multiple rounds of selection with kinetic challenge, partitioning and amplification from a random library of modified ssDNA (Gold et al. 2010; Vaught et al. 2010). Advantages of SOMAmers over antibodies include exceptional thermostability in solution, lower molecular weight, higher multiplexing capabilities, chemical stability to heat, drying and solvents, reversible renaturation, ease of reagent manufacturing, consistent lot-to-lot performance and lower cost. SOMAmers have been generated to >1000 human proteins and are the basis for the SOMAscan proteomic platform developed by SomaLogic to measure these proteins simultaneously and with high accuracy in a small (0-1 ml) blood sample. The application of this highly multiplexed assay has led to the discovery of biomarkers in various areas of medicine (Gold et al. 2012). With respect to microbial proteins, we have previously reported on the characterization of SOMAmers...
for *Clostridium difficile* toxins and shown the wide range of potential applications of these binding agents (Ochsner et al. 2013).

Binding agents to specific components on the surface of bacteria can be valuable diagnostic tools useful for different detection platforms. Staining of surface antigens for immunofluorescence microscopy has been demonstrated using antibody–fluorophore conjugates to detect relatively low numbers of *Staph. aureus* cells over time in *in vivo* infection models (Timofeyeva et al. 2014). Short peptides as specific ligands to the *Staph. aureus* cell surface have been identified by phage-display, and a synthetic consensus peptide (SA5-1) was able to detect approx. 100 CFU ml$^{-1}$ in a spiked biological sample using fluorescent quantum dots (Rao et al. 2013).

*Staphylococcus aureus* proteins useful for the selection of binding reagents for whole cells or secreted factors include MSCRAMMs (Microbial surface components recognizing adhesive matrix molecules), SERAMs (secretable expanded repertoire adhesive molecules), and extracellular toxins and immune evasion factors (Gill et al. 2005; Speziale et al. 2009). It is possible to use whole bacterial cells for SELEX (Cao et al. 2009), or surface-associated proteins extracted from cells with LiCl, lysostaphin, or 2% SDS (Palma et al. 1998; Hussain et al. 2001; Roche et al. 2003), or released by trypsin shaving (Ythier et al. 2012). We focused on well-conserved *Staph. aureus*-specific cell surface proteins that are known to be expressed in abundance and under most growth conditions.

The *Staph. aureus* cell envelope, cell wall-associated proteins and mechanisms for protein attachment, are quite well understood (Dreisbach et al. 2011). The ten surface-associated proteins for which we generated SOMAmers include SpA, ClfA, ClfB, FnbA, FnbB, SasD, IsdA, IsdB, IsdC and IsdH. All of these proteins are attached to the cell wall via sortase-mediated cleavage between the threonine and the glycine of the LPXTG sortase motif (Schneewind et al. 1995) and become amide-linked to the pentaglycine cross-bridge of peptidoglycan (Marraffini et al. 2006). As our goal was to obtain binding agents to *Staph. aureus* cells, we produced recombinant proteins that represent the surface-exposed domains but lack the signal sequences and the repeat regions of the cell wall-embedded domain. *Staphylococcus aureus* protein A (SpA) is present on the bacterial surface as well as secreted into the extracellular milieu (Sorum et al. 2013) and represents an attractive diagnostic target as it is well conserved in among *Staph. aureus* but absent in non-pathogenic staphylococci. A potent immune evasion factor, SpA, binds the Fc region of antibodies and the Fab regions of the B-cell receptor (Falugi et al. 2013; Kobayashi and Deleo 2013). ClfA and ClfB are structurally related fibrinogen-binding proteins (McDevitt et al. 1997; Ni Eidhin et al. 1998). ClfB is one of the key factors responsible for adherence to desquamated epithelial cells of the anterior nares and is typically produced during early exponential phase of growth (Ni Eidhin et al. 1998). FnbA and FnbB adhere to components of the extracellular matrix, both fibronectin and elastin, and are important for colonization of host tissues during infection (Roche et al. 2004). SasD is a putative adhesion protein with unknown physiological role (Roche et al. 2003; Ythier et al. 2012). Four of the proteins belong to the iron-responsive surface determinant (Isd) system that is induced in *Staph. aureus* under iron-limiting conditions and is important for capture of haeme from haemoglobin (IsdB, IsdH) and its transport (IsdA, IsdC) across the cell wall (Mazmanian et al. 2003; Grigg et al. 2010).

