Supplemental Information

Rapid Acquisition of High-Affinity DNA Aptamer Motifs Recognizing Microbial Cell Surfaces Using Polymer-Enhanced Capillary Transient Isotachophoresis

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1. PectI selection

1.1 Experimental

1.1.1 Reagents. Tris(hydroxymethyl)aminomethane (>99.8% purity, Sigma-Aldrich, Tokyo, Japan) and glycine (>99% purity, Sigma, Kanagawa, Japan) were employed as buffer reagents. Polyethyleneoxide (PEO) 600000 as a running buffer additive in PectI was purchased from Sigma-Aldrich.

The ssDNA pool was prepared by dissolving 5'-fluorescein-labeled 88-mer randomized ssDNA (5'-/56-FAM/GCA ATG GTA CGG TAC TTC CN-N_{45}-CAA AAG TGC ACG CTA CTT TGC TAA-3'), purchased from Integrated DNA Technologies (Coralville, USA), into ultrapure water. A fluorescein-labeled forward primer (5'-FAM- GC AAT GGT ACG GTA CTT CC -3') and a 5'-biotinylated reverse primer (5'-Bio-TT AGC AAA GTA GCG TGC ACT TTT G -3') were employed in the PCR amplification procedure, The forward primer provided for fluorescent detection of amplified ssDNA in PectI-LIF and the reverse primer provided for immobilization of amplified dsDNA onto magnetic beads coated with streptavidin for purification. The randomized ssDNA pool solution used for selection (1×10^{-5} mol/L ssDNA, 10 mmol/L NaCl, 50 mmol/L-13.5 mmol/L Tris-HCl at pH 8.6) was annealed in a thermal cycler at 95 ˚C for 5 min prior to cooling to 20 ˚C for 60 min.

A PCR enzyme kit with TaKaRa Ex Taq polymerase (Takara Bio, Shiga, Japan) for PCR amplification and a MinElute PCR purification kit (Qiagen, Tokyo, Japan) for purification of the amplified dsDNA, were used according to the manufacturers' protocols.

Escherichia coli (BL21 competent cell), *Bacillus subtilis* (*Bs* 168) and *Saccharomyces cerevisiae* including emulsifier and L-ascorbic acid (Nissin Foods, Tokyo, Japan) were used as model bacteria. For incubation of *E. coli* and *B. subtilis*, LB medium was employed at 120 rpm for 8 hours at 37 ˚C. The bacteria were purified by three-time centrifugation (6000 rpm, 3min) in ultrapure water or buffer solution before use.

1.1.2 Instruments. To produce ultrapure water (>18.2 MΩ), a Direct Q3 UV system was employed (Millipore, Tokyo, Japan). For PectI selection of ssDNA-bacterial cell complexes, an Agilent G7100 capillary electrophoresis system (Agilent Technologies, CA, USA) was used with an in-house modification to accommodate a USB2000+ second fiber optic UV/Vis detector (Ocean Optics, FL, USA) located at the capillary cassette such that effective lengths from inlet to detector were 31.2 (2^{nd} detector) and 41.5 cm (original detector). Total length and internal diameter of the fused-silica capillary (GL Science, Tokyo, Japan) were 50 cm and 100 μm, respectively. A Wako WK-0232 thermal cycler (Wako Pure Chemical Industries, Osaka, Japan) was employed for PCR amplification. To conduct polyacrylamide gel electrophoresis experiments, an AE-6200 vertical electrophoresis system equipped with an AE-8155 power supply (ATTO, Tokyo, Japan) was used.

1.1.3 Procedure. The sample solutions for PectI selection were prepared by mixing sufficient volumes of stock solutions to produce a final mixture containing 1.0×10^{-6} mol/L randomized ssDNA pool, 8×10^{7} cfu/mL *E. coli* or 1×10^{7} cfu/mL *B. subtilis*, 50 mmol/L-13.5 mmol/L Tris-HCl, and 0.0125% PEO 600000 at pH8.5. For *S. cerevisiae*, a simple counter selection was conducted prior to the PectI selection, which involved mixing a 1.0×10^{-7} mol/L DNA library solution with 8×10^{9} cfu/mL *E. coli* in a 50 mmol/L-13.5 mmol/L Tris-HCl buffer solution at pH 8.6, followed by centrifugation (6000 rpm for 300 s) after incubation for 1 hour. The resulting supernatant of approximately 8×10^{-8} mol/L DNA pool was directly mixed with 5×10^{5} cfu/mL *S. cerevisiae* to be subjected to PectI selection according to the above mentioned procedure for positive selection.

Before partitioning of ssDNA-bacterial cell complexes, the precise detection position of the in-house equipped (second) detector on the capillary was measured using electroosmotic flow in capillary zone electrophoresis (CZE) This procedure was necessary because it was difficult to directly measure the position of the second detector due to the fact that the light path was completely hidden in a detector box (while the precise position of the preset detector was easily measured by a slide caliper). The electroosmotic flow (EOF) mobility, μ_{EOF}, was determined according to the following equation (S1), after injection of electroneutral marker such as methanol in capillary zone electrophoresis:

$$\mu_{EOF} = \frac{L_T L_{eff\,i}}{V}\left(\frac{1}{t_{EOF}}\right) \qquad \text{(S1)}$$

Here, L_T, $L_{eff\,i}$, V and t_{EOF} represent the total capillary length (50 cm), the effective capillary length (i = 1 or 2; and L_{eff1} = 41.5 cm for the preset detector), the applied voltage and the detection time of the neutral marker, respectively. After measurement of μ_{EOF} using the detection time at the first preset detector, the L_{eff2} for the second detector was determined to be 31.2 ± 0.1 cm (N = 3) using the detection

time at the second detector and the μ_{EOF} value previously obtained, since the EOF rate is constant in CZE mode.

