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ORIGINAL ARTICLE

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

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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*.

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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 (FnbA, FnbB) 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_0t 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 multicopy clones and families that shared common sequence patterns. Representative clones were screened in affinity assays (Figure S2b), and the K_d'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

Table 1 SOMAmer reagents for *Staphylococcus aureus* cell surface proteins, with affinity (K_d) shown for the original full-length sequences obtained in SELEX

	SOMAmer characterization, length and fraction of C5-modified bases				
Target	SeqID	Modified	K_d (nmol l^{-1})	Length (nt)	% mod dU
SPA	4520–8	NapdU	0·22	40	30
SPA	4531–56	TrpdU	0·03	39	30·8
ClfA	4503–73	BndU	0·79	40	37·5
ClfA	4522–5	TrpdU	0·35	39	30·8
ClfB	4504–27	BndU	1·35	40	47·5
ClfB	4511–67	NapdU	3·90	40	40
ClfB	4523–79	TrpdU	0·47	40	32·5
FnbA	4726–44	NapdU	4·38	40	20
FnbA	4745–51	TrpdU	0·63	40	25
FnbB	4506–13	BndU	4·73	39	52·5
FnbB	4516–29	NapdU	0·63	40	27·5
FnbB	4527–83	TrpdU	0·84	40	32·5
IsdA	4727–62	NapdU	0·73	40	27·5
IsdA	4746–3	TrpdU	0·16	40	30
IsdB	4728–7	NapdU	0·14	40	22·5
IsdB	4747–90	TrpdU	1·98	40	22·5
IsdC	4507–52	BndU	0·15	40	45
IsdC	4517–71	NapdU	0·08	40	30
IsdC	4528–22	TrpdU	0·07	40	35
IsdH	4731–69	NapdU	1·30	40	27·5
SasD	4730–3	NapdU	2·17	40	25

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. 10^4 cells per well (10^5–10^6 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 SOMAmers (Figure S2c). Similar binding characteristics were observed for the ClfA and ClfB SOMAmers. In contrast, most of the FnbA and FnbB SOMAmers that strongly bound to *Staph. aureus* also had some affinity to *Staph. epidermidis* and *Staph. haemolyticus*. SOMAmers 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 SOMAmers 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_d's. Thus, the radiolabel filter binding assays, where the SOMAmers 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 SOMAmers 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 concentrations of 10 nmol l^{-1} or above were required for efficient capture of *Staph. aureus* at such low cell densities (Fig. 1b). SOMAmers were able to bind selectively to *Staph. aureus* cells in mixed cultures that contained *Staph. aureus*, *Staph. epidermidis* and *E. coli* each at 10^5–10^6 CFU ml^{-1}. The best performing binding agents were SpA 4520-8 (PCGGCPPCGGGPACCPAPPAPCGGPPPA GCCCAGPCAGAA; P=NapdU) and ClfA 4503-73 (AZCZ GGZZCAAAGZGGCGAZZGGGCAZCZGGZZZZZAAGZ; 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 5×10^2 CFU ml^{-1} (Fig. 1c) to 5×10^9 CFU ml^{-1} (Fig. 1d).

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

Capture of *Staph. aureus* cells proved useful for downstream 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 SOMAmers allowed the faster detection by qPCR of the SOMAmers 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 SOMAmers and coated with amplifiable SpA SOMAmers 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 SOMAmers. 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 SOMAmers 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-

