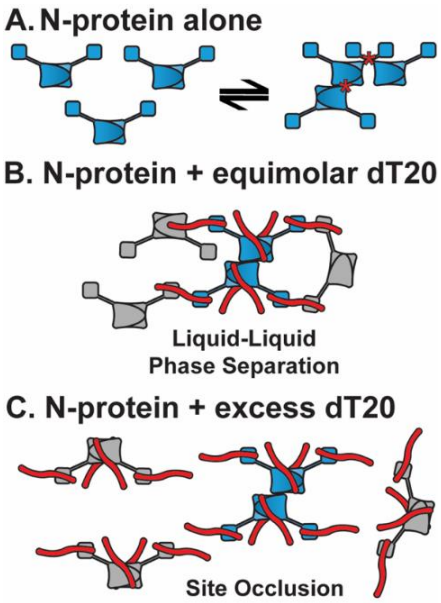
QCB Evenings

Tuesday, April 23rd • 5:30 – 6:30 PM • SI001

A narrow ratio of nucleic acid to SARS-CoV-2 N-protein enables phase separation.

Patrick Laughlin, Zlotnick Lab

SARS-CoV-2 Nucleocapsid protein (**N**) is a viral structural protein that packages the 30kb genomic RNA inside virions and forms condensates within infected cells through liquid-liquid phase separation (**LLPS**). N, in both soluble and condensed forms, has accessory roles in the viral life cycle including genome replication and immunosuppression. The ability to perform these tasks depends on phase separation and its reversibility. The conditions that stabilize and destabilize N condensates and the role of N-N interactions are poorly understood. We have investigated LLPS formation and dissolution in a minimalist system comprised of N protein and an ssDNA oligomer just long enough to support assembly. The short oligo allows us to focus on the role of N-N interaction. We have developed a sensitive FRET assay to interrogate LLPS assembly reactions from the perspective of the oligonucleotide. We find that N alone can form oligomers but that oligonucleotide enables their assembly into a three-dimensional phase. At a ~1:1 ratio of N to oligonucleotide LLPS formation is maximal. We find that a modest excess of N or of nucleic acid causes the LLPS to break down catastrophically. Under the conditions examined here assembly has a critical concentration of about 1 μM. The responsiveness of N condensates to their environment may have biological consequences. A better understanding of how nucleic acid modulates N-N association will shed light on condensate activity and could inform antiviral strategies targeting LLPS.



Structure of HIV-1 RRE stem-loop II identifies two conformational states of the high-affinity Rev binding site.

Jerricho Tipo, Choi Lab

During HIV infection, specific RNA-protein interaction between the Rev response element (RRE) and viral Rev protein is required for nuclear export of intron-containing viral mRNA transcripts. Rev initially binds the high-affinity site in stem-loop II, which promotes oligomerization of additional Rev proteins on RRE. Here, we present the crystal structure of RRE stem-loop II in distinct closed and open conformations. The high-affinity Rev-binding site is located within the three-way junction rather than the predicted stem IIB. The closed and open conformers differ in their non-canonical interactions within the three-way junction, and only the open conformation has the widened major groove conducive to initial Rev interaction. Rev binding assays show that RRE stem-loop II has high- and low-affinity binding sites, each of which binds a Rev dimer. We propose a binding model, wherein Rev-binding sites on RRE are sequentially created through structural rearrangements induced by Rev-RRE interactions.

Free pizza and drinks! **All students and postdocs are welcome!**