

Supplementary Materials: Binding of RNA by the Nucleoproteins of Influenza Viruses A and B

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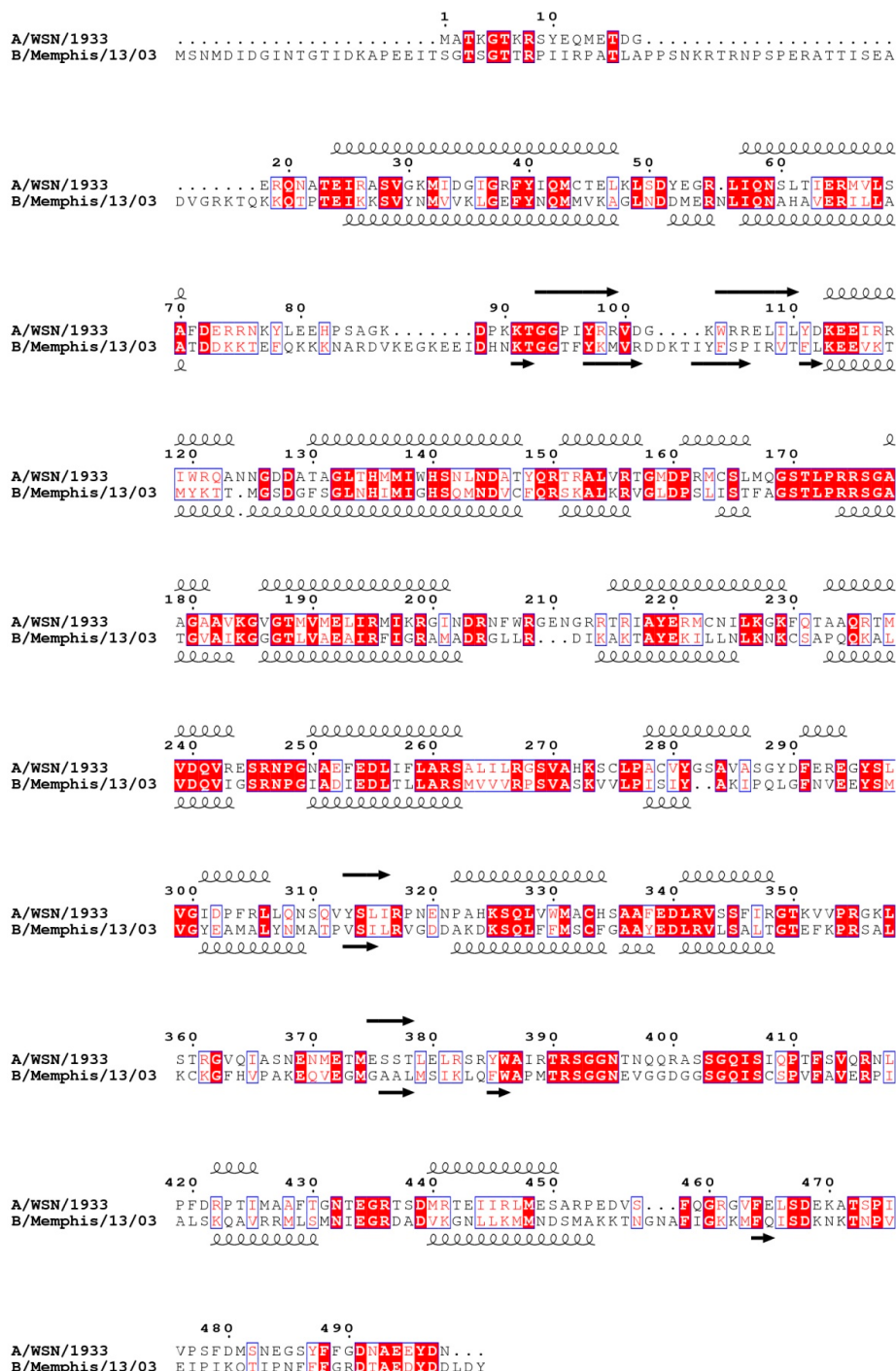