As a proof-of-concept and to assess their efficiency, the SOMAmers generated against *Staph. aureus* cell surface-associated proteins were used to capture and detect *Staph. aureus* using qPCR and also to directly detect the cells by flow cytometry.

**Results and discussion**

**SELEX with *Staphylococcus aureus* proteins**

All 10 recombinant *Staph. aureus* cell surface proteins were found in the soluble fraction when over-expressed in *Escherichia coli*. Sequential affinity chromatography on Ni-NTA agarose and Streptactin sepharose yielded 0.1–1.5 mg of each protein in >95% purity (Figure S1). Eight rounds of SELEX were performed with these proteins, using three separate ssDNA libraries, and C$_0$ reassociation kinetics indicated a reduction of sequence complexity. Pool affinity assays confirmed the successful selection of SOMAmers for a total of 22 pools obtained with BndU, NapdU or TrpdU modified ssDNA, with pool affinities in the range of 0.13–8.90 nmol l$^{-1}$. Specific binding to *Staph. aureus* cells, but no binding to *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *E. coli* or *Pseudomonas aeruginosa*, was observed, as shown for a subset of the SOMAmer pools for SpA and ClfB (Figure S2a).

Alignment of sequences determined for 48 clones from each pool showed multiplicity clones and families that shared common sequence patterns. Representative clones were screened in affinity assays (Figure S2b), and the $K_a$’s of the best SOMAmers were in the range of 0.03–2.17 nmol l$^{-1}$ (Table 1).

**Capture of *Staphylococcus aureus* cells with cell surface directed SOMAmers**

SOMAmers binding affinities to purified *Staph. aureus* proteins correlated well with the observed binding to...
whole bacteria. Two of the SpA-NapdU clones (4520-8 and 4520-9) and three of the SpA-TrpdU clones (4531-55, 4531-56, 4531-94) were able to bind whole cells of all *Staph. aureus* strains tested, with a detection limit of approx. $10^3$ cells per well ($10^4$–$10^6$ cells ml$^{-1}$) in a radiolabel filter binding assay. Binding to *Staph. epidermidis* or *Staph. haemolyticus* cells was not observed, indicating good specificity of these SOMAmerers (Figure S2c). Similar binding characteristics were observed for the ClfA and ClfB SOMAmerers. In contrast, most of the FnBA and FnBB SOMAmerers that strongly bound to *Staph. aureus* also had some affinity to *Staph. epidermidis* and *Staph. haemolyticus*. SOMAmerers directed to the Isd proteins, in particular IsdC, showed strong and specific binding to *Staph. aureus* cells, and signals were enhanced when the bacteria had been grown under iron-limiting conditions. SasD SOMAmerers failed to bind whole cells, although it is not clear whether this is due to the rather modest affinity or due to low expression levels of this surface protein (data not shown).

