Introduction

In retroviruses, the initiation of reverse transcription is primed by a cellular tRNA that is encapsidated in viral particles. tRNA\(^{\text{Lys}}\) is the natural primer of all immunodeficiency viruses, including the type 1 human immunodeficiency virus (HIV-1). Indeed, the tRNA is strongly bound to the genomic RNA through Watson–Crick base-pairing of its 18 5′-terminal nucleotides with the complementary viral primer binding site (PBS) [for reviews, see [1,2]]. The annealing of tRNA\(^{\text{Lys}}\) to the PBS requires the action of the viral capsid protein (NCp7) that acts as an RNA chaperone [3,4,5]. HIV-1 NCp7 is a short basic protein with two zinc-finger domains that destabilizes base-pairing in the primer RNA without opening its structure [6]. Addition of a viral template containing the PBS results in RNA-RNA complex formation and significant structural changes in both RNAs. Both basic and zinc-finger domains of NCp7 are required for proper annealing of the tRNA-RNA complex. The basic domains help to destabilize the base-pairing in the four-way junction of the tRNA structure whereas the zinc-finger domains disrupt the ternary interactions within the tRNA molecule [6,7,8,9].

The specificity for tRNA\(^{\text{Lys}}\) as the primer of reverse transcription is strictly maintained in HIV-1 evolution. Earlier experiments from several groups have shown that altering the PBS sequence alone is not sufficient to stably switch tRNA usage [10,11,12]. To improve the understanding of the exclusive usage of tRNA\(^{\text{Lys}}\) as primer by HIV-1, the search for the determinants of specific tRNA\(^{\text{Lys}}\) selection, other than the PBS, was the subject of extensive studies [13,14,15,16,17,18,19,20,21,22,23,24,25,26,27]. Additional contacts between tRNA\(^{\text{Lys}}\) and the viral RNA have been naturally considered as potential secondary determinants and were proposed to play a role in reverse transcription [20,28,29,30].

The different interactions (reviewed in [31]) are located in the PBS domain of the viral RNA and include an A-rich loop, a C-rich region and the primer activation signal (PAS). The A-rich loop interaction was found to play a role in the initiation of reverse transcription of the HIV-1 MAL strain but not in the NLA.3 or HXB2 isolates. By adaptation of both the PBS and PAS motifs, the HIV-1 leader could be changed to accommodate tRNA\(^{\text{Lys}}\) as the primer of reverse transcription in vitro [16]. Additional studies mutating the PAS sequence in the viral RNA showed that the PAS is critically involved in stimulating tRNA\(^{\text{Lys}}\)-primed reverse transcription initiation in the HIV-1 HXB2 and NL4.3 strains [17,18]. Indeed, the PAS is an 8-nucleotide sequence in the U5 region (nucleotides 123–130, Figure 1) that was proposed to be involved in a base-pairing interaction with a region located in the T-arm of tRNA\(^{\text{Lys}}\) (the anti-PAS region: nucleotides 48–55, Figure 1). This interaction would thus lead to the formation of a higher order RNA structure that is suitable for stimulating initiation of reverse transcription. Indeed, the annealing of the tRNA primer to the
PBS requires the opening of the acceptor and T stems of tRNA\textsuperscript{Lys\_3}, cloverleaf structure, thus releasing the anti-PAS motif (Figure 1). However, no direct evidence of the PAS/anti-PAS association can be obtained by probing experiments [20,32,33]. Nevertheless, one strong argument in favour of its existence during the viral replication cycle is the very strong conservation of the PAS-like tRNA–tRNA interactions among all HIV-1 isolates, but also in SIV isolates, and even possibly in all retroviruses [16,17,34]. An attractive model for the regulation of HIV reverse transcription has been proposed by the group of Ben Berkhour. The PAS sequence is initially masked in the viral RNA through base-pairing in the U5 leader stem (Figure 1B). Later, this sequence anneals with the anti-PAS sequence in the primer (RNA), which stimulates the reverse transcription initiation. Altogether, the presence of the PAS enhancer motif, initially masked and repressed by base-pairing, would provide a unique mechanism for positive and negative regulation of HIV-1 reverse transcription [18].

