View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by University of Birmingham Research Portal

UNIVERSITY OF BIRMINGHAM

Research at Birmingham

Mutation of M13 Bacteriophage Major Coat Protein for Increased Conjugation to Exogenous Compounds.

Tridgett, Matthew; Lloyd, James R.; Kennefick, Jack; Moore-Kelly, Charles; Dafforn, Timothy

DOI:
10.1021/acs.bioconjchem.8b00307

License:
None: All rights reserved

Document Version
Peer reviewed version

Citation for published version (Harvard):
Tridgett, M, Lloyd, JR, Kennefick, J, Moore-Kelly, C & Dafforn, T 2018, 'Mutation of M13 Bacteriophage Major Coat Protein for Increased Conjugation to Exogenous Compounds.' Bioconjugate Chemistry, vol. 29, no. 6, pp. 1872-1875. https://doi.org/10.1021/acs.bioconjchem.8b00307

Link to publication on Research at Birmingham portal

Publisher Rights Statement:
This document is the Accepted Manuscript version of a Published Work that appeared in final form in Bioconjugate Chemistry, copyright © American Chemical Society after peer review and technical editing by the publisher.
To access the final edited and published work see http://dx.doi.org//10.1021/acs.bioconjchem.8b00307

General rights
Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

• Users may freely distribute the URL that is used to identify this publication.
• Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
• User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
• Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy
While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Download date: 13. Aug. 2019

UNIVERSITY OF BIRMINGHAM

Research at Birmingham

Mutation of M13 Bacteriophage Major Coat Protein for Increased Conjugation to Exogenous Compounds.

Tridgett, Matthew; Dafforn, Timothy; Moore-Kelly, Charles

Document Version
Peer reviewed version

Citation for published version (Harvard):
Tridgett, M, Dafforn, T & Moore-Kelly, C 2018, 'Mutation of M13 Bacteriophage Major Coat Protein for Increased Conjugation to Exogenous Compounds.' Bioconjugate Chemistry, pp. 1872-1875.

Link to publication on Research at Birmingham portal

General rights
Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

• Users may freely distribute the URL that is used to identify this publication.
• Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
• User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
• Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy
While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Download date: 26. Jun. 2018

Mutation of M13 Bacteriophage Major Coat Protein for Increased Conjugation to Exogenous Compounds

Matthew Tridgett,* James R. Lloyd, Jack Kennefick, Charles Moore-Kelly and Timothy R. Dafforn*

School of Biosciences, University of Birmingham, Edgbaston, Birmingham, West Midlands, B15 2TT, UK

*Matthew Tridgett e-mail: mxt1332@bham.ac.uk. Timothy R. Dafforn e-mail: t.r.dafforn@bham.ac.uk.

Abstract

Over the past ten years there has been an increasing interest in the conjugation of exogenous compounds to the surface of M13 bacteriophage. M13 offers a convenient scaffold for the development of nano-assemblies with useful functions, such as highly specific drug delivery and pathogen detection. However, the progress of these technologies has been hindered by the limited efficiency of conjugation to the bacteriophage. Here we generate a mutant version of M13 with an additional lysine residue expressed on the outer surface of the M13 major coat protein, pVIII. We show that this mutation is accommodated by the bacteriophage and that up to a further 520 exogenous groups can be attached to the bacteriophage surface via amine-directed conjugation. These results could aid the development of high payload drug delivery nano-assemblies and pathogen detection systems with increased sensitivity.

M13 bacteriophage (M13) is a filamentous, non-lytic coliphage that was first isolated around fifty years ago.[1] M13 has a simple and highly efficient genome with only eleven genes, two of which overlap entirely with neighboring genes whilst producing discrete gene products.[2-5] The coat of M13 comprises ~2700 copies of the major coat protein, pVIII and five copies each of the minor coat proteins pIII, pVI, pVII and pIX. The pVIII coat is arranged as stacked pentameric rings, giving M13 a cylindrical shape with a high aspect ratio of ~6 nm by ~1000 nm.[6-8] The 50 amino acid major coat protein, pVIII, is almost entirely alpha-helical and is arranged with its carboxy-terminus associated with the single-stranded circular DNA at the core of the virion, and its amino-terminus surface-exposed.[9]

