

Supplemental Material

CBE—Life Sciences Education

Tobler *et al.*

Supplementary Materials

Understanding Randomness on a Molecular Level: A Diagnostic Tool

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Appendix A: Molecular Randomness Concept Inventory

A1. MRCI in English

Each item was shown on an individual page. There was no time limit to answer the question. Participants were advised to work alone and without any auxiliaries. Bold answers correspond to the correct answer.

R1. *Adapted from Champagne Queloz et al. (2017), originally published by Garvin-Doxas and Klymkowsky (2008) (BCI item 17)*

How can it be ensured that a molecule binds its correct partner and avoids incorrect interactions?

- a. The two binding partners send signals to each other.
- b. The binding partners have sensors with which they check for incorrect binding.
- c. The correct binding results in a lower energy than incorrect binding does.**
- d. Correctly bound molecules fit together perfectly like puzzle pieces.

R2. *Adapted from Champagne Queloz et al. (2017), originally published by Garvin-Doxas and Klymkowsky (2008) (BCI item 18)*

If two molecules are non-covalently bound to each other, how can they be separated again?

- a. Only if a chemical reaction has changed the structure of one of the molecules, the two molecules can separate again.
- b. Collisions with other molecules could separate them.**
- c. The complex must be broken down.
- d. They must bind to another molecule.

R3. Adapted from Champagne Queloz et al. (2017), originally published by Garvin-Doxas and Klymkowsky (2008) (BCI item 25)

Imagine an ADP molecule in a bacterial cell. How might this molecule get to an ATP synthase to be completed and form an ATP molecule?

- a. The ATP synthase would simply grab the ADP molecule.
- b. Because of its electronegativity, it would be attracted to ATP synthase.
- c. It would be actively pumped to the right place.
- d. The molecule arrives at the ATP synthase by random movements.**

R4. Strongly adapted from Fisher et al., (2011) (ODCA item 3)

During diffusion in a human cell, molecules generally move from high to low concentration. This is because

- a. densely packed molecules want to move to an area with more space.
- b. the random movement of molecules in a liquid leads to their uniform distribution.**
- c. the molecules tend to move until they are evenly distributed and then stop moving.
- d. the probability is higher that molecules will repel each other.

R5. Adapted from Couch et al. (2015) (MBCA item 12)

The synaptic cleft separates two neurons. The presynaptic neuron secretes a neurotransmitter that binds specifically to a membrane receptor of the postsynaptic neuron.

Which explanation of how the ligand can cross the cleft is most likely true?

- a. The receptor pulls the neurotransmitter across the synaptic cleft.
- b. Charged regions on the neurotransmitter and receptor attract each other.
- c. A transport protein carries the neurotransmitter across the synaptic cleft to the postsynaptic neuron.
- d. The neurotransmitter sometimes moves toward and sometimes away from the receptor.**

R6.

Proteins, which are transported into the nucleus after translation, carry a nuclear localization signal on their surface. This nuclear localization signal is transported into the cell nucleus with the help of a nuclear pore complex. How does the to-be imported protein find the nuclear pore complex?

- a. The nuclear localization signal directs the to-be imported protein through the cytosol to the nuclear pore complex.
- b. Cytoplasmic transport proteins recognize proteins with nuclear localization signals and bring them to the nuclear pore complex.
- c. The to-be imported protein moves around the cell undirected until the nuclear localization signal encounters a nuclear pore complex and is imported.**
- d. Proteins with a nuclear localization signal are attracted to the nuclear pore complex because of their positive charge.

R7.

A faulty aminoacyl-tRNA synthase attaches an aspartate to the tRNA molecule instead of the amino acid glutamate. Which statement about the resulting effects in translation is true?

- a. Because an incorrect amino acid residue is attached to the tRNA molecule, it is no longer attracted to the ribosome, and the protein is not formed.
- b. Even if incorrect amino acid residues are bound to the tRNA molecule, such molecules are actively attracted to the ribosome by the cytosol, and the wrong amino acid is incorporated into the protein.
- c. tRNA molecules are directionally pumped to ribosomes regardless of whether or not they have the correct amino acid bound.
- d. None of the above answers are true.**

R8.

For transcription to occur in bacteria, the RNA polymerase complex must first find the promoter region on the DNA. Which statement about this process is true?