Figure S1. Sequence alignment of the nucleoproteins of influenza A and B viruses. The alignment has been made with the sequences of the two human influenza A and influenza B viruses used for this work: A/WSN/1933 (UniProtKB access number: Q1I2B5) and B/Memphis/13/03 (UniProtKB access number: Q5V8X3). The secondary sequences elements shown over and below the sequences alignment correspond to A/NP, A/WSN/1933 (PDB ID: 2IQH) and B/NP, B/Managua/4577.01/2008 (PDB ID: 3TJ0). The sequence identity between these two B/NP is 98.6%. The alignment has been done using Clustal W [46] and drawn with ESPrict [47].

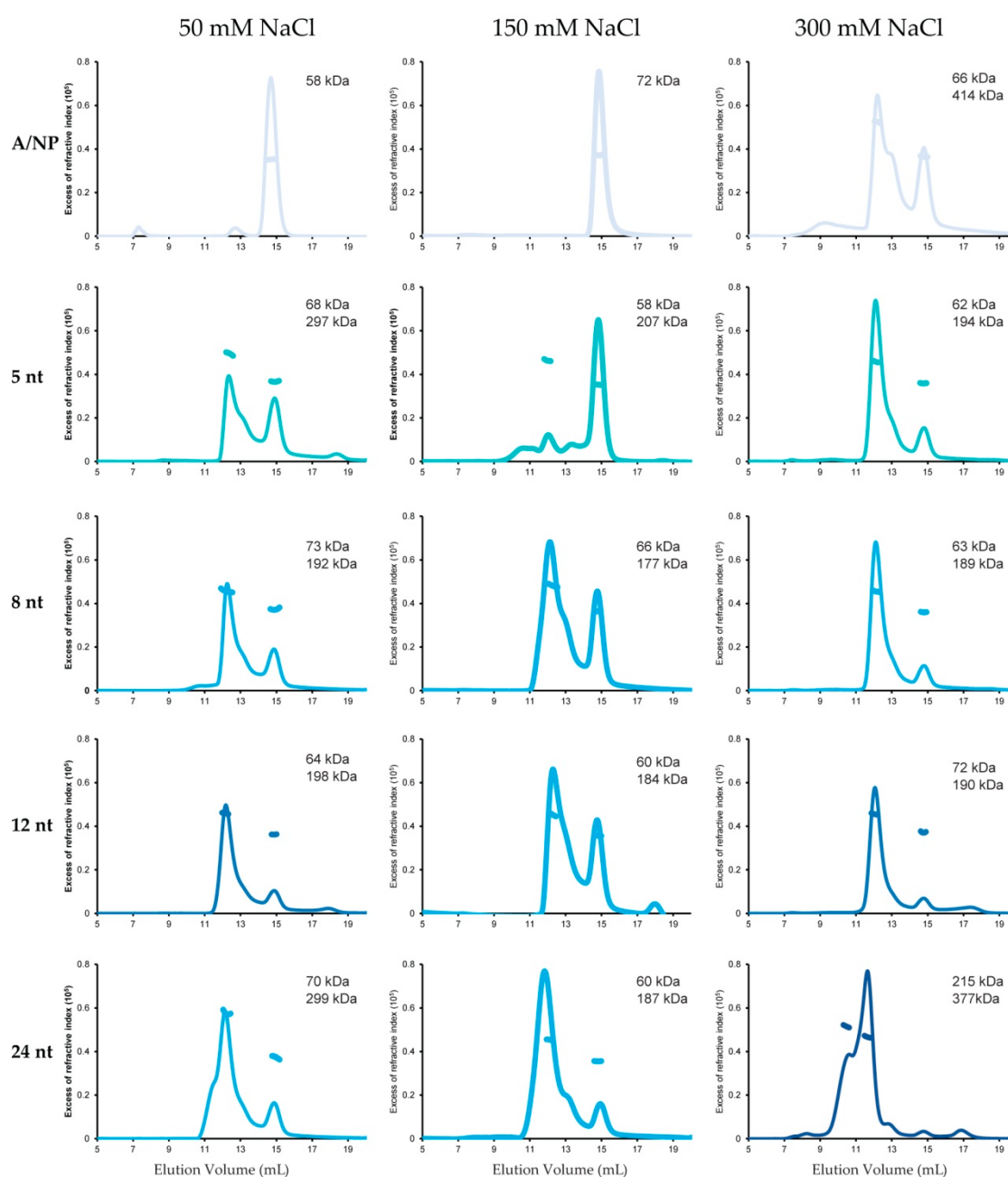


Figure S2a. Oligomeric states of influenza A virus nucleoprotein according to RNA length by size exclusion chromatography-multi-angle laser light scattering (SEC-MALLS) analysis. SEC-MALLS analysis were performed on a Superdex 200 increase 10/300 GL column equilibrated with 20 mM Tris-HCl pH 7.5 and different concentrations of NaCl (from 50 to 300 mM). Protein was injected alone or in complex (ratio 1:1) with short 5'phosphate-polyUC-OH^{3'} RNAs (from 5 to 24 nt). Complexes were incubated 1 h at room temperature before injection. SEC-MALLS chromatograms are represented with the refractive index as a back trace and molecular weights are estimated at the left.

