

The Use of Electrochemically Generated Reagents for Solid Phase Synthesis

COMBIMATRIX

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Company Profile

- Located near Seattle, WA
- Founded in 1995. Publicly traded since December '02
- 70 employees with diverse technical backgrounds
- Creates enabling technology for biotech and defense industries
 - CMOS microarray biochips
 - Instrumentation and informatics
 - Micro and nano scale processes
- Core technologies protected by 4US and 10 foreign patents with over 78 US and foreign patents pending
- First commercial products launched in March 2004

PRODUCTS



CustomArray chips and chip design services

Semiconductor chips that are programmed to synthesize microarrays according to customers specifications

12K

Sectored 4X2k

90K

Benchtop CustomArray Synthesizer

Automated system for the synthesis of DNA biochips

Electrochemical Detection system

Electrical sensing of hybridization/ligand binding to CustomArray microarray

CMOS Based Arrays of Electrodes



Surface of Semiconductor Arrays

902 12000 94000

92µm 44µm 25µm

Platinum microelectrodes
Silicon nitride insulation

Smaller Electrodes

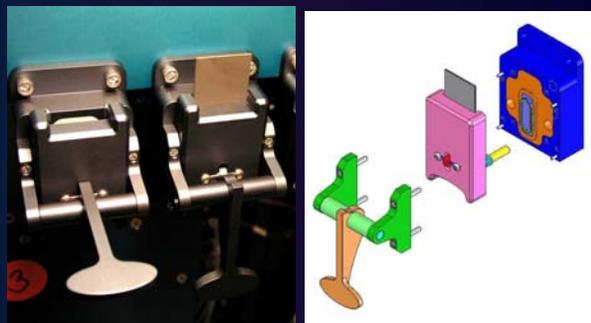
- 5 µm Diameter Vias
- Signal from hybridized target
- Smallest Edge-to-Edge Distance: 2.65mm
- Smallest Unit Cell: 58.5µm²
- Potential Density: 1,709,400/cm²

SEM of CombiMatrix Electrode Array

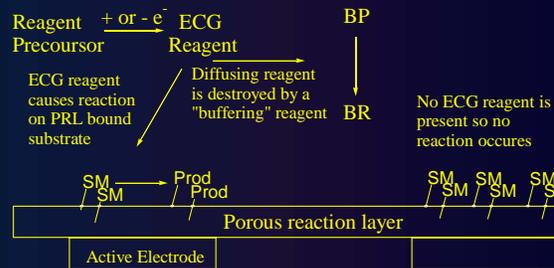
- One of many Porous Reaction Layers used by CombiMatrix

Another of our Porous Reaction Layers

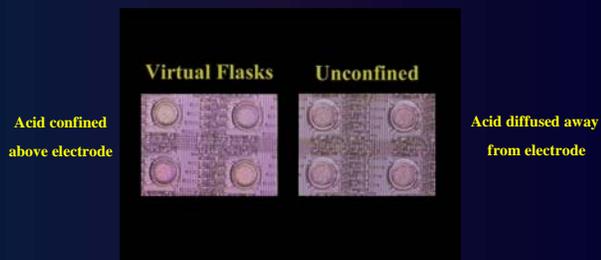
Macro to Micro Interface: Synthesis Chamber



