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# 1 **Specific interaction between DSPHTELP peptide and various functional groups**

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19



## 1 Abstract

2 M13 bacteriophages serve as a versatile foundation for nanobiotechnology due to their  
3 unique biological and chemical properties. The polypeptides that comprise their coat proteins,  
4 specifically pVIII, can be precisely tailored through genetic engineering. This enables the  
5 customized integration of various functional elements through specific interactions, leading to  
6 the development of innovative hybrid materials for applications such as energy storage,  
7 biosensing, and catalysis. Notably, a certain genetically engineered M13 bacteriophage variant,  
8 referred to as DSPH, features a pVIII with a repeating DSPHTELP peptide sequence. This  
9 sequence facilitates specific adhesion to single-walled carbon nanotubes (SWCNTs), primarily  
10 through  $\pi$ - $\pi$  and hydrophobic interactions, though the exact mechanism remains unconfirmed.  
11 In this study, we synthesized the DSPHTELP peptide (an 8-mer peptide) and analyzed its  
12 interaction forces with different functional groups across various pH levels using a Surface  
13 Forces Apparatus (SFA). Our findings indicate that the 8-mer peptide binds most strongly to  
14  $\text{CH}_3$  groups ( $W_{\text{ad}} = 13.74 \pm 1.04 \text{ mJ m}^{-2}$  at pH 3.0), suggesting that hydrophobic interactions  
15 are indeed the predominant mechanism. These insights offer both quantitative and qualitative  
16 understanding of the molecular interaction mechanisms of the 8-mer peptide and clarify the  
17 basis of its specific interaction with SWCNTs through the DSPHTELP M13 bacteriophage.

18



## 1 Introduction

2 The M13 bacteriophage, belonging to the filamentous bacteriophage family, is characterized  
3 by approximately 2,700 copies of the major coat protein (pVIII) and is terminated at each end  
4 with about five pairs of four distinct minor proteins (pIII, pVI, pVII, and pIX).<sup>1,2</sup> Owing to its  
5 biocompatibility, efficient production, target-specific reactivity, high surface-to-volume ratio,  
6 and flexibility, the M13 bacteriophage stands out as an ideal candidate for assembling and  
7 fabricating a variety of functional nanomaterials.<sup>3-5</sup> Notably, phage display technology enables  
8 the facile and versatile modification of the M13 phage to bind selectively to target substances,  
9 facilitating the creation of tailor-made functional nanoparticles for epitope mapping, naive  
10 binding peptide selection, and drug discovery.<sup>6-11</sup>

11 Due to these advantages, researchers have employed the M13 bacteriophage in a wide array  
12 of applications, including rechargeable batteries that integrate peptides for binding active  
13 materials,<sup>12-15</sup> extracellular matrix-based cell platforms,<sup>16-19</sup> piezoelectric devices,<sup>20</sup> sensors<sup>21-</sup>  
14 <sup>27</sup> and etc. Specifically, DSPH phages within the M13 bacteriophage, characterized by a  
15 repeating DSPHTELP sequence in the pVIII coat proteins, have shown high specificity in  
16 adhering to single-walled carbon nanotubes (SWCNTs). This specificity has been harnessed  
17 for highly efficient applications in dye-sensitized solar cells (DSSC),<sup>28</sup> sodium,<sup>29</sup> and lithium-  
18 ion batteries,<sup>30</sup> and second near-infrared (NIR) window fluorescence imaging.<sup>31</sup> Despite the  
19 broad utility, the precise adhesion mechanisms, which is critical to the success of these  
20 applications, largely remain speculative, with a clear understanding of the biomolecular  
21 interaction mechanisms posing a challenge for the systematic design of efficient target material  
22 binding. Therefore, we investigated the interaction between the DSPHTELP peptide and  
23 various functional groups to understand the adhesion mechanism of this specific sequence and  
24 analyze the similarity and the difference against the actual DSPH phage's adhesion mechanism.



1 The poor understanding is attributed to the intricate nature of biomolecule interaction  
2 mechanisms, which may involve a complex interplay of hydrogen bonding, van der Waals  
3 forces, electrostatic interactions, steric repulsion, hydration forces, cation- $\pi$ , and hydrophobic  
4 interactions.<sup>32</sup> In recent studies utilizing a Surface Forces Apparatus (SFA), we explored the  
5 interactions between the M13 bacteriophage (specifically DSPHTELP-peptide) and various  
6 functionalized self-assembled monolayers (SAMs) to shed light on the interaction mechanisms,  
7 identifying  $\pi$  and hydrophobic interactions as predominant.<sup>33</sup> However, definitive conclusions  
8 were elusive due to the potential involvement of other coat proteins in the adhesion process  
9 and significant phage swelling at higher pH levels ( $\sim 8.5$ ), which emphasized steric over other  
10 interactions.

11 To mitigate these uncertainties observed in our previous work, this study synthesizes and  
12 examines the interactions of only the DSPHTELP-peptide (an 8-mer peptide mirroring the  
13 sequence of the pVIII protein, but devoid of other bacteriophage biomolecules such as other  
14 coat proteins and nucleotides). We analyzed the interactions between this peptide and four  
15 differently functionalized self-assembled monolayers (SAMs) — carboxylic (-COOH), amine  
16 (-NH<sub>2</sub>), methyl (-CH<sub>3</sub>), and phenyl (-C<sub>6</sub>H<sub>5</sub>) — using an SFA. Our findings reveal the peptide's  
17 strongest adhesion to CH<sub>3</sub>-SAMs, underscoring the pivotal role of hydrophobic interactions in  
18 molecular binding processes.

## 20 **Experimental Methods**

### 21 **Materials**

22 All the amino acids (Aspartic Acid; **D**, Serine; **S**, Proline; **P**, Histidine; **H**, Threonine; **T**,  
23 Glutamic Acid; **E**, Leucine; **L**) required for the peptide synthesis and O-(Benzotriazol-1-yl)-



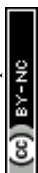
1 *N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) were purchased from Apex Bio,  
2 Houston and Chem-Impex, Chicago. Piperidine and diisopropyl ethyl amine were purchased  
3 from Alfa Aesar. Dimethylformamide was purchased from Thermo Fisher Scientific. 3-  
4 (triethoxysilyl)propyl isocyanate, 10-carboxy-1-decanethiol (95%) for COOH-SAM, 11-  
5 amino-1-undecanethiol hydrochloride (99%) for NH<sub>2</sub>-SAM, 1-undecanethiol (98%) for CH<sub>3</sub>-  
6 SAM and 2-phenylethanethiol (98%) for C<sub>6</sub>H<sub>5</sub>-SAM were purchased from Sigma-Aldrich.