Very recently, using FRET spectroscopy, Beerens et al. [35] obtained new results that are consistent with a model for tRNA annealing that involves a secondary interaction between the tRNA\textsuperscript{A\_3} molecule and the PAS sequence. In this study, the tRNA\textsuperscript{A\_3}/PAS interaction appears to be dynamic and stimulated by the nucleocapsid protein [35].

In the present report, the PAS/anti-PAS interaction and the role of the nucleocapsid protein have been directly investigated \textit{in vitro} at a single base-pair resolution using the nucleocapsid protein [35].

The PAS/anti-PAS interaction can involve a secondary interaction between the tRNA\textsuperscript{A\_3} and the PAS sequence. In this study, the tRNA\textsuperscript{A\_3}/PAS interaction appears to be dynamic and stimulated by the nucleocapsid protein [35].

The PAS/anti-PAS interaction can also involve a secondary interaction between the tRNA\textsuperscript{A\_3} and the PAS sequence. In this study, the tRNA\textsuperscript{A\_3}/PAS interaction appears to be dynamic and stimulated by the nucleocapsid protein [35].

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Figure 1. Secondary structure of A) the human tRNA\textsubscript{Lys}\textsubscript{3}, primer of the HIV-1 reverse transcription, in blue the sequence complementary to the PBS and in red the anti-PAS sequence, B) the PBS domain within the HIV-1 HXB2 isolate, in blue the PBS sequence, in red the PAS sequence and in green the sequences complementary to the PAS sequence, the nucleotides that are different between HXB2 and NL4.3 are indicated in orange, and C) the duplexes corresponding to the tRNA\textsubscript{Lys}\textsubscript{3}/PBS and to the tRNA\textsubscript{Lys}\textsubscript{3}/PAS complexes.

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Figure 2. Secondary structures of RNA used in this study. A) the recombinant tRNA<sub>Lys</sub><sup>3</sup> expressed in E. coli, B) the hairpin encompassing PAS sequence, the double-stranded PAS (PAS<sub>ds</sub>) with the numbering of nucleotides in the HXB2 isolate, C) the single-stranded PAS sequence (PAS<sub>ss</sub>) and D) the PBS. A cartoon symbolizing the secondary structure of these RNAs is drawn next their names.
doi:10.1371/journal.pone.0064700.g002

Figure 3. Tests of annealing of PAS<sub>ss</sub> to tRNA<sub>Lys</sub><sup>3</sup>. A) Schematic drawing of the experimental procedure, B) Superimposition of two TROSY experiments recorded at 15°C showing the imino groups of tRNA<sub>Lys</sub><sup>3</sup> (0.1 mM) alone (in black, reference spectrum) and after the heat-annealed procedure with 1 equivalent of PAS<sub>ss</sub> (red spectrum). The arrows indicate peaks corresponding to the PAS/anti-PAS complex described in Figure 7.
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The PAS Signal Can Anneal to tRNA\(^{Lys}\)_3

The nucleocapsid protein can promote the annealing of PAS to the anti-PAS part of tRNA\(^{Lys}\)_3 if the PBS is also annealed to tRNA\(^{Lys}\)_3

From a thermodynamic point of view, the annealing of tRNA\(^{Lys}\)_3 to the PBS is strongly favoured (Figure 5, \(\Delta G = -36.2\) kcal/mol and \(T_m = 92^\circ\)C). In addition, the annealing of tRNA\(^{Lys}\)_3 to the PBS will partially open the tRNA structure and thus increase the accessibility to the anti-PAS sequence. Therefore, in order to investigate whether the PBS could influence the annealing of the PAS sequence with tRNA\(^{Lys}\)_3, we decided to conduct the heat-annealed procedure in the presence of the PBS. Figure 6 shows the NMR footprint experiment resulting from this procedure. The assignment of tRNA\(^{Lys}\)_3/PBS imino groups was previously published [7]. The imino groups of tRNA\(^{Lys}\)_3 (black spectrum – Figure 6) have disappeared to give rise to imino groups of tRNA\(^{Lys}\)_3 involved in base-pairing with the PBS (red spectrum – Figure 6). A NOESY experiment (data not shown) substantiates that the PAS\(_{ds}\) is still free as its imino groups are still observable at the chemical shifts of the imino groups of the free PAS\(_{ds}\), like in Figure 4. In conclusion, when we mixed tRNA\(^{Lys}\)_3 to the PBS and to the PAS\(_{ds}\) at the same time, the heat-annealed complex is composed of the PBS bound to tRNA\(^{Lys}\)_3 and the PAS remains free in solution (Figure 6). This complex is stable and does not change with time.

