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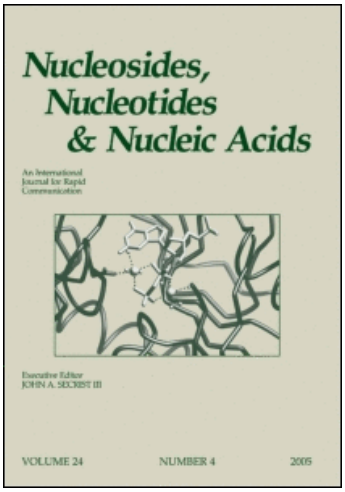

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ACCELERATION OF NUCLEIC ACID HYBRIDIZATION ON DNA MICROARRAYS DRIVEN BY pH TUNABLE MODIFICATIONS

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ACCELERATION OF NUCLEIC ACID HYBRIDIZATION ON DNA MICROARRAYS DRIVEN BY pH TUNABLE MODIFICATIONS

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ABSTRACT

A series of peptides containing histidine residues were designed as potential hybridization rate enhancers within a polymeric matrix of DNA microarrays. The polymeric matrix modified with these peptides showed strong attraction to DNA molecules under conditions of induction. DNA probes on the peptide-modified sites rapidly hybridized to their complementary targets with single base pair mismatch discrimination.

Recently, a collaboration between Motorola BioChip Systems and Genometrix was initiated to study kinetics of nucleic acid hybridization on DNA microarrays built on a polymeric matrix. The primary objective of our research has been to develop a "smart" polymeric matrix with a hybridization rate enhancement feature. It has been known that hybridization on DNA microarrays is impeded by diffusion of target molecules, particularly on those microarrays made out of porous matrices (1). Since nucleic acids are strong polyelectrolytes with negatively charged phosphodiester backbones, it is very reasonable to assume that a positively charged matrix will speed up the transport of nucleic acid molecules to their designated locations. Corey et al. reported that attaching an oligonucleotide to the lysine rich positively charged surface of staphylococcal nuclease increased its association constant (k_a) by 12,000 fold (2). Furthermore, the hybridization rate was enhanced 48,000 fold by an oligonucleotide conjugated with a lysine rich peptide (3). This enhancement

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Table 1. Sequences and Isoelectric Points* (pI) of Peptides, and Fluorescent Partition Data of Oligonucleotide Targets within the Polyacrylamide Film Containing pH Tunable Modifications

Modification	Sequence	pI	Relative Fluorescence
0	Background	–	14 ± 0.8
1	His-Phe-Glu-Gly	5.50	26.6 ± 3.8
2	His-Phe-Gly	7.19	35.0 ± 1.6
3	His-His-Phe-Glu	6.48	39.0 ± 1.6
4	His-His-Glu	6.48	38.3 ± 1.7
5	His-Glu	5.50	26.0 ± 1.4
6	His-Phe-Glu-Asn	5.50	31.3 ± 2.0
7	Histamine	–	45.0 ± 0.8
8	Histidine	7.19	36.3 ± 0.9

*Isoelectric points were calculated using Protein Calculator on www.scripps.edu/∼cdputnam/protcalc.html.

Experiment conditions: Cy3 labeled oligonucleotide targets in 150 mM N_aHCO_3 buffer at pH = 8.0 were allowed to bind for approximately 30 seconds to the array printed with spot modifications. Binding buffer was 5 mM sodium phosphate at pH 5.2. No binding is observed if pH is 7.0 or if the COOH groups are not capped with EDC + ethanolamine.

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was attributed to an increase in the effective concentration of DNA targets near the probe by electrostatic attraction.

