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Assembly and Characterization of Oligonucleotide-Polycation Complexes

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Abstract

We have characterized bulk complexes of oligonucleotides with poly-L-lysine (p(L)K) in an attempt to better understand the behavior of polyelectrolytes in micelles' complex cores made from the same materials. These biocompatible polymers can be synthesized with well-defined lengths and low polydispersities, which allows us to effectively vary polymer length together with parameters like DNA hybridization state, salt concentration, charge ratio, and urea concentration to explore a wide parameter space, and exert greater control over the phase and stability of the final complexes. We used methods including optical microscopy, fluorescence microscopy and FRET, circular dichroism, and IR spectroscopy to characterize the phase and behavior of these complexes.

Introduction

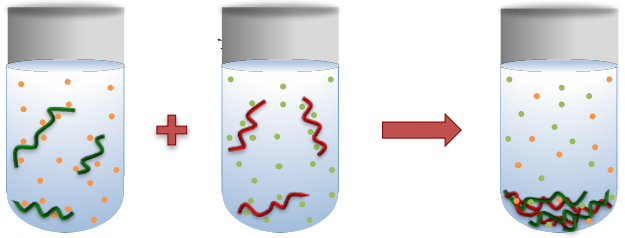


Fig. 1: Interaction of oppositely-charged polymers produces macrophase-separated complexes

Phase control of complexes

- Double-stranded DNA forms solid precipitates, while single-stranded DNA forms liquid coacervates when mixed with poly-L-lysine.
- The addition of NaCl causes charge screening, and eventually melts solid precipitates into liquid coacervates.
- At higher salt concentrations, complexes dissolve completely. We can quantify this behavior by measuring the free DNA in solution.

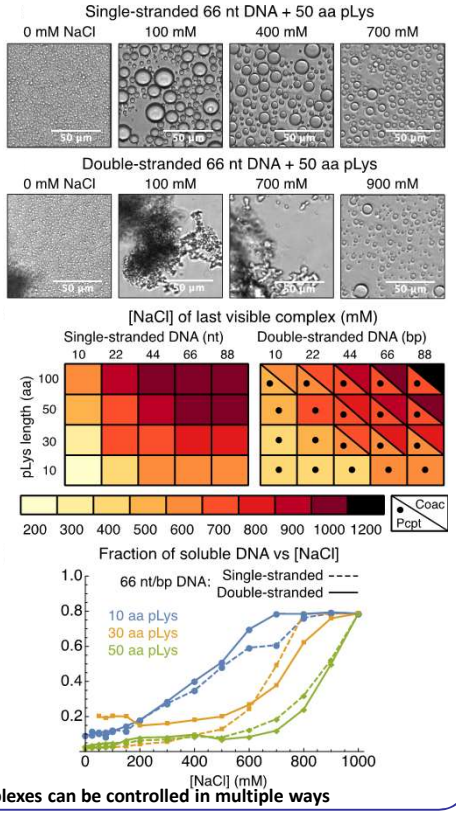


Fig.2: The phase of the complexes can be controlled in multiple ways

Nucleic acids remain competent for hybridization in complexes

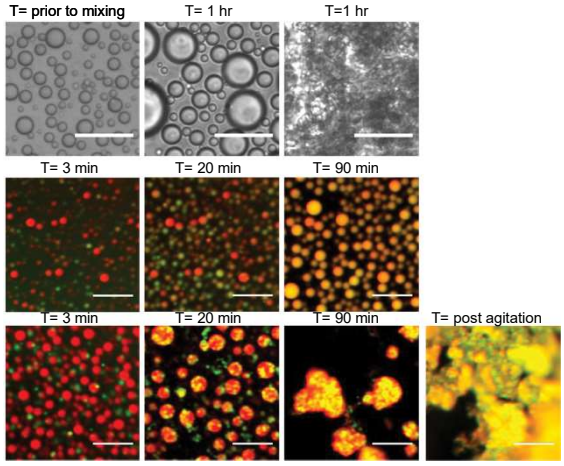
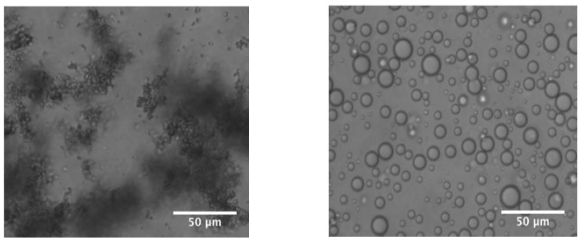


Fig. 3: Fluorescence microscopy can be used to follow DNA hybridization. Dyes colocalize in the complexes as nucleic acids are exchanged between them. Complementary nucleic acid strands hybridize and turn liquid coacervates into solid precipitates. Scale bars are 50μm.

Nucleic acid charge density can determine phase of complexes

22 nt mDNA + 22 nt DNArc + p(L)K 50 (77% charge density)

Double-stranded 22 nt mDNA + p(L)K 50 (55% charge density)



- Using methyl-phosphonated nucleic acid backbones allows us to decrease the charge density of single and double-stranded DNA.
- When the charge density of double-stranded DNA is lowered to 55% of its original value, liquid coacervates are formed instead of solid precipitates.

Fig. 4: Nucleic acid charge density controls complex phase

Conclusions

We have successfully characterized the phase of bulk complexes of oligonucleotides with poly-L-lysine over a wide range of polymer lengths, salt concentrations, and charge densities. Single-stranded DNA was observed to form liquid coacervates, while double-stranded DNA formed solid precipitates. We have also demonstrated control over the phase, solid or liquid, of the complexes. Nucleic acid hybridization can be used to convert liquid coacervates into solid precipitates, and increasing salt concentrations can convert solid precipitates into liquid coacervates. This work can contribute to improved assembly design using these biomolecules, improves our fundamental understanding of nucleic acid interactions with polycations, and could be used as an indicator of DNA sequence recognition in a sensor format.

Acknowledgement

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