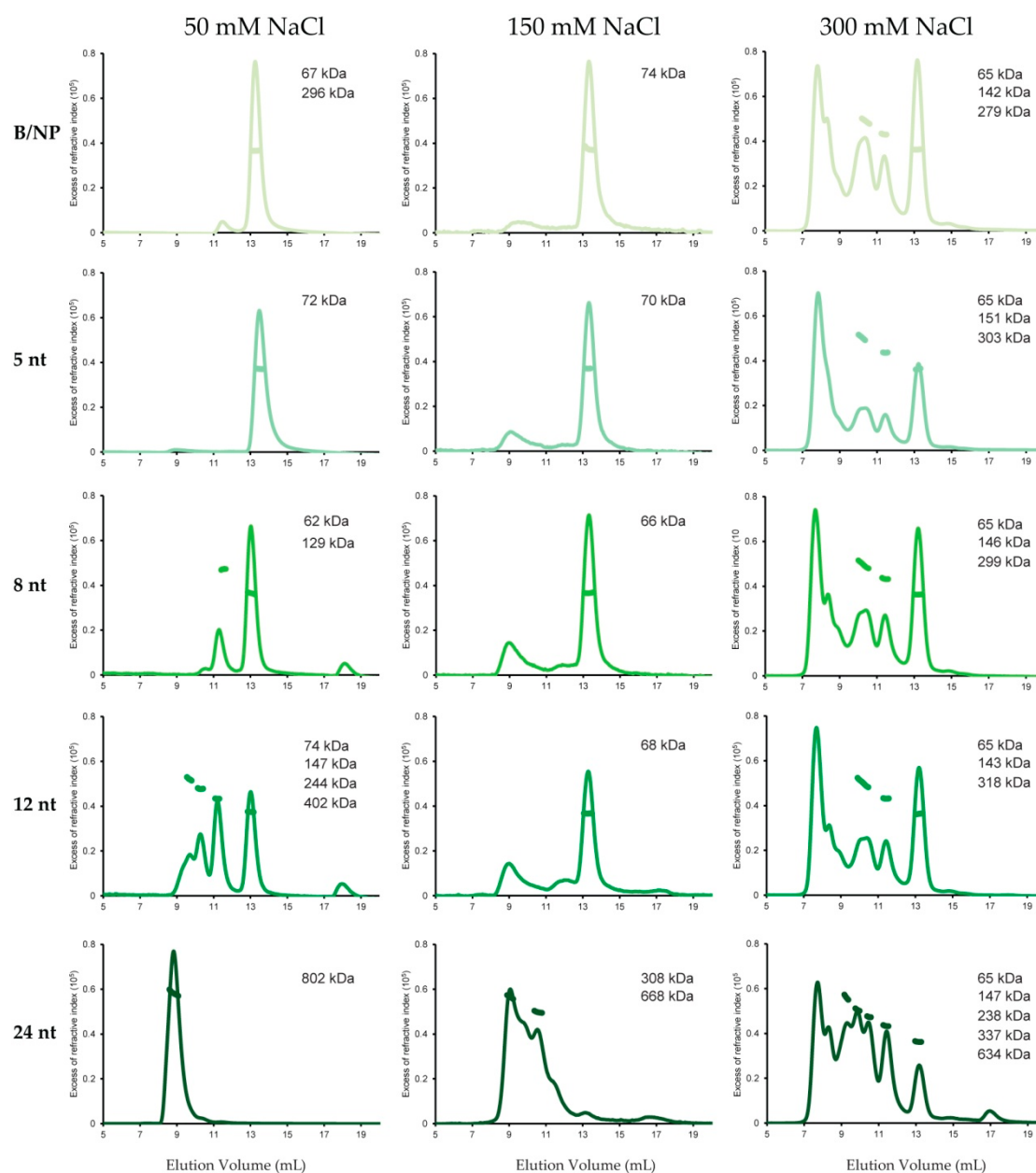


Figure S2b. Oligomeric states of influenza B virus nucleoprotein according to RNA length by SEC-MALLS analysis. SEC-MALLS analysis were performed on a Superdex 200 increase 10/300 GL column equilibrated with 20 mM Tris-HCl pH 7.5 and different concentrations of NaCl (from 50 to 300 mM). Protein was injected alone or in complex (ratio 1:1) with short $5'$ phosphate-polyUC-OH $3'$ RNAs (from 5 to 24 nt). Complexes were incubated 1 h at room temperature before injection. SEC-MALLS chromatograms are represented with the refractive index as a back trace and molecular weights are estimated at the left.