In PectI, the migration velocity of each zone changes due to the transition of migration mode from isotachophoresis (ITP) to CZE. Since the velocity is constant in CZE, the constant velocity was measured only after completion of the transition between migration modes, using dual detectors. For PectI selection (partitioning of ssDNA-cell complexes), a migration buffer of 50 mmol/L-300 mmol/L Tris-Gly and 0.0125% PEO was employed. The applied voltage and detection wavelength were 20 kV and 270 nm, respectively. The sample solution was injected by pressure from the cathodic (inlet) end, at 50 mbar for 20 s (with a corresponding injection volume of 500 nL, as determined based on the Hagen-Poiseuille equation). The apparent flow velocity, *v,* was calculated using equation S2 (see below). The transition to CZE mode after ITP mode was confirmed by measuring whether the current value was constant. The precise elution time, t_{elut}, at the outlet of the target zone was calculated using equation S3 along with the observed *v* value:

$$v = \frac{L_{eff1} - L_{eff2}}{t_1 - t_2} \quad \text{(S2)}$$

$$t_{elut} = \frac{L_T - L_{eff1}}{v} + t_1 \quad \text{(S3)}$$

Here, t_1 and t_2 are the detection times of the target zone at the first preset and the second modified detector. The practical partitioning of the ssDNA-bacterial cell peak was conducted using intervals of 12 and 18 s before and after the peak maximum, respectively, using t_{elut}. This procedure involved pausing the applied voltage, exchanging the inlet vial to a fraction collection vial (which contained 20 μL of the buffer solution into which the fraction was collected), and then reapplying the voltage.

The precisely partitioned fraction was then amplified and purified by a PCR kit and a purification kit, respectively, according to the manufacturers' procedures. Briefly, 50 μL of a mixture containing 0.2 mmol/L dNTP, 600 nmol/L 5'-FAM-forward primer (5'-FAM-GC AAT GGT ACG GTA CTT CC-3') and 5'-Bio-reverse primer (5'-Bio-TT AGC AAA GTA GCG TGC ACT TTT G-3'), 1 unit Taq polymerase including buffering reagents, and 1× Ex Taq buffer, was placed in a thermal cycler for PCR amplification. The following temperature cycle was employed in PCR after pre-heating to 95 ˚C for 5 min: heating to 95 ˚C and holding for 10 s for denaturing, followed by cooling to 50 ˚C and holding for 30 s for annealing, followed by heating to 72 ˚C and holding for 10 s for elongation. This cycle was repeated 25 times, after which, the vial was cooled to 4 ˚C from 72 ˚C and held for 2 min. The products were purified by the MinElute PCR purification kit (Qiagen, Tokyo, Japan) using a purification column. The amplification was confirmed by polyacrylamide gel electrophoresis (PAGE).

The purified dsDNA was then converted to ssDNA using a VERITAS Dynabeads MyOne Streptavidin C1 magnetic bead kit.

2. Confirmation of the absence of DNA contamination in ssDNA-cell complex fraction

2.1 Experimental

The procedure of partitioning was identical to that the previous section except for the bacteria; that is , 1.0×10^{-6} mol/L randomized ssDNA pool, 50 mmol/L-13.5 mmol/L Tris-HCl and 0.0125% PEO 600000 at pH8.5, with no added bacteria cells. The partitioning was conducted with precise 11.5 s intervals: 1.1σ-4.2σ, 5.7σ-8.8σ, 10.3σ-13.4σ, 15.0σ-18.1σ and 20.9σ-24.0σ distant from the ssDNA pool peak, where σ represents the standard deviation of the ssDNA peak (see Figure S1). The partitioned fraction was subjected to PCR amplification prior to the detection of DNA by PAGE with ethidium bromide staining. The PCR amplification was conducted two times to determine trace levels of ssDNA. In our case, the product of the first 15-cycle PCR was diluted 100-fold prior to the second 15-cycle PCR.

2.2 Results

Figure S1 shows the electropherogram of the DNA pool obtained using PectI. The represented fractions were partitioned, followed by PCR amplification. According to our calculations, when the migration time of the partitioning interval is distant from the free DNA peak by more than 6.5σ, not even a single molecule from the DNA zone may have co-migrated with the partitioned zone, based on a first approximation by applying a Gaussian distribution to the DNA peak (where the number of injected DNA molecules was 6×10^{11} in 500 nL, and $\sigma = 3.7 \pm 0.3$ s ($N = 5$)). After PCR, no ssDNA was detected in any of the fractions except in fraction #1(1.1σ-4.2σ) (see Figure S1-B). Since the detection limit after the second PCR was about 10 fmol/L of ssDNA under our experimental conditions, the ssDNA concentration in the partitioned samples (fraction #2-5, with resulting sample volumes of 20 μL) must be less than 10 fmol/L, i.e. less than 10^5 molecules in the fractions distant by more than 5.4σ. This fact agrees well with our estimation (no DNA contamination at more than 6σ). Hence, it can be concluded that virtually no contamination by unbound ssDNA occurs in our PectI selection procedure.

