



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Peptide-tags for enhanced DNA microarray performance

Citation for published version:

Schulze, H, Ross, AJ, Ember, SWJ, Luby, J, Khondoker, M, Giraud, G, Ciani, I, Tlili, C, Papale, D, Terry, JG, Mount, AR, Walton, AJ, Crain, J, Ghazal, P, Bachmann, TT & Campbell, CJ 2011, 'Peptide-tags for enhanced DNA microarray performance', *Faraday Discussions*, vol. 149, pp. 201-210.
<https://doi.org/10.1039/c005491g>

Digital Object Identifier (DOI):

[10.1039/c005491g](https://doi.org/10.1039/c005491g)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Faraday Discussions

Publisher Rights Statement:

Copyright C2010 Royal Society of Chemistry. All rights reserved.

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



Post-print of peer-reviewed article published by the Royal Society of Chemistry.

Published article available at: <http://dx.doi.org/10.1039/c005491g>

Cite as:

Schulze, H., Ross, A. J., Ember, S. W. J., Luby, J., Khondoker, M., Giraud, G., Ciani, I., Tlili, C., Papale, D., Terry, J. G., Mount, A. R., Walton, A. J., Crain, J., Ghazal, P., Bachmann, T. T., & Campbell, C. J. (2011). Peptide-tags for enhanced DNA microarray performance. *Faraday Discussions*, 149, 201-210.

Manuscript received: 13/07/2010; Accepted: 23/07/2010; Article published: 15/10/2010

Peptide-tags for enhanced DNA microarray performance**

Holger Schulze,¹ Alan J. Ross,¹ Stuart W.J. Ember,¹ Julie Luby,¹ Mizanur Khondoker,¹ Gerard Giraud,² Ilenia Ciani,³ Chaker Tlili,³ Davide Papale,³ Jon Terry,⁴ Andrew Mount,³ Anthony J Walton,⁴ Jason Crain,^{2,5} Peter Ghazal,¹ Till T. Bachmann¹ and Colin J. Campbell^{1,3,*}

^[1]Division of Pathway Medicine, Chancellor's Building, College of Medicine and Veterinary Medicine, University of Edinburgh, Edinburgh EH16 4SB, Scotland, UK

^[2]School of Physics and Astronomy, University of Edinburgh, Edinburgh, EH9 3JZ, Scotland, UK.

^[3]EaStCHEM, School of Chemistry, Joseph Black Building, University of Edinburgh, Edinburgh, EH9 3JJ, Scotland, UK.

^[4]Institute of Integrated Systems, School of Engineering, The University of Edinburgh, Edinburgh EH9 3JF, Scotland, UK.

^[5]National Physical Laboratory, Hampton Road, Teddington, UK.

^[*]Corresponding author; C.J.C. e-mail: colin.campbell@ed.ac.uk, tel: +44 (0)131 242 647
fax: +44 (0)131 242 6244

^[**]This work was supported by ITI Techmedia (Glasgow, Scotland, UK) under the BioSensing Platform program. We would like to thank Mark Bradley and Juan Jose Diaz-Mochon for technical advice. CC is supported by an EaStCHEM fellowship.

Electronic supplementary information (ESI):

Calibration plots obtained with differentially functionalized HCMV and HCV probes hybridized with Cy3-labelled complementary targets. See DOI: <http://dx.doi.org/10.1039/c005491g>

Keywords:

DNA microarray; probe functionalization; hybridization efficiency; peptide-tag

Abstract

The DNA microarray is a powerful tool for gene expression analysis and genotyping studies in research and diagnostic applications. A high sensitivity and short time-to-result are prerequisites for practical applications in the clinic. The hybridization efficiency of DNA microarrays depend on the probe density and the probe orientation and thus their accessibility for target molecules. In order to find an optimal probe immobilization procedure a set of different oligonucleotide modifications was tested on epoxy silane functionalized glass slides. It was found that histidine-tagged oligonucleotides resulted in the highest amount of bound probe and by far the best hybridization efficiencies. The detection limit obtained with histidine-tagged probes was up to two orders of magnitude lower compared to commonly used probe modifications. In order to further investigate the binding mechanism of histidine-tags towards functionalized glass substrates a set of different peptide-tags with and without free terminal amino-groups and with different amino acid compositions was tested. The results indicate an impact of the terminal amino group on the covalent surface binding and of aromatic amino acid residues on the enhanced hybridisation efficiency.

