Electronic Supplementary Material (ESI) for RSC Advances.

Characterization of structure-function relationship of a novel salt-resistant antimicrobial peptide, RR12

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Supporting information

Molecular modeling of RR12 in complex with SDS micelle based on the PRE results

The determined solution of RR12 was used as the starting conformation for the molecular modeling. The SDS micelle with 60 molecules was built, minimized and equilibrated by the Micelle Maker web server (http://micelle.icm.uu.se/main.php). The GROMACS topology file for SDS coordinates (SDS.itp) was obtained from web site of Bioinformatics Group of the International Scientific-Educational Center (http://bioinformatics.am/downloads/). Based on the results of our PRE studies, the structure of RR12 was initially docked into the SDS micelle with the residues (R1-L8) buried and C-terminal residues (R9-R12) outside the micelle. Subsequently, the built peptide-micelle complex was subjected to a brief molecular dynamic simulation performed by GROMACS v4.6.7[1, 2]. The peptide-micelle coordinates were converted to GROMACS format and placed in a 100 $Å^3$ periodic box of spc216 waters and then neutralized with sodium and chloride counter ions. The RR12 peptide was further position constrained and underwent the conjugated gradient minimization to remove any interfering contacts. After that, the simulation was carried out at 313 K with the modified force filed for lipids (http://www.gromacs.org/topologies/uploaded_force_fields/ffgmx_lipids.tar.gz). The remaining parameters were set as previous studies[3] with a shorten simulation time of 2 ns to obtain the model of peptide-micelle complex in which the orientation of RR12 corroborates with the observations of PRE experiments.

Figures

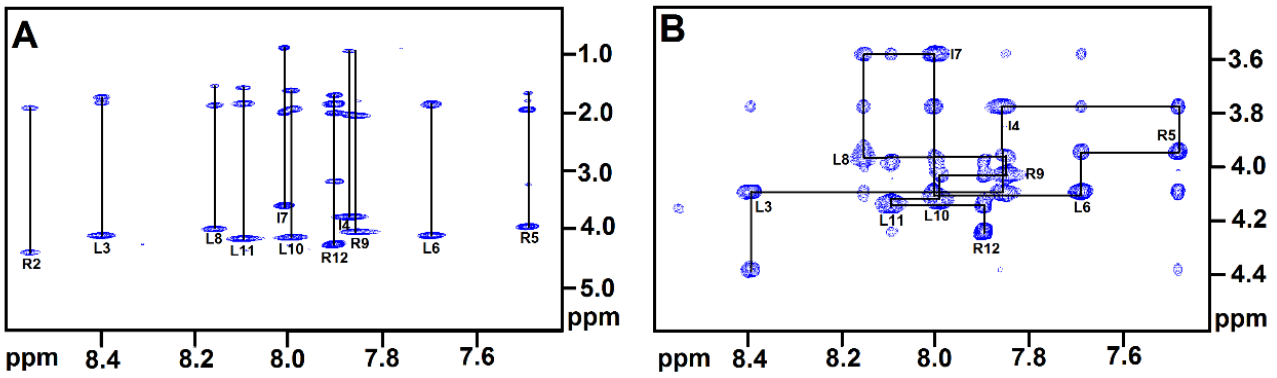

Fig. S1. (A) An 800 MHz TOCSY spectrum recorded at 60 ms. (B) The finger-print region of the NOESY spectrum (mixing time, 150 ms). In (A) and (B), peaks are labelled at the positions of the NH-C$_\alpha$H cross-peaks.

