Highly specific, multiplexed isothermal pathogen detection with fluorescent aptamer readout

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Monday, February 7th, 3:30 – virtual presentation

Zoom Link:
https://ucf.zoom.us/j/95114459377?pwd=aDIzUjhxZjNkRHZZV1YybEprcXVQdz09

Meeting ID: 951 1445 9377
Passcode: 699971

Host: Yulia Gerasimova

Isothermal, cell-free, synthetic biology-based approaches to pathogen detection leverage the power of tools available in biological systems, such as highly active polymerases compatible with lyophilization, without the complexity inherent to live-cell systems Nucleic Acid Sequence Based Amplification (NASBA) is one well-known example. Despite the reduced complexity associated with cell-free systems, side reactions are a common characteristic of these systems. As a result, these systems often exhibit false positives from reactions lacking an amplicon. Here we show that the inclusion of a DNA duplex lacking a promoter and unassociated with the amplicon, fully suppresses false positives, enabling a suite of fluorescent aptamers to be used as NASBA tags (Apta-NASBA). Apta-NASBA has a 1 pM detection limit and can provide multiplexed, multicolor fluorescent

readout. Furthermore, Apta-NASBA can be performed using a variety of equipment, for example a fluorescence microplate reader, a qPCR instrument, or an ultra-low-cost Raspberry Pi-based 3D-printed detection platform employing a cell phone camera module, compatible with field detection.Isothermal, cell-free, synthetic biology-based approaches to pathogen detection leverage the power of tools available in biological systems, such as highly active polymerases compatible with lyophilization, without the complexity inherent to live-cell systems Nucleic Acid Sequence Based Amplification (NASBA) is one well-known example. Despite the reduced complexity associated with cell-free systems, side reactions are a common characteristic of these systems. As a result, these systems often exhibit false positives from reactions lacking an amplicon. Here we show that the inclusion of a DNA duplex lacking a promoter and unassociated with the amplicon, fully suppresses false positives, enabling a suite of fluorescent aptamers to be used as NASBA tags (Apta-NASBA). Apta-NASBA has a 1 pM detection limit and can provide multiplexed, multicolor fluorescent readout. Furthermore, Apta-NASBA can be performed using a variety of equipment, for example a fluorescence microplate reader, a qPCR instrument, or an ultra-low-cost Raspberry Pi-based 3D-printed detection platform employing a cell phone camera module, compatible with field detection.