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# Chapter 59

## Expression, Purification and Functional Characterization of Tectonin 2 from *Laccaria bicolor*: a Six-Bladed beta-Propeller Lectin Specific for O-Methylated Glycans

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**Running Head:** Expression, purification and functional characterization of Lb-Tec2

### Abstract

Tectonins are conserved defense proteins of innate immune systems featuring a  $\beta$ -propeller fold. Tectonin 2 from *Laccaria bicolor*, Lb-Tec2, is the first fungal representative of the tectonin superfamily that has been described. In-depth characterization revealed a specificity for O-methylated glycans and identified a unique sequence motif and binding site architecture underlying this unusual specificity. This chapter provides information on how to produce and purify recombinant Lb-Tec2, characterize its interaction with O-methylated glycans and demonstrate its biological function.

**Keywords:**  $\beta$ -Propeller, Non-self recognition, O-Methylated glycans, Defense effector, Nematotoxic lectin, Innate immunity.

### 1 Introduction

Tectonins represent a large family of proteins widely distributed from bacteria to humans. These proteins share the occurrence of multiple

TECPR domains, each of which forms one blade in a  $\beta$ -propeller fold [1]. Tectonins can be classified into two subgroups depending on whether they comprise six or more TECPR repeats, respectively. The first group is mainly found in bacteria, fungi, amoebae, limulidae, sponges and fishes while the second has diverged to a far more complex domain architecture and essentially occurs in insects, nematodes and mammals [1, 2]. Tectonins recognize non-self epitopes at the surface of pathogens and predators and by this means act as effectors of innate immunity, the sole line of defense in many organisms. Certain tectonins bind to conserved carbohydrate components in the cell wall of invaders, *e.g.*, outer membrane lipopolysaccharides (LPS) in bacteria [3, 4]. Upon target binding, tectonins can induce signalling and activation of cellular pathways, *e.g.*, phagocytosis in case of the horseshoe crab L6 lectin or selective autophagy as described for human TECPR1 [3, 5]. In contrast, other members of the tectonin superfamily act as toxins. One example is Tectonin 2 from the mushroom *Laccaria bicolor*, referred to as Lb-Tec2, which is nematotoxic and agglutinates bacteria [6].

Characterization of Lb-Tec2 revealed a defense effector role that depends on the recognition of *O*-methylated glycans on the surface of soil microbes and predators [6]. Methylation is an occasional secondary modification of glycans that occurs in various organisms but is absent in mammals [7]. Recognition of this modification therefore allows tectonins to target a wide range of invaders. In solution, binding of Lb-Tec2 to 2-*O*-methyl-L-fucoside, 3-*O*- or 4-*O*-methyl-D-mannoside is of low affinity (millimolar range) as determined by isothermal microcalorimetry (ITC). However, a 60-fold increase in avidity was observed for methylated glycans immobilized on a surface as demonstrated by surface plasmon resonance (SPR), exemplifying the importance and benefits of multivalent lectin-carbohydrate interactions [2, 6]. The synthesis of a specific selenated methylated glycan allowed the determination of the crystal structure of recombinant Lb-Tec2 in complex with the ligand [8]. Structure-function relationships could thus be established: in accordance with the number of TECPR domains, tectonins from the first subgroup form a six-bladed  $\beta$ -propeller [2, 9]. Furthermore, co-crystal structures of Lb-Tec2 with methylated ligands revealed a highly conserved binding site architecture specifically designed for the recognition of methylated glycans. The identified conserved methylation recognition motif can now be used to identify lectins with this specificity and fold in other genomes using the PropLec server in the Unilectin database [10]. With regard to quaternary structure, the Lb-Tec2 crystal structure revealed a unique tetrameric organisation that was confirmed by small angle X-ray scattering (SAXS) measurements in solution [2]. The resulting high multivalency (24 binding sites) is probably essential for the toxicity of this lectin.

The following sections will describe the recombinant expression of Lb-Tec2 in *Escherichia coli* and its purification by affinity chromatography. Different protocols to study its affinity in solution or on surface are provided. Finally, bioassays to evaluate Lb-Tec2 nematotoxicity and bacterial agglutination as well as *in vitro* binding to target proteins by lectin blotting are described.

## 2 Materials

Prepare all solutions using analytical grade reagents and ultrapure water and then filter on 0.22  $\mu\text{M}$  membrane. Follow waste disposal regulations. Protein concentration is determined at 280 nm using a molecular weight of 23,795 daltons and a theoretical molar extinction coefficient of 91,900  $\text{M}^{-1} \text{cm}^{-1}$ .

