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A protein purification card game develops subject knowledge and transferable skills

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Abstract

Games can help learners of all ages retain knowledge and build skills such as critical thinking. As such, they could be a useful tool in supporting practical training at the university level.

This paper describes the development, use, and evaluation of a card game based on protein purification techniques, for a large cohort of undergraduates studying Molecular Biology and related subjects. Game materials are available and can be adapted for a different audience. Players collect cards representing chromatography columns, buffers, and elution methods by drawing cards from a common deck, and discard them in order to separate a single protein from a mixture of molecules with different properties (represented by a separate set of cards).

Surveys showed that the game met its three goals: to assess, increase, and apply subject knowledge; to develop skills in communication and experimental planning; and to provide a fun experience. It was more successful for those students who enjoy games and puzzles in their spare time than for those who do not, correlating with the "gamer" group finding it easier to understand and remember the rules of the game. Thus, games can form a useful part of biochemistry teaching practice.

Keywords: biochemistry, practicals, game-based learning, transferable skills, experimental planning

Introduction: game-based learning

For many educators seeking to engage a diverse student population (Vita, 2001) in experience-based learning (Kolb, 1984; Ruben, 1999), activities such as digital games, role play, simulations, and card or board games can be an attractive option (Franklin, Peat and Lewis, 2003; Lean *et al.*, 2006). The work of Piaget and followers has established that play is a central part of learning for young children (*e.g.* Piaget, 1952), but a growing body of evidence shows that games and play can also be very helpful in adult learning.

Integrating a wide variety of active learning activities, including games, into the curriculum can prevent boredom in learners; games in particular induce a "flow state" where we do not notice the time passing (Mirvis, Csikszentmihalyi and Csikzentmihaly, 1991; Spiegel *et al.*, 2008) and therefore spend more time on task. Games are generally judged by students to be more enjoyable than "traditional" teaching (*e.g.* Steinman and Blastos, 2002; Franco-Mariscal, Oliva-Martínez and Almoraima Gil, 2014).

This paper will concentrate on educational board games and card games, which can have a variety of uses. They are especially effective when used for revision and consolidation of knowledge delivered previously (*e.g.* Spandler, 2016; Cavalho, Beltramini and Bossolan, 2019), and have been shown in some cases to lead to an increase in test scores (*e.g.* Steinman and Blastos, 2002; Neef *et al.*, 2011; Gutierrez, 2014; Pennington, Sears and Clegg, 2014). Generic skills such as interaction with other students, use of appropriate vocabulary, self-evaluation of learning, and critical can also be developed through playful teaching methods thinking (Franklin, Peat and Lewis, 2003; Antunes, Pacheco and Giovanela, 2012; Bridge, 2014; Qian and Clark, 2016; Azizan *et al.*, 2018; Franco and DeLuca, 2019).

The use of games has been especially widely reported in medical and allied disciplines (*e.g.* Steinman and Blastos, 2002; Ogershok and Cottrell, 2004; Bochennek *et al.*, 2007; Akl *et al.*, 2008; Valente *et al.*, 2009; Rose, 2011; Struwig, Beylefeld and Joubert, 2013; Whittam and Chow, 2017), and is becoming fairly widespread throughout the biologies (*e.g.* Cohen *et al.*, 1989; Lewis, Peat and Franklin, 2005; Spiegel *et al.*, 2008; Jaipal and Figg, 2009; Miralles *et al.*, 2013; Takemura and Kurabayashi, 2014; Dunitz, Shields and Hall, 2014; Gutierrez, 2014; Vulcu and Heirwegh, 2015; Coil, Ettinger and Eisen, 2017; Luttikhuizen, 2018; Cavalho, Beltramini and Bossolan, 2019). This paper describes the design, use, and evaluation of a card game simulation of protein purification for undergraduate students studying Molecular Biology and related subjects.

Designing a game as part of a lab module

The example of game-based teaching described here forms part of a laboratory-based practical module, a quintessential part of the science degree. Practical work can provide students with a model of the scientific process and genuine enquiry, and to consolidate specific content covered elsewhere in the curriculum. However, often this opportunity is not well used, with "cook book" labs, involving prescriptive instructions being prevalent (Mccomas, 2005). This activity adds elements of experimental planning to complement existing sessions, as part of an iterative process to improve practical training in a Molecular Biology department and make it more inquiry-based (Brownell *et al.*, 2012).

The game concerns the purification of proteins based on their molecular weight (size exclusion chromatography), electric charge (ion exchange chromatography), or specific recombinant epitopes (affinity chromatography). Protein purification is a central element of many molecular biology research projects, with researchers facing a bewildering array of possibilities in a multi-step process (Gräslund *et al.*, 2008). Our first-year students carry out very simple ion exchange, size exclusion, and affinity purification chromatography experiments in the practical lab. I decided to add an activity to better simulate a real-life situation, where several steps are generally needed in order to purify a protein of interest from a complex mixture. I chose to do this using a game to allow students to go through many different iterations of experimental planning in a short space of time.

My intentions in designing the game were threefold:

1. to improve students' knowledge and understanding of this particular laboratory technique. This was at the centre of game design, as players need to actively use their knowledge in order to decide which cards to use, all the while receiving feedback from other players.
2. to develop students' skills in communication and experimental design. The process followed in the game models in a simple and playful manner some of the principles of experimental planning applied in a research lab (see similar work by Strom and Barolo (2011)), where a researcher selects the best approach from a range of possibilities: players need to decide which cards to keep and which to discard, and therefore to consider which cards will be useful in the future. The game also aims to develop communication skills by requiring players to justify each purification step, using specific scientific vocabulary.
3. to provide the class with an enjoyable activity. It was hoped that this approach would be fun for the students, providing them with a pleasant learning experience that would help them to stay engaged (Steinman and Blastos, 2002; Franco-Mariscal, Oliva-Martínez and Almoraima Gil, 2014).

Game play is summarised in the "How to Play" instructions provided to students (Figure 1), and more fully in Supplementary Material. Game materials consist of two types of cards (examples in Figure 1): protein cards and separation cards. The aim of the game is for each player to use the properties of different separation cards to gradually isolate their randomly assigned "favourite protein" from an individual pool of cards representing proteins with different molecular weights, isoelectric points, and recombinant epitope tags. Proteins were chosen because they were already familiar to students from lectures and practicals; a different selection of diverse proteins could easily be chosen to appeal to a different audience. There are three different classes of separation cards, in a single communal deck. In a manner similar to the traditional card game Rummy (*Rummy: Understanding the Rules and Starting a Game*, no date), players draw and discard separation cards from a communal deck; they can then combine one column, one buffer, and one elution card to discard protein cards with certain properties from their personal protein mixture. Column cards represent four different purification methods (size exclusion (G50/G75), anion and cation exchange, and affinity purification (Nickel)). Buffer cards representing different pHs provide versatility to the ion exchange steps. Finally, cards representing different elution methods were included (simple flow-through collection, imidazole, or high salt (NaCl) concentration).

