

## Measurement of the adhesive force between a single *Klebsiella pneumoniae* type 3 fimbria and collagen IV using optical tweezers

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### Abstract

Type 3 fimbriae are important adhesive filaments that assist *Klebsiella pneumoniae* to establish an infection. Different MrkD adhesin variants on the fimbriae are known to display distinct adherence capability for the bacteria to bind extracellular matrix proteins, although the difference has not been determined physically. For this reason, the adhesive force between type 3 fimbriae and collagen IV were measured using optical tweezers. The measured force data displayed a periodic histogram thus Fourier analysis was applied to group it to extract the adhesive force of a single molecular pair. Specifically, we showed that grouping should begin with an offset at the first half of the period. Finally, we first present the adhesive force between each *mrkD*<sub>V2</sub>-, *mrkD*<sub>V3</sub>-, and *mrkD*<sub>V4</sub>-expressed fimbriae and collagen IV is 2.03, 3.79, and 2.87 pN, respectively. This result can be referred to further research on *mrkD* allelic effect on bacteria infection.

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**Keywords:** Adhesive force; Type 3 fimbriae; Grouping offset; *Klebsiella pneumoniae*; Optical tweezers; Fourier analysis

*Klebsiella pneumoniae* is an opportunistic pathogen that causes complicated urinary tract infection, pneumonia, septicemia, and liver abscess in immunocompromised patients [1,2]. The ability of many *K. pneumoniae* clinical isolates to colonize respiratory and urinary epithelia has been attributed to the presence of many adhesive molecules, including type 3 fimbriae [3]. The fimbriae, encoded by a multigene operon, contain the major subunit MrkA that forms the shaft of the fimbriae and MrkD adhesin [4]. On the tip of the fimbriae, MrkD adhesin is responsible

for mediating erythrocyte agglutination in a mannose independent way [5] and could affect biofilm formation of the bacteria [6]. Although the receptor for the adhesin has yet to be identified, in vitro research indicates that bacteria expressing type 3 fimbriae are able to bind to type IV and V collagen, which may be exposed on exfoliated and denuded epithelial surfaces during infections [7].

Our analysis of *K. pneumoniae* clinical isolates from Taiwan has revealed the presence of four *mrkD* alleles, namely *mrkD*<sub>V1</sub> (GenBank Accession No. AY225462), *mrkD*<sub>V2</sub> (GenBank Accession No. AY225463), *mrkD*<sub>V3</sub> (GenBank Accession No. AY225464), and *mrkD*<sub>V4</sub> (GenBank Accession No. AY225465). To investigate the effect of the allelic variation on binding activity of the fimbriae, a type 3

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fimbriae display system carrying, respectively, each of the MrkD adhesin variants was constructed in *Escherichia coli* JM109. The availability of these type 3 fimbriae-producing *E. coli* strains provides a useful mean to address many fundamental questions concerning bacteria–extracellular matrix protein interaction.

The adhesion strength of a fimbria to its target molecules is conventionally determined by incubating the testing bacteria to a substrate such as collagen and counting the number of the bacteria remaining attached to the substrate after several washes [8]. However, because the number of fimbrial filaments varies considerably among different bacterial cells and in different growth conditions, this assay could only determine the average binding activity between a given bacterial population and the target molecules. To determine precisely the direct interacting force between a fimbria and its target molecules, more sophisticated techniques and strategies must be adopted.

Among many techniques that may be used to investigate biomolecule interaction, optical tweezers [9] have been thought to be one of the most effective methods and has been applied successfully in related questions [10–14]. By detaching a biomolecule-coated bead from either another biomolecule-coated surface or cell, the adhesive force of the biomolecular pair is measured. A major problem that is commonly encountered is that the adhesive force magnitude of each biomolecular pair may be different from others, and some of the measured forces could be a result of several molecular pairs. This creates the possibility that a force data measured from a group of molecular pairs displays a continuous and periodic histogram. Thus, Fourier analysis was applied to extract the period of the force elements in the histogram [11]. With the period, the force elements are grouped into several clusters that are related to the number of biomolecular pairs. The mean force in each cluster against the number of biomolecular pairs is plotted, and the slope of the regression line estimates the adhesive force of a single biomolecular pair. However, Fourier analysis provides no information of the grouping offset, which is noticed to affect the estimation of the adhesive force in our preliminary data analysis. Therefore, we modified the method in the grouping of histogram with an offset at the first half of the grouping period from a physics intuition.

