

# Advancements in Electric Field Directed Self-Assembly Of Bio-Derivatized Nanoparticle Structures

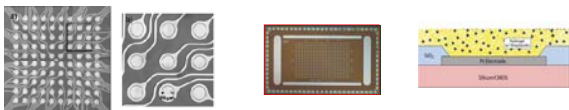
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## INTRODUCTION

One of the challenges in nanotechnology is the development of fabrication technologies that will lead to cost effective nanomanufacturing processes. Recently we have developed an electronic microarray process for the rapid and highly parallel directed self-assembly of protein and DNA derivatized nanoparticles into multi-layer structures. This process allows 3D structures with more than forty alternating nanoparticle layers to be completed in less than one hour. We have now shown the ability to incorporate active enzymes into the nanoparticle layers while retaining enzyme functionality. While the application of a DC electric field has proven very effective in fabrication of nanoparticle structures, there are other applications that can benefit from an AC electric field directed assembly. Although low conductivity medium provides optimal particle transport in a DC field, most biological functionality requires higher salt concentrations (therefore higher solution conductivity). In order to fabricate DNA nanoparticle structures in a DC field, the DNA hybridization between nanoparticle layers requires cationic stabilization without the addition of excessive ions. This was solved using zwitterionic histidine. Our recent advances using AC fields (dielectrophoresis) have allowed us to manipulate nanoparticles in higher conductivity solutions including 1x TBE and 1x PBS. Using this method we can construct structures without the use of special buffers while better maintaining biological functionality of the derivatized nanoparticles. We now intend to extend this technique to advance the layer-by-layer self-assembly process in high conductivity solutions.

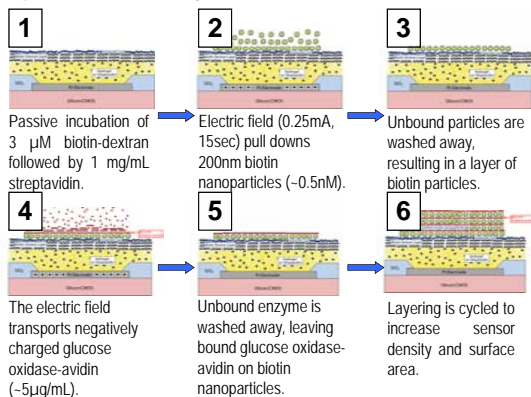
## MATERIALS AND METHODS

### CMOS Microarrays for DC/AC Electric Field Directed Assembly



This figure depicts the CMOS microarray chips used in the electric field directed assembly experiments. On the left is a 100 site array with a zoomed in 3x3 section; this device is used in conjunction with a function generator for AC dielectrophoretic assembly. On the right is a 400 site microarray used for DC directed assembly. The figure also shows a cross sectional view of an individual 50µm diameter electrode.

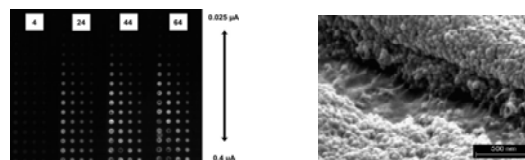
### Example of Assembly Procedure



## DC Directed Assembly

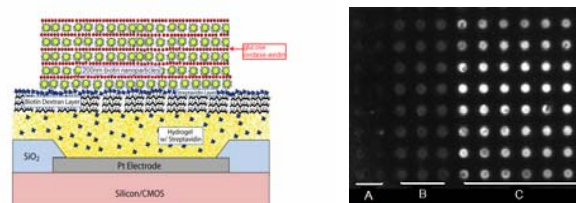
### DC assembly of Nanoparticles through Complementary DNA

Neutravidin-fluorescent nanoparticles were conjugated to complementary biotinylated ssDNA sequences and were subsequently assembled in a DC electric field in 100mM histidine buffer to more than 40 layers high. Zwitterionic histidine is required to stabilize the DNA hybridization in the low conductance solution.



