SUPPORTING INFORMATION

Structural rearrangements in mRNA upon its binding to human 80S ribosomes revealed by EPR spectroscopy

Alexey A. Malygin,1,4 Dmitri M. Graifer,1,4 Maria I. Meschaninova,1 Alia G. Venyaminova,1 Ivan Timofeev,2,4 Andrey A. Kuzhelev,3,4 Olesya A. Krumkacheva,2,4 Matvey V. Fedin,*,2,4 Galina G. Karpova*,1,4 and Elena G. Bagryanskaya*,3,4

1Institute of Chemical Biology and Fundamental Medicine SB RAS, pr. Lavrentjeva 8, Novosibirsk, 630090, Russia;
2International Tomography Center SB RAS, Institutskaya str. 3a, Novosibirsk, 630090, Russia;
3N. N. Vorozhtsov Novosibirsk Institute of Organic Chemistry SB RAS, pr. Lavrentjeva 9, Novosibirsk, 630090, Russia;
4Novosibirsk State University, Pirogova Str. 2, Novosibirsk, 630090, Russia

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I. PELDOR/DEER data

Figure S1A shows the four-pulse DEER sequence used. In this method, selective excitation of the spectrum at the observation and pumping frequencies is required. Fig. S1B shows Q-band echo-detected (ED) spectrum for MR and the positions of the observation and pumping pulses in DEER experiments. The modulation depth of Electron Spin Echo (ESE) signal ($\lambda$, indicated in Fig. S4) in DEER experiments in general is determined by the number of spin pairs (relative to total amount of spin-labels) and by a power of the pulses used.

![Image](https://example.com/image.png)

**Figure S1.** (A) Four-pulse DEER sequence/ (B) Typical echo-detected EPR spectrum for studied samples at Q-band at 50 K. The positions of probe and pump pulses are indicated.

Figure S2 shows the obtained PELDOR time traces prior to background correction for studied samples. The Q-band spectrometer used had a low microwave power and EN5107D2 resonator, so it was impossible to use pulses shorter than 20 ns, as well as to overcouple resonator and increase the excitation bandwidth. This resulted in a relatively low modulation depth in our DEER experiments, and its absolute value is not sufficiently reliable. However, some trends in the study of series of MR samples were noticed.
Figure S2. Original (before background correction) four-pulse Q-band DEER traces (intensity is normalized) of studied samples. Lines with noise correspond to the experimental data. Solid lines show the baseline function obtained using DeerAnalysis2013.

Figure S3 compares PELDOR time traces obtained after their scaling to the modulation depth. The time traces for MR, its binary complexes and complexes 1, 2, 3 are significantly different indicating that differences in the distance distribution are not due to the experimental accuracy. At the same time, complexes 3, 4 and 3* have similar time traces after scaling.

Figure S3. Background corrected four-pulse Q-band DEER traces (intensity is normalized) of studied samples after scaling to the modulation depth (left), and corresponding distance distributions (right).
Figure S4 compares the modulation depths for the isolated MR and its complexes. The obtained $<r_{\text{DEER}}>=2.5 \text{ nm}$ for the isolated MR is in good agreement with the expected distance between the terminal nucleotides in the four-spin system duplexes formed by two MR molecules (See Fig. 1 in the main text), where two of three possible distances (2-3 nm, corresponding to labels on the opposite ends of a duplex) are available for measurement by DEER, and one (1-2 nm, relating to spin labels located on the same side of the duplex) by CW EPR.

Figure S4. Background corrected four-pulse Q-band DEER traces (intensity is normalized) of studied samples. Lines with noise correspond to the experimental data. Solid lines show the best fits obtained using DeerAnalysis2013.

In general case, DEER signal of N number of spins coupled by dipole-dipole interaction at $T\to\infty$ is described as [Bode B. E., J. Am. Chem. Soc., 2007, 129 (21), pp 6736–6745]:

$$F(T\to\infty)=F_0(1-\lambda)^{N-1},$$

where $\lambda$ is the modulation depth of ESE signal, $F_0$ is the ESE signal in DEER time traces at $T=0$ (the maximum signal), $F(T\to\infty)$ is the ESE signal at the end of DEER time traces.