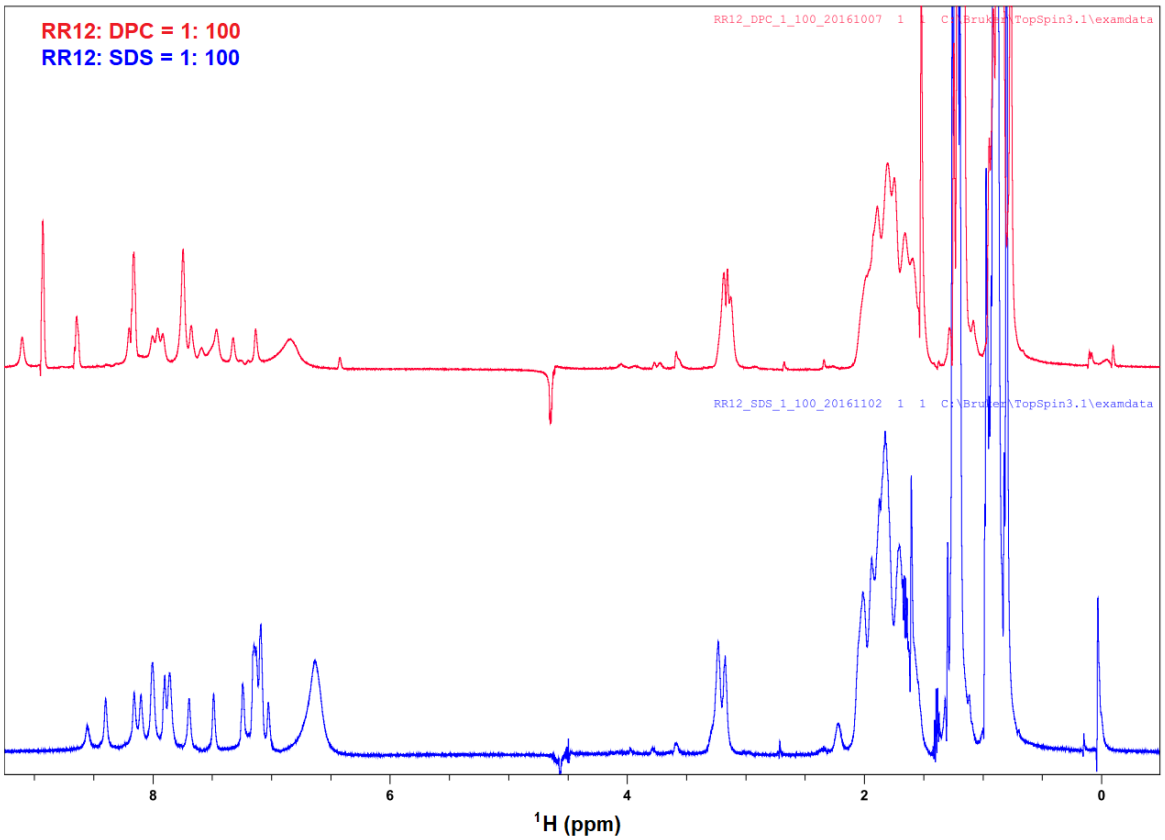

Fig. S2. 1D NMR spectra of RR12 recorded in DPC and SDS micelles.