- a. **The holoenzyme of the bacterial RNA polymerase complex moves along the DNA without a specific target until it encounters and binds to the promoter region.**
- b. Specific transcription initiation factors bound to the promoter regions seek and recruit RNA polymerase complexes moving freely in the cytoplasm.
- c. The RNA polymerase complex diffuses freely in the nucleoplasm until the holoenzyme of this complex is pumped to a promoter region to bind.
- d. Promoter regions of genes to be transcribed attract the RNA polymerase complex due to its positive charge, causing it to bind to DNA.

R9.

The sodium-potassium pump transports sodium and potassium ions against their concentration gradients across the membrane. How do the ions get to the transmembrane protein?

- a. For sodium ions to be transported across the membrane, potassium ions must first be brought to the sodium-potassium pump.
- b. ATP must be hydrolyzed so that there is enough energy to recruit sodium ions from the extracellular space to the transmembrane protein.
- c. Sodium and potassium ions are attracted to the transmembrane protein because of their electronegative charge.
- d. **Due to collisions with other molecules, the ions move to the sodium-potassium pump.**

A2. MRCI in German

Each item was shown on an individual page. There was no time limit to answer the question. Participants were advised to work alone and without any auxiliaries. Bold answers correspond to the correct answer.

R1. *Adapted from Champagne Queloz et al. (2017), originally published by Garvin-Doxas and Klymkowsky (2008) (BCI item 17)*

Wie kann sichergestellt werden, dass ein Molekül seinen richtigen Partner bindet und falsche Interaktionen vermieden werden?

- a. Die beiden Bindungspartner senden Signale zueinander aus.
- b. Die Bindungspartner haben Sensoren, mit denen sie falsche Bindungen überprüfen.
- c. Durch die richtige Bindung wird eine niedrigere Energie erreicht als durch die falsche.**
- d. Richtig gebundene Moleküle passen wie Puzzleteile perfekt zueinander.

R2. *Adapted from Champagne Queloz et al. (2017), originally published by Garvin-Doxas and Klymkowsky (2008) (BCI item 18)*

Wenn zwei Moleküle nicht-kovalent aneinandergelassen sind, wie können sie dann wieder getrennt werden?

- a. Nur wenn die Struktur eines der Moleküle durch eine chemische Reaktion verändert worden ist, können sich die beiden Moleküle wieder trennen.
- b. Sie könnten durch Zusammenstöße mit anderen Molekülen getrennt werden.**
- c. Der Komplex muss abgebaut werden.
- d. Sie müssen an ein weiteres Molekül binden.

R3. *Adapted from Champagne Queloz et al. (2017), originally published by Garvin-Doxas and Klymkowsky (2008) (BCI item 25)*

Stellen Sie sich ein ADP-Molekül in einer Bakterienzelle vor. Wie könnte dieses Molekül zu einer ATP-Synthase gelangen, so dass es zu einem ATP Molekül ergänzt werden kann?

- a. Die ATP-Synthase würde sich das ADP Molekül einfach greifen.
- b. Aufgrund seiner Elektronegativität würde es von der ATP-Synthase angezogen.
- c. Es würde aktiv an die richtige Stelle gepumpt werden.
- d. Das Molekül gelangt durch zufällige Bewegungen zur ATP-Synthase.**

R4. Strongly adapted from Fisher et al., (2011) (ODCA item 3)

Während der Diffusion in einer menschlichen Zelle bewegen sich Moleküle generell von hoher zu tiefer Konzentration. Dies ist, weil

- a. dicht gedrängte Moleküle sich in ein Gebiet mit mehr Raum bewegen wollen.
- b. die zufällige Bewegung von Molekülen in einer Flüssigkeit zu deren gleichmässigen Verteilung führt.**
- c. die Moleküle dazu tendieren, sich zu bewegen bis sie gleichmässig verteilt sind und dann aufhören, sich zu bewegen.
- d. die Wahrscheinlichkeit höher ist, dass sich Moleküle abstossen.

R5. Adapted from Couch et al. (2015) (MBCA item 12)

Zwei Neuronen sind durch den synaptischen Spalt voneinander getrennt. Das präsynaptische Neuron sekretiert einen Neurotransmitter, der spezifisch an einen Membranrezeptor des postsynaptischen Neurons bindet. Welche Erklärung, wie der Ligand den Spalt überwinden kann, trifft am ehesten zu?

- a. Der Rezeptor zieht den Neurotransmitter durch den synaptischen Spalt.
- b. Geladene Regionen des Neurotransmitters und des Rezeptors ziehen sich gegenseitig an.
- c. Ein Transportprotein transportiert den Neurotransmitter durch den synaptischen Spalt zum postsynaptischen Neuron.
- d. Der Neurotransmitter bewegt sich manchmal auf den Rezeptor zu und manchmal von ihm weg.**

R6.