According to this formula, the dipolar oscillations should be deeper for the duplex (MR)$_2$ compared to the monomer MR fixed in the ribosomal channel. This is exactly what is observed in our DEER experiments, where modulation of observed spin echo oscillation decreases as the fraction with $<r_{\text{DEER}}>$~2.5 nm disappears (complex 3).

Since the conversion of dipolar evolution functions to distance distributions is an ill-posed problem, we studied the influence of baseline correction on the obtained distance distribution. The greatest effect of baseline correction is expected for the peak at 4.9 nm, since such distance is relatively long for the time traces recorded for 2.6 microseconds. All PELDOR time traces were...
background corrected using DeerAnalysis automatic fitting assuming three-dimensional homogeneous spin distribution. Starting time of background fitting appears to be the crucial parameter of simulations. To validate the distance distributions we analyzed their behavior upon variation of starting time for the background fitting near the optimal one (t_{opt}) where t_{opt} was obtained automatically. Figure S5 shows the validation results for all studied samples except for isolated MR, as the corresponding distribution manifests no deviation from one obtained with automatic background correction. We used the shape of Pake pattern of background corrected time trace in order to evaluate the correctness of baseline subtraction. Starting time variation was restricted to threshold behavior of Pake patterns. In particular, we analyzed the intensity of zero frequency component in order to track the over- or under- baseline subtraction (the spike and the hole in the center of the Pake pattern, correspondingly).

Distance distributions manifest appearance of long-range features upon an increase of the starting time for background fitting. For example, for complexes 1-3 an increase of starting time leads to the onset of obviously wrong distances at ~8.0 nm – the upper limit of available distance range. Thus, the optimal time was determined as the maximum value at which peaks at the edge of the distribution were not observed.

![Distance distributions](image-url)

**Figure S5.** Distance distribution obtained upon variation of starting time for the background fitting near the optimal one (t_{opt}, obtained automatically). Insets depict the corresponding Pake patterns. Optimal times of background correction are indicated in parentheses above the corresponding data. Gray lines display heights of the 4.9 nm peak in distance distribution obtained with t_{opt}.
Most important, all peaks analyzed in this work are steadily present in the calculated distribution upon variation of $t_{opt}$ value. Moreover, integral values of peaks at 4.9 nm for complexes 1-3 change by less than 2% of total area upon variation of the starting time. Gray lines in Fig. S5 display heights of the 4.9 nm peak in distance distribution obtained with optimal starting time $t_{opt}$.

Figure S5 clearly shows that the difference in distance distribution for complexes 1 and 3, as well as for complexes 2 and 3, exceeds the uncertainties introduced by background correction. This allows us to ensure that tRNA directed to the E site (complex 3) increases the amount of MR bound at the mRNA channel to a higher extent compared to the case where tRNA is directed to the A site (complex 2). At the same time, the obtained distance distributions for complexes 3 and 3* should be considered as identical. This allows us to conclude that the effect of a non-cognate tRNA directed to the E site is almost the same as that of the cognate tRNA.
II. Continuous wave EPR spectra at 300 K

Continuous wave (CW) EPR experiments were carried out at X-band (9 GHz) at 300 K using a commercial Bruker EMX spectrometer. Experimental settings were as follows: sweep width 6 mT; microwave power 20 μW; modulation frequency 100 kHz; modulation amplitude 0.05 mT; time constant 81.92 ms; number of points 1024; number of scans 32.

It is well known that the shape of the nitroxide spectrum strongly depends on its mobility, as well as on the character of motion (whether it is isotropic or to some extent anisotropic). Figure S6 compares room-temperature X-band CW EPR spectra of MR with those of its binary complexes.

![Figure S6](image.png)

**Figure S6.** Room temperature X-band CW EPR spectra for MR and its binary complexes (indicated in the legend).

All spectra were simulated using EasySpin [www.easyspin.org] software and slow-motion regime to describe label mobility; the parameters of calculation are given in Table S1. In order to fit experimental spectrum, we assumed the presence of a set of fractions characterized by different rotational correlation times of nitroxide as well as by different line widths and orienting potentials.