Subsequently, we added two equivalents of NCP7 to this mixture and left it for several hours at 37°C. Interestingly, new signals in addition to those originating from the tRNA\(^{Lys}\)_3/PBS complex further appeared in the NMR footprint experiment (Figure 7) indicating the formation of new base-pairs. Because the PBS and the PAS sequences are not labelled, only the imino groups of tRNA\(^{Lys}\)_3 are observable in \(^1\)H-\(^15\)N TROSY experiments whereas all the imino signals from tRNA\(^{Lys}\)_3, PBS and PAS are observable in \(^1\)H-\(^1\)H NOESY experiments. Moreover, N1 of Gs and N3 of Us and Ts can be easily distinguished by their \(^15\)N chemical shift. Therefore, we could easily assign the four new signals to three \(^15\)N-labelled guanines and one \(^15\)N-labelled pyrimidine involved in new base-pairings and belonging to tRNA\(^{Lys}\)_3. A NOESY experiment enabled us to assign the new signals to the imino groups of the PAS/anti-PAS complex as highlighted on Figure 7C. In short, the G\(_{52}\)-U\(_{126}\) pair is readily assigned since the \(^15\)N-labelled G at 10.93 ppm in the proton dimension is connected through an intense NOE with a non-labelled U at 12.27 ppm as expected for a G-U pair. Both nucleotides are connected with a \(^15\)N-labelled guanine at 13.44 ppm that is further connected to the T resonating at 14.10 ppm. In addition, the \(^15\)N chemical shift of T\(_{54}\) at 159.16 ppm and the sequence of the PAS/anti-PAS complex further supports that this peak corresponds to the T\(_{54}\) of tRNA\(^{Lys}\)_3 involved in the complex. The last signal corresponding to a guanine in the TROSY spectrum (12.85 ppm-147.04 ppm) was assigned to G\(_{51}\) since its imino proton gives an NOE to both imino protons of the G\(_{52}\)-U\(_{126}\) pair. The imino groups carried by the PAS sequence (U\(_{128}\), G\(_{129}\), G\(_{130}\)) were more difficult to assign since they are not labelled and therefore do not give rise to peaks in the TROSY experiment. We proceeded using a stepwise strategy: first we identified in the NOESY experiment of the mixture (Figure 7) the cross-peaks originated from the PAS\(_{ds}\) alone and that from the tRNA\(^{Lys}\)_3/PBS complex using previously recorded NOESY experiments (Figure S5). Then, the comparison of these three NOESY experiments revealed imino cross-peaks originating from the tRNA/PAS complex (black peaks alone). In short, we assigned U\(_{128}\) to 13.63 ppm, G\(_{129}\) to 13.26 ppm and G\(_{130}\) to 12.37 ppm by superimposing and comparing these three NOESY experiments (Figure S5). Only the G\(_\Psi\)-base-pair, located at one extremity of the PAS/anti-PAS interaction region, was not observed in our experiments. These data clearly demonstrate for the first time the existence of a PAS/anti-PAS association at a single base-pair resolution.

It is interesting to notice that in addition to the signals of the PBS annealed to tRNA\(^{Lys}\)_3, the signals of the free PAS\(_{ds}\) are still observable in the NOESY experiment, and that even though...
tRNA\textsubscript{\textit{Lys}\textsubscript{3}} and PAS have been mixed at a 1:1 ratio. It is easily observable on the GU pairs. The free and the bound PAS contain each a GU pair that gives rise to an intense peak appearing at a different chemical shift for the free and the bound PAS (Figure 7C). These characteristic GU peaks represent therefore a clear signature that not all the PAS\textsubscript{ds} has annealed to tRNA\textsubscript{\textit{Lys}\textsubscript{3}}. The comparison of the intensity of these two GU cross-peaks in the NOESY experiment indicates that approximately 50% of the

**Figure 5. Thermodynamic stability of the interaction involving the PBS (in blue) or the PAS (in red) sequences.** \(\Delta G\) values calculated with UNAFold [57] are indicated in kcal/mol and the number of base pairs are indicated in parenthesis. The melting temperature \(T_m\) was calculated by UNAFold and the melting temperature \(T_m\) was measured by UV spectroscopy (Figure S5). See also Figure S1 for details on the different sequence and secondary structures used to model the intra-molecular interaction within the MAL and HXB2 isolates.