### 8 **Synthesis of DSPHTELP-Peptide**

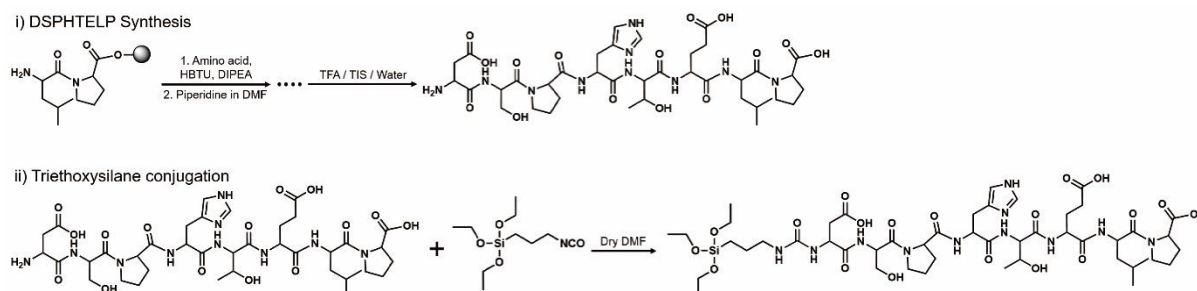
9 The peptide was synthesized using the standard Fmoc-(9-fluorenylmethoxycarbonyl) solid-  
10 phase peptide synthesis (SPPS) method, scaled to 0.106 mmol. Rink amide MBHA resin was  
11 used. The Fmoc on the resin and each amino acid were removed by a DMF/piperidine mixture.  
12 In each step of synthesis Fmoc-protected amino acid was added using *O*-(Benzotriazol-1-yl)-  
13 *N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) and diisopropyl ethyl amine  
14 (DIPEA) as a coupling agent. Finally, the peptide was cleaved from the resin using cleavage  
15 cocktail (TFA/Triisopropylsilane/Deionized water mixture (9.5: 0.5: 0.5)) and products will be  
16 precipitated in cold ether (**Fig. 1**). Precipitates were purified using high-performance liquid  
17 chromatography (HPLC, Agilent Technologies, USA) with a C18 reverse column in  
18 Acetonitrile/water mixture (**Fig. S1**).

### 20 **DSPHTELP-Peptide Conjugation with Triethoxysilane**

21 Purified DSPHTELP peptide (20 mg, 0.0224 mmol) was dissolved in anhydrous DMF (1  
22 mg/mL). Then, 3-(triethoxysilyl)propyl isocyanate (6 mg, 0.0224 mmol) was added in to the  
23 solution. After stirring at room temperature for overnight, crude products were purified by size



1 exclusion chromatography. The synthesis of peptides will be confirmed with Matrix-Assisted  
2 Laser Desorption/Ionization (MALDI-TOF/TOF, Ultraflex III) as depicted in **Fig. S2**.



5 **Fig. 1.** Schematic representation for the synthesis of 8-mer peptides. 8-mer peptides were  
6 synthesized by the solid-phase peptide synthesis as a 0.106 mmol scale. The N-terminal of  
7 synthesized amino acid backbones was conjugated with a triethoxysilane in anhydrous DMF  
8 solvent.

### 10 Preparation of DSPHTELP-Peptide Surface on Mica

11 A cleaved clean mica (Grade #1, S&J Trading, Floral Park, NY, USA) with a back-silver  
12 coating of approximately 50 nm thickness was adhered to a cylindrical disk using UV glue for  
13 50 minutes. The back-silvered mica attached to the disk was treated with O<sub>2</sub> plasma at 100W  
14 for 3 minutes.<sup>34</sup>

15 The synthesized triethoxysilane-DSPHTELP peptide solution (100 μL) in DMF solvent (1  
16 mg/mL) was diluted in 20 mL of DMF. The 50 μL of peptide solution was dropped around the  
17 treated mica immersed in the 20 mL of DMF solvent at 700 rpm. After 1 hour, weakly bound  
18 peptides were removed using DMF solvent and dried with nitrogen gas. The evenly coated  
19 peptide surface was confirmed via Veeco multimode V<sub>AFM</sub> in standard tapping mode (**Fig.**  
20 **S3**).



## 1 **Preparation of Self-Assembled Monolayer (SAMs) Films with Different End-Functional**

### 2 **Groups**

3 Surfaces with four different functional groups were prepared by using alkanethiol  
4 monolayers deposited on atomically smooth gold surfaces. Initially, the smooth gold surface  
5 (~45 nm in thickness) was prepared through electron-beam evaporation on cleaved clean mica  
6 (Grade #1, S&J Trading, Floral Park, NY, USA). Subsequently, the gold-coated surface was  
7 affixed to a cylindrical glass disk (Radius,  $R \sim 2$  cm) using UV glue (NOA 81, Norland Products,  
8 NJ, USA), gold surface down, and allowed to cure for 50 minutes.<sup>35, 36</sup> The mica was gently  
9 peeled off in ethanol solution glued surface, leaving behind atomically smooth gold surface.  
10 The gold-coated disk was then immersed in a 1 mM alkanethiol-ethanol solution for 18 hours.  
11 The strong gold-sulfur interaction facilitated the effective binding of each self-assembled  
12 monolayer (SAM) to the gold surface.<sup>33, 37, 38</sup> To eliminate excess SAM molecules, a low-  
13 intensity sonication was applied for 30 seconds, followed by washing with ethanol and nitrogen  
14 gas drying.<sup>39</sup>

### 16 **Measurement of Interaction Forces Using an SFA**

17 The interaction between the 8-mer peptide and each functionalized SAM was measured  
18 using a surface forces apparatus (SFA2000, Surforce LLC, Santa Barbara, USA). The SFA is  
19 an instrument that directly measures the absolute distance and interaction forces between two  
20 surfaces with resolutions of 0.1 nm and 10 nN, respectively.<sup>34</sup> The SFA offers the advantage  
21 of measuring a wide range of forces, and through a careful analysis of the force-distance curves,  
22 it can provide valuable insights on the interaction mechanism. Consequently, the SFA has been  
23 effectively utilized to quantify the interaction forces between various surfaces coated with  
24 proteins<sup>40</sup>, lipid bilayers<sup>41</sup>, biopolymers<sup>42-45</sup>, and supramolecules<sup>46</sup>.