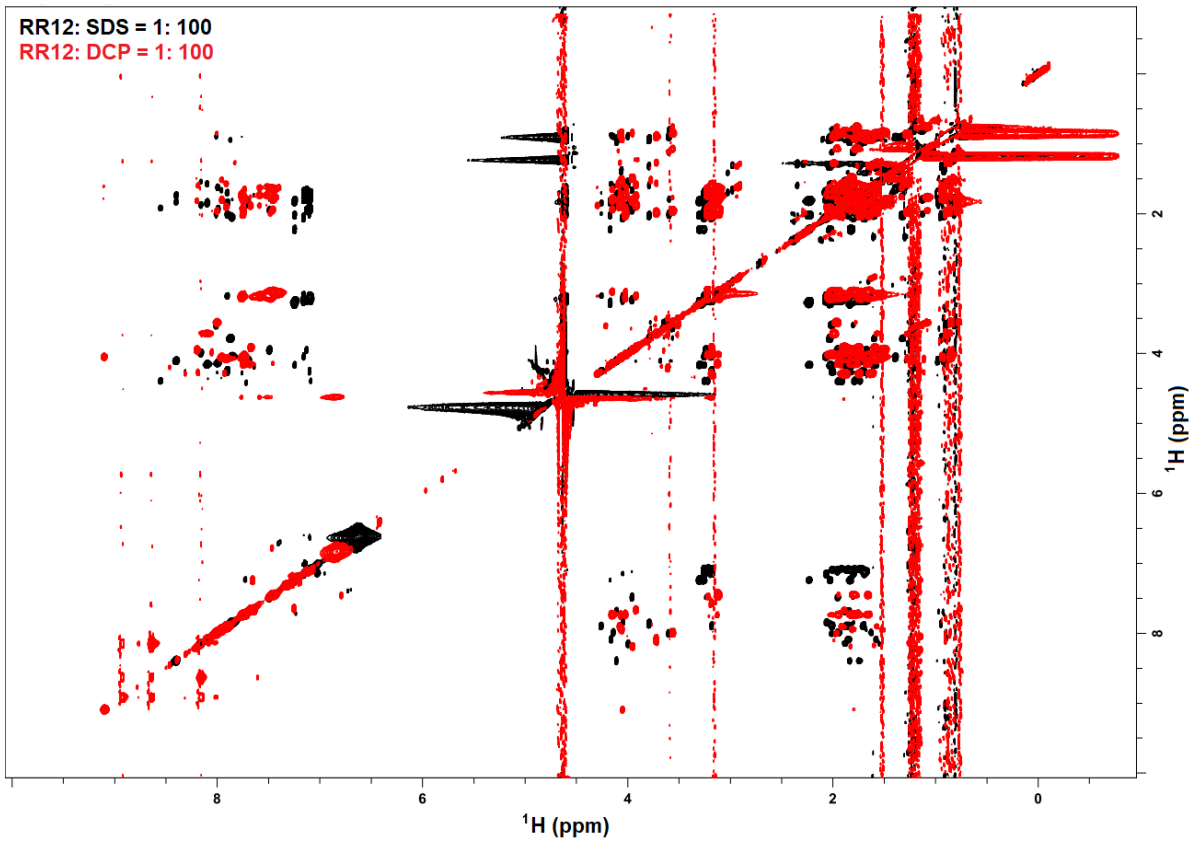

Fig. S3. The overlapped 2D-TOCSY spectra of RR12 recorded in DPC and SDS micelles.