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**Figure 6. Test of annealing of PAS\textsubscript{ds} to tRNA\textsubscript{\textit{Lys}\textsubscript{3}} in presence of the PBS using the heat-annealed procedure.** A) Schematic drawing of the annealing procedure, B) Superimposition of two TROSY experiments recorded at 15°C showing the imino groups of tRNA\textsubscript{\textit{Lys}\textsubscript{3}} alone (in black, reference spectrum) and after the heat-annealed procedure with 1 equivalent of PBS and PAS\textsubscript{ds} (red spectrum).

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PAS<sub>ds</sub> is annealed to tRNA<sup>Lys</sup><sub>3</sub>. Whatever the salt concentration, the amount of NCp7 (not more than 4 equivalents to avoid aggregation), we did not succeed in obtaining more than 50% of PAS/anti-PAS complex. We could not rule out that the amount of NCp7 added in our samples is not sufficient to reach higher amount of the PAS/anti-PAS complex. Briefly, we want here to mention that no exchange cross-peaks between individual imino protons in the free and annealed form of the PAS RNA are observed in the different NOESY spectra (Figure 7 and S3). This absence is most probably attributable to a rapid exchange of the unprotected imino protons with the solvent during the interconversion between the free and bound forms of the PAS. In conclusion, the PAS<sub>ds</sub> signal can anneal to tRNA<sup>Lys</sup><sub>3</sub> when the PBS is present under the action of the nucleocapsid protein. In contrast with the annealing of the PBS to tRNA<sup>Lys</sup><sub>3</sub>, only a fraction of the PAS (<~50%) is annealed to tRNA<sup>Lys</sup><sub>3</sub>.

For the single-stranded PAS sequence, we cannot quantify the amount of PAS<sub>ss</sub>/anti-PAS complex that is formed, since the free single-stranded PAS<sub>ss</sub> has no observable imino proton. However, we investigated the possibility to make this complex with the heat-annealing or the NCp7-mediated procedures. Interestingly, the heat-annealed complex is present in sufficient amount to give observable NMR signals when 1 equivalent of PAS<sub>ss</sub> is mixed with equimolar quantity of tRNA<sup>Lys</sup><sub>3</sub> and PBS (Figure S4). The addition of PAS<sub>ss</sub> at 5 equivalents did not change the resulting NMR footprint. The addition of two equivalents of NCp7 protein led to the same results suggesting that NCp7 would be crucial for destabilizing the base-pairings within PAS<sub>ds</sub>, but would be dispensable in the case of an unpaired PAS sequence, as seen here with the PAS<sub>ss</sub> RNA. Indeed, the destabilization of RNA helices is a well-known feature of the NCp7 chaperone activity [46,47,48,49]. For this reason, we ultimately investigated the interaction between NCp7 and PAS<sub>ds</sub> by 1D <sup>1</sup>H NMR.
Interestingly, we observed an important broadening of all imino proton NMR signals indicating that NCp7 binds non-specifically to PAS₃ and is likely to induce a destabilization of the hairpin (Figure S6).

Discussion

In this report, we provide direct evidence at a molecular level and with a single base pair resolution of base-pairings between the PAS in the viral RNA and the anti-PAS in tRNA^{16S}. In addition, we showed that NCp7 promotes the annealing of the PAS sequence to its complementary anti-PAS sequence within the primer tRNA^{16S}. Importantly, we were unable to simulate the action of the nucleocapsid protein with a simple heat-annealing procedure when the PAS sequence is engaged in a double-stranded region, accentuating the importance of NCp7 in this RNA structural rearrangement. Our results are in agreement with a recent study [35] showing a two-fold increase of annealed tRNA^{16S} molecules to the template viral RNA containing the PAS sequence in the presence of NCp7 compared to a heat-annealing procedure. Moreover, differences between viral RNA/ tRNA^{16S} complex promoted by heat or by NCp7 have been noticed, in particular, the conformational heterogeneity of this complex becomes smaller in the presence of NCp7 [35]. This study thus suggests that NCp7 facilitates tRNA annealing to the PAS motif. Our work provides further and complementary evidences at the level of single base-pairs, regarding the formation of the PAS/tRNA^{16S} complex using small fragments of viral RNA.