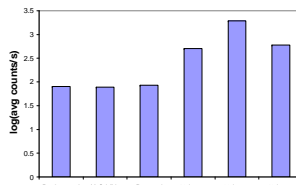
The figure above depicts the same section of array after 4, 24, 44, and 64 layers have been addressed. Within each time group, there is a current ramp from 0.025µA to 0.4µA and a time ramp from right to left of 0, 10, 15, 20, and 30 seconds deposition time. It can be seen that higher currents and prolonged deposition times lead to more rapid degradation of the layers. Also shown is an SEM image of a 40 layer structure of nanoparticles.

### Fabrication of Enzyme-linked Nanoparticle Layers



- A) No field
- B) Field on ONLY when 200nm biotin nanoparticles addressed
- C) Field on when BOTH biotin nanoparticles and glucose oxidase-avidin is addressed.

This figure shows that the diagrammed assembly process is required for proper formation of enzyme-nanoparticle layers. Section A indicates that fluorescent nanoparticles do not passively bind. In addition, the results from sections B and C demonstrate that the glucose oxidase-avidin addressing step is necessary for layer fabrication.



Enzyme-nanoparticle layers retain activity after assembly and removal from chip. There may be a loss of functionality when the enzyme is layered and bound to nanoparticles. In addition, non-uniform layer formations and layer loss during washes may attribute to variations in signal.

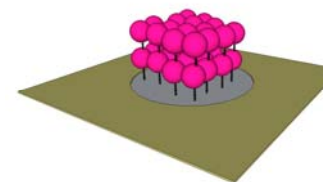
This procedure has successfully been repeated using streptavidinated horseradish peroxidase as well as an alternating pattern of streptavidin-HRP and glucose oxidase-avidin on the same electrode site.

## AC Directed Assembly

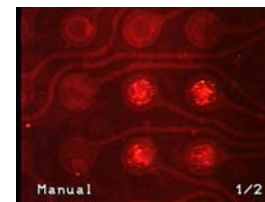
### AC Dielectrophoretic Assembly of DNA Nanoparticles

Recent work in our lab has shown the ability to use AC dielectrophoresis to separate nanoparticles and cells in higher conductivity solutions. We now investigate the assembly of complementary ssDNA-nanoparticles in a buffer solution of 1xTBE and 20mM NaCl. This method now circumvents the necessity of a zwitterionic histidine buffer and directs assembly parameters of nanostructures towards more physiological conditions.

Unlike DC electrophoresis, AC dielectrophoresis imparts a force on the particle in solution dependant on the gradient of the electric field and the relative permittivity of the particle to the medium. This allows uncharged particles to be manipulated in the electric field; a huge advantage over DC electrophoresis. Moreover, AC dielectrophoresis allows for much higher voltages with mitigated electrolysis, thus allowing for the usage of higher conductivity buffers that are more suitable for biological applications.



This figure is a simplified depiction of the ssDNA nanoparticle assembly atop an individual electrode. ssDNA is first immobilized onto the hydrogel surface through biotin-streptavidin interaction. Subsequently, under the influence of the AC electric field, the ssDNA nanoparticles are pulled down to the high field region, the electrode surface, where they bind to the complementary ssDNA immobilized on the surface. Subsequent layers are added as aforementioned. Figure not to scale.



This figure depicts the concentration of 40nm neutravidin polystyrene nanoparticles conjugated with biotin-ssDNA atop a 2x2 electrode section. The ssDNA nanoparticles are drawn to the high field region (the electrodes). The nanoparticles are under the influence of a 20Vpp, 10kHz AC sine wave applied for 10 minutes. The remaining electrodes in the figure were not turned on and therefore do not attract any ssDNA nanoparticles.

## CONCLUSIONS & FUTURE WORK

Electric field assisted self-assembly represents an example of combining some of the best aspects of "top-down" and "bottom-up" technologies into viable process for the hierarchical assembly and integration of nanocomponents into 3D structures. The process is now being used for the fabrication of bio/chem-sensor devices and in-vivo therapeutic/drug delivery devices; it may also prove useful for many nanoelectronic, nanophotonic, energy conversion (fuel cells, photovoltaics, batteries) and nanocomposite material applications.