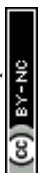


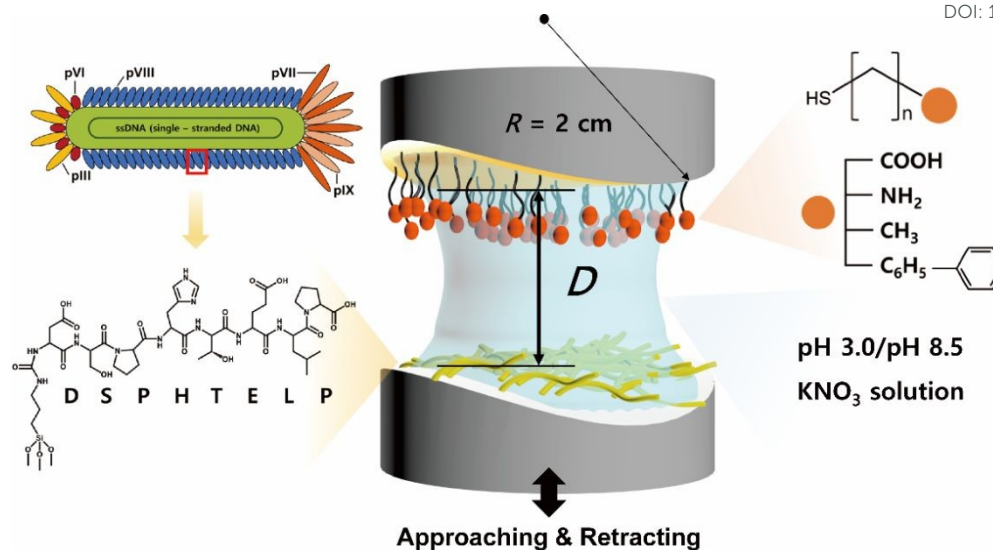


1 The disk coated with SAM was mounted at the top of the chamber, while the disk coated  
2 with peptide was mounted at the bottom in a cross-cylindrical geometry. 60  $\mu\text{L}$  of filtered buffer  
3 solution (pH 3.0 or pH 8.5  $\text{KNO}_3$ ) was injected between the surfaces and equilibrated for 30  
4 minutes (**Fig. 2**). Two surfaces are brought into contact by using a fine-control motor at an  
5 approach velocity of  $\sim 5$  nm/s. Then the short (5 sec) or long (1 hr) contact time ( $t_c$ ) was allowed  
6 to check the molecular rearrangement during the contact, followed by separation of two  
7 surfaces at a separation velocity of  $\sim 5$  nm/s.

8 The absolute distance ( $D$ ) between two surfaces was determined using multiple beam  
9 interferometry (MBI) by observing the fringes of equal chromatic order (FECO).<sup>47</sup> The  
10 interaction forces ( $F$ ) were measured by observing the deflection of the lower surface's double  
11 cantilevered spring with a spring constant of 2451.7 N/m. The adhesion force,  $F_{\text{ad}}$ , was defined  
12 as the absolute value of the lowest  $F$ , before jump-out, where  $R$  is the radius of the cylindrical  
13 disk, typically around 2 cm.<sup>42, 48</sup> The  $F_{\text{ad}}$  was converted into interaction energy per unit of  
14 contact area ( $W_{\text{ad}} = 2F_{\text{ad}}/3\pi R$ ) according to the Johnson-Kendall-Roberts model<sup>49</sup> which is  
15 typically applied to soft materials with significant deformations. All experiments were  
16 conducted at room temperature ( $T \sim 23$  °C) and repeated at least three times.

17





1

2 **Fig. 2.** Schematic of surface forces apparatus (SFA) for measuring interaction forces between  
 3 8-mer peptide layer (bottom surface) and four different alkanethiols SAM layers (top surface)  
 4 at various conditions.

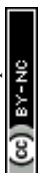
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## 6 Results and discussion

### 7 Interaction Force Measurements between DSPHTELP-Peptide and SAMs

8 The binding constant,  $K_a$ , is generally used to quantitatively compare the binding between  
 9 two molecules<sup>50</sup>, and can be measured using Surface Plasmon Resonance (SPR) or Isothermal  
 10 Titration Calorimetry (ITC). However, the goal of this study is to evaluate the “adhesion energy  
 11 per unit area” of the 8-mer peptide to surfaces with various functional groups and compare it  
 12 with our previous work<sup>33</sup>, which was conducted using a Surface Forces Apparatus (SFA).

13 We utilized the SFA to measure the force-distance profiles between a synthesized 8-mer  
 14 peptide and four types of functionalized SAMs at two pH levels: low (pH 3.0) and high (pH  
 15 8.5). The selection of SAMs with distinct terminal functional groups allows for the exploration  
 16 of various interaction forces (e.g., hydrogen bonding,  $\pi$ - $\pi$ , cation- $\pi$ , hydrophobic, and  
 17 electrostatic interactions) which are pivotal in understanding the material interaction



1 mechanisms.<sup>33, 45, 51, 52</sup>

2 The charge states of both the SAMs and the peptide (pI value: ~4.1) are known to vary with  
3 pH, necessitating the analysis at both low and high pH for a holistic view of the interaction  
4 mechanisms. Notably, the charge variations of amino acids within the peptide, influenced by  
5 pH changes, are critical to this analysis.<sup>53-55</sup> Among the seven amino acids present in the 8-  
6 mer peptide, aspartic acid (**D**), glutamic acid (**E**), and histidine (**H**) exhibit ionizable  
7 characteristics, which lead to fluctuations in charge dependent on the pH environment. As  
8 depicted in **Fig. 3f** and **4f**, **H** ( $pK_a \sim 6.00$ ) carries a positive charge at pH 3.0, while it becomes  
9 neutral at pH 8.5. On the other hand, **D** ( $pK_a \sim 3.65$ ) and **E** ( $pK_a \sim 4.25$ ) are neutral at pH 3.0  
10 but carry a negative charge at pH 8.5. Proline (**P**,  $pK_a \sim 1.99$ ) retains a negative charge on its  
11 carboxyl group at both pH 3.0 and pH 8.5.<sup>33</sup>

12 Furthermore, to consider the potential molecular rearrangement of peptides upon contact  
13 with SAM surfaces, we introduced both short and long contact times ( $t_c$ ). This approach is  
14 based on the hypothesis that molecules at the interface may reorganize into more energetically  
15 favorable configurations, a phenomenon observed as an increase in adhesion energy with  
16 prolonged  $t_c$ <sup>33, 56</sup>.