Proteine, die nach der Translation in den Zellkern transportiert werden, tragen auf ihrer Oberfläche ein Kernlokalisierungssignal. Dieses wird mit Hilfe eines Kernporenkomplexes in den Zellkern geschleust. Wie findet das zu importierende Protein den Kernporenkomplex?

- a. Das Kernlokalisierungssignal leitet das zu importierende Protein durch das Zytosol zum Kernporenkomplex.
- b. Zytoplasmatische Transportproteine erkennen Proteine mit Kernlokalisierungssignal und bringen diese zum Kernporenkomplex.
- c. Das zu importierende Protein bewegt sich ungerichtet in der Zelle umher, bis das Kernlokalisierungssignal auf einen Kernporenkomplex trifft und importiert wird.**
- d. Proteine mit einem Kernlokalisierungssignal werden aufgrund ihrer positiven Ladung vom Kernporenkomplex angezogen.

R7.

Eine fehlerhafte Aminoacyl-tRNA Synthase hängt anstatt der Aminosäure Glutamat ein Aspartat an das tRNA-Molekül an. Welche Aussage über die daraus folgenden Auswirkungen bei der Translation stimmt?

- a. Da ein falscher Aminosäure-Rest am tRNA-Molekül gebunden ist, wird dieses vom Ribosom nicht mehr angezogen und das Protein wird nicht gebildet.
- b. Auch wenn falsche Aminosäure-Reste am tRNA-Molekül gebunden sind, werden solche tRNA-Moleküle aktiv vom Ribosom durch das Zytosol angezogen und die falsche Aminosäure in das Protein eingebaut.
- c. tRNA-Moleküle werden unabhängig davon, ob sie die richtige Aminosäure gebunden haben oder nicht, gerichtet zu Ribosomen gepumpt.
- d. Keine der genannten Antworten trifft zu.**

R8.

Damit die Transkription in Bakterien stattfinden kann, muss der RNA-Polymerase-Komplex zuerst die Promoter-Region auf der DNA finden. Welche Aussage zu diesem Prozess stimmt?

- a. **Das Holoenzym des bakteriellen RNA-Polymerase-Komplexes bewegt sich ohne spezifisches Ziel entlang der DNA, bis es auf die Promotor-Region trifft und daran bindet.**
- b. Spezifische an die Promotor-Regionen gebundenen Transkriptions-Initiationsfaktoren suchen und rekrutieren von dort sich im Zytoplasma frei bewegenden RNA-Polymerase-Komplexe.
- c. Der RNA-Polymerase-Komplex diffundiert frei im Nukleoplasma umher, bis das Holoenzym dieses Komplexes an eine Promotor-Region gepumpt wird und so binden kann.
- d. Promotor-Regionen von Genen, die transkribiert werden sollen, ziehen den RNA-Polymerase-Komplex aufgrund dessen positiven Ladung an, wodurch dieser an die DNA bindet.

R9.

Die Natrium-Kalium-Pumpe transportiert Natrium- und Kalium-Ionen gegen ihre Konzentrationsgradienten über die Membran. Wie gelangen die Ionen zum Transmembranprotein?

- a. Damit Natrium-Ionen über die Membran transportiert werden können, müssen zuerst Kalium-Ionen an die Natrium-Kalium-Pumpe gebracht werden.
- b. ATP muss hydrolysiert werden, damit genug Energie vorhanden ist, um Natrium-Ionen aus dem extrazellulären Raum zum Transmembranprotein zu rekrutieren.
- c. Natrium- und Kalium-Ionen werden aufgrund ihrer elektronegativen Ladung vom Transmembranprotein angezogen.
- d. **Aufgrund von Kollisionen mit anderen Molekülen bewegen sich die Ionen zur Natrium-Kalium-Pumpe.**

A3. References

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Appendix B: Supplementary Figures

