

Materials Design Analysis Reporting (MDAR) **Checklist for Authors**

The MDAR framework establishes a minimum set of requirements in transparent reporting mainly applicable to studies in the life sciences.

eLife asks authors to provide detailed information within their article to facilitate the interpretation and replication of their work. Authors can also upload supporting materials to comply with relevant reporting guidelines for health-related research (see EQUATOR Network), life science research (see the BioSharing Information Resource), or animal research (see the ARRIVE Guidelines and the STRANGE Framework; for details, see *eLife's* Journal Policies). Where applicable, authors should refer to any relevant reporting standards materials in this form.

For all that apply, please note where in the article the information is provided. Please note that we also collect information about data availability and ethics in the submission form.

Materials:

| Newly created materials | Indicate where provided: section/figure legend | N/A |
|--|---|-----|
| The manuscript includes a dedicated "materials availability statement" providing transparent disclosure about availability of newly created materials including details on how materials can be accessed and describing any restrictions on access. | | N/A |

| Antibodies | Indicate where provided: section/figure legend | N/A |
|--|---|-----|
| For commercial reagents, provide supplier name, catalogue number and <u>RRID</u> , if available. | | N/A |

| DNA and RNA sequences | Indicate where provided: section/figure legend | N/A |
|---|---|-----|
| Short novel DNA or RNA including primers, probes: Sequences should be included or deposited in a public repository. | 5 RNA duplexes were used in this study whose sequences are mentioned in Supplementary Information file in "Supplementary File 2: RNA sequences used to study interaction with dsRBDs." | |

| Cell materials | Indicate where provided: section/figure legend | N/A |
|--|---|-----|
| Cell lines: Provide species information, strain. Provide accession number in repository OR supplier name, catalog number, clone number, OR RRID. | | N/A |

| Primary cultures: Provide species, strain, sex of origin, genetic modification status. | | N/A |
|--|--|-----|
|--|--|-----|

| Experimental animals | Indicate where provided: section/figure legend | N/A |
|---|---|-----|
| Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository OR supplier name, catalog number, clone number, OR RRID. | | N/A |
| Animal observed in or captured from the field: Provide species, sex, and age where possible. | | N/A |

| Plants and microbes | Indicate where provided: section/figure legend | N/A |
|--|---|-----|
| Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens). | | N/A |
| Microbes: provide species and strain, unique accession number if available, and source. | | N/A |

| Human research participants | Indicate where provided: section/figure legend) or state if these demographics were not collected | N/A |
|--|--|-----|
| If collected and within the bounds of privacy constraints report on age, sex, gender and ethnicity for all study participants. | | N/A |

Design:

| Study protocol | Indicate where provided: section/figure legend | N/A |
|---|---|-----|
| If the study protocol has been pre-registered, provide DOI. For clinical trials, provide the trial registration number OR cite DOI. | | N/A |

| Laboratory protocol | Indicate where provided: section/figure legend | N/A |
|---|---|-----|
| Provide DOI OR other citation details if detailed step-by-step protocols are available. | | N/A |

| Experimental study design (statistics details) * | | - |
|---|--|-----|
| For in vivo studies: State whether and how the following have been done | Indicate where provided: section/figure legend. If it could have been done, but was not, write "not done" | N/A |
| Sample size determination | | N/A |
| Randomisation | | N/A |
| Blinding | | N/A |
| Inclusion/exclusion criteria | | N/A |