Thermodynamic analysis

Thermodynamic considerations are important to properly describe and understand these large structural rearrangements involving three different RNA molecules. The formation of the complex between tRNA^{16S} and the PBS is largely thermodynamically favoured (Figure 5, \( \Delta G = -36.2 \text{ kcal/mol, } T_m = 92^\circ \text{C} \)). As a result, the tRNA/PBS complex can easily be promoted by both the heat-annealed and the NCp7 procedures [7].

The situation concerning the formation of the binary complex between tRNA^{16S} and the PAS is very different. tRNA^{16S} on its own is more stable than paired with the PAS sequence. Indeed, the free energy (\( \Delta G \)) reaches \(-22 \text{ kcal/mol} \) with a melting temperature \( T_m \) calculated by UNAFold above 80°C whereas for the PAS/anti-PAS complex, \( \Delta G \) is only \(-8.8 \text{ kcal/mol} \) with a \( T_m \) around 40°C (Figure 5). In addition, the free PAS is already involved within the viral RNA in quite stable secondary structures \( \Delta G = -4.8 \text{ kcal/mol, } T_m = 47^\circ \text{C for the MAL isolate and } \Delta G = -8.9 \text{ kcal/mol, } T_m = 42^\circ \text{C for the HXB2 strain; Figure 5} \). As a result, the tRNA/PAS binary complex is clearly disfavoured and we showed that it cannot be promoted neither by the heat-annealed nor the NCp7-mediated procedures (Figure S2).

The situation is different in the case of the binary complex between tRNA^{16S} and the single-stranded PAS molecule (PAS₃). Indeed, in this case, the thermodynamic parameters of the tRNA/ PAS complex have not changed, but the PAS sequence cannot fold on its own and only 6 bp (Figure 5 and 1A) at the level of the tRNA^{16S} T-arm need to be disrupted. However, the T-arm is stacked on the acceptor arm forming a long helix (12 bp) that further stabilizes this region. This could explain why the single-stranded PAS can only be partially annealed to the primer tRNA^{16S} (Figure 3).

Finally, the situation governing the formation of the ternary complex between tRNA^{16S}, the PBS, and the PAS is of interest. The tRNA^{16S}/PBS complex is thermodynamically greatly favoured thereby freeing the tRNA^{16S} anti-PAS sequence. The PAS sequence within the HXB2 viral RNA or annealed with the anti-PAS region of tRNA^{16S} are of similar thermodynamic stability. Altogether, these thermodynamic considerations can help explaining why the association of the PAS sequence with the anti-PAS region of tRNA^{16S} can only be observed in the presence of the PBS (Figure 6), unless one uses the single-stranded PAS molecule (Figure S4). Indeed, as NCp7 acts as an RNA chaperone, it helps remodelling RNA structures towards the most stable assembly. Here, the strand exchange property of NCp7 is definitely crucial to promote the formation of the ternary complex (tRNA^{16S}/PBS/PAS) that was shown to stimulates the initiation of reverse transcription [17,18]. For the PAS/anti-PAS interaction, the fact that the intra-molecular PAS association within the viral RNA and the inter-molecular association with tRNA^{16S} are of comparable stability (see Figure 5), can very likely explain why we did observed a 50/50 mixture of the PAS sequence annealed with tRNA^{16S} or with “itself” in the PAS₃ hairpin.