Figure S1

Bland-Altman Plot for assessing test-retest reliability

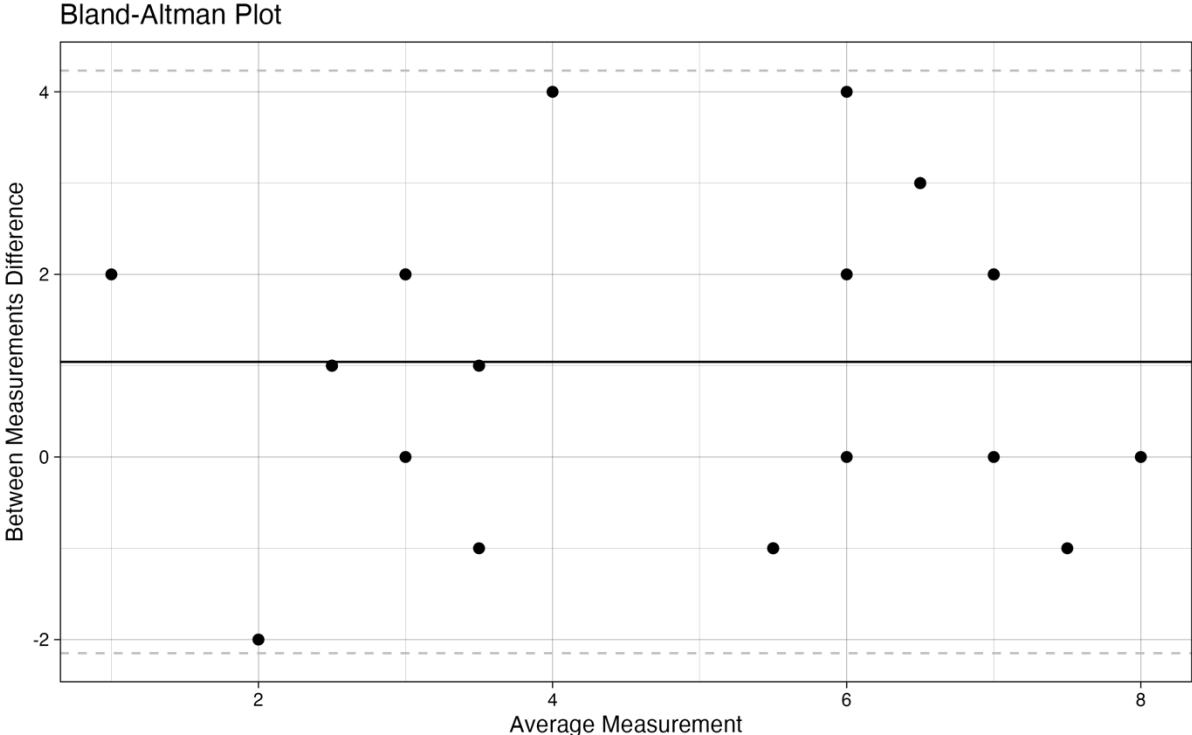
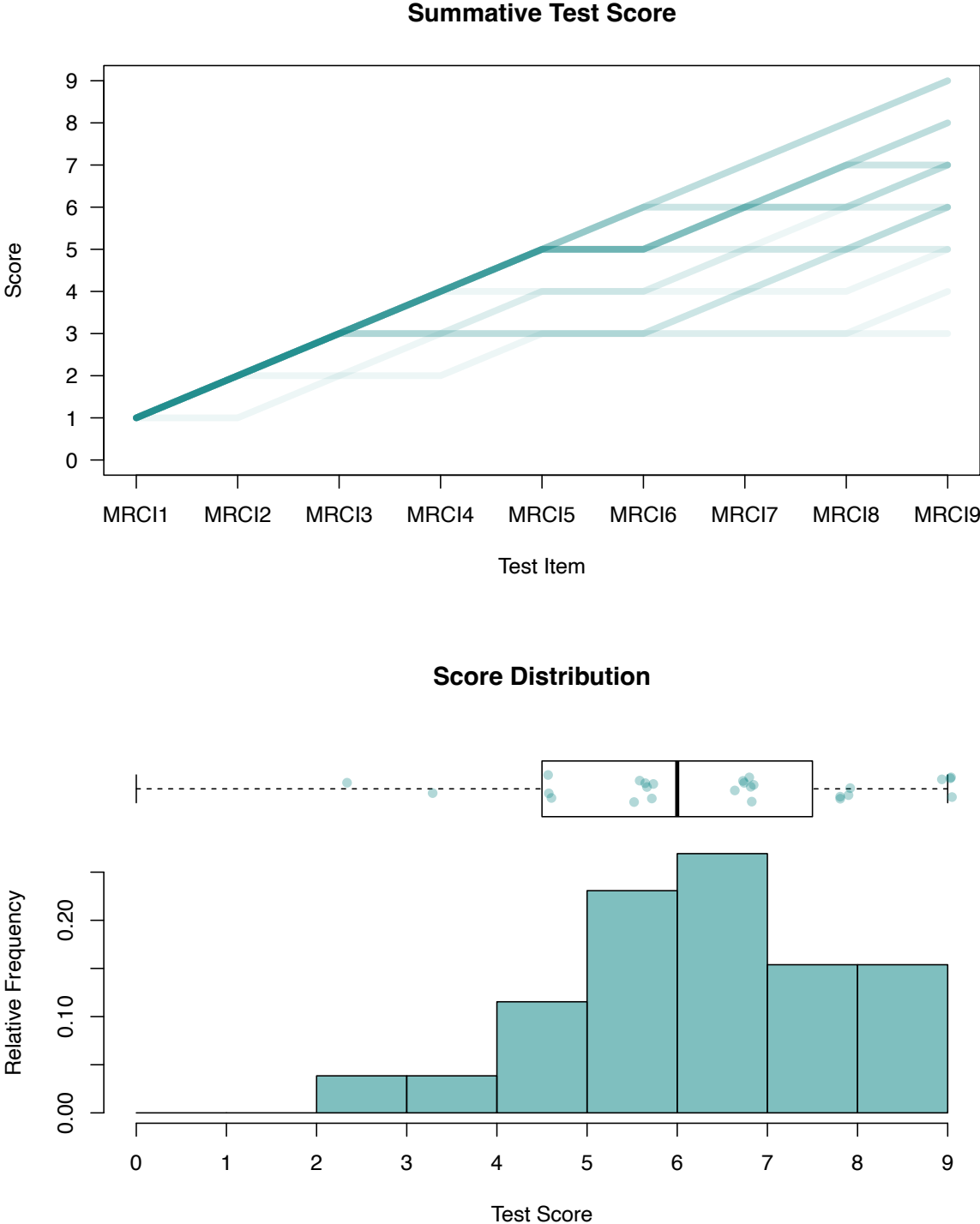