### 2.1 Production of Recombinant Lb-Tec2

1. Incubation shaker with cooling option.
2. 250 mL and 3 L baffled Erlenmeyer flasks.
3. pET24-Lb-Tec2 plasmid obtained according to [6].
4. *Escherichia coli* BL21(DE3) chemically competent cells (*see Note 1*).
5. Lennox L Broth Base (LB). Dissolve 20 g of premixed powder in 980 mL of  $\text{dH}_2\text{O}$  and autoclave 20 min at 121 °C.
6. Dry block heater at 42 °C.
7. LB-Agar. Dissolve 1.6 g of premixed powder in 50 mL of  $\text{dH}_2\text{O}$  and autoclave 20 min at 121 °C. Add the appropriate antibiotic after cooling to 60 °C and pour in sterile Petri dish. Leave the plates to solidify and flip them to avoid condensation. Store at 4 °C in a plastic bag until use.
8. 50 mg/mL kanamycin sulfate (*see Note 2*).
9. 1 M Isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG).
10. 50 mL conical centrifuge tubes and 1 L polycarbonate centrifuge bottle (Nalgene).
11. Centrifuge with adapted rotors for 1 L bottle or 50 mL conical tubes.

### 2.2 Purification of Recombinant Lb-Tec2

1. 0.45  $\mu\text{m}$  PES syringe filter.
2. C10/10 column with flow adapter (GE Healthcare) or Empty Econo-Pac® Chromatography Columns (Bio-Rad Ltd.).
3. 100 mM phenylmethylsulfonyl fluoride (PMSF, *see Note 3*).
4. French press (SLM Aminco; SLM Instruments).
5. Sepharose CL-6B matrix.
6. Rotating wheel.

7. Binding buffer: 100 mM HEPES/NaOH pH 7.5 (*see Note 4*). Store at 4 °C.
8. Elution buffer: 100 mM HEPES/NaOH pH 7.5 and 400 mM GlcNAc (*see Note 4*). Store at 4 °C.
9. 10 × 8 cm 12% SDS-PAGE gel with vertical gel electrophoresis unit and universal power supply.
10. PD-10 desalting column (GE Healthcare Life Sciences) or Snakeskin dialysis tubing 3500 MWCO (Thermo Fisher Scientific).
11. Microsep 10K (Pall).

## **2.3 Isothermal Titration**

### **Microcalorimetry**

1. MicroCal iTC200 microcalorimeter (Malvern Panalytical).
2. Methylated ligands (synthesis described in [2, 6, 8]).
3. GlcNAc (Carbosynth).

## **2.4 Surface Plasmon**

### **Resonance**

1. Biacore X100 (GE Healthcare Life Sciences).
2. CM5 sensor chips (GE Healthcare Life Sciences).
3. HBS-ET buffer (10 mM HEPES/NaOH pH 7.5, 150 mM NaCl, 3 mM EDTA, and 0.05% Tween 20).
4. Streptavidin from *Streptomyces avidinii* (Sigma-Aldrich).
5. Amine coupling Kit (GE Healthcare Life Sciences).
6. 1 M ethanolamine.
7. Biotinylated 4-*O*-methyl- $\alpha$ -D-mannopyranose (4MeMan, synthesis described in [2]).
8. 1 M NaCl, 50 mM NaOH.
9. 50% isopropanol, 50 mM NaOH, and 1 M NaCl.
10. 1 M NaCl.

## **2.5 Caenorhabditis elegans**

### **Biotoxicity Assay**

1. Synchronous population of *C. elegans* L1 larvae [11, 12].
2. Bacterial culture of recombinant *E. coli* expressing Lb-Tec2 and vector control (incubated at 23 °C upon induction).
3. Sterile phosphate-buffered saline buffer (PBS) pH 7.4.
4. 96-well plate (TPP).
5. Dark incubator at 20 °C.
6. Microscope (*e.g.*, Zeiss Axiovert 25).

## **2.6 Localization of Lb-Tec2-binding in C. elegans**

1. TAMRA succinimidyl ester or Alexa Fluor 488 5-sulfodichlorophenol (SDP) ester (Molecular Probes).

2. PD-10 desalting column (GE Healthcare Life Sciences).
3. S-basal: dissolve 5.85 g NaCl, 1 g K<sub>2</sub>HPO<sub>4</sub>, 6 g KH<sub>2</sub>PO<sub>4</sub> and 1 mL of cholesterol (5 mg/mL in ethanol), in 1 L of water. Sterilize by autoclaving.
4. Microscope (e.g. Zeiss Axiophot with a 10× or 20× objective and AxioVision 4.8).