17 **Figure 3** and **Figure 4** illustrate the approaching and retracting force-distance profiles of the  
18 peptide and SAMs at pH 3.0 and 8.5, respectively. At pH 3.0, the peptide is overall neutral  
19 carrying one positive and one negative charge (**Fig. 3f**). The measured adhesion energies are  
20 reported as follows (**Table 1**): COOH-SAM ( $W_{ad} = 0.33 \pm 0.12 \text{ mJ m}^{-2}$  at  $t_c = 5 \text{ s}$ ,  $W_{ad} = 0.50 \pm$   
21  $0.19 \text{ mJ m}^{-2}$  at  $t_c = 1 \text{ h}$ ); NH<sub>2</sub>-SAM ( $W_{ad} = 4.78 \pm 0.60 \text{ mJ m}^{-2}$  at  $t_c = 5 \text{ s}$ ,  $W_{ad} = 6.72 \pm 0.91 \text{ mJ}$   
22  $\text{m}^{-2}$  at  $t_c = 1 \text{ h}$ ); CH<sub>3</sub>-SAM ( $W_{ad} = 11.79 \pm 0.42 \text{ mJ m}^{-2}$  at  $t_c = 5 \text{ s}$ ,  $W_{ad} = 13.74 \pm 1.04 \text{ mJ m}^{-2}$  at  
23  $t_c = 1 \text{ h}$ ); C<sub>6</sub>H<sub>5</sub>-SAM ( $W_{ad} = 4.34 \pm 0.20 \text{ mJ m}^{-2}$  at  $t_c = 5 \text{ s}$ ,  $W_{ad} = 5.84 \pm 0.48 \text{ mJ m}^{-2}$  at  $t_c = 1$   
24  $\text{h}$ ). Based on these results, the adhesion energy at pH 3.0 was the highest against CH<sub>3</sub>-SAM,  
25 followed by NH<sub>2</sub>-, C<sub>6</sub>H<sub>5</sub>-, and COOH- SAMs. Conversely, at pH 8.5 (**Fig. 4f**), where the

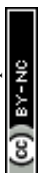


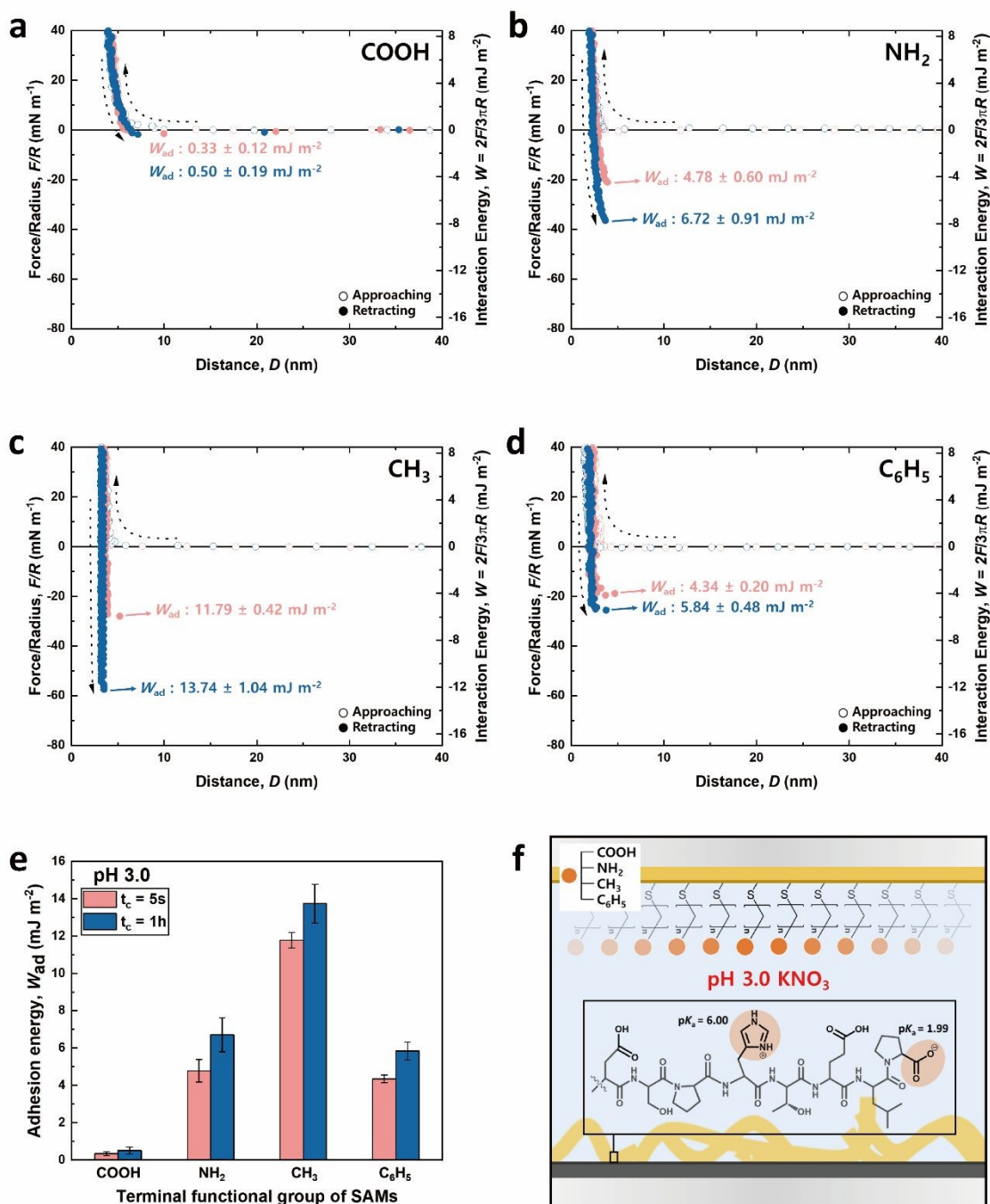
1 peptide is negatively charged, the adhesion energy decreased across all SAMs compared to pH  
 2 3.0, with a similar preference order, showing the strongest adhesion with CH<sub>3</sub>-SAM and the  
 3 weakest with COOH-SAMs. The adhesion energies at pH 8.5 are detailed as follows (**Table**  
 4 **1**): COOH-SAM ( $W_{\text{ad}} = 0.08 \pm 0.04 \text{ mJ m}^{-2}$  at  $t_c = 5 \text{ s}$ ,  $W_{\text{ad}} = 0.24 \pm 0.12 \text{ mJ m}^{-2}$  at  $t_c = 1 \text{ h}$ );  
 5 NH<sub>2</sub>-SAM ( $W_{\text{ad}} = 2.43 \pm 0.58 \text{ mJ m}^{-2}$  at  $t_c = 5 \text{ s}$ ,  $W_{\text{ad}} = 5.03 \pm 0.90 \text{ mJ m}^{-2}$  at  $t_c = 1 \text{ h}$ ); CH<sub>3</sub>-  
 6 SAM ( $W_{\text{ad}} = 4.20 \pm 0.70 \text{ mJ m}^{-2}$  at  $t_c = 5 \text{ s}$ ,  $W_{\text{ad}} = 10.94 \pm 1.32 \text{ mJ m}^{-2}$  at  $t_c = 1 \text{ h}$ ); C<sub>6</sub>H<sub>5</sub>-SAM  
 7 ( $W_{\text{ad}} = 0.46 \pm 0.08 \text{ mJ m}^{-2}$  at  $t_c = 5 \text{ s}$ ,  $W_{\text{ad}} = 0.59 \pm 0.20 \text{ mJ m}^{-2}$  at  $t_c = 1 \text{ h}$ ). These results  
 8 highlight the significant influence of pH on the peptide's adhesion energy with different SAMs,  
 9 underscoring the role of charge in mediating these interactions.

