A model of factors determining students' ability to interpret external representations in biochemistry

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ABSTRACT

The aim of this research was to develop a model of factors affecting students' ability to interpret external representations (ERs) in biochemistry. The study was qualitative in design and was guided by the modelling framework of Justi and Gilbert (2002). Application of the process outlined by the framework, and consultation with relevant literature, led to the expression of a Venn model and to the formulation of operational definitions for seven component factors of the model namely, the conceptual (C), reasoning (R), representation mode (M), reasoning-mode (R-M), reasoning-conceptual (R-C), conceptual-mode (C-M) and conceptual-reasoning-mode (C-R-M) factors. To validate the model, nine students were interviewed using a specially designed *three-phase single interview technique* (3P-SIT) to investigate their interpretation of three ERs, representing antibody-antigen interaction. The data was analysed by induction, where response patterns emerged naturally rather than being predisposed. The results verified the validity of the expressed model and its component factors. We suggest that the model has a range of potential applications including, as a tool for framing researchers' thinking about students' difficulties with, and interpretation of scientific ERs and for the design of strategies to improve learning with ERs.

INTRODUCTION

Much research in science education and educational psychology has centred on the role and effectiveness of external representations (ERs) in the learning and teaching of science. ERs are pictorial and graphical depictions of phenomena in the external world (e.g. Lohse, Walker, Biolsi, & Rueter, 1991) that contain spatial relationships and topographical markings. ERs can be distinguished from internal representations (e.g. mental models), which are an archetype of the mind (Zhang & Norman, 1994). Over the years, science education research studies on students' interpretation of ERs have focused on a wide range of ERs, including,static pictures, drawings, graphs, photographs, maps, flowcharts, scientific models, computer-based visuals and diagrams.

The literature contains numerous reports of the importance of ERs for promoting student learning, understanding and visualization ability. For example, Harrison & Treagust (2000) have suggested that ERs are important tools for constructing knowledge, Peña and Quílez (2001) have stated that ERs are valuable for communicating and integrating scientific concepts, while Kozma (2003) has shown that ERs can support a flexible understanding of scientific phenomena. Additional studies on scientific ERs have focussed on the various factors that might influence ER interpretation and,

therefore, cause potential difficulties for students. For example, Lowe (2003, 1996) has shown that learners' interpretation of meteorological ERs is often influenced by salient characteristics of the visual displays, rather than on an underlying appreciation of what the external features represent. This can often lead to a 'surface-level' interpretation of the ER. In support of this finding, Kozma and Russell (1997) have shown that novices' understanding of ERs used in chemistry is largely determined by the surface features of the ERs and therefore, learners often struggle to associate external features of the ERs with deeper conceptual explanations. In addition, Roth (2002), Cheng, Lowe and Scaife (2001) and Winn (1993) have all suggested that the interpretation of scientific ERs is compromised if students lack the domain-specific knowledge (e.g. knowledge about the graphical conventions) associated with the ER. In a study that investigated learners' use of domain-specific diagrams during reasoning, Kindfield (1993/1994) demonstrated the co-evolution of pictorial skill and biological understanding, and showed that scientific understanding can be negatively affected if either is neglected.

Apart from findings concerned with conceptual and cognitive aspects of ER-interpretation, other authors have published results which demonstrate that the topographical and graphical make-up of the ERs themselves also has a marked influence on the quality of student learning with ERs. For example, Dwyer (1967) and, more recently, Mayer (2003, 1997) have shown that different ER combinations, as well as varying media environments, can have a profound influence on student learning which, in some cases (e.g. Pozzer & Roth, 2003) could result in ER-related learning difficulties. Furthermore, Treagust, Chittleborough, & Mamiala (2002) have indicated that students' difficulties with ERs could be related to the failure of teachers to explicitly describe the strengths and limitations of scientific ERs. This problem could be largely due to naïve assumptions by science instructors that what 'works' for experts will automatically be beneficial for novices, without any formal confirmation by research (e.g. Scaife & Rogers, 1996). In fact, science education research has shown that students' interpretation of ERs can sometimes induce alternative conceptions and reasoning difficulties. For instance, studies by Ametller and Pintó (2002) and Stylianidou, Ormerod and Ogborn (2002) have documented examples of learning difficulties with pupils' interpretation of ERs that depict energy. In addition, work by Peña and Quílez (2001) has revealed students ER-related difficulties with sun, earth and phases of the moon concepts.

In comparison with the extensive literature concerning students' interpretation of ERs in the scientific disciplines described above, very limited research has been carried out on students' processing of ERs in a biochemistry education context (Schönborn & Anderson, 2006; Richardson

& Richardson, 2002). This is surprising, given the extent and diversity of ER forms that the modern biochemistry student is required to process and interpret (e.g. Schönborn, Anderson, & Mnguni, 2007). Nevertheless, examples of recent studies available in this context are as follows. In a study concerned with the effectiveness of analogy use in tertiary biochemistry textbooks, Orgill and Bodner (2006) have found that textbook authors do not always present pictorial analogies in the most effective manner nor do they describe the potential limitations of such ERs . As a result, upon relating the visual ER to the necessary target, they suggest that some biochemistry students may develop misconceptions. Furthermore, work by Patrick, Carter and Wiebe (2005) has revealed particular reasoning difficulties relating to learners' interpretation of ERs depicting DNA replication. These are important research contributions to a field that must deal with the rapid expansion of knowledge and entry of biochemical ERs into educational settings. Therefore, since biochemical ERs also have the potential to cause alternative conceptions and erroneous reasoning, there remains an ongoing need to further investigate the potential causes and sources behind such learning difficulties.. This is of extreme pedagogical importance if we are to obtain an understanding of the cognitive dimensions associated with students' processing and interpretation of ERs in biochemistry and, if we want such research to be of use to workers in other fields of science education as well.

In response to the motivation framed above, we conducted an initial study (Schönborn, Anderson, & Grayson, 2002) which led to the preliminary identification of at least three factors that could cause student difficulties with the interpretation of ERs in biochemistry namely, students' *reasoning* ability (termed 'R' in this study), students' understanding of the *concepts* of relevance to the ER (termed 'C'), and the nature of the *mode* in which the desired phenomenon is represented by the ER (termed 'M'). In continuation of this endeavour, the study reported in this article aims to further investigate and model these and other possible factors affecting ER interpretation in biochemistry by addressing the following research questions: 1) How can the C, R and M factors be incorporated into, and expressed as, an appropriate model? 2) How can empirical data be obtained to investigate the nature of the factors and the validity of the model? 3) What practical applications will the model have and will it be generalisable to all ERs in science? To address these questions, the modelling process of Justi and Gilbert (2002) was used to express a model of factors affecting students' ability to interpret ERs in biochemistry. Subsequently, a specially designed *three-phase single interview technique* (3P-SIT) was used to generate empirical data to validate the model.