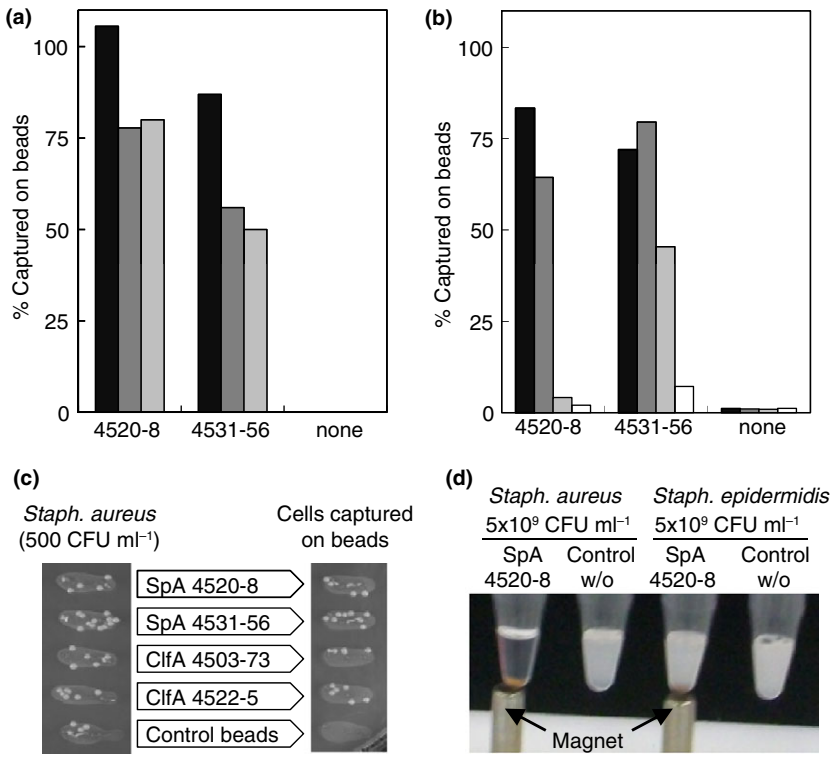

Figure 1 Capture of *Staphylococcus aureus* bacteria with SpA SOMAmers immobilized on paramagnetic beads. The efficiency of cell capture was calculated via quantitative culture of the beads. (a) SOMAmer concentration was fixed at 20 nmol l^{-1} to capture cells in a 0·1-ml sample. (■) 5000 CFU, (■) 500 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).

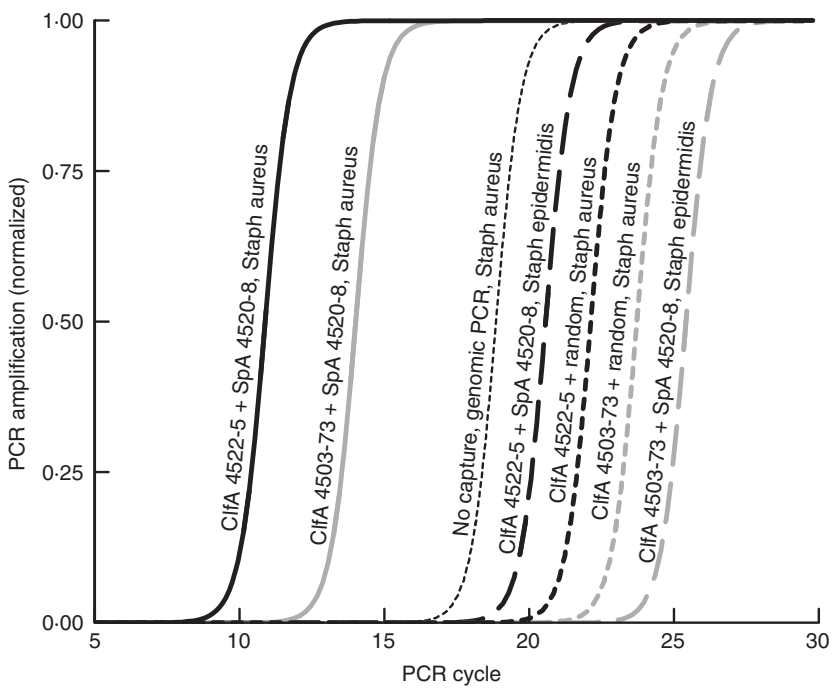

Figure 2 SOMAmer-based capture of *Staphylococcus aureus* and signal amplification via qPCR of SOMAmers bound to highly abundant cell surface components compared with qPCR of single genomic copies. Nonamplifiable, biotinylated ClfA SOMAmers 4522-5 (black lines) or 4503-73 (grey lines) were used for capture 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}.