Using the game

This activity was used as part of a two-hour "analysis session" for the entire first-year cohort studying in a Molecular Biology department (degree titles such as Biochemistry, Genetics, and Microbiology). Analysis sessions, held in a lecture theatre, form part of the core practical module, and consist of exercises and group activities to complement concepts covered in the lab. The session was staffed by three postgraduate teaching assistants and one member of academic staff. Teaching assistants were given the game cards and rules a few days in advance, and all the teaching staff had a training meeting the day before the session where they played the game together.

The session plan was as follows. Firstly, a short presentation recapped the different protein purification methods, which students had encountered in the lab previously. Then, a rules explanation was given, including photographs and diagrams showing different stages of the game on PowerPoint slides. Finally, students played the game for about an hour in groups of five, which allowed between five and ten rounds; this repetition aimed to allow students to gain mastery of the rules, and to experience a number of different purification scenarios. Students recorded at least one of their purification schemes in their lab book towards the end of the session.

General impressions from teaching staff were that the session ran well and that students were engaged with the activity. Some groups were very competitive, which perhaps did not lend itself very well to reflective thinking about protein purification; however, groups mostly stayed on task for the duration of the class. Several students asked to take the game away as a revision aid, indicating that they found the game useful and/or enjoyable.

Evaluation of the game

Methodology

Student perceptions of the session were measured using a brief online survey (Appendix 1) administered at the end of the session. The survey questions investigated whether the three main goals of the activity had been met: 1) development of specific subject knowledge; 2) development of transferrable skills; 3) enjoyment.

The activity was evaluated twice, following teaching sessions in the Spring semester of 2017 and 2019 (the session did not run in 2018 due to industrial action). At the end of the teaching session, students were asked to fill in a Google Form using their own mobile devices. Students without a suitable device were able to either use a peer's device or the instructor's laptop, or to access the survey later. Ethics approval was obtained through the University of Sheffield and consent to participate in the study was granted by signing a paper consent form; the first question of the online survey provided confirmation that students had read and signed the information sheet and consent form. Questions were all on the five-point Likert scale (Strongly Agree to Strongly Disagree), with space for free comments at the end of the survey. Data was analysed using stacked bar charts and nonparametric statistical tests (Mann-Whitney U test for comparing two groups, or Kruskal-Wallis test for comparing more than two).

This method of data collection yielded a good response rate: in 2017, 139 responses were gathered from a total class size of 198 and estimated attendance of roughly 170, and in 2019 53 responses were gathered from a total class size of 140 and estimated attendance of roughly 120. Data from 2017 and 2019, and from male and female respondents (data collected for 2019 only: 20 female and

32 male students) were compared by Mann-Whitney test and no statistically significant differences were found for any survey question. Therefore, all responses were pooled for the subsequent analysis.

I was interested in whether students' experience of games and gaming in a social context affected their reaction to the activity. The final question on the survey was, "I often play board games / do puzzles in my spare time" (responses summarised in Table 1). One might expect that individuals who choose to engage in such activities for fun would react differently to an educational card game than those who do not. Therefore, respondents who replied Strongly Agree or Agree to this question were grouped together as "Gamers" (83 students), and those who replied Disagree or Strongly Disagree were grouped together as "Non-gamers" (74 students). These two groups could then be compared to each other and to the class as a whole ("Whole group" or "All"; this group also includes respondents who answered "Neither agree nor disagree").

Analysis

Subject knowledge

The first goal of the activity was to develop students' specific subject knowledge in the area of protein purification (Figure 2). The first set of questions in the survey concerned students' perceptions of this: "the game helped me to assess my knowledge and understanding of protein purification techniques" (Figure 2a); "the game helped me to increase my knowledge and understanding of protein purification techniques" (Figure 2b); and "the game helped me to apply my knowledge and understanding of protein purification techniques" (Figure 2c). Respondents were overwhelmingly in agreement with all these questions, with 93%, 85%, and 92% Agree or Strongly Agree responses, respectively. Free comments also showed that students found the activity useful in developing their knowledge of the topic: for instance, "*I was able to identify a misconception about how gel filtration chromatography actually occurs*"; "*The game was extremely useful and definitely solidified my knowledge on the experimental theory*". However, it should be noted that development of knowledge was only measured using student perceptions; further investigations measuring students' knowledge directly would be necessary to make any definitive statements on this.

Thus, students perceived the activity as being useful in developing subject-specific knowledge - specifically in assessing and applying rather than increasing their knowledge. As the content had in fact already been covered elsewhere and in an introductory lecture at the start of the session, one would not necessarily expect students to be exposed to many new concepts during gameplay. These results fit in well with findings from other contexts showing that games can be a good revision method (*e.g.* Spandler, 2016; Cavalho, Beltramini and Bossolan, 2019) and provide an active learning tool to explore material that had already been taught by other means.

Students who enjoy games and puzzles in their spare time perceived the activity as more useful in developing their subject knowledge than those who do not. Although the percentage of Agree plus Strongly Agree responses is roughly equivalent, Gamers had a higher percentage of Strongly Agree responses as compared to Non-Gamers in all cases (39% compared to 22% for assessing knowledge and understanding; 34% compared to 20% for increasing knowledge and understanding; and 50% compared to 35% for applying knowledge and understanding). A Mann-Whitney test shows that the responses of Gamers and Non-Gamers are significantly different from each other for all three prompts (P-values of 0.035, 0.049, and 0.032, respectively).

Transferrable skills

The second goal of the activity was to develop students' transferable skills (Figure 3). The first skill I was interested in was verbal communication, covered by the survey prompt, "the game helped me discuss scientific concepts with my peers" (Figure 3a); 77% of students agreed or strongly agreed with this statement. The second skill that was assessed in the survey was experimental planning, using the prompt, "the game helped me think strategically about experimental design (Figure 3b); 72% of respondents agreed or strongly agreed with this. Thus, students perceived some value in the activity in improving their communication and experimental design skills.

Students who play games in their spare time found this activity more beneficial in the development of generic skills than those who do not. 83% of Gamers vs 70% of Non-Gamers agreed or strongly agreed that the game helped them discuss scientific concepts with their peers, and 81% of Gamers vs 61% of Non-Gamers agreed or strongly agreed that it helped them think strategically about experimental design. These differences were found to be highly statistically significant using a Mann-Whitney test ($P = 0.002$ and $P < 0.001$, respectively).