Figure S2

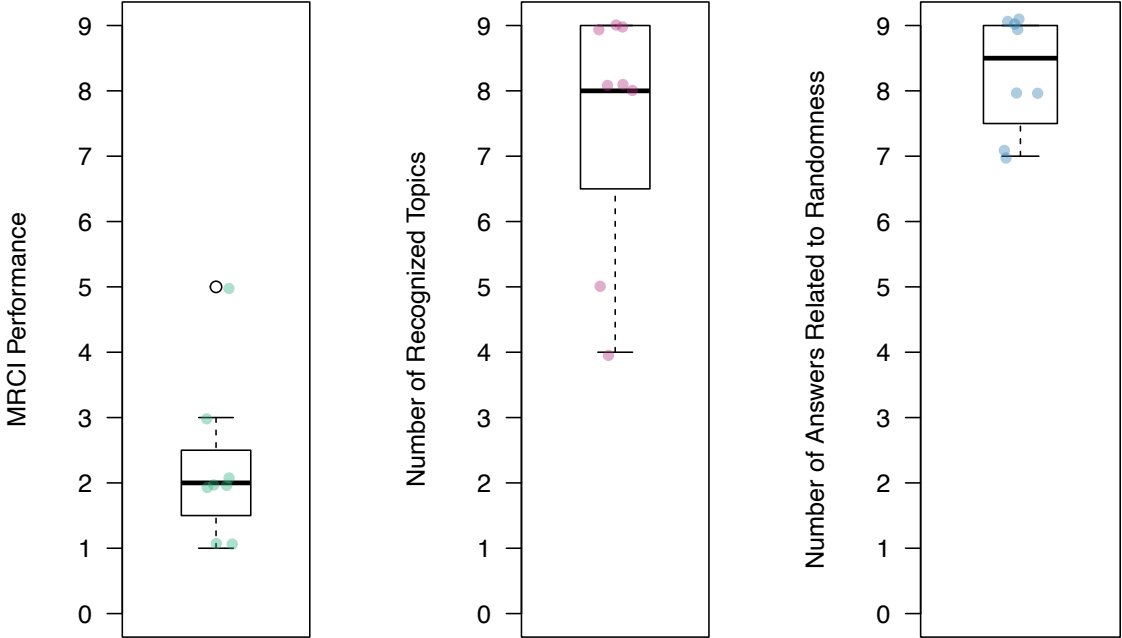
Summative MRCI test scores of the doctoral students' performance assessment



Note. The figure on top indicates the summative test score over the different test items. The figure below shows the performance distribution. Dots in the boxplot plot indicate individual participants.