METHODS

Description of the participants and the external representations used in the study

The study was conducted from 2001 to 2002 with nine biochemistry students, at the University of KwaZulu-Natal, South Africa, who had all completed a third-year level module on immunology. Each of the nine students was interviewed three times, one interview per each of three different ERs (Figure 1)[1] giving a total of 27 interviews conducted in the study. Two of the three ERs (Figure 1 A and B) were obtained from the textbook (Roitt, 1997) prescribed for the immunology module, while a colleague provided the remaining ER (Figure 1 C). The sequence in which each ER was interpreted by each participant across each of the three separate interviews was totally random. In other words, one participant may have received Figure 1 A in their first interview, followed by Figure 1 B in their second and Figure 1 C in their third, while another participant may have received the reverse order, or other possible combinations. We purposefully administered the ERs randomly so as to minimise any sequence or knowledge transfer effects from one ER to the next across the sample of participants.

Insert Figure 1 about here

The three ERs used in the study (Figure 1 A - C) are multiple representations of antibody-antigen interaction that fall on a real to abstract continuum (e.g. Pozzer & Roth, 2003; Wheeler & Hill, 1990; Alesandrini, 1984; Fry, 1981; Dwyer, 1967). The electron micrograph (Figure 1 A) can be considered a 'real' depiction of antibody and antigen interaction, the space-filling model (Figure 1 B) a 'semipictorial' (stylised) representation of antibody-antigen interaction and the graphical plot (Figure 1 C) an 'abstract' portrayal of antibody-antigen interaction. The electron micrograph (Figure 1 A) shows trimer and pentamer complexes formed when Y-shaped immunoglobulin G

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[1] Figure 1 A was reprinted from the Journal of Molecular Biology, 27, Valentine, R.C. and Green, N.M., Electron Microscopy of an Antibody-Hapten Complex, Plate II, Copyright (1967), with permission from Elsevier and reproduced from Roitt's Essential immunology, 9th ed., Roitt, I.M., p. 84, figure 5.5, Copyright (1997), by permission of Blackwell Science, Inc. Figure 1 B was reprinted with permission from Science, 233, Amit, A.G., Mariuzza, R.A., Phillips, S.E.V. and Poljak, R.J., Three-dimensional structure of an antigen-antibody complex at 2.8 Å resolution p. 749, Copyright 1986 AAAS and reproduced from Roitt's Essential immunology, 9th ed., Roitt, I.M., p. 45, figure 3.1, Copyright (1997), by permission of Blackwell Science, Inc.

(IgG) antibodies bind to the divalent hapten dinitrophenyl (DNP) (Roitt, 1997; Valentine & Green, 1967). Figure 1 B represents a three-dimensional, space-filling display of the binding of an antigen (lysozyme protein) to a 'Fab' fragment of an IgG antibody molecule (Roitt, 1997; Amit, Mariuzza, Phillips, & Poljak, 1986). Lastly, Figure 1 C is a Cartesian graph of the quantitative results obtained from an enzyme-linked immunosorbent assay (ELISA) (J. G. Jackson, personal communication, June 16, 2000) of the binding interaction between antibody and antigen molecules. Each of the four curves represents results obtained at different weeks of an immunisation schedule. Absorbance at 405 nm is plotted against the negative logarithm of antibody concentration. The presentation of Figure 1 C to students also included insertion of the letter 'P' on the 'Week 12' (blue) curve at an approximate coordinate (black square) of 0.33 on the y-axis and 1.75 on the x-axis. A further letter 'Q' and black square were inserted just after the 'peak' of the 'Week 12' (blue) curve.

This paper shall refer to each of the ERs in Figure 1 as 'ER A', 'ER B' and 'ER C', respectively. During the interviews, both the coloured versions of the ERs and their captions were supplied to students, but only one ER plus caption was supplied at a time for each interview. We acknowledge that this journal does not print in colour. Since the use of colour in the ERs was an important component of our study, where relevant we have provided colour keys and further descriptions in the Figure captions pertaining to the use of colour. Captions supplied to students were as in Figure 1 but excluded the additional explanations concerning the original colours of the graphical features. In addition, for ER B, the original statement, 'In the third frame, both molecules have been rotated 90^0 about a vertical axis and contact residues are shown in red and Gln 121 in light purple' (Roitt, 1997, p. 84), was removed as we wished to gauge students' own interpretations in this regard.

Development of the model and operational definitions of component factors

Justi and Gilbert (2002) have proposed and implemented a 'model of modelling' framework concerned with the role of modelling in the learning and teaching of science. Although the purpose of their modelling framework is centred on stimulating science teachers to become capable 'modellers' of scientific phenomena, we saw the value of their underlying modelling rationale as an extremely useful tool for guiding our *process* of developing and expressing the model described in this paper. An outline of the modelling framework devised by Justi and Gilbert (2002, p. 371) is presented in Figure 2 and discussed below.

Insert Figure 2 about here

The modelling process involved a five-stage cyclical process (Figure 2). Firstly, the *purpose* and nature of the model was decided upon, based on the previous identification of three factors (C, R and M) by the authors (Schönborn et al., 2002), the authors' prior knowledge and experience of student difficulties with ERs, other potential factors that might be important, and a thorough analysis of the literature on learning and teaching with ERs in science (Schönborn, 2005). Secondly, a *mental model* of all the factors was produced and thirdly, the mental model was externalised as an *expressed model* (Figure 2). Fourthly, conduction of various *thought experiments*, as well as extensive discussion of the expressed model between the authors helped formulate the operational definitions of the component factors and decide on any necessary modifications to the *expressed* model. Stages 2 – 4 were repeated several times so as to optimise and reach agreement between authors as to the nature of the expressed model and the operational definitions of its component factors. Fifthly, the expressed model and its factors were empirically tested (Figure 2) and validated, employing the methods described below.

Empirical validation of the model

A clinical instrument termed the *three-phase single interview technique* (3P-SIT) and designed and piloted by Schönborn (2005) was used in the current study to empirically validate the model and the operational definitions of its component factors. The development of 3P-SIT was informed by other science education research literature on clinical interview methods such as those described in physics (e.g. Ametller & Pintó, 2002; Duit, Roth, Komorek, & Wilbers, 2001), astronomy (e.g. Bakas & Mikropoulos, 2003), biology (e.g. Flores, 2003; Simonneaux, 2000) chemistry (e.g. Furió-Más, Calatayud, Guisasola, & Furió-Gómez, 2005; Sumfleth & Telgenbüscher, 2001) and mathematics (e.g. Merenluoto & Lehtinen, 2004).

The overall rationale of 3P-SIT is that it is a post-Piagetian clinical interviewing method, which is semi-structured, neutral and flexible in design (e.g. Nicoll, 2003; Duit et al., 2001). Thus, interview questions (also termed 'probes' as the questions are used to probe for student understanding and reasoning patterns) can be modified according to student response patterns that emerge during the interview session (e.g. White & Gunstone, 1992; Posner & Gertzog 1982). Empirical data

generated from 3P-SIT was used to investigate the nature of the factors of the model, to formulate clear operational definitions for each component factor of the model, to test the validity of the model and, to establish the *nature of interaction* between the factors of the model. In brief, each single interview conducted with each participant comprised three interview Phases. Phase 1 of 3P-SIT investigates a student's conceptual knowledge, represented by the ER, *prior* to the student being exposed to the ER; while during Phase 2 students' reasoning processes and any changes in their conceptual knowledge during the interpretation of the ER are probed. Lastly, Phase 3 requires students to evaluate and critique the ER itself, thereby allowing the researchers to gain knowledge about the ER mode. The ER mode is also evaluated by experts such as scientists, researchers and graphic artists to supplement the student data. Each phase of 3P-SIT is described in greater detail below.