Student experience

The third goal of the session was to provide students with an enjoyable activity (Figure 4). This was probed by the prompt, "I enjoyed playing the game" (Figure 4a). On the whole, students reported enjoying the session, with 79% of the whole group agreeing or strongly agreeing with the statement. Free comments from the survey confirm that some students found the activity extremely enjoyable; for example, "*Loved it*"; "*SO MUCH FUN*". It is important to also note that some students did not report finding the activity enjoyable, with 6% of respondents disagreeing or disagreeing strongly with the statement.

I investigated whether the extensive rule set of the game impacted on the student experience using the survey prompt, "the game was simple to understand and play" (Figure 4b). Learning and remembering complicated game rules could be off-putting to some students and detract from their overall experience. Responses were mixed, with only 56% of the group agreeing or strongly agreeing with the statement. Free comments highlighted the difficulty for some students in learning the rules of the game (*"The game wasn't really simple but as you played it you did understand it […]"*), and offered some suggestions for streamlining gameplay, for instance playing in smaller groups.

To probe whether enjoyment of the activity is linked to understanding and remembering the rules of the game, respondents who answered Agree or Strongly Agree to "The game was simple to understand and play" were compared to those who answered Disagree or Strongly Disagree. These groups were highly significantly different from each other as confirmed by Mann-Whitney test, with a P-value <0.001. Therefore, finding the game simple correlates with enjoyment. It is therefore tempting to speculate that difficulty learning the game is preventing some students from engaging with it. The way the activity is introduced should be considered to improve the experience for these learners. Simplification of the rules would be another option, as long as this did not come at the expense of simplifying the scientific problems the students address during the activity.

Student experience of the activity was the area where external experience of games and puzzles made the most difference, with Gamers as a group finding the activity both more enjoyable and simpler to play than Non-Gamers (90% vs 64% and 61% vs 50%, respectively). This difference between the two groups was confirmed by Mann Whitney tests ($P < 0.001$ and $P = 0.007$, respectively). The cause and effect is not obvious here: perhaps the Gamer group find game rules intuitive to

understand, and therefore choose to play games in their spare time, or maybe they find it easier to learn new rules thanks to their higher experience levels.

Conclusion

Overall, the analysis presented here suggests that the game broadly met its aims of improving subject knowledge, developing transferable skills, and providing an enjoyable experience for learners, with students who play games and do puzzles in their spare time perceiving more benefit from the session than those who do not. Although students on the whole found this activity useful and enjoyable, it is not known how this compares to other types of teaching: how much would students report enjoying the average lecture, for example? Future research could question the same group of learners at the end of different types of teaching session, thus drawing more useful conclusions about the success of different activities in engaging students.

In conclusion, despite the caveats discussed above, the activity described was an overall success and might fit well into others' teaching of similar subjects; the full game materials and resources for teachers can be found in the Supplementary Material.

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Appendix 1. Survey questions

1. I have read, understood, and signed the information sheet and consent form (these will remain available on the [module VLE]) survey can only be submitted if the participant has clicked “yes”

The following questions are all scored on a five-point Likert scale (Strongly agree / Agree / Neither agree nor disagree / disagree / Strongly disagree), and could be left unanswered:

2. The game helped me to ASSESS my knowledge and understanding of protein purification techniques

3. The game helped me to INCREASE my knowledge and understanding of protein purification techniques

4. The game helped me to APPLY my knowledge and understanding of protein purification techniques

5. The game helped me discuss scientific concepts with my peers

6. The game helped me think strategically about experimental design

7. The game was simple to understand and play

8. I enjoyed playing the game

9. I often play board games / do puzzles in my free time

10. (2019 only) I would describe my gender as [female / male / prefer not to say / other with option to write in an answer]

11. Please use this space to make any additional comments (Free text box)

Table 1. Count of students who gave each response to the prompt, “I often play board games / do puzzles in my spare time”. The 83 individuals who responded Strongly Agree or Agree were grouped together as Gamers and the 74 individuals who responded Disagree or Strongly Disagree were grouped together as Non-Gamers for the rest of the analysis.

Response	Students	
Strongly Agree	27	83 “Gamers”
Agree	56	
Neither Agree Nor Disagree	35	
Disagree	59	74 “Non-Gamers”
Strongly Disagree	15	
Total	**192**	

Figure 1. Game materials. Re-cap sheet provided to students, showing examples of the playing cards and a summary of the rules of the game. A slightly modified version of this is included in the Supplementary Material.

Figure 2. Responses to prompts regarding subject-specific knowledge.

Prompts were, "The game helped me to assess (a); increase (b); or apply (c) my knowledge and understanding of protein purification techniques". The percentage of students who gave each response is shown from the whole group ("All"; left), and split into students who reported playing games in their spare time ("Gamers"; middle), and those who did not ("Non-gamers"; right).

