Chapter 14

Using the QCM Biosensor-Based T7 Phage Display Combined with Bioinformatics Analysis for Target Identification of Bioactive Small Molecule

Yoichi Takakusagi, Kaori Takakusagi, Fumio Sugawara, and Kengo Sakaguchi

Abstract

Identification of target proteins that directly bind to bioactive small molecule is of great interest in terms of clarifying the mode of action of the small molecule as well as elucidating the biological phenomena at the molecular level. Of the experimental technologies available, T7 phage display allows comprehensive screening of small molecule-recognizing amino acid sequence from the peptide libraries displayed on the T7 phage capsid. Here, we describe the T7 phage display strategy that is combined with quartz-crystal microbalance (QCM) biosensor for affinity selection platform and bioinformatics analysis for small molecule-recognizing short peptides. This method dramatically enhances efficacy and throughput of the screening for small molecule-recognizing amino acid sequences without repeated rounds of selection. Subsequent execution of bioinformatics programs allows combinatorial and comprehensive target protein discovery of small molecules with its binding site, regardless of protein sample insolubility, instability, or inaccessibility of the fixed small molecules to internally located binding site on larger target proteins when conventional proteomics approaches are used.

Key words T7 phage display, Affinity selection, Biopanning, Peptide, QCM, Biosensor, Bioinformatics, RELIC

1 Introduction

Phage display is a molecular biological tool that can be used to generate functional molecules, such as bioactive peptide agonists or antagonists, drug delivery tags, specific antibodies, and others [1]. Unlike other proteomics approaches, phage display has a number of useful features that make it possible to efficiently identify the binding partner of specific bait of interest. The principle of the technology is based on fusing nucleotide sequences of random peptides to that of a phage coat protein, which enables display of the chimeric protein on the phage surface. Various foreign peptides, even from humans or plants, can be displayed on the phage capsid

Friedrich Fauser and Martin Jonikas (eds.), *Plant Chemical Genomics: Methods and Protocols*, Methods in Molecular Biology, vol. 1795, https://doi.org/10.1007/978-1-4939-7874-8_14,

by inserting their cDNAs into specific region of phage genome. This characteristic allows facile determination of the amino acid sequence by simply sequencing the DNA of the corresponding phage. By selection with the substrate of interest immobilized on a solid support as bait, phages selectively recognizing the substrate are subsequently enriched by repeated rounds of biopanning (interaction, washing, elution, and amplification) [2–5].

Although phage display itself is a well-established technique, several technical limitations often hampered the success of selection. For example, nonspecific binding of phages to solid support or phage distribution bias during the amplification process is included. In 1990s, T7Select System (Novagen) emerged as an innovative technique that allows display of up to 1200 a.a. of foreign polypeptides [6, 7]. This allows application of T7 phage display for identification of drug target protein [2–4]. However, size limitations or misfolding of proteins as well as absence in posttranscriptional modifications of proteins displayed on the capsid seem to limit the comprehensive screening of small molecule-binding protein. Significantly, unlike the detection of protein-protein interactions, interaction between small molecule and protein displayed on capsid may be often hampered by immobilization of small molecules onto solid support during biopanning. This is because the smaller molecular size of fixed compounds may lose degree of freedom necessary for docking with protein, or be inaccessible to the binding site internally located in larger target proteins than the compounds.

To address these technical issues, we established a series of strategies using random peptide T7 phage library from synthetic DNA. The affinity selection is assisted with a quartz-crystal microbalance (QCM) apparatus for selection platform. QCM is a biosensor whose shear modulus of quartz (AT-cut) decreases in proportion to the increase of mass on the gold electrode attached on the quartz by vapor deposition. The interaction on the gold electrode (mass increase) is, therefore, detected as a decrease of oscillatory resonance frequency in real time. This QCM device comprises a buffer-filled cuvette, which is constantly stirred, where the sensor chip is immersed. The guest molecule is then injected into the cuvette to analyze the interaction on the gold electrode [8–10]. The use of this platform to T7 phage display enables monitoring the binding of phages to small molecule in real time and facilitates selection of small molecule-recognizing weak affinity short peptides without repeated rounds of biopanning [11–18]. Furthermore, the bioinformatics analysis of the subset of the small molecule-recognizing peptides allows evaluation of the series of process from affinity selection to target identification by similarity search of amino acid sequence, even with its binding site that is comprised of intrinsically disordered regions of weakly recognizing small molecule [17, 19]. Beyond technical limitations or issues in pro-

tein sample preparation, this method is applicable to target identification of any small molecules of interest under the identical protocol, even membrane-associated receptors [11, 20] or plant enzymes [12] whose identification is somewhat challenging in other experimental approaches. Furthermore, combinatorial and high-throughput discovery of small molecule-binding candidate proteins is feasible by using the same subset of small molecule-recognizing short peptides and bioinformatics analysis [11].

2 Materials

Use reagents of special grade or higher unless otherwise noted.