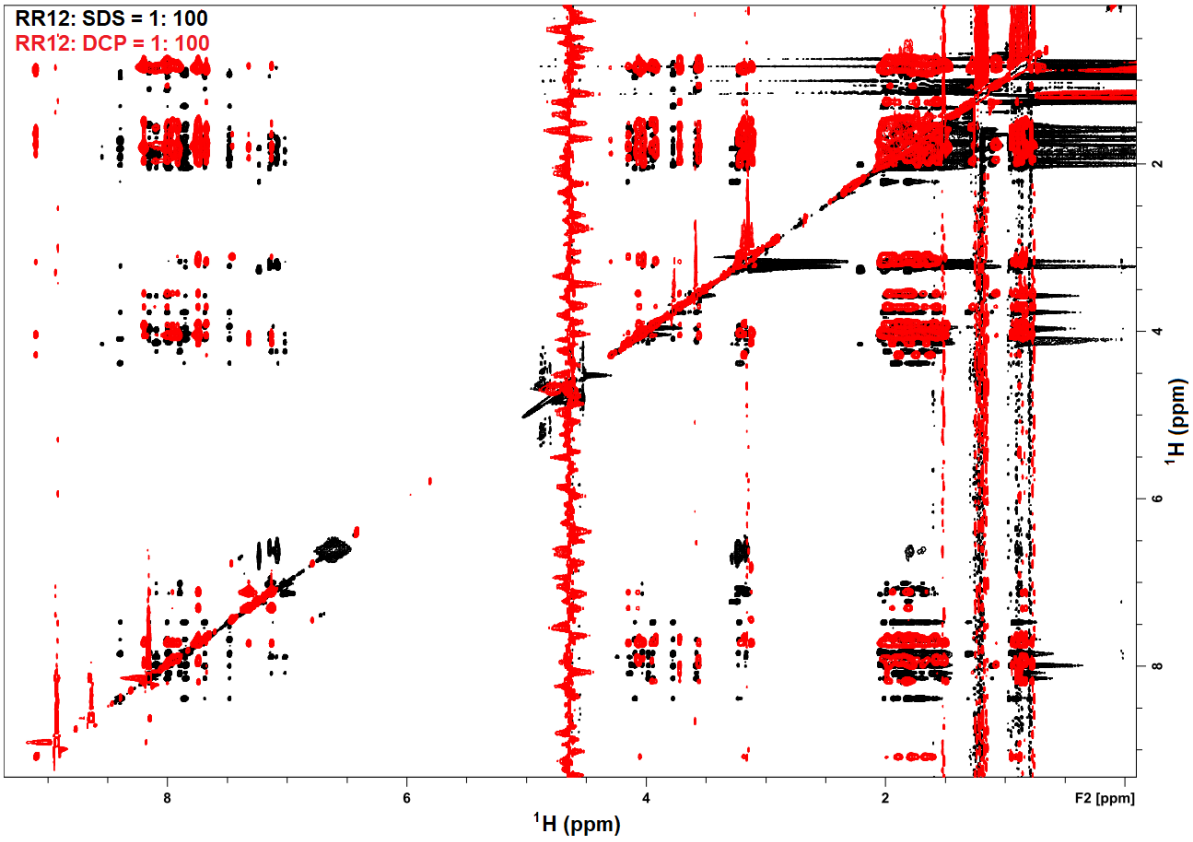

Fig. S4. The overlapped 2D-NOESY spectra of RR12 recorded in DPC and SDS micelles.