In this paper, we report the result of using optical tweezers to measure the interaction force between different MrkD adhesin variants and collagen IV for the first time. We demonstrated that the grouping begins with an offset at the first half of the period by examining the least mean square error (MSE) of the averaged adhesive force as a function of the offset. Finally, the adhesive force between various fimbriae that expressed various *mrkD* alleles and collagen IV is successfully presented.

## Materials and methods

*Preparation of recombinant E. coli displaying type 3 fimbriae of K. pneumoniae.* The type 3 fimbriae of *K. pneumoniae* were displayed on the

surface of *E. coli* JM109, a non-type 3 fimbriae-producing strain, by transformation of a plasmid carrying the complete *mrk* operon including one of the four *mrkD* variants. The plasmids used in the study include *pmrkABCD*<sub>V1F</sub>, *pmrkABCD*<sub>V2F</sub>, *pmrkABCD*<sub>V3F</sub>, and *pmrkABCD*<sub>V4F</sub>. Plasmid *pmrkABC*, which was incapable of producing fimbriae, was used as a negative control. Prior to the adhesive force measurement, the *E. coli* transformants were grown in GCAA medium (with 100 µg/ml ampicillin antibiotics) at 37 °C for 20 h, for an optimal expression of type 3 fimbriae [15].

*Preparation of collagen-coated beads.* Polystyrene beads (Polysciences, Inc. Warrington, PA, USA) of 1 µm in diameter were added into 500 µl PBS (phosphate buffer saline: 137 mM NaCl, 2.7 mM KCl, 10 mM Na<sub>2</sub>HPO<sub>4</sub>, and 2 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.4) containing 10 µg collagen and then incubated at 4 °C for 16 h. The beads were then blocked with the blocking reagent (2% BSA in PBS) at room temperature for 1 h. After blocking, the beads were washed and re-suspended in 100 ml PBS. BSA-coated beads were also prepared similarly, with the replacement of the collagen to BSA solution.

*Optical tweezers.* A diode pumped solid-state laser (1 W, 1064 nm, IRCL-1064-1000-L, CrystaLaser, USA) was used as the light source of the optical tweezers. Through a 12× beam expander, the laser beam was guided into an inverted microscope (DMIRB, Leica, Germany) and focused on the sample by an objective lens (100× oil, NA = 1.25, N PLAN, Leica, Germany). The specimen holder of the sample was mounted on a PZT stage (Tritor 100 CAP, piezosystem Jena, Germany) with a minimum step size of 1.2 nm and a maximum travel range of 80 µm during its closed operation. When a bead was trapped by the optical tweezers, the forward scattered laser light was collected by using a condenser (20×, NA = 0.5, Plan Fluor, Nikon, Japan). In addition, the forward scattering pattern of the collected light on the back focal plane of the condenser was imaged on a quadrant photodiode (QPD, G6849, Hamamatsu, Japan) by a lens (*f* = 50 mm). The voltage signals output from the QPD were processed by a preamplifier and then a main amplifier (Öffner, MSR-Technik, Germany) with a maximum amplification factor of 500 and a cut-off frequency of 1 MHz. Subsequently, a DAQ card (NI-6115, National Instrument, USA) was used to record the voltage signals with a sampling rate up to 10 MHz for a simultaneous acquisition of four channels. A bright-field illumination was achieved by using a halogen lamp through a lens and the condenser. The images were taken by using a CCD camera (12v1E, Mintron, Taiwan) with a filter blocking the unwanted laser light.

The trapping force was calculated by multiplying the trapping stiffness and the displacement of a trapped bead. The stiffness of the optical tweezers was calibrated by using power spectrum method [16]. The displacement of the bead was derived from the signal of QPD. Since the unit of the QPD signal was in voltage, a conversion parameter of the QPD signal to the bead's displacement was also calibrated by using the power spectrum method [16]. The stiffness and the conversion parameter of each bead were calibrated in advanced to each adhesive force measurement.

*Force measurement using optical tweezers.* The bacteria with type 3 fimbriae were adhered and fixed on a coverslip for 30 min. A collagen IV-coated bead was trapped still by the optical tweezers. Then, the coverslip was shifted by using the PZT stage so that one of the bacteria upon it can be moved toward the trapped bead. Once the bacterium contacted the collagen-coated bead, the coverslip was shifted immediately to detach the bacterium from the bead. During the detachment, the optical tweezers exerts a trapping force balance to the adhesive force and the position of the bead is displaced. The farther the displacement of the trapped bead, the larger the trapping force on the bead. Then, once the trapping force was larger than the adhesive force, the trapped bead was pulled away from the bacterium.