2.1 QCM Biosensor

1. The QCM apparatus: 27-MHz AffinixQ, AffinixQ$_N$ (Initium Inc. Tokyo, Japan) (*see* **Note 1**).
2. Sensor chip (SiO_2, 0.06 mm thick, 9 mm in diameter, 64 mm^2; Au, 0.1 mm thick, 2.5 mm in diameter, 4.9 mm^2).
3. 10 mL Glass cuvette.
4. Stir magnet (*see* **Note 2**).
5. Reaction buffer: 10 mM Tris–HCl, 200 mM NaCl, pH 7–8 (*see* **Note 3**).
6. 1% Sodium dodecyl sulfate (SDS).
7. Piranha solution (concentrated H_2SO_4:30% H_2O_2 = 3:1): Prepare just prior to use.
8. Small molecule that forms self-assembled monolayer (SAM) (*see* **Note 4**).

2.2 T7 Phage-Displayed Random Peptide Library

1. The oligonucleotide and primer (custom oligonucleotides):
   Oligonucleotide 1: 5′-GGG GAT CCG AAT TCT $(NNK)_{15}$ TGA AAG CTT CTC GAG GG-3′ (0.056 pM) (*see* **Note 5**).
   Oligonucleotide 2: 5’-CCC TCG AGA AGC TTT CA-3′ (0.56 pM).
2. T7Select10-3 *Eco*R I/*Hind* III Vector Arms (0.5 μg/μL) (Novagen): Store at 4 °C.
3. 2.5 mM dNTP mix (TaKaRa): Store at −20 °C.
4. Klenow DNA polymerase I (5 U/μL) (USB Corporation): Store at −20 °C.
5. *Eco*R I (15 U/μL): Store at −20 °C.
6. *Hind* III (20 U/μL): Store at −20 °C.
7. 10× NEBuffer 2.1 (NEW ENGLAND BioLabs) (*see* **Note 6**).
8. T7 Packaging Extracts (Novagen): Store at −80 °C.

9. Ligation-convenience kit (NIPPON GENE): Store at −20 °C (*see* **Note 7**).
10. BLT5615 Glycerol Stocks (Novagen): Store at −80 °C.
11. Isopropylthio-β-galactoside (IPTG).

2.3 Plaque Formation

1. Luria-Bertani (LB) medium containing 50 μg/mL sodium carbenicillin: 10 g/L Bacto tryptone, 5 g/L yeast extract, 10 g/L NaCl, 50 mg sodium carbenicillin; for plates, add 15 g/L agar. Autoclave for 20 min.
2. Top agarose: 10 g/L Bacto tryptone, 5 g/L yeast extract, 5 g/L NaCl, 6 g/L agarose. Autoclave for 20 min.
3. Phage extraction buffer: 20 mM Tris–HCl, pH 8.0, 100 mM NaCl, 6 mM $MgSO_4$.

2.4 PCR and DNA Purification, Sequencing

1. Primer forward: 5′-TGC TAA CTT CCA AGC GGA CC-3′.
2. Primer reverse: 5′-AAA AAC CCC TCA AGA CCC GTT TA-3′.
3. 2.5 mM dNTP mix (TaKaRa).
4. 10× Ex Taq buffer (TaKaRa).
5. Ex Taq DNA polymerase (5 U/μl) (TaKaRa): Store at −20 °C (*see* **Note 8**).
6. ExoSAP-IT (USB Corporation): Store at −20 °C.
7. ABI PRISM® BigDye™ Terminator Cycle Sequencing Kit (Applied Biosystems).
8. ABI PRISM® 3100 or other capillary sequencer (Applied Biosystems).

2.5 Items for Bioinformatics Analysis

1. PC with a Windows platform.
2. Receptor ligand contacts (RELIC): Stand-alone program [21, 22].

2.5.1 Quantitative Assessment of Parent or Affinity-Selected Peptide Populations

(a) **AADIV**: Calculates the frequency of occurrence of each of the 20 amino acids at each recombinant insert position, as well as the overall position-independent frequency of each amino acid within that set of peptide sequences. Also roughly estimates the sequence diversity of a display library by statistical sampling method based upon sequences obtained from a limited number of randomly sampled members of the library.

(b) **INFO**: Provides mathematical measure of the probability of observing a particular peptide sequence by random chance (i.e., nonspecific binding) as opposed to by selection for a specific property (affinity to small molecule).

(c) **MOTIF1**: Searches for three continuous amino acid sequence motifs within a peptide population.

(d) **MOTIF2**: Searches for patterns of three amino acids and does not allow conservative amino acid substitutions, but does allow identical gap lengths.

2.5.2 Analysis of Single- or Multiple-FASTA Sequences

(e) **MATCH**: Identifies any stretches of amino acid residues within a particular protein that exhibit significant similarity to a group of affinity-selected peptides. Outputs as cluster diagram and cumulative similarity plot calculated from a modified BLOSUM62 matrix with a short window (5–6 amino acids in length).

(f) **HETEROalign**: Visualizes cumulative similarity score to a PDB file (*see* **Note 9**).

(g) **FASTAskan**: Lists proteins with high similarity to a peptide population (*see* **Note 10**).

(h) **FASTAcon**: Identifies proteins from a population with short consensus sequences.

3 Methods

Carry out all procedures at room temperature unless otherwise specified.