The number of target molecules per cell is unknown for any of these surface proteins, and expression levels may vary depending on growth conditions and growth phase. However, assuming 1000 copies per cell and using $10^7$ CFU ml$^{-1}$ would represent a target concentration of 20 pmol l$^{-1}$, which is at or below the typical SOMAmer $K_a$. Thus, the radiolabel filter binding assays, where the SOMAmerers are present at low concentrations of 10–20 pmol l$^{-1}$, are limited to relatively high cell densities. To drive the binding reaction, we used higher concentrations (20 nmol l$^{-1}$) of biotinylated SpA SOMAmerers as capture agents attached to beads and were able to detect as few as 50 cells in a 0.1-ml sample (Fig. 1a). SOMAmer concentration of 10 nmol l$^{-1}$ or above were required for efficient capture of *Staph. aureus* at such low cell densities (Fig. 1b). SOMAmerers were able to bind selectively to *Staph. aureus* cells in mixed cultures that contained *Staph. aureus*, *Staph. epidermidis* and *E. coli* each at $10^2$–$10^6$ CFU ml$^{-1}$. The best performing binding agents were SpA 4520-8 (PCGGCPPCGBPAPCCPAPPAPCGGPPPA GCCCAGPCAGAA; P=NapdU) and ClfA 4503-73 (AZCZ GGZZCAAAGZGCGAZGGCAZCGZGZGZZZZAAAGZ; Z=BndU), demonstrating low nonspecific binding comparable to random sequence modified SOMAmer controls. Capture of *Staph. aureus* on paramagnetic SOMAmer beads was efficient over a wide range of cell densities, from $5 \times 10^2$ CFU ml$^{-1}$ (Fig. 1c) to $5 \times 10^9$ CFU ml$^{-1}$ (Fig. 1d).

### Enhanced detection of *Staphylococcus aureus* using SOMAmer-based enrichment

Capture of *Staph. aureus* cells proved useful for down-stream detection by PCR, either for enrichment of the sample when cell densities were low, or to remove PCR inhibitors. Coating of the *Staph. aureus* cell surface with full-length, amplifiable SOMAmerers allowed the faster detection by qPCR of the SOMAmerers compared with qPCR of a genomic target, as each cell contained hundreds of copies of the target surface component for detection, compared with a single genome. In the example shown in Fig. 2, *Staph. aureus* cells were captured with nonamplifiable ClfA SOMAmerers and coated with amplifiable SpA SOMAmerers or random sequence SOMAmer controls, followed by qPCR using SOMAmer-specific primers. Separately, the cells were lysed and subjected to qPCR using *Staph. aureus*-specific genomic primers, which was clearly less efficient compared with qPCR of bound SOMAmerers. This is obvious from the observed shift by up to eight cycles in qPCR detection, from 10 cycles for SOMAmer qPCR to 18 cycles for genomic qPCR, which is consistent with a ratio of several hundred copies ($2^8 = 256$) of surface-bound SOMAmerers to only a single genome. The method of ClfA SOMAmer capture and SpA SOMAmer detection was specific for *Staph. aureus* cells, because *Staph. epidermidis* cells that do not possess ClfA or SpA did not result in any SOM-
Amer amplification above background. Capture of bacteria on beads followed by detection with SOMAmers not only enabled enrichment from low cell density suspensions, but also allowed the efficient removal of PCR inhibitors. Excess salt (e.g. 1 mol l\(^{-1}\) NaCl or 0.5 mol l\(^{-1}\) KCl), detergents (e.g. 0.5% Na-deoxycholate) or solvents (e.g. 5% isopropanol) are known PCR inhibitors (Abu Al-Soud and Radstrom 1998; Schrader et al. 2012), and

Figure 1 Capture of *Staphylococcus aureus* bacteria with SpA SOMAmers immobilized on paramagnetic beads. The efficiency of cell capture was calculated via quantitative culture of the beads. (a) SOMAmer concentration was fixed at 20 nmol l\(^{-1}\) to capture cells in a 0.1-ml sample. (■) 5000 CFU, (■) 500 CFU and (■) 50 CFU. (b) Cell density was fixed at 6600 CFU in a 0.1-ml sample, and the capture SOMAmer concentrations were varied. (■) 32 nmol l\(^{-1}\), (■) 10 nmol l\(^{-1}\), (■) 3.2 nmol l\(^{-1}\) and (■) 1 nmol l\(^{-1}\). Monitoring of capture efficiency by semiquantitative culture at low cell density (c) or by visually apparent decrease in turbidity at high cell density (d).