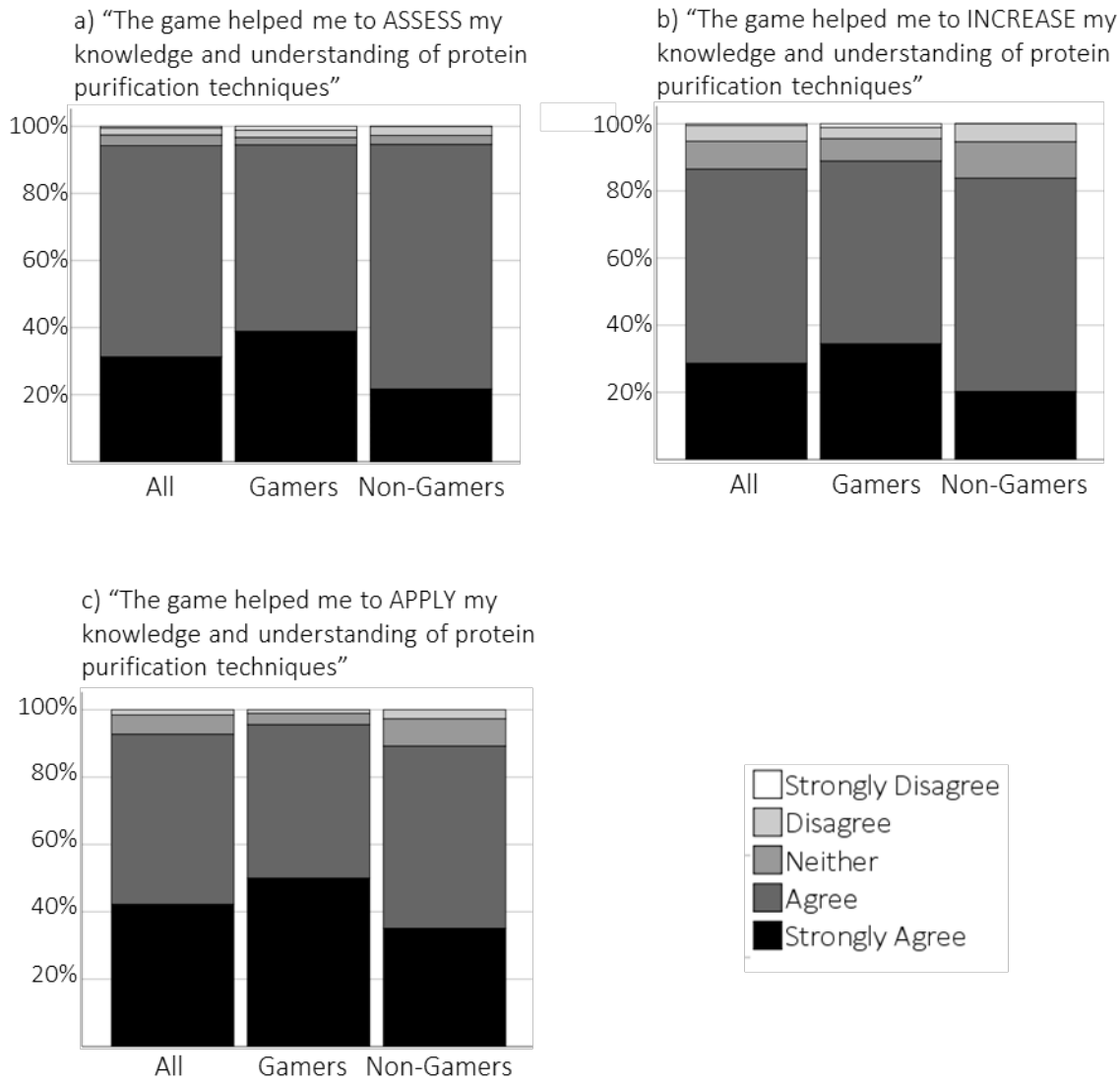


Figure 3. Responses to prompts regarding science skills. Prompts were, "The game helped me discuss scientific concepts with my peers (a), or think strategically about experimental design" (b). The percentage of students who gave each response is shown from the whole group ("All"; left), and split into students who reported playing games in their spare time ("Gamers"; middle), and those who did not ("Non-gamers"; right).

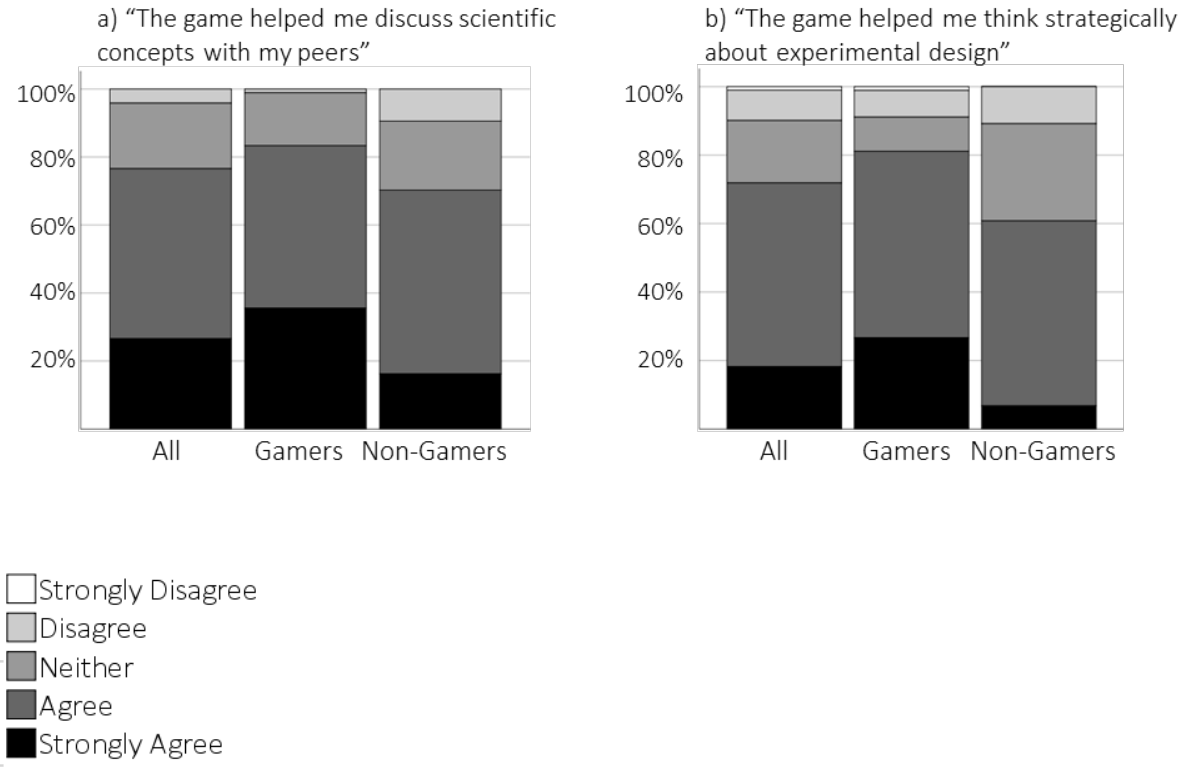
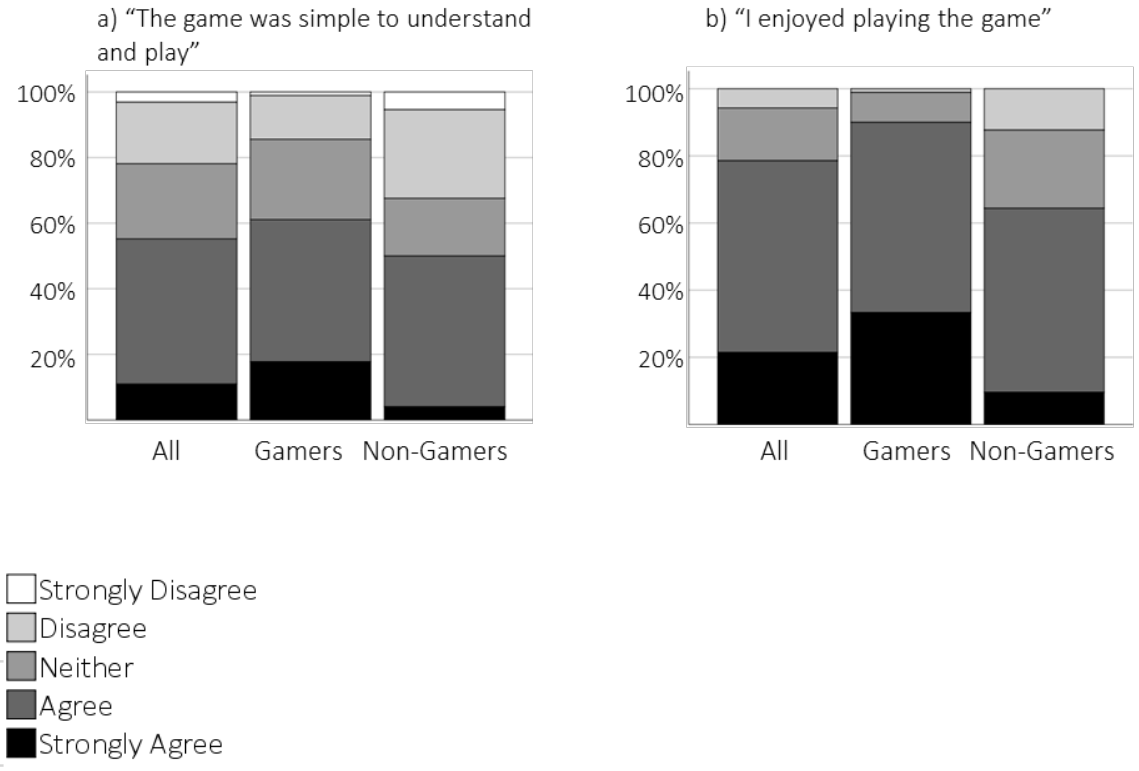