3.1 Construction of T7 Phage Library (Fig. 1)

1. For the preparation of a duplex DNA library, oligonucleotide 1 (0.056 pM) and oligonucleotide 2 (0.56 pM) are mixed with Klenow buffer, heated to 95 °C for 5 min, and annealed by slowly cooling the mixture to 37 °C (*see* **Note 11**).
2. The single-stranded regions are converted to duplex DNA by continuing the incubation at 37 °C for 2 h in the presence of dNTPs and Klenow DNA polymerase I (*see* **Note 12**).
3. After the reaction, double-stranded DNA is recovered by EtOH precipitation. The obtained DNA insert is double-digested with *Eco*R I and *Hind* III restriction enzyme (*see* **Note 13**).
4. The DNA insert (0.02–0.06 pmol) is ligated with the T7 select 10-3b *Eco*R I/*Hind* III Vector Arms (0.5 μg; 0.02 pmol) (*see* **Note 14**).
5. The 5 μL of product is finally incubated with 25 μL T7 phage packaging extract at 22 °C, for 2 h.
6. After the incubation, the reaction is stopped by adding 270 μL of LB medium and then 20 μL chloroform is added and mix gently by inversion.

3.2 Plaque Counting

1. Culture the host *E. coli* BLT5615 in 20 mL LB/carbenicillin medium at 37 °C, overnight. Add the 2 mL of the overnight culture to 18 ml LB/carbenicillin medium and culture at

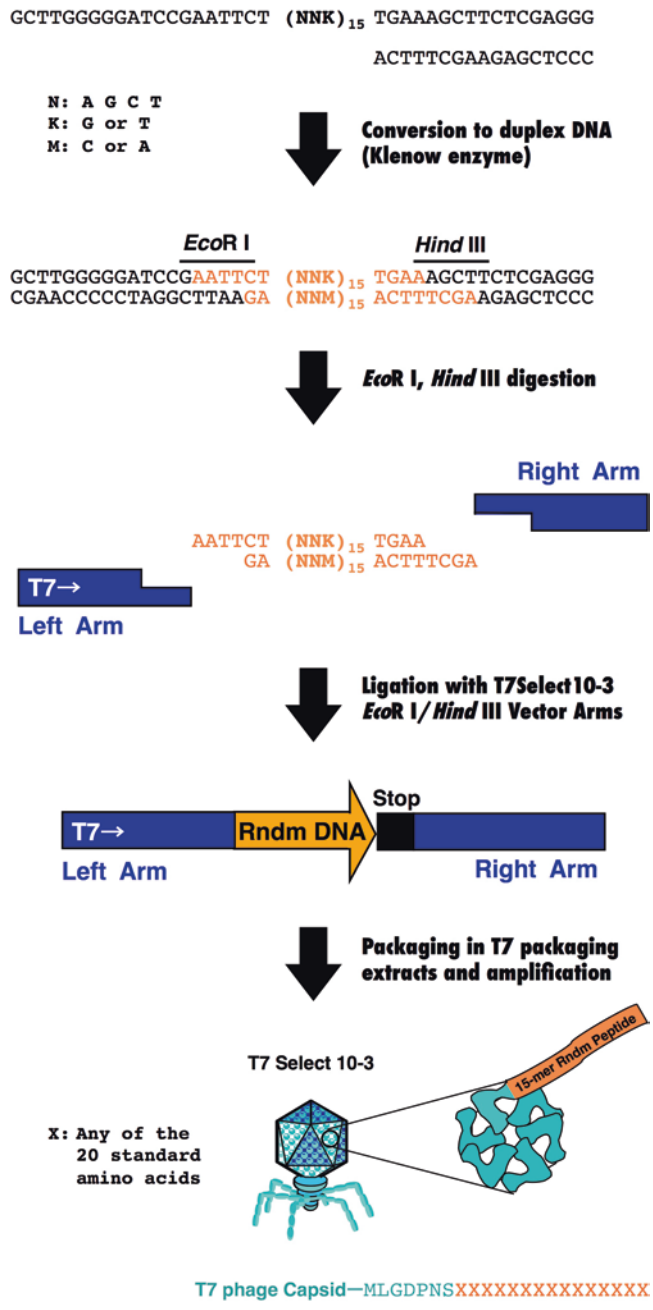

Fig. 1 Construction for T7 phage-displayed random peptide library

37 °C by shaking to an OD_{600} of 0.5–1.0. Add 1 mM of IPTG (final) and continue shaking for 30 min.

2. Dilute the 10–20 μL of packaging solution with 10^{-2} to 10^{-6} using LB medium or phage extraction buffer (*see* **Note 15**).

3. Mix each 100 μL with 200 μL of BLT5615 culture and 3 ml of prewarmed top agarose and then spread onto LB/carbenicillin plate. Leave the plate at room temperature until the agar solidifies, and then incubate at 37 °C for 2.5–3 h.
4. Count the plaques on each dish and calculate the pfu in packaging solution. The primary titer of this T7 phage library will be 1–2 × 10^7 pfu/mL following the protocol in Subheading 3.1.