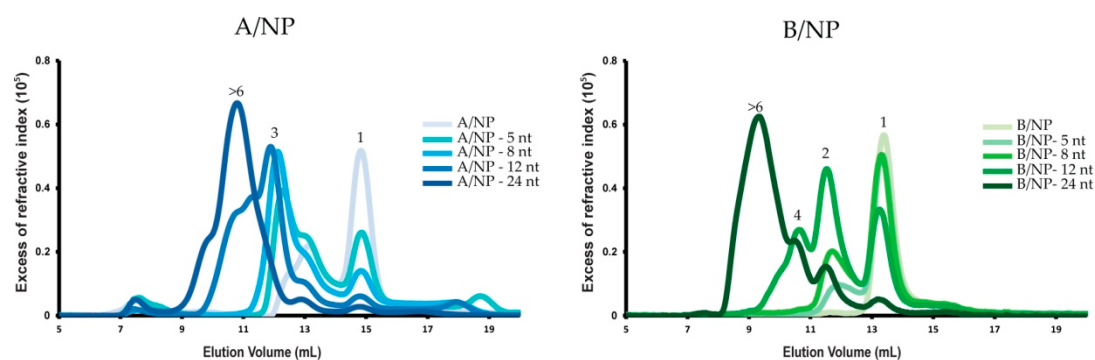


Figure S3. Oligomeric state of nucleoproteins of influenza A and B viruses using polyUC-FAM^{3'} RNAs by SEC-MALLS analysis. SEC-MALLS analysis were performed on a Superdex 200 increase 10/300 GL column equilibrated with 20 mM Tris-HCl pH 7.5 at 150 mM NaCl. The protein was injected alone or in complex (ratio 1:1) with a single short ^{5'}phosphate-polyUC-FAM^{3'} RNA (5 to 24 nucleotides). The complexes were incubated 1 h at room temperature before injection. Peaks are labeled 1 for monomer, 2 for dimer, 3 for trimer, 4 for tetramer and 6 for hexamer.