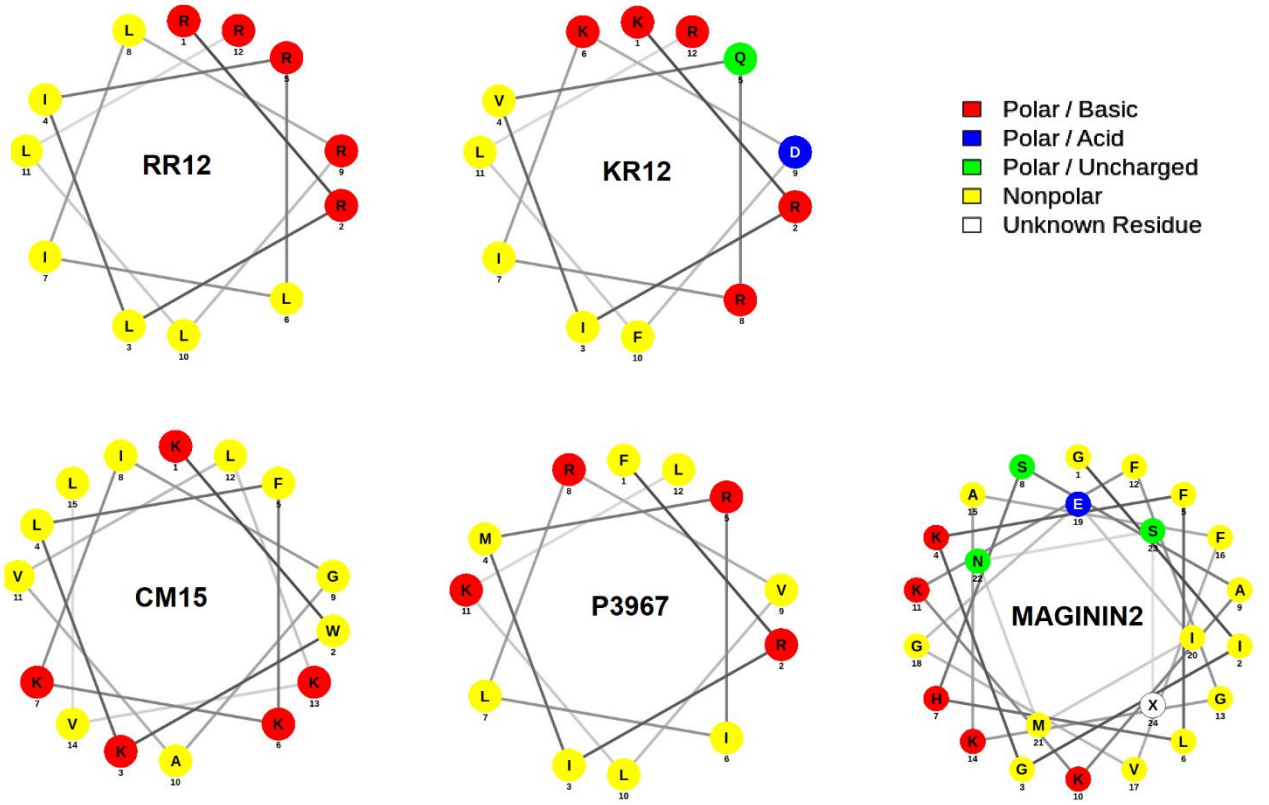

Fig. S5. Helical wheels projections of the AMPs compared with RR12. The helical wheels of the presented AMPs were draw by using NetWheels (http://lbqp.unb.br/NetWheels/).

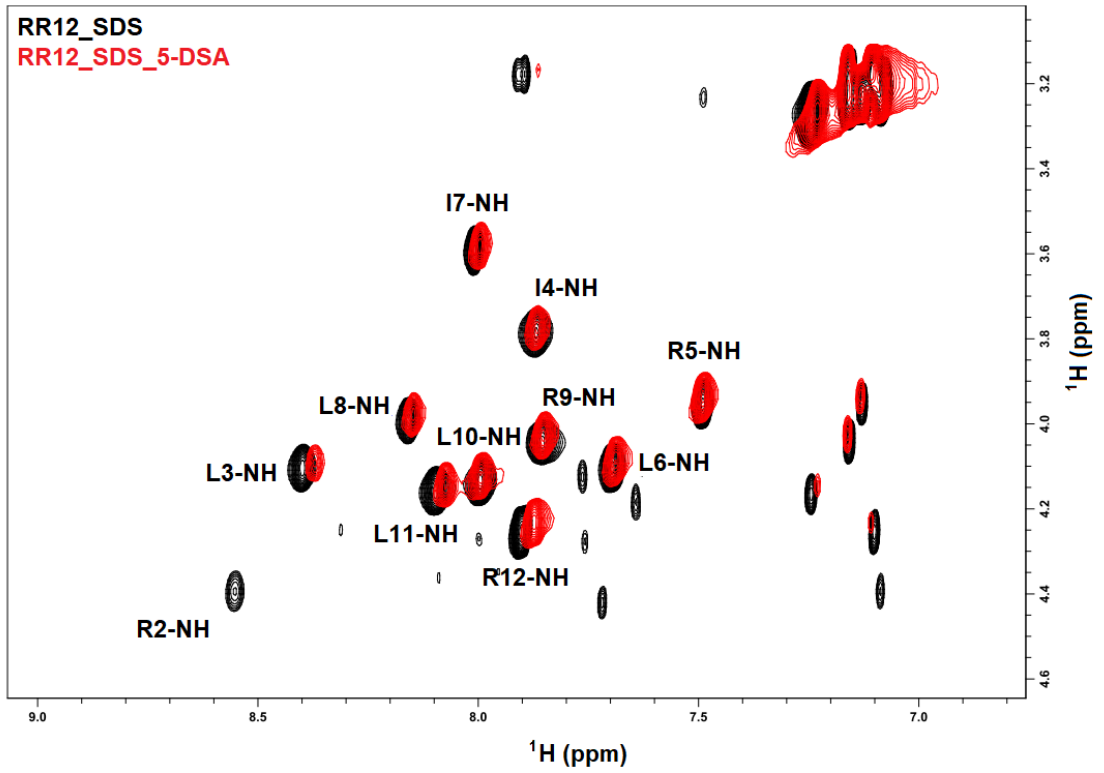

Fig. S6. PRE-NMR spectra of RR12 in SDS micelle alone and with 5-DSA. PRE induced by 5-DSA. Amide region of a TOCSY spectrum in the absence (black) and presence (red) of 5-DSA.