Figure S3

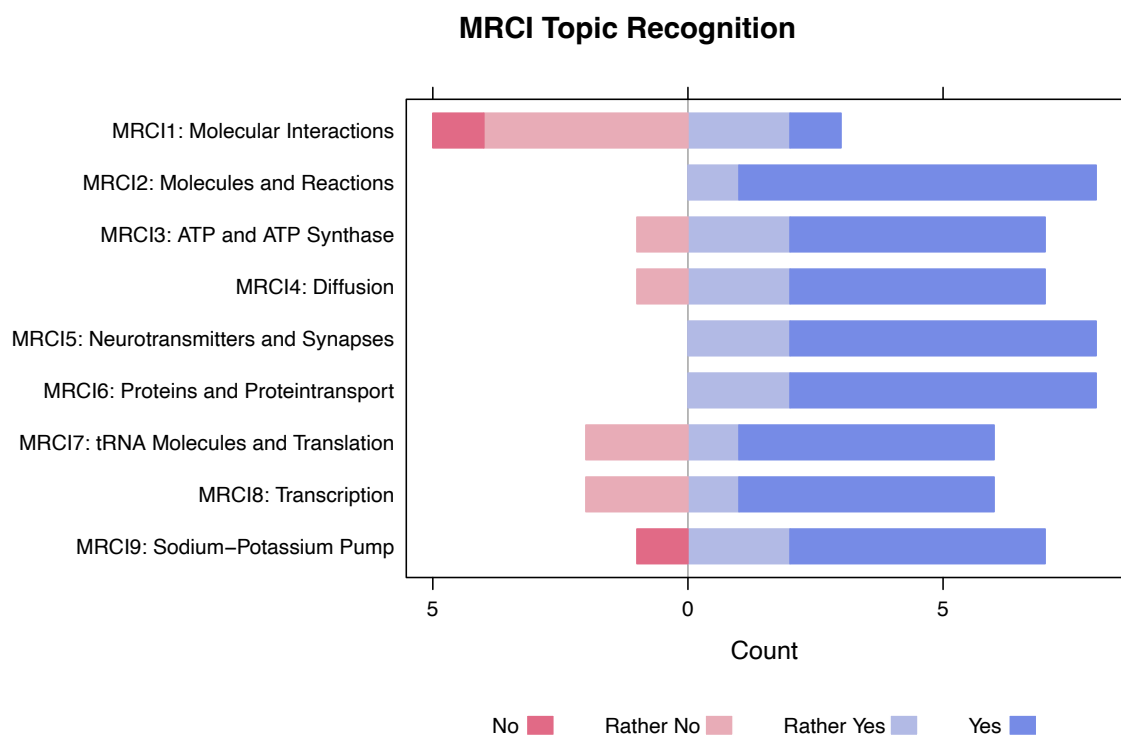
Descriptive plots of interview participants' answers



Note. Individual dots indicate the performance of individual participants.

Figure S4

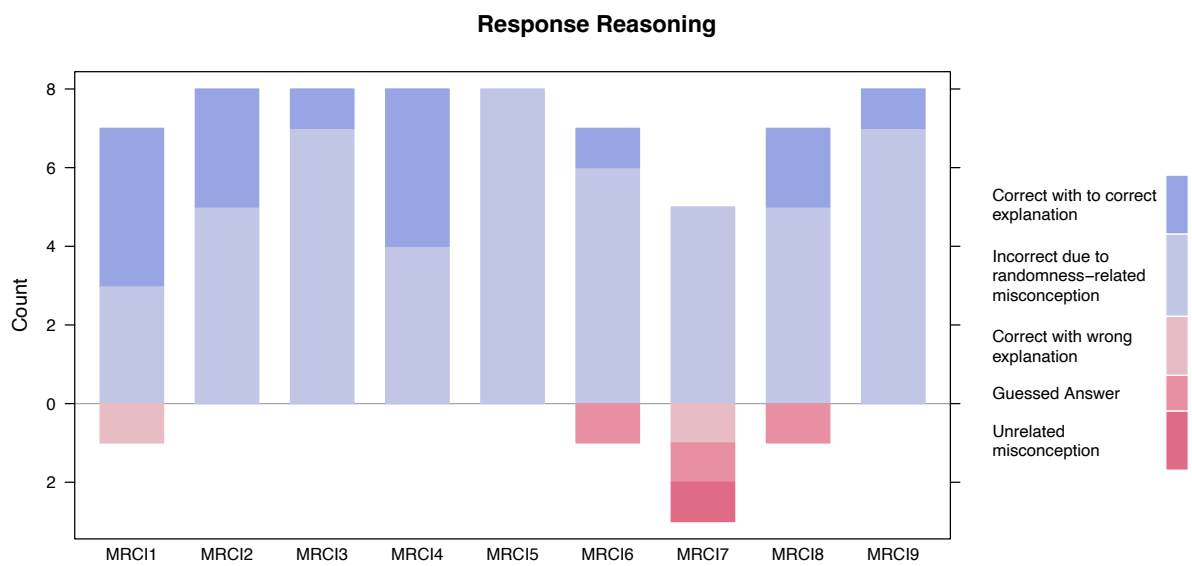
MRCI topic recognition



Note. Each topic on the left indicates the contexts of the indicated MRCI question. Students were asked, “For each topic, please indicate if you have heard of that topic before” on a 4-point Likert scale. Count data indicates the number of students who had selected the particular answer.