Chemistry Overview

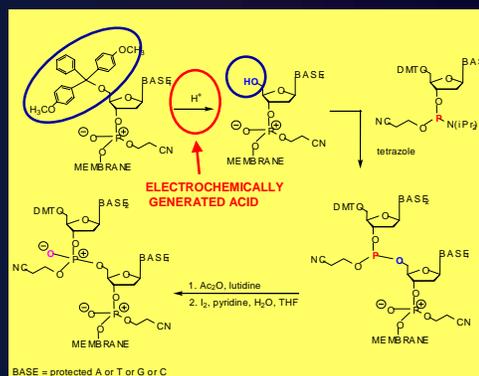


Proton Confinement

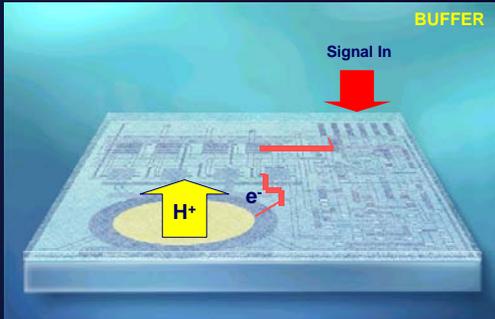


Bromophenol Blue dye added for illustrative purposes

Use of Electrochemically Generated Protons For DNA Synthesis

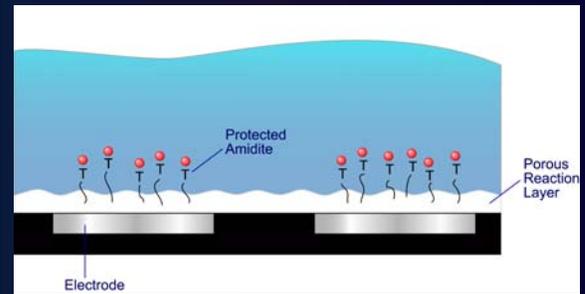


Electrochemical Synthesis (one electrode shown)

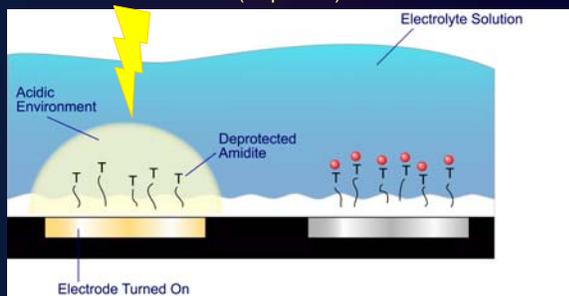


Applying a potential drives REDOX reaction to produce protons

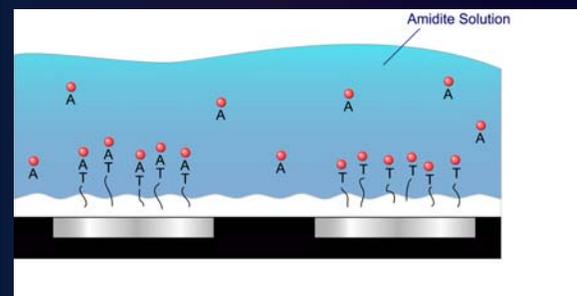
Start of Synthesis



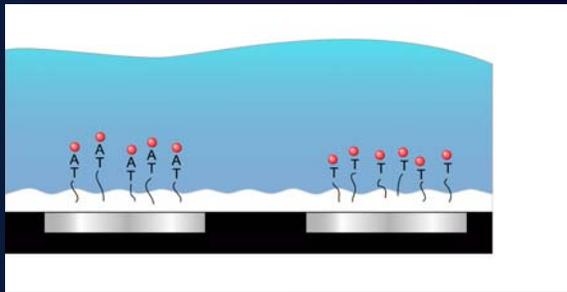
Electrochemically Detritylate (Deprotect)



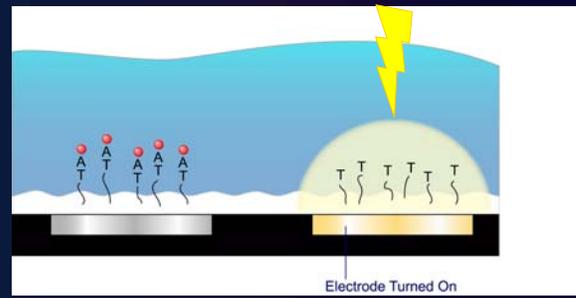
Couple



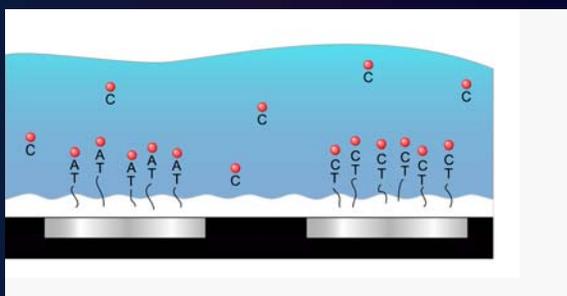
Wash and repeat process
with sequential amidite exposure