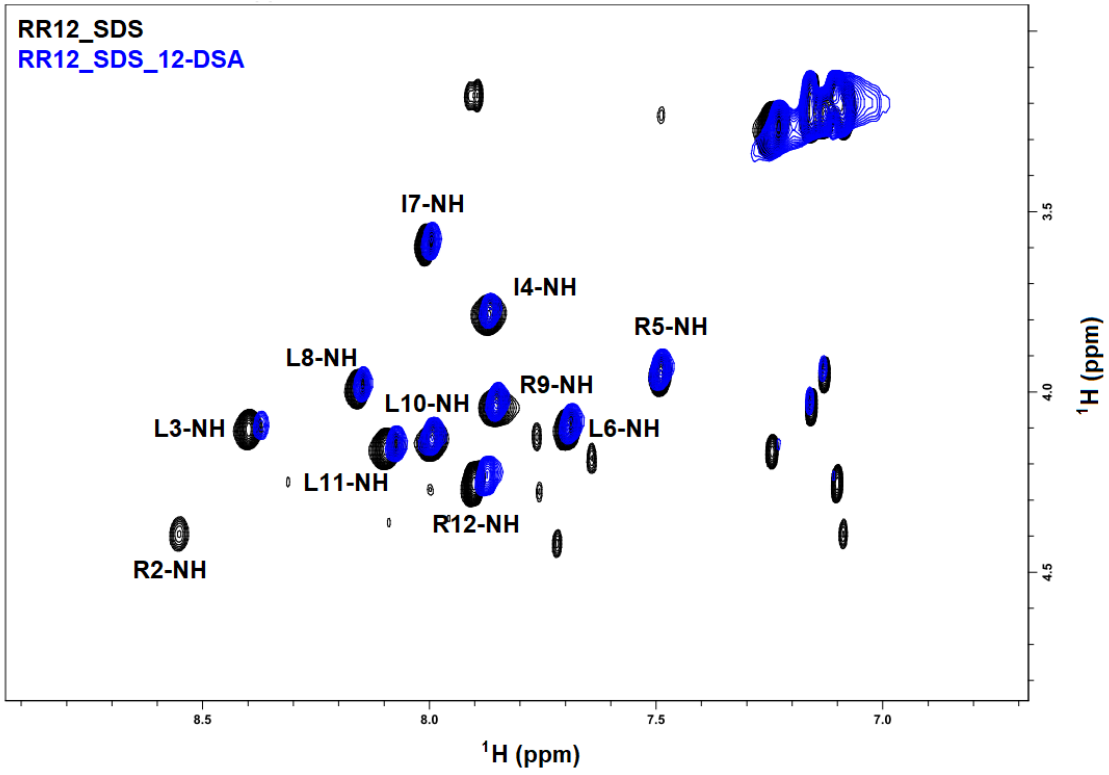

Fig. S7. PRE-NMR spectra of RR12 in SDS micelle alone and with 12-DSA. PRE induced by 12-DSA. Amide region of a TOCSY spectrum in the absence (black) and presence (blue) of 12-DSA.