Phase 1: Generating data corresponding to students' conceptual knowledge

Phase 1 of 3P-SIT, which requires approximately 20 – 30 minutes of interviewer-student engagement, is concerned with exposing students' conceptual understanding about a scientific idea *prior* to being exposed to any ER (e.g. Figure 1). The rationale of Phase 1 is that at first, initial probing is of a free-response nature, followed by specific questions that are posed to the student as deeper patterns of interest emerge (e.g. Furió-Más et al., 2005; Rubin & Rubin, 1995). The following free-response probe was used at the start of Phase 1 in all 27 interviews to probe students' conceptual understanding prior to being exposed to any ER.

> I: Today I would like us to talk about antibody molecules… [long pause][2]… take your time and start thinking about these types of molecules. Take as much time as you want, don't rush, just relax and think

[2] In the transcript text, interviewer is designated 'I' and student 'S'. Words included between square brackets are inserted for the purposes of adjusting an immediately previous word or phrase for scientific or grammatical clarity. An ellipse between square brackets designates an excluded section of transcript text while an ellipse used within the transcript text designates a sudden change in thought, slight pause, or verbal interruption. In addition, included between square brackets are abbreviations used to describe tacit physical gestures, drawing behaviours and additional verbal outputs of students during data collection. The abbreviations serve as a nomenclature with which to present data corresponding to students' observable and explicit behaviours. For this study, the abbreviations used in the transcript text were as follows: Ab, antibody; Ag, antigen; ax., X or Y axis of a Cartesian plane; beg., begins; b. site(s), antigen binding site(s) on antibody; bot., bottom; conc., concentration; Fab, fragment antigen-binding; gen, generates; Gln, glutamine; grad., gradient; H, heavy chain; lt, on the left or on left hand side; L, light chain; lyso., losozyme; rt, on the right or on right hand side; wk, week number.

> about them for a while [long pause]. Try to imagine it; an immunoglobulin molecule… think about everything you know about these types of molecules [long pause]… slowly, let your thoughts flow… [silence]. When you feel like telling me something about these molecules, go ahead… speak slowly and clearly, there is no rush… [after a while]… Ok, what are you thinking about now… tell me slowly and clearly, take your time.

During students' responses to the probe above, the interviewer waits for responses to emerge naturally. Following this, the interviewer delves deeper into the student's conceptual understanding. The subsequent probes do not follow any pre-determined sequence and are solely dependent on the nature of the responses elicited by the student (e.g. Ametller & Pintó 2002; Posner & Gertzog 1982). Interestingly, it was found that in *all* cases, participants spontaneously requested to draw their own diagrams to form part of their responses during Phase 1. This activity was encouraged whenever such a request was made. Overall, the data collected in Phase 1 of 3P-SIT is a measure of the conceptual understanding that a student would *bring* to an ER (e.g. Cheng et al., 2001; Lowe, 1996) when required to respond to questions about an ER of interest, during Phase 2 (see below).

Phase 2: Generating data corresponding to students' reasoning processes

Following Phase 1 of the 3P-SIT interview, the student is then exposed to a particular ER (e.g. Figure 1 A, B or C), which marks the beginning of Phase 2. Phase 2 requires between thirty and forty minutes and has the primary objective of probing a student's reasoning processes and any changes in their conceptual knowledge, *during* the interpretation of a scientific ER. The researcher uses semi-structured questions to first probe for surface-level reasoning and then more demanding questions to probe for evidence of deep-level reasoning. In so doing, the researcher aims to establish the way in which subjects link their interpretations of an ER to their conceptual knowledge (obtained from Phase 1) and how they go about reasoning with the ER, and the markings contained within the ERs, to acquire meaning. In other words, the probes designed for Phase 2 aim to induce the student into making sense of the graphical markings and visual-spatial features on the ER such as conventions, visual icons, spatial arrangements, topography and the representation of abstraction, while also inducing the student to associate their interpretations of the ER with their already existing conceptual knowledge.

The rationale behind the design of Phase 2 of the interview is that the semi-structured probes are used to first probe for 'surface-level' reasoning (e.g. Lowe, 1993; Chi, Feltovich, & Glaser, 1981) and increasingly more demanding questions to probe for evidence of 'deeper-level' reasoning (e.g.

Lowe, 1993; Chi et al., 1981). Such an approach allowed the interviewer to observe the slow constructive process of ER interpretation by the student. In this regard, as the interviewer progressed through the probes, the student was required to steadily increase their level of engagement with the ER, as the probes became more cognitively demanding. In addition, the authors felt that this approach allowed for both a useful and valid means for tracing any changes in students' ER-reasoning processes as the interview phase developed. The content contained within the probes for Phase 2 was informed by the authors' informal visual analysis of ER A, B and C for any potential and, therefore, suspected interpretation difficulties that students may have shown (see Schönborn, 2005).

When commencing with Phase 2, the interviewer first gave each student approximately two to three minutes with which to familiarise themselves with the ER and its Figure caption. As part of this, the interviewer pointed out the relevant Figure caption to the participant (Figure 1) and read it out aloud. As suggested above, the probes comprising Phase 2 of the interview were pitched as progressing from a 'surface-level' to a 'deeper-level' of necessary student engagement. A surface-level of engagement can best be described as a process of *extracting information* (Kindfield, 1993/1994) from ER features that are salient or stand out (e.g. Lowe, 2003). In order to respond to the probes successfully, the student is required to 'extract' visual information from the particular ER by making sense of the graphical markings and visual-spatial features on the ER such as pictorial conventions, visual icons, spatial arrangements, topography and the representation of abstraction. In contrast, a deeper level of engagement can be described as a process of *extracting meaning* (Kindfield, 1993/1994) from ER features that are not salient (e.g. Kozma, 2003; Lowe, 2003, 1996). In order to respond to the probe fruitfully, the student is required to gradually associate their interpretations of the ER with their already existing conceptual knowledge. This process requires students to use the ER *and* engage their own conceptual knowledge to successfully reason with the ER. Sometimes, such 'deeper-level' type probes incorporated more specific think-aloud tasks (e.g. Lewalter, 2003; Bowen, 1994), in which students were induced to generate their own diagrams (e.g. Glynn, 1997) when interpreting an ER. Obtaining this data was a major feature of the present study and, where possible, students were prompted to succinctly explain the diagrams that they generated. These types of probes aimed to attain information pertaining to how a student reasoned with an ER or made *use* of it to solve a problem (e.g. Cox & Brna, 1995; Koedinger & Anderson, 1990; Larkin & Simon, 1987). In this regard, the authors have noted that recent researchers place great emphasis on the collection of *non-verbal* data in order to obtain more precise inferences about ER processing (e.g. Gobert, 2000; Gobert & Clement, 1999). Here, the

'drawing' of mental models can be seen as a diagnostic tool that can help researchers get a better idea of students' cognitive structures (e.g. Glynn, 1997). As part of the diagram-generating probes, the interviewer also noted students' tacit behaviours used to convey their reasoning processes (e.g. Gall, Borg, & Gall, 1996) such as pointing, indicating to, constructing, annotating and modifying of students' generated diagrams (e.g. Kindfield, 1993/1994).