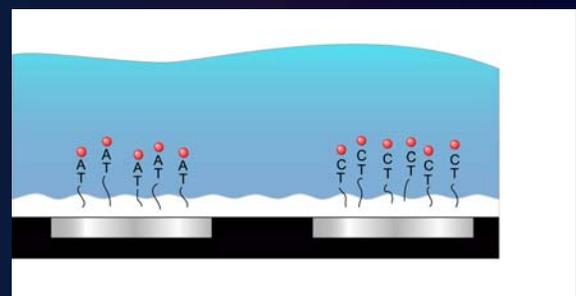
Deblock



Couple



Wash



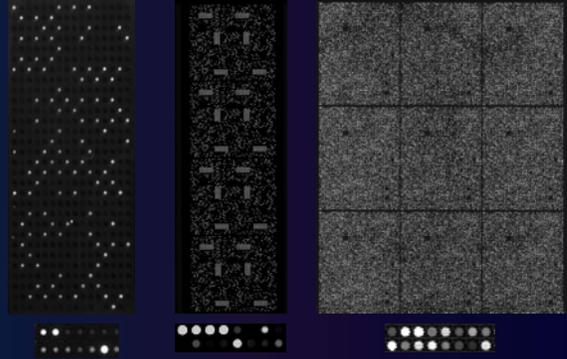
DNA chip Synthesizer



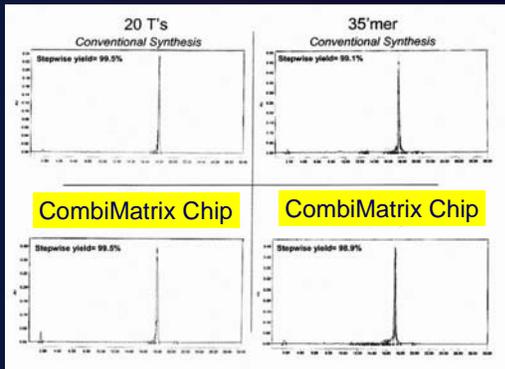
- Automates the 600 or so steps of synthesis:
 - Chips, reagents, chip layout files
- Every chip carries a unique barcode which can be traced to the silicon wafer manufacturing batch and is used to track the layout of the oligonucleotides synthesized on the chip
- Every synthesis run is logged to record which chemicals were exposed to the chip, in what order and the amplitude and frequency of electrical current applied to each electrode.

CustomArray Versions

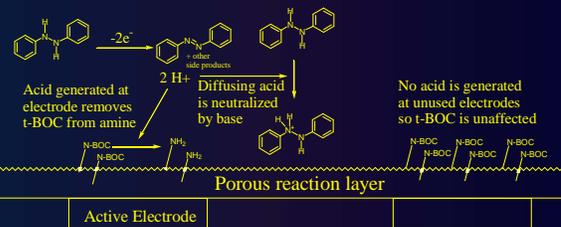
1k 12k 90k



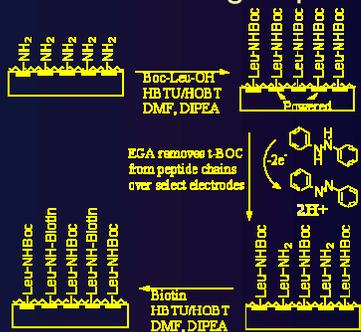
HPLC analysis of oligos cleaved of the chip (Destructive testing)