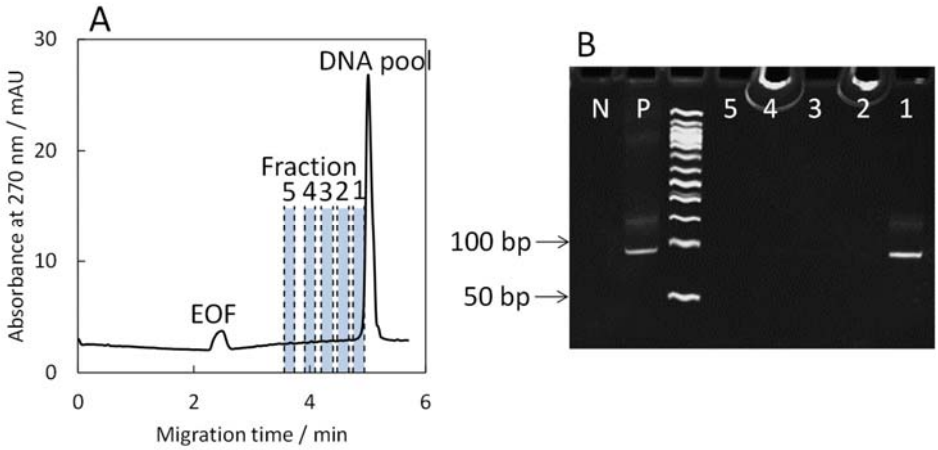

Figure S1 Partitioning intervals (A) and ethidium bromide-stained PAGE gel for amplified samples after the second PCR amplification (B). (A) The fraction numbers correspond to each 11.5-s wide partitioning interval: **1**, 1.1σ-4.2σ; **2**, 5.7σ-8.8σ; **3**, 10.3σ-13.4σ; **4**, 15.0σ-18.1σ; **5**, 20.9σ-24σ. Sample: 1.0×10^{-6} mol/L randomized ssDNA, 50 mmol/L-13.5 mmol/L Tris-HCl and 0.0125% PEO 600000 at pH8.5. (B) The lane number corresponds to the fraction number in Figure S1-A. **P**, positive control (1.0×10^{-11} mol/L ssDNA library); **N**, negative control. Gel concentration, 12%T, 3.3%C; 1× TBE buffer; voltage, 250 V.

3. Sequencing and bioinformatics of selected DNA pool for E. coli and S. cerevisiae

3.1 Experimental

Sequencing using Next Generation Sequencer (NGS). Aliquots from the selected DNA library pools were used as additional PCR template to prepare samples for sequencing. For PCR amplification, the reagents and the procedures were the same as described in section 1.1, except for primers, annealing temperature and cycle number. A 2 μL solution of the DNA templates obtained by PectI selection was diluted by a factor of 100 and served to PCR amplification with unmodified primer (15 cycles and annealing at 56.3 ˚C). Amplified DNA library (with a 5 μL of the sample solution diluted by a factor of 100) were tagged to the adaptor with barcode region and sequencing primer binding region (forward primer: 5'-CCA TCT CAT CCC TGC GTG TCT CCG ACT CAG CTA AGG TAA CGA TGC AAT GGT ACG GTA CTT CC-3', reverse primer: 5'-CCT CTC TAT GGG CAG TCG GTG ATT TAG CAA AGT AGC GTG CAC TTT TG-3', PCR product: 5'-CCA TCT CAT CCC TGC GTG TCT CCG ACT CAG CTA AGG TAA CGA TGC AAT GGT ACG GTA CTT CC-N_{45}-CAA AAG TGC ACG CTA CTT TGC TAA ATC ACC GAC TGC CCA TAG AGA GG-3'). Then, the PCR products were used as templates for PCR (5 cycles with annealing at 62.7 ˚C) with normal short

primers (forward primer: 5'-CCA TCT CAT CCC TGC GTG TC-3', reverse primer: 5'-CCT CTC TAT GGG CAG TCG GT-3') to obtain sufficient amount of tagged DNA sample. To confirm the yield and purity, 12 % agarose gel electrophoresis was conducted for all PCR products. The final PCR products were purified with MinElute PCR purification kit (Qiagen, Tokyo, Japan) to remove primers and undesired byproducts. An Ion PGM system (Life Technologies, California, USA) was employed for sequencing of the selected DNA sequences. Beads preparation and sequencing were performed according to the Ion PGM user guides (Publication Number MAN0007220, Rev.5.0 and MAN0007273, Rev.3.0, respectively) with Ion PGM Template OT2 200 Kit, Ion PGM Sequencing 200 Kit v2, and Ion 314 Chip Kit v2 (Life technologies, California, USA).

3.1.2 Sequence analysis using ClustalX and MEME software. Sequenced data was exported as FASTAQ files and analyzed using the trimming, extract and count tools in CLC Genomics Workbench (CLC bio, Aarhus, Denmark). After trimming 5', 3' primer regions, the desired products with lengths from 43 to 47 were used for the next analysis. A set of sequence reads obtained from NGS was input into ClustalX software with a default parameter setting. A complete set of all sequences was analyzed with each preset of 4000 sequences repeatedly analyzed, since more than 6000 sequences could not be analyzed per a program run. Searches were conducted after gathering output data into one file, to identify highly analogous sequences (families). Some typical sequences included in families were employed for binding experiments (see section 4).

All sequences of families were analyzed by MEME4.10.1 (Multiple Em for Motif Elicitation) software to search for and rate contiguous (contig) sequences. The motifs thus found were used as inputs for a FIMO (Find Individual Motif Occurrences) scan in the MEME Suite package (version 4.10.1) to analyze the population of the motifs in all sequences. Since each run in MEME could process no more 1000 sequences, the scan results of each 1000-sequence sub-set were put together to determine the population (the number of sequence containing a motif found in FIMO/all sequences).

3.2 Results

The determined sequences, families and motifs are represented in Figures S2 and S3, and in Tables S1-S4. From these results it is obvious that certain unique sequences (or motifs) were enriched by the PectI selection with one-time CE partitioning.