## 2.7 Lectin Blot

1. Target protein(s) of interest, e.g., *C. elegans* protein extract.
2. EZ Link Sulfo-NHS-SS-Biotin (Pierce).
3. PD-10 desalting column (GE Healthcare Life Sciences).
4. Vectastain® ABC-alkaline phosphatase kit (AK-5000, Vector laboratories).
5. BCIP/NBT substrate (B1911, Sigma-Aldrich).
6. SDS-PAGE gel with vertical gel electrophoresis unit and universal power supply.
7. Nitrocellulose membrane and blotting equipment.
8. Washing buffer (PBS-T): phosphate-buffered saline pH 7.4. (PBS) containing 0.1% Tween 20.
9. Blocking buffer: 5% Bovine Serum Albumin (BSA) in PBS-T.

## 2.8 Bacterial Agglutination Assay

1. Bacterial strains of interest.
2. 100 mM HEPES/NaOH pH 7.5.
3. U-shaped 96-well microtiter plate.
4. Bovine Serum Albumin (BSA).
5. Microscope (e.g., Leica MZ125 stereomicroscope).

## 3 Methods

All procedures are performed at room temperature unless otherwise stated.

### 3.1 Production of Recombinant Lb-Tec2

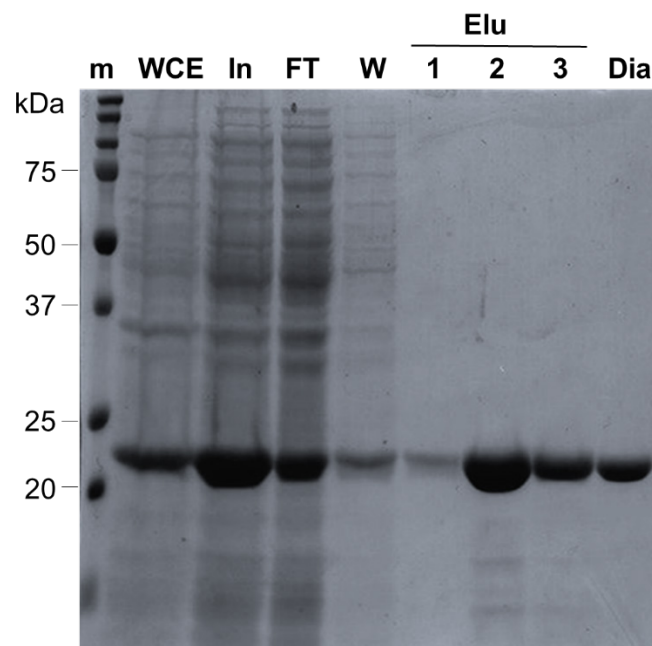
1. Transform *E. coli* BL21(DE3) competent cells with the pET24-Lb-Tec2 plasmid by heat shock. Add 1 µL of plasmid to an aliquot of competent cells and incubate 5 minutes on ice. Heat shock the cells at 42 °C for 45 s before placing the tube on ice for 2 min. Add 500 µL of LB and shake horizontally in an incubator for 45 min at 37 °C. Spread 100 µL on LB-agar plate supplemented with 50 µg/mL kanamycin and incubate overnight at 37 °C.
2. Inoculate 30 mL LB + 50 µg/mL kanamycin with a single colony of transformed cells. Cultivate overnight in an incubation shaker at 37 °C.
3. Inoculate 1 L LB + 50 µg/mL kanamycin with the overnight preculture and grow at 37 °C at 150 rpm until OD<sub>600nm</sub> = 1. Set incubator

temperature to 23 °C, induce protein expression with 0.5 mM IPTG and cultivate for 16 h (*see Note 5*). Take a sample for SDS-PAGE (*see Fig. 1*, WCE: whole cell extract).

4. Harvest the cells by centrifugation for 15 min at 16,000 ×g at room temperature.
5. Resuspend the pellet in 20 ml dH<sub>2</sub>O and transfer to a 50 mL conical centrifuge tube. Spin down for 8 min at 5,000 rpm, 4 °C.
6. Freeze pellet in liquid nitrogen and store at -20 °C.

### 3.2 Purification of Recombinant Lb-Tec2

- 1 Resuspend the cell pellet in 20 mL cold HEPES/NaOH 100 mM pH 7.5 containing 1 mM PMSF.
- 2 Lyse the cells using a French press and keep the lysate on ice (*see Fig.1*, WCE: whole cell extract and **Note 6**).
3. Clear the lysate by centrifugation for 30 min at 24,000 ×g and 4 °C.
4. Filter the supernatant on a 0.45 μm PES syringe filter. Take a sample for SDS-PAGE (*see Fig. 1*, In: input).