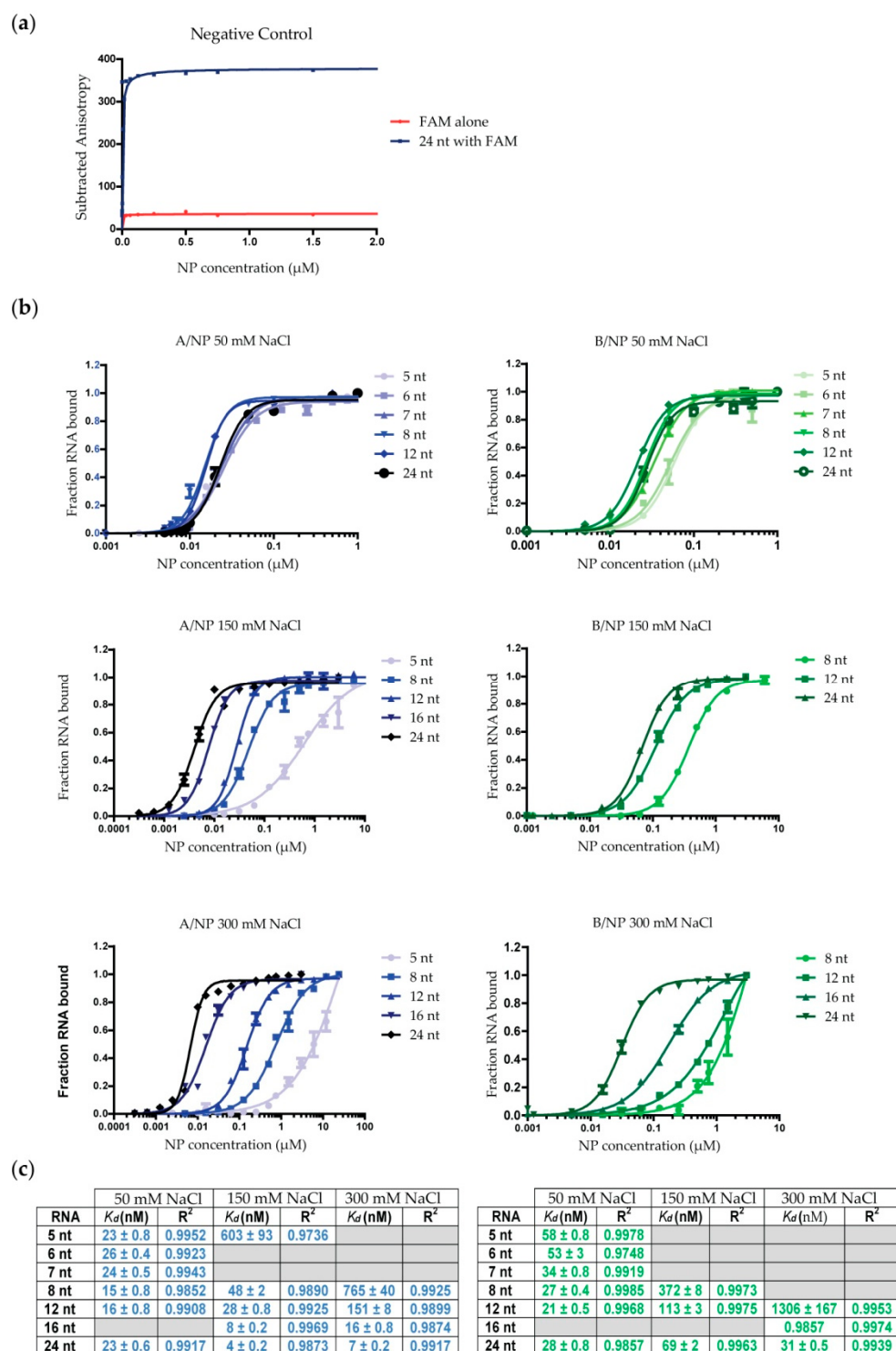


Figure S4. Titration measurements against RNAs by fluorescence anisotropy. All the experiments have been made in triplicate in 20 mM Tris-HCl pH 7.5, 5 mM β -mercaptoethanol (β -ME) and different salt concentrations (from 50 to 300 mM NaCl). The mixes were incubated 5 min at room temperature; (a) Binding titration of A/NP at 50 mM NaCl using 6-fluorescein amidite (FAM) alone; (b) Binding titration of A/NP and B/NP at 50, 150 and 300 mM NaCl using different length of short RNAs (from 5 to 24 nt); (c) Table of the different values obtained from the titrations.