

Supplemental Materials

Hardening of Respiratory Syncytial Virus Inclusion Bodies by Cyclopamine Proceeds through Perturbation of the Interactions of the M2-1 Protein with RNA and the P Protein

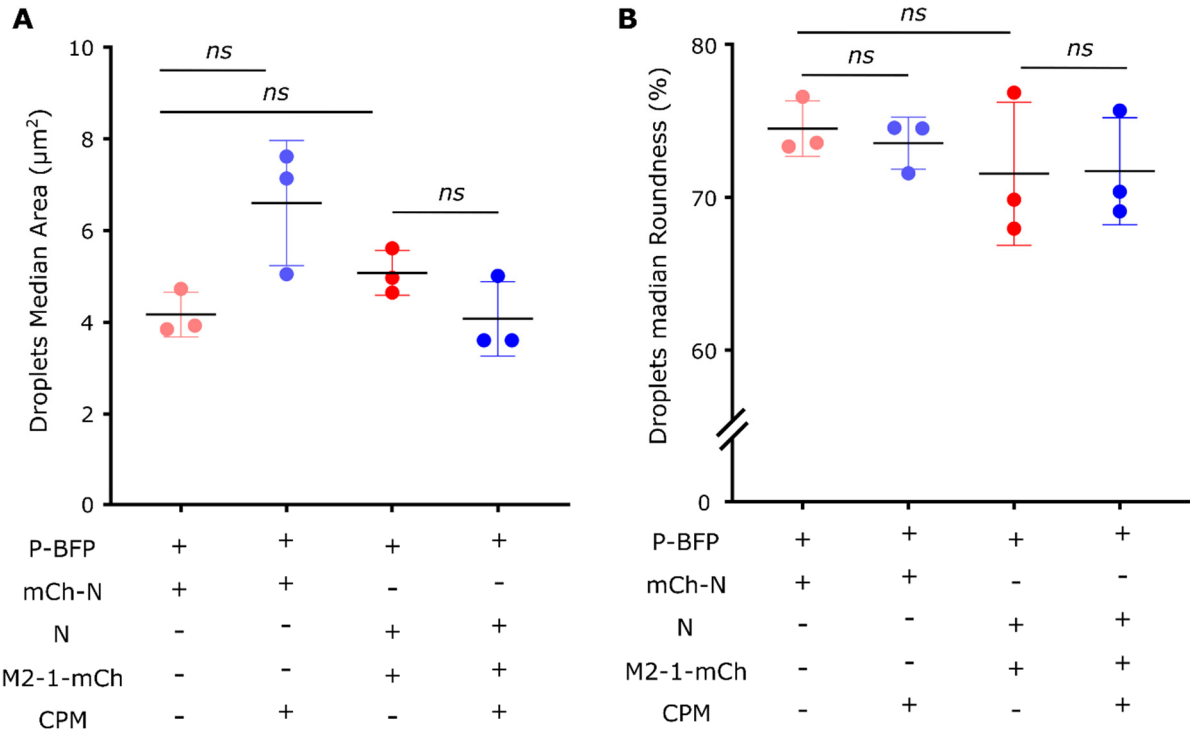


Figure S1. Droplets size is not altered by M2-1 or CPM addition. Size and roundness of condensates were quantified on the Icy software using the Analysis Particles function and a size threshold at $>3 \mu\text{m}^2$. Imaging was performed under a Leica SP8 microscope using the 63x oil-immersion objective. Artefactual values from aggregates and pairs of condensates fusing together were filtered-out using a 0.9 sphericity cut-off. These parameters were measured on 500 to 1,500 condensates per experiment. Median area (A) and roundness (B) from three independent experiment are plotted, central bars indicate mean \pm SD. Statistical analysis were done using Kolmogorov-Smirnov test; *ns*: not significant.

