**Targeting G-quadruplexes in the rhinovirus genome by Pyridostatin inhibits uncoating and highlights a critical role for sodium ions.**

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Supplementary Figure S1

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**Supplementary Fig. S1 QGRS-mapper analysis of the RV-A2 genome.** Analysis of the genomic sequence of RV-A2 (X02316.1), displaying putative QGRS with G-scores ≥ 10 within non-overlapping windows of 30 nucleotides. The positions of the oligonucleotides G11 and G20 derived from the RV-A2 sequence are indicated. (On top) cartoons displaying the predicted conformation of the G11 and G20 GQs. Note the long loop and the zero-loop (red) in G11.

Supplementary Figure S2



**Supplementary Fig. S2 NMR.** (a) HDX assay: G11 (250 µM in 10 mM sodium phosphate (pH 7.4), 100 mM KCl, and 10 % D2O) was kept for ca 7 h at 37 °C. During this time, 30 1H-NMR spectra were acquired and averaged in three blocks of ten spectra each from 1-2.5 h (bottom), 2.5-5 h (middle), and 5-7.5 h (top). (b) One-dimensional 1H-NMR spectra of G11 and G20, both at 250 µM in 10 mM sodium phosphate (pH 7.4), 100 mM KCl. The chemical shift region characteristic of imino protons involved in Hoogsteen base pairing (Salmon coloured box) and the Watson-Crick base pair region (Olive coloured box) are indicated. NMR spectra were measured at 298 K (– PDS) and after the addition of PDS to 0.5 mM (i.e., a stoichiometry of 1:2) final concentration (+ PDS).

Supplementary Figure S3

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**Supplementary Fig. S3 The PDS association curve and model of a PDS dimer.** PDS was dissolved at 15 – 1000 µM in 10 mM sodium phosphate (pH 7.4), 100 mM KCl and the optical density (OD) determined in a scan mode. The wavelength of the maximum OD was plotted against the respective concentrations of the drug, and a curve was fitted to the data by nonlinear regression using GraphPad Prism version 8.0.0. Three independent measurements were performed (n = 3). The 3D molecular stick models of monomeric and dimeric PDS are displayed next to the graph. They were obtained by an *in silico* molecular docking with the charged form of PDS and represent the lowest energy structure in water in each instance.

Supplementary Figure S4

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**Supplementary Fig. S4 Molecular docking analysis.** Best docking pose for the interaction between model GQ RNA (pdb: 6JJH) and PDS in the monomeric form (a, b) and PDS in the dimeric form (c, d). Shown are the two distinct binding modes (groove-loop region and π-π stacking to one end of the G-quartet) in each instance when viewed from the front (left panels) and top of the GQ (right panels). The G-quadruplex RNA backbone (ribbon) with the bases (sticks) are fitted in the van der Waals surface of the model, while PDS is in stick representation in beige and cyan colours. Spheres in purple are the K+ ions. Hydrogen, oxygen, and nitrogen atoms are in white, red, blue colours, respectively.

Supplementary Figure S5

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**Supplementary Fig. S5 Gel migration of GQ-forming ribooligonucleotides in the presence of K+.** Samples of the synthetic ribooligonucleotides miniTERRA, G11 and G20 were prepared in 100 mM potassium phosphate buffer (pH 7.4) and subjected to an electrophoretic separation on a non-denaturing 12 % polyacrylamide gel supplemented with 150 mM KCl for 3 h at 50 V and 4 °C. The migration positions of the respective intermolecular (= dimeric) GQ (only G11) and intramolecular (= monomeric) GQs are indicated.

Supplementary Figure S6



**Supplementary Fig. S6** **PaSTRy analysis of RV-A2 preincubated with 20 µM PDS as in Fig. 1.** The virus preparation was heated in a real-time PCR machine from 25 to 95 °C at a 1.5 °C/min ramp rate. Samples were excited at 541 nm, and the emission intensity was determined at 560 nm at each 0.5°C temperature increase. The data were plotted against the temperature (upper panel), and the first derivative is displayed (lower panel). Ton indicates the temperature of the onset of SYTO 82 accessibility of the encapsidated RNA for each condition; T50 indicates the temperature of 50 % SYTO 82 accessibility; Tmax refers to the temperature of the maximum of SYTO 82 accessibility, correlating with the massive conversion of native RV-A2 into A particles. From that temperature onward, RNA is progressively released from the capsids. Each line represents the mean and standard deviation of three independent assays (n = 6).

Supplementary Figure S7

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**Supplementary Fig. S7 AFM imaging of RNA cores** **plus PDS in Na+ or K+ containing buffers.** RV-A2 RNA cores were incubated with PDS at a final concentration of 20 µM in 100 mM sodium or potassium phosphate buffer (pH 7.4) (upper and lower panels respectively) for 10 min at 25 °C and applied to a freshly cleaved mica and imaged. Viral RNA likely at an intermediate stage of the conformational change triggered by PDS only in the presence of sodium is indicated by an asterisk. White arrows denote aggregates of the viral RNA.

Supplementary Figure S8

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**Supplementary Fig. S8** **DSF analysis of RV-A2 ex virion RNA in Na+ or K+ containing buffers.** RV-A2 ex virion RNA prepared by capsid proteolysis with proteinase K in 100 mM sodium or potassium buffer (pH 7.4) were supplemented with SYTO 82 (5 µM, final concentration) and heated in a real-time PCR machine from 25 to 95°C at a 1.5°C/min ramp rate. The samples were excited at 541 nm, and the emission intensity was determined at 560 nm at each 0.5°C temperature increase. The obtained data were plotted against the temperature. BSA instead of ex virion RNA and treated similarly was employed as a control to detect any interference of contaminant proteins with the SYTO 82 signal during the temperature ramping. Each line represents the mean of three independent assays (n = 3).

Supplementary Figure S9



**Supplementary Fig. S9 nanoDSF analysis of RV-A2 in sodium- or potassium-containing buffers.** A suspension of RV-A2 in 100 mM sodium or potassium buffer (pH 7.4) was heated from 25 to 95°C at a ramp rate of 1°C/min, with one fluorescence measurement per 0.044°C. The normalised ratio of the recorded emission intensities (Em350nm/Em330nm) represents the change in the tryptophan fluorescence intensity as well as the shift of the emission maximum to higher wavelengths (“red-shift”) was plotted as a function of the temperature (upper panel). The lower panel shows a magnification of the salmon coloured region in the upper panel. The curves of the averaged Em350nm/Em330nm values were smoothed to facilitate the visualisation. Each line represents the mean of three independent assays (n = 3).