In our experiment, every single adhesive event was measured by moving a bacterium in contact with a trapped bead from the bacterium's side. The contact time was about one second. The velocity of the stage was 1 µm/s during the entire measuring process. A typical record of the bead's displacement during the measurement is illustrated in Fig. 1. In Fig. 1, the position of the bead along with time exhibits a triangle-like shape. The peak of the triangle indicates when the trapping force is just sufficient to

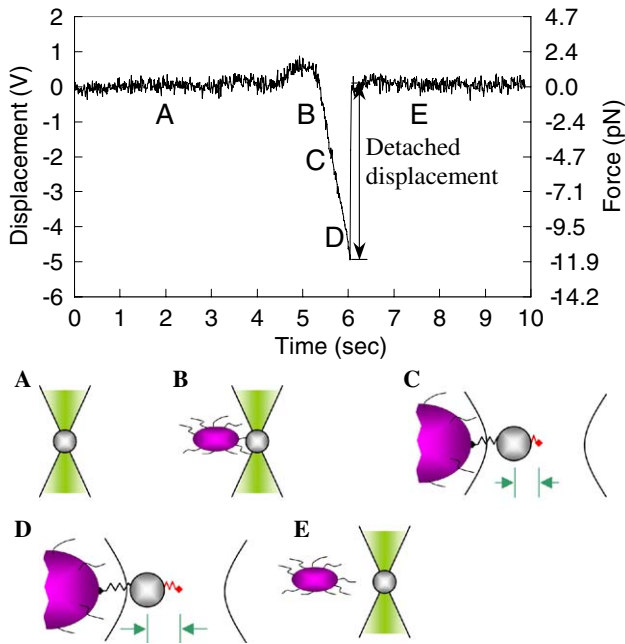


Fig. 1. A schematic presentation describing the adhesive force measurement. (A) The trapped bead is under a confined Brownian motion. (B) The bead is adhered on a single fimbria. The rise in force is due to over push of the bacterium on the bead. (C) and (D) The bead is being pulled away from the trapping center. The farther the displacement of the trapped bead to the trapping center, the larger the trapping force on the bead. (E) The trapping force is larger than the adhesive force so that the bead is detached from the bacterium and trapped back to the trapping center. The QPD signal is the same as the one in (A). The data is sampled with 10 kHz, and averaged by each 100 sample points. The left vertical axis represents the bead's displacement recorded by the QPD (units in voltage). The right vertical axis represents the resulting adhesive force (units in pico-Newton). The adhesive force is calculated by multiplying the displacement when the bead is detached from the bacterium (in V), the conversion parameter (in nm/V), and the stiffness of the optical tweezers (in N/m).

detach the bead from the bacterium. The adhesive force is obtained accordingly.

**Data analysis.** The measured force data were plotted in a histogram. It was thought that the adhesive force could be a result from the formation of multiple fimbria–collagen pairs, thus the force elements in the histogram displayed into a series of clusters. The series of clusters were distributed periodically because the mean force associated with each cluster was an integer multiple of the mean force of the lowest cluster. Thus, Fourier analysis was applied to extract the period to group the force elements in the histogram. The analysis was presented via a periodogram as described [11]. A noticeable peak in the periodogram would directly indicate the initial guess of the period. However, it was noticed that Fourier analysis provided no information of the grouping offset. Without any available method, we thought from the nature of a periodic histogram. With a physics intuition, the mean forces of the first, second, ... clusters in a periodic histogram should represent the adhesive forces of single, double, ... pairs, respectively. For a histogram with a period of  $T$ , grouping with an offset of  $1/2 T$  led to a grouping of  $1/2 T \sim 3/2 T$ ,  $3/2 T \sim 5/2 T$ , and ... etc. The mean force of each cluster thus approximated to  $T$ ,  $2 T$ ,  $3 T$ , ... , which exactly represented the adhesive force of single, double, triple, ... fimbria–collagen pairs. Therefore, the force elements were grouped with an offset at the first half of the period. Finally, the force elements were grouped according to a period and a resulting offset extracted from Fourier analysis. The sequence of the clusters corresponded with the number of fimbria–collagen pairs. The mean force in each cluster against the number of pairs was plotted, and a regression line

was fitted. The slope of the regression line then indicated the adhesive force of a single fimbria–collagen pair.