Figure 2 SOMAmer-based capture of *Staphylococcus aureus* and signal amplification via qPCR of SOMAmers bound to highly abundant cell surface components compared with qPCR of single genomic copies. Nonamplifiable, biotinylated Cia SOMAmers 4522-5 (black lines) or 4503-73 (grey lines) were used for capture of *Staph. aureus* (solid lines) or *Staph. epidermidis* (dashed lines), followed by detection with amplifiable SpA SOMAmer 4520-8. Random SOMAmer library was used as negative control for detection (dotted lines). PCR amplification of a genomic target (sasD gene) is shown for reference (dotted hairline), using the same cell titre of \(10^8\) cells ml\(^{-1}\).
direct genomic PCR failed unless the cells were captured first to remove these agents (Table S2).

Direct detection of *Staphylococcus aureus* by flow cytometry

Fluorescence-labelled SpA SOMAmers 4520-8 and 4531-56 were used to assess SOMAmers efficiency for the detection of *Staph. aureus* by flow cytometry. Both SOMAmers performed well in staining whole *Staph. aureus* cells.

While 100% of the cells were already stained at a low SpA SOMAmers concentration of 0·07 μmol l⁻¹, the mean fluorescence intensities increased substantially at higher reagent concentrations of 0·7 and 7 μmol l⁻¹ (Fig. 3a). The data are consistent with the expected increase in saturation of highly abundant cell surface components with the fluorescence-labelled SOMAmers. The minimal concentration of SpA SOMAmers to ensure a precise discrimination by flow cytometry between the negative populations (unstained cells) and the positive populations (stained cells) can be set up at 0·07 μmol l⁻¹ (Fig. 3b).

To define the optimal binding time, a time-course assay was performed. After only a 5-min binding reaction, 100% of the cells were already stained in the presence of 7 μmol l⁻¹ SpA SOMAmers (Fig. 4a). By increasing the binding time to 15 and 30 min, the fluorescence intensities were improved about twofold and threefold, respectively, leading to a better discrimination between the negative population and the positive population. This increase in fluorescence intensity was less pronounced when the cells were labelled with a lower SpA SOMAmer concentration (0·7 μmol l⁻¹), likely due to limiting SOMAmer concentration (Fig. 4b).

The SpA SOMAmers developed in this study proved to have a high staining efficiency for *Staph. aureus*. In our experimental conditions, the optimal detection of
Staph. aureus by flow cytometry was achieved in a simple one-step procedure using a low reagent concentration (0.7 µmol l⁻¹) and a short binding time (15 min). DNA aptamers have already been reported for the flow cytometry-based detection of Staph. aureus using single or combined aptamers (Cao et al. 2009) or for the detection of Salmonella typhimurium using aptamers generated by whole-cell SELEX (Dwivedi et al. 2013). However, the percentages of aptamer-labelled cells obtained in these studies did not approach 100%, and the fluorescence intensities were weaker compared with the fluorescence intensities obtained with SOMAmers.

In this study, we show that the developed SOMAmers proved useful for the selective capture and enrichment of Staph. aureus cells from low cell density suspensions. Furthermore, fluorescence-labelled SpA SOMAmers demonstrated great efficiency for the direct detection of Staph. aureus cells by flow cytometry.

SOMAmers represent an interesting type of reagents to detect whole bacterial cells. New SOMAmers could be generated against a broader range of micro-organisms to develop new diagnostic tools for pathogen detection. Potential applications include targeted monitoring, quantitative testing and surveillance of nonsterile products, food, water and environmental samples for microbial contamination. Given the ease of generating these specific, stable and high-affinity binding reagents and producing them synthetically, SOMAmers are attractive for biodetection, biosurveillance and food safety. The superior multiplexing capability of SOMAmer reagents compared with antibodies allows the assembly of screening platforms as well as of specific panels.