Functional groups within the pVIII coat of M13 can be targeted for the chemical conjugation of exogenous molecules to the bacteriophage to create nano-assemblies with non-biological functions.[10] For amine-directed conjugation, it is the amine group of the amino-terminus of pVIII, and to a lesser extent the ε-amine group of the lysine residue at position eight that are targeted for conjugation of exogenous compounds.[11] Conjugated species include antibiotics,[12] anti-cancer drugs,[13] chromophores,[11,14,15] NMR contrast agents,[16] antibodies and aptamers. However each of these studies have identified a common issue with using M13. In all cases the level of conjugation to the virion surface limited the performance of the product. This limitation was starkly demonstrated in the use of M13 to target folate receptors on the surface of cancer cells by conjugating folate to the surface of M13. In this case multivalency was said to play a role in strengthening the bond between the conjugated molecules and their target,[11] meaning that increasing conjugation of folate to the surface of the M13 would have increased the effectiveness of the reagent. We have observed the same limitation in our laboratory where we aim to use dye-labeled M13 as the basis of a diagnostics test.[17] Our experience is that the number of dye molecules on the bacteriophage surface is directly related to the eventual sensitivity of the assay. We have studied the conjugation of wild type M13 using the model dye 4-chloro-7-nitrobenzofurazan (NBD-Cl). NBD-Cl is a relatively small dye meaning that steric effects on conjugation should be kept to a minimum. Our studies have shown that conjugation efficiencies of approximately 1200 dye molecules per virion are the maximum that can be achieved. This result agrees with previous studies on this subject.[11,15] We suspect that this upper limit indicates that despite its small size NBD-Cl conjugation is still limited by steric hindrance from other side chains that surround the conjugation site.

As smaller dyes are not readily available one solution to this problem would be to introduce additional conjugation sites onto the surface. Presented here is a systematic study of the effect of substituting additional lysine residues into a variety of positions on the solvent-exposed face of pVIII. For each mutant the effect on viral viability is assessed and if the mutation is non-lethal then the conjugation efficiency is also quantified. Using this approach we were able to introduce an additional lysine residue – and thereby an additional ~2700 amine groups to the overall surface of M13 – to the amino-terminal region of bacteriophage M13 major coat protein pVIII (Figure 1), imparting an increased capacity for amine-directed conjugation of exogenous compounds. To prove the utility of this new mutant form of M13, conjugation of a range of dyes (including NBD-Cl, Cyanine3 (Cy3), Tetramethylrhodamine iosthiocyanate (TRITC) and Cyanine5 (Cy5)) were tested for their conjugation efficiency to bacteriophage. These

data showed that in all cases dye conjugation efficiency was improved in the mutant bacteriophage expressing an additional Lys residues compared to the wild type, with up to 520 additional exogenous chromophores being bound.

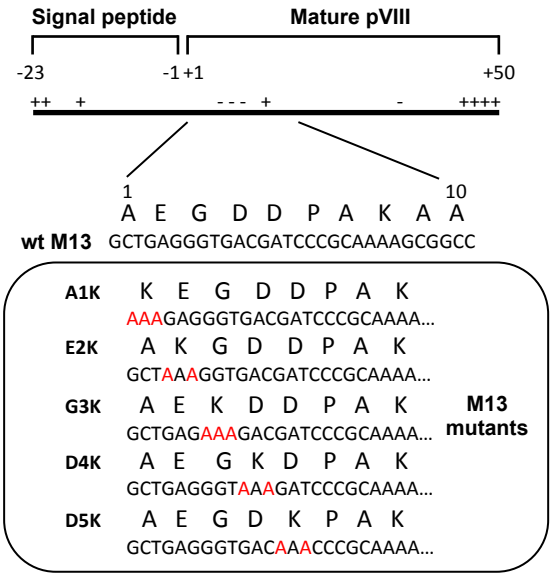

Figure 1. Scheme for the generation of M13 mutants displaying additional lysine residues. Schematic of bacteriophage M13 procoat. Positively charged residues labeled with '+', and negatively charged residues labeled with '-'. Nucleotide and amino acid sequences of the amino-terminal region of mature pVIII, both wild type (wt) and mutant. Alterations to nucleotide sequence displayed in red.

During the M13 bacteriophage replication cycle, amino acid residues +1 to +20 of M13 procoat (Figure 1) are translocated across the *Escherichia coli* (*E. coli*) inner membrane from the cytoplasm to the periplasm, allowing proteolytic cleavage to occur, producing mature pVIII and allowing the assembly of the M13 virion. Of the mutations attempted during the production of mutant M13, we observed that all those that caused a net positive change in the charge of pVIII were non-viable (Table 1). This is a significant issue as the conjugation systems often used rely on the presence of the positively charged amine on the side chain of lysine. The observation of this abolition of viability is consistent with the findings of Schuenemann *et al*. (1999),[18] who showed that prior to the assembly of M13 inside its host, the proton motive force across the inner membrane of *E. coli* inhibits the translocation of positively charged residues from the cytoplasm to the periplasm, thus preventing completion of the M13 replicative cycle when additional positive charge is added to the amino terminal region of pVIII.