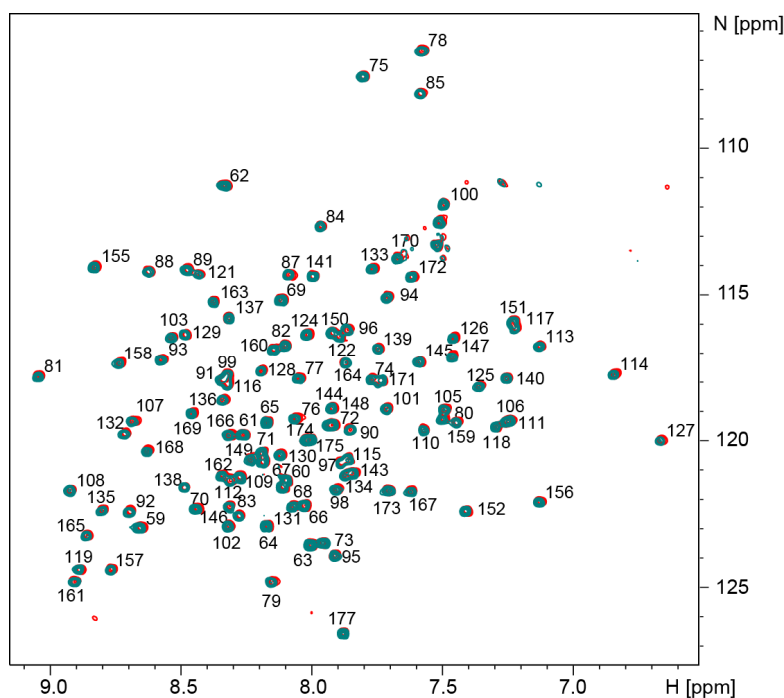


Figure S2: CPM does not bind to M2-1core with high affinity. ^1H - ^{15}N BTRSY spectra of 100 μM ^{15}N -labeled M2-1core domain without (red contours) and with one molar equivalent of CPM (teal contours) are superimposed. Measurements were done at 700 MHz ^1H frequency and at a temperature of 298K. Addition of CPM does not induce significant chemical shift or intensity perturbations, indicating that CPM does not bind to M2-1core. Amide signals are annotated with the corresponding residue number.

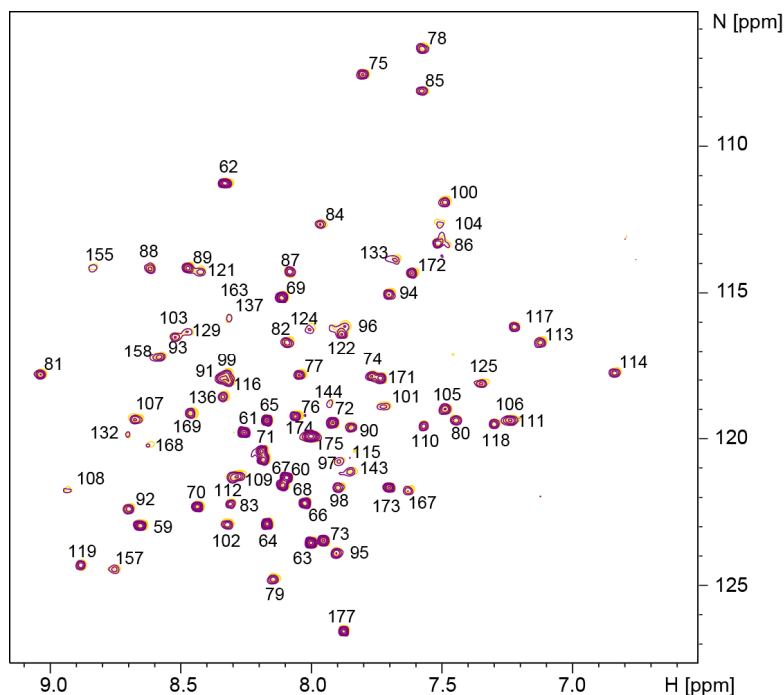


Figure S3: CPM does not perturb the complex between ^{15}N -labeled M2-1core domain and the FITC-P₉₅₋₁₁₂ peptide. ^1H - ^{15}N BTROSY spectra of ^{15}N -labeled wild-type M2-1core domain (100 μM) in complex with FITC-P₉₅₋₁₁₂ peptide in a 0.25:1 ratio before (yellow) and after (purple) addition of 1 molar equivalent of CPM (100 μM final concentration). Measurements were done at 700 MHz ^1H frequency and at a temperature of 298K.

