# SUPPLEMENTARY MATERIAL

## Solution structure of $tRNA^{Val}$ from refinement of homology model against residual dipolar coupling and SAXS data

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**Description of the NCS terms used in the refinement**

During stage 1, the NCS term acted between the X–ray structure of $tRNA^{Phe}$ (PDB code 1EHZ) and the homology model. The non-crystallographic symmetry (NCS) term operates over heavy atoms that include backbone (C1', C2', C3', C4', C5', O2', O3', O4', O5', P, O1P, O2P) for nucleotides 1:76 and bases for those nucleotides where the sequences of $tRNA^{Phe}$ and $tRNA^{Val}$ were identical (1, 3, 7-15, 18-25, 27,28,32,33,38,42,43,45,46,48,53-61,66,70,73). The residual NCS restraint violation was 0.33 Å. The specification for the stage 1 NCS restraint term in Xplor/CNC syntax is:

```
!initialize all
do (B=0.0) (all)
! select those for which the PHE/VAL sequences match
do (B=1.0) (segi TVAL and resi  1    and not chemical H*)
do (B=1.0) (segi TVAL and resi  3    and not chemical H*)
do (B=1.0) (segi TVAL and resi  7:15 and not chemical H*)
do (B=1.0) (segi TVAL and resi 18:25 and not chemical H*)
do (B=1.0) (segi TVAL and resi 27:28 and not chemical H*)
do (B=1.0) (segi TVAL and resi 32:33 and not chemical H*)
do (B=1.0) (segi TVAL and resi 38    and not chemical H*)
do (B=1.0) (segi TVAL and resi 42:43 and not chemical H*)
do (B=1.0) (segi TVAL and resi 45:46 and not chemical H*)
do (B=1.0) (segi TVAL and resi 48    and not chemical H*)
do (B=1.0) (segi TVAL and resi 53:61 and not chemical H*)
do (B=1.0) (segi TVAL and resi 66    and not chemical H*)
do (B=1.0) (segi TVAL and resi 70    and not chemical H*)
do (B=1.0) (segi TVAL and resi 73    and not chemical H*)
do (B=1.0) (segi TPHE and resi  1    and not chemical H*)
do (B=1.0) (segi TPHE and resi  3    and not chemical H*)
do (B=1.0) (segi TPHE and resi  7:15 and not chemical H*)
do (B=1.0) (segi TPHE and resi 18:25 and not chemical H*)
do (B=1.0) (segi TPHE and resi 27:28 and not chemical H*)
do (B=1.0) (segi TPHE and resi 32:33 and not chemical H*)
do (B=1.0) (segi TPHE and resi 38    and not chemical H*)
do (B=1.0) (segi TPHE and resi 42:43 and not chemical H*)
do (B=1.0) (segi TPHE and resi 45:46 and not chemical H*)
do (B=1.0) (segi TPHE and resi 48    and not chemical H*)
do (B=1.0) (segi TPHE and resi 53:61 and not chemical H*)
do (B=1.0) (segi TPHE and resi 66    and not chemical H*)
do (B=1.0) (segi TPHE and resi 70    and not chemical H*)
do (B=1.0) (segi TPHE and resi 73    and not chemical H*)
! select backbone atoms for all
do (B=1.0) (segi TVAL and resi 1:73 and (name C1' or name C2' or name C3' or name C4' or name
C5' ))
do (B=1.0) (segi TVAL and resi 1:73 and (name O2' or name O3' or name O4' or name O5' ))
do (B=1.0) (segi TVAL and resi 1:73 and (name P  or name O1P or name O2P ))

do (B=1.0) (segi TPHE and resi 1:73 and (name C1' or name C2' or name C3' or name C4' or name
C5' ))
do (B=1.0) (segi TPHE and resi 1:73 and (name O2' or name O3' or name O4' or name O5' ))
do (B=1.0) (segi TPHE and resi 1:73 and (name P  or name O1P or name O2P ))

ncs restraints
  group equi (segi TVAL and resi  1:76 and attr B=1.0) equi
             (segi TPHE and resi  1:76 and attr B=1.0) weight-ncs=10.0 end

 ?

end
```

During stage 2, the NCS term acted between the homology model obtained from stage 1 and the model that is being refined against RDC and SAXS data. Atom selections include all atoms within each individual restraint term. Specific NCS terms and their violations are listed below.