Table 1. Effect of mutation on major coat protein charge and bacteriophage viability.

Mutation	Δ charge[a]	Viable? Y/N[b]
A1K	+1	N
E2K	+2	N
G3K	+1	N
D4K	+2	N
D5K	+2	N
$A1K_{N12D}$	0	Y
$A1K_{G3E}$	0	N
$G3K_{N12D}$	0	Y
$G3K_{A1E}$	0	Y

[a] Per pVIII monomer; [b] As determined by DNA sequencing

Applying the findings of Schuenemann *et al*. (1999),[18] the mutations that resulted in a change in charge of +1 per pVIII monomer (A1K and G3K) were repeated alongside a secondary mutation that would cancel out the addition of a positive charge by the addition of a negatively charged residue (asparagine at position 12 to aspartic acid, N12D). The two mutants resulting from this approach, $A1K_{N12D}$ and $G3K_{N12D}$, were viable (Table 1). Conjugation experiments using NBD-Cl were carried out to examine whether the new mutant forms of M13 could be labeled with a higher number of exogenous groups than wild type. Unfortunately UV-vis spectroscopy showed that neither $A1K_{N12D}$ nor $G3K_{N12D}$ increased the level of conjugation despite the addition of an additional lysine residue. Examination of the structure of the M13 coat suggests strongly that introduction of a negatively charged aspartate group at position 12 (D12) could lead to the formation of a salt bridge between D12 and the Lys residue at position eight (K8), altering the orientation of K8.[19] K8 contains one of the two solvent accessible amine groups in wild type M13 that are derivatized during conjugation reactions. It seems likely that if the introduction of an aspartate at position 12 is leading to an ion bridge then this will inhibit conjugation of K8, meaning that one conjugation site was added at positions one or three whilst another was effectively removed from position eight (Figure S1) leading to no improvement in conjugation efficiency. To overcome this issue two alternative mutations (at positions 1 or 3) were made in an attempt at balancing the charge of an introduced lysine while not forming a salt bridge with the wild type K8. Analysis of these mutants found that $A1K_{G3E}$ was non-viable however $G3K_{A1E}$ was found to be viable by plaque assay with sequencing showing that viability was not the result of genetic reversion (Table 1). To test the conjugation efficiency of $G3K_{A1E}$ compared to wild type M13, the two bacteriophage variants were separately

combined with NBD-Cl before removing unreacted dye by size exclusion chromatography. UV-vis analysis was used to quantify the number of dye molecules bound to the surface of M13 as the two entities have clear and distinct absorbance spectra. M13 has a band at 269 nm with an extinction coefficient of 3.84 $mg^{-1}.cm^{-2}$,[20] while NBD-Cl when bound to an amine group absorbs at 475 nm with an extinction coefficient of 18293 $M^{-1}.cm^{-1}$ (Figure S2). When the two reaction products were compared by UV-vis analysis, the peaks attributable to dye were more prominent in the sample that contained $G3K_{A1E}$ than wild type M13 (Figure 2 a), indicating that there was more dye bound to the mutant than the wild type. Quantification of the number of NBD groups bound to each type of bacteriophage indicated that there were 1201 ± 12 NBD groups per wild type virion (consistent with our findings that this was the upper limit of NBD-Cl conjugation to the wild type bacteriophage, Figure S3) and 1491 ± 20 NBD groups per $G3K_{A1E}$ virion, a significant increase ($p < 0.0001$) of 290 dye molecules per virion (Figure 2 a and e). When the experiment was repeated with Cy3 in place of NBD-Cl, the absorbance spectra of the dye when bound to the different bacteriophage variants did not match closely, indicating dye aggregation (Figure 2 b, solid lines), which in the case of H-aggregation causes hypochromicity, thus suggesting that the absorbance values did not represent the true concentration of the dye bound to the bacteriophage variants, making proper comparison of the conjugation efficiencies unreliable. To overcome this, the samples were measured again in the presence of Triton X-100 at a 1/1000 (v/v) concentration to prevent dye aggregation. Analysis of the resulting spectra (Figure 2 b, dashed lines) revealed 1417 ± 28 Cy3 groups per wild type virion compared to 1937 ± 320 Cy3 groups per $G3K_{A1E}$ virion, a significant increase ($p < 0.05$) of 520 dye molecules per virion (Figure 2 e). When the conjugation was repeated with TRITC, UV-vis analysis revealed 359 ± 8 TRITC groups per wild type virion compared to 672 ± 65 TRITC groups per $G3K_{A1E}$ virion, a significant increase ($p = 0.001$) of 313 TRITC groups per virion (Figure 2 c and e). Finally, when the conjugation experiment was repeated with Cy5, as with Cy3, the spectra of the dye bound to the different bacteriophage samples did not match closely (Figure 2 d, solid lines), indicating dye aggregation, thus Triton X-100 was added and the UV-vis measurements were repeated. Analysis of the resulting spectra (Figure 2 d, dashed lines) revealed 614 ± 160 Cy5 groups per wild type virion compared to 1067 ± 24 Cy5 groups per $G3K_{A1E}$ virion, a significant increase ($p = 0.008$) of 453 Cy5 groups per virion (Figure 2 d and e). Furthermore, gel electrophoresis in denaturing conditions confirmed dye conjugation to bacteriophage (Figure S4).