| Sample definition and in-laboratory replication | Indicate where provided: section/figure legend | N/A |
|--|---|-----|
| State number of times the experiment was replicated in the laboratory. | The number of times the ITC calorimetry experiments are replicated is mentioned under the section "Materials and Methods" sub-section "Isothermal titration calorimetric binding assays" which states "All isothermal titration calorimetry (ITC) experiments were performed using a MicroCal PEAQ-ITC calorimeter (Malvern Panalytical, Malvern, UK) operating at 25°C. The final RNAs and protein solutions used for the assays were prepared in buffer D. The D12 dsRNA was used at a concentration of 10 or 20 μ M in the sample cell. TRBP2-dsRBD1 concentration was varied from 5–19 folds of RNA, whereas, in the case of TRBP2-dsRBD2, it was varied from 10–18 folds. The first injection was 0.4 μ l (discarded for data analysis), which was followed by eighteen 2 μ l injections. All the ITC data was measured in triplicate. Data were fitted with a single-site binding model using the MicroCal PEAQ-ITC analysis software (Malvern Panalytical, Malvern, UK) to extract the equilibrium dissociation constant (Kd), stoichiometry (n), and change in enthalpy (Δ H). The final values of the thermodynamic parameters are given as the average of triplicate measurements (Supplementary File 10)." | |
| Define whether data describe technical or biological replicates. | | N/A |

| Ethics | Indicate where provided: section/submission form | N/A |
|---|---|-----|
| Studies involving human participants: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval. | | N/A |
| Studies involving experimental animals: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval. | | N/A |
| Studies involving specimen and field samples: State if relevant permits obtained, provide details of authority approving study; if none were required, explain why. | | N/A |

| Dual Use Research of Concern (DURC) | Indicate where provided: section/submission form | N/A |
|--|---|-----|
| If study is subject to dual use research of concern regulations, state the authority granting approval and reference number for the regulatory approval. | | N/A |

Analysis:

| Attrition | Indicate where provided: section/figure legend | N/A |
|--|--|-----|
| Describe whether exclusion criteria were pre-established. Report if sample or data points were omitted from analysis. If yes, report if this was due to attrition or intentional exclusion and provide justification. | The exclusion criteria of not considering certain residues while analysis are mentiond in the following residues of TRBP2- dsRBD2 are mentioned in the following Sections/Figure legends: 1) Section: "Materials and Methods" sub-section "Protein overexpression and purification":first paragraph which states "Treatment with TEV protease during purification resulted in non-native Ser-Asn-Ala residues at the N-terminal to TRBP2-dsRBD2 (154-234), which were excluded from all the NMR-based dynamics studies" | |
| | 2) Figure legend: "Figure 3: Spin relaxation parameters (A) R ₁ , (B) R ₂ , and (C) [¹ H]- ¹⁵ N nOe plotted against the residues for (left panel) apo TRBP2-dsRBD2 and (right panel) D12-bound TRBP2-dsRBD2. Experiments were recorded on a 600 MHz NMR spectrometer at 298 K. The secondary structure of TRBP2-dsRBD2 has been mentioned | |

at the top, and the RNA-binding region of the protein has been marked in grey vertical columns. Average R_1 , R_2 , and $[^{1}H]^{-15}N$ -nOe of the core residues (159-227 aa) at 600 MHz is depicted in the green bar, and at 800 MHz is depicted in the red bars."

3) Supplementary Figure legend: "Figure 3-figure supplement 1: A) Longitudinal relaxation rates (R_1) , B) transverse relaxation rates (R_2) , and heteronuclear [1H]-15N-nOe, as measured for common residues of TRBP2-dsRDB2 on 600 MHz (black) and 800 MHz (grey) magnetic fields at 298 K plotted against residue numbers for both fields. Average $R_{\rm L}$ R_{2} , and $[^{1}H]-^{15}N-nOe$ of the core residues (159-227 aa) at 600 MHz is depicted in the green bar, and at 800 MHz is depicted in the grey bar (average is calculated only for the common residues that could be analyzed between the data measured at two magnetic fields). The secondary structure of the protein has been shown on the top, and three RNA-binding regions have been highlighted using vertical grey bars."

4) Supplementary file legend: "Supplementary File 11: Nuclear spin relaxation data for RNA-bound TRBP2-dsRBD2 recorded at 600 MHz and 800 MHz NMR spectrometer. Data for some residues is missing in this table due to line-broadening issues in the corresponding experiments."