Figure 4 Detection of Staphylococcus aureus by flow cytometry after staining of 10⁷ cells with fluorescence-labelled 4531-56 SpA SOMAmer at (a) 7 µmol l⁻¹ or at (b) 0.7 µmol l⁻¹. Comparison of different staining times (n = 3). Mean fluorescence intensities are represented in columns and percentages of stained cells as line graph.
Aptamers for bacterial cells

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Materials and methods

Purification of Staphylococcus aureus targets

Relevant portions of the genes encoding the desired targets or target domains were PCR-amplified from Staph. aureus NRS384 (USA300) genomic DNA with primers shown in Table S1 and cloned into pCR-Script SK+ (Stratagene, La Jolla, CA). The clfA, clfB, fnbA, sasD, isdA, isdB, isdC and isdB genes were transferred as BamHI-SacI cassettes into the expression vector pET-51b (EMD-Millipore, Billerica, MA) that harbours an aminoterminal Strep-tag and a carboboxterminal His10-tag. One of the targets, fnbB, was cloned as and NdeI-BamHI fragment into pET-14b (EMD-Millipore), which harbours an aminoterminal His10-tag. The plasmids were sequenced to verify the gene identity and proper gene fusion of the cloned DNA fragment with the vector-encoded sequences for the His-tag and Streptag.

The recombinant proteins were over-expressed in E. coli BL21(DE3) or in BL21(DE3)/pLysE (EMD-Millipore). Conditions for optimal expression of soluble proteins were optimized with respect to growth temperature (25–37°C) and induction time (4–15 h). Cells from 0-1 to 0-8 l cultures were lysed with 10 ml BugBuster/Benzonase reagent (EMD-Millipore). The recombinant, His10-/Strep-tagged proteins were purified from the soluble fraction via sequential affinity chromatography on Ni-NTA agarose and Superflow™ agarose (EMD-Millipore). Native staphylococcal protein A was purchased from VWR and was biotinylated with NHS-PEG4-biotin (Pierce Bio-technology, Rockford, IL). Protein concentrations were determined using the Quick Start Bradford Protein Assay Kit (Bio-Rad, Hercules, CA).

SOMAmer selection

Separate libraries with 5-benzylaminocarbonyl-dU (BndU), 5-naphthylmethyl-aminocarbonyl-dU (NapdU) and 5-tryptaminocarbonyl-dU (TrpdU) were used for SELEX with the Staph. aureus proteins. Each selection started from 1 nmol (10^{14}–10^{15}) sequences containing 40 consecutive randomized positions flanked by fixed sequences required for PCR amplification. SELEX was performed essentially as described (Gold et al. 2010; Vaught et al. 2010; Ochsner et al. 2013). Buffer SB18T was used throughout SELEX and subsequent binding assays, consisting of 40 mmol l^{-1} HEPES pH 7.5, 0-1 mol l^{-1} NaCl, 5 mmol l^{-1} KCl, 5 mmol l^{-1} MgCl2 and 0-05% Tween-20. Eight rounds of selection were carried and, beginning with round 2, a kinetic challenge with 10 mmol l^{-1} dextran sulphate was performed to favour slow off-rates. Counter-selection was performed with 10 μl of 5 mg ml^{-1} Dynabeads® Talon™ (Life Technologies, Carlsbad, CA) containing 4 μg mg^{-1} Hexa-His (AnaSpec) or with MyOne Streptavidin C1 beads (Life Technologies, Carlsbad, CA) to discourage selection of bead- or tag-binding SOMAmers and with a protein competitor mixture consisting of 10 μmol l^{-1} each of prothrombin, casein and human albumin to remove SOMAmers with potential for nonspecific binding. Partitioning of the SOMAmer–target complexes was achieved with paramagnetic Dynabeads® Talon™ (Invitrogen) that bind the His10-tag on the recombinant proteins or with MyOne Streptavidin C1 beads (Life Technologies) for the biotinylated SpA. Selected sequences were eluted from the bead-bound targets with 80 μl of 40 mmol l^{-1} NaOH, neutralized with 20 μl of 160 mmol l^{-1} HCl and PCR-amplified using KOD EX DNA polymerase (Invitrogen-Life Technologies). Modified DNA for the next round was prepared with KOD EX DNA polymerase via primer extension from the antisense strand of the PCR products and purified as described (Gold et al. 2010).

