Localizing DNA into Peptide Liquid-Liquid Coacervates

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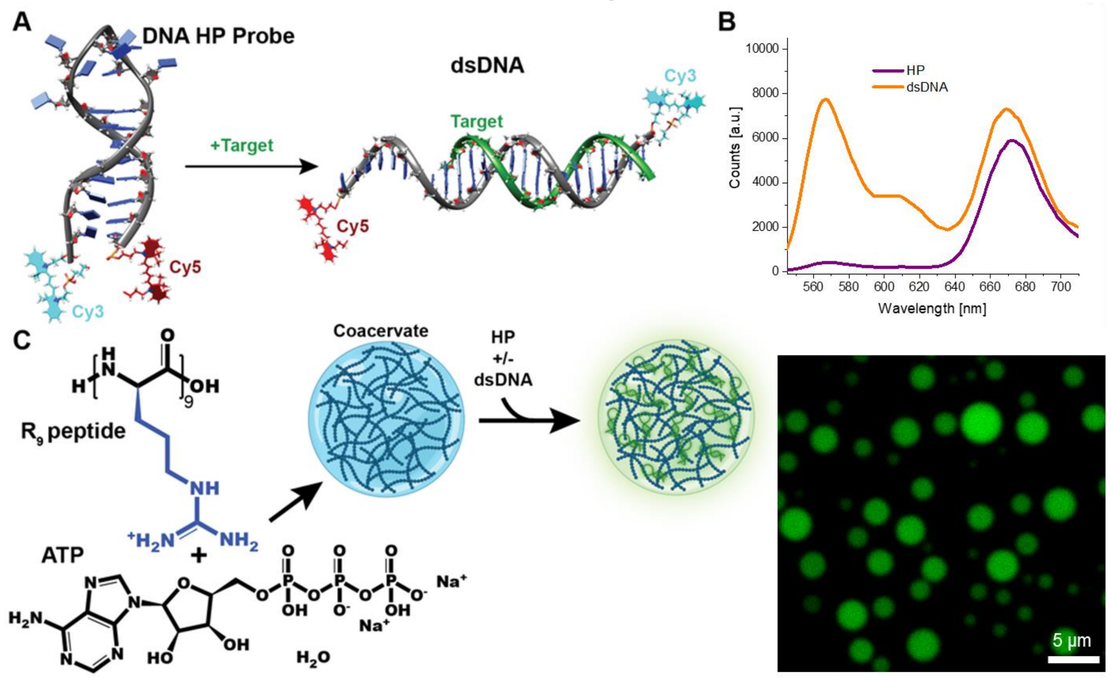
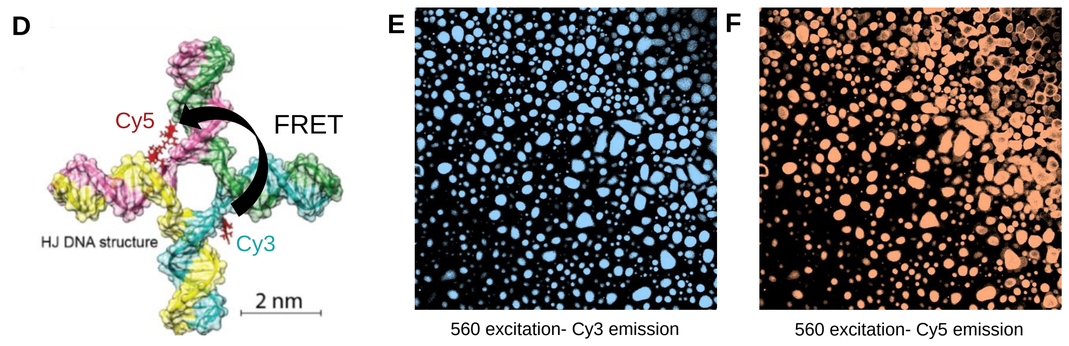
Motivation

DNA biosensing and nanotechnology are combined with peptide based liquid-liquid phase separations, known as coacervates. Coacervates are neither pure homogenous liquid phase nor a heterogeneous aggregate, displaying varying degrees of order inside a mostly disordered system. Coacervates are of keen interest, where they function as a membraneless organelle, co-localizing DNA nanostructures and biosensors within coacervates allows for enhanced concentration and combinatorial function.