**Supplementary Table S1.** NCS terms and violations during stage 2 of structure refinement.

| NCS Restraint | Restraint violation, Å |
|---|---|
| All sequential pairs [a] | 0.254±0.107[b] |
| Secondary structure base parings | |
| G1/C72 | 0.3268 |
| G2/C71 | 0.2952 |
| G3/C70 | 0.1650 |
| U4/A69 | 0.2414 |
| G5/C68 | 0.2628 |
| A6/U67 | 0.1682 |
| U7/A66 | 0.3101 |
| G10/C25 | 0.2177 |
| C11/G24 | 0.1456 |
| U12/A23 | 0.2874 |
| C13/G22 | 0.5013 |
| U29/A41 | 0.3459 |
| C30/G40 | 0.3514 |
| C31/G39 | 0.2180 |
| C32/A38 | 0.3800 |
| U33/A35 | 0.1946 |
| G49/C65 | 0.2564 |
| G50/U64 | 0.1995 |
| C51/G63 | 0.1630 |
| G52/C62 | 0.1833 |
| G53/C61 | 0.2517 |
| U54/A58 | 0.2919 |
| U55/G57 | 0.1986 |
| Tertiary structure (long-range base pairings) | |
| G15/C48 | 0.2329 |
| G18/U55 | 0.2794 |
| G18/G57 | 0.2230 |
| U55/A58 | 0.2890 |
| C27/G43 | 0.3298 |
| C28/G42 | 0.6334 |
| U8/A14/A21 | 0.4676 |
| A9/A23 | 0.2506 |
| C13/G22 | 0.5013 |
| G22/G46 | 0.2073 |

[a] Total of 60 sequential terms (all except for 7/8, 15/16, 16/17, 17/18, 19/20, 20/21, 46/47, 47/48, 58/59, 59/60, 60/61, 73/74, 4/75, 75/76).
[b] Average and standard deviation over the set of 60 restraints.