DNA reassociation kinetic analysis (C0t) of selected DNA from rounds 3 through 8 was used for the assessment of sequence convergence during the later rounds, indicating increased abundance of some sequences or sequence families. SOMAmer pools that demonstrated good affinity (Kd ≤10 nmol l^{-1}) in solution binding radioassays (see below) were cloned, and the sequences of 48 clones per pool were determined. Up to 12 individual SOMAmers were chosen based on sequence patterns and diversity and prepared enzymatically for further characterization.

Synthetic SOMAmers were prepared as 48- to 50-mers at 1 μmol scale via standard phosphoramidite chemistry and HPLC purified. They contained a 5'biotin-dA or 5'fluorescein-biotin-dA and an inverted dT nucleotide at the 3' end (3'IdT) for added stability to 3' to 5' exonucleases.

SOMAmer equilibrium and whole-cell radiolabel binding assays

SOMAmers were properly folded via heating for 5 min at 95°C, followed by cooling to room temperature over a 10- to 15-min period, prior to binding assays.

Affinities (Kd's) were determined in equilibrium solution binding assays of radiolabelled SOMAmers (10–20 pmol l^{-1}) with serially diluted proteins (0-001–100 nmol l^{-1}) and Zorbax PSM-300A (Agilent Technologies, Santa Clara, CA) resin for partitioning onto filter plates as described (Gold et al. 2010).

Prior to cloning, the SOMAmer pools were also tested for specific binding to Staph. aureus, using Staph. epidermidis, Staph. haemolyticus, Strep. pyogenes, Ent. faecalis, E. coli and Ps. aeruginosa as controls in 2-h equilibrium binding assays. Cell densities ranged from 10^5–10^8 CFU ml^{-1}, and 0-1 nmol l^{-1} dextran sulphate and
0.35 mol l⁻¹ NaCl was added to the binding buffer to reduce nonspecific background. In addition, individual SOMAmers were screened for binding to eight different Staph. aureus strains belonging to different lineages, including NRS382, NRS383, NRS384, NRS123, NRS385, NRS386, NRS103 (NARSA) and ATCC 29213 (ATCC).

Cell capture assays and detection via culture or qPCR
Biotinylated SOMAmers were prepared enzymatically via primer extension, using PBDC primers (5’-photocleavable biotin, D-spacer and cy3). For immobilization, 1 pmol of PBDC SOMAmers were added to 20 μl MyOne Streptavidin Cl beads (10 mg ml⁻¹) and shaken for 15 min, resulting in approx. 90% efficiency of immobilization based on cv3 measurements in the noncaptured supernatant fraction. Bacteria were grown for 16 h at 35°C in LB broth cultures or on tryptic soy agar with 5% sheep blood and 0.1 mmol l⁻¹ dipyridyl to create iron-limiting conditions. Cell suspensions containing up to 10⁸ bacteria in 50 μl SB18T were added to the capture beads. After incubation with shaking for 1 h at 37°C, the beads were washed and resuspended in 50 μl SB18T. Cells in the noncaptured supernatant, wash fraction and on the beads were enumerated by quantitative plating of serial dilutions onto LB agar. Capture efficiency via quantitative culture was also determined in mixed populations and over a range of cell densities (10⁵–10⁷ CFU ml⁻¹).

Capture of Staph. aureus cells was also achieved with 25 nmol l⁻¹ of synthetic, biotinylated SOMAmers (50 mers) attached to paramagnetic SA beads (15 min, 37°C, with intermittent shaking). The beads were washed twice with 100 μl of SB18 to remove any unbound cells and resuspended in 50 μl SB18. Full-length, amplifiable SOMAmers were added (50 μl of 20 nmol l⁻¹), and the beads were incubated for 15 min at 37°C with intermittent shaking to allow coating of the cells with these surface-component-specific SOMAmers. After washing five times for 2 min each with 100 μl of SB18/1 mmol l⁻¹ dextran sulphate/0.01% Tween-20 and twice with 100 μl of SB18, bound SOMAmers were eluted, cleaned up on primer capture beads and used for qPCR with primers specific for the 5’ and 3’ fixed regions as described (Gold et al. 2010). For direct genomic PCR, cells were boiled for 15 min in lysis buffer (10 mmol l⁻¹ Tris-HCl pH 8/1 mmol l⁻¹ EDTA/0.5% Triton X-100), and one-fifth volume of cleared lysate was used as template.