DNA biosensing

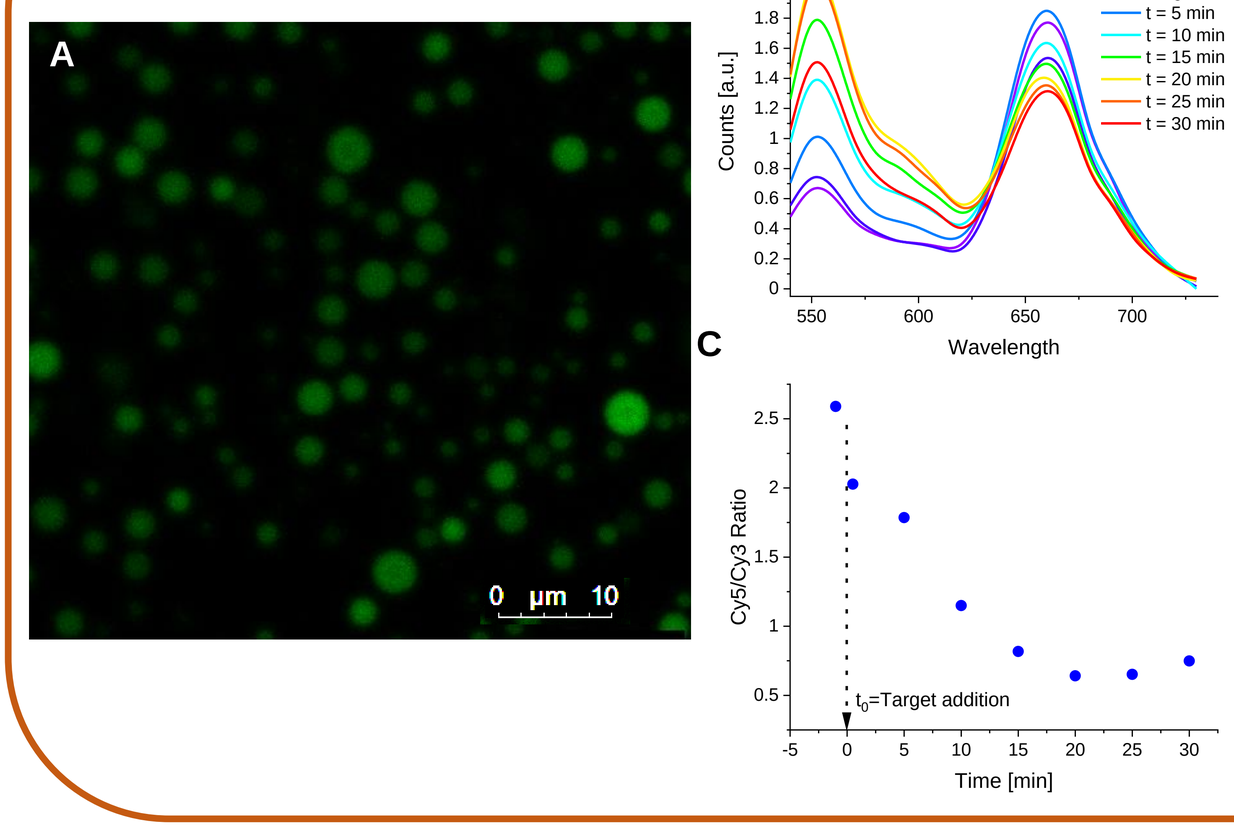


DNA Nanostructures



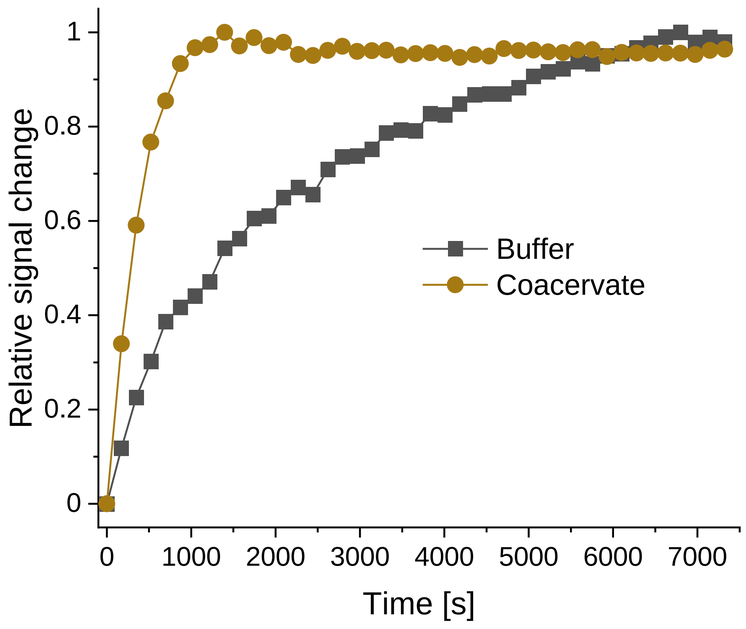
A) DNA molecular beacon (MB) in the hairpin (HP) conformation, where addition of nucleotide target transforms it into the double stranded (dsDNA) form. The MB is composed of a Cy3 donor and Cy5 acceptor FRET pair. B) Fluorescence spectra of the two MB conformations. C) Combination of polyarginine peptide (R_9) and ATP results in coacervate formation. Addition of the MB allows for sequestration of the DNA into the coacervate, resulting in localization of the fluorescence within the coacervate as seen in the fluorescent micrograph. D) 4-strand DNA nanostructure called a holiday junction (HJ) with a Cy3-Cy5 FRET pair integrated. The FRET signal from the Cy5 will only be observed if the structure is fully formed. E) Fluorescence microscope image of R_9-ATP coacervate with HJ DNA nanostructure. Excitation of Cy3 dye and observed Cy3 emission. F) Image from left with Excitation of Cy3 dye and observed Cy5 emission, confirming FRET and DNA nanostructure stability within coacervate.

DNA Biosensor within Coacervate Characterization

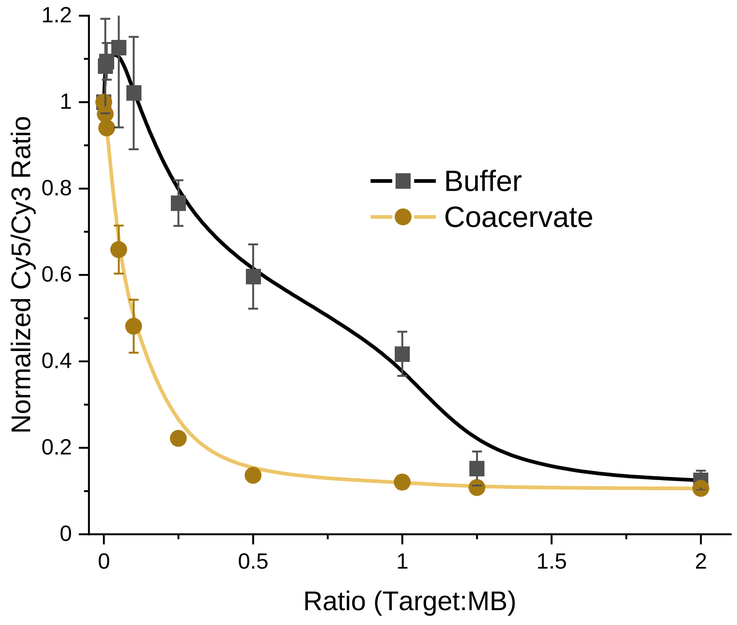


A) Fluorescence microscopy of R_9-ATP coacervate with MB biosensor.
B) Spectra from microscopy images in (A) obtained after addition of Target strand.
C) Fluorescence emission ratio through time after target addition.
D) Fluorescence emission of DNA biosensing in buffer solution.
E) Same concentration of dyes as in (D) but within coacervates. Fluorescence intensity enhanced ~3-fold, improves signal-to-noise of biosensing.
F) FRAP experiment showing that the DNA within the coacervate system is dynamic.

Results



Biosensing with coacervates has a 4.4 ± 1.7 times quicker response time



- **Biosensing with coacervates has a > 20-fold decrease in Limit-of-Detection (LOD)**
- **Biosensing with coacervates has a ~ 8-fold improvement on Signal-to-Noise (S/N)**

Conclusions

- Coacervates sequester oligonucleotides based on charge. Local concentration imcreased 3-4 orders of magnitude.
- Order of addition does not modify the results.
- Kinetics of DNA hybridization are improved
- Greater viscosity within coacervates enhances dye fluorescence, resulting in greater S/N
- Greater local concentration drastically decreases the LOD of oligonucleotide biosensing.

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