The pool of semi-structured probes designed for each of the three ERs and which were also used in preliminary studies (e.g. Schönborn, 2005) are available from the authors. For the purposes of this article, specific contents of the interview questions pertaining to Phase 2 of 3P-SIT are presented in the 'Results and Discussion' section where relevant.

Phase 3: Generating data corresponding to the mode of representation

Phase 3 of the 3P-SIT interview takes about fifteen to twenty minutes and requires students to evaluate and critique the ER in response to semi-structured probes. In so doing, the responses to such probes help the authors generate data about the role and effect of the graphical markings and features of the ER such as conventions, icons, colour, artistic devices, labels and captions on students' reasoning processes. In other words, the rationale behind Phase 3 is that the responses help the researchers measure the nature or influence of the ER *in isolation*, i.e. the role and effect of the *representation mode* on students' reasoning processes. This student data can also be compared to that from experts' evaluation of the same ER conducted independently of the student interview. All the probes utilised in Phase 3 were similar across all three ERs. The probes used for all 27 interviews were as follows:

> Is there anything on the ER in particular that you don't understand of find confusing?
>
> What do you think this ER is not showing? Explain your answer.
>
> Consider yourself a diagram designer or textbook author. If you could change this ER in any way, what would you do to improve it, if anything?
>
> Do you think this is a good and clear representation? Give reasons for your answer.
>
> Comment on these types of representations in general, and your feelings on interpreting them.

A further design characteristic of the Phase 3 probes is that they require the student to think critically about the ER and to apply a subjective 'rating' of its usefulness. In this way, the probes induce metacognitive and reflective behaviours (e.g. Ward & Wandersee, 2002; Case, Gunstone, &

Lewis, 2001) in that students are required to 'take a step back' in an effort to evaluate the ER objectively. As for all Phases of 3P-SIT, the interviewer pursues the patterns of interest applicable to a particular ER by delving deeper into a student's emerging responses while refraining from leading or biasing a student into a particular response (e.g. Ametller & Pintó 2002). Overall, empirical data generated during Phase 3 of 3P-SIT can provide useful information about the role of graphical and pictorial markings on students' interpretation of the ERs and thereby help the researcher identify how the external nature of the ER *per se* influences ER interpretation. In other words, the effect of the *mode* in which the desired scientific phenomenon is represented in the ER on students' reasoning processes. Once Phase 3 of 3P-SIT has been completed, the interview session is closed.

Analysis of the 3P-SIT interview data

All 27 interviews (9 participants x 3 ERs) were both audiotaped and videotaped (e.g. Hull, 2003; Ametller & Pintó, 2002; Pavlinic, Buckley, Davies, & Wright, 2001; Sumfleth & Telgenbüscher, 2001; Buckley, 2000; Simonneaux, 2000). The data collected consisted of 27 video segments, 27 audio-transcripts, 134 student-generated diagrams (SGDs) and 27 researcher-generated field note items. Data was analysed by means of a qualitative, iterative and inductive method in which categories of responses emerged from the data themselves, rather than being pre-determined (e.g. Grayson, Anderson, & Crossley, 2001), and in which patterns were uncovered and 'made explicit from embedded information' (Lincoln & Guba, 1985, p. 203). In this regard, our analysis of the data could best be described as a 'descriptive synthesis' rather than a process of data reduction (McMillan & Schumacher, 1993, p. 480) and follows a post-positivistic approach to data treatment.

The following general seven-step process, not necessarily in a linear manner, was used to analyse the data and relate it to the various factors affecting ER interpretation. Firstly, the interviewer made paper-based field notes consisting of any relevant issues that were observed while the interview was in progress. Secondly, each audiotape was transcribed and the data electronically assigned to categories corresponding to Phases 1, 2 and 3 of 3P-SIT. Thirdly, the authors used analytic induction of the transcripts, to formulate common patterns of student responses into categories. During this process, in addition to the field notes, the authors made further notes on the printed transcripts. Fourthly, the authors analysed the diagrams that were generated by the respondents. Analysis of these SGDs further facilitated the diagnosis of students' reasoning processes and the

extent of their conceptual understanding (e.g. Glynn, 1997; Kindfield, 1993/1994). This approach is supported by other workers in which students' *drawing* of their mental images has proved to be a powerful way of measuring thought processes and ways of reasoning (e.g. Beilfuss, Dickerson, Libarkin, & Boone, 2004; Reiss & Tunnicliffe, 2001; Gobert, 2000; Gobert & Clement, 1999).

Fifthly, the authors used the video footage to supplement the electronic transcripts with additional information pertaining to students' interpretation of the ERs (e.g. 'pointing' on the ER and other tacit knowledge). This allowed the authors to gain more information about students' mental processing of the ERs. The ER-related observable behaviours, that were inserted into the transcripts, were those such as students' specific sequences of diagram construction; students' modification, annotation or rejection of their diagrams; and, their gestures such as 'pointing' and 'indicating' on the diagram and various other observable behaviours (e.g. Kindfield, 1993/1994; Lowe, 1993).

Sixthly, the interrelationships between the data across the 3P-SIT phases were investigated in an attempt to measure how successfully the ER was interpreted and, whether sound or unsound learning had occurred after exposure to the ER. The success of the *interpretation* was measured by comparing the student's conceptual knowledge *after* exposure to the ER (Phase 2) to the conceptual (propositional) knowledge represented by the ER. Evidence of any *learning* from the ER was measured by comparing the student's conceptual knowledge *after* exposure to the ER (Phase 2) to the student's prior knowledge, obtained during Phase 1. Through the latter, it could also be determined whether the construction of a new conception, an alternative conception or a modification of an existing conception had taken place. Furthermore, through the above comparative analysis, we could monitor how existing conceptions *modulated* reasoning with a particular ER (e.g. Cheng et al., 2001; Lowe, 1996), especially when the ER was novel to a student. Lastly, by comparing data generated from Phase 3 with that of Phase 2, insight could be gained into how the actual visual-spatial markings on the ER influenced and modulated students' reasoning processes.

Seventhly, similar categories and patterns of difficulties obtained from transcripts and SGDs across the three ERs were pooled and analysed in order to identify difficulty categories that were common to students regardless of the nature of the ER. For example, we investigated evidence of particular reasoning (e.g. analogical reasoning) and conceptual patterns (e.g. misconceptions about antibody binding sites) among all students, regardless of the ER in question.