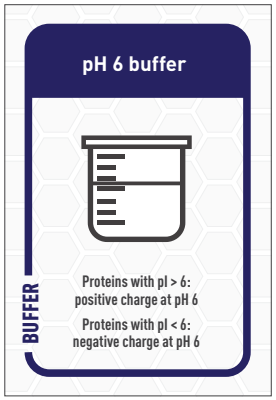
Figure 4. Responses to prompts regarding game play. The prompts were, "The game was simple to understand and play" (a), or "I enjoyed playing the game" (b). The percentage of students who gave each response is shown from the whole group ("All"; left), and split into students who reported playing games in their spare time ("Gamers"; middle), and those who did not ("Non-gamers"; right).

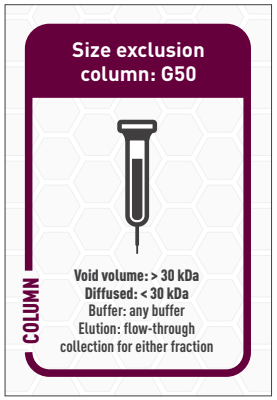


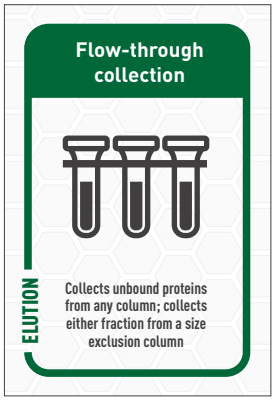
Supplementary Material

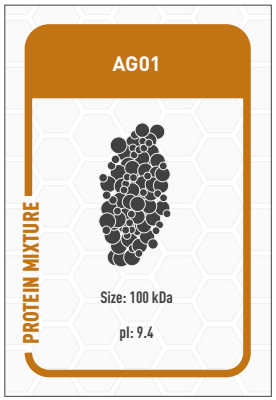
PROTEIN PURIFICATION CARD GAME

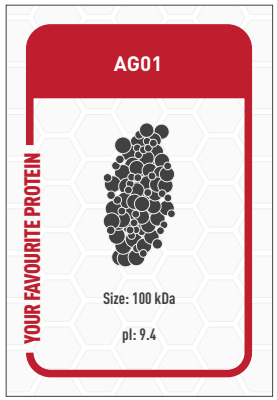
SAMPLE CARDS











HOW TO PLAY

The aim of the game is to separate your favourite protein from the rest of the protein mixture, using a series of purification steps. For each purification step you need an appropriate column, buffer, and elution method card.

1. You are playing in groups of five. Each player has the same six (**orange**) protein mixture cards. Each player picks one (**red**) "your favourite protein" card at random. This is the protein they are trying to separate from all the others, and replaces the "protein mixture" version of that protein. It may not be removed from the protein mixture. Players should place their six protein cards face-up.

2. Each player is dealt five separation cards (columns (**purple**), buffers (**blue**), or elution methods (**green**)). This hand of cards is separate from the protein mixture cards. The rest of the deck is placed face-down somewhere everyone can reach it. A communal discard pile forms next to this deck.

3. Each player takes a turn to do the following:

EITHER play a set of column-buffer-elution cards (see paragraph below)

OR discard one or two cards

THEN draw cards from the deck until you have five in your hand.

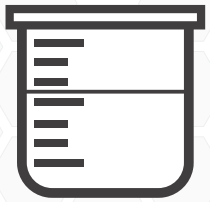
When a set of three separation cards is played, protein cards are removed from that player's mixture according to the properties of the separation cards. The group that are removed from the mixture should be placed face-down. These are no longer in play for that player (but other players' protein mixtures remain unaffected). Why each protein is in each group should be justified to the other players.

4. If the end of the separation deck is reached, the discard pile should be shuffled to form a new deck.

5. The winner is the first player to remove all the proteins apart from their red "favourite protein" from their mixture. The other players should keep on going until everyone has finished.