11 **Table 1.** Adhesion energies between 8-mer peptide and functionalized SAM surfaces at various  
 12 conditions.

Adhesion energy, $W_{\text{ad}}$ (mJ m <sup>-2</sup> )				
	pH 3.0		pH 8.5	
	5 s	1 h	5 s	1 h
COOH	$0.33 \pm 0.12$	$0.50 \pm 0.19$	$0.08 \pm 0.04$	$0.24 \pm 0.12$
NH <sub>2</sub>	$4.78 \pm 0.60$	$6.72 \pm 0.91$	$2.43 \pm 0.58$	$5.03 \pm 0.90$
CH <sub>3</sub>	$11.79 \pm 0.42$	$13.74 \pm 1.04$	$4.20 \pm 0.70$	$10.94 \pm 1.32$
C <sub>6</sub> H <sub>5</sub>	$4.34 \pm 0.20$	$5.84 \pm 0.48$	$0.46 \pm 0.08$	$0.59 \pm 0.20$

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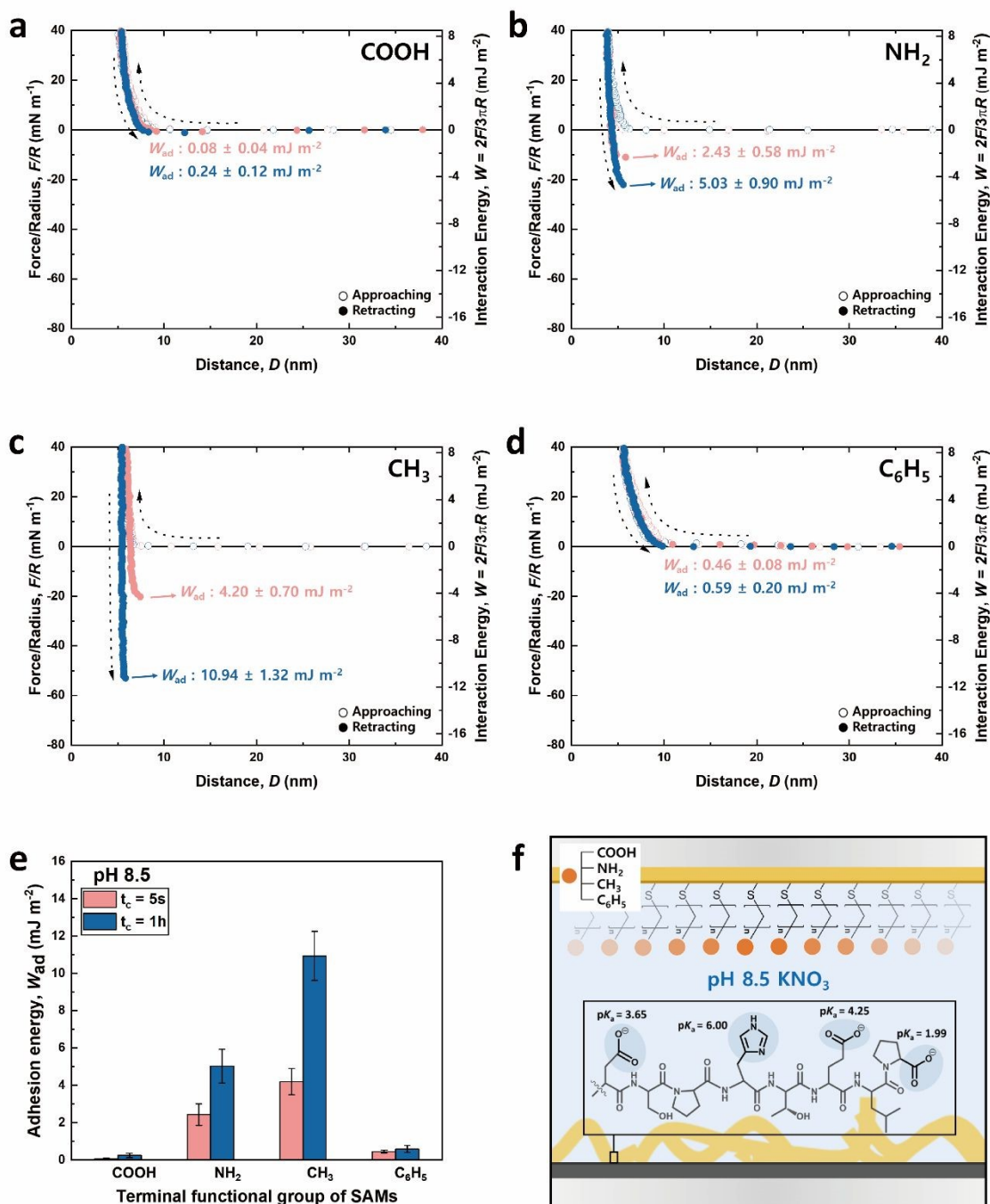


