# Electron-beam Patterned PEG Microgels for DNA Detection

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**Introduction**

Focused electron beam is used to create surface-patterned biotinylated poly(ethylene glycol) (PEG) microgels to which molecular beacon[1] (MB) probes are immobilized via biotin-streptavidin (SAv) binding. We hypothesized that immobilizing MB probes to highly hydrated microgels will maintain the high performance typical of untethered MB probes free in solution. Monte Carlo (MC) simulations of electron-polymer interactions show that the microgel mesh size resulting from focused radiation crosslinking is highly non-uniform and gradually approaches infinity at the microgel-water interface. This structure is important because the extremely liquidlike environment at the diffuse microgel surface reduces the compromising effects that hard surfaces can exert on MB secondary conformation, accessibility of nucleic acid targets to the MBs, and the efficiency of energy transfer between the MB fluorophore and quencher. We assess the performance of microgel-tethered MBs using beacons designed to distinguish between methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*. We show that each e-beam patterned microgel presents approximately 11,800 such beacons and that each beacon on average occupies an area of microgel surface corresponding to an individual SAv molecule. The signal-to-background ratio we measure ranges from 40-50, substantially higher than many other surface-tethering approaches. Furthermore, this platform exhibits both low non-specific background and high specific fluorescence when the microgel-tethered MBs are exposed to multiple targets, and it thus promises to lend itself well to high-sensitivity, self-reporting oligonucleotide based assays.

**Materials & Methods**

Biotin-PEG-Biotin (5000 g/mol) solution was spin-casted on a silicon wafer and formed a 65 nm polymer film. A Zeiss Auriga FIB-SEM CrossBeam Work Station equipped with a Nanometer Pattern Generation System was used to radiate the PEG film at 2 keV to generate the microhydrogel arrays.[2] The uncrosslinked polymers were dissolved and washed off by methanol. Through SAv-biotin route, the patterned microgels were subsequently functionalized with 3 types of MBs, in which *mecA, spa and bac16s* sequences (35-40 bases) were incorporated respectively. Their corresponding oligonucleotide targets were synthesized. Hybridizations were all carried at 50 $^{o}$C in PCR buffer. Fluorescence images were collected by Nikon E1000 fluorescence microscopy.

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[1] S. Tyagi and F. R. Kramer, Nat. Biotechnol. **14**, 303 (1996)
[2] X. Dai, W. Yang, E. Firlar, S. Marras and M. Libera, Soft Matter, DOI:10.1039/C1SM06702H (2012)

## Results & Discussion

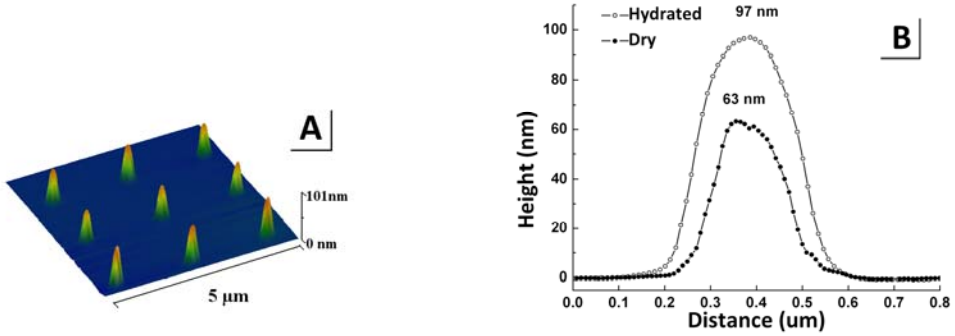


*Figure 1:* (A) AFM image of surface-patterned microgels formed by focused-electron-beam crosslinking of a biotinylated PEG thin film on a silanized silicon substrate; (B) line profile of microgel height in the dry and hydrated states.

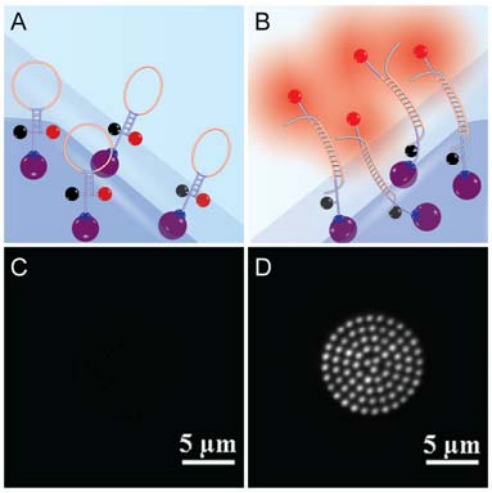


*Figure 2:* When no complementary targets are present (A), the molecular beacon assumes a hairpin conformation and no fluorescence is emitted. Hybridization of a molecular beacon probe to its complementary target (B) results in a conformational change of the molecular beacon, restoring fluorescence. Fluorescence images of a PEG microgel array functionalized with *mecA* molecular beacons before (C) and after (D) hybridization to its target are presented

*Table 1:* Signal-to-background ratios of MB probes in solution and tethered to surface-patterned microgels.

| Molecular beacon | In solution | Microgel tethered molecular beacons |
|---|---|---|
| *mecA* | 100.2 ± 1.5 | 45.6 ± 8.1 |
| *spa* | 106.7 ± 1.5 | 61.1 ± 6.9 |
| *bac16S* | 124.8 ± 6.1 | 48.5 ± 4.0 |

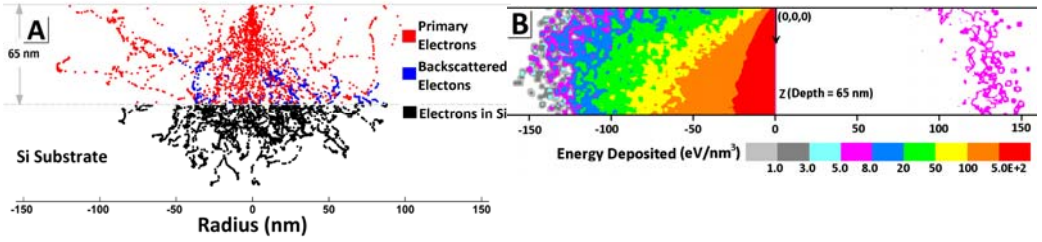


*Figure 3:* An MC simulation of 2 keV electrons incident on a 65 nm PEG film showing: (A) a Z-R section of the trajectories from a 100 electron simulation; (B) the energy deposition for a 320,000 electron simulation. The right half isolates only those voxels with the threshold energy for crosslinking.