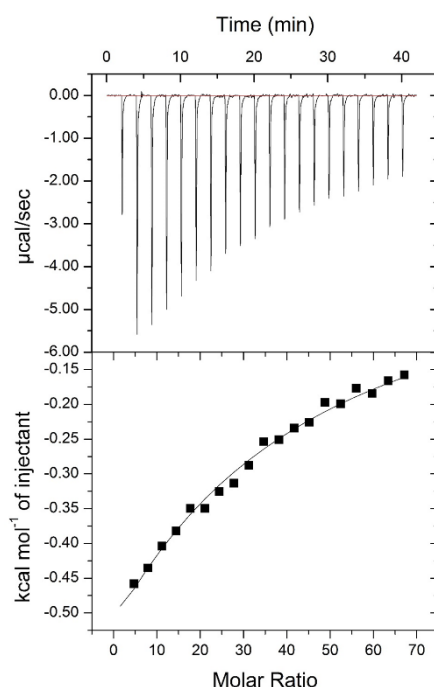
**Fig. 1.** Coomassie-stained SDS-PAGE gel of samples from Lb-Tec2 purification. m: marker, WCE: whole cell extract, In: input, FT: flow-through, W: wash, Elu: eluate. Dia: after dialysis. 12% SDS-PAGE gel.

5. Apply the supernatant to 4 mL of Sepharose CL-6B equilibrated with the binding buffer (*see Note 7*). After incubation for 1 h at 4 °C on a rotating wheel, load on an empty column, collect the flow-through and wash the resin with 10 column volumes of binding buffer. Take a sample of the flow-through and the wash for SDS-PAGE (*see Fig. 1*, FT and W, respectively).

- Apply 4 mL of elution buffer to the column and incubate for 10 min before elution of bound Lb-Tec2. Take a sample for SDS-PAGE (see Fig. 1, Elu1). Repeat twice (Elu2 and Elu3). Analyze fractions by electrophoresis on a 12% SDS-PAGE gel.
- Combine eluates containing Lb-Tec2 and exchange the buffer to the binding buffer using a PD-10 desalting column according manufacturer recommendations or by dialysis using a snakeskin dialysis tubing with 3500 MWCO at 4 °C. Take sample for SDS-PAGE (see Fig. 1, Dia).
- Concentrate to the desired value by centrifugation using Microsep 10K (Pall).
- Store at 4 °C.

### 3.3 Isothermal Titration Microcalorimetry

- Dilute the protein to 150  $\mu\text{M}$  in 20 mM HEPES/NaOH pH 7.5, degas and load in the 200  $\mu\text{L}$  sample cell at 25 °C (see **Note 8**).
- Dilute the methylated ligand to 50 mM or GlcNAc to 100 mM in 20 mM HEPES/NaOH pH 7.5, degas and load in the injection syringe.
- Perform the titration at 25 °C with 20-30 injections of 2  $\mu\text{L}$  of ligand (4 s duration each, 120 s interval between additions) while stirring at 1,000 rev/min (see Fig. 2, thermogram).



**Fig. 2.** Typical thermogram and corresponding integrated titration curve representing the binding of Lb-Tec2 to allyl 4-*O*-methyl- $\alpha$ -D-mannopyranoside resulting in a  $K_d$  of 17.3 mM.