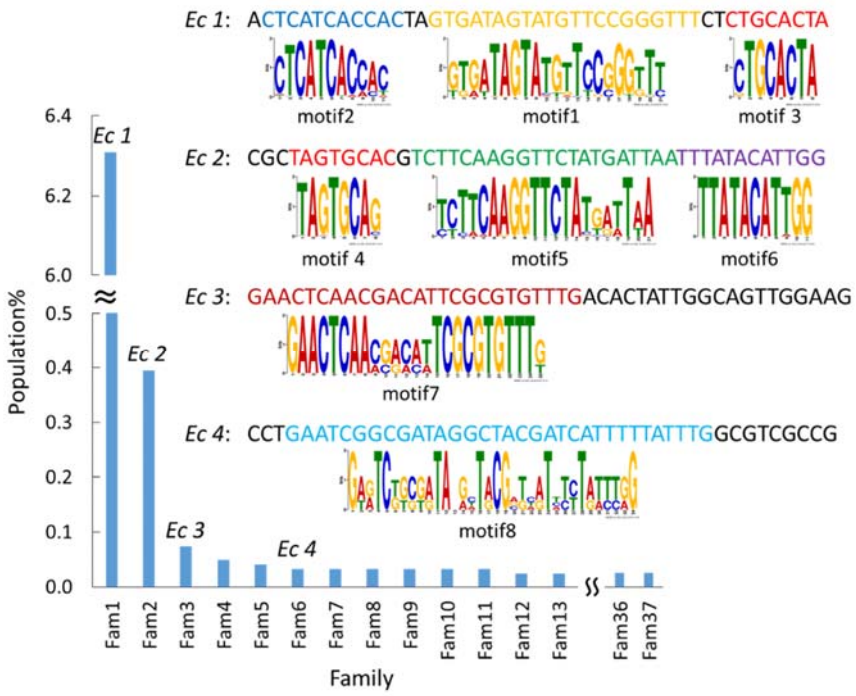

Figure S2 Distribution of families and motifs for *E. coli*, found using ClustalX and MEME software, respectively.

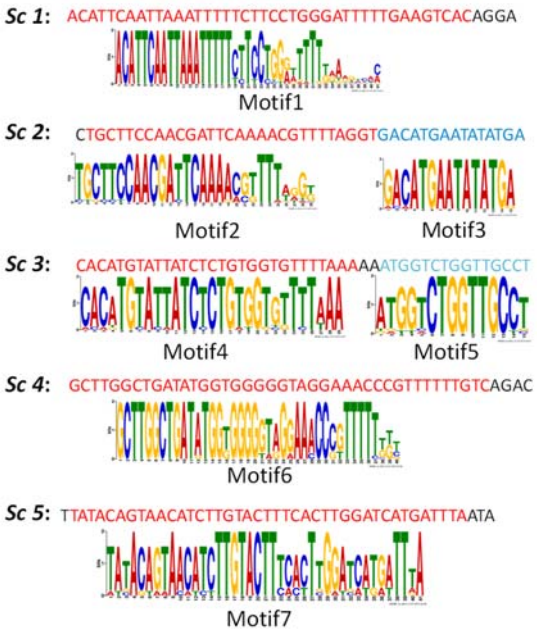

Figure S3 Motifs for *S. cerevisiae*, found using MEME software (see also Table S4 and S5 for the population and dissociation constants, respectively).

Table S1: Results of sequence analysis of DNA pool selected for *E. coli* using ClustalX.[a]

	Number of sequence	Population of family in the selected pool (%)	Typical sequence	K_d / nmol/L
Fam1	768	6.3	*Ec 1*	106 ± 9
Fam2	48	0.39	*Ec 2*	27 ± 4
Fam3	9	0.074	*Ec 3*	9 ± 6

[a] Families, which included more than 9 analogue sequences, were selected.

Table S2: Results of sequence analysis of DNA pool selected for *S. cerevisiae* using ClustalX.[a]

	Number of sequence	Population of family in the selected pool (%)	Typical sequence	K_d / nmol/L
Fam1	16	0.014	*Sc 1*	390 ± 170
Fam2	16	0.014		
Fam3	16	0.014		
Fam4	15	0.013	*Sc 2*	170 ± 60
Fam5	15	0.013	*Sc 3*	100±40
Fam6	15	0.013		
Fam7	15	0.013		
Fam8	14	0.012	*Sc 4*	760 ± 370
From Fam9 to 12	14	0.012		
From Fam13 to 17	13	0.011		
From Fam18 to 32	12	0.010		
From Fam33 to 46	11	0.010		
Fam47	10	0.009	*Sc 5*	30 ± 10
From Fam48 to 90	10	0.009		
From Fam91 to 140	9	0.008		

[a] Families, which included more than 9 analogue sequences, were selected in this table.

Table S3: Results of motif search conducted on DNA pool selected for *E. coli* using MEME and FIMO software.[a]

Motif	Base number	Theoretical population of the sequences possessing the motif in randomized ssDNA pool (%)	Population of the sequences possessing the motif in the selected DNA pool (%)	Family (typical sequence) possessing the motif
1	21	5.7×10^{-10}	7.0	Fam1 (*Ec 1*)
2	11	8.3×10^{-4}	5.6	Fam1 (*Ec 1*)
3	8	5.8×10^{-2}	3.5	Fam1(*Ec 1*)
4	8	5.8×10^{-2}	0.85	Fam2(*Ec 2*)
5	29	5.9×10^{-15}	1.3	Fam2(*Ec 2*)
6	11	8.3×10^{-4}	0.75	Fam2 (*Ec 2*)
7	25	1.9×10^{-12}	0.24	Fam3 (*Ec 3*)
8	41	1.0×10^{-22}	0.21	Fam6 (*Ec 4*)

[a] see Figure S2 and Table S5 for the sequences of motifs and the dissociation constants, respectively.