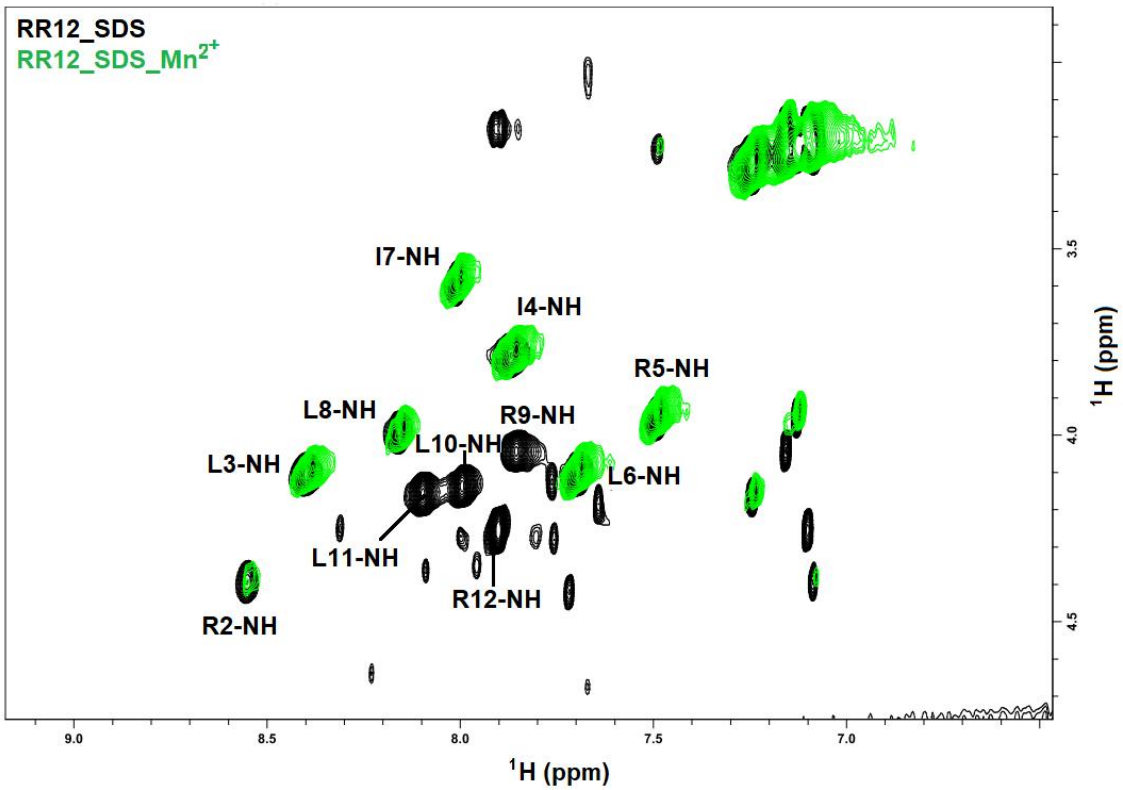

Fig. S8. PRE-NMR spectra of RR12 in SDS micelle alone and with Mn^{2+} ions. PRE induced by Mn^{2+} ions. Amide region of a TOCSY spectrum in the absence (black) and presence (green) of 0.5mM Mn^{2+} ions.

Tables

Table1. The NMR chemical shift table of RR12.

