PERFORMANCE REPORT

LRIR: FA9550-05-1-0424

Title: DNA Computing

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A microscopy system was acquired for molecular detection using molecular motor-driven nanodevices. This state of the art system incorporates a motorized stage that can move to successive adjacent fields of view, and stitch the digital color photos for analysis. The detection system utilizes the molecular motor attached to a slide and a gold nanorod that is visible by dark-field microscopy. Assembly of each nanodevice is dependent upon the presence of one molecule of target that bridges between motor and nanorod. Zeptomole sensitivity of target DNA is achieved without PCR or other means of amplification via molecular motor-dependent rotation. We have now demonstrated simultaneous, multiplexed detection of sequence specific DNA, RNA, and proteins unique to MRSA on the same platform with three separate colors of gold nanorods each having a different functionalization. We have also demonstrated the ability to differentiate between Staphylococcus aureus and MRSA as well as detection of target from crude cell lysate. We have written algorithms that can analyze the stitched digital photos of fields of view, correctly identify red, green, and yellow nanorods used to detect DNA, RNA, and proteins, respectively, then rapidly quantify the amount of each target present. Assembly of a commercial prototype of the detection device is underway.
**TASK A: DNA COMPUTING TO SOLVE OPTIMIZATION PROBLEMS**

*Funded by AFOSR and DARPA*

DNA hybridization was used to make probabilistic computations to identify the optimal path for fully connected asymmetric Traveling Salesman Problems in four steps: (1) Answer generation; (2) Answer sorting; (3) Answer Readout; (4) Optimal Answer Identification. The DNA concentration of starting sequences was controlled to allow the entire answer space to be sampled, with the constraint that the probability of forming a particular answer sequence was directly proportional to its optimality. We first used this procedure to solve an asymmetric 10 city TSP with small concentration differences of starting sequences, which tested the ability to find optimal paths among many closely suboptimal answers out of ~3.3 million possible answers.

We now report the ability to identify the optimal answer of a fully asymmetric 15 City TSP for which \( \sim 1.3 \times 10^{12} \) possible solutions exist. Starting DNA concentrations were used that clearly favored formation of one optimal answer, in contrast to the 10 city TSP problem. The high abundance of a subset of DNA to form the optimal answers relative to suboptimal answers increased the difficulty of optimal answer sorting and readout due to problems in PCR signal amplification. This was averted by generating enough initial answer without PCR amplification to allow purification of correct answers. Under these conditions it was possible to identify the optimal answer to the 15 City TSP by inspection directly from raw data, and is the largest problem ever solved by DNA computing. An answer pool for a 20 City TSP was also successfully generated, and answer-sorting completed. Answer analysis is underway.

We have created a novel “double-padlock” probe to increase the speed and accuracy during answer determination. This will allow the use of RT-PCR to rapidly quantitate amounts of city pairings formed in the answer sequences. Construction has also begun of 3D DNA structures for DNA computations. These structures are being designed to: (1) eliminate incorrect answer formation that consumes valuable DNA starting material, and (2) use thermodynamic stability to drive optimal answer generation in order to minimize formation of suboptimal answers. We have used a supercomputing cluster to optimize the thermodynamic stability of proposed 3D DNA structures prior to synthesis of components and assembly.

**TASK B: MICROSCOPY SYSTEM FOR MOLECULAR DETECTION USING NANODEVICES**

*Funded by AFOSR, DARPA, and DURIP*

A microscopy system was acquired for molecular detection using molecular motor-driven nanodevices. This state of the art system incorporates a motorized stage that can move to successive adjacent fields of view, and stitch the digital color photos for analysis. The detection system utilizes the molecular motor attached to a slide and a gold nanorod that is visible by dark-field microscopy. Assembly of each nanodevice is dependent upon the presence of one molecule of target that bridges between motor and nanorod. Zptomole sensitivity of target DNA is achieved without PCR or other means of amplification via molecular motor-dependent rotation. We have now demonstrated simultaneous, multiplexed detection of sequence specific DNA, RNA, and proteins unique to MRSA on the same platform with three separate colors of gold nanorods each having a different functionalization. We have also demonstrated the ability to differentiate between *Staphylococcus aureus* and MRSA as well as detection of target from crude cell lysate. We have written algorithms that can analyze the stitched digital photos of fields of view, correctly identify red, green, and yellow nanorods used to detect DNA, RNA, and proteins, respectively, then rapidly quantify the amount of each target present. Assembly of a commercial prototype of the detection device is underway.
Publications during past year:

Patents during past year:
1. Frasch, W.D., Spetzler, D. and York, J. “Methods for Generating a Distribution of Optimal Solutions to Nondeterministic Polynomial Optimization Problems”, Provisional filed
3. Frasch, W.D. and Spetzler, D. “Self-Regulating Answer Formation in DNA Computing via 3D Assembly of DNA”, Provisional Filed
4. Frasch, W. D. and Xiong, F. “Methods to Use a Double Lock Probe for Rapid and Quantitative Answer Determination of Nondeterministic Polynomial Optimization Problems, Provisional Filed.
Invited Presentations during this Past Year:
1. U.S. Army Research Office, Life Sciences Division, Research Triangle Park, NC
   “DNA Computing”
2. Edgewood Chemical Biological Center, U. S. Army RDECOM, Edgewood, MD,
   “Single-Molecule Detection of DNA via Sequence-Specific Links between F1-ATPase
   Motors and Gold Nanorod Sensors”
3. University of Pittsburgh, Department of Structural Biology, “The F1Fo ATP synthase-
   two molecular motors with two intertwined mechanisms.”
4. 13th International Meeting on DNA Computing (DNA13), Memphis, TN,
   “Probabilistic DNA Computing Solution to a Fully Connected 10-City Traveling
   Salesman Problem.”
5. FASEB Summer Research Conference on Transport ATPases: Genomics,
   Mechanisms, and Relevance to Diseases, Saxtons River, VT “Mechanism of Torque
   Generation by \textit{E. coli} Biomolecular Motor.” F1ATPase
6. Gordon Research Conference on Molecular and Cellular Bioenergetics, Andover, NH,
   “Mechanism of Torque Generation by \textit{E. coli} Biomolecular Motor.” F1ATPase
7. University of Illinois Urbana Champaign, Department of Biophysics, “Biomolecular
   motor-powered nanodevices for DNA detection, and their application for DNA
   Computing.”
8. University of Illinois Urbana Champaign, Department of Biochemistry, “Single
   Molecule Studies to Investigate the Rotary Mechanism of the F1Fo molecular motor.”
9. University of North Carolina-Chapel Hill, NC, Department of Biochemistry and
   Biophysics, “Single Molecule Studies to Investigate the Rotary Mechanism of the F1Fo
   molecular motor.”

Awards Past Year:
Best Graduate Student Paper at DNA 13 Conference awarded to David Spetzler, PhD
student in my lab.

Scientific or Technological Transitions this Year:
New Funding from Science Foundation Arizona- Small Catalytic Business Grant for
Biosensing Project.

Founded Zeptometrics, a new biotechnology company, beginning to build a prototype of
a commercial instrument for Biosensing using the technology developed by AFOSR.
Initial instrument design will be for rapid detection of \textit{Staphylococcus aureus} and MRSA.
Ultimate goal is to build a hand-held device.

Began hospital trials using the device on patient samples.