Column	Separates proteins by	Buffer	Bound proteins	Elute bound proteins with	Unbound proteins	Elute unbound proteins with
Nickel	His tag	Any – doesn't matter	His-tagged	Imidazole	No His tag	Flow-through collection
G50	Size	Any – doesn't matter	<30 kDa	Flow-through collection	>30 kDa	Flow-through collection
G75	Size	Any – doesn't matter	<80 kDa	Flow-through collection	>80 kDa	Flow-through collection
Anion exchange	pI	Choose one depending on what you want to discard	Negative at this pH	NaCl	Positive at this pH	Flow-through collection
Cation exchange	pI	Choose one depending on what you want to discard	Positive at this pH	NaCl	Negative at this pH	Flow-through collection

pH 6 buffer



BUFFER

Proteins with pI > 6:
positive charge at pH 6

Proteins with pI < 6:
negative charge at pH 6

pH 6 buffer

BUFFER

Proteins with pI > 6:
positive charge at pH 6

Proteins with pI < 6:
negative charge at pH 6

pH 6 buffer

BUFFER

Proteins with pI > 6:
positive charge at pH 6

Proteins with pI < 6:
negative charge at pH 6

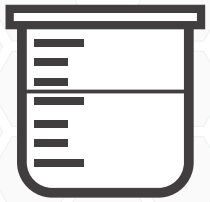
pH 6 buffer

BUFFER

Proteins with pI > 6:
positive charge at pH 6

Proteins with pI < 6:
negative charge at pH 6

pH 7 buffer



BUFFER

Proteins with pI > 7:
positive charge at pH 7

Proteins with pI < 7:
negative charge at pH 7

pH 7 buffer

BUFFER

Proteins with pI > 7:
positive charge at pH 7

Proteins with pI < 7:
negative charge at pH 7

pH 7 buffer

BUFFER

Proteins with pI > 7:
positive charge at pH 7

Proteins with pI < 7:
negative charge at pH 7

pH 7 buffer

BUFFER

Proteins with pI > 7:
positive charge at pH 7

Proteins with pI < 7:
negative charge at pH 7

pH 8 buffer

BUFFER

Proteins with pI > 8:
positive charge at pH 8

Proteins with pI < 8:
negative charge at pH 8

pH 8 buffer

BUFFER

Proteins with pI > 8:
positive charge at pH 8

Proteins with pI < 8:
negative charge at pH 8

pH 8 buffer

BUFFER

Proteins with pI > 8:
positive charge at pH 8

Proteins with pI < 8:
negative charge at pH 8

pH 8 buffer

BUFFER

Proteins with pI > 8:
positive charge at pH 8

Proteins with pI < 8:
negative charge at pH 8

pH 9 buffer

BUFFER

Proteins with pI > 9:
positive charge at pH 9

Proteins with pI < 9:
negative charge at pH 9

pH 9 buffer

BUFFER

Proteins with pI > 9:
positive charge at pH 9

Proteins with pI < 9:
negative charge at pH 9

pH 9 buffer

BUFFER

Proteins with pI > 9:
positive charge at pH 9

Proteins with pI < 9:
negative charge at pH 9

pH 9 buffer

BUFFER

Proteins with pI > 9:
positive charge at pH 9

Proteins with pI < 9:
negative charge at pH 9

Nickel column

COLUMN

Binds His-tags.
Buffer: any buffer
Elution: imidazole to collect bound OR flow-through collection to collect unbound

Nickel column

COLUMN

Binds His-tags.
Buffer: any buffer
Elution: imidazole to collect bound OR flow-through collection to collect unbound

Nickel column

COLUMN

Binds His-tags.
Buffer: any buffer
Elution: imidazole to collect bound OR flow-through collection to collect unbound

Nickel column

COLUMN

Binds His-tags.
Buffer: any buffer
Elution: imidazole to collect bound OR flow-through collection to collect unbound

Anion exchange column

COLUMN

Binds negative proteins.
Buffer: choose one based on pIs you have
Elution: NaCl to collect bound OR flow-through collection to collect unbound

Anion exchange column

COLUMN

Binds negative proteins.
Buffer: choose one based on pIs you have
Elution: NaCl to collect bound OR flow-through collection to collect unbound

Anion exchange column

COLUMN

Binds negative proteins.
Buffer: choose one based on pIs you have
Elution: NaCl to collect bound OR flow-through collection to collect unbound

Anion exchange column

COLUMN

Binds negative proteins.
Buffer: choose one based on pIs you have
Elution: NaCl to collect bound OR flow-through collection to collect unbound

Size exclusion column: G50

COLUMN

Void volume: > 30 kDa
Diffused: < 30 kDa
Buffer: any buffer
Elution: flow-through collection for either fraction

Size exclusion column: G50

COLUMN

Void volume: > 30 kDa
Diffused: < 30 kDa
Buffer: any buffer
Elution: flow-through collection for either fraction

Size exclusion column: G50

COLUMN

Void volume: > 30 kDa
Diffused: < 30 kDa
Buffer: any buffer
Elution: flow-through collection for either fraction

Size exclusion column: G50

COLUMN

Void volume: > 30 kDa
Diffused: < 30 kDa
Buffer: any buffer
Elution: flow-through collection for either fraction

Size exclusion column: G75

COLUMN

Void volume: > 80 kDa
Diffused: < 80 kDa
Buffer: any buffer
Elution: flow-through collection for either fraction

Size exclusion column: G75

COLUMN

Void volume: > 80 kDa
Diffused: < 80 kDa
Buffer: any buffer
Elution: flow-through collection for either fraction

Size exclusion column: G75

COLUMN

Void volume: > 80 kDa
Diffused: < 80 kDa
Buffer: any buffer
Elution: flow-through collection for either fraction

Size exclusion column: G75

COLUMN

Void volume: > 80 kDa
Diffused: < 80 kDa
Buffer: any buffer
Elution: flow-through collection for either fraction

Cation exchange column

COLUMN

Binds positive proteins.
Buffer: choose one based on pls you have
Elution: NaCl to collect bound OR flow-through collection to collect unbound

1 M NaCl

ELUTION

High salt concentration: elutes proteins from anion and cation exchange columns

Cation exchange column

COLUMN

Binds positive proteins.
Buffer: choose one based on pls you have
Elution: NaCl to collect bound OR flow-through collection to collect unbound

1 M NaCl

ELUTION

High salt concentration: elutes proteins from anion and cation exchange columns

Cation exchange column

COLUMN

Binds positive proteins.
Buffer: choose one based on pls you have
Elution: NaCl to collect bound OR flow-through collection to collect unbound

1 M NaCl

ELUTION

High salt concentration: elutes proteins from anion and cation exchange columns

Cation exchange column

COLUMN

Binds positive proteins.
Buffer: choose one based on pls you have
Elution: NaCl to collect bound OR flow-through collection to collect unbound

1 M NaCl

ELUTION

High salt concentration: elutes proteins from anion and cation exchange columns

500 mM imidazole

ELUTION

Imidazole: elutes proteins from Nickel columns

500 mM imidazole

ELUTION

Imidazole: elutes proteins from Nickel columns

500 mM imidazole

ELUTION

Imidazole: elutes proteins from Nickel columns

500 mM imidazole

ELUTION

Imidazole: elutes proteins from Nickel columns

Flow-through collection

ELUTION

Collects unbound proteins from any column; collects either fraction from a size exclusion column

Flow-through collection

ELUTION

Collects unbound proteins from any column; collects either fraction from a size exclusion column