## Results

The histograms of successful fimbriae–collagen adhesion events are shown in the left column of Fig. 2. The bin width in the histograms was set at 0.1 pN because our force measurement resolution was slightly below this level. The maximal force element was set at 20 pN to include all our data points. A slight different setting of the bin width and the maximal force element did not affect the result of Fourier analysis. The periodograms are shown in the right column of Fig. 2. From the periodogram, the period to group the histogram was extracted. Accordingly, the force histogram was grouped with an offset at the first half of the period. At last, plots of the mean force of each cluster against the number of fimbria–collagen pairs are shown in Fig. 3. The adhesive force between each single fimbria and collagen IV was obtained from the slopes of the regression lines in Fig. 3. The data of *mrkD<sub>v1</sub>*-expressed fimbriae is not shown because they exhibited weak adhesion in both optical tweezers experiments and collagen-binding assay (data not shown).

The control strain *E. coli* JM109 [pmrkABC] displayed no fimbriae on its surface, and adhered poorly to the bead coated with 2% bovine serum albumin (BSA; data shown in supplemental material). JM109 [pmrkABC] also exhibited poor adhesion with collagen IV in collagen-binding assay (data shown in supplemental material). These results illustrate that our adhesive events were indeed come from the interaction between the fimbriae and collagen IV.

Our periodograms, unlike those in Ref. [11], reveal multiple peaks that make it difficult to determine the period to group the histogram. To select proper peaks in our periodograms to group the histograms, there are two foregoing constraints. First, all adhesive elements should be included in the grouping since they were all effective. Second, the grouping should begin with an offset at the first half of the period.

In Fig. 2(D), the periodogram of *mrkD<sub>v2</sub>* displays a noticeable peak at 2 pN, which leads to an offset at 1 pN. The selected period and offset make the grouping includes all the force elements in the data of *mrkD<sub>v2</sub>*. In Fig. 2(E), the periodogram of *mrkD<sub>v3</sub>* shows two noticeable peaks at 4 and 10 pN. However, the peak at 10 pN leads to an offset at 5 pN, in which these grouping parameters do not make the grouping include all the force elements. Consequently, the period and the offset to group the force elements in the data of *mrkD<sub>v3</sub>* are selected as 4 and 2 pN, respectively. In Fig. 2(F), the periodogram of *mrkD<sub>v4</sub>*, the noticeable peak at 5 pN is not selected due to the same reason that it does not satisfy the constraints. The top at 2.9 pN thus becomes the selected grouping period with and resulting offset at 1.45 pN.

Accordingly, the histograms of *mrkD<sub>v2</sub>*, *mrkD<sub>v3</sub>*, and *mrkD<sub>v4</sub>* were grouped into 6, 5, and 7 clusters, respectively. The adhesive force shows a satisfactory linear relation to