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

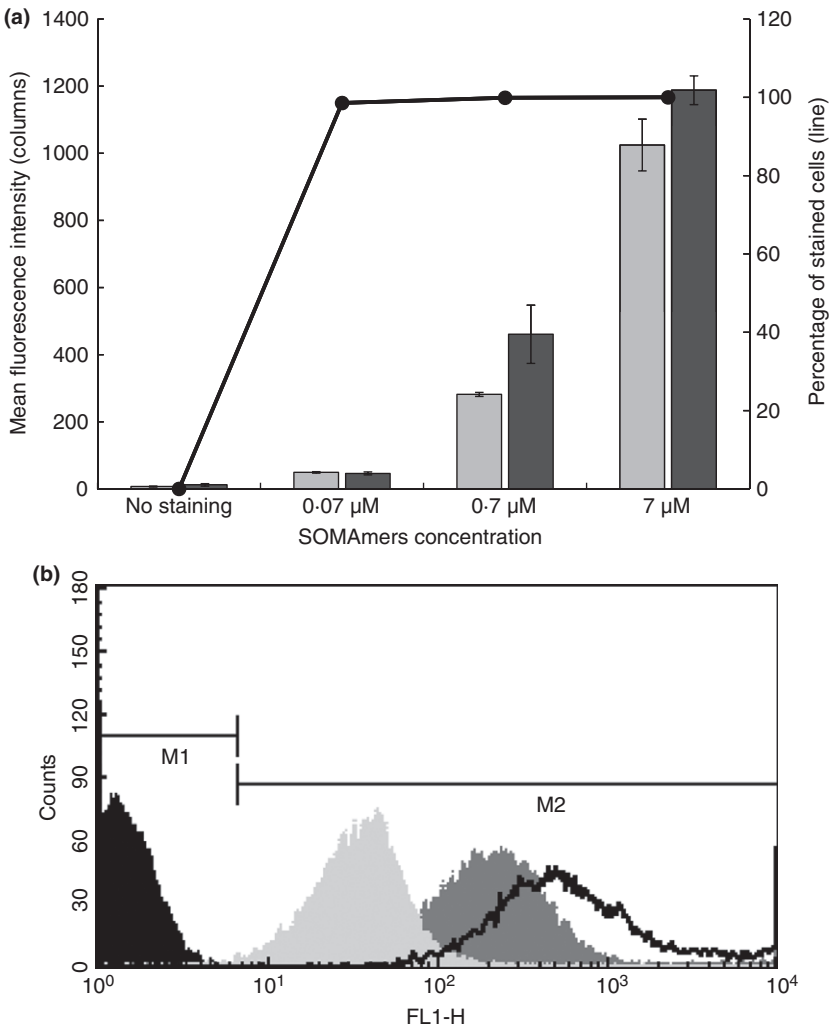

Figure 3 Detection of *Staphylococcus aureus* by flow cytometry after staining of 10^7 cells with different concentrations of fluorescence-labelled 4520-8 and 4531-56 SpA SOMAmers (15-min binding reaction). (a) Representation of the mean fluorescence intensities (columns) and the percentages of stained cells (line) obtained at different SOMAmers concentrations (n = 3). (▒) mean fluorescence intensity with 4520-8, (■) mean fluorescence intensity with 4531-56 and (—●—) percentages of stained cells with either 4520-8 or 4531-56 (identical data). (b) Example of flow cytometry results obtained with 4531-56 SpA SOMAmer. (■) Unstained cell population; cell population stained with (▒) 0·07 μmol l^{-1}, (■) 0·7 μmol l^{-1} and in thick black line 7 μmol l^{-1}. M1 and M2 intervals have been set up to include in M1 the fluorescence signal of the control unstained cells (autofluorescence). Therefore, cell populations located in the M2 interval are considered as stained.