The specification for the stage 2 NCS restraint term in Xplor/CNS syntax is:

```
set message=on echo=on end
do (B=0.0) (all)

do (B=1.0) (segi TVAL and resi  1:73)
do (B=0.0) (segi TVAL and resi  7 and (name O3' ))
do (B=0.0) (segi TVAL and resi  8 and (name P   or name O1P or name O2P or name O5' or name C5'
or name H5' or name H5''))
do (B=0.0) (segi TVAL and resi 48 and (name O3' ))
do (B=0.0) (segi TVAL and resi 49 and (name P   or name O1P or name O2P or name O5' or name C5'
or name H5' or name H5''))

do (B=1.0) (segi STG1 and resi  1:73)
do (B=0.0) (segi STG1 and resi  7 and (name O3' ))
do (B=0.0) (segi STG1 and resi  8 and (name P   or name O1P or name O2P or name O5' or name C5'
or name H5' or name H5''))
do (B=0.0) (segi STG1 and resi 48 and (name O3' ))
do (B=0.0) (segi STG1 and resi 49 and (name P   or name O1P or name O2P or name O5' or name C5'
or name H5' or name H5''))

ncs restraints
! *** sequential stackings ***
  group equi (segi TVAL and resi  1: 2 and attr B=1.0) equi (segi STG1 and resi  1: 2 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi  2: 3 and attr B=1.0) equi (segi STG1 and resi  2: 3 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi  3: 4 and attr B=1.0) equi (segi STG1 and resi  3: 4 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi  4: 5 and attr B=1.0) equi (segi STG1 and resi  4: 5 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi  5: 6 and attr B=1.0) equi (segi STG1 and resi  5: 6 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi  6: 7 and attr B=1.0) equi (segi STG1 and resi  6: 7 and attr
B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and resi  8: 9 and attr B=1.0) equi (segi STG1 and resi  8: 9 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi  9:10 and attr B=1.0) equi (segi STG1 and resi  9:10 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 10:11 and attr B=1.0) equi (segi STG1 and resi 10:11 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 11:12 and attr B=1.0) equi (segi STG1 and resi 11:12 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 12:13 and attr B=1.0) equi (segi STG1 and resi 12:13 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 13:14 and attr B=1.0) equi (segi STG1 and resi 13:14 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 14:15 and attr B=1.0) equi (segi STG1 and resi 14:15 and attr
B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and resi 18:19 and attr B=1.0) equi (segi STG1 and resi 18:19 and attr
B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and resi 21:22 and attr B=1.0) equi (segi STG1 and resi 21:22 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 22:23 and attr B=1.0) equi (segi STG1 and resi 22:23 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 23:24 and attr B=1.0) equi (segi STG1 and resi 23:24 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 24:25 and attr B=1.0) equi (segi STG1 and resi 24:25 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 25:26 and attr B=1.0) equi (segi STG1 and resi 25:26 and attr
B=1.0) weight-ncs=100.0 end
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  group equi (segi TVAL and resi 26:27 and attr B=1.0) equi (segi STG1 and resi 26:27 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 27:28 and attr B=1.0) equi (segi STG1 and resi 27:28 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 28:29 and attr B=1.0) equi (segi STG1 and resi 28:29 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 29:30 and attr B=1.0) equi (segi STG1 and resi 29:30 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 30:31 and attr B=1.0) equi (segi STG1 and resi 30:31 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 31:32 and attr B=1.0) equi (segi STG1 and resi 31:32 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 32:33 and attr B=1.0) equi (segi STG1 and resi 32:33 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 33:34 and attr B=1.0) equi (segi STG1 and resi 33:34 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 34:35 and attr B=1.0) equi (segi STG1 and resi 34:35 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 35:36 and attr B=1.0) equi (segi STG1 and resi 35:36 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 36:37 and attr B=1.0) equi (segi STG1 and resi 36:37 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 37:38 and attr B=1.0) equi (segi STG1 and resi 37:38 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 38:39 and attr B=1.0) equi (segi STG1 and resi 38:39 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 39:40 and attr B=1.0) equi (segi STG1 and resi 39:40 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 40:41 and attr B=1.0) equi (segi STG1 and resi 40:41 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 41:42 and attr B=1.0) equi (segi STG1 and resi 41:42 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 42:43 and attr B=1.0) equi (segi STG1 and resi 42:43 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 43:44 and attr B=1.0) equi (segi STG1 and resi 43:44 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 44:45 and attr B=1.0) equi (segi STG1 and resi 44:45 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 45:46 and attr B=1.0) equi (segi STG1 and resi 45:46 and attr
B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and resi 49:50 and attr B=1.0) equi (segi STG1 and resi 49:50 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 50:51 and attr B=1.0) equi (segi STG1 and resi 50:51 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 51:52 and attr B=1.0) equi (segi STG1 and resi 51:52 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 52:53 and attr B=1.0) equi (segi STG1 and resi 52:53 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 53:54 and attr B=1.0) equi (segi STG1 and resi 53:54 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 54:55 and attr B=1.0) equi (segi STG1 and resi 54:55 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 55:56 and attr B=1.0) equi (segi STG1 and resi 55:56 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 56:57 and attr B=1.0) equi (segi STG1 and resi 56:57 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 57:58 and attr B=1.0) equi (segi STG1 and resi 57:58 and attr