RESULTS AND DISCUSSION

Development of the model

In a preliminary study (Schönborn et al., 2002), we identified three factors affecting students' ability to interpret ERs in biochemistry. These are, students' *reasoning* ability (R factor), students' understanding of the *concepts* of relevance to the ER (C factor), and the nature of the *mode* in which the desired phenomenon was represented by the ER (M factor). The aim of this study was to further investigate these and other possible factors affecting ER interpretation and to incorporate them into a model, using the modelling process of Justi and Gilbert (2002) (Figure 2). Regarding the purpose of the model, it was decided that it should serve as a tool with which to frame researchers' and educators' thinking on the factors that affect a student's ability to interpret a scientific ER in biochemistry. The model was first conceptualised as a mental model and then externalised as an expressed model. Initially, the model was expressed as a *triarchic* model, in which the three vertices of a triangle represented the three factors, C, R and M. Subsequently, upon further thought experimentation, we realised that the triarchic model needed to be modified and expanded by a further four factors (R-C, R-M, C-M and C-R-M) to account for the *interaction* between each of the three original factors. This was because we realised that, for example, reasoning (R) could not occur without having *something to reason with*; in this case, with one's own conceptual knowledge of the ER (which we now represented as factor R-C) or with the features of the ER itself (represented as factor R-M). In addition, we realised that the nature of the propositional knowledge represented by the ER would be an important interactive factor affecting ER interpretation and termed this factor C-M. Finally, we decided that the interaction between all three original factors (C-R-M) could appropriately represent a student's ability to engage all factors of the model in order to successfully interpret an ER. Taking into account the above seven factors, we proposed that a Venn diagram would more accurately represent the interactive nature of the model than the triarchic representation would, and thus expressed the model presented in Figure 3.

Insert Figure 3 about here

Formulation of operational definitions for factors of the expressed model

The modelling framework of Justi and Gilbert (2002) (Figure 2), as well as extensive consultation with the literature, enabled detailed operational definitions for each factor of the expressed model (Figure 3) to be formulated. The rationale behind, and description of each definition is as follows.

We defined the conceptual factor (C) of the model (Figure 3) as the existing conceptual understanding and prior conceptual knowledge (of relevance to the ER in question) that a student holds *before* exposure to any ER. Therefore, it embodies a student's preconceptions, conceptions, conceptual frameworks, mental models and alternative conceptions (conceptual difficulties) that a student 'brings' to the ER. The C-factor was validated from data obtained from Phase 1 of 3P-SIT.

We defined the reasoning factor (R) (Figure 3) as representing those *cognitive processes* that a student employs when reasoning with the ER and with his/her own conceptual knowledge of relevance to the ER. More specifically, factor R represents a student's total reasoning ability that s/he has available for interpreting the ER, i.e. the skills needed to decode and perceive visual markings on an ER (e.g. Ward & Wandersee, 2002; Bennett & Flach, 1992), to access and retrieve conceptual knowledge from long term into working memory (e.g. Baddeley, 1992) in order to perform ER-related reasoning; and, to assimilate or accommodate information that is first perceived from an ER and then incorporated into already existing knowledge. Factor R also represents both sound reasoning and any reasoning difficulties with the ER or with a student's own conceptual knowledge, including surface-level reasoning; inappropriate analogical reasoning; knowledge transfer; and, translation between ERs.. Therefore, factor R includes the student's ability to reason with *both* the ER (see R-M below) and his/her conceptual knowledge (see R-C below) of relevance to the ER (see C-M below). Our definition stems from a constructivist paradigm, which suggests that cognitive mechanisms associated with R are not passive (e.g. von Glasersveld, 1989) in that reasoning is an active process (e.g. Treagust et al., 2002; Bruner, 1986), characterised by students' *constant* selection, organisation, integration and encoding of information (e.g. Mayer, 2003, 1997). Unlike a conceptual difficulty, which is context-dependent, a *reasoning difficulty* is *independent* of context (e.g. Grayson et al., 2001) and can be observed in multiple scientific content areas. For instance, localised reasoning is an example of an ER-related reasoning difficulty identified in the contexts of electricity (Cohen, Eylon, & Ganiel, 1983) and metabolism (Anderson, Crossley, & Grayson, 1999). With respect to the model, a reasoning difficulty can span across several ERs within a specific context (e.g. across antibody-antigen binding; Figure 1), across several ERs from different contexts (e.g. across antibody-antigen binding *and* the particulate nature of matter), across ERs in an even larger context (e.g. across biochemistry or physics) or, across science as a whole.

Factor R was evaluated by combining data obtained for the R-C and R-M interactive factors (see below) from Phases 1 and 2 of 3P-SIT.

The representation *mode* factor (M) of the model (Figure 3) encapsulates the actual external nature of the ER. By the external nature of the ER, we mean the characteristics of the ER such as the graphical and diagrammatic features, the spatial arrangement of the ER elements, ER conventions, visual icons, visual cues, artistic devices, colour, topography, level of abstraction, symbols, labels, captions, and so on. Factor M can be considered distinct from both C and R, since it does not depend on any human constituent during the interpretation process and remains constant unless the ER is modified (e.g. during animation or the advancement of science). Factor M can be evaluated, by experts such as scientists, researchers and graphic artists as well as students, in isolation from interpretation of the ER during Phase 3 of 3P-SIT.

The interactive factor, defined as the relationship between the reasoning (R) and conceptual (C) factor, and termed R-C (Figure 3),, represents cognitive processes such as when a student accesses, selects, retrieves, actively adjusts, processes or adds to their existing knowledge. Therefore, R-C is indicative of a student's ability to reason with his/her conceptual knowledge of relevance to the ER because, in effect, students are *using* the collection of their concepts to 'think about something' or to 'solve a task'. Congruently, within R-C, cognitive processes such as assimilation and accommodation of knowledge can also be represented. This is because a student may add to, or adjust, their conceptual structure, especially when concepts are constructed that were not part of an existing conceptual framework. Factor R-C also includes the ability to perform cognitive processes such as analogical reasoning, knowledge transfer, inductive and deductive reasoning. It includes both sound reasoning and unsound/inappropriate reasoning difficulties. Factor R-C was evaluated through Phases 1 and 2 of 3P-SIT.

The R-M interactive factor (Figure 3) between the representation mode (M) factor and the reasoning (R) factor exemplifies a student's ability to decipher, process and reason with the ER and its graphical features. For instance, when reading an ER, a student will employ perceptual mechanisms and cognitive processes such as recognition and organisation of patterns, decoding and deciphering of shapes and colours (e.g. Bennett & Flach, 1992), visuo-spatial operations (e.g. Lord, 1990) and visualisation operations (McCormick, DeFanti, & Brown, 1987) to distinguish relationships and translate between ER features (e.g. Lowe, 1993), and to mentally organise the topographical information on the ER (e.g. Ward & Wandersee, 2002; Mayer, 1997). The R-M

factor includes both sound reasoning and unsound/inappropriate reasoning difficulties and students' inability to perform any of the above cognitive processes. Factor R-M was validated from data obtained from Phase 2 of 3P-SIT.

The C-M interactive factor of the model (Figure 3) was defined as representing the nature of the conceptual (propositional) knowledge represented by the ER and its symbolism. This includes the extent, complexity and soundness of the knowledge represented by the ER. It also includes both the conceptual knowledge that is communicated through, or represented by, the graphical markings and symbolism used to construct the ER, and knowledge of the meaning of the symbolism and conventions employed in the ER to communicate the science. For example, one aspect of the scientific meaning of the '**x**' symbolism in Figure 1 C is that it is an x, y coordinate. Data used to evaluate factor C-M was obtained from surrounding text, captions and expert evaluation of the ER and the knowledge represented by the ER, in terms of extent, complexity and soundness. Thus, evaluation of factor C-M is obtained in isolation from students' interpretation of the ER.