3.3 Liquid Lysate Amplification

1. Count the number of BLT5615 cells in culture solution by measuring OD_{600} (*see* **Note 16**) and mix with packaging solution at an MOI of 0.001 (i.e., 1000 cells for each pfu).
2. Incubate with shaking at 37 °C for 3–4 h until lysis is observed.
3. Clarify the lysate by spinning at 8000 × *g* for 10 min. Decant the supernatant into a sterile bottle. The phage library will be amplified up to 1–2 × 10^{10} pfu/mL using *E. coli* (BLT5615) as the host strain (Fig. 1) (*see* **Note 17**).

3.4 QCM Sensor Chip Preparation

1. Attach a ceramic sensor chip on the oscillator of a 27-MHz QCM apparatus, and record the intrinsic frequency in the air phase before compound immobilization.
2. After detaching the chip, drop a 20 μL aliquot of a small-molecule derivative solution (1 mM in 70% EtOH) onto the gold electrode of the ceramic sensor chip and leave for 1 h under a humid and shaded atmosphere at room temperature.
3. Wash the surface of the electrode for 10 min in reaction buffer, which is stirred at 1000 rpm at 25 °C.
4. Set up the sensor chip for the QCM apparatus and record the decreased frequency in the air phase to measure the immobilized small-molecule amount. The small-molecule derivative will be immobilized around 10,000 Hz (300 ng).

3.5 QCM-Biosensor-Based One-Cycle Biopanning (Fig. 2)

1. Immerse the QCM sensor into the cuvette containing 8 mL of buffer (stirred at 1000 rpm) and then fully stabilize.
2. Inject an aliquot of 8 μL of a T7 phage library (1–2 × 10^{10} pfu/mL) into the cuvette (final 1–2 × 10^7 pfu/mL).
3. Monitor the frequency changes, caused by binding to the small molecule immobilized on the gold electrode surface, for 10 min.
4. Dislodge the sensor chip from oscillator and drop the 20 μL of log-phase host *E. coli* (BLT5615) solution onto the gold electrode and then incubate at 37 °C for 30 min on shaker for the recovery of DNA of bound T7 phages (*see* **Note 18**).
5. Add another 200 μL of LB medium to the resulting solution and then subject to plaque isolation.