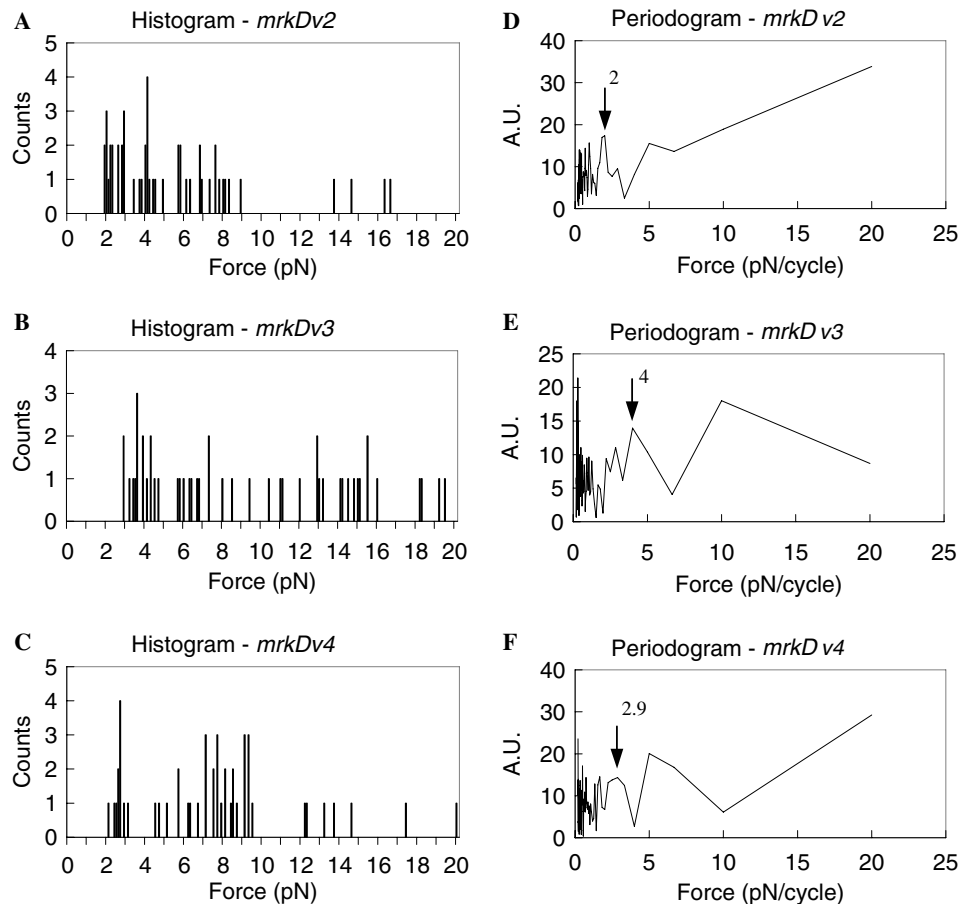


Fig. 2. The histograms (A–C) and the periodograms (D–F) of *mrkD<sub>v2</sub>*-, *mrkD<sub>v3</sub>*-, and *mrkD<sub>v4</sub>*-expressed fimbriae adhered to collagen IV. The numbers of experimental events are 51, 48 and 53, respectively.

the number of fimbriae–collagen pairs, as indicated in Fig. 3. In Fig. 3(A), the means  $\pm$  standard deviations of the data points are  $2.33 \pm 0.38$ ,  $4.06 \pm 0.39$ ,  $6.16 \pm 0.5$ ,  $7.90 \pm 0.5$ ,  $14.10 \pm 0.58$ , and  $16.41 \pm 0.2$  pN, respectively. In Fig. 3(B) the means  $\pm$  standard deviations of the data points are  $4.06 \pm 0.95$ ,  $7.38 \pm 1.04$ ,  $12.01 \pm 1.11$ ,  $14.92 \pm 0.64$ , and  $18.75 \pm 0.66$  pN. In Fig. 3(c), the means  $\pm$  standard deviations of the data points are  $2.64 \pm 0.27$ ,  $5.92 \pm 0.90$ ,  $8.39 \pm 0.69$ ,  $12.22 \pm 0.04$ ,  $13.78 \pm 0.73$ ,  $17.37$ , and  $19.95$  pN. The estimated adhesive force  $\pm$  error is the slope  $\pm$  the mean squared error (MSE) of the regression line. As a result, we present for the first time the adhesive force between collagen IV and each of the type 3 fimbriae, which expressed with various *mrkD* alleles, *mrkD<sub>v2</sub>*, *mrkD<sub>v3</sub>*, or *mrkD<sub>v4</sub>* are  $2.03 \pm 0.03$  pN,  $3.79 \pm 0.12$  pN, and  $2.87 \pm 0.15$  pN, respectively. It is shown that the *mrkD<sub>v3</sub>*-containing fimbriae exhibited the largest adhesive force on collagen IV whereas *mrkD<sub>v1</sub>*-containing fimbriae showed almost no adhesion.

## Discussions

In this study, we proposed that the grouping of a histogram begins with an offset of  $1/2$  T in data analysis.

To verify this, the MSE of the regression line against offset is presented as shown in Fig. 4. The most reasonable offset in terms of precise force estimation should occur at the least MSE. As shown in Fig. 4, the least MSE occurs at the offset of  $1/2$  T in both curves for *mrkD<sub>v2</sub>*- and *mrkD<sub>v3</sub>*-carried fimbriae. As for the curve of the *mrkD<sub>v4</sub>*-carried fimbriae, although the least MSE occurs at an offset of  $3/10$  T, the MSE at an offset of  $1/2$  T is still relative low. We thus conclude that the most reasonable offset occurs  $1/2$  T. Further, the largest difference of the adhesive force was about 27% in terms of different applying offset in our experiment (data shown in supplementary material).

In our experience, longer contact periods increase the overall adhesive force, presumably due to the formation of multiple fimbria–collagen pairs. In this experiment, the contact period of the fimbriae and collagen was kept short to ensure fewer fimbria–collagen pairs formation. Thus, the histograms shown in Fig. 2 were grouped only into several clusters. Otherwise a peak at short period in terms of grouping into more clusters would be selected. Correspondingly, our data analysis processes as well as the selected peaks shown in Fig. 2 were reasonable.