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2 **Fig. 3.** Approaching and retracting force-distance profiles between 8-mer peptide and  
 3 functionalized SAM surfaces at pH 3.0. Four different functionalized SAMs are utilized: (a) -  
 4 COOH, (b) -NH<sub>2</sub>, (c) -CH<sub>3</sub>, (d) -C<sub>6</sub>H<sub>5</sub>. (e) Bar graphs show the adhesion energy of 8-mer  
 5 peptide against four different SAMs as a function of contact time; average ± standard error bars  
 6 ( $n > 3$ ). (f) A peptide schematic showing the charged state of amino acids at pH 3.0.

7





1

2 **Fig. 4.** Approaching and retracting force-distance profiles between 8-mer peptide and  
 3 functionalized SAM surfaces at pH 8.5. Four different functionalized SAMs are utilized: (a) -  
 4 COOH, (b) -NH<sub>2</sub>, (c) -CH<sub>3</sub>, (d) -C<sub>6</sub>H<sub>5</sub>. (e) Bar graphs show the adhesion energy of 8-mer  
 5 peptide against four different SAMs as a function of contact time; average  $\pm$  standard error bars  
 6 ( $n > 3$ ). (f) A peptide schematic showing the charged state of amino acids at pH 8.5.

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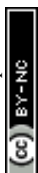


## Interaction Force between DSPHTELP-Peptide and COOH-SAM

At pH 3.0, COOH-SAM ( $pK_a$  value:  $\sim 5.5$ ) exposes COOH without deprotonation, allowing it to function as both hydrogen bond donors and acceptors. This characteristic facilitates hydrogen bonding with the peptide, especially due to the presence of hydrogen bond-capable functional groups in aspartic acid (**D**), serine (**S**), histidine (**H**), threonine (**T**), and glutamic acid (**E**). However, at pH 8.5, COOH-SAM undergoes deprotonation to form  $\text{COO}^-$ , functioning only as hydrogen bond acceptors. Concurrently, the peptide acquires negative charge, predominantly due to the **D**, **E**, and proline (**P**), diminishing the probability of hydrogen bonding and amplifying electrostatic repulsion with the  $\text{COO}^-$  group. Consequently, adhesion to COOH-SAM is reduced at pH 8.5 ( $W_{\text{ad}} = 0.24 \pm 0.12 \text{ mJ m}^{-2}$ ), compared to that at pH 3.0 ( $W_{\text{ad}} = 0.50 \pm 0.19 \text{ mJ m}^{-2}$ ). Nevertheless, as illustrated in **Fig. 3e** and **4e**, COOH-SAM demonstrates the weakest interaction energy among the four SAM types, suggesting that hydrogen bonding plays a minimal role in peptide adhesion.

## Interaction Force between DSPHTELP-Peptide and $\text{NH}_2$ -SAM

At pH 3.0, the terminal  $\text{NH}_2$  group of  $\text{NH}_2$ -SAM, with a  $pK_a$  value around 7.5, predominantly exists in its protonated form ( $\text{NH}_3^+$ ,  $\sim 100\%$ ), serving as a cation source and hydrogen bond donor. Therefore, the protonated amine can engage in cation- $\pi$  interactions with **H**, which acts as a  $\pi$ -source, and hydrogen bonding with **D**, **S**, **H**, **T**, and **E**. Moreover, the negatively charged carboxyl group of **P** ( $pK_a=1.99$ ), can participate in electrostatic attraction with  $\text{NH}_3^+$ . As the pH increases to 8.5, the Henderson-Hasselbalch equation<sup>57, 58</sup> predicts a sharp decline in the proportion of protonated amine (to about 9%), markedly reducing cation- $\pi$  interactions. Nonetheless, the rise in the concentration of unprotonated  $\text{NH}_2$  groups, capable of acting as both hydrogen bond donors and acceptors, possibly fosters enhanced hydrogen bonding with



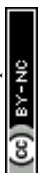
1 the peptide's amino acids. Additionally, due to the presence of negatively charged **D**, **E**, and **P**  
2 residues in the peptide imparts an overall negative charge to it, facilitating electrostatic  
3 interactions. As a result, the adhesion energy was measured as  $W_{\text{ad}} = 6.72 \pm 0.91 \text{ mJ m}^{-2}$  at pH  
4 3.0 and  $W_{\text{ad}} = 5.03 \pm 0.90 \text{ mJ m}^{-2}$  at pH 8.5.

### 6 **Interaction Force between DSPHTELP-Peptide and CH<sub>3</sub>-SAM**

7 CH<sub>3</sub>-SAM (water contact angle  $\sim 101.0 \pm 0.8^\circ$ ) displays the highest hydrophobicity among  
8 the four SAMs tested, making it an optimal candidate for assessing hydrophobic interactions.  
9 In our experiments, CH<sub>3</sub>-SAM showed the most substantial adhesion energy at both pH levels  
10 studied (**Fig. 3e** and **4e**), underscoring the primacy of hydrophobic interactions in binding of  
11 the DSPHTELP peptide. This high level of interaction is attributed mainly to the hydrophobic  
12 nature of **L** with its methyl group, and **P** with its ring structure. Moreover, the adhesion energy  
13 was notably higher at pH 3.0 ( $W_{\text{ad}} = 13.74 \pm 1.04 \text{ mJ m}^{-2}$ ) compared to that at pH 8.5 ( $W_{\text{ad}} =$   
14  $10.94 \pm 1.32 \text{ mJ m}^{-2}$ ). This variance can be attributed to the peptide's charge states at different  
15 pH levels; it is neutral at pH 3.0, enhancing hydrophobic interactions, whereas at pH 8.5, the  
16 peptide carries a negative charge, which slightly diminishes these hydrophobic interactions.  
17 These findings suggest that the synthesized 8-mer peptide, despite containing only three non-  
18 polar amino acids (one **L** and two **Ps**), exhibits strong hydrophobic interactions due to the  
19 presence of hydrophobic moieties. Previous research has also confirmed that even net-  
20 hydrophilic substances can participate in strong hydrophobic interactions if hydrophobic  
21 moieties are present.<sup>45, 59</sup>

