

# Supplementary Figures

## Energetic and structural features of SARS-CoV-2 N-protein co-assemblies with nucleic acids

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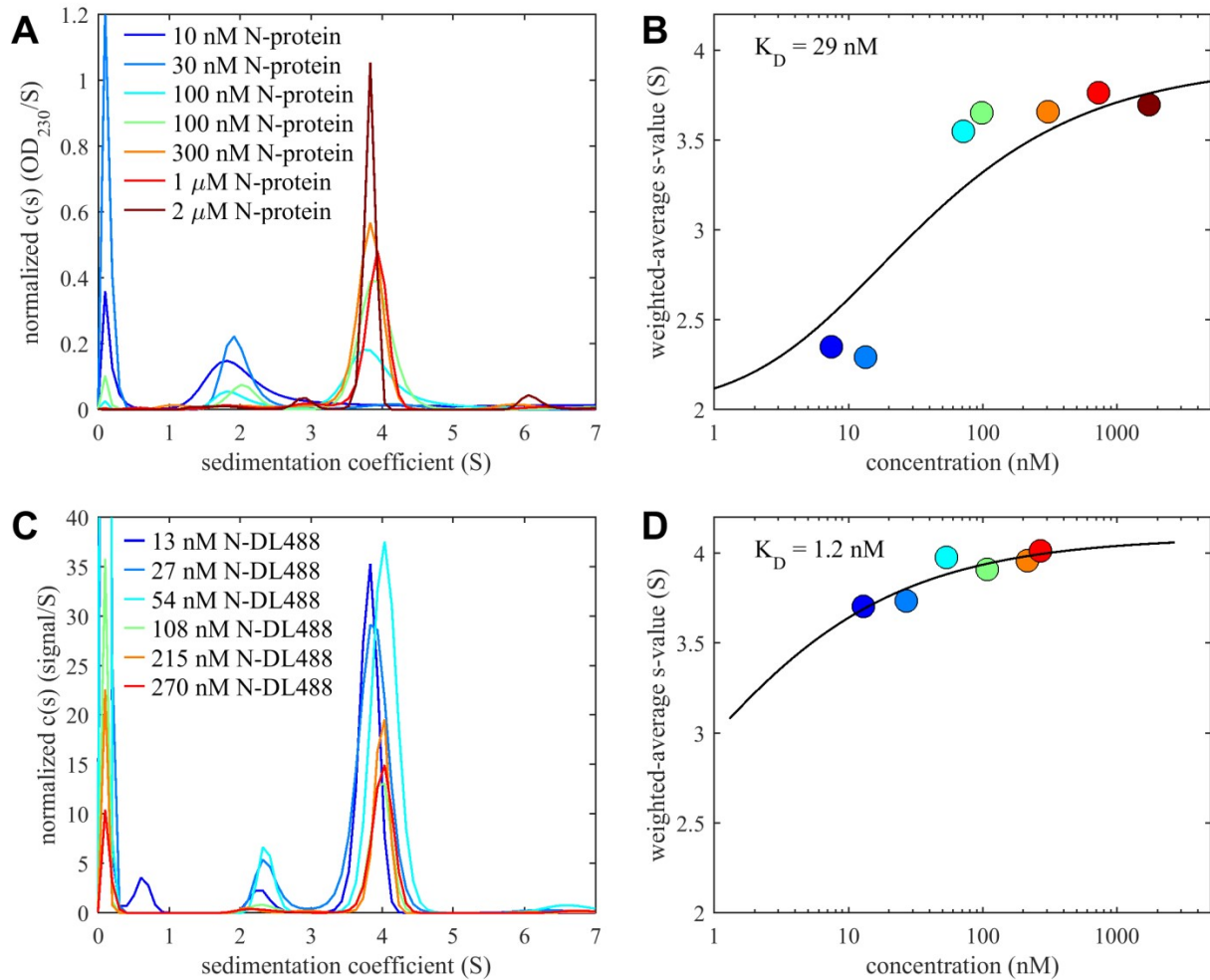
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**Figure S1: N-Protein Dimer Dissociation**