B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and resi 61:62 and attr B=1.0) equi (segi STG1 and resi 61:62 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 62:63 and attr B=1.0) equi (segi STG1 and resi 62:63 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 63:64 and attr B=1.0) equi (segi STG1 and resi 63:64 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 64:65 and attr B=1.0) equi (segi STG1 and resi 64:65 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 65:66 and attr B=1.0) equi (segi STG1 and resi 65:66 and attr
B=1.0) weight-ncs=100.0 end
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  group equi (segi TVAL and resi 66:67 and attr B=1.0) equi (segi STG1 and resi 66:67 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 67:68 and attr B=1.0) equi (segi STG1 and resi 67:68 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 68:69 and attr B=1.0) equi (segi STG1 and resi 68:69 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 69:70 and attr B=1.0) equi (segi STG1 and resi 69:70 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 70:71 and attr B=1.0) equi (segi STG1 and resi 70:71 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 71:72 and attr B=1.0) equi (segi STG1 and resi 71:72 and attr
B=1.0) weight-ncs=100.0 end
  group equi (segi TVAL and resi 72:73 and attr B=1.0) equi (segi STG1 and resi 72:73 and attr
B=1.0) weight-ncs=100.0 end

! ***  base pairs ***
  group equi (segi TVAL and (resi  1 or resi 72) and attr B=1.0)
        equi (segi STG1 and (resi  1 or resi 72) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi  2 or resi 71) and attr B=1.0)
        equi (segi STG1 and (resi  2 or resi 71) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi  3 or resi 70) and attr B=1.0)
        equi (segi STG1 and (resi  3 or resi 70) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi  4 or resi 69) and attr B=1.0)
        equi (segi STG1 and (resi  4 or resi 69) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi  5 or resi 68) and attr B=1.0)
        equi (segi STG1 and (resi  5 or resi 68) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi  6 or resi 67) and attr B=1.0)
        equi (segi STG1 and (resi  6 or resi 67) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi  7 or resi 66) and attr B=1.0)
        equi (segi STG1 and (resi  7 or resi 66) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 10 or resi 25) and attr B=1.0)
        equi (segi STG1 and (resi 10 or resi 25) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 11 or resi 24) and attr B=1.0)
        equi (segi STG1 and (resi 11 or resi 24) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 12 or resi 23) and attr B=1.0)
        equi (segi STG1 and (resi 12 or resi 23) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 13 or resi 22) and attr B=1.0)
        equi (segi STG1 and (resi 13 or resi 22) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 29 or resi 41) and attr B=1.0)
        equi (segi STG1 and (resi 29 or resi 41) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 30 or resi 40) and attr B=1.0)
        equi (segi STG1 and (resi 30 or resi 40) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 31 or resi 39) and attr B=1.0)
        equi (segi STG1 and (resi 31 or resi 39) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 32 or resi 38) and attr B=1.0)
        equi (segi STG1 and (resi 32 or resi 38) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 33 or resi 35) and attr B=1.0)
        equi (segi STG1 and (resi 33 or resi 35) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 49 or resi 65) and attr B=1.0)
        equi (segi STG1 and (resi 49 or resi 65) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 50 or resi 64) and attr B=1.0)
        equi (segi STG1 and (resi 50 or resi 64) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 51 or resi 63) and attr B=1.0)
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        equi (segi STG1 and (resi 51 or resi 63) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 52 or resi 62) and attr B=1.0)
        equi (segi STG1 and (resi 52 or resi 62) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 53 or resi 61) and attr B=1.0)
        equi (segi STG1 and (resi 53 or resi 61) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 54 or resi 58) and attr B=1.0)
        equi (segi STG1 and (resi 54 or resi 58) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 55 or resi 57) and attr B=1.0)
        equi (segi STG1 and (resi 55 or resi 57) and attr B=1.0) weight-ncs=100.0 end

! *** long-range base pairs ***



  group equi (segi TVAL and (resi 15 or resi 48) and attr B=1.0)
        equi (segi STG1 and (resi 15 or resi 48) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 55 or resi 18) and attr B=1.0)
        equi (segi STG1 and (resi 55 or resi 18) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 57 or resi 18) and attr B=1.0)
        equi (segi STG1 and (resi 57 or resi 18) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 55 or resi 58) and attr B=1.0)
        equi (segi STG1 and (resi 55 or resi 58) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 27 or resi 43) and attr B=1.0)
        equi (segi STG1 and (resi 27 or resi 43) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 28 or resi 42) and attr B=1.0)
        equi (segi STG1 and (resi 28 or resi 42) and attr B=1.0) weight-ncs=100.0 end


! *** conserved triplets ***
! U8-A14-A21
  group equi (segi TVAL and (resi  8 or resi 14 or resi 21) and attr B=1.0)
        equi (segi STG1 and (resi  8 or resi 14 or resi 21) and attr B=1.0) weight-ncs=100.0
end

! A9-U12-A23
  group equi (segi TVAL and (resi  9 or resi 23) and attr B=1.0)
        equi (segi STG1 and (resi  9 or resi 23) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 12 or resi 23) and attr B=1.0)
        equi (segi STG1 and (resi 12 or resi 23) and attr B=1.0) weight-ncs=100.0 end

! C13-G22-G46
  group equi (segi TVAL and (resi 13 or resi 22) and attr B=1.0)
        equi (segi STG1 and (resi 13 or resi 22) and attr B=1.0) weight-ncs=100.0 end

  group equi (segi TVAL and (resi 22 or resi 46) and attr B=1.0)
        equi (segi STG1 and (resi 22 or resi 46) and attr B=1.0) weight-ncs=100.0 end

 ?

end
```