Table S4: Results of motif search conducted on DNA pool selected for *S. cerevisiae* using MEME and FIMO software.[a]

Motif	Base number	Theoretical population of the sequences possessing the motif in randomized ssDNA pool (%)	Population of the sequences possessing the motif in the selected DNA pool (%)	Family (typical sequence)possessing the motif
1	41	1.0×10^{-22}	0.24	Fam1 (*Sc 1*), Fam33
2	29	5.9×10^{-15}	0.24	Fam4 (*Sc 2*), Fam9
3	15	2.9×10^{-6}	0.22	Fam4 (*Sc 2*)
4	29	5.9×10^{-15}	0.38	Fam5 (*Sc 3*), Fam9, Fam18, Fam91
5	15	2.9×10^{-6}	0.48	Fam5 (*Sc 3*), Fam92, Fam93
6	40	5.0×10^{-22}	0.11	Fam8 (*Sc 4*), Fam48
7	41	1.0×10^{-23}	0.14	Fam19, Fam35, Fam47(*Sc 5*), Fam94

[a] see Figure S3 and Table S5 for the sequences of motifs and the dissociation constants, respectively.

4. Binding assay and fluorescence microscopy for selected DNA aptamers with E. coli and S. cerevisiae

4.1 Experimental

The selected DNA pool and sequenced DNA, which were labeled by fluorescein (purchased from Integrated DNA Technologies), were subjected to binding assays using PectI-LIF. A Beckman Coulter P/ACE MDQ CE system equipped with a laser-induced fluorescence detector and an Ar-ion laser module (λ_{ex} = 488 nm) (Brea, CA, USA) was employed for the binding assays involving ssDNA aptamer-bacterial cell complexes. A fused silica capillary of 100 μm i.d., 50.2 cm total length and 40 cm effective length (GL Science, Tokyo, Japan) was employed. A migration buffer composed of 50 mmol/L-300 mmol/L Tris-glycine with 0.0125% PEO 600000 was employed. The sample solution of 0-2.0 μmol/L ssDNA, 8×10^7 cfu/mL *E. coli*, 50 mmol/L-13.5 mmol/L Tris-HCl and 0.0125% PEO 600000, was hydrodynamically injected (1 psi*13 sec), and then a separation voltage of 20 kV was applied. See Figure 2B and Figure S6 for typical results using PectI-LIF.

In addition to PectI-LIF experiments, binding assays by manual binding-free separation (washing by buffer) followed by fluorescence spectroscopy were also conducted. A FP-6300 fluorescence spectrophotometer (JASCO, Tokyo, Japan) was used to obtain the fluorescence spectra. The sample solutions of 0-2 μmol/L sequenced 5'-FAM-DNA (or the selected DNA pool), 8×10^7 cfu/mL *E. coli* or 2×10^6 cfu/mL *S. cerevisiae* and 50 mmol/L-13.5 mmol/L Tris-HCl (pH 8.6), were incubated for 1 hour, followed by centrifugation (6000 rpm, 3 min). Fluorescence spectra of the resulting supernatant were recorded for free ssDNA (unbound to bacterial cells). The obtained precipitate of cells was collected and washed by buffer solution three times, accompanied by centrifugation. A 1 mol/L NaOH solution was added to the precipitate to release cell-bound ssDNA with centrifugation (13000 rpm, 10 min). The recovered supernatant was subjected to fluorescence spectroscopy (1 cm cuvette, λ_{ex} = 488 nm, λ_{em} = 500-600 nm, 700 V photomultiplier voltage) to detect the ssDNA formally bound to the cell surface. Typical results from binding assay by fluorescence spectroscopy are shown Figure S4.

The dependence of peak area in PectI-LIF or fluorescence intensity in fluorometry, F_{obs}, on DNA concentration, $[DNA]_0$, was fitted to Equation S4 (where the DNA concentration represents a large excess compared to the number of binding sites), which is widely used to determine dissociation constants:

$$F_{obs} - F_0 = \frac{F_{max}\,[DNA]_0}{K_d + [DNA]_0} \qquad \text{(S4)}$$

Here, F_0 and F_{max} represent the fluorescence signals when no DNA is present and at its maximum

value. The dissociation constants for DNA aptamer-microbe cell complexes determined according to this equation are summarized in Table S5.

The binding of DNA aptamer was also confirmed by fluorescence microscopy. Mixtures of 4×10^8 cfu/mL *E. coli* or 1×10^7 cfu/mL *S. cerevisiae*, with 1.0×10^{-7} mol/L DNA aptamer and 50 mmol/L-13.5 mmol/L Tris-HCl were incubated for 1 hour to allow for binding, followed by thrice washing with 50 mmol/L-13.5 mmol/L Tris-HCl buffer and centrifugation (6000 rpm, 3 min). The recovered precipitate of bacteria cells was resuspended into the buffer solution (50 mmol/L-300 mmol/L Tris-HCl, pH 8.6) and was examined by fluorescence microscopy using an Olympus FV1000D confocal laser scanning microscope (Tokyo, Japan) with excitation at 473 nm by a semiconductor laser.

