Supplementary Data

Solution structure of stem-loop α of the hepatitis B virus
post-transcriptional regulatory element

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Selected strips from 2D homo- and 3D heteronuclear NOE spectra of HSLAap. (A) Strip displaying the contacts of G13 H1 to the Watson-Crick base paired C10 amino protons H41/42. (B) Strip displaying the the H1 contacts of G17 to the Watson-Crick base paired C7 amino protons H41/42. (C) Strip from the 3D ($^1$H,$^1$H,$^{13}$C)-NOESY-HSQC recorded for the aromatic region of HSLAap showing the H8 NOE contacts of G6. Multiple NOE contacts for one cross peak here were assigned based on the individual cross peaks at the carbon plane of the respective ribose CH group in the 3D ($^1$H,$^1$H,$^{13}$C)-NOESY-HSQC. Asterisks indicate peaks arising from resonances centered at a neighbouring plane.
Supplementary Figure S2

Imino region of the 2D \((^1H,^1H)-\text{NOESY}\) of HSLAap. 1D spectrum of the same region on top. Connectivities are indicated by lines and the assignments are indicated with the residue type and number.
Sequential walk (red lines) indicated by the H6/8\textsubscript{r}-H1'\textsubscript{r}-H6/8\textsubscript{r+1} connectivities in the 3D $^{1}$H,$^{1}$H,$^{13}$C)-NOESY-HSQC recorded for the ribose region of HSLAap. Strips corresponding to the individual C1'H1' carbon planes are labelled by nucleotide name and number. Blue square: weak crosspeak detectable at lower threshold.
Supplementary Figure S4

Comparison of experimentally determined residual dipolar couplings (RDC$_{\text{measured}}$) with those back-calculated (RDC$_{\text{calculated}}$) using PALES for the HSLAap NMR solution structure.; RDCs are given in Hz for the imino $^{15}$NH groups of the helical region and G13 of the apical loop. Black: regression line; red: diagonal.
(\(^{1}H,^{13}C\)-CT-HSQC of HSLAap. The C1’H1’ region is shown; residue numbers are indicated.

Note that the signals for the loop residues (C10, A11, G12, G13, U14) and the bulged G6 (residue number in red) are well separated from those of the helical residues.
NOE contacts between H8 and H1' protons of G residues in HSLAap as detected in the 3D ($^1$H,$^1$H,$^{13}$C)-NOESY-HSQC recorded for the aromatic region of HSLAap. Strips corresponding to the individual C8H8 carbon planes are labelled by nucleotide name and number. All strips were plotted at the same intensity level for the C8H8 diagonal peaks..
Relaxation study:

$T_1$, $T_{1\rho}$ and $^{1}H$-$^{13}C$ steady-state heteronuclear NOE (HETNOE) were measured using gradient-enhanced pulse sequences to minimise water saturation (Farrow et al., 1994). $T_1$ and $T_{1\rho}$ relaxation data for aromatic (sugar moieties) were typically collected as 2048 (2048) in t2 per 128 (256) complex points in t1 with 16 (16) transients, respectively. HETNOE experiments were recorded as 2048 (t2) per 300 (t1) real data points, with 96 transients per increment. The $^1H$ and $^{13}C$ spectral widths for aromatic and sugar regions were 13, 30 and 60 ppm, respectively. 12 experiments were performed for $T_1$ measurements with increasing values for the relaxation delay (0, 0.01, 0.02, 0.1, 0.25, 0.3, 0.5, 0.6, 0.75, 0.9, 1.0 and 1.5 s). $T_{1\rho}$ data sets were obtained using the following relaxation delays: 0, 0.005, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1 s. As control for estimation of peak intensity uncertainties and for evaluation of the experimental error the zero value experiment was run in triplicate for each relaxation set. The recycle delay between transients was set to 3 seconds in the pulse sequences for $T_1$ and $T_{1\rho}$ measurements. In the HETNOE experiments a 5 s recycle delay was used with or without proton saturation. The uncertainty in the HETNOE values was set to 5% of their values (Viles et al., 2001). The relaxation data were Fourier transformed after application of a cosine-squared apodisation function to yield a matrix of 2048*1024 data points. $T_1$ and $T_{1\rho}$ rates were determined by fitting the peaks intensities at multiple relaxation delays to the equation $I = I_0 \exp(-\tau/T_1)$ (Viles et al., 2001) using RELAXFIT (Fushman et al., 1997). The data were analysed using a model-free dynamics formalism (Lipari & Szabo, 1982a,b) as implemented in ROTDIF (Walker et al., 2004) and DYNAMICS (Fushman et al., 1997). A CSA value for C1’ of 40 ppm was used representing a weighted mean between the values for C3’-endo and C2’-endo ribose puckers found in DFT calculations (Dejaegere & Case, 1998) and experimental data (Bryce et al., 2005). For the
aromatic C6 of pyrimidines and for the aromatic C2 and C8 of purines CSA values according to Shajani & Varani (2005) were used.

Supplementary Figure S7

Order parameter $S^2$ per residue of HSLAap derived from the relaxation study. Values derived from the aromatic carbons are given in blue, values from ribose C1’ in magenta. Data points are omitted for residues for which no reliable extraction of experimental data due to e.g. signal overlap or ridges was possible.
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