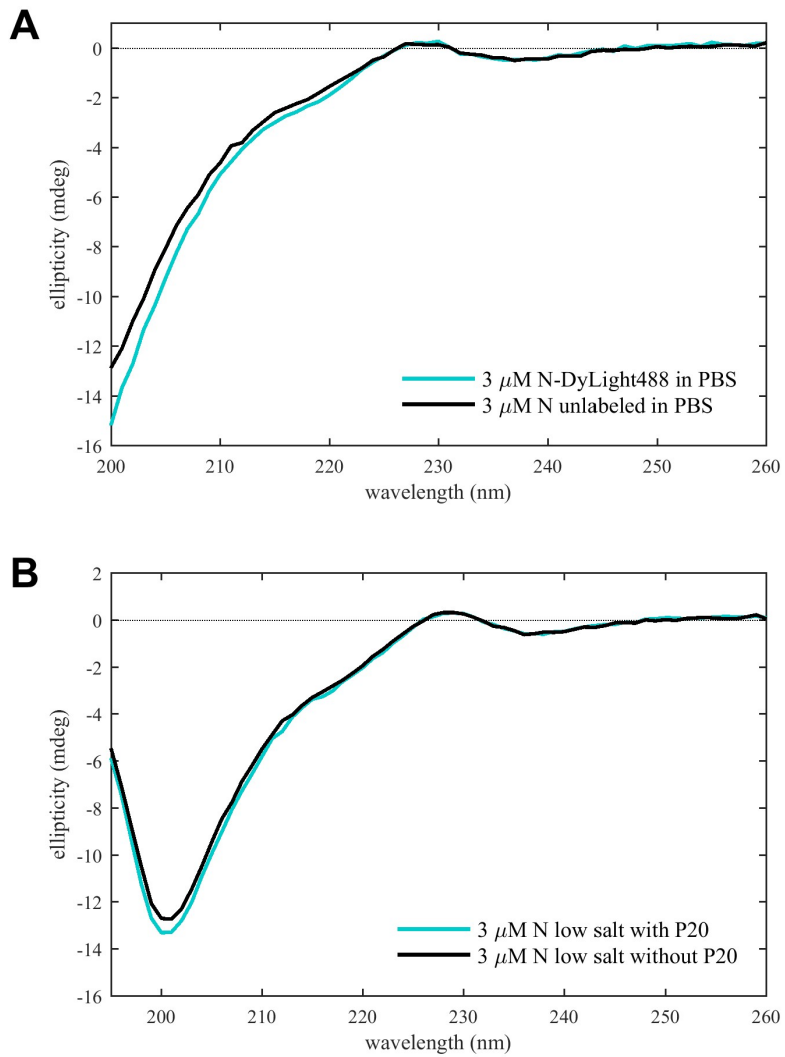
(A) Sedimentation coefficient distributions of N-protein in PBS supplemented with 0.005% surfactant P20, obtained from SV-AUC experiments using far-UV detection at 230 nm. Shown are traces labeled with nominal loading concentrations (including a replicate at 100 nM).

(B) Isotherm of weight-average s-values of N-protein with P20 shown in Panel A, and best-fit monomer-dimer model. Concentrations are determined from integrated sedimentation boundary amplitudes.

(C) Sedimentation coefficient distributions of DyLight488-labeled N-protein in PBS based on SV-AUC using fluorescence detection (MacGregor et al., 2004; Zhao et al., 2013).

(D) Isotherm of weight-average s-values of DyLight-488-labeled N-protein in Panel C, and best-fit monomer-dimer model.

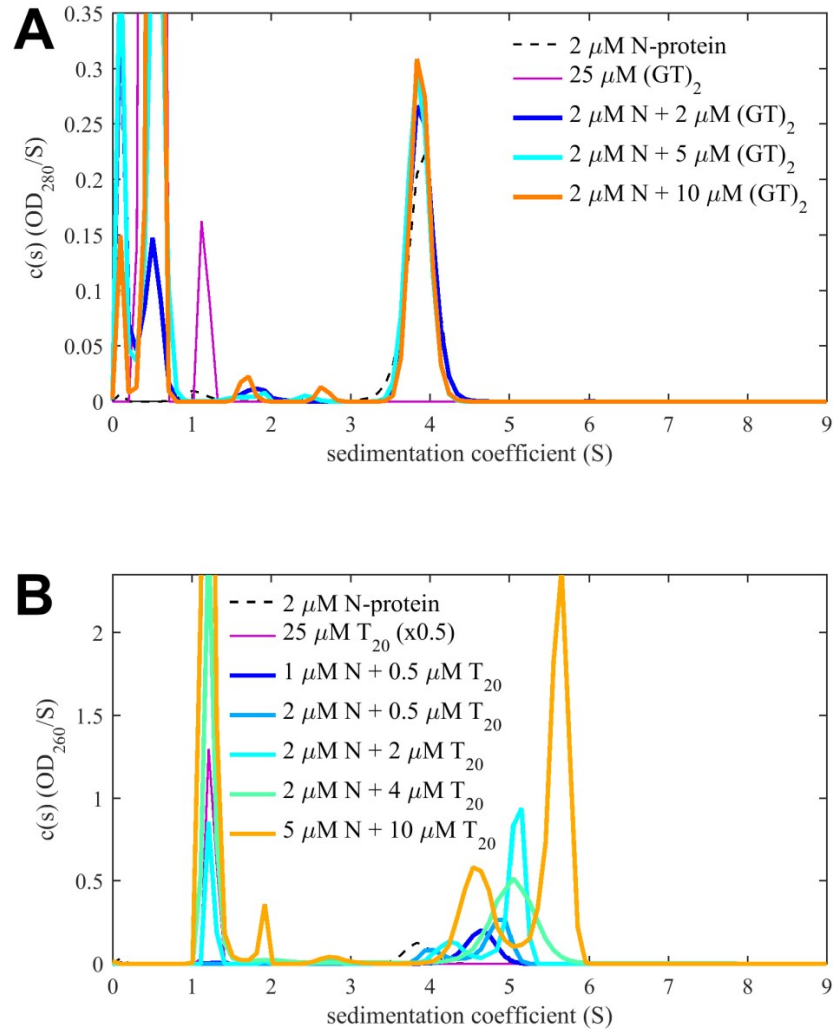
Figure S2: Circular Dichroism Spectra of N-protein with surfactant P20 or with fluorescent tag