**Structure refinement starting from the idealized A-form model instead of the tRNA$^{Phe}$ structure 1EHZ.**

In order to test whether our refinement approach is also applicable to a more general case, where less prior structural information were available, a starting model was also generated based on the known base-pairing, and assuming A-form helical geometry for stretches of Watson-Crick base-paired nucleotides. The program INSIGHTII (MSI) was used to generate four A-form helices with the sequences of the acceptor (1-7, 49-53, 61-72) and anticodon (residues 10-13, 22-31, 39-43) arms. These helical regions were then superimposed on the X-ray structure for tRNA$^{Phe}$ and the A-form helices were replaced for the corresponding regions in the X-ray structure. This model was then energy minimized in XPLOR. A number of covalent bonds cannot be accommodated during the above model building procedure. Therefore, additional steps were taken to address this issue, while keeping this model very close to its initial configuration. Specifically, proper covalent linkages were introduced during a 2000-step Powell minimization in which only the bonds, angles, impropers, and non-bonded interaction were active, followed by three successive stages of a simulated annealing protocol. During simulated annealing of the initial A-form model, three separate NCS terms to the INSIGHTII-generated model for nucleotide stretches 1-7&49-73, 8-31&39-48, and 32-38 were enforced in order to essentially keep these regions frozen to those of the INSIGHTII model. An additional simulated annealing run was performed on the resulting models with active energy terms for bonds, angles, impropers, non-bonded interactions and database base-pairing, stacking, and dihedral angle potentials. In order to ensure reasonable geometries for the non-Watson-Crick paired nucleotides (15/48, 50/64, 53/61, 54/58, and triplets 8/14/21, 9/12/23,

and 13/22/46), which are insufficiently represented in the empirical conformational database, these seven sets were restrained by NCS terms relative to the coordinates of these nucleotides in the $tRNA^{Phe}$-based starting model. From this point on, structure refinement proceeded as described in the main text for Stage 2.