The C-R-M interactive factor of the model (Figure 3) represents a student's ability to successfully interpret and/or learn from the ER. This includes the student's ability to engage *all* factors of the model by using reasoning skills (R) to reason with both their conceptual knowledge (C and R-C) of relevance to the ER and with the symbolism and features of the ER itself (R-M), to make sense of the graphical features of the ER (M) and visualise the conceptual knowledge represented by the ER (C-M). For example, the process could take the following form. Upon reading the ER (M), the individual deciphers and decodes the visual information on the ER (R-M) and, in so doing, links and integrates their interpretation into, and filters their interpretation through (R-C), already existing current knowledge (C). The outcome of this process could result in the construction of a unique conception (R-C) consistent with accepted scientific knowledge (C-M) or an erroneous conception, inconsistent with a scientific worldview (e.g. von Glasersveld 1989; Osborne and Wittrock, 1983). Hence this scenario would depend on a combination of all three factors (C-R-M), during which all factors comprising the model would, at some time or other, be engaged resulting in the student achieving some measure of interpretation and/or learning from the ER. Therefore, the C-R-M factor is measured by how correctly the ER is interpreted and the improvement in understanding and/or development of alternative conceptions that occurs after exposure to the ER. The success of the *interpretation* of the ER, and of *any learning* from the ER, is measured by comparing the student's conceptual knowledge after exposure to the ER (Phase 2) to the conceptual knowledge represented by the ER (i.e. C-M) and to the student's prior knowledge (C), respectively.

Empirical validation of the model and its factors

The following empirical data, employing 3P-SIT, was used to validate the expressed model (Figure 3) and its component factors and test the appropriateness of the operational definitions for each factor. Even though extensive empirical data was obtained with the ERs, in the interests of brevity, this paper contains only selected examples of relevant data.

Validation of the Conceptual Factor (C)

Phase 1 of 3P-SIT allowed us to obtain students' prior conceptual understanding of antibody structure and antibody-antigen interaction *before* exposure to any ER. For example, the following student quotation and corresponding SGD (Figure 4a) shows a sound scientific understanding of the bivalent nature of antibody-antigen binding:

> S: …you'd have two binding regions that look the same on an antibody molecule …and … they'll [binding regions] recognise the same antigen.[3]

Insert Figure 4 about here

In contrast to the above, various students showed a range of conceptual difficulties. In one example, three students erroneously thought that an antibody only had one possible binding site for an antigen and that this site was the entire 'V' cleft of the Y-shaped antibody, instead of the two variable binding domains. In a related finding, two other students showed an interesting variation of this conceptual difficulty. As illustrated in the following quotation and corresponding SGD (Figure 4b) from one of the students, even though the student accurately represented *both* antigen binding sites (see two black circles), s/he nevertheless still believed that the antigen binds into the V-cleft of the antibody.

> I: …where are the actual binding sites on the antibody molecule?
> S: Ok… [S beg. to gen. Figure 4b]… these are your binding sites here [inserts black circular shaped sites on Ab]… the components within these two domains are responsible for recognising antigen.
> I: … show me where the antigen would be when there is an antigen-antibody complex.
> S: Ok… these are the binding sites [points to black circular shapes]… the antigen will basically fit in between here… between these domains [draws elongated antigen fitting into V-cleft of Ab]…
> I: …what are these regions over here [points just below circular binding domains on Figure 4b]?

3

> S: I would say they are also part of the binding domains, because this is where the antigen binds to [indicates entire V-cleft and inserts 'brackets' on lt and rt of Ab]…

Thus the above student's conception of antibody-antigen binding encompasses the misunderstanding that only a single antibody can bind to a single antigen and that *both* the two binding sites and the 'V' cleft are simultaneously responsible for recognising a single antigen. The ideas of *specificity* and *recognition* between antibody and antigen were very pronounced amongst participants. For example, complementing students' explanations of antibody-antigen binding in Phase 1 were statements such as, 'a key unlocking a specific lock', 'complementary shapes', 'two-piece puzzle', 'specific fit', 'fit into a pocket', 'compatibility', 'an upside down pyramid which tries to fit into the V-cleft' and 'join perfectly'.

A possible source of the above misconception could be students' understanding of the 'lock-and-key' analogy, used by instructors and textbooks to describe specific binding interactions between biomolecules (e.g. enzyme-substrate binding). The analogy emphasises that for a *fit* between biomolecules to occur, both participating elements must have a complementary and specific shape (e.g. Stryer, 1995). The following student quote from Phase 1 illustrates a succinct expression of the analogy:

> S: …It's a very specific interaction between antigen and antibody. The antibody has to be specific to the epitope found on the antigen, which is with regard to the lock-and-key mechanism... It [Ab] has to fit properly otherwise it [Ab] won't bind. So, it [binding] actually has to be compatible…

The lock-and-key metaphor was very ingrained in all students' conceptual understanding of antibody-antigen binding. Although, Fischer (1894) first used the lock-and-key metaphor to exclusively describe enzyme-substrate interaction, the metaphor can also be applied to antibody-antigen binding since the structural basis of binding is synonymous in both cases (e.g. Roitt, 1997; Amit et al., 1986). However, for enzyme-substrate reactions, typical lock-and-key ERs usually show the simple situation of a *single* enzyme binding to a *single* substrate (e.g. Ritter, 1996). When applying the analogy to the context of antibody-antigen binding it is possible, therefore, that students may have thought that each IgG molecule can only bind one rather than two antigen molecules. Also, some students' may have interpreted the analogy literally (e.g. Orgill & Bodner, 2004), instead of taking it to be only a *representation* of reality (e.g. Wheeler & Hill, 1990), resulting in the alternative conception of antigen binding into the 'V-cleft' of an antibody. Another possible source of the binding misconception is that students may have been associating their ideas of antibody-antigen binding with a single ligand binding into a single receptor site, as in the case

when a peptide binds within the cleft of an MHC molecule (e.g. Roitt, 1997). Finally, some students may have simply lacked any other explanatory models to describe binding, other than the lock-and-key analogy. For instance, Koshland's (1963) notion of an induced-fit between antibody and antigen was found to be absent from students' conceptual knowledge, with only a single student exposing the idea. Instead, students seemed to only expose conceptual knowledge relating to the 'physical fit' between antibody and antigen and not other stereospecific considerations such as the role of amino acid side chains or intermolecular forces such as hydrophobicity, hydrogen bonding and electrostatic interaction during binding. Overall, data from Phase 1 of 3P-SIT allowed us to obtain information pertaining to the conceptual understanding that students' 'brought' to the ER. In this regard, and as shall be further supported later with respect to the interactive factors of the model, the above examples confirmed the importance of students' prior knowledge, i.e. the conceptual factor (C), as one component of the expressed model affecting students' ability to interpret an ER (Figure 3).