Our study has been directed to the modification of a polymeric matrix with peptides. As listed in Table 1, a series of peptides containing histidine residues was designed as hybridization rate enhancers, which as well as histidine and histamine, were incorporated into a polymeric matrix at the addressed sites. Since these peptides are polyampholytes with pI values between 5.5–7.2, the modified matrix is allowed to modulate its electrostatic nature between the positive and negative polarity through subtle changes in pH. The polymeric matrix used in this study was a polyacrylamide film bearing NHS* esters photolithographically coated on a glass slide. Peptides and monomeric molecules were printed on the activated polyacrylamide film by robotic deposition, and the excess NHS esters blocked with ethanolamine in the presence of EDC†. As a result, each of these modification molecules was covalently attached to the film in an isolated location through formation of amide bonds. As can be seen from Table 1, all of these molecules demonstrated partitioning of DNA target molecules to specific addresses of modification under conditions of induction. The peptides containing two histidines as well as the monomers histidine and histamine showed the most robust attraction to DNA molecules. A critical step to the successful attraction was to attenuate the free carboxylate groups in the

*NHS: N-hydroxysuccinimide.

†EDC: (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride).

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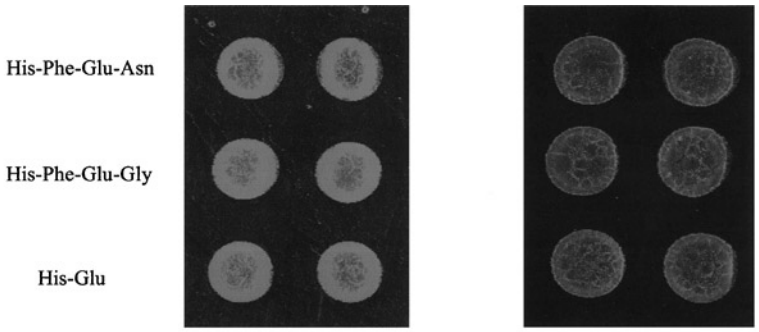
polymeric matrix. Since there was approximately 10% of active NHS ester in the blank polymeric matrix, a significant portion of carboxylate groups remained in the matrix due to hydrolysis of the ester. To neutralize this negatively charged component, EDC was introduced to the blocking solution to activate the carboxylates, which in turn were converted to N-hydroxyethylcarboxamide by ethanolamine. Without the EDC treatment, no inducible effects were observed with any of these modifications, presumably due to the overwhelming density of carboxylate groups within the polymeric matrix.

Three peptides were selected to assay specificity of hybridization within the peptide-modified matrix. The peptides were co-printed on the polymeric substrate with DNA probes representing a perfect match and a single base mismatch with respect to the target nucleic acid. As shown in Figure 1, the oligonucleotide arrays on the modified background rapidly hybridized to their target under the low pH and low salt conditions, while having a discrimination ratio of approximately 2.5:1 between perfect match and single base pair mismatch after washing with a pH $\sim$ 8.0 buffer.

We also studied the gene expression on the peptide-modified matrix. Preliminary data has shown that the expression signal intensity was doubled on average when the polymeric film was simply flooded with a peptide solution after the probes were printed (data not shown).

In summary, we have demonstrated a novel approach to perform hybridization within a charged matrix where the rate of hybridization was controlled by pH and ionic strength. Heller and coworkers reported that under an electric field, DNA molecules were rapidly transported to the positive electrodes within a minute (4)



Experimental details: DNA oligonucleotide probes co-printed with peptide modifications at equimolar (10 uM) concentrations. Target conc. = 10 nM, binding time = 1 min., in ~ 2mM NaPO4, pH 5.0; Wash time = 1 hour, 50 mM NaPO4, pH 8.0; Imaging performed using Packard Instrument laser scanner using CY3 optics. Quantitation of signal intensities with OptiQuant software from scanner

Figure 1. Hybridization of fluorescent target onpeptide-modified polymeric matrix.

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(5). One of the limitations of this technology is that it can only be applied to microelectronic arrays. As a chemical alternative, a polymer with pH tunable charges is unique material that might be exploited in fabrication of a new generation of DNA microarrays.

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