Regularization of the initial A-form $tRNA^{Val}$ model yielded a structure that exhibited an all-atom rmsd of 2.3 Å relative to the $tRNA^{Phe}$-based stage 1 structure, and 3.0 Å with respect to the final Pf1/MSA/SAXS-refined structure (PDB code 1K4C). The model exhibited Pf1 RDC cross-validation statistics of $Q^{free}$ = 53%. The relative orientation of the two arms in this regularized A-form model is similar to the $tRNA^{Phe}$-based stage 1 structure, but inclusion of the Pf1 and MSA RDCs opens the inter-arm angle to 105°, slightly larger than observed for the $tRNA^{Phe}$-based starting structure. Subsequent refinement, which also includes SAXS data, reduces the rms difference in atomic coordinates relative to the refined $tRNA^{Phe}$-based model to about 2 Å, but does not significantly impact the inter-arm angle (Supplementary Table S2). Cross-validation statistics at $Q^{free}$ = 28% are obtained by leaving out one Pf1 RDC at a time. Thus, the procedure described in the main text, starting from the high resolution X-ray structure of the $tRNA^{Phe}$ structure (58% identity), yields better statistics than when starting from a more general model that simply assumes idealized A-form helices and base triplets that mimic those seen in X-ray structures of tRNA. Starting from a cruder model, on the other hand, shows a larger improvement in cross validation statistics upon refinement, but does not reach as low a $Q^{free}$ factor as the starting model based on $tRNA^{Phe}$.

**Supplementary Table S2.** Structural statistics for the A-form based models

| | Regularized A-form model | Pf1/MSA refined model | Pf1/MSA/SAXS refined model |
|---|---|---|---|
| **Deviations from experimental data** | | | |
| Pf1 RDCs (Hz) | 4.1 | 2.0 | 2.3 |
| MSA RDCs (Hz) | 0.36 | 0.34 | 0.37 |
| SAXS data fit ($\chi$) | 1.85 | 2.39 | 1.10 |
| **Deviations from idealized geometry** | | | |
| Bond lengths (Å) | 0.0056 | 0.0057 | 0.0057 |
| Bond angles (°) | 0.71 | 0.72 | 0.71 |
| Impropers (°) | 0.59 | 0.59 | 0.59 |
| Molprobity clash score [a] | 4.1 | 4.5 | 4.1 |
| **Structural parameters** | | | |
| rmsd (Å) to stage 1 model all heavy atoms, nt. 1-72 | 2.3 | 2.7 | 3.3 |
| rmsd (Å) to Pf1/MSA/SAXS stage 2 model all heavy atoms, nt. 1-72 | 3.0 | 2.5 | 2.1 |
| Inter-arm opening angle (°) | 86 | 105 | 106 |

[a] Clashes per 1000 atoms

**Supplementary Table S3**. Imino $^{15}$N-$^{1}$H RDCs of tRNA$^{Val}$ aligned by Pf1 and by magnetic field

| | $^{1}D_{NH}$, Hz (Pf1) | $^{1}D_{NH}$, Hz (MSA)[a] |
|---|---|---|
| G1 | -8.0 | -1.4 |
| G2 | 8.8 | 0.1 |
| G3 | 12.4 | |
| U4 | 5.1 | 0.8 |
| G5 | -21.1 | -1.2 |
| U7 | -7.6 | -1.1 |
| $s^4$U8 | -1.5 | -0.8 |
| G10 | -13.2 | |
| U12 | -0.8 | -0.2 |
| G15 | -22.2 | -1.2 |
| G22 | 4.9 | |
| G24 | -9.5 | -0.7 |
| U29 | -18.8 | -1.1 |
| G39 | -14.2 | |
| G40 | -22.9 | -2.4 |
| G42 | -6.1 | |
| G43 | 2.2 | |
| $m^7$G46 | -21.0 | -1.7 |
| G49 | 21.6 | 0.1 |
| G50 | 17.1 | 0.3 |
| G52 | -23.5 | -1.9 |
| G53 | -12.7 | |
| T54 | 3.2 | -0.1 |
| Ψ55 (N3) | 15.4 | 1.2 |
| G63 | 2.4 | 0.5 |
| U64 | 20.4 | 1.1 |
| U67 | -17.4 | -1.2 |

[a] values obtained by averaging $^{1}J_{NH}$(800)-$^{1}J_{NH}$(500) and values ($^{1}J_{NH}$(800)-$^{1}J_{NH,\ calc}$(0))/1.641 reported previously (Table 1S of Ying et al. J. Biomol. NMR 39: 91-96, 2007).