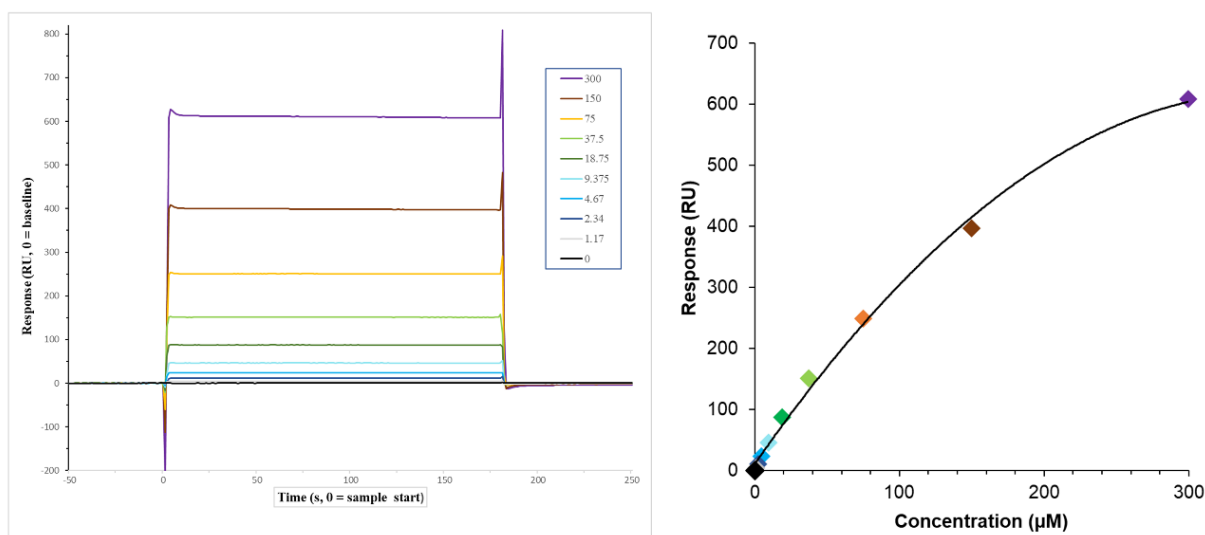
- Analyze the data (see Fig. 2, integration curve and **Note 8**).
- Perform at least duplicates of each titration.



6. Measure the heat of ligand dilution by titrating the ligand in the buffer. Subtract it from the experimental data if it is significant following data analysis software instructions.

### 3.4 Surface Plasmon Resonance

1. Use a flow rate of 30  $\mu\text{L}/\text{min}$  for injections and 10  $\mu\text{L}/\text{min}$  for washing steps on a Biacore X100 at 25  $^{\circ}\text{C}$ . Protein and ligand are all diluted in HBS-ET buffer.
2. Activate channels 1 and 2 of CM5 sensor chip by injection of a fresh mix of EDC/NHS followed by a solution of streptavidin at 100  $\mu\text{g}/\text{mL}$  in 10 mM sodium acetate pH 4.5 according to the classical amine protocol recommended by the manufacturer.
3. Inactivate remaining reacting species with an injection of 1 M ethanolamine.
4. After washing with 120  $\mu\text{L}$  of 1 M NaCl plus 50 mM NaOH, immobilize biotinylated 4MeMan on channel 2 by injection of 140  $\mu\text{L}$  of 0.1 mg/mL 4MeMan-biotin and wash with 50% isopropanol, 50 mM NaOH and 1 M NaCl as described in the wizard protocol (*see Note 9*).
5. Dilute Lb-Tec2 in a two-fold cascade starting from 300  $\mu\text{M}$  to 1  $\mu\text{M}$ .
6. Inject 130  $\mu\text{L}$  of each Lb-Tec2 concentration and the buffer control (lectin concentration 0  $\mu\text{M}$ ) with an association time of 180 s and a dissociation time of 180 s.
7. Regenerate by washing with 1 M NaCl in both channels for 30 s.
8. Subtract binding signal of control channel 1 from channel 2 binding signal for data analysis using the steady state affinity fit (Fig. 3, *see Note 9*).



**Fig. 3.** Sensorgrams obtained for binding of Lb-Tec2 to 4-*O*-methyl- $\alpha$ -D-mannopyranose for a protein concentration range of 0–300  $\mu\text{M}$  with steady-state affinity fit resulting in a  $K_d$  of 286  $\mu\text{M}$ .