Validation of the Reasoning Factor (R)

Upon analysis of the data generated with 3P-SIT, we identified at least five different reasoning mechanisms associated with students' interpretation of ERs. Firstly, some students employed *surface-level reasoning* (Chi et al., 1981) when processing the graphical markings on the ERs. These students interpreted the ER markings literally and at face value, without considering the deeper meaning of the markings (e.g. Ametller & Pintó, 2002; Cheng et al., 2001; Lowe, 1993). As Kozma (2003) and Olivier (2001) have pointed out, students who employ surface-level reasoning rely heavily on perceptual processes when interpreting ERs, rather than on deeper knowledge structures. Secondly, our data suggested that some students performed *inappropriate analogical reasoning* when interpreting the ERs (e.g. Orgill & Bodner, 2006; Sumfleth & Telgenbüscher, 2001). As introduced during validation of the C factor above, this was found to be the case especially when students struggled to use the lock-and-key analogy as a tool with which to explain the nature of antibody-antigen binding. Thirdly, some students engaged in *inappropriate transfer* (Salomon & Perkins, 1989) when interpreting the ERs. Here, the students inappropriately transferred a particular biochemical concept (e.g. destruction of invading pathogens) from the context of cellular immune responses to the context of primary antibody-antigen binding. Fourthly, and related to the former, some students found it difficult to *translate* between different ERs, which all represent the same concept or phenomenon. In particular, these students could not map between one ER and another (e.g. Ainsworth, 2006), probably because students treated each ER as a unique

situation, instead of viewing all the ERs as being multiple representations of the same scientific concept (e.g. Gobert & Clement, 1999; Ainsworth, Bibby, & Wood, 1998). Fifthly, we also discovered what we have termed the apparent *superimposing* of one concept upon another. Here, some students tended to fuse two or more distinctively different concepts together into a single explanative model, leading to the moulding of scientifically inaccurate conceptions. The superimposing of concepts could be related to a recent finding by Grayson (2004), who has referred to a similar phenomenon in the context of electric circuit ERs in that some students struggled to *disentangle* the distinctively different concepts of current and energy from one another. Empirical data that we suggest validates the five abovementioned reasoning processes can be found under R-M and R-C below. Since both the R-M and R-C factors can be considered *subsets* of the R factor (Figure 3), R cannot be validated in isolation (i.e. without 'something' to reason with).

Validation of the Reasoning-Mode (R-M) Factor

The following are examples of empirical data generated from Phase 2 of 3P-SIT that demonstrated unsound and sound reasoning with ER B (Figure 1), respectively:

> I: What does this plate over here represent [points to frame c in ER B]?
> […]
> S: …interaction [between the antibody and the lyso.] caused the glutamine to break down and join with the antibody [points on frame c]. The antibody is actually working on the glutamine [circular pointing on frame c]… the antibody is probably responding to the lysozyme… the antibody is breaking down the molecule [lyso.]… that is how you get this glutamine [points to red spheres on frame c].
>
> S: … this is the antigen [points to lyso. in frame c]… the lysozyme… it shows how it fits onto that molecule [points to Fab in frame c]. So, this is the paratope [points correctly on Fab on frame c] and that is the epitope [points correctly on lyso. on frame c]. And, this [points to red spheres on lyso. and Fab in frame c] shows the position of the molecules that facilitate that association.

The first student above showed an unsound interpretation of ER B by suggesting that the single red glutamine molecule, represented on frames 'a' and 'b' (ER B), had in some manner been degraded so as to produce the scenario that appears on frame 'c' of ER B. This suggests that the student was interpreting the red 'spheres' on the ER superficially and that an over reliance on the graphical markings had resulted in surface-level processing (Lowe, 1993; Chi et al., 1981), rather than on a deeper appreciation of what the markings actually meant (e.g. Cheng et al., 2001; Olivier, 2001). That is, instead of interpreting the numbered red spheres in frame 'c' as contact amino acid residues between antibody and antigen during binding (see second quote above), the student erroneously attributed a digestive process to the 'increase' in the number of red spheres in frame 'c'. Thus, the

student inappropriately decoded the symbolism used to represent the amino acids involved in binding.

In addition to the above examples,, eight of the nine respondents struggled to accurately visualise the biochemical structures portrayed in ER B. Whereas the space-filling display (ER B) only represents a single 'arm' or Fab fragment of IgG, these students visualised it as the *complete* Y-shaped antibody. This is illustrated by the following example of a SGD (Figure 5a) and accompanying verbal explanation.

Insert Figure 5 about here

I: In terms of structure, what is being shown on this representation [ER B]?
S: …you can see the antibody structure… one can see that it consists of the two chains [H and L]… it is actually two heavy chains [points to bot. two 'groups' of blue spheres making up the H-chain simultaneously in frame a] and two light chains [points to top two 'groups' of yellow spheres making up the L-chain simultaneously in frame a].
[…]
I: …Could you relate the markings that you've drawn on paper [Figure 5a] to what is visually represented on the actual diagram [ER B]?
S: … that's [points to Ag depicted in top rt of Figure 5a] your antigen there [points to green lyso. in frame a]. This would be your epitope [points to oval shape on Ag on Figure 5a], your actual region of binding, which is glutamine, so that is your red part [points on ER B], that little blob sort of part sticking out [red Gln in frame a]. Then, these [points to each 'group' of blue spheres on Fab in frame a] are your two heavy chains [points to each lower part of H chains on Figure 5a]... these are your two light chains [points to each 'group' of yellow spheres on Fab in frame a and then to each light chain on Figure 5a].
I: If I were to bind an antigen over there [points to lt binding site on Figure 5a], how would that look [in ER B]?
S: What I'm thinking is that it [Ag] would actually come in from this side [points to lt of Fab on frame a], so it would actually be more or less a mirror image of this molecule [points to green lyso. on frame a], but on that side [points to lt of Fab on frame a].

Based on the extract above, as well as an analysis of the student's observable behaviours, such as pointing and indicating to different components on ER B in addition to the SGD (Figure 5a), it was clear that the Fab arm represented in the ER was interpreted as an entire Y-shaped antibody. This was further supported when the student described how ER B would appear if another antigen had bound to the other antigen binding-site, depicted in the SGD (Figure 5a). In this case, the student has attributed the general shape and topography of the visually grouped 'clusters' of spheres in the ER to the visualisation of a complete and upright Y-shaped antibody. One possible source for this reasoning is that the student could not distinguish between, and organise, the visual information on the ER appropriately (e.g. Kozma & Russell, 1997; Bennett & Flach, 1992). As a result, the student erroneously translated (e.g. Brna, Cox, & Good., 2001; Gobert & Clement, 1999; Ainsworth et al., 1998) between the ER portrayed in Figure 1 B and his/her mental models of other more common

textbook ERs that portray antibodies as upright and complete Y-structures. In contrast to the above student, the following quote constitutes evidence of sound processing of ER B with respect to the structural components represented by the ER.

> S: …Basically, on this structure [ER B], you'll be representing one arm of your molecule. You have two of these [Fab arms] on your entire antibody molecule… it [ER B] is just showing one arm.