CW EPR spectra of isolated MR were simulated assuming the admixture (25%) of free spin label with the isotropic motion and the rotational correlation time $\tau_c = 0.16$ ns. In addition to the fast rotating fraction, the spectra contain 75% of slow mobile fraction with $\tau_c = 0.63$ ns and anisotropic motion in the orienting potential. Orienting potential is characterized by parameters for motional anisotropy $\lambda_{2,0}$ and $\lambda_{2,2}$, representing coefficients in front of spherical harmonic terms $Y_{20}$ and $Y_{22}$, respectively. In this model, the spin label is partially ordered within its local environment, and the correlation time relates to the local mobility of the label (not to the rotation of molecule as a whole) that can be high compared to the mobility of the whole system. Non-zero $\lambda_{2,0}$ and $\lambda_{2,2}$ parameters indicate a partial ordering of spin label within its local environment occurring due to the covalent attachment to the model RNA.
Figure S7. Room-temperature X-band CW EPR spectra for studied samples (indicated in the legend) and their simulations (red lines).

Table S1. The parameters for simulations shown in Fig. S7. For spin-labeled MR we used $g=\{2.0083 \ 2.0061 \ 2.0022\}$ and $A=\{0.58 \ 0.58 \ A_{zz}\}$ mT in all simulations; the values of $A_{zz}$ were varied.

| Sample                  | $A_{zz}$ mT | Fraction weight [Gauss Lorentz], mT | $\tau_c$, ns | $[\lambda_{20} \ \lambda_{22}]$ |
|-------------------------|-------------|------------------------------------|--------------|-----------------------------------|
| MR                     | 3.61        | 0.30 [0.14 0.01]                   | 0.63         | [-0.2 -0.5]                       |
|                         |             | 0.45 [0.50 0.10]                   | 0.63         | [-0.2 -0.5]                       |
|                         |             | 0.25 [0.12 0.01]                   | 0.16         | -                                 |
| Complex 1              | 3.66        | 0.23 [0.14 0.01]                   | 0.16         | [-0.1 -0.3]                       |
|                         |             | 0.77 [0.20 0.10]                   | 2.24         | [-0.5 -1.0]                       |
| Complex 2              | 3.66        | 0.19 [0.14 0.01]                   | 0.16         | [-0.1 -0.3]                       |
|                         |             | 0.81 [0.20 0.10]                   | 2.24         | [-0.5 -1.0]                       |
| Complex 3              | 3.66        | 0.15 [0.14 0.01]                   | 0.16         | [-0.1 -0.3]                       |
|                         |             | 0.85 [0.20 0.10]                   | 2.24         | [-0.5 -1.0]                       |
| Complex 4              | 3.66        | 0.16 [0.14 0.01]                   | 0.16         | [-0.1 -0.3]                       |
|                         |             | 0.84 [0.20 0.10]                   | 2.24         | [-0.5 -1.0]                       |
| Complex 3 (tRNA$^{Phe}$ at the E site) | 3.66 | 0.10 [0.14 0.01]                   | 0.16         | [-0.1 -0.3]                       |
|                         |             | 0.90 [0.20 0.10]                   | 2.24         | [-0.5 -1.0]                       |

Herewith 45% out of 75% of attached spin labels have Gaussian line broadening with the full width at half maximum about 0.5 mT. Such broadening appears due to dipolar coupling of spin labels separated by about 1-2 nm in duplex formed by two MR (See Fig.1 in the main text). There are also
25% out of 75% of spin labels for which EPR line is described by an anisotropic slow motion, but without additional broadening. Most likely these labels refer to a singly-labeled MR and the formation of the duplex with such MR does not induce EPR line broadening for one of three nitroxides.

According to DEER data (see Fig.4 of the main text), the addition of tRNA$^{\text{Phe}}$ to the MR solution leads to the onset of new conformation of MR with $<r_{\text{DEER}}>$~3.6 nm, which was assigned to MR interacting with tRNA$^{\text{Phe}}$ by its UUC triplet. Taking into account the data obtained from simulation of CW EPR spectra of isolated MR, it is necessary to assume that the spectra of MR•tRNA$^{\text{Phe}}$ contains at least four fractions with different weights (unbound labels, attached labels with and without broadening in MR duplexes, labels in MR bound to tRNA$^{\text{Phe}}$). In fact, fitting of the CW EPR spectrum of nitroxide at X-band using such a large number of components does not lead to a unique solution. A simple comparison of the two spectra shows (Fig. S6) that the addition of tRNA$^{\text{Phe}}$ to the MR solution has a small impact on the CW EPR spectra, whereas DEER experiments are more informative and allowed us to detect a new conformation of MR.