Figure S5

Response reasoning



Note. The participants' reasonings during the interview phase were coded and aggregated. The reasons for choosing a particular were classified into the five categories indicated in the legend on the right side of the plot.

Appendix C: Applied R Packages

C1. R packages list

The following R packages were used in alphabetical order:

- add2ggplot (Li & Ryo, 2020)
- behavdata (Tobler, 2022)
- data.table (Dowle & Srinivasan, 2021)
- dplyr (Wickham et al., 2022)
- eRm (Mair & Hatzinger, 2007)
- GGally (Schloerke et al., 2021)
- ggplot2 (Wickham, 2016)
- ggpubr (Kassambara, 2020)
- ggsci (Xiao, 2018)
- HH (Heiberger, 2022)
- hrbrthemes (Rudis, 2020)
- knitr (Xie, 2014)
- lattice (Sarkar, 2021)
- latticeExtra (Sarkar & Andrews, 2022)
- mclust (Fraley et al., 2022)
- osfr (Wolen & Hartgerink, 2020)
- poLCA (Linzer & Lewis, 2011)
- psych (Revelle, 2021)
- TAM (Robitzsch et al., 2021)
- tidyLPA (Rosenberg & van Lissa, 2021)
- tidyverse (Wickham, 2021)
- TOSTER (Lakens, 2017)
- viridis (Garnier, 2021)

C2. References

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Appendix D: Cognitive Laboratory Interview Materials

D1. Word protocol of the interviews

The original protocol was presented in German as the study was conducted in this version. To illustrate the word protocol in this publication, we automatically translated the protocol to English by using the DeepL software environment (v. 3.5.251434).

Greeting and introduction

Hello [name].

Thank you for having the time to participate in the study today. Why don't we get right into the study? So that we all see the same thing all the time, I ask you to open the following link [send link in chat]. Take a moment to read through the information. Let me know if you have any questions and when you are ready to proceed.

Interview Setup

In this study, I am interested in finding out what your ideas and thought processes are that come to mind when answering the questions. In particular, I am interested in your rationale for why you chose a specific answer or why not. Therefore, I will ask you to tell me everything you think and what the reasons are for your thoughts. In other words, your job is to think out loud when you answer the questions. By thinking out loud, I mean that you tell me everything as you work through the task. Don't worry about making perfectly polished sentences or justifying your thoughts. What I really want to hear are your thoughts while solving the task.

If you need to think silently for a moment for yourself, that's fine. Please just let me know what went through your mind afterwards. I may also ask you from time to time to give me some better reasoning or to ask you why you are choosing a certain answer. I realize that thinking out loud can be weird, especially at first. If it helps you, you can also try imagining that you were alone. I'm also not going to evaluate your answers as to their accuracy. It's really just to see how first-year students think about biological processes. Before we start the study, we will do a practice assignment together. Before we get started: Do you have any questions?

Good. First, I'll ask you to share your screen with me, so we both know what questions you're answering. I will record our conversation to write this down later. The audio and video files will only be used for this and nothing else. However, if you prefer, you can turn off your camera. The important thing is that I hear what you say.

Practice exercise

Now let's get started with this first exercise.

Inputs for more detailed explanations:

- *Can you explain to me why you chose / excluded this answer choice?*
- *Can you explain to me what you think this question is asking?*
- *Can you explain how you arrived at your answer?*

Good. Is everything clear so far? Then let's start with the tasks. You can just start thinking out loud when you are ready.

Data collection

[Students solve MRCI alone. If needed, the interviewer can ask the input questions above.]

End of the task phase

Great, we've reached the end of the think-aloud tasks. In this last part, there will be a few more questions that I will ask you to answer. These are just for information to statistically describe the people were that participated. You can also stop the screen sharing now, and I will stop the recording. You can just work on the last tasks for yourself. Just let me know when you get to the end of the study.

Wrap Up and end of the study

Very good, that's it. Do you have any questions?