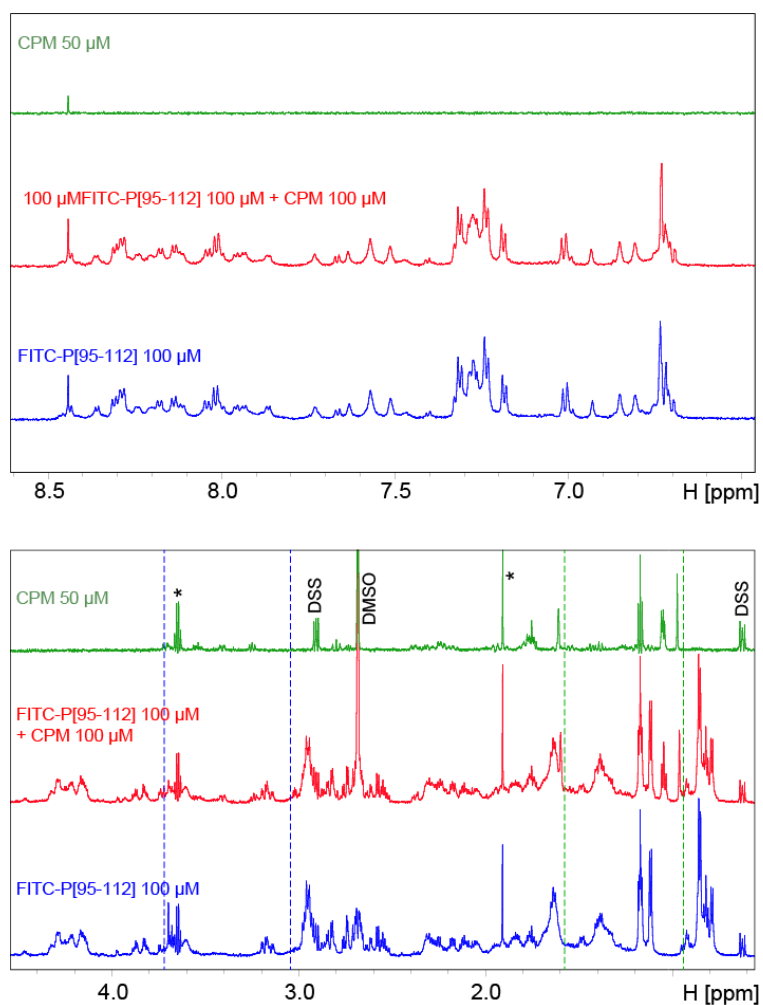


Figure S4: Analysis of the interaction between FITC-P₉₅₋₁₁₂ and CPM by NMR. 1D ¹H NMR spectra were acquired with water suppression by excitation sculpting at 700 MHz ¹H frequency and at a temperature of 298K for FITC-P₉₅₋₁₁₂ alone (100 μM), CPM alone (50 μM) and an equimolar mixture of both (100 μM both), under the same buffer conditions (phosphate saline at pH 6.8). Samples with CPM contained 0.8 % DMSO-d₆. 50 μM DSS was used as a chemical shift reference. The DSS signal at 0 ppm is not shown, but the DSS signals around 0.65 and 2.9 ppm are indicated. Impurities contained in the buffer are indicated by a star. Vertical lines indicate displacement of CPM (green) FITC-P₉₅₋₁₁₂ (blue) signals.

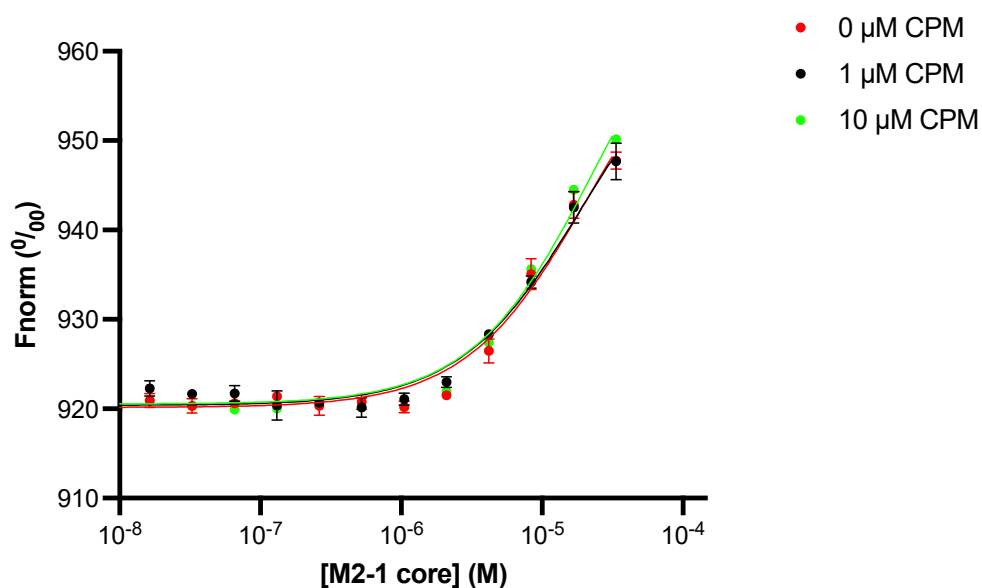


Figure S5: CPM does not affect the affinity of the complex between M2-1 core domain and P₉₀₋₁₁₂ peptide. Microscale Thermophoresis analysis of the titration of 100 nM FITC-P₉₀₋₁₁₂ peptide with an increasing amount of