(A) Comparison of CD spectra in PBS of unlabeled and DyLight488-labeled protein.

(B) Comparison of CD spectra of unlabeled N-protein in 12 mM  $\text{KH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ , 3 mM KCl, 10 mM NaCl, pH 7.4 and in the same buffer supplemented with 0.005% P20.

**Figure S3: Oligonucleotide binding to N-protein in high ionic strength buffer**



(A) Sedimentation coefficient distributions of mixtures of N-protein and (GT)<sub>2</sub> in PBS show no increase in s-value and no significant increase in boundary amplitudes with (GT)<sub>2</sub> concentration.

(B) Concentration series of N-protein with T<sub>20</sub> in PBS shows significant binding, but at strongly reduced level than in low salt buffer, exhibiting lower complex sedimentation coefficients.

**Figure S4: Reversibility of droplet formation of N-protein with T<sub>20</sub>.**

Widefield microscopy image of 5  $\mu$ M N-protein with 10  $\mu$ M T<sub>20</sub> before (A) and after (B) twofold dilution.

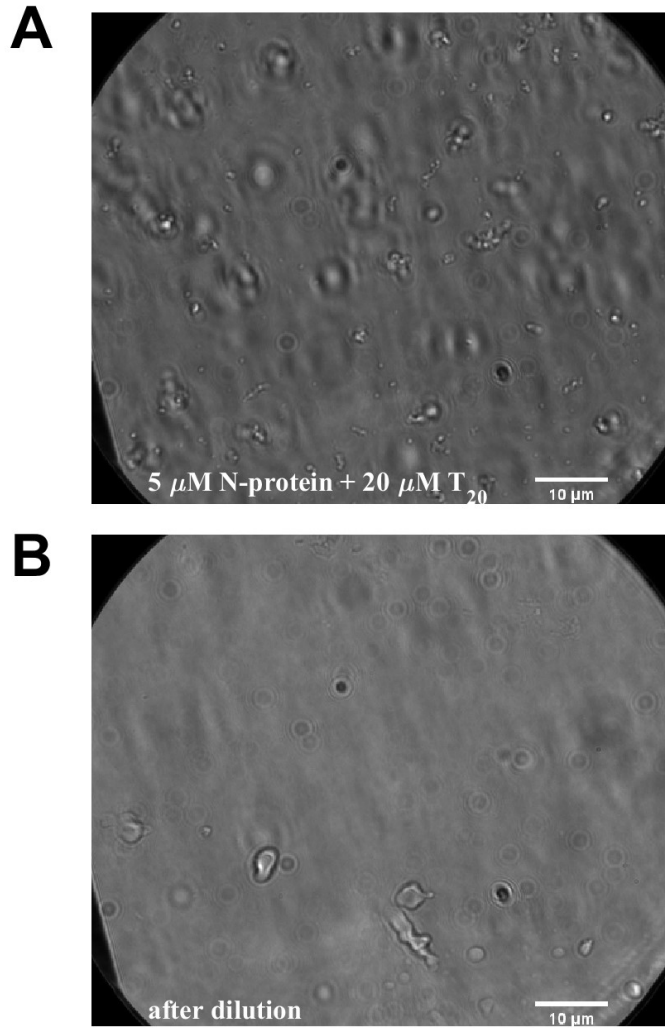
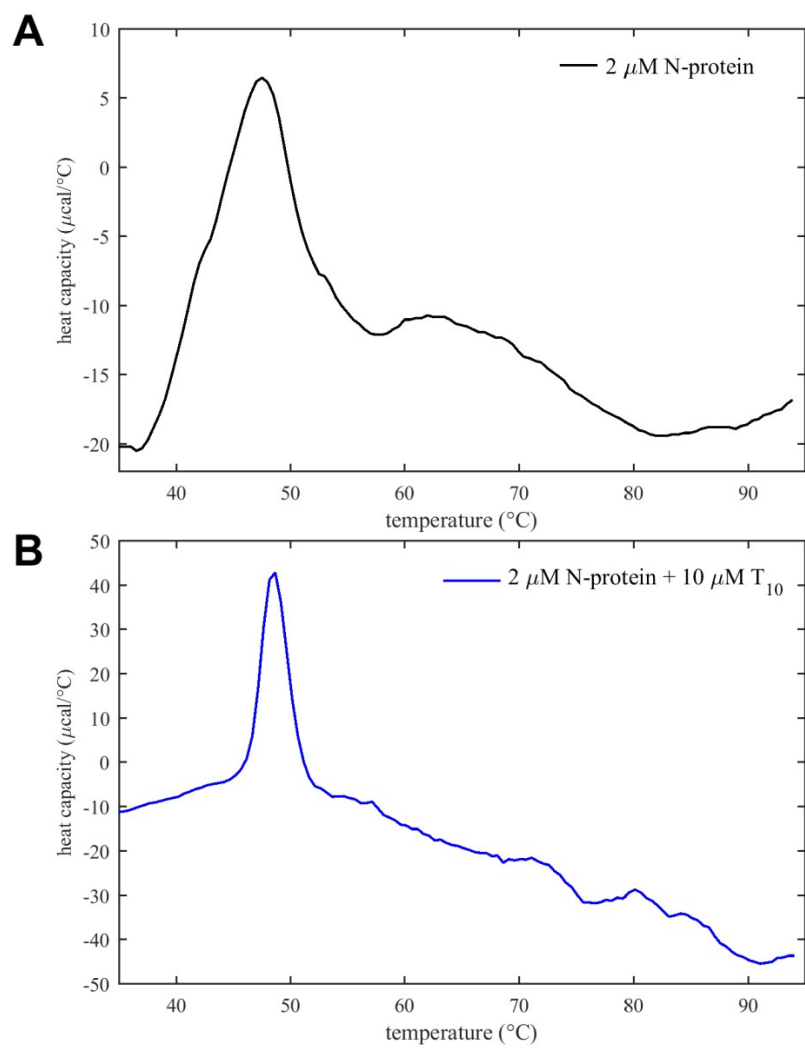
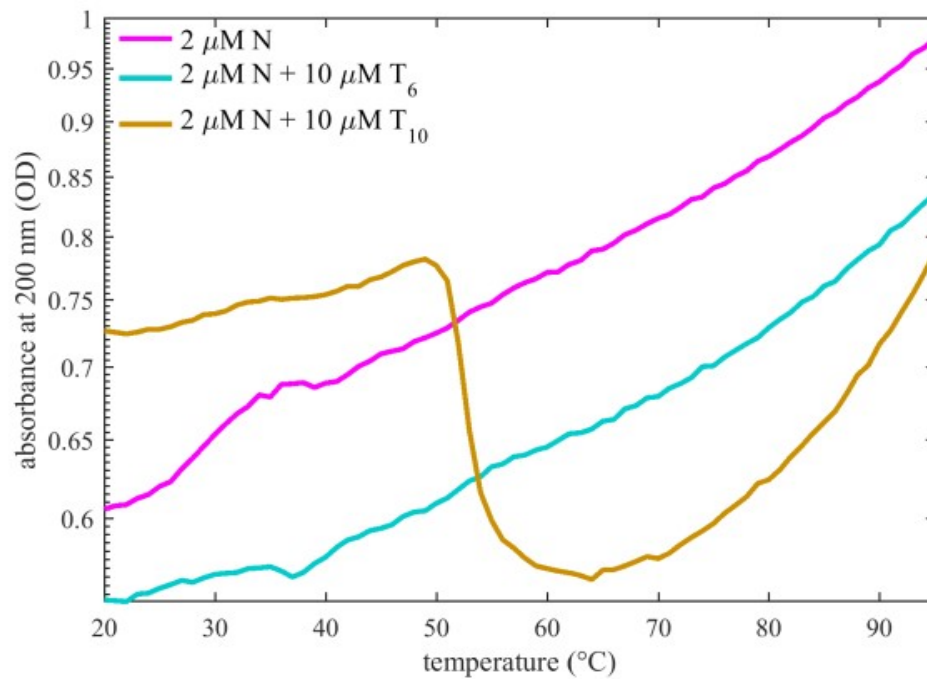


Figure S5: Differential scanning calorimetry of N-protein with T<sub>10</sub>.



**Figure S6: Far-UV absorbance during temperature scan**

Absorbance traces at 200 nm recorded during CD temperature scans in **Figure 7**.



**Figure S7: Absorbance spectrum of N-protein**

Shown is a 1:10 dilution of N-protein stock solution after dialysis against working buffer.