Main text

DNA and protein microarrays are powerful tools for clinical diagnostics as they enable the detection of a high number of biomarkers in parallel with the possibility to multiplex relevant information like cell surface antigens ⁽¹⁾, pathogen detection ⁽²⁾, virulence factors, antibiotic resistance or gene expression on a single test ⁽³⁻¹⁰⁾. The production methods for DNA microarrays are well established and mainly use either in-situ synthesis or spotting of oligonucleotide detection probes ⁽¹¹⁻¹⁴⁾. Several microarrays have been developed for the identification of pathogens, such as bacteria, fungi and viruses ⁽¹⁵⁻²⁰⁾. A prerequisite for their practical application is a high sensitivity and short time-to-result. There are different approaches to increase the sensitivity of DNA microarrays e.g. by decreasing the reaction volume in microfluidic systems and integrated lab-on-a-chip devices, as summarized in recent reviews ⁽²¹⁻²⁴⁾. The efficiency and speed of DNA target binding to probe molecules immobilized on a solid support can also be improved in the standard DNA microarray slide format by optimizing the mixing conditions during hybridization, e.g. by the means of automated hybridization stations ⁽²⁵⁾. Another parameter which has a major impact on the hybridization efficiency in solid phase systems is the surface chemistry and the type of probe binding. Noncovalent approaches, such as the use of polylysine coated slides, which rely on electrostatic interaction of the negative charged DNA backbone with the positively charged slide surface ⁽²⁶⁾ are relatively simple to use but covalent, terminal attachment of the probe molecules to the slide surface is preferable in terms of probe layer stability. The most common slide types used for covalent attachment of oligonucleotide probes on DNA microarrays are glass substrates functionalized either with aldehyde or epoxy silane functional

groups ⁽²⁷⁻²⁹⁾. We focused our studies on epoxy silane modified glass slides as they have been shown to be an excellent matrix for various microarray applications, e.g. for SNP detection and are commercially available with a high quality standard ^(3;7). For epoxy silane modified surfaces, the most commonly used probe modifications are amino functional groups ⁽²⁹⁾, whereas there are also examples of thiol modified oligonucleotides used on epoxy silane coated slides ⁽³⁰⁻³²⁾.

In this study we tested uncommon peptide tags as new probe modifications for covalent attachment of oligonucleotide probes onto epoxy silane coated substrates. We compared the amount of immobilized peptide tagged probes with that of other modifications using fluorescence-labeled probes and measured the influence of the different probe modifications on the hybridization efficiency using an HCV viral load assay.

Experimental section

Reagents

Oligonucleotide probes derived against human cytomegalovirus (HCMV) ⁽¹⁵⁾ and hepatitis C virus (HCV) ⁽³³⁾ with different modifications were purchased from Metabion (Martinsried, Germany) and Eurogentec (Seraing, Belgium). Peptide tagged oligonucleotides were obtained from Eurogentec (Seraing, Belgium) (see ***Table 1*** on the next page →)

Oligo Type	Sequence (5' - 3')	5' modification	3'
HCMV	CAAATACCGTGGGACGACACGCACCGG CAGTGCGCAGGCAGCGTCGGACACAAC ACGCTTACGGCCCTCAAACT	biotin	-
HCMV	CAAATACCGTGGGACGACACGCACCGG CAGTGCGCAGGCAGCGTCGGACACAAC ACGCTTACGGCCCTCAAACT	NH2 (C6)	-
HCMV	CAAATACCGTGGGACGACACGCACCGG CAGTGCGCAGGCAGCGTCGGACACAAC ACGCTTACGGCCCTCAAACT	SH (C6)	-
HCMV	CAAATACCGTGGGACGACACGCACCGG CAGTGCGCAGGCAGCGTCGGACACAAC ACGCTTACGGCCCTCAAACT	NH2-His-His-His-His-His- His-Cys	-
HCMV	CAAATACCGTGGGACGACACGCACCGG CAGTGCGCAGGCAGCGTCGGACACAAC ACGCTTACGGCCCTCAAACT	-	-
HCMV	CAAATACCGTGGGACGACACGCACCGG CAGTGCGCAGGCAGCGTCGGACACAAC ACGCTTACGGCCCTCAAACT	biotin	Cy3
HCMV	CAAATACCGTGGGACGACACGCACCGG CAGTGCGCAGGCAGCGTCGGACACAAC ACGCTTACGGCCCTCAAACT	NH2 (C6)	Cy3
HCMV	CAAATACCGTGGGACGACACGCACCGG CAGTGCGCAGGCAGCGTCGGACACAAC ACGCTTACGGCCCTCAAACT	SH (C6)	Cy3
HCMV	CAAATACCGTGGGACGACACGCACCGG CAGTGCGCAGGCAGCGTCGGACACAAC ACGCTTACGGCCCTCAAACT	NH2-His-His-His-His-His- His-Cys	Cy3
HCMV target	AGTGTTGAGGGCCGTAAGCGTGTGTGT CCGACGCTGCCTGCGCACTGCCGGTGCG TGTCGTCCCACGGTATTTG	Cy3	-
HCV	TCGCAAGCACCCCTATCAGGCAGTACCAC AAGGCCTTTCGC	NH2-His-His-His-His-His- His-Cys	-
HCV	TCGCAAGCACCCCTATCAGGCAGTACCAC AAGGCCTTTCGC	NH-C(O)-CH3-His-His- His-His-His-His-Cys	-
HCV	TCGCAAGCACCCCTATCAGGCAGTACCAC AAGGCCTTTCGC	NH2-Tyr-Tyr-Tyr-Tyr- Tyr-Tyr-Cys	-
HCV	TCGCAAGCACCCCTATCAGGCAGTACCAC AAGGCCTTTCGC	NH2-Lys-Lys-Lys-Lys- Lys-Lys-Cys	-
HCV	TCGCAAGCACCCCTATCAGGCAGTACCAC AAGGCCTTTCGC	NH2-Ala-Ala-Leu-Ala- Leu-Ala-Cys	-
HCV	TCGCAAGCACCCCTATCAGGCAGTACCAC AAGGCCTTTCGC	NH2 (C6)	-
HCV	TCGCAAGCACCCCTATCAGGCAGTACCAC AAGGCCTTTCGC	SH (C6)	-
HCV	TTCGCAAGCACCCCTATCAGGCAGTACCA CAAGGCCTTTCGC	NH2 (C6)	-
HCV	TTTTTCGCAAGCACCCCTATCAGGCAGTAC CACAAGGCCTTTCGC	NH2 (C6)	-
HCV	TTTTTTTCGCAAGCACCCCTATCAGGCAG TACCACAAGGCCTTTCGC	NH2 (C6)	-
HCV	TTTTTTTTTCGCAAGCACCCCTATCAGGC AGTACCACAAGGCCTTTCGC	NH2 (C6)	-
HCV target	GCGAAAGGCCTTGTGGTACTGCCTGATA GGGTGCTTTCGA	Cy3	-