Group	Atom	Nuclear	Shift	SDev	Group	Atom	Nuclear	Shift	SDev
R1	HA	^{1}H	4.16	0.003	**I7**	HB	^{1}H	2.015	0.005
R1	HB	^{1}H	2.222	0	**I7**	HG2	^{1}H	0.896	0.007
R1	HB2	^{1}H	2.22	0.003	**I7**	HN	^{1}H	8.003	0.003
R1	HB3	^{1}H	2.016	0.002	**I7**	HN	^{15}N	118.42	0.01
R1	HD#	^{1}H	3.244	0	**L8**	HA	^{1}H	3.985	0.006
R1	HD2	^{1}H	3.287	0.003	**L8**	HB#	^{1}H	1.876	0.008
R1	HD3	^{1}H	3.245	0	**L8**	HG	^{1}H	1.54	0.003
R1	HE	^{1}H	7.236	0.001	**L8**	HN	^{1}H	8.153	0.004
R1	HG#	^{1}H	1.821	0.003	**L8**	HN	^{15}N	118.3	0.002
R2	HA	^{1}H	4.389	0.003	**R9**	HA	^{1}H	4.035	0.005
R2	HB#	^{1}H	1.932	0.006	**R9**	HB#	^{1}H	2.047	0.004
R2	HD#	^{1}H	3.248	0	**R9**	HD2	^{1}H	3.236	0.006
R2	HE	^{1}H	7.079	0.001	**R9**	HD3	^{1}H	3.169	0.002
R2	HN	^{1}H	8.549	0.001	**R9**	HE	^{1}H	7.148	0.001
R2	NHε	^{15}N	124.05	0.01	**R9**	HG2	^{1}H	1.796	0.007
L3	HA	^{1}H	4.103	0.007	**R9**	HG3	^{1}H	1.699	0.01
L3	HB2	^{1}H	1.825	0	**R9**	HN	^{1}H	7.852	0.003
L3	HB3	^{1}H	1.751	0	**R9**	HN	^{15}N	118.68	0.02
L3	HG	^{1}H	1.731	0	**R9**	NHε	^{15}N	124.86	0.01
L3	HN	^{1}H	8.396	0.002	**L10**	HA	^{1}H	4.13	0.008
L3	HN	^{15}N	120.99	0.02	**L10**	HB#	^{1}H	1.932	0
I4	HA	^{1}H	3.779	0.003	**L10**	HG	^{1}H	1.611	0.003
I4	HG2	^{1}H	0.934	0.01	**L10**	HN	^{1}H	7.995	0.003
I4	HN	^{1}H	7.862	0.004	**L10**	HN	^{15}N	119.7	0
I4	HN	^{15}N	117.07	0.01	**L11**	HA	^{1}H	4.143	0.01
R5	HA	^{1}H	3.947	0.003	**L11**	HB#	^{1}H	1.864	0.004
R5	HB#	^{1}H	1.941	0.005	**L11**	HG	^{1}H	1.58	0.012
R5	HD#	^{1}H	3.228	0.002	**L11**	HN	^{1}H	8.096	0.004
R5	HE	^{1}H	7.119	0	**L11**	HN	^{15}N	117.63	0.01
R5	HG#	^{1}H	1.663	0	**R12**	HA	^{1}H	4.25	0.004
R5	HG2	^{1}H	1.802	0.005	**R12**	HB2	^{1}H	2.002	0.008
R5	HG3	^{1}H	1.672	0.005	**R12**	HB3	^{1}H	1.843	0.006
R5	HN	^{1}H	7.483	0.002	**R12**	HD#	^{1}H	3.173	0.003
R5	HN	^{15}N	118.46	0.01	**R12**	HE	^{1}H	7.091	0.001
R5	NHε	^{15}N	124.43	0.02	**R12**	HG#	^{1}H	1.702	0.003

L6	HA	^{1}H	4.1	0.005	**R12**	HH1#	^{1}H	7.134	0.001
L6	HB#	^{1}H	1.843	0	**R12**	HH2#	^{1}H	7.019	0.001
L6	HN	^{1}H	7.697	0.034	**R12**	HN	^{1}H	7.896	0.002
L6	HN	^{15}N	120.34	0.02	**R12**	HN	^{15}N	117.51	0.01
I7	HA	^{1}H	3.584	0.006	**R12**	NHε	^{15}N	124.64	0.02

References

1. D. Van Der Spoel, E. Lindahl, B. Hess, G. Groenhof, A. E. Mark and H. J. Berendsen, *J Comput Chem*, 2005, **26**, 1701-1718.
2. B. Hess, C. Kutzner, D. van der Spoel and E. Lindahl, *J Chem Theory Comput*, 2008, **4**, 435-447.
3. T. S. Tseng, S. H. Wang, T. W. Chang, H. M. Wei, Y. J. Wang, K. C. Tsai, Y. D. Liao and C. Chen, *PLoS One*, 2016, **11**, e0164597.