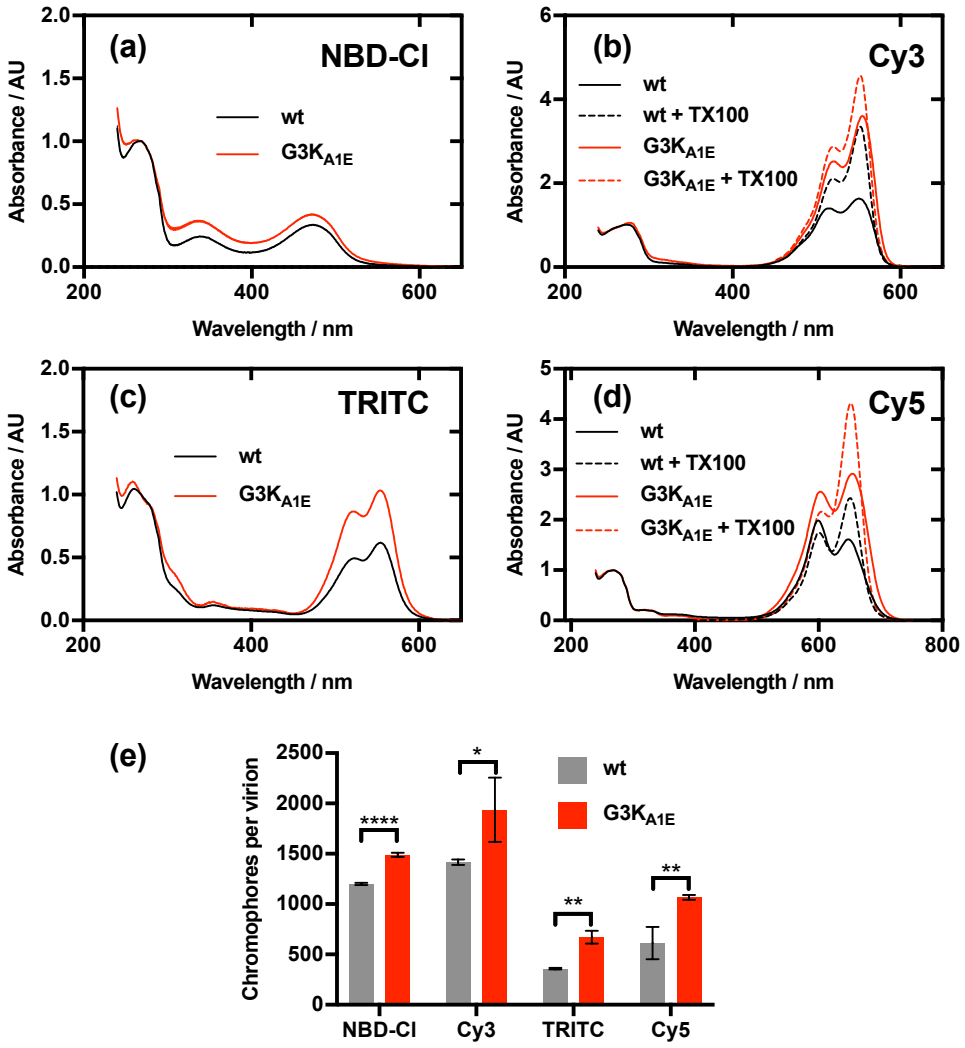

Figure 2. Conjugation of exogenous chromophores to M13 variants (n=3, error bars represent one standard deviation). (a) Absorbance spectra of wild type (wt) and G3K$_{A1E}$ M13 bacteriophage following conjugation to (a) NBD-Cl, (b) Cy3, (c) TRITC and (d) Cy5. (e) Conjugation efficiencies of wt and G3K$_{A1E}$ M13 bacteriophage to dyes indicated in the figure. Where Triton X-100 was used, the values used to calculate conjugation efficiency were those from the spectra measured in the presence of Triton X-100.