Flow cytometry assays
SOMAmers to Protein A (SpA) were synthesized as 48-mers containing a 5’ABiT (biotin and fluorescein), for use in flow cytometry experiments. Activity was confirmed for 4520-8 (Kd = 0.17 nmol l⁻¹) and 4531-56 (Kd = 0.09 nmol l⁻¹). SOMAmers were properly folded via heating for 5 min at 95°C, followed by cooling to room temperature over a 10- to 15-min period, prior to staining and flow cytometry assays.

Trycase Soy Broth (TSB; bioMérieux, Craponne, France) was inoculated with a frozen-preserved culture of Staph. aureus DSM 1104 (or ATCC 25923) and incubated overnight at 32-5°C with shaking. Overnight culture was then subcultured in fresh TSB at 32-5°C with shaking until the working culture reached an OD₆₀₀ of 0.8 (approx. 10⁸ bacteria ml⁻¹) and divided into aliquots of approx. 10⁷ cells per tube. Bacteria were harvested by centrifugation at 10 000 g for 2 min. Pellets were resuspended in 100 μl of PBS/25 mmol l⁻¹ MgCl₂ containing a range of SOMAmers concentrations (0.07–7 pmol l⁻¹). After incubation at room temperature for 5, 15 or 30 min, the bacteria were centrifuged at 10 000 g for 2 min and the pellets were resuspended with PBS/25 mmol l⁻¹ MgCl₂. This washing step was repeated twice. Controls of unstained bacteria were included by following the same protocol without adding SOMAmers. Unstained and stained bacteria were then analysed by flow cytometry. All experiments were performed with a FACS Calibur™ flow cytometer (Becton Dickinson Biosciences; Le Pont de Claix, France) equipped with an air-cooled 15 mW argon-ion laser emitting at 488 nm. The green fluorescence was collected in the FL1 channel (530 ± 30 nm) as logarithmic signal. The mean fluorescence intensity and the percentage of fluorescent cells (n = 10 000 in the defined gate) occurring as a consequence of SOMAmers binding were determined in these assays. Data from the FACS Calibur™ were analysed using the BD CellQuest™ software (Becton Dickinson Biosciences).

Acknowledgements
We thank Sheela Waugh, Tim Fitzwater, Sheri Wilcox, Jeff Carter, Steve Wolk and Larry Gold for technical expertise, SOMAmder synthesis, quality control and scientific guidance. We also acknowledge Mathilde Arnault and Mathieu Brette for excellent technical assistance in flow cytometry experiments. This project was self-funded by SomaLogic, Inc., and Merck Millipore.

Conflict of Interest
N. Janic and U. Ochsner are employees and shareholders of SomaLogic, Inc. A. Baumstummler and D. Lehmann are employees at Merck Millipore.
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Supporting Information
Additional Supporting Information may be found in the online version of this article:

**Figure S1** SDS-PAGE analysis of cell surface-associated Staph. aureus proteins over-expressed in recombinant form in E. coli and purified by affinity chromatography on Ni-NTA agarose and Streptactin Sepharose.

**Figure S2** Radiolabel affinity binding assays with individual SOMAmers from SELEX pool 4520 NapdU and 4531 TrpdU using purified SpA protein serially diluted from 0-001–100 nmol l⁻¹ (a) and whole cells diluted to 10⁷, 10⁶, 10⁵, and 10⁴ CFU ml⁻¹ (b).

**Table S1** Amplification and cloning of genes encoding Staphylococcus aureus cell surface proteins.

**Table S2** Effect of cell capture for removal of PCR inhibitors.