The measurement of adhesion force in several other bacteria have been reported previously [10–14] and the

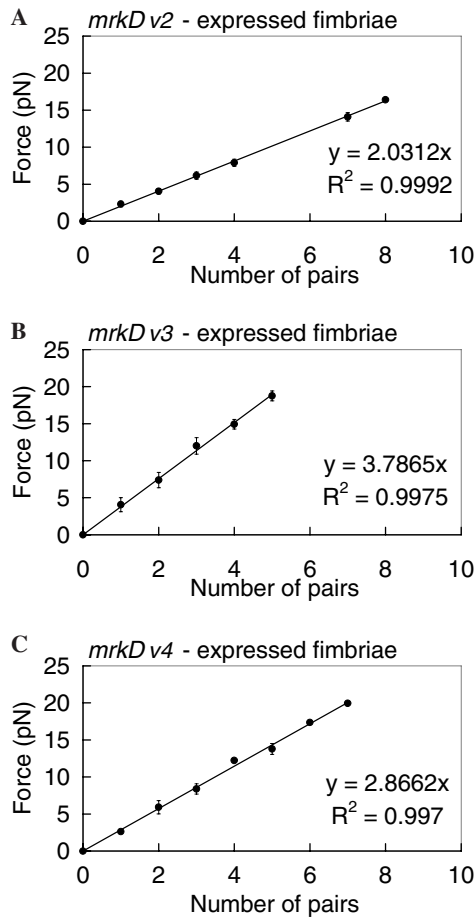


Fig. 3. The adhesive force against the number of (A) *mrkD*<sub>v2</sub>, (B) *mrkD*<sub>v3</sub>, (C) *mrkD*<sub>v4</sub>-expressed fimbriae adhered to collagen IV.

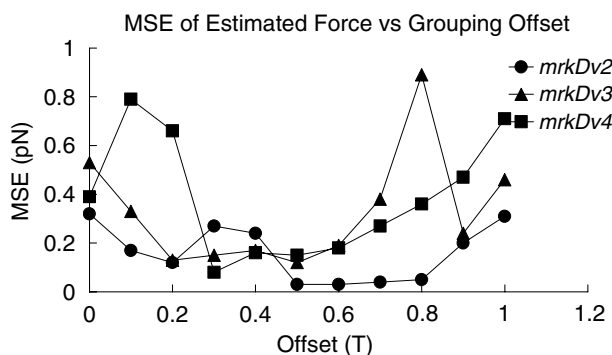


Fig. 4. The mean square error (MSE) against the grouping offset.

magnitude of the interacting force varies significantly depending on the type of molecular pairs. In the case of *Staphylococcus* sp. [11–13], the adhesive force measured between extracellular matrix (ECM) molecules fibronectin and a series of *Staphylococcus* strains is in the range of 16–25 pN. Unlike fimbriae in Gram-negative bacteria that extend out from the cell, the protein mediating the adhesion of the staphylococcal cells to fibronectin is covalently anchored on the bacterial cell wall and spread all over the surface. Thus, the adhesion forces measured in these studies are

surely the sum of multiple components on the bacterial surface and therefore are much larger than that of our results. Another study that is more related to ours was the measurement of interacting force between a single type 1 pilus and an  $\alpha$ -C-mannoside ligand [10]. The adhesive force obtained in the study was 1.7 pN, which is close to our data of the adhesive force between type 3 fimbriae and collagen IV.

In summary, by using a set of high resolution optical tweezers, we have demonstrated that the sequence variation of the adhesins could affect their binding force to the receptor. Although the biological meaning of the phenomenon remains to be investigated, the information can be referred to further research on the connection between adhesion and infection of bacteria such as how bacteria sustain the shearing force in gastric and urinary systems. In data analysis, we verified that the grouping should begin with an offset at the middle of the first period by selecting the least MSE of the regression line. The adhesive force measurements are reliable because the errors of the measured forces are in the range of the system resolution. Finally, as slight differences between the collagen binding forces exerted by different MrkD adhesin variants could be distinguished, we believe that our force measurements are reliable and have a high resolution. Thus, the optical tweezers and the analytical process set-up in our laboratory can also be applied to study other biomechanical processes that involve subtle differences.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.bbrc.2006.08.190](https://doi.org/10.1016/j.bbrc.2006.08.190).

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