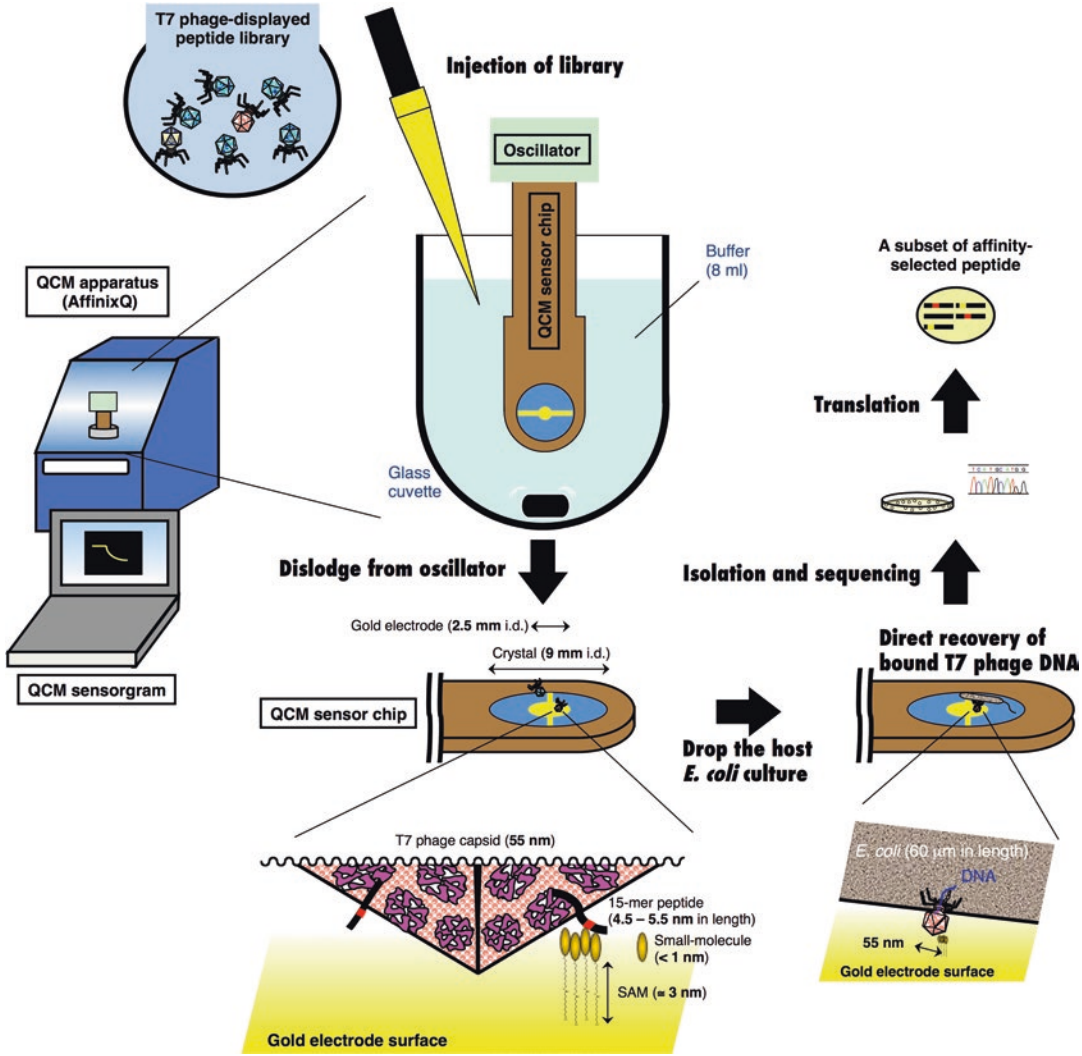

Fig. 2 Schematic representation of QCM biosensor-based T7 phage display

6. Dilute the recovered phage solution (contains 5×10^5 to 5×10^6 pfu/mL of T7 phages on average) from 10^1 to 10^6 times.
7. Mix a 100 μL aliquot of each solution with 200 μL of log-phase host *E. coli* solution and 3 mL of prewarmed top agarose, and then seed onto an LB/carbenicillin plate.
8. Incubate the plate at 37 °C for 3 h to form individual phage plaque.

3.6 PCR and DNA Sequencing

1. Randomly pick plaques from LB plates, and dissolve each in 50 μL of phage extraction buffer.

2. Disrupt the phages by heating the extract to 65 °C for 10 min. Amplify the peptide-encoding region by PCR using the forward primer and the reverse primer (*see* **Note 19**).
3. Treat the 5 μL of products with 2 μL of ExoSAP-IT (digested at 37 °C for 15 min and then inactivated at 80 °C for 15 min) and precipitate the DNA by 70% EtOH precipitation.
4. Sequence the products using ABI Prism BigDye Terminator Cycle Sequencing Kit and on an ABI Prism3100 Genetic Analyzer according to the manufacturer's protocols.

3.7 After Maintenance of QCM Sensor Chip

1. After the bound T7 phage recovery, swab the gold electrode surface with 1% sodium dodecyl sulfate.
2. Treat with 5 μL of piranha solution for 5 min (*see* **Note 20**).
3. Wash with dH_2O, and then dry up.

3.8 Bioinformatics Analysis Using RELIC Programs (Figs. 3 and 4)

1. Summarize amino acid sequence of affinity-selected peptides or proteins in a text file (.txt) with FASTA format and execute according to instructions of each program.
2. On MATCH or FASTAskan, calculate the scores between peptides from parent library and protein(s) and subtract the ones between affinity-selected peptides and corresponding protein(s) to remove the background noise from nonspecific binding peptides or sequences matched by chance.

4 Notes

1. Other cuvette-type QCM devices are applicable, even a QCM apparatus of flow injection type [11].
2. This ceramic sensor chip, glass cuvette, and stir magnet are designed for 27-MHz AffinixQ series (Initium Inc., Japan).
3. The contents of reaction buffer will be freely changeable. The different kinds or concentrations of salt, presence of detergent (e.g., 0.1–0.5% Tween 20), or tuning solution pH will affect the charge or conformation of peptides displayed on T7 phages and may influence the interaction with small molecule. T7 phage is not stable to acidic conditions below about pH 4.0 [6]. The temperature of the solution can also be changed on this apparatus (0.1~50 °C). However, the QCM sensor is the most stable at room temperature (around 25 °C).
4. Immobilization kit is commercially available. The SAM immobilization enhances the sensitivity of QCM at least thrice greater than that using avidin-biotin immobilization (piggyback or mass enhancer effect) [12]. The introduced position of linker in small molecule will affect the quality of information content in peptide population selected by the small molecule.