Similar results were obtained with binary complex of MR and ribosomes in the absence of tRNA. The DEER data (Fig.4 of the main text) demonstrated the appearance of new MR conformation with $<r_{\text{DEER}}>$~3.6 nm in the presence of ribosomes in comparison with the isolated MR. Spectral resolution of CW EPR is not sufficient to track these changes and reliably separate all conformations. It is necessary to note that samples containing ribosomes were dissolved in D$_2$O, whereas samples MR and MR•tRNA contained more viscous mixture of D$_2$O and glycerol-d$_8$. Therefore, narrower CW EPR lines were observed for the spectra of the MR•ribosome complex in comparison with the spectra of the isolated MR.

According to DEER data in the presence of ribosomes and tRNA, there is an equilibrium between MR in duplex form (MR)$_2$, MR associated with ribosomes outside of the mRNA binding channel and MR bound to the channel. The analysis of CW EPR spectra (Figure S7) does not allow accurate characterization of all these fractions, although we performed a qualitative analysis of the spectra and simulated them using only two components: the fast fraction with $\tau_c = 0.16$ ns and the slow fraction with $\tau_c = 2.24$ ns.

Figure S8 compares EPR spectra for complexes 1-4 after normalization to their second integrals. The weight of the slow fraction increased when tRNAs were added to the solution, therefore this EPR signal was assigned to MR fixed in mRNA-binding site. It is noteworthy that the weights calculated from CW EPR spectra of ribosome-bound MR strongly correlate with those obtained by DEER (Table S2). The quantitative discrepancies between CW EPR and DEER data appear because the broader CW EPR line simulated with $\tau_c = 2.24$ ns actually contains the contribution of another
broad signal from MR duplexes, as was discussed above. As soon as DEER signal of MR duplexes decreases (upon addition of tRNAs directed to the ribosomal P and/or to the A and E sites), the discrepancy between CW EPR and DEER data becomes smaller. Thus, the weights obtained by CW EPR are the upper limit of weights of MR fixed at the ribosomal mRNA binding channel. We would like to note that the obtained CW EPR data at X-band cannot be used for reliable quantitative analysis of the binding event, because it is practically challenging to distinguish more than two fractions. Therefore, we used CW EPR data herewith only in order to demonstrate that, in general, these data do not contradict to the data obtained by PELDOR.

**Figure S8.** Room-temperature X-band CW EPR spectra of complexes 1-4 after normalization to the second integral.

**Table S2.** The ratio of MR fixed in ribosomal channel obtained by DEER and CW EPR spectroscopy.

| Sample             | Ribosome-bound MR ratio obtained by DEER | Ribosome-bound MR ratio obtained by CW EPR |
|--------------------|----------------------------------------|------------------------------------------|
| Complex 1          | 0.46                                    | 0.77                                     |
| Complex 2          | 0.59                                    | 0.81                                     |
| Complex 3          | 0.74                                    | 0.85                                     |
| Complex 4          | 0.71                                    | 0.84                                     |
| Complex 3 (tRNA\(^\text{Phe}\) at the E site) | 0.81                                     | 0.90                                     |

The analysis of \(\lambda\) parameters of orienting potential allows obtaining information on anisotropy of the label motion. For each sample, the set of parameters \(\lambda\) is principally the same (\(\lambda_{2,2}<\lambda_{2,0}<0\)), indicating the similar nitroxide label orientations with variable degree of anisotropy. Figure S9 shows graphical representation of population distribution of nitroxide label orientation and
compares anisotropies of motion for isolated MR and MR fixed at the ribosomal mRNA binding site. In both cases, nitroxide label orients predominantly along Y-axis. Comparing two depicted distributions, it can also be seen that the spin labels of MR fixed at ribosomal mRNA binding site undergo rotational motion that is more restricted than that in solution.