4.2 Results

Typical results of a binding assay for *S. cerevisiae* are shown in Figure S4, and the resulting calculated dissociation constants for *E. coli* and *S. cerevisiae* are summarized in Table S5. As shown in Figure 2B (in the main text) and Figure S4, the selected sequences apparently bound with their microbe cell targets in a one-to-one complexation, in contrast to the lack of binding observed for the unselected DNA pool with the microbes. Since the dissociation constant determined by PectI-LIF agreed well with that determined by fluorescence spectroscopy (106 ± 9 and 100 ± 30 nmol/L for *Ec 1* with *E. coli* by PectI-LIF and spectroscopy, respectively: see Table S5), it was indicated that the values determined by both methods are appropriate.

The results of fluorescence imaging are shown in Figure S5. Each DNA aptamer for *E. coli* and *S. cerevisiae* showed strong fluorescence when the DNA aptamer was mixed with the relevant microbe cells (Figure S5-b and f), while no emission was observed for mixture of microbe cells with unselected DNA pool (Figure S5-c and g) (while very low emission was observed without DNA aptamer in fact, it was due to autofluorescence of microbe cell, which intensity is substantially lower compared with the emission of DNA aptamer-cell complex) or DNA aptamer for different species (Figure S5-d and h).

Crossover binding experiments were conducted for *S. cerevisiae* with aptamers of *E. coli*, and *vice versa*, and for another strain of *E. coli* (BW25113) by fluorescence spectroscopy (Figure S7).

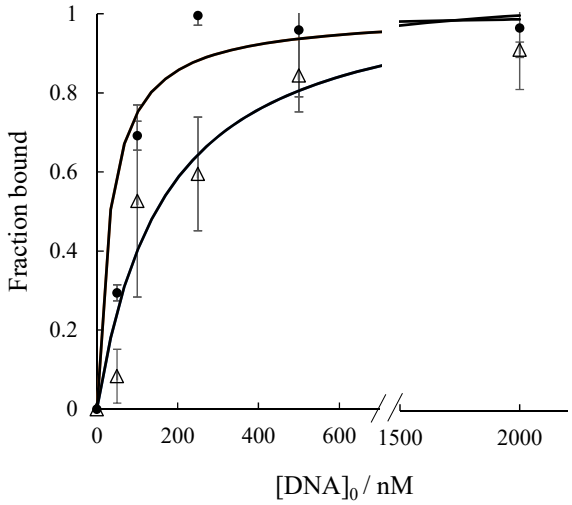

Figure S4 Typical results of binding assay for *S. cerevisiae* with DNA aptamer (△, *Sc 2*; •, *Sc 5*) by fluorescence spectroscopy. Sample: 2×10^6 cfu/mL *S. cerevisiae*; 0-2 μmol/L DNA aptamer (*Sc 2* or *Sc 5*); 50 mmol/L-13.5 mmol/L Tris-HCl.

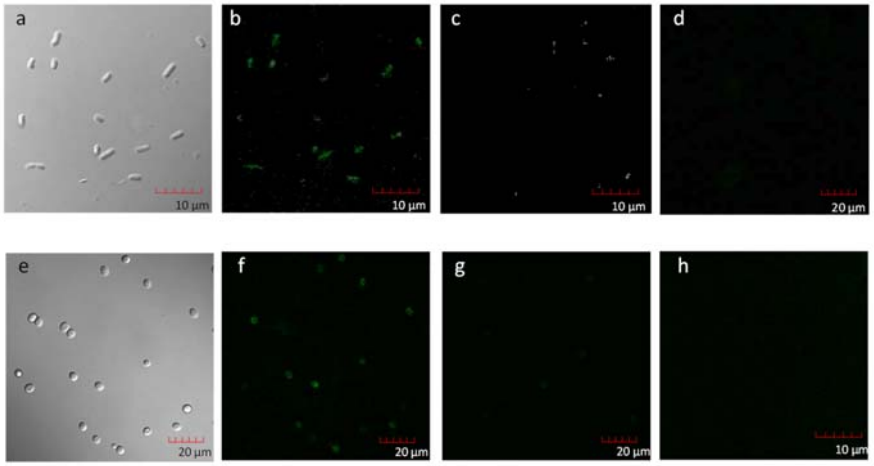

Figure S5 Fluorescence (b-d and f-h) and differential interference contrast images (a and e) for mixtures of microbe cells (*E. coli* and *S. cerevisiae*) with DNA. Samples: a and b, 4×10^8 cfu/mL *E.coli*, 1.0 μmol/L *Ec 3*; c, 4×10^8 cfu/mL *E.coli*, 1.0 μmol/L randomized ssDNA library; d, 1×10^7 cfu/mL *S. cerevisiae*, 1.0 μmol/L *Ec 3*; e and f: 1×10^7 cfu/mL *S. cerevisiae*, 1.0 μmol/L *Sc 5*; g, 1×10^7 cfu/mL *S. cerevisiae*, 1.0 μmol/L ssDNA pool; h, 4×10^8 cfu/mL *E. coli*, 1.0 μmol/L *Sc 5*.