22

23





## Interaction Force between DSPHTELP-Peptide and C<sub>6</sub>H<sub>5</sub>-SAM

At low pH, the peptide carries a positive charge due to **H**, thus serving as a source for cation- $\pi$  interactions. This interaction mechanism contributes to a robust adhesion with C<sub>6</sub>H<sub>5</sub>-SAM at pH 3.0 ( $W_{\text{ad}} = 5.84 \pm 0.48 \text{ mJ m}^{-2}$ ). However, at the higher pH of 8.5, while **H** loses its positive charge and becomes neutral, it retains its  $\pi$ -structure, allowing for the possibility of  $\pi$ - $\pi$  interactions. As a result, the adhesion energy at pH 8.5 is markedly lower ( $W_{\text{ad}} = 0.59 \pm 0.20 \text{ mJ m}^{-2}$ ) than pH 3.0. This difference emphasizes the significance of cation- $\pi$  interactions (with the peptide acting as a cation source) and suggests that  $\pi$ - $\pi$  interactions contribute minimally to the overall adhesion energy.

## Effect of contact time on the adhesion energy

Previous studies have commonly observed an increase in adhesion energy with prolonged contact time among biomolecules and polymers.<sup>42, 60-62</sup> Consistent with these findings, our system displayed higher adhesion energies at longer contact times ( $t_c = 1 \text{ h}$ ) compared to a shorter ones ( $t_c = 5 \text{ s}$ ) across both pH levels, 3.0 and pH 8.5. This trend suggests that the peptide undergoes a gradual reorientation or structural rearrangement to optimize its binding affinity towards the SAM surfaces.

Moreover, the rise in adhesion energy attributable to extended contact was more pronounced at pH 8.5 than at pH 3.0. This phenomenon can be attributed to several factors: 1) At the elevated pH, aspartic acid (**D**) and glutamic acid (**E**) acquire a negative charge, inducing electrostatic repulsion between these amino acids. This repulsion may expand the structure of the peptide, increasing the mobility of the peptide. 2) A more substantial hydration layer may form around the peptide at higher pH levels, as the ionized segments of the peptide engage

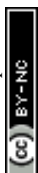


1 more intensely with water molecules. The substantial hydration layer formed at higher pH  
2 levels can initially impede adhesion by preventing direct contact between the peptide and the  
3 SAM. Over time, however, this layer may undergo changes or compression during prolonged  
4 contact, contributing to increase in adhesion energy.

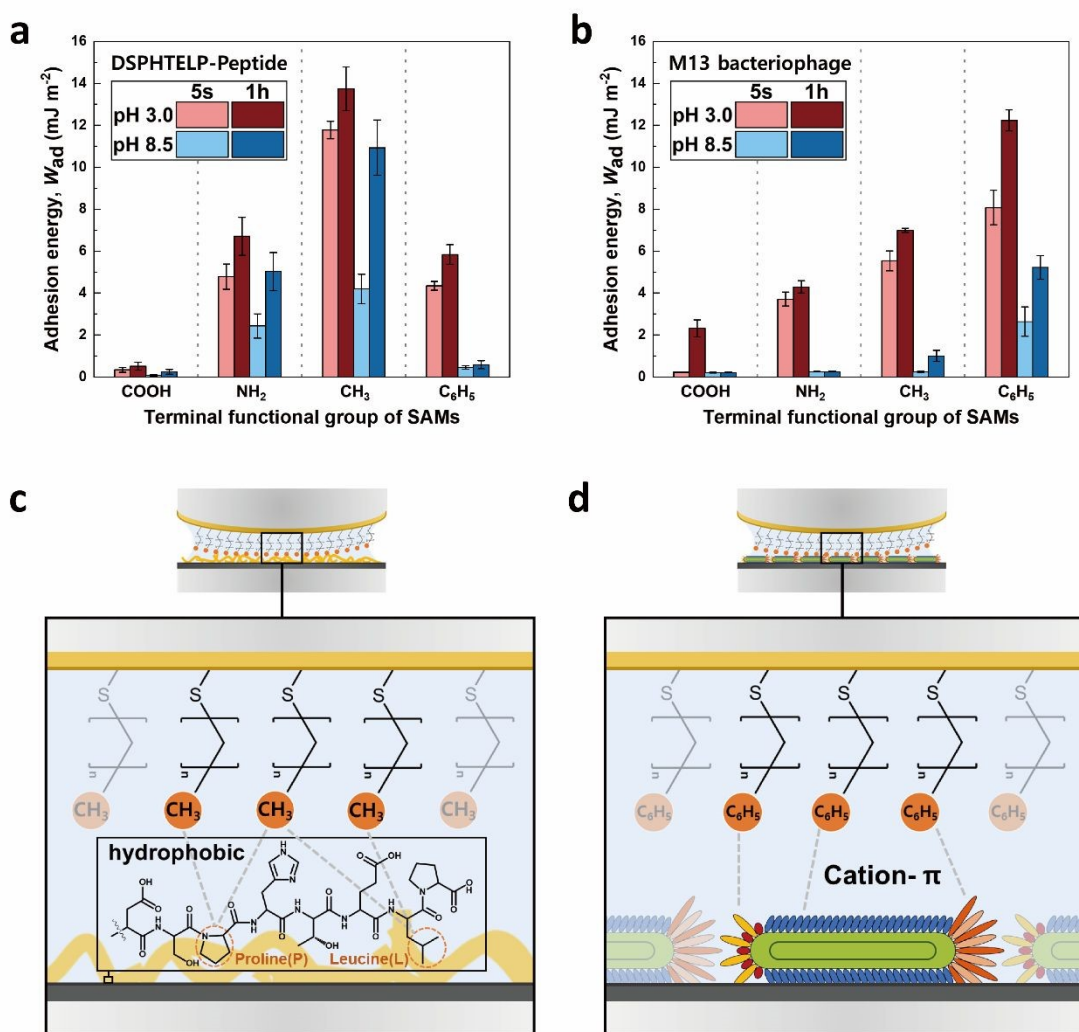
### 6 **Comparing the Interaction mechanism of DSPHTELP-Peptide and M13 bacteriophage**