Figure S9. Character of nitroxide label motion pictured as normalized population distribution of NO’ moiety orientation. Color indicates population density. XY plane is associated with location of biopolymer, Z-axis – with average orientation of NO’ moiety. Left figure illustrates distribution for spin label residing in the ribosome with $[\lambda_{2,0} \lambda_{2,2}] = [-0.5 -0.1]$, right figure – in solution with $[\lambda_{2,0} \lambda_{2,2}] = [-0.3 -0.3]$. 
III. Phase memory time $T_m$ measurements

Measurements of electron phase memory time $T_m$ ($T_m \sim T_2$) were performed at Q-band at $T=50 \text{ K}$. $T_m$ was measured using two-pulse Electron Spin Echo (ESE) sequence with pulse lengths of 22/44 ns for $\pi/2$ and $\pi$ pulses, respectively. ESE was measured at field position corresponding to the observation pulses in DEER experiments.

Figure S10 shows the two-pulse echo decays measured for studied samples and their fittings obtained by monoexponential analysis and yielding the $T_m$ values. In case of non-exponential decay (isolated MR and MR+tRNA$^{\text{Phe}}$ mixture) the $T_m$ values were estimated as the time at which the spin echo signal decreases by a factor of $e=2.718$. The ESE decay for nitroxide radical at temperatures lower than 80 K is mainly determined by the electron-nuclear spin-spin interactions between radical and surrounding matrix protons (nuclear spin diffusion mechanism). In order to maximize the $T_m$ value, all experiments were carried out in deuterated solvents. The ribosome carries lot of protons; therefore the complex formation of MR with the ribosome should accelerate the relaxation rates, since the HFI with protons is $\sim 6.5$ times greater than that with the deuterons. Thus, the observed shorter $T_m$ values for complexes 1-5 and MR+80S compared to isolated MR indicate binding of MR to ribosomes. It is interesting that $T_m$ value for MR+tRNA$^{\text{Phe}}$ is greater than that for MR. This fact is in good agreement with the assumption that MR tends to form the duplex (MR)$_2$. Upon addition of tRNA$^{\text{Phe}}$, the duplex formed by two MR dissociates into monomers due to interaction of UUC triplet with the tRNA, which results in an increase of a number of solvent deuterium molecules near spin-label and thus increases the $T_m$ value.

![Figure S10](image)

**Figure S10.** Two-pulse echo decays at 50 K at Q-band for studied samples and obtained $T_m$ values.
IV. Electron Spin Echo Envelope Modulation (ESEEM) study

ESEEM experiments were carried out at 50 K at Q-band using commercial Bruker ELEXSYS E580 EPR spectrometer equipped with an EN5107D2 resonator. All measurements were done at the magnetic field corresponding to the ESE maximum. Three-pulse ESE sequence $\pi/2 - \tau - \pi/2 - T - \pi/2 - \tau - \text{echo}$ with the four-step phase cycling was used; $\pi/2$-pulse duration was 22 ns. The $\tau$-delay of 190 ns was optimized to achieve the maximum modulation depth due to the $^2$H nuclei. The background was removed by division by an exponential function.

Deuterium ESEEM is one of informative approaches for studying possible non-specific binding between spin-labeled MR and 80S ribosomes, which might compete with the specific binding of MR at the ribosomal mRNA channel. For this purpose, deuterium solvent was used, whereas all other biological components were not enriched and contained lot of protons. The deuterium modulation depth in three-pulse ESEEM experiments was analyzed. In the absence of ribosome and tRNA, the amount of deuterons in the vicinity of the spin label should be maximum possible, therefore one expects deepest deuterium modulation for MR. Upon addition of any other proton-containing component (80S ribosome), the deuterium modulation should decrease if any kind of supramolecular (specific or non-specific) complex is formed. Other way around, the absence of changes in deuterium ESEEM upon addition of proton-containing components would imply the absence of the complexation with MR.

![Graph showing ESEEM time traces](image.png)

**Figure S11.** Three-pulse ESEEM time traces at Q-band at 80 K for the studied samples (as indicated in the plot) normalized to the spin echo intensity.
Figure S11 shows three-pulse ESEEM time traces recorded at 80 K at Q-band for four samples: isolated MR, ribosomal complexes without tRNA and those containing tRNAs at P and E sites. The maximum deuterium modulation depth is indeed detected for the isolated MR. With 80S ribosomal complexes with tRNAs, a pronounced decrease in the modulation depth is observed, in agreement with anticipated formation of complexes where MR is bound at the ribosomal channel. The same decrease in the modulation depth is found for the sample of the MR-80S ribosome mixture without tRNA, indicating a formation of binary complexes of the ribosome with MR.