5) Figure legend: "Figure 7: Conformational exchange perturbations in core TRBP2dsRBD2 in the presence of D12 RNA. (A) Δk_{ex} (D12-bound – apo) TRBP2dsRBD2 plotted against residue numbers. The secondary structure has been shown on the top, and three RNA-binding regions have been highlighted using vertical grey bars. Only residues having significant perturbation ($\Delta k_{ex} > 10$ kHz) have been plotted, where an increase is shown in red, and a decrease is shown in blue, (B) An increase in k_{ex} (red) and a decrease (blue) in the presence of D12 RNA indicated on the backbone of the CS-Rosetta structure of apo TRBP2dsRBD2. The RNA-binding residues have been depicted in stick mode in the tertiary structure."

| Statistics | Indicate where provided: section/figure legend | N/A |
|--|---|-----|
| Describe statistical tests used and justify choice of tests. | | N/A |

| Data availability | Indicate where provided: section/submission form | N/A |
|--|---|-----|
| For newly created and reused datasets, the manuscript includes a data availability statement that provides details for access (or notes restrictions on access). | | N/A |
| When newly created datasets are publicly available, provide accession number in repository OR DOI and licensing details where available. | | |
| If reused data is publicly available provide accession number in repository OR DOI, OR URL, OR citation. | | N/A |

| Code availability | Indicate where provided: section/figure legend | N/A |
|--|--|-----|
| For any computer code/software/mathematical algorithms essential for replicating the main findings of the study, whether newly generated or re-used, the manuscript includes a data availability statement that provides details for access or notes restrictions. | DOI: 10.1007/BF00197809; DOI: 10.1093/bioinformatics/btu830; ISBN 3-85600-112-3; DOI: 10.1093/bioinformatics/btu166 | |
| Where newly generated code is publicly available, provide accession number in repository, OR DOI OR URL and licensing details where available. State any restrictions on code availability or accessibility. | | N/A |
| If reused code is publicly available provide accession number in repository OR DOI OR URL, OR citation. | | N/A |

Reporting:

The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives.

| Adherence to community standards Indicate where provided: section/figure legend | N/A |
|---|-----|
|---|-----|

* We provide the following guidance regarding transparent reporting and statistics; we also refer authors to <u>Ten common statistical mistakes to watch out for when writing or reviewing a manuscript</u>.

Sample-size estimation

- You should state whether an appropriate sample size was computed when the study was being designed
- You should state the statistical method of sample size computation and any required assumptions
- If no explicit power analysis was used, you should describe how you decided what sample (replicate) size (number) to use

Replicates

- You should report how often each experiment was performed
- You should include a definition of biological versus technical replication
- The data obtained should be provided and sufficient information should be provided to indicate the number of independent biological and/or technical replicates
- If you encountered any outliers, you should describe how these were handled
- Criteria for exclusion/inclusion of data should be clearly stated
- High-throughput sequence data should be uploaded before submission, with a private link for reviewers provided (these are available from both GEO and ArrayExpress)

Statistical reporting

- Statistical analysis methods should be described and justified
- Raw data should be presented in figures whenever informative to do so (typically when N per group is less than 10)
- For each experiment, you should identify the statistical tests used, exact values of N, definitions of center, methods of multiple test correction, and dispersion and precision measures (e.g., mean, median, SD, SEM, confidence intervals; and, for the major substantive results, a measure of effect size (e.g., Pearson's r, Cohen's d)
- Report exact p-values wherever possible alongside the summary statistics and 95% confidence intervals. These should be reported for all key questions and not only when the p-value is less than 0.05.

Group allocation

- Indicate how samples were allocated into experimental groups (in the case of clinical studies, please specify allocation to treatment method); if randomization was used, please also state if restricted randomization was applied
- Indicate if masking was used during group allocation, data collection and/or data analysis