Table S5 Experimentally determined dissociation constants of selected ssDNA aptamer-microbe cell complexes.[a,b]

	Species	Sequence	K_d
Ec_1	*E. coli*	ACTCATCACCACTAGTGATAGTATGT TCCGGGTTTCTCTGCACTA	106 ± 9 nmol/L[c] 100 ± 30 nmol/L[d]
Ec_2	*E. coli*	CGCTAGTGCACGTCTTCAAGGTTCT ATGATTAATTTATACATTGG	27 ± 4 nmol/L[c]
Ec_3	*E. coli*	GAACTCAACGACATTCGCGTGTTTG ACACTATTGGCAGTTGGAAG	9 ± 6 nmol/L[c]
Ec_4	*E. coli*	CCTGAATCGGCGATAGGCTACGATC ATTTTTATTTGGCGTCGCCG	40 ± 13 nmol/L[c]
Randomized library	*E. coli*	NNNNNNNNNNNNNNNNNNNNN NNNNNNNNNNNNNNNNNNNNN	7 ± 5 μmol/L[c]
Sc_1	*S. cerevisiae*	ACATTCAATTAAATTTTTCTTCCTGG GATTTTTGAAGTCACAGGA	390 ± 170 nmol/L[d]
Sc_2	*S. cerevisiae*	CTGCTTCCAACGATTCAAAACGTTT TAGGTGACATGAATATATGA	170 ± 60 nmol/L[d]
Sc_3	*S. cerevisiae*	CACATGTATTATCTCTGTGGTGTTTT AAAAAATGGTCTGGTTGCCT	100 ± 40 nmol/L[d]
Sc_4	*S. cerevisiae*	GCTTGGCTGATATGGTGGGGGTAGG AAACCCGTTTTTTGTCAGAC	760 ± 370 nmol/L[d]
Sc_5	*S. cerevisiae*	TTATACAGTAACATCTTGTACTTTC ACTTGGATCATGATTTAATA	30 ± 10 nmol/L[d]

[a] the sequences in two primer regions were omitted. [b] N = 6-7 (three times for each point). [c] obtained by PectI-LIF. [d] obtained by fluorescence spectrometry.

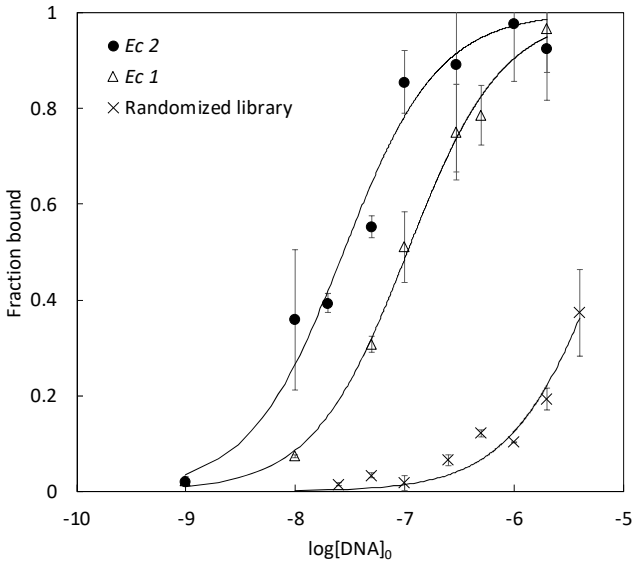

Figure S6 Binding curves for DNA apatamer-*E. coli* cell complex in logarithmic scale. The conditions are the same as in Figure 2B.

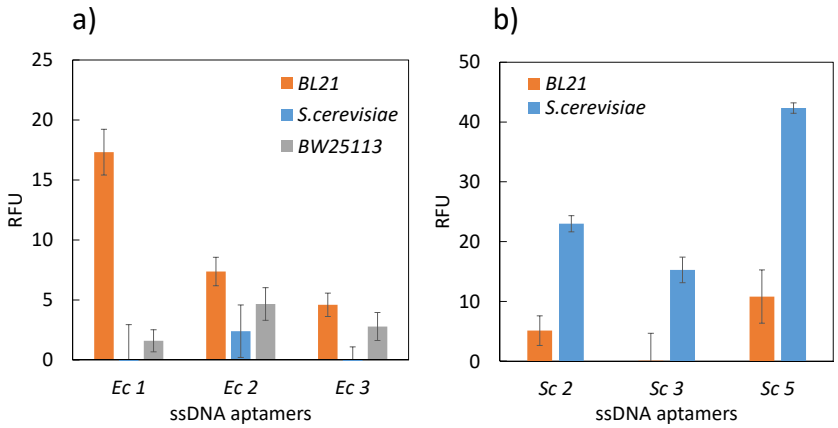

Figure S7 Selectivity of *E. coli*-binding DNA aptamers (*Ec 1*, *Ec 2* and *Ec 3*) towards *S. cerevisiae* and the other *E. coli* strain (BW25113) (a), and selectivity of *S. cerevisiae*-binding DNA aptamers (*Sc 2*, *Sc 3* and *Sc 5*) towards *E. coli* (b). Samples: 8×10^7 cfu/mL bacterial cells (*E.coli* BL21, BW25113 or *S. cerevisiae*), 250 nmol/L DNA.

5. Dissociation of DNA aptamer-E. coli cell complex in PectI-LIF

5.1 Experimental

For PectI-LIF, the sample solution including 1.0×10^{-6} mol/L DNA aptamer (FAM-*Ec 1*,

annealed before use), 8×10^7 cfu/mL *E. coli*, and 50 mmol/L-13.5 mmol/L Tris-HCl was prepared. The sample solution was analyzed by PectI-LIF using the same conditions as described previously for the binding assay (see section 4.1), except for the use of additional external pressure (0-0.2 psi) at the capillary outlet, in order to forcibly change migration velocity to obtain a different detection time for the DNA-cell complex peak; that is, the reaction time of dissociation was changed with applied pressure. Since the dissociation process occurs throughout migration, the peak areas with different detection times were pursued.