Table 1. List of oligonucleotide used in this study.

DNA microarray fabrication

Modified oligonucleotides were spotted in 1x Schott Nexterion spot buffer (20 μ M) on Schott Nexterion Slides E (epoxy silane modified surface) with a Microgrid II spotter using 200 μ m solid pins. Thiol-modified oligonucleotides were spotted in 1x Schott Nexterion spot buffer containing 5 mM Tris(2-carboxy-ethyl)phosphine hydrochloride (TCEP) to cleave the mercapto-ethyl protection group. TCEP containing spotting solutions were incubated for 30 min at RT before printing. The oligonucleotide probe molecules were immobilized by incubating the slides in a humidity chamber for 1 h followed by storage over night at room temperature (RT) under dry conditions. The slides were then washed with 0.1% TritonX-100 solution under constant mixing for 5 min at RT, with 1 mM HCl solution for 4 min, with 100 mM KCl solution for 10 min, and with deionized water for 1 min. The slides were blocked with 50 mM ethanolamine + 0.1% sodium dodecyl sulfate (SDS) in 0.1 M Tris buffer (pH 9) for 15 min at 50 °C. After blocking the slides were washed in deionized water for 1 min and then dried by centrifugation (2 min at 1000 rpm).

Arrays of Cy3-labeled probes were spotted in 3x sodium chloride, sodium citrate (SSC) solution (450 mM NaCl + 45 mM Na-citrate) on Erie Scientific Superchip epoxy silane slides (Thermo Fisher Scientific, Waltham, USA) with a Microgrid II spotter using 200 μ m solid pins. Probes were immobilized 60 min after printing by UV crosslinking at 350 mJoule with a UV crosslinker device (UVP, Upland, USA). Slides were blocked with 1% (w/v) bovine serum albumin in 3x SSC solution for 10 min, followed by 15 min wash in water and four additional rinsing steps in water. Slides were then dried by centrifugation (2 min at 800 g) with an Eppendorf centrifuge 5810 R (Eppendorf, Hamburg, Germany).

Hybridization and washing

Arrays were hybridized with 50 μ L Cy3-labelled 40mer target solution in 4xSSC buffer + 0.01% SDS with an Agilent 8 well gasket slide in an Agilent hybridization oven at 55 °C under agitation (rotation speed 4). After hybridization the arrays were washed with 2x SSC + 0.2% SDS solution for 10 min at RT under constant mixing, with 2x SSC solution for 10 min at RT, and with 0.2x SSC for 10 min. After dipping into water the slides were dried by centrifugation (2 min at 800 g).

Hybridization with HCMV target was performed in a Tecan HS400Pro automated hybridization station (Tecan, Maennedorf, Switzerland). Slides were washed for 2 min with 0.1% Triton-X-100 solution, 2 min with 10 pM HCl solution, 5 min with 100 mM KCl solution, and 1 min with water, respectively. Blocking with 50 mM ethanolamine solution was performed for 10 min at 50 °C, followed by 1 min washing with water. Hybridization was performed at 55 °C for 2 h. Slides were

then washed with 2x SSC + 0.1 % SDS for two times 2 min, with 0.2 x SSC for 2 min and with water for 30 sec. Slides were automatically dried by nitrogen flow for 2 min.

Image acquisition and data analysis

Fluorescence images were generated with a Tecan LS Reloaded fluorescence scanner (Tecan, Maennedorf, Switzerland) with excitation at 532 nm and emission at 575 nm at PMT 200 (chosen to maximise signal without pixel saturation). Quantification of fluorescence signal intensities was performed with the Quantarray software (Perkin Elmer, Waltham, MA) using the Histogram quantification method. For further analysis, the mean signal intensity minus local background intensity was processed with Excel (Microsoft Corp., Redmond, USA) and the mean and standard deviation of all replicates were calculated. The detection limit was determined using the mean of the fluorescence intensity of the negative control probe plus three times the standard deviation.