Thank you very much for your participation!

I will email you the voucher right after this interview.

Have a great day, and thank you again for participating.

D2. Analysis protocol

To analyze the verbal reports, the following analysis protocol was applied:

1. Analysis model determination

The analysis model chosen for this interview study was the question feature model that allows focusing on individual items (Willis, 2015).

2. General coding of utterances

Students' utterances were coded into different categories to differentiate between on-task (question-related) and off-task (as interjections or unrelated statements). Thereby, the following codes were used:

- Description of (own) actions (verbally)
- Interjection (e.g., «ehm», «hmm»)
- Interviewer (statements of the interviewer)
- Non-verbal interaction
- Related (to the question)
- Unrelated statement

3. Classify stage of answer

The stage of understanding of the given answers was then classified according to the five-step model of verbal report data (Leighton, 2017; Tourangeau et al., 2000). This model can be summarized as described below (paraphrased from Leighton, 2017). The five categories are marked in italics.

- i. *Interaction* with the question
- ii. *Comprehension* of the question
- iii. *Retrieval* of knowledge from memory
- iv. Integration of the retrieved information to allow *judgment* of whether the retrieved information is sufficient to give a response
 - a. Information is sufficient → Go to step v.
 - b. Information is insufficient → Go to step iii.
- v. Give a *response* to the question

4. Specific coding and aggregation

Those statements that have been identified as *response* statements in the verbal reports were then coded according to the guidelines by Creswell and Creswell (2005). Thereby, the following steps were considered (paraphrased from Creswell and Creswell, 2005):

- i. Read through a few verbal reports and identify different statements (topics)
- ii. Examine whether these topics also emerge in a sub-sample of other reports
- iii. Make a list of all identified topics and assign code names
- iv. Assign these codes to all verbal reports
- v. If new codes emerge over time, repeat the procedure
- vi. Aggregate the codes

The coding scheme is shown in appendix D3.

5. Draw conclusions

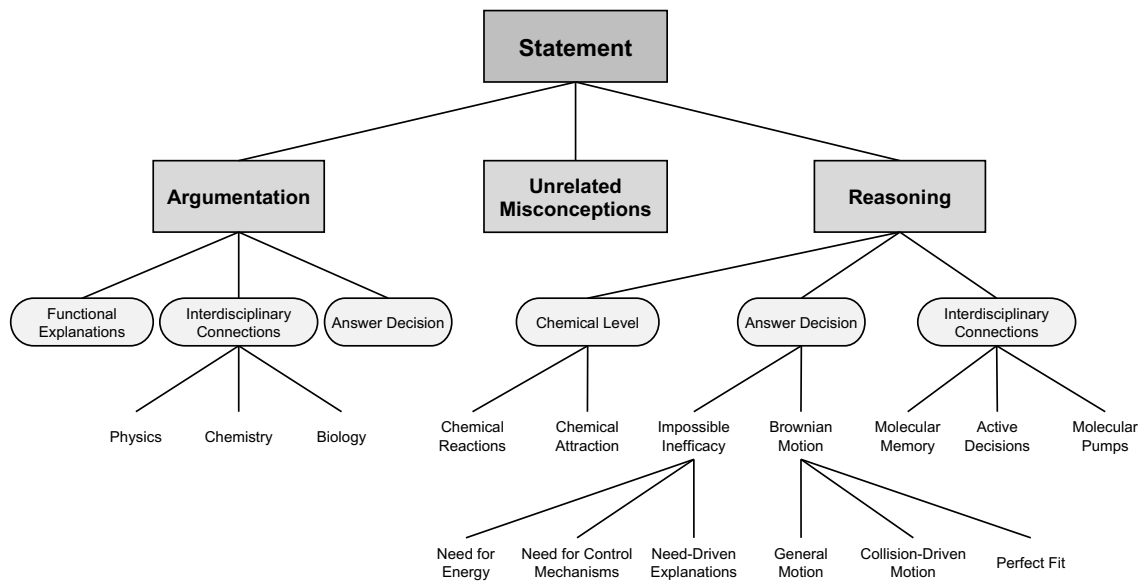
Conclusions are drawn based on the coding and aggregated data to inform about the reasons for which individual answers were chosen.

6. Categorize decision

The final decision regarding which answer was chosen was classified according to five categories:

- i. Correct answer and correct explanation
- ii. Correct answer but wrong explanation
- iii. Incorrect answer and randomness misconception
- iv. Incorrect answer but due to a non-randomness-related misconception
- v. Guessing

D3. Coding scheme



D4. References

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