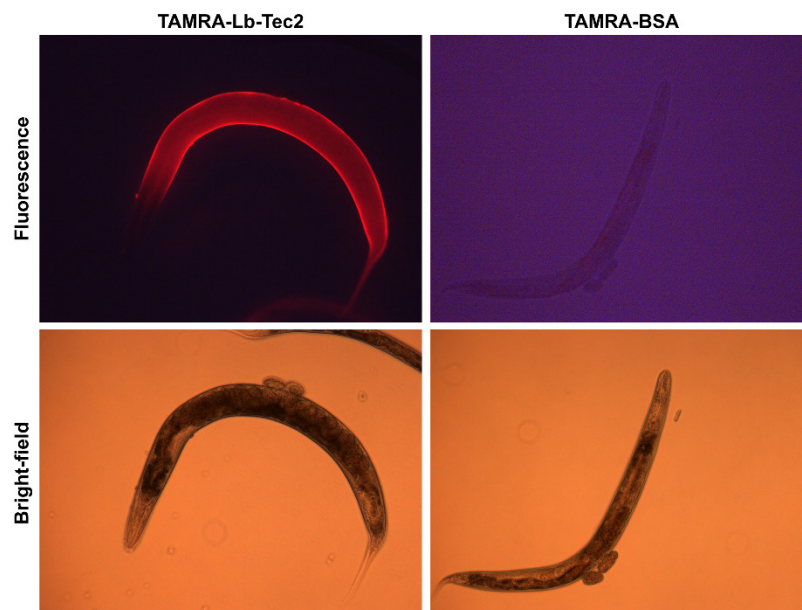
### 3.5 *C. elegans* Biotoxicity Assay

1. Prepare eggs from gravid hermaphrodites as described in [12]. Transfer to unseeded Nematode Growth Media (NGM) plate and incubate at 23 °C overnight for hatching in order to obtain a nearly synchronous population of L1 larvae.
2. Wash L1 larvae from the plate using sterile milliQ water and collect them in a sterile 15 mL screw cap tube. Count larvae using a microscope and adjust the number of larvae to 30 per 20  $\mu$ L.
3. Resuspend *E. coli* cells expressing Lb-Tec2 in sterile PBS buffer pH 7.5 to OD<sub>600</sub> = 2 after pelleting them by centrifugation for 5 min at max. rpm. Use *E. coli* transformed with empty pET24a vector as control.
4. Mix 80  $\mu$ L of bacterial suspension with 20  $\mu$ L of L1 larvae in a 96-well plate (TPP).
5. Allow the worms to feed on the bacteria at 20 °C in the dark.
6. Quantify the number of animals and percentage of individuals reaching the L4 stage after 48 h using a microscope.
7. Perform five independent replicates for each treatment.

### 3.6 Localization of Lb-Tec2-binding in *C. elegans*

The described procedure is used to assess binding of Lb-Tec2 to the cuticle of *C. elegans* (see **Note 10**).

1. Label purified Lb-Tec2 with TAMRA ester (or Alexa Fluor 488 SDP ester) according to manufacturer's instructions.

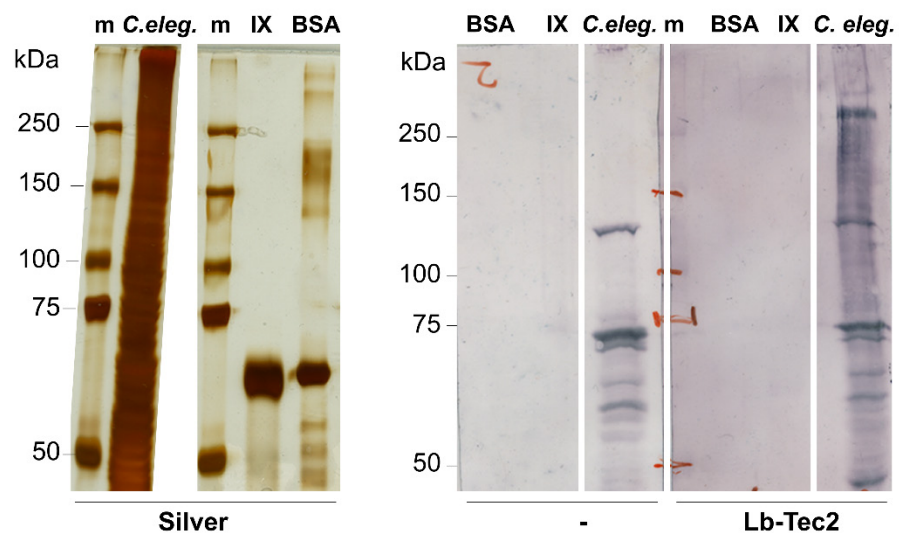


**Fig. 4.** Binding of TAMRA-Lb-Tec2 to the cuticle of *C. elegans pmk-1(km25)* (WT). Fluorescence (*Upper*) and bright-field (*Lower*) images are shown. TAMRA-BSA was used as negative control. An exposure time of 140 ms was used for fluorescent images.

2. Remove the excess of free label using a PD-10 desalting column and 100 mM HEPES/NaOH pH 7.5.
3. Harvest nematodes from NGM plates, wash with S-basal and incubate for 10 min in 50  $\mu$ L S-basal containing 2  $\mu$ g/mL TAMRA-Lb-Tec2 in the dark.
4. Wash the worms three times with S-basal for 10 min each and anesthetize them with 10 mM levamisol.
5. Transfer to a glass slide coated with 1% agarose for microscopy.
6. Acquire phase-contrast and fluorescence images (Fig. 4).