Results and discussion

Comparative probe binding

Commercial epoxy silane coated glass slides were chosen as microarray substrate for their competitive advantages over other slide types. The electrophilic epoxy silane group forms stable covalent bonds with DNA probe molecules which are modified with a terminal nucleophilic functional group without the need of any additional chemicals. Aldehyde coated slides form less stable bonds or require an additional chemical reduction step with sodium borohydride to form stable bonds^(29;34). In this study we examine the influence of different probe modifications on probe binding to the slide and on assay performance. The relative amount of immobilized probe molecules with different functional groups was determined with a set of 3' fluorescently labeled probes. These probe modification included amino, thiol, biotin and modification with an oligopeptide containing six histidines at their 5' end. Histidine and biotin-tags were included in the study because we initially tested streptavidin and Nickel-NTA coated glass slides but found them to give non satisfactory results (data not shown). We chose a probe sequence specific for HCMV, which has been previously used in a gene expression array⁽¹⁵⁾. **Figure 1** shows the relative fluorescence intensities measured at spots of Cy3-labeled oligonucleotides with different modifications. The highest fluorescence intensities and thus the highest amount of bound probe were obtained with histidine-tagged probes, followed by thiol modified, amino modified and biotinylated probes.

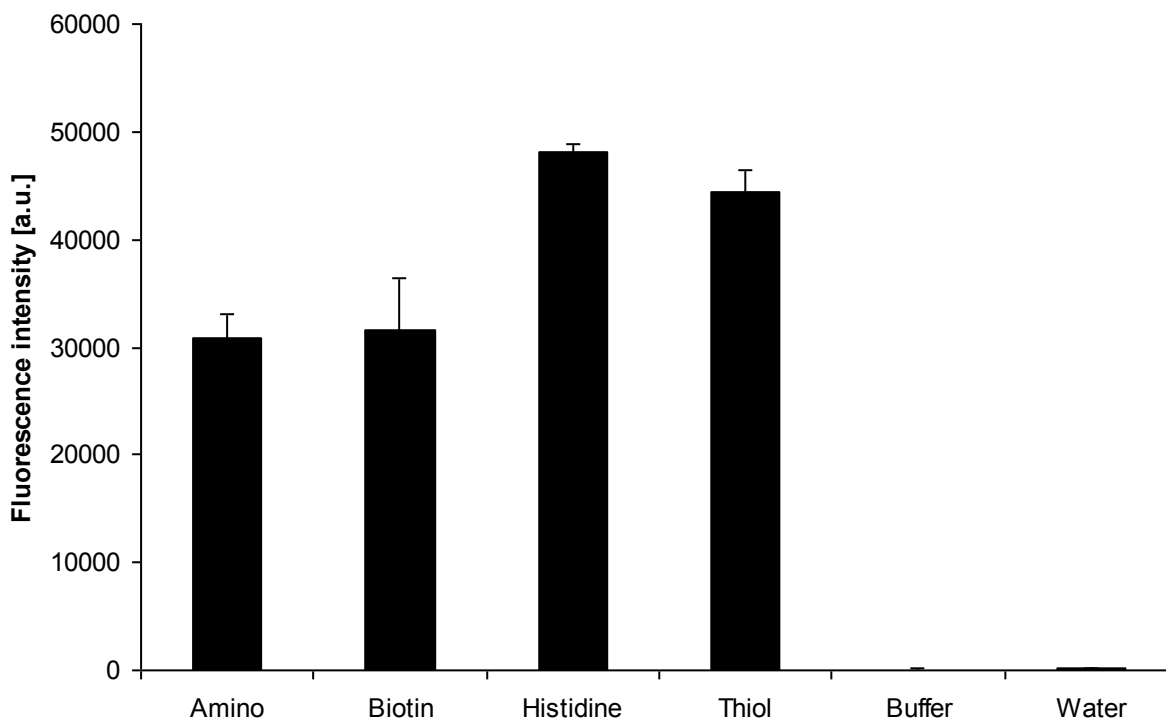


Figure 1. Mean fluorescence intensities of differentially functionalized, Cy3-labelled HCMV oligonucleotides (20 μ M) spotted on epoxy silane slides. Histidine = 6x histidine; n = 30.

Hybridization efficiency

The impact of different probe modifications on the hybridization efficiency was tested with unlabelled, but differently modified 75mer probes, which (after immobilization) were hybridized with Cy3-labeled 75mer target molecules complementary to the probe molecules. The type of probe immobilization had a dramatic impact on the hybridization efficiency as can be seen in **Table 2** and supplementary Figure S1. By far the highest sensitivity with a detection limit of 1 pM was obtained with histidine-tagged probes. Unmodified probes were least sensitive towards the target with a detection limit of 100 pM. This low sensitivity can be explained with a probe immobilization via the DNA backbone and not via terminal reactive groups causing the probe to be less accessible for the target. The detection limit obtained with amino modified probes is consistent with that obtained by Zammatteo et al for HCMV. However, they used more than three times longer amino functionalized probes (255 bp) on aldehyde coated glass slides ⁽²⁹⁾.

In contrast, a peptide tag consisting of six histidine residues with a free terminal amino group improved the hybridization efficiency compared to commonly used amino modification. This was a rather unexpected result as this is, to the best of our knowledge, the first example of the use of a

histidine-tag for covalent attachment of oligonucleotides on a solid support. Up to now histidine-tags have predominantly been used for affinity purification or affinity binding of peptides to microarray slides^(35;36). It has been reported previously that imidazole residues, the building block of histidine, react with epoxy functional groups forming a covalent linkage⁽³⁷⁾. Thus, the covalent linkage between the histidine-tag and the epoxy silane surface can either be caused by the terminal amino group or the imidazole residues or a combination of both effects.