7 The adhesion observed between the 8-mer peptide and M13 bacteriophage, although similar,  
8 exhibited noticeable differences despite sharing the identical DSPHTELP sequence, as  
9 illustrated in **Fig. 5**. The differences in adhesion with NH<sub>2</sub>- and CH<sub>3</sub>-SAMs under both pH 3.0  
10 and pH 8.5 conditions can be explained by the following factors:

- 11 1) The differences in adhesion compared to M13 bacteriophage at both NH<sub>2</sub>- and CH<sub>3</sub>-  
12 SAMs, under pH 3.0 and pH 8.5, can be attributed to differences in size and density. The  
13 M13 bacteriophage, when coated as a monolayer on mica, showed a density of about 30  
14 units per  $\mu\text{m}^2$ , with coverage reaching approximately 82%. This indicates a less dense  
15 coating of the M13 bacteriophage (**Fig. S4**)<sup>33</sup>, compared to the 8-mer peptide, which, due  
16 to its smaller size, forms a dense layer without significant void spaces (**Fig. S3**). The  
17 denser arrangement of the peptide enhances its interaction with the SAM through  
18 increased hydrophobic and cation- $\pi$  interactions, attributed mainly to the presence of **L**,  
19 **P**, and **H**. Particularly at pH 8.5, the peptide maintains adhesion to both NH<sub>2</sub>- and CH<sub>3</sub>-  
20 SAMs, unlike the M13 bacteriophage, which exhibits limited adhesion likely due to  
21 swelling.
- 22 2) The reduced adhesion of the 8-mer peptide compared to the M13 bacteriophage on C<sub>6</sub>H<sub>5</sub>-  
23 SAM is attributed to difference in amino acid mobility. Unlike the M13 bacteriophage



peptide, which is oriented along the pVIII and connected to ssDNA, the 8-mer peptide directly attaches to the mica surface. At pH 3.0, **H** in the peptide carries a positive charge, facilitating its binding to the negatively charged mica. However, upon binding to the mica surface, **H** can no longer act as a cation source for cation- $\pi$  interactions, resulting in the peptide's lower adhesion to C<sub>6</sub>H<sub>5</sub>-SAM. In contrast, for the M13 bacteriophage, histidine remains relatively free, as it is situated away from the mica surface. This greater freedom of histidine appears to enhance the effectiveness of the cation- $\pi$  interaction between the M13 bacteriophage and C<sub>6</sub>H<sub>5</sub>-SAM.



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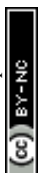
1 **Fig. 5.** Bar graph shows the adhesion energy on (a) 8-mer peptide and (b) M13 bacteriophage  
2 layers with four different SAMs<sup>33</sup>, depending on contact time and pH; average  $\pm$  standard error  
3 bars ( $n = 3$  in each group). Schematic representations of interaction between (c) CH<sub>3</sub>-SAM and  
4 8-mer peptide, (d) C<sub>6</sub>H<sub>5</sub>-SAM and M13 bacteriophage, respectively.

## 6 Conclusion

7 In this study, we successfully synthesized 8-mer peptide (DSPHTELP), designed to mimic  
8 the coat protein (pVIII) of DSPH M13 bacteriophages. Our investigation into the interaction  
9 mechanisms, facilitated by measuring the forces between the synthesized peptide and four  
10 different functionalized self-assembled monolayers (SAMs: COOH-, NH<sub>2</sub>-, CH<sub>3</sub>-, C<sub>6</sub>H<sub>5</sub>-) using  
11 a Surface Forces Apparatus (SFA), led to several notable conclusions:

- 12 1) The adhesion order of the peptide with SAMs (CH<sub>3</sub>- > NH<sub>2</sub>- > C<sub>6</sub>H<sub>5</sub>- > COOH-)  
13 highlights hydrophobic interaction as the primary force, especially influenced by  
14 Leucine (**L**) and Proline (**P**), with histidine (**H**) playing a crucial role in cation- $\pi$   
15 interactions.
- 16 2) Adhesion strengths were higher at pH 3.0 compared to pH 8.5 across all SAMs. This  
17 difference is attributed to the neutral charge of the peptide at pH 3.0, which enhances  
18 its hydrophobic properties. Additionally, **H** becomes positively charged at lower pH,  
19 facilitating cation- $\pi$  interactions. The observation of increased adhesion over longer  
20 contact times suggests a beneficial structural rearrangement and reorientation of the  
21 peptide for optimal binding.

22 These findings not only provide detailed quantitative insights into the molecular interaction  
23 mechanisms but also highlight the DSPHTELP sequence's utility at higher pH levels — a



1 scenario where M13 bacteriophages previously faced limitations. The implications of this  
2 research extend to the design and development of innovative materials in fields such as nano-  
3 biomaterials and biomedicine, offering foundational knowledge for future advancements. In  
4 upcoming future works, we plan to investigate the effect of individual amino acids by inserting  
5 a point mutation (e.g., DSSHTELS), and also study other sequences, such as VSGSSPDS  
6 which are known to specifically adhere to gold.<sup>63, 64</sup>

## 8 **Author contributions**

9 The research was conceived by all authors. Experiments were performed by H. K., and S. J.  
10 with the aid of J. K. The research was supervised by J. R., J.-H. R., and D. W. L. All authors  
11 contributed to the writing of the manuscript and the ESI.<sup>†</sup>

## 13 **Conflicts of interest**

14 There are no conflicts to declare.

## 16 **Acknowledgements**

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18 2023R1A2C2004762, RS-2023-00208386) and 2022 PRIMA QUEBEC-NRF Joint Research  
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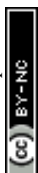


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## DATA AVAILABILITY STATEMENT

The data supporting this article have been included as part of the Supplementary Information.