Second Method for ECG of Protons



Use of ECG Protons to Remove the t-BOC group



Use of ECG Protons to Construct a Peptide Array

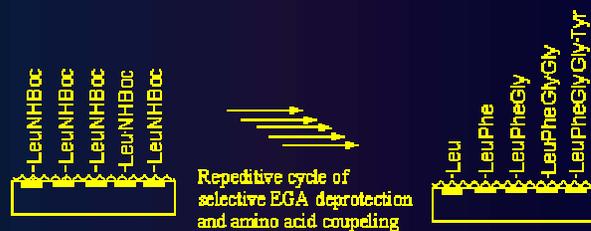


Image of Peptide Array After Labeling with Texas Red Antibody

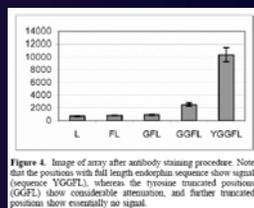
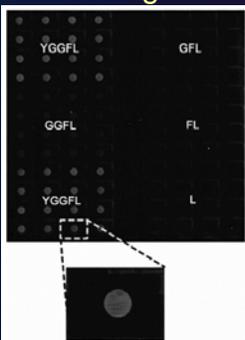
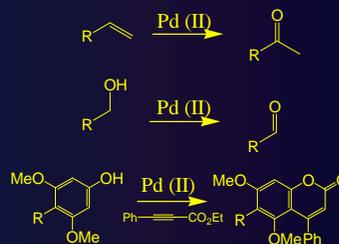


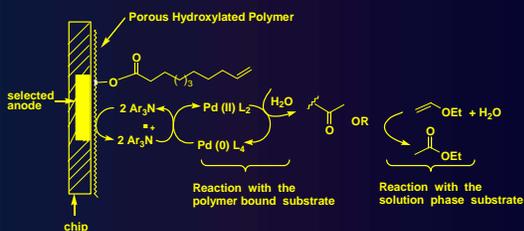
Figure 4. Image of array after antibody staining procedure. Note that the positions with full length endospine sequence show signal (sequence YGGFL), whereas the tyrosine truncated positions (GGFL) show considerable attenuation, and further truncated positions show essentially no signal.

J. Combi. Chem. 2005 p 637-640

Electrochemically Generated Reagent: Palladium (II)

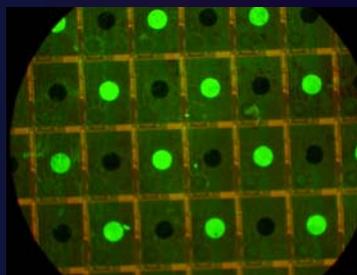


Use of Electrochemically generated Pd (II) in an Array Based Wacker Reaction

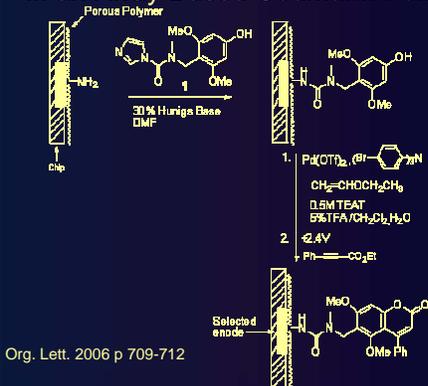


JACS 2004 p 6212-13

Example of an Antibody Labeled Array Derivatized by Pd (II)



Use of Electrochemically generated Pd (II) in an Array Based Coumarin Formation



Example of an Antibody Labeled Coumarin Array

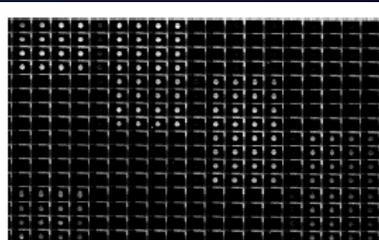
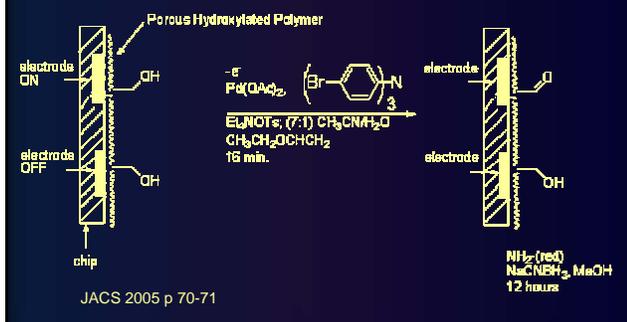
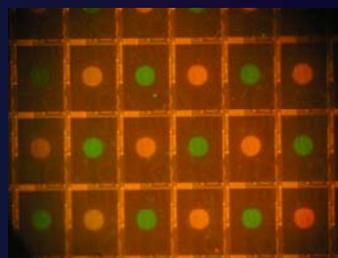


Figure 1. "Zigzag" pattern of electrodes used for site-selective coumarin synthesis.