Dissociation of the DNA-cell complex was also observed in fluorescence spectroscopy. The sample solution of 500 nmol/L DNA aptamer, FAM-*Ec 1*, 8×10^7 cfu/mL *E. coli*, and 50 mmol/L-13.5 mmol/L Tris-HCl was incubated for 30 min at room temperature to form the *Ec 1*-*E. coli* complex, followed by centrifugation (6000 rpm, 3 min). The resulting precipitate of microbe cells (with DNA complex) was resuspended in 50 mmol/L-13.5 mmol/L Tris-HCl buffer solution. This preparation cycle was repeated three times. A very low concentration of ssDNA (less than $(1.8 \pm 0.9) \times 10^{-10}$ mol/L) in the resulting supernatant was confirmed by fluorescence spectrometry (λ_{ex} = 488 nm, λ_{em} = 500-600 nm, 700 V photomultiplier voltage, where the detection limit of FAM-labeled DNA was 3.8×10^{-10} mol/L). Judging from the dissociation constant of *Ec 1* with *E. coli* (K_d = 100 nmol/L), the dissociation reaction of the *Ec 1*-*E. coli* complex was facilitated by the sample preparation process just described. After 30 or 60 min standing time, the mixture was centrifuged to recover the supernatant and precipitate in order to be able to detect dissociated and bound DNA, respectively. The precipitate (with its bound DNA) was mixed with 1 mol/L NaOH in order to force the dissociation of *Ec 1* from *E. coli*, prior to centrifugation (13000 rpm, 10 min) to recover the formally bound *Ec 1* into the supernatant, which was then detected by fluorescence spectroscopy (1 cm cuvette, λ_{ex} = 488 nm, λ_{em} = 500-600 nm, 700 V photomultiplier voltage).

5.2 Results

For PectI-LIF, the detection time (reaction time) in the range of 10-20 min was observed with pressure, as shown in Figure S8. The peak area corrected by detection time was seen to decrease slightly with increasing detection time. The rate of this area decrease was 21% at a maximum detection time of 18 min. If a first-order dissociation reaction rate is presumed (whereby $A = A_0 \exp(-kt)$, and A, A_0, k and t represent the corrected peak area, the peak area at $t = 0$, the dissociation rate constant, and the detection time, respectively), then the dissociation rate constant was estimated to be $(2 \pm 4) \times 10^{-4}$ s^{-1} or lower (N = 3, and the half life corresponds to 58 min).

The slow dissociation was also confirmed by fluorescence spectrometry. Figure S9 shows fluorescence spectra for the *Ec 1* aptamer-*E. coli* cell complex after three washings. Dissociation should occur due to the very low concentration ($< 2 \times 10^{-10}$ mol/L) of free *Ec 1*, according to the K_d value (100 nmol/L) (see section 5.1). After 30 and 60 min standing, the strong emission of *Ec 1* bound

to cells was still observed and the intensity of these samples left to stand was almost identical to the just-prepared sample (with a standing time of 0 min). Thus, the fact that fluorescence spectroscopy revealed no dissociation over the course of one hour agrees with the other results pertaining to dissociation kinetics obtained by PectI-LIF. These facts obtained from CE-based methods and spectroscopic experiments suggest that *Ec 1* remained bound to the cell without dissociation for 60 min; i.e. the binding is inert (kinetically stable).

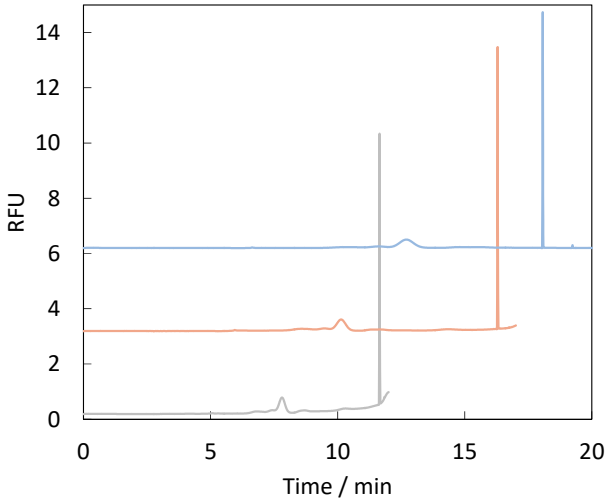

Figure S8 Typical electropherograms for a mixture of FAM-labeled *Ec 1* aptamer with *E. coli* obtained by applying different external pressure (0-0.2 psi) in order to obtain different detection times for the *Ec 1*-*E. coli* complex peak.

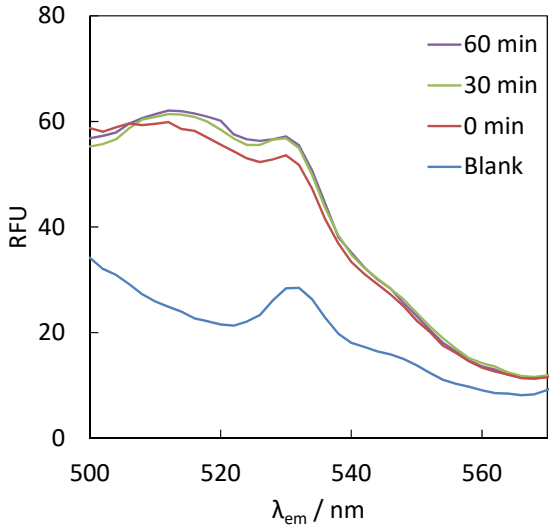

Figure S9 Fluorescence spectra of a mixture of FAM-labeled *Ec 1* aptamer with *E. coli* after three washings. Sample, [*Ec 1*] = 500 nmol/L (K_d = 100 nmol/L) (no *Ec 1* in blank sample); [*E. coli*] = 8 × 10^8 cfu/mL; 50 mmol/L-15 mmol/L Tris-HCl, pH8.6. λ_{ex} = 488 nm. Sample solutions were measured after 0-60 min standing in buffer solution.