### 3.7 Lectin Blot

1. Biotinylate purified Lb-Tec2 with EZ Link Sulfo-NHS-SS-Biotin according to manufacturer's instructions and desalt on a PD-10 column in 100 mM HEPES/NaOH pH 7.5.
2. Separate target proteins, *e.g.*, *C. elegans* protein extracts, on an SDS-PAGE gel. Include additional lanes of protein extracts as a negative control to check for unspecific binding during the detection procedure.
3. Transfer proteins to a nitrocellulose membrane by semi-dry blotting (*see Note 11*).
4. Block the membrane with 5% BSA in PBS-T for 16 h at 4  $^{\circ}$ C on a shaker.
5. Rinse the membrane with PBS-T and cut it to obtain a separate piece of membrane for the negative control.
6. Incubate the membrane with biotinylated Lb-Tec2 in PBS-T at a concentration of 5  $\mu$ g/mL for 1 h at room temperature on a shaker. Incubate the negative control in PBS-T without lectin.



**Fig. 5.** Lectin blot. A protein extract from *C. elegans pmk-1* (*C. eleg.*) and purified human factor IX (IX) was probed with Lb-Tec2. Bovine serum albumin (BSA) was included as negative control. (A) Silver-stained SDS-PAGE gel of the proteins. (B) Blot of the same proteins probed with Lb-Tec2 or a mock control (-). m: marker. 7% SDS-PAGE gel.

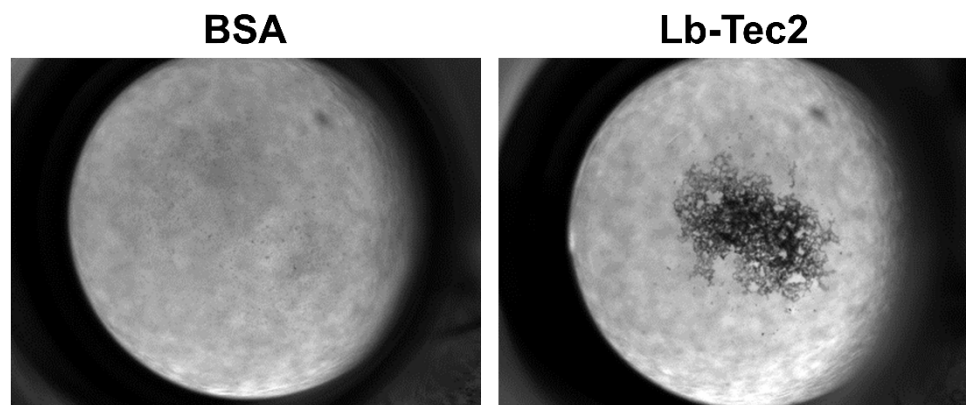
7. Rinse the membrane and wash three times for 20 min with PBS-T on a shaker.
8. Incubate with streptavidin-linked alkaline phosphatase for 1 h at room temperature on a shaker. This solution is obtained by diluting Reagent A and B of a Vectastain® ABC-alkaline phosphatase kit 150-fold in PBS-T.
9. Wash the membrane three times for 5 min in PBS-T on a shaker.
10. Overlay the membrane with BCIP/NBT substrate and develop to the desired intensity (not shaking).
11. Stop the color reaction with two washes in water and air-dry the membrane.
12. Take a picture (Fig. 5).

### 3.8 Bacterial Agglutination

#### Assay.

##### 3.8.1 Assay with Purified Protein

1. Grow *E. coli* O8:K<sup>-</sup> in LB at 37 °C until OD<sub>600</sub> = 0.7–1, wash with PBS and resuspend to an OD<sub>600</sub> of 2.5. (*E. coli* strains O8<sup>-</sup>:K<sup>-</sup>, O9a:K<sup>-</sup>, and O9a<sup>-</sup>:K<sup>-</sup> may be included as negative controls.)
2. Mix 10 µL of bacteria with 40 µL Lb-Tec2 in 100 mM HEPES/NaOH pH 7.5 at a final protein concentration of 15–500 µg/mL.
3. Take images after a 10–20 min incubation at room temperature using a stereomicroscope (Fig. 6).
4. Use BSA as a negative control.



**Fig. 6.** Agglutination of *E. coli* O8 by purified Lb-Tec2 at a final concentration of 5 µg/mL in a 96 well plate. BSA was used as a negative control.