We have shown that the introduction of additional lysine residues into the coat of M13 adversely affects viral propagation. This results from an increase in positive charge on pVIII that interferes with the viral assembly process. This observation is consistent with results obtained by: 1) Merzlyak and Lee (2009),[20] who – when attempting to

introduce up to six Lys residues to the amino-terminal region of pVIII in a partial library approach - found that they were able to introduce only one additional Lys residue and that the attempted introduction of 2-6 Lys residues only yielded plaques due to deletions or wild type DNA; 2) Schuenemann *et al*. (1999),[18] who observed that the proton motive force across the inner membrane of *E. coli* prevents the translocation of M13 immature pVIII when positively charged residues are added via mutagenesis, thus halting M13 replication; and 3) Kuzmicheva *et al*. (2008),[22] who observed an under-representation of positively charged residues in pVIII phage display, suggesting that there is a disadvantage to the expression of such residues. Applying these observations, we designed rescue mutations in an effort to allow the expression of an additional Lys residue in pVIII. While the introduction of a second, charge-neutralising mutation alongside A1K (N12D) and G3K (N12D or A1E) allowed the propagation of these mutations (albeit with a reduced titer compared to wild type, Figure S5), which were non-viable when introduced to a wild type background, not all rescue mutations allowed propagation ($A1K_{G3E}$), demonstrating that this approach is not always effective, suggesting that there are determinants of M13 viability that are as yet not understood. Following the generation of the mutants, their conjugation efficiencies were determined by binding to NBD-Cl, Cy3, TRITC and Cy5. Of the three mutants generated, only $G3K_{A1E}$ showed an increase in conjugation efficiency compared to wild type M13.

We have shown that the addition of lysine residues to the coat of M13 is not a trivial process and that certain rules need to be followed to ensure that the virus is viable and that conjugations sites are available. By following these rules we have developed a mutant strain of M13 that is viable and has increased conjugation efficiency above wild type. As a number of studies have cited that their progress was limited by conjugation efficiency, we propose that this mutant form of M13 could find use in the development of new – or the improvement of existing – technologies that rely on the conjugation of exogenous compounds to the outer surface of M13 bacteriophage.

Associated content

Supporting Information. Conjugation of dyes to M13 bacteriophage and quantification, effect of N12D mutation on conjugation efficiency, determination of 4-chloro-7-nitrobenzofurazan extinction coefficient, optimization of conjugation to wild type M13 bacteriophage, gel analysis of dye conjugation and plaque-forming ability of bacteriophage mutants.

Author information

Corresponding Authors

*E-mail: mxt133@bham.ac.uk

*E-mail: t.r.dafforn@bham.ac.uk

ORCID

Matthew Tridgett: 0000-0003-2375-5433

Timothy R. Dafforn: 0000-0003-2257-6679

Acknowledgements

This work was supported by the Midlands Integrative Biosciences Training Partnership funded by the Biotechnology and Biological Sciences Research Council (BB/M01116X/1).

Notes

The authors declare no competing financial interest.

References

(1) Hofschneider, P. (1963). [Discovery of Bacteriophage M13]. *Naturforsch B. Chem. Biochem. Biophys. Biol.*, 18b, 203-205.

(2) Kokoska, R. J. and Steege, D. A. (1998). Appropriate expression of filamentous phage f1 DNA replication genes II and X requires RNAse E-dependent processing and separate mRNAs. *Journal of Bacteriology*. 180(12), 3245-3249.

(3) Haigh, N. G. and Webster, R. E. (1999). The pI and pXI assembly proteins serve separate and essential roles in filamentous phage assembly. *Journal of Molecular Biology*. 293, 1017-1027.

(4) Rapoza, M. P. and Webster, R. E. (1995). The products of gene I and the overlapping in-frame gene xi are required for filamentous phage assembly. *Journal of Molecular Biology*. 248, 627-638.