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

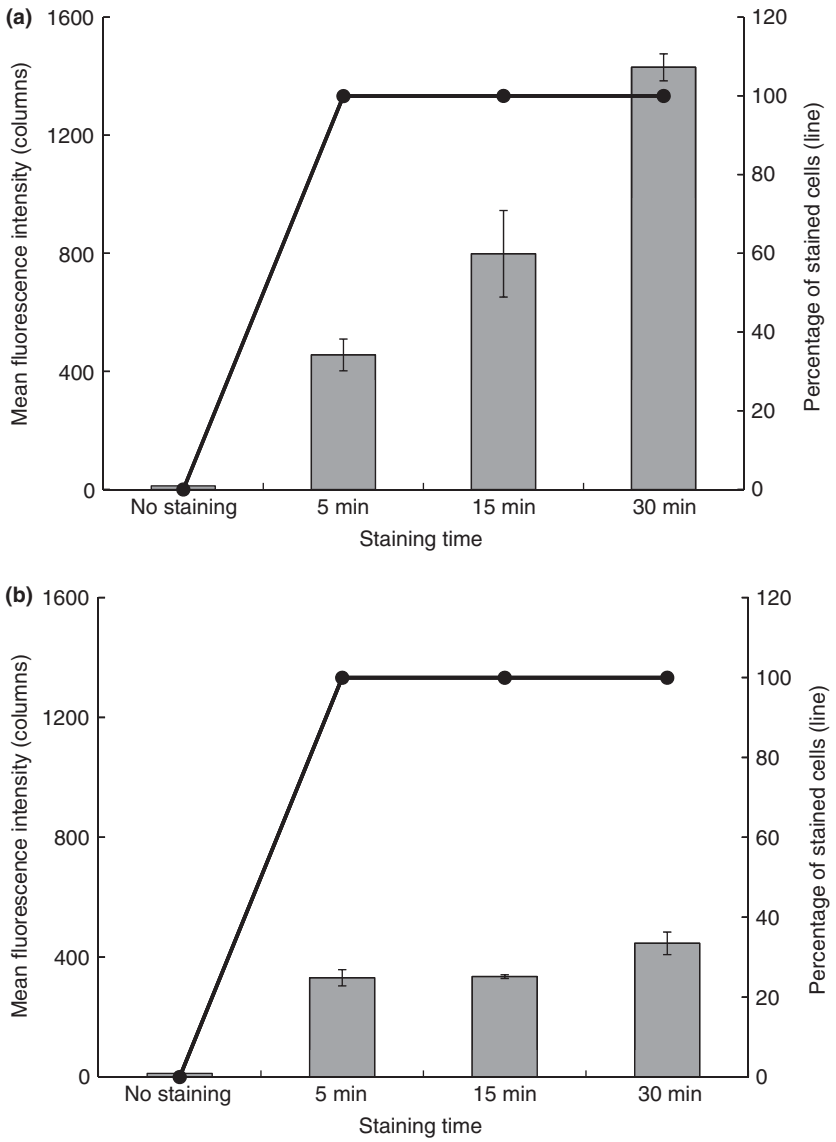

Figure 4 Detection of *Staphylococcus aureus* by flow cytometry after staining of 10^7 cells with fluorescence-labelled 4531-56 SpA SOMAmer at (a) 7 μmol l^{-1} or at (b) 0·7 μmol l^{-1}. Comparison of different staining times (n = 3). Mean fluorescence intensities are represented in columns and percentages of stained cells as line graph.

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.

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 *isdH* genes were transferred as *Bam*HI-*Sac*I cassettes into the expression vector pET-51b (EMD-Millipore, Billerica, MA) that harbours an aminoterminal Strep-tag and a carboxyterminal His_{10}-tag. One of the targets, *fnbB*, was cloned as and *Nde*I-*Bam*HI fragment into pET-14b (EMD-Millipore), which harbours an aminoterminal His_{10}-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 Strep-tag.

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, His_{10}-/Strep-tagged proteins were purified from the soluble fraction via sequential affinity chromatography on Ni-NTA agarose and Strep•Tactin® Superflow™ agarose (EMD-Millipore). Native staphylococcal protein A was purchased from VWR and was biotinylated with NHS-PEG4-biotin (Pierce Biotechnology, 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} $MgCl_2$ 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 His_{10}-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 (C_0t) 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 (K_d ≤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 SOMAmer 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 (K_d'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 mmol 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 C1 beads (10 mg ml^{-1}) and shaken for 15 min, resulting in approx. 90% efficiency of immobilization based on cy3 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 10^6 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 (10^1–10^7 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′ABflT (biotin and fluorescein), for use in flow cytometry experiments. Activity was confirmed for 4520-8 (K_d = 0·17 nmol l^{-1}) and 4531-56 (K_d = 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.

Trypcase 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 OD_{600} of 0·8 (approx. 10^8 bacteria ml^{-1}) and divided into aliquots of approx. 10^7 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} $MgCl_2$ containing a range of SOMAmers concentrations (0·07–7 μmol 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} $MgCl_2$. 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 FACSCalibur™ 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 FACSCalibur™ were analysed using the BD CellQuest™ software (Becton Dickinson Biosciences).

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Conflict of Interest

N. Janjic 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 10^7, 10^6, 10^5, and 10^4 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.