Flow-through collection

ELUTION

Collects unbound proteins from any column; collects either fraction from a size exclusion column

Flow-through collection

ELUTION

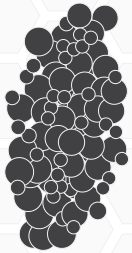
Collects unbound proteins from any column; collects either fraction from a size exclusion column

Flow-through collection

ELUTION

Collects unbound proteins from any column; collects either fraction from a size exclusion column

AG01



PROTEIN MIXTURE

Size: 100 kDa

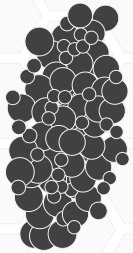
pI: 9.4

Flow-through collection

ELUTION

Collects unbound proteins from any column; collects either fraction from a size exclusion column

AG01



PROTEIN MIXTURE

Size: 100 kDa

pI: 9.4

Flow-through collection

ELUTION

Collects unbound proteins from any column; collects either fraction from a size exclusion column

AG01

PROTEIN MIXTURE

Size: 100 kDa

pI: 9.4

Flow-through collection

ELUTION

Collects unbound proteins from any column; collects either fraction from a size exclusion column

AG01

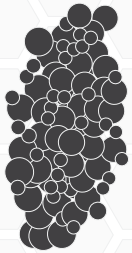
PROTEIN MIXTURE

Size: 100 kDa

pI: 9.4

PROTEIN MIXTURE

AG01



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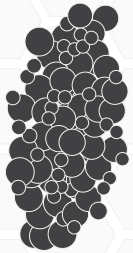
PROTEIN MIXTURE

His-RNAseA



Size: 13 kDa

pI: 7.8

PROTEIN MIXTURE

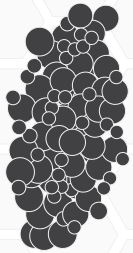
His-RNAseA

Size: 13 kDa

pI: 7.8

PROTEIN MIXTURE

His-RNAseA



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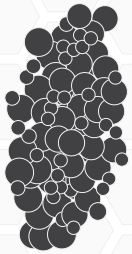
PROTEIN MIXTURE

His-RNAseA



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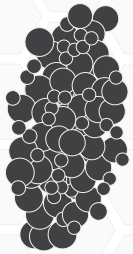
PROTEIN MIXTURE

His-RNAseA

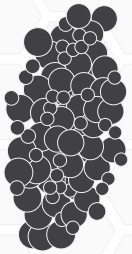


Size: 13 kDa

pI: 7.8

PROTEIN MIXTURE

Ferritin

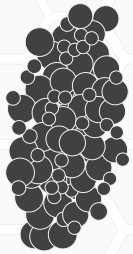


Size: 450 kDa

pI: 5.5

PROTEIN MIXTURE

Ferritin

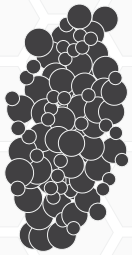


Size: 450 kDa

pI: 5.5

PROTEIN MIXTURE

Ferritin

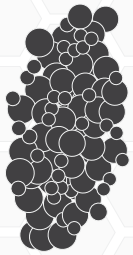


Size: 450 kDa

pI: 5.5

PROTEIN MIXTURE

Ferritin



Size: 450 kDa

pI: 5.5

PROTEIN MIXTURE

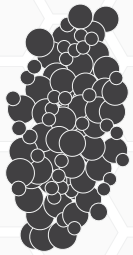
Ferritin

Size: 450 kDa

pI: 5.5

PROTEIN MIXTURE

Haemoglobin

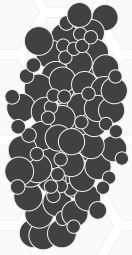


Size: 64 kDa

pI: 6.9

PROTEIN MIXTURE

Haemoglobin

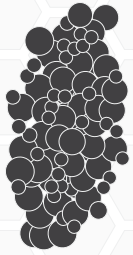


Size: 64 kDa

pI: 6.9

PROTEIN MIXTURE

Haemoglobin

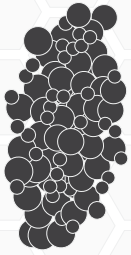


Size: 64 kDa

pI: 6.9

PROTEIN MIXTURE

Haemoglobin

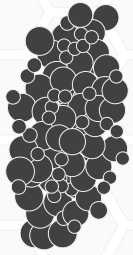


Size: 64 kDa

pI: 6.9

PROTEIN MIXTURE

Haemoglobin

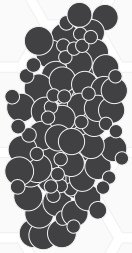


Size: 64 kDa

pI: 6.9

PROTEIN MIXTURE

Aryl sulphatase

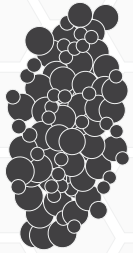


Size: 44 kDa

pI: 4.75

PROTEIN MIXTURE

Aryl sulphatase

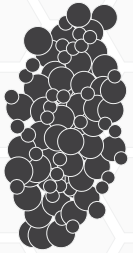


Size: 44 kDa

pI: 4.75

PROTEIN MIXTURE

Aryl sulphatase

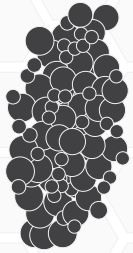


Size: 44 kDa

pI: 4.75

PROTEIN MIXTURE

Aryl sulphatase

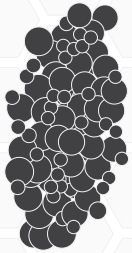


Size: 44 kDa

pI: 4.75

PROTEIN MIXTURE

Aryl sulphatase

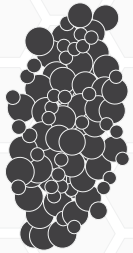


Size: 44 kDa

pI: 4.75

PROTEIN MIXTURE

Cytochrome C



Size: 12 kDa

pI: 10

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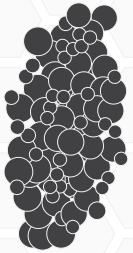

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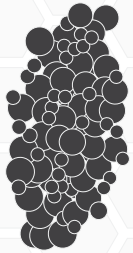

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PROTEIN MIXTURE

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YOUR FAVOURITE PROTEIN

Aryl Sulphatase

Size: 44 kDa

pI: 4.75

YOUR FAVOURITE PROTEIN

His-RNAseA

Size: 13 kDa

pI: 7.8

YOUR FAVOURITE PROTEIN

Ferritin

Size: 450 kDa

pI: 5.5

YOUR FAVOURITE PROTEIN

Haemoglobin

Size: 64 kDa

pI: 6.9

YOUR FAVOURITE PROTEIN

Cytochrome C

Size: 12 kDa

pI: 10

YOUR FAVOURITE PROTEIN

AG01

Size: 100 kDa

pI: 9.4

Protein purification card game: notes for teachers

Dr Rebecca Barnes, Department of Molecular Biology and Biotechnology, University of Sheffield

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The aims of this session are 1) to consolidate knowledge about different methods of protein purification, and 2) to develop students' experimental design skills. It was designed for Level 1 undergraduates in the Department of Molecular Biology and Biotechnology at the University of Sheffield as part of their core laboratory module. They had already been presented with most of the different purification methods in lecture modules and through lab sessions on ion exchange and size exclusion chromatography. This game would be suitable for use in a tutorial setting or, with appropriate support, in a large group divided into groups of ~5.