(5) Van Wezenbeek, P. M. G. F., Hulsebos, T. J. M., and Schoenmakers, J. G. G. (1980). Nucleotide sequence of the filamentous bacteriophage M13 DNA genome: comparison with phage fd. *Gene*. 11, 129-148.

(6) Lee, J., Song, J., Hwang, M., and Lee, K. (2013). Nanoscale bacteriophage biosensors beyond phage display. *International Journal of Nanomedicine*, 8, 3917-3925.

(7) Marvin, D. A. (1998). Filamentous phage structure, infection and assembly. *Current Opinions in Structural Biology*. 8(2), 150-158.

(8) Morag, O., Sgourakis, N. G., Baker, D., and Goldbourt, A. (2015). The NMR-Rosetta capsid model of M13 bacteriophage reveals a quadrupled hydrophobic packing epitope. *Proceedings of the National Academy of Sciences*. 112(4), 971-976.

(9) Armstrong, J., Hewitt, J. A., and Perham, R. N. (1983). Chemical modification of the coat protein in bacteriophage fd and orientation of the virion during assembly and disassembly. *The EMBO Journal*. 2(10), 1641-1646.

(10) Bernard, J. M. L. and Francis, M. B. (2014). Covalent strategies for the covalent modification of filamentous phage. *Frontiers in Microbiology*. 5(734), 1-7.

(11) Li, K., Chen, Y., Li, S., Nguyen, H., Niu, Z., You, S., Mello, C., Lu, X., and Wang, Q. (2010). Chemical modification of M13 bacteriophage and its application in cancer cell imaging. *ACS Bioconjugate Chemistry*, 21, 1369-1377.

(12) Yacoby, I., Chamis, M., Bar, H., Shabat, D., and Benhar, I. (2006). Targeting antibacterial agents by using drug-carrying filamentous bacteriophages. *Antimicrobial Agents and Chemotherapy*, 50(6), 2087-2097.

(13) Suthiwangcharoen, N., Li, T., Li, K., Thompson, P., You, S., and Wang, Q. (2011). M13 bacteriophage-polymer nanoassemblies as drug delivery vehicles. *Nano Research*, 4(5), 483-493.

(14) Carrico, Z. M., Farkas, M. E., Zhou, Y., Hsiao, S. C., Marks, J. D., Chokhawala, H., Clark, D. S., and Francis, M. B. (2012). N-terminal labelling of filamentous phage to create cancer marker imaging agents. *ACS Nano*. 6, 6675-6680.

(15) Hilderbrand, S. A., Kelly, K. A., Niedre, M., and Weissleder, R. (2008). Near infrared fluorescence-based bacteriophage particles for ratiometric pH imaging. *Bioconjugate Chemistry*. 19(8), 1635-1639.

(16) Palaniappan, K. K., Ramirez, R. M., Bajaj, V. S., Wemmer, D. E., Pines, A., and Francis, M. B. (2013). Molecular imaging of cancer cells using a bacteriophage-based 129Xe NMR biosensor. *Angewandte Chemie International Edition in English*. 52, 4849-4853.

(17) Pacheco-Gómez, R., Kraemer, J., Stokoe, S., England, H., Penn, C., Stanley, E., Rodger, A., Ward, J., Hicks, M., and Dafforn, T. (2012). Detection of pathogenic bacteria using a homogeneous immunoassay based on shear alignment of virus particles and linear dichroism. *Analytical Chemistry*, 84, 91-97.

(18) Schuenemann, T. A., Delgado-Nixon, V. M., and Dalbey, R. E. (1999). Direct evidence that the proton motive force inhibits membrane translocation of positively charged residues within membrane proteins. *Journal of Biological Chemistry*. 274(11), 6855-6864.

(19) Morag, O., Abramov, G. and Goldbourt, A. (2011). Similarities and differences within members of the Ff family of filamentous bacteriophage viruses. *The Journal of Physical Chemistry*. 115, 15370-15379.

(20) Berkowitz, S. A. and Day, L. A. (1976). Mass, length, composition and structure of the filamentous bacterial virus fd. *Journal of Molecular Biology*. 102, 531-547.

(21) Merzlyak, A., and Lee, S. (2009). Engineering phage materials with desired peptide display: Rational design sustained through natural selection. *Bioconjugate Chemistry*, 20, 2300-2310.

(22) Kuzmicheva, G., Jayanna, P., Sorokulova, I., and Petrenko, V. (2008). Diversity and censoring of landscape phage libraries. *Protein Engineering, Design and Selection*, 22(1), 9-18.

For Table of Contents only