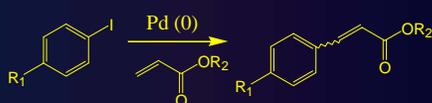
Use of Electrochemically generated Pd (II) in an Array Based Hydroxyl Oxidation



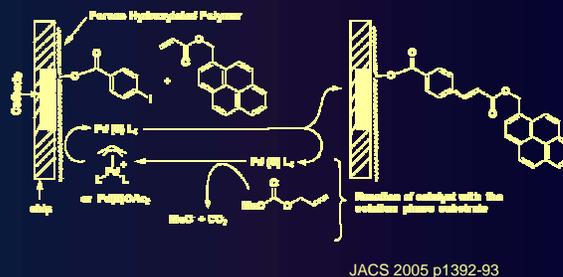
Example of Flours Selectively Immobilized by Reductive Amination



Electrochemically Generated Reagent: Palladium (0)



Example of the use of Palladium (0): Chip Based Heck Reaction



Example of Pyrene Labeled Array After Reaction

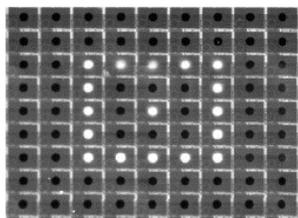


Figure 1. 1-Pyrenemethylacrylate was deposited at selected microelectrodes on an active semiconductor chip by using the electrodes as cathodes to reduce Pd(II) to Pd(0). The Pd(0) triggered a Heck reaction between the substrate and an aryl iodide on the chip's surface. Bright spots indicate electrodes that were activated.⁹

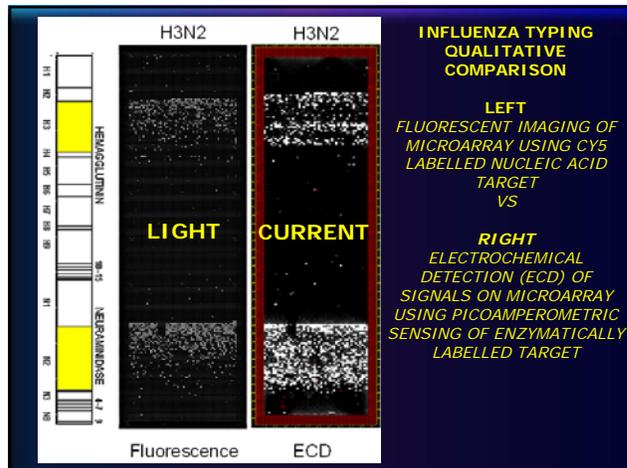
Electrodes can be useful for more than synthesis:

- Since electrodes are an integral part of the array they can be used for sensing once their job during synthesis is complete. HRP assay has allowed non-optical reading of DNA arrays
- Measurements of conductivity have been able to detect antibody binding.



•Low current sensor (100pA-low nA range)
(essentially a multiplex REDOX sensor with a microelectrode array element)

•Faraday cage
•Currents per electrode read off the chip



CV analysis of Coumarin array

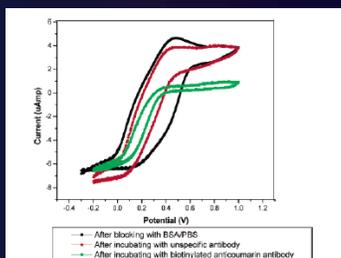


Figure 2. Cyclic voltammetry of ferrocene acetic acid on Pt electrodes with coumarin linked to a polymer through a single thymidine having an amino ethoxy ethyl terminating group. All measurements were performed in PBS buffer and the concentration of the ferrocene acetic acid was 8 mM.

Our Team:

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 - Kevin D. Moeller
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