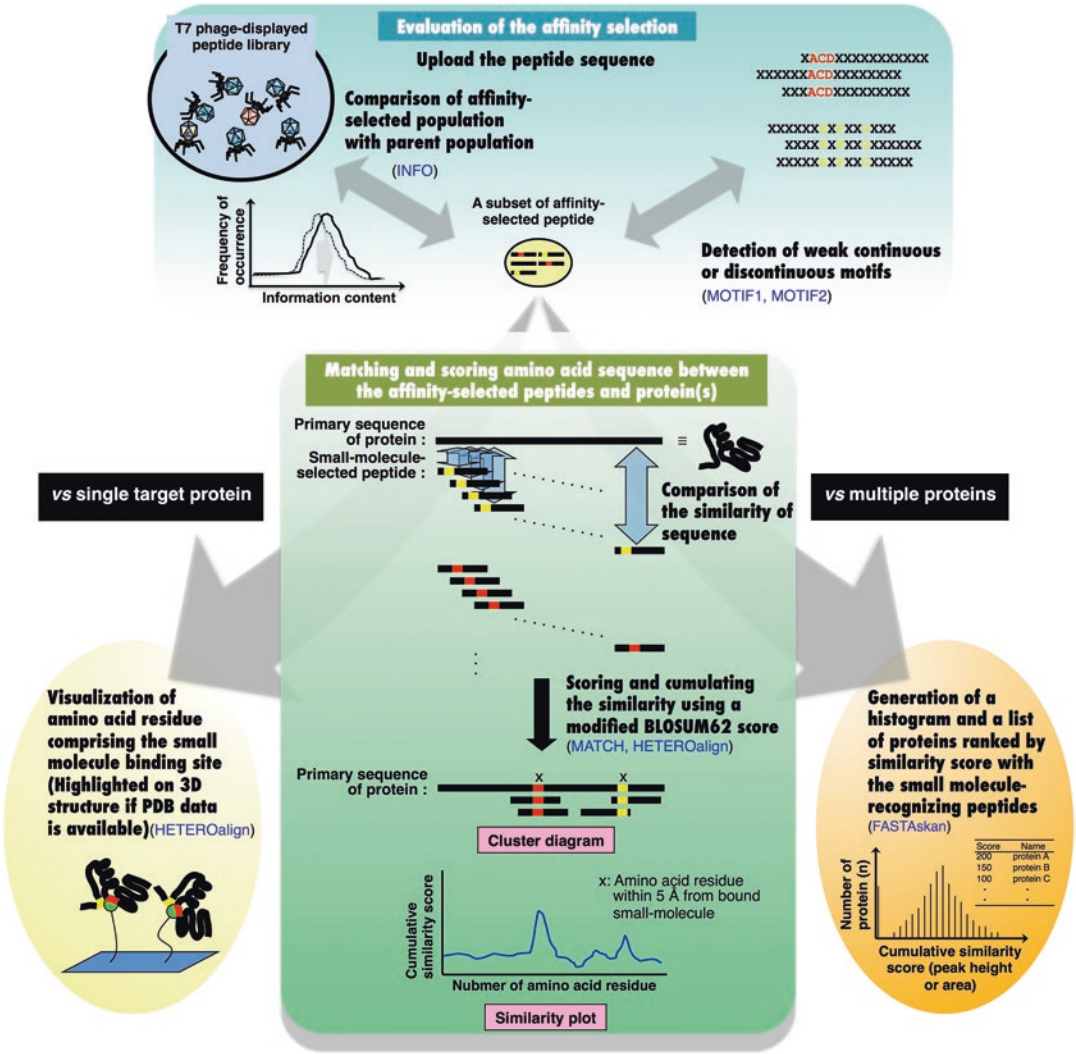

Fig. 3 Qualitative and quantitative assessment of peptide populations and analysis of similarity with single or multiple proteins

5. The NNK base sequence can reduce the emergence rate of stop codon to one-third (TAG only); nonetheless it comprehensively encodes standard 20 amino acids. In general, 7–15 mer peptide libraries are used. Statistical consideration in terms of designing library length will contribute to the reduction of background noise generated by matching between selected peptides and proteins by chance.
6. NEB buffer allows double digestion with *Eco*R I and *Hind* III and shorten the steps.
7. According to the manufacturer's protocol, use of this buffer allows efficient ligation on 5–30 min of incubation at