<i>Modification</i>	<i>Detection limit [pM]</i>
none	100
Amino	10
Thiol	10
Biotin	10
Histidine-Tag	1

Table 2. Detection limit obtained with different probe modifications against fluorescence labeled HCMV target.

Different peptide tags

In order to investigate the influence of the peptide-tags on the surface binding in more detail, we used a set of different peptide tags with aromatic and aliphatic amino acids. Additionally, we also added a histidine-tagged oligonucleotide with an acyl group blocked terminal histidine (AcHis) to probe the influence of the terminal amino group. These tests were performed with HCV viral load probes to ensure that the effect was not specific to the HCMV probe. As can be seen in **Figure 2**, histidine-tagged probes resulted again in the highest sensitivity. Oligonucleotides, which were functionalized with six tyrosine residues yielded in comparably high hybridization efficiency, which indicates an impact of aromatic amino acid residues on the favorable target binding. The aliphatic amino acids alanine and leucine resulted in a reduced sensitivity compared to that obtained with histidine and tyrosine modified probes. The reduced sensitivity of histidine-tagged probes without terminal amino group clearly indicate the impact of this terminal amino group to the covalent attachment of the peptide tags to the epoxy silane coated microarray slides. Tests with different amounts of 40mer fluorescence labeled HCV targets revealed a detection limit two order of magnitude lower when using a histidine-tagged compared to amino modified probes (see **Table** and supplementary material Figure S2).

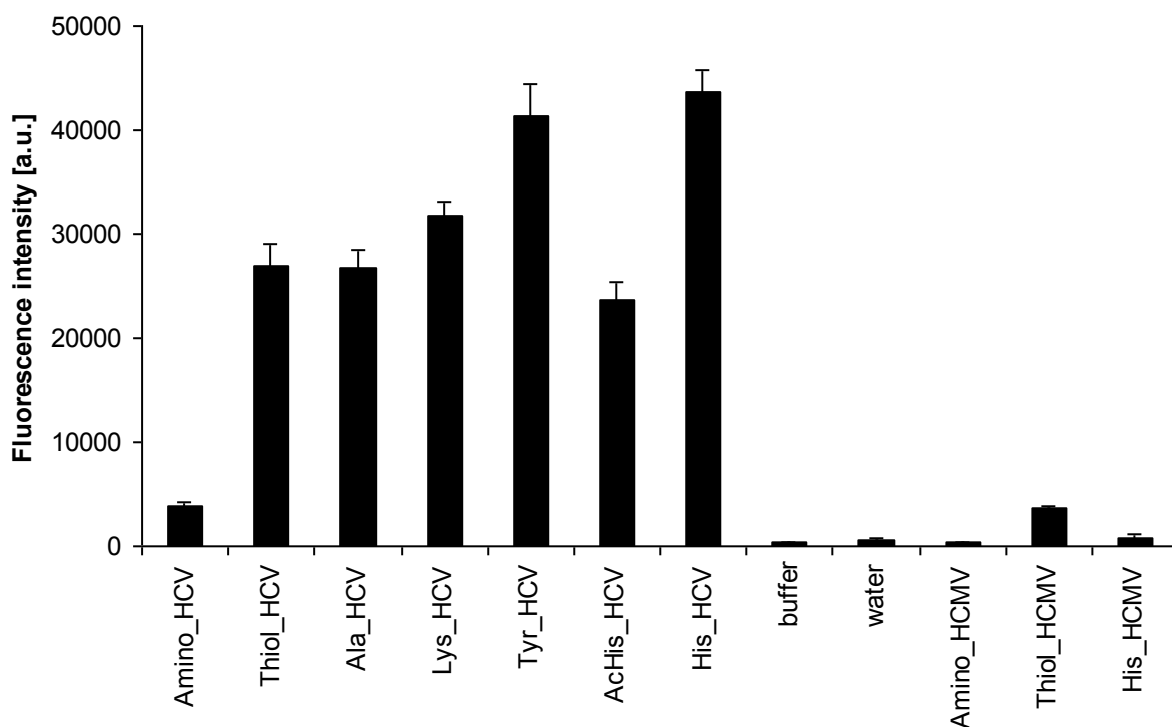


Figure 2. Mean fluorescence intensities of differentially functionalized probes (20 μ M) on epoxy silane slides hybridized with 10 pM Cy3-labelled HCV target; Ala = 6x alanine; Lys = 6x lysine; Tyr = 6x tyrosine; AcHis = 5 histine + terminal histidine with acetylated terminal amino group; His = 6x histidine; n = 5.

<i>Modification</i>	<i>Detection limit [pM]</i>
Amino	10
Thiol	1
Alanine/Leucine-Tag	1
Lysine-Tag	0.1
Tyrosine-Tag	0.1
Acetylated-Histidine-Tag	1
Histidine-Tag	0.1

Table 3. Detection limit obtained with different probe modifications against fluorescence labeled HCV target.