Relevance for HIV-1 initiation of reverse transcription

Obviously, the use of small viral RNA fragments and the absence of reverse transcriptase cannot reproduce all the events occurring during the initiation of HIV-1 reverse transcription. But, replacing our new findings in the context of previous works showing that the PAS sequence stimulates the initiation of HIV-1 reverse transcription [17,18] is, however, very informative. First, we confirmed that the PAS/anti-PAS interaction requires the annealing of tRNA^{16S} to the PBS thus, these new base-pairings will not drive the annealing of tRNA^{16S} to the PBS. Consequently, our results are in agreement with works that demonstrated that the PAS sequence had no effect on the hybridization of the tRNA primer on the PBS [18]. Secondly, the interaction between tRNA^{16S} and the PAS motif is dynamic [35], and probably short-lived during the reverse transcription, since the reverse transcriptase must penetrate the PAS/anti-PAS helix during the early elongation phase [50]. Our observation that only a fraction of the PAS sequence is annealed to tRNA^{16S} is definitely related to its low relative stability, its dynamic behaviour and might certainly be put in relation with its transient nature in vivo. It also probably explains why chemical probing did not succeed to uncover this interaction [32,33]. Interestingly, in the case of the A-rich loop of the viral RNA of MAL isolates that interacts with the anticodon loop of tRNA^{16S}, NMR did not succeed in observing it [7,38] whereas a number of chemical probing studies did [20,21,22,51]. Thirdly, the PAS sequence in the HIV-1 genome is occluded by base-pairing in the U5 leader stem, and thus, needs to be free for the interaction with the tRNA primer. We showed that the nucleocapsid, as expected, plays a major role to promote its annealing to the anti-PAS sequence, most probably by destabilizing the PAS duplex and promoting strand exchange between the viral RNA and tRNA^{16S}.

Finally, the presence of the PAS motif that is temporarily repressed by base-pairing provides a unique mechanism for regulation of HIV-1 reverse transcription. It was speculated that this mechanism may preclude premature reverse transcription in virus-producing cells such that the vRNA genome is copied only after it is appropriately packaged into virions [18,32,53]. Because NCp7 is released from the Gag precursor protein during maturation of virion particles, this mechanism will ensure the precise timing for activation of reverse transcription. tRNA packaging and annealing to the PBS in viral particles is not dependent on Gag processing [54], whereas efficient tRNA primer extension is. PAS accessibility may therefore regulate reverse transcription in the viral life cycle.
Conclusions

We showed in this report that base-pairings between the PAS in the viral RNA and the anti-PAS in tRNA$^{Lys}_3$ can be observed by NMR under certain conditions. In our hands, the PAS/anti-PAS interaction is positively influenced by several factors: (i) the annealing of tRNA$^{Lys}_3$ to the PBS, which results in the partial opening of the tRNA structure and therefore increases the accessibility to the anti-PAS sequence; (ii) the presence of the nucleocapsid chaperone protein, which might destabilize the initial PAS base-pairing within the viral RNA and favour RNA strand exchange. On the other hand, it is disfavoured by the molecular inaccessibility of the PAS sequence as a result of its embedment in a base-paired region within the viral RNA. Altogether, the PAS/anti-PAS interaction appears to be a new illustration of the chaperone activity of the NCp7 protein. In addition, our data highlight the power of NMR experiments to map global RNA folds and demonstrate that NMR is a powerful tool to characterize annealing processes in detail.

Materials and Methods

Sample Preparation

$^{15}$N-labelled tRNA$^{Lys}_3$ was expressed in E. coli (JM101TR strain) from a recombinant plasmid and purified as previously described [39]. Small fragments of HIV viral RNA namely the PBS (18 nucleotides), PAS$_{sh}$ (17 nucleotides) and PAS$_{sa}$ (8 nucleotides) were purchased from Dharmacon research with 2'-O-bis (acetoxymethoxy)-methyl (ACE) protection. The samples were deprotected following manufacturer instructions and lyophilised. They were then dissolved in a phosphate buffer (10 mM, pH 6.5) with 50 mM KCl. The PBS and the PAS$_{ds}$ hairpin were phylised. They were then dissolved in a phosphate buffer (10 mM, pH 6.5) with 50 mM KCl. The PBS and the PAS$_{ds}$ hairpin were then dissolved in a phosphate buffer (10 mM, pH 6.5) and the PBS was added to RNA samples and the mixture was then heated at 37°C for 30 min to several hours. Both procedures were performed in the NMR buffer (10 mM KPO$_4$ pH 6.5) containing 50 mM KCl or 125 mM KCl. The same results were obtained at both salt concentrations.