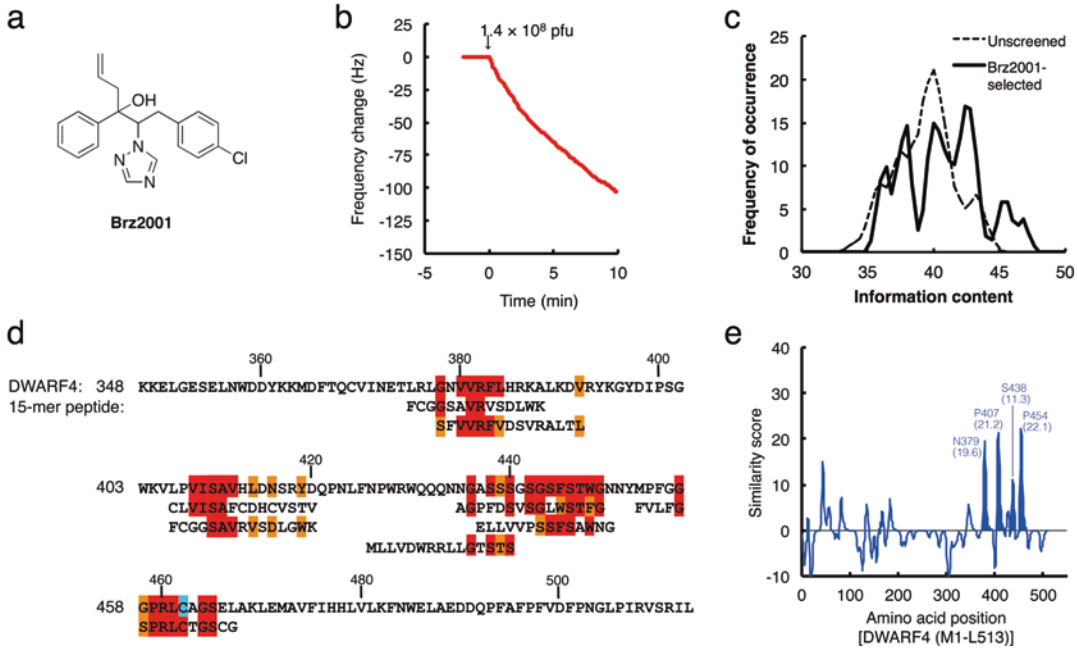

Fig. 4 Example results for the QCM biosensor-based T7 phage display and RELIC programs using Brz2001/DWARF4 system in plants. (**a**) Chemical structure of Brz2001, a specific DWARF4 inhibitor in plant hormone brassinosteroid biosynthesis. (**b**) A representative QCM sensorgram obtained by an affinity selection of peptide on the QCM apparatus (AffinixQ) using the Brz2001 derivative and a random peptide T7 phage library (8 μL; 1.4×10^8 pfu). The Brz2001-immobilized ceramic sensor chip generated by SAM was attached to the QCM apparatus. After injecting the T7 phage library at the indicated concentration, the frequency decrease was monitored for 10 min. 1 Hz = 0.62 ng/cm^2. (**c**) Upward shift in the information profile of the 15-mer random peptide library following affinity selection against Brz2001. INFO program executed using 103 of random peptides chosen without affinity selection (unscreened, dotted line) and 26 of Brz2001 affinity-selected peptides (bold line). (**d**) Cluster diagram between Brz2001-selected 26 of peptides and DWARF4 at the portion of maximal similarity (generated by MATCH program). Residues exhibiting identity or similarity to the protein sequence are highlighted in red or orange. The axial ligand of C462 coordinated with iron ion is shown in cyan. (**e**) Similarity plot. Scores for the sequence of DWARF4 (M1-L513) against the sequence of 26 peptides selected for affinity to Brz2001. Similarity scores of 103 of randomly selected peptides have been subtracted from these scores to remove library bias. Figures (**b**), (**d**), and (**e**) reproduced from [12] with permission from Mary Ann Liebert, Inc.

16 °C. The reaction time should be optimized according to the packaging efficiency.

8. Other Taq polymerases and buffers are available.
9. Currently unavailable.
10. Any protein database can be available in FASTA format (e.g., UniProt (http://www.uniprot.org/), DrugBank (https://www.drugbank.ca/), or other databases in addition to originally defined dataset in .txt).

11. Combine in a sterile, RNase-free 1.5 mL Eppendorf tube:

10 μL	Oligonucleotide 1 (10 pmol/μL)
10 μL	Oligonucleotide 2 (100 pmol/μL)
200 μL	10× Klenow buffer
1570 μL	Nuclease-free water
1790 μL	Total volume

Dispense into PCR tube (89.5 μL × 20), heat at 95 °C for 5 min on a PCR apparatus, and gradually cool to 37 °C.

12. Add 10.5 μL/tube of the following mixture:

200 μL	dNTP (2.5 mM)
1 μL (1 U)	Klenow enzyme (diluted to 1 U/μL by 1× Klenow buffer)
9 μL	Nuclease-free water
210 μL	Total volume

Incubate at 37 °C for 2 h.

13. Double-digest with *Eco*R I and *Hind* III by adjusting the following mixture:

1–2 μg	DNA
5 μL (75 U)	*Eco*R I
5 μL (100 U)	*Hind* III
10 μL	10× NEBuffer 2.1
x μL	Nuclease-free water
100 μL	Total volume

Incubate at 37 °C for 2 h. In addition to phenol/chloroform extraction and EtOH precipitation, purification of the DNA by gel filtration column chromatography dramatically enhances ligation and packaging efficiency.

14. Ligate the DNA with E/H Vector Arms by adjusting the following mixture:

0.02–0.06 pmol	DNA (E/H digested)
0.02 pmol (0.5 μg)	T7Select10-3 E/H Vector Arms
2.5 μL	2× Ligation mix
x μL	Nuclease-free water
5 μL	Total volume

Incubate at 16 °C for 5 min to 16 h.

15. LB medium is also available.
16. When the absorbance measured at OD_{600} is 0.5, the number of *E. coli* in the culture is 2×10^8 cells per mL.
17. The library peptides cannot be displayed on the capsid until T7 phages are amplified by the host infection after the packaging.
18. T7 phage is capable of binding the host *E. coli* even if the capsid protein is involved in binding to the bait.
19. Adjust the following mixture:

0.3 μL	Phage extract solution
0.05 μL	Forward primer (100 pmol/μL)
0.05 μL	Reverse primer (100 pmol/μL)
1 μL	10×Ex Taq DNA polymerase buffer
1 μL	dNTP (2.5 mM)
0.05 μL	Ex Taq (5 U/μL)
7.55 μL	Nuclease-free water
10 μL	Total volume

Amplify the part of gene10 region encoding affinity-selected peptide. PCR condition; 25 cycles each of 94 °C for 60 s, 50 °C for 30 s, and 72 °C for 30 s.

20. Treatment with piranha solution longer than 5 min erodes the sensor chip.

Acknowledgements

This work is partly supported by JSPS KAKENHI Grant Number 19380066 (F.S.), 19750145 (Y.T.), and 17K01363 (Y.T.).