Spacer effect

We also tested a range of poly thymine spacers from one to nine additional thymine residues at the 5' end of the specific probe sequence in order to find out if the superior performance of the peptide tags

is only related to a spacer effect. The size of a peptide consisting of six amino acids is in the range between 1 and 2 nm. A DNA double helix of 10 nucleotide bases is in the order of 3.4 nm long⁽³⁸⁾. This means that a peptide tag of six amino acids should have a comparable spacer length to a polyT spacer of three to six thymine residues. **Figure 3** shows that the superior effect of the histidine and tyrosine tag can not be explained only by a spacer effect. The peptide tags seem to result in an optimized orientation of the surface bound probe molecules making them more accessible for the target molecules in solution and thus increasing the sensitivity of the DNA microarray.

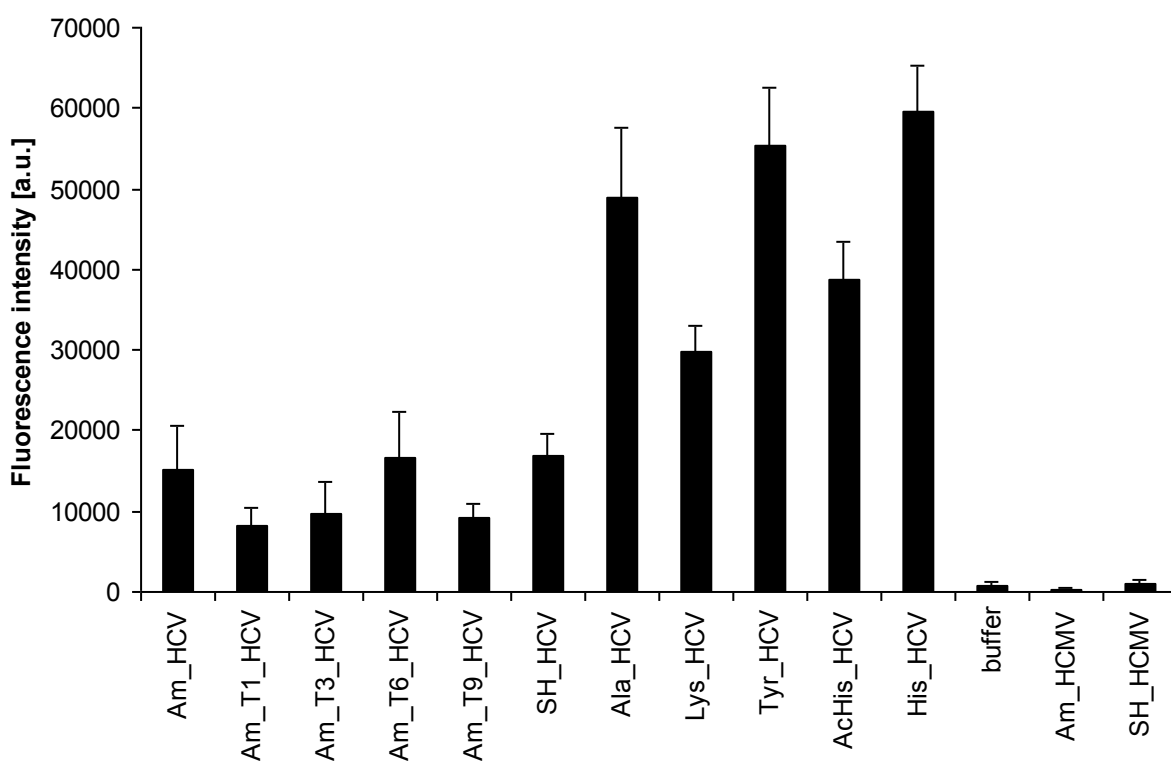


Figure 3. Mean fluorescence intensities after hybridization with 100 pM Cy3-labelled HCV target on an array of differentially functionalized probes (20 μ M) on epoxy silane slides. Am = amino; T1 = 1 thymine spacer; T3 = 3 thymine spacer; T6 = 6 thymine spacer; T9 = 9 thymine spacer; SH = thiol; Ala = 6x alanine; Lys = 6x lysine; Tyr = 6x tyrosine; AcHis = 5 histine + terminal histidine with acetylated terminal amino group; His = 6x histidine. n = 5.

Conclusion

We have shown in this study that peptide tags, especially histidine-tags, used as a new type of oligonucleotide probe modification can substantially increase the sensitivity of DNA microarrays by

up to two orders of magnitude. We have shown that this effect was not limited to a certain probe sequence. Similar results were obtained with HCMV and HCV probes. Tests of different type of peptide tags with aliphatic and aromatic amino acid residues revealed an impact of aromatic amino acids on the enhanced sensitivity and an impact of the terminal amino group on the surface binding. Histidine-tags without a free terminal amino group showed substantially reduced hybridization efficiency. This influence of the terminal amino group is an indication for a directed terminal immobilization of the histidine-tagged probe molecule. This 10 – 100 times higher sensitivity of histidine-tagged probes compared to commonly used amino modified oligonucleotides could have a substantial impact on the use of DNA microarrays for practical applications such as clinical diagnostics.