Thermodynamic stabilities and melting temperatures reported in Figure 5 were calculated using the UNAFold web server (http://mfold.rna.albany.edu/) [57]. The melting temperature of PAS$_{sh}$ was measured by UV spectroscopy measuring the absorbance at 260 nm from 15°C to 80°C (Figure S5).

NMR spectroscopy

NMR data were measured on a Bruker Avance DRX600 spectrometer equipped with a TCI cryoprobe. 1D $^1$H NMR spectra were recorded using a watergate sequence for solvent suppression [58]. For samples at a concentration of 0.1 mM, 512 scans were usually acquired. $^1$H-$^{15}$N TROSY experiments [36,37] were carried out with 128 t1 increments and 2048 t2 data points per increment. The t1 dimension was acquired with the echo-antiecho method. The spectral widths were 3600 Hz and 1900 Hz in the proton and the nitrogen dimensions, respectively. NOESY experiments with a mixing time of 150 ms were recorded at 15°C using a watergate sequence for solvent suppression [58]. 256 t1 increments and 2048 t2 data points per increment were recorded. The t1 dimension was acquired with the States-TPPI method. The spectral widths were 12600 Hz in both dimension. Spectra were processed using TOPSPIN (Bruker) and analysed with TOPSPIN or CcpNMR (http://www.ccpn.ac.uk/).

The interaction between $^{15}$N-tRNA$^{Lys}_3$ and others partners were carried out at a concentration of 0.45 mM when we needed to acquire a NOESY experiment and at a concentration of 0.1 mM when only a TROSY experiment was necessary to analyze the RNA complex in the NMR sample.

Supporting Information

Figure S1 RNA sequences used to model the secondary structure context of the PAS sequence within A) the MAL isolate and B) the HXB2 isolate. These RNA fragments were used to derive the thermodynamic parameters for the PAS sequence within the two different viral RNA isolates (See Figure 5). (TIF)

Figure S2 Tests of annealing of PAS$_{sh}$ to tRNA$^{Lys}_3$. A) Schematic drawing of the experiment, B) Superimposition of two TROSY experiments recorded at 15°C showing the imino groups of tRNA$^{Lys}_3$ (0.1 mM) alone (red spectrum) and after the heat-annealed procedure with 1 equivalent of PAS$_{sh}$ (red spectrum). (TIF)

Figure S3 Superimposition of three NOESY experiments recorded at 15°C with a mixing time of 150 ms showing the imino-imino region, in blue for PAS$_{sh}$, in red for a mixture of tRNA$^{Lys}_3$, PAS$_{sh}$ and PBS for which the annealing between the tRNA$^{Lys}_3$/PBS was promoted, in black for a mixture of tRNA$^{Lys}_3$, PAS$_{sh}$, PBS, NCp7 for which the annealing tRNA$^{Lys}_3$/PBS/PAS$_{sh}$ was promoted (same experiment as Figure 7). The NOESY cross-peaks for GU base-pairs within PAS$_{sh}$, and tRNA$^{Lys}_3$/PAS complex are indicated. The assignment of the imino groups within the PAS/anti-PAS complex is indicated in purple for $^{15}$N-signals and the assignment for black cross-peaks corresponding to non-labelled imino groups are in orange. (TIF)
Figure S4 Annealing of PASna to tRNA^{3Lys}_{3} in the presence of PBS. Superimposition of two TROSY experiments recorded at 15°C showing the imino groups of tRNA^{3Lys}_{3} (0.1 mM), in black: in complex with PBS (1 equivalent) and PASna (1 equivalent) after the heat-annealed procedure; and in red: in complex with PBS (1 equivalent) and PASna (1 equivalent) after the NCP7-mediated procedure. Only the GC regions are shown since the T54 imino group was not observed in these experiments. (TIF)

Figure S5 UV melting curve of the PASds. (TIF)

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Conceived and designed the experiments: DS PB CT. Performed the experiments: DS PB FB CT. Analyzed the data: DS PB CT. Contributed reagents/materials/analysis tools: DS PB FB CT. Wrote the paper: DS PB CT.
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