There are two types of cards in this game: protein cards (orange or red) and separation cards (purple, blue, or green). Each person should have six protein cards (the same for each player), and each group should have one set of "your favourite protein" cards and one deck of separation cards. One of the proteins will be nominated "your favourite protein"; it is the aim of the game to separate this protein from all the others in the mixture (*i.e.*, the "favourite protein" should be the only one remaining face-up). This will probably take several steps.

Players should use sets of separation cards to remove proteins from the mixture; they need to collect a column, an appropriate buffer, and an appropriate elution method. (I have been quite lax about the buffer requirements to keep the game moving even if this is not the most true-to-life.) Players take it in turns to discard cards from their hand and replace them with ones from the deck (one or two at a time). By discarding a set of separation cards they can remove some proteins from the mixture. Full rules are below.

When I used it in my teaching, the game was played by all ~180 Level 1 students in a large lecture theatre, but of course it would work well in a tutorial also. I ran the session with three demonstrators (previously, I had provided extensive training to them and spent some time playing the game with them). We just walked around keeping the students on task and settling any disagreements on which fraction proteins should be in. I asked the students to write a couple of their purification schemes in their lab book as a record of the session.

I ran the two-hour session as follows:

1. A short lecture recapping the different protein purification methods

2. Rules explanation using PowerPoint showing different stages of the game.

3. Split students into groups of five and play the game for about an hour.

I hope you find this game useful and somewhat entertaining. I would love to hear how you get on with it! Please email me at r.barnes@sheffield.ac.uk. I would also be interested in hearing from anyone interested in collaborating on making similar teaching materials, for any discipline.

Happy gaming!
Rebecca Barnes

How to play

The aim of the game is to separate your favourite protein from the rest of the protein mixture, using a series of purification steps. For each purification step you need an appropriate column, buffer, and elution method card.

1. The game is played in groups of five. Each player has the same six (orange) protein mixture cards. Each player picks one (red) "your favourite protein" card at random. This is the protein that this player is trying to separate from all the others, and replaces the "protein mixture" version of that protein, *i.e.* that is the final protein that will remain after step-wise removal of the other, contaminating proteins. Players should place their six protein cards face-up.

2. Each player is dealt five separation cards (columns (purple), buffers (blue), or elution methods (green)). This hand of cards is separate from the protein mixture cards. The rest of the deck is placed face-down somewhere everyone can reach it. A communal discard pile forms next to this deck.

3. Each player takes a turn to do the following:

EITHER play a set of column-buffer-elution cards (see paragraph below)
OR discard one or two cards from their hand
THEN draw cards from the deck until they have five in their hand.

When a set of separation cards is played, protein cards are removed from that player's mixture according to the properties of the separation cards played. The group that are removed from the mixture should be placed face-down. These are no longer in play for that player (but other players' protein mixtures remain unaffected). Why each protein is in each group should be justified to the other players.

4. If the end of the separation deck is reached, the discard pile should be shuffled to form a new deck.

5. The winner is the first player to remove all the proteins apart from their red "favourite protein" from their mixture. The other players should keep on going until everyone has finished.

NB, it is crucial that cards be well shuffled before the game begins. This can be achieved by "pile shuffling" – for instance, making separate piles containing one of each type of separation card and two non-consecutive Flow-through collection cards before combining all the piles into one stack from which the cards are handed out to the players.

Some notes on the different columns:

Size exclusion columns: G50 and G75. Separate proteins by size. You can use any buffer card – in this range, pH should not affect binding. In this game, we are separating proteins into two groups: those that elute in the void volume, and those that diffuse into the matrix and are retained.
G50: proteins >30 kDa are eluted first in the void volume, proteins <30 kDa are retained by the column.

G75: proteins >80 kDa are eluted first in the void volume, proteins <80 kDa are retained by the column.

To collect either the void volume or the bound proteins, you should use the generic "flow-through collection" card.

Ion exchange columns: anion exchange and cation exchange. Separate proteins according to their pI.
Anion exchange column is positively charged and therefore binds to negatively charged proteins. Positively charged proteins do not bind to the column. On the other hand, a cation exchange column is negatively charged and therefore binds to positively charged proteins.

Whether a particular protein is positively or negatively charged depends on the pH of the buffer: if the pI is higher than the pH of the buffer then the protein is positive, but if the pI is lower than the pH of the buffer then it is negative.

If you wish to collect the bound proteins, you should use salt: use an NaCl card. If you wish to collect the unbound proteins, you should use the generic "flow-through collection" card.

Nickel column: binds to His-tagged proteins. Proteins without a His tag do not bind the column. You can use any pH of buffer – in this range, pH should not affect binding. If you wish to collect the bound proteins, you should use imidazole: use an imidazole card. If you wish to collect the unbound proteins, you should use the generic "flow-through collection" card. (it's true that it can be easy to win if you have the His-tagged protein as your favourite protein - I think this is part of what we are trying to teach. The rules could be adapted so players with this protein as their favourite have to sit out a few turns before they can start playing while they are "making their cell line"), or one could reduce the number of Nickel and Imidazole cards in the deck.

Column	Separates proteins by	Buffer	Bound proteins	Elute bound proteins with	Unbound proteins	Elute unbound proteins with
Nickel	His tag	Any	His-tagged	Imidazole	No His tag	Flow-through collection
G50	Size	Any	<30 kDa	Flow-through collection	>30 kDa	Flow-through collection
G75	Size	Any	<80 kDa	Flow-through collection	>80 kDa	Flow-through collection
Anion exchange	pI	Choose one depending on what you want to discard	Negative at this pH	NaCl	Positive at this pH	Flow-through collection
Cation exchange	pI	Choose one depending on what you want to discard	Positive at this pH	NaCl	Negative at this pH	Flow-through collection