References

- [1] Campbell, C. J., O'Looney, N., Chong, K. M., Robb, J. S., Ross, A. J., Beattie, J. S. et al. (2006) Cell interaction microarray for blood phenotyping. *Anal. Chem.* 78, 1930-8.
- [2] Burgess, S. T., Kenyon, F., O'Looney, N., Ross, A. J., Chong, K. M., Beattie, J. S. et al. (2008) A multiplexed protein microarray for the simultaneous serodiagnosis of human immunodeficiency virus/hepatitis C virus infection and typing of whole blood. *Anal. Biochem.* 382, 9-15.
- [3] Barl, T., Dobrindt, U., Yu, X., Katcoff, D.J., Sompolinsky, D., Bonacorsi, S. et al. (2008) Genotyping DNA chip for the simultaneous assessment of antibiotic resistance and pathogenic potential of extraintestinal pathogenic *Escherichia coli*. *Int. J. Antimicrob. Agents.* 32, 272-7.
- [4] Grimm, V., Ezaki, S., Susa, M., Knabbe, C., Schmid, R. D., Bachmann, T. T. (2004) Use of DNA microarrays for rapid genotyping of TEM beta-lactamases that confer resistance. *J. Clin. Microbiol.* 42, 3766-74.
- [5] Strommenger, B., Schmidt, C., Werner, G., Roessle-Lorch, B., Bachmann, T. T., Witte, W. (2007) DNA microarray for the detection of therapeutically relevant antibiotic resistance determinants in clinical isolates of *Staphylococcus aureus*. *Mol. Cell. Probes.* 21, 161-70.
- [6] Yu, X., Susa, M., Knabbe, C., Schmid, R. D., Bachmann, T.T. (2004) Development and validation of a diagnostic DNA microarray to detect quinolone-resistant *Escherichia coli* among clinical isolates. *J. Clin. Microbiol.* 42, 4083-91.
- [7] Yu, X., Susa, M., Weile, J., Knabbe, C., Schmid, R. D., Bachmann, T. T. (2007) Rapid and sensitive detection of fluoroquinolone-resistant *Escherichia coli* from urine samples using a genotyping DNA microarray. *Int. J. Med. Microbiol.* 297, 417-29.
- [8] Hoheisel, J. D. (2006) Microarray technology: beyond transcript profiling and genotype analysis. *Nature Reviews Genetics* 7, 200-10.
- [9] Campbell, C. J., Ghazal, P. (2004) Molecular signatures for diagnosis of infection: application of microarray technology. *J. Appl. Microbiol.* 96, 18-23.
- [10] Giraud, G., Schulze, H., Bachmann, T. T., Campbell, C. J., Mount, A. R., Ghazal, P. et al. (2009) Fluorescence Lifetime Imaging of Quantum dot Labeled DNA Microarrays. *Int. J. Mol. Sci.* 10, 1930-41.
- [11] Palacios, G., Quan, P. L., Jabado, O. J., Conlan, S., Hirschberg, D. L., Liu, Y. et al. (2007) Panmicrobial oligonucleotide array for diagnosis of infectious diseases. *Emerg. Infect. Diseases.* 13, 73-81.

- [12] Cleven, B. E., Palka-Santini, M., Gielen, J., Meembor, S., Kronke, M., Krut, O. (2006) Identification and characterization of bacterial pathogens causing bloodstream infections by DNA microarray. *J. Clin. Microbiol.* 44, 2389-97.
- [13] Cheung, G. Y. C., Dickinson, P., Sing, G., Craigon, M., Ghazal, P., Parton, R. et al. (2008) Transcriptional responses of murine macrophages to the adenylate cyclase toxin of *Bordetella pertussis*. *Microb. Pathog.* 44, 61-70.
- [14] Burteau, S., Bogaerts, P., de Mendonca, R., Ireng, L., Berhin, C., Hiffe, J. et al. (2008) Design and validation of a low density array (Nosochip) for the detection and identification of the main pathogenic bacteria and fungi responsible for nosocomial pneumonia. *Europ. J. Clin. Microb. & Infect. Dis.* 27, 17-27.
- [15] Chambers, J., Angulo, A., Amaratunga, D., Guo, H. Q., Jiang, Y., Wan, J. S. et al. (1999) DNA microarrays of the complex human cytomegalovirus genome: Profiling kinetic class with drug sensitivity of viral gene expression. *J. Virol.* 73, 5757-66.
- [16] Hsia, C. C., Chizhikov, V. E., Yang, A. X., Selvapandiyar, A., Hewlett, I., Duncan, R. et al. (2007) Microarray multiplex assay for the simultaneous detection and discrimination of hepatitis B, hepatitis C, and human immunodeficiency type-1 viruses in human blood samples. *Biochem. Biophys. Res. Com.* 356, 1017-23.
- [17] Leinberger, D. M., Schumacher, U., Autenrieth, I. B., Bachmann, T. T. (2005) Development of a DNA microarray for detection and identification of fungal pathogens involved in invasive mycoses. *J. Clin. Microb.* 43, 4943-53.
- [18] Leinberger, D. M., Grimm, V., Rubtsova, M., Henn, A., Schroppel, K., Wichelhaus, T. A. et al. (2007) Diagnostic DNA chips to detect-Lactam resistance in the clinic. *Int. J. Med. Microbiol.* 297, 17.
- [19] Wiesinger-Mayr, H., Vierlinger, K., Pichler, R., Kriegner, A., Hirschl, A. M., Presterl, E. et al. (2007) Identification of human pathogens isolated from blood using microarray hybridisation and signal pattern recognition. *BMC Microbiol.* 7, 78.
- [20] Burteau, S., Bogaerts, P., de Mendonca, R., Ireng, L., Berhin, C., Hiffe, J. et al. (2008) Design and validation of a low density array (Nosochip) for the detection and identification of the main pathogenic bacteria and fungi responsible for nosocomial pneumonia. *Eur. J. Clin. Microbiol. Infect. Dis.* 27, 17-27.
- [21] Schulze, H., Giraud, G., Crain, J., Bachmann, T. T. (2009) Multiplexed optical pathogen detection with lab-on-a-chip devices. *J. Biophot.* 2, 199-211.