##### 3.8.2 Assay with Soluble Protein Extract

5. Grow 50 mL cultures of BL21(DE3) expressing Lb-Tec2 at 23 °C. Wash cells with 100 mM HEPES/NaOH pH 7.5 and resuspend in 1

- mL to OD<sub>600</sub> of 20. Use cells harboring empty pET24a vector as negative control (*see Note 12*).
6. Lyse cells with 1 g of 0.5-mm glass beads in four consecutive homogenization steps with a FastPrep FP120 (Thermo Savant) for 45 s at level 6.5.
  7. Centrifuge the homogenate at 12,000 ×g for 30 min.
  8. Mix 10 μL of bacteria with 40 μL of the supernatant. Use HEPES buffer as a negative control.
  9. Take images after 1 h incubation at room temperature using a stereomicroscope.

#### 4 Notes

1. *E. coli* BL21 Star (DE3) cells can also be used for Lb-Tec2 expression. Cells were made chemically competent according to standard protocols using CaCl<sub>2</sub> and 100 μL aliquots were stored at -80 °C.
2. Kanamycin sulfate is a CMR substance; wear gloves and use a microbiological safety hood for manipulations.
3. PMSF is cytotoxic and should be manipulated with precaution. Dissolve powder in 100% ethanol and store at -20 °C in 100 μL aliquots. Dilute to the desired concentration just prior to use since it is quite unstable in aqueous solutions. PMSF may be replaced by the cCOMPLETE, mini protease inhibitor cocktail (Roche).
4. Purification of Lb-Tec2 may also be performed using 50 mM HEPES/NaOH pH 7.5 or 100 mM HEPES/NaOH pH 7.5, 150 mM NaCl. The purified protein is also stable in Tris pH 8.0.
5. The concentration of IPTG used for induction can be reduced to 0.1 mM.
6. Cells can also be lysed using a one-shot table top cell disruptor (Constant Systems Ltd.) at 1.9 MPa. In this case, addition of 1 μL of endonuclease such as Benzonase or Denarase at 250 units/μL to the resuspended cell pellet, and incubation for 30 minutes on a rotating wheel is recommended to improve cell lysis and reduce sample viscosity. Alternatively, sonication on ice may be used for cell lysis; long pulses or high energy should be avoided to prevent overheating and denaturation. WCE need to be sonicated before SDS-PAGE gel.
7. Sepharose CL-6B matrix can be replaced by Sepharose 4B (Sigma-Aldrich) for Lb-Tec2 purification. It was successfully used for the purification of the carp fishlectin FEL [9] and Lb-Tec2. Use fresh resin for each purification to avoid saturation. For Sepharose 4B, 5 mL of resin are recommended to purify Lb-Tec2 from 1 L of *E. coli* culture. Resin can be prepacked in C10/10 column and adapted to low or medium pressure chromatography system. Loading should be at low flowrate to increase purification yield since the affinity for the resin is

weak. That is why incubation with the resin and later packing of the resin is recommended.

8. Measurements can also be done in 100 mM HEPES/NaOH pH 7.5 supplemented with 150 mM NaCl without influence on the affinity as described in [6]. Use the software provided by the manufacturer to analyze data. Origin 5.0 or older versions are commonly used as analysis software for data measured on ITC200. New Microcal PEAK-ITC (Malvern Panalytical) may also be used in combination with its associated analysis software. For complexed titration and bias free thermogram integration, a procedure has been setup using NITPIC, SEDPHAT and GUSI as described in [13]. Since the affinity is low, the titration is done in excess of ligand and one site model is used with the stoichiometry (n) blocked at 6 (number of binding sites available). No sigmoidal curves could be obtained; values for entropy (S) and enthalpy (H) thermodynamic parameters should therefore be pondered accordingly and not considered for c-values below 0.01, ( $c = [\text{protein}]/K_d$ ).
9. It is recommended to prepare a low-density sensor chip (128 resonance units of sugar immobilized). Even with such a chip, the sensorgrams do not permit analysis in the kinetic mode since the association and dissociation rates are very fast and form straight lines (Fig. 3).  $K_d$  was measured according to the affinity fit protocol of the analysis software.
10. Intestinal binding may be evaluated as previously described [14]. For this purpose, L4 staged *C. elegans* are incubated with *E. coli* and TAMRA-Lb-Tec2 in S-basal for 24 h. Worms are then transferred to plates seeded with *E. coli* OP50 for 2 h and screened for TAMRA fluorescence thereafter.
11. Wet blotting using Mini Trans-Blot® Cell system and universal power supply (Bio-Rad Ltd) may alternatively be used for small gels. This system provides better transfer from gel to blot but is slower and consumes more buffer than semi-dry blotting.
12. The bacterial agglutination assay may be performed with soluble protein extracts instead of purified proteins if the respective protein cannot easily be purified. Alternatively, 100 mM HEPES/NaOH pH 7.5, 150 mM NaCl may be used.

## References

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