References

1. Smith GP, Petrenko VA (1997) Phage display. Chem Rev 97:391–410
2. Piggott AM, Karuso P (2016) Identifying the cellular targets of natural products using T7 phage display. Nat Prod Rep 33:626–636
3. Takakusagi Y, Takakusagi K, Sugawara F, Sakaguchi K (2010) Use of phage display technology for the determination of the targets for small-molecule therapeutics. Expert Opin Drug Discov 5:361–389
4. Kuroiwa Y, Takakusagi Y, Kusayanagi T, Kuramochi K, Imai T, Hirayama T, Ito I, Yoshida M, Sakaguchi K, Sugawara F (2013) Identification and characterization of the direct interaction between methotrexate (MTX) and high-mobility group box 1 (HMGB1) protein. PLoS One 8:e63073
5. Piggott AM, Kriegel AM, Willows RD, Karuso P (2009) Rapid isolation of novel FK506 binding proteins from multiple organisms using gDNA and cDNA T7 phage display. Bioorg Med Chem 17:6841–6850
6. Novagen (2009) T7 Select® System Manual. Novagen TB178:1009JN
7. Novagen (2009) OrientExpressTM cDNA Manual. Novagen TB247:1109JN
8. Speight RE, Cooper MA (2012) A survey of the 2010 quartz crystal microbalance literature. J Mol Recognit 25:451–473
9. Becker B, Cooper MA (2011) A survey of the 2006–2009 quartz crystal microbalance biosensor literature. J Mol Recognit 24:754–787
10. Cooper MA, Singleton VT (2007) A survey of the 2001 to 2005 quartz crystal microbalance biosensor literature: applications of acoustic physics to the analysis of biomolecular interactions. J Mol Recognit 20:154–184
11. Takakusagi K, Takakusagi Y, Suzuki T, Toizaki A, Suzuki A, Kawakatsu Y, Watanabe M, Saito Y, Fukuda R, Nakazaki A, Kobayashi S, Sakaguchi K, Sugawara F (2015) Multimodal biopanning of T7 phage-displayed peptides reveals angiomotin as a potential receptor of the anti-angiogenic macrolide Roxithromycin. Eur J Med Chem 90:809–821
12. Takakusagi Y, Manita D, Kusayanagi T, Izaguirre-Carbonell J, Takakusagi K, Kuramochi K, Iwabata K, Kanai Y, Sakaguchi K, Sugawara F (2013) Mapping a disordered portion of the Brz2001-binding site on a plant monooxygenase, DWARF4, using a quartz-crystal microbalance biosensor-based T7 phage display. Assay Drug Dev Technol 11:206–215
13. Kusayanagi T, Tsukuda S, Shimura S, Manita D, Iwakiri K, Kamisuki S, Takakusagi Y, Takeuchi T, Kuramochi K, Nakazaki A, Sakaguchi K, Kobayashi S, Sugawara F (2012) The antitumor agent doxorubicin binds to Fanconi anemia group F protein. Bioorg Med Chem 20:6248–6255
14. Takakusagi Y, Takakusagi K, Ida N, Takami M, Matsumoto Y, Kusayanagi T, Nakabayashi T, Aoki S, Murata H, Ohta K, Sugawara F, Sakaguchi K (2011) Binding region and interaction properties of sulfoquinovosylacylglycerol (SQAG) with human vascular endothelial growth factor 165 revealed by biosensor-based assays. Med Chem Commun 2:1188–1193
15. Takakusagi Y, Takakusagi K, Sugawara F, Sakaguchi K (2009) [Validation of small-molecule/protein interactions by the T7 phage display strategy using a quartz-crystal microbalance device]. Tanpakushitsu Kakusan Koso 54:1203–1209
16. Takakusagi Y, Kuramochi K, Takagi M, Kusayanagi T, Manita D, Ozawa H, Iwakiri K, Takakusagi K, Miyano Y, Nakazaki A, Kobayashi S, Sugawara F, Sakaguchi K (2008) Efficient one-cycle affinity selection of binding proteins or peptides specific for a small-molecule using a T7 phage display pool. Bioorg Med Chem 16:9837–9846
17. Takakusagi Y, Kuroiwa Y, Sugawara F, Sakaguchi K (2008) Identification of a methotrexate-binding peptide from a T7 phage display screen using a QCM device. Bioorg Med Chem 16:7410–7414
18. Takakusagi Y, Takakusagi K, Kuramochi K, Kobayashi S, Sugawara F, Sakaguchi K (2007) Identification of C10 biotinylated camptothecin (CPT-10-B) binding peptides using T7 phage display screen on a QCM device. Bioorg Med Chem 15:7590–7598
19. Rodi DJ, Janes RW, Sanganee HJ, Holton RA, Wallace BA, Makowski L (1999) Screening of a library of phage-displayed peptides identifies human bcl-2 as a taxol-binding protein. J Mol Biol 285:197–203
20. Rodi DJ, Agoston GE, Manon R, Lapcevich R, Green SJ, Makowski L (2001) Identification of small molecule binding sites within proteins using phage display technology. Comb Chem High Throughput Screen 4:553–572
21. Makowski L (2011) Quantitative analysis of peptide libraries. In: Petrenko VA, Smith GP (eds) Phage nanobiotechnology. RSC Publishing, Cambridge, pp 33–54
22. Mandava S, Makowski L, Devarapalli S, Uzubell J, Rodi DJ (2004) RELIC—a bioinformatics server for combinatorial peptide analysis and identification of protein-ligand interaction sites. Proteomics 4:1439–1460