- [22] Easley, C. J., Karlinsey, J. M., Bienvenue, J. M., Legendre, L. A., Roper, M. G, Feldman, S. H. et al. (2006) A fully integrated microfluidic genetic analysis system with sample-in-answer-out capability. *Proc. Natl. Acad. Sci. U S A* 103, 19272-7.
- [23] Vanderhoeven, J., Pappaert, K., Dutta, B., Vanhummelen, P., Baron, G. V., Desmet, G. (2004) Exploiting the benefits of miniaturization for the enhancement of DNA microarrays. *Electrophor.* 25, 3677-86.
- [24] Ligler, F. S. (2009) Perspective on Optical Biosensors and Integrated Sensor Systems. *Anal. Chem.* 81, 519-26.
- [25] Vanderhoeven, J., Pappaert, K., Dutta, B., Van Hummelen, P., Desmet, G. (2005) DNA microarray enhancement using a continuously and discontinuously rotating microchamber. *Anal. Chem.* 77, 4474-80.
- [26] Taylor, S., Smith, S., Windle, B., Guiseppi-Elie, A. (2003) Impact of surface chemistry and blocking strategies on DNA microarrays. *Nucl. Aci. Res.* 31, e87.
- [27] Auburn, R. P., Kreil, D. P., Meadows, L. A., Fischer, B., Matilla, S. S., Russell, S. (2005) Robotic spotting of cDNA and oligonucleotide microarrays. *Trends Biotech.* 23, 374-9.
- [28] Dawson, E. D., Reppert, A. E., Rowlen, K. L., Kuck, L. R. (2005) Spotting optimization for oligo microarrays on aldehyde-glass. *Anal. Biochem.* 341, 352-60.
- [29] Zammateo, N., Jeanmart, L., Hamels, S., Courtois, S., Louette, P., Hevesi, L. et al. (2000) Comparison between different strategies of covalent attachment of DNA to glass surfaces to build DNA microarrays. *Anal. Biochem.* 280, 143-50.
- [30] Sethi, D., Kumar, A., Gupta, K. C., Kumar, P. (2008) A Facile Method for the Construction of Oligonucleotide Microarrays. *Bioconjug. Chem.* 19, 2136-43.
- [31] Mahajan, S., Sethi, D., Seth, S., Kumar, A., Kumar, P., Gupta, K. C. (2009) Construction of Oligonucleotide Microarrays (Biochips) via Thioether Linkage for the Detection of Bacterial Meningitis. *Bioconjug. Chem.* 20, 1703-10.
- [32] Preininger, C., Sauer, U., Dayteg, J., Pichler, R. (2005) Optimizing processing parameters for signal enhancement of oligonucleotide and protein arrays on ARChip Epoxy. *Bioelectrochem.* 67, 155-62.
- [33] Ember, S. W. J. , Ross, A. J., Schulze, H., Luby, J., Bachmann, T. T., Crain, J. et al. Clinical applications of DNA and protein microarrays for the serodiagnosis of HCV infection. In preparation.

- [34] Sobek, J., Aquino, C., Schlapbach, R. (2007) Quality considerations and selection of surface chemistry for glass-based DNA, peptide, antibody, carbohydrate, and small molecule microarrays. *Methods Mol. Biol.* 382, 17-31.
- [35] Khan, F., He, M. Y., Taussig, M. J. (2006) Double-hexahistidine tag with high-affinity binding for protein immobilization, purification, and detection on Ni-nitrilotriacetic acid surfaces. *Anal. Chem.* 78, 3072-9.
- [36] Wingren, C., Steinhauer, C., Ingvarsson, J., Persson, E., Larsson, K., Borrebaeck, C. A. K. (2005) Microarrays based on affinity-tagged single-chain Fv antibodies: Sensitive detection of analyte in complex proteomes. *Proteomics* 5, 1281-91.
- [37] Shi, S. H., Yamashita, T., Wong, C. P. (1999) Development and characterization of imidazole derivative cured bisphenol A epoxy materials for flip-chip underfill applications. *Proc. Int. Symp. Advanced Packaging Materials: Processes, Properties and Interfaces, Braselton, Ga*, Mar 14-17 1999, 317-24.
- [38] Mandelkern, M., Elias, J. G., Eden, D., Crothers, D. M. (1981) The Dimensions of Dna in Solution. *J. Mol. Biol.* 152, 153-61.