In addition to the above, and part of generating responses during Phase 2 of 3P-SIT, students were asked how the ELISA graph (ER C) would appear if absorbance results for week one hundred were plotted on the same curve. Realistically, at week one hundred, the experimental serum obtained from the laboratory animal would show an antibody concentration very close to pre-immune levels (green/'Pre-Immune' curve on ER C), due to the lack of experimental antigen, which is needed to stimulate antibody production. An example of a verbal response demonstrating sound reasoning with the graph in terms of this scenario is shown in the first extract below. In contrast, two students thought that the absorbance value for week 100 would be higher than for week twelve. This reasoning is demonstrated by the SGD (Figure 5b) and accompanying second quotation from one of the students below:

> I: Consider that we stopped the experiment…at week one hundred, we took another sample, and we did a plot, how would that look here [on ER C]?
> […]
> S: It will be something like the pre-immune… because… there won't be antigens in your system to make you produce antibodies… or increase your antibody production.

> I: Say they [the researchers] had finished taking readings and had finished the experiment. Then, they plotted for say, week 100, how would the graphs [ER C] look then?
> S: It would probably be a higher value than twelve, with bigger absorbance values…[student proceeds to draw Figure 5b]… here we have week twelve [curve labelled '12' on Figure 5b]. When I'm looking at this graph [ER C], I would think that week 100 would be somewhere up there [curve labelled '100' on Figure 5b], with a similar effect [in comparison with wk 12 curve in ER C]… with it [curve] going higher then coming down.

A possible source of the above difficulty is that the students may have placed greater emphasis on the visual relationships on the graph (ER C) rather than engaging their knowledge of ELISA concepts to consider the biochemical implications of ceasing booster injections (e.g. Kozma, 2003; Roth, 2002). For instance, these students used the visual trend of a 'higher' graph corresponding to a higher week number to solve the task. Thus, they were reasoning in a linear manner, with the graphical data playing a dominant role in their interpretations, rather than thinking deeply about the related and underlying biochemistry. As a result, the students relied heavily on the graphical markings to interpret the ELISA curves. Overall, the above examples suggest that students' understanding of ER B and C was largely influenced by a surface-level interpretation of the

graphical features (e.g. Lowe, 1993; Egan & Schwartz, 1979). In turn, we suggest that the data provides evidence for the tenets of the R-M factor, and therefore, serves to validate the R-M factor of the expressed model (Figure 3).

Validation of the Reasoning-Conceptual (R-C) Factor

Since Phase 1 of 3P-SIT allowed us to first establish the nature and extent of a student's prior knowledge (Factor C) of relevance to the ER before actually seeing the ER, in Phase 2 we aimed to establish the extent to which the student engaged this conceptual knowledge when subsequently interpreting an ER. For example, when interpreting ER A, the following student was shown to rely heavily on his/her unsound conceptual understanding (measured in Phase 1) to interpret the ER during Phase 2 of the interview. This is demonstrated by the first extract below obtained during Phase 1, followed by a subsequent SGD (Figure 6a) and corresponding verbal commentary generated during Phase 2 of the same interview:

S: … antibodies… they form complexes with the antigen in order to destroy it or engulf it […] they [Ab and Ag] will form like a lock and key mechanism and join... the antibody has certain compounds in it… that infiltrate the antigen, when it [Ab] engulfs it [Ag] […] this little antibody infiltrates the antigen and releases little granules that contain the digestive enzyme and then these things degrade the whole antigen into smaller things…

Insert Figure 6 about here

S: … step four [labelled '4' on Figure 6a], they [Ab's] form a trimer. The different antibodies bind to three sites ['V-clefts']…and then they [Ab's] join… to form a trimer.
I: What would happen after [step] four [on Figure 6a]?
S: The antibody has done its function of removing this hapten [Ag] molecule…it [hapten] gets broken down and destroyed.
I: Ok. Between [step] four and five ['4' and '5' on Figure 6a] what is going on?
S: Ok, [step] four…once antibody has bound onto the hapten [Ag] molecule, um… they [Ab's] start their action … the granules… they move in and then they [granules from Ab] start destroying the hapten [Ag] molecule…

Based on the SGD (Figure 6a) and interview extracts obtained from the student above, three reasoning processes are of relevance to this study. Firstly, the student is clearly demonstrating inappropriate analogical reasoning by using the ingrained lock-and-key analogy from his/her conceptual knowledge (from Phase 1) to facilitate reasoning with ER A during Phase 2 of 3P-SIT. As displayed by Figure 6a, the student has applied the lock-and-key analogy by inserting the hapten (antigen) molecule into the centre of the trimer. Unfortunately, the student is not utilising the analogy in the appropriate manner and is thus displaying erroneous analogical reasoning (e.g.

Sumfleth & Telgenbüscher, 2001) when interpreting ER A. In support of this finding, Orgill and Bodner (2004) have reported that biochemistry students often lack clear ideas as to the purpose of analogies and how to use them as learning or reasoning tools. Secondly, the student is inappropriately transferring concepts (e.g. Salomon & Perkins, 1989) reserved for cellular immune functions to the domain of primary interaction. In this regard, it is a cellular immune response that is responsible for 'killing' and 'digesting' the antigen (e.g. Simonneaux, 2000) and not the primary response, as suggested by the student above. Thirdly, the student is selecting at least two misconceptions from his/her prior knowledge (see Phase 1 quote above) to interpret the ER. Specifically, we suggest that selection of the misconception that the antibody is the agent that destroys the antigen, as well as the misconception that the antigen binds into the V-cleft of the antibody (both from Phase 1), had a very pronounced effect on the way the student reasoned with their conceptual knowledge to make sense of ER A during Phase 2.

In contrast to the above data, consider the following interview extract and accompanying SGD above (Figure 6b) from a student who showed sound reasoning with his/her conceptual knowledge when interpreting ER A during Phase 2 of the interview:

> S: …the divalent hapten is going to attract an antibody from each side [indicates with trimer on ER A]…It actually agglutinates and forms a clump […] I've drawn the hapten as a sphere [Figure 6b], so I've actually drawn the fragment antigen binding-site as… a little curve to fit the sphere [points to top rt b. sites on Figure 6b]…
> I: Ok, so where would a lock-and-key interaction happen here [Figure 6b]?
> S: Um, well on both sides of the hapten [Ag]. Because, if you see here [indicates on Figure 6b], it would happen on this side and on this side [indicates with bot. hapten/Ag on Figure 6b]. So, there'd be like two lock-and-key interactions on both sides.

Clearly, the above student was able to select and engage sound scientific conceptual knowledge, as well as successfully apply his/her knowledge of the lock-and-key analogy to interpret ER A during Phase 2. In so doing, the student correctly suggested that linking between antibodies and the divalent antigen allows agglutination to occur rather than, as in the case of the previous student, the antibody itself being responsible for elimination of the antigen.

A further intriguing situation was one where some students were found to fuse two distinctly different concepts together when attempting to interpret ER A. For example, one student struggled to explain the difference between the lock-and-key analogy as an *analogy* and the actual binding *mechanism* between antigen and antibody. The SGD provided in Figure 6c and corresponding verbal commentary are testament to this interpretation.

I: Can you represent antigen on your diagram [S previously generated Figure 6c]?
S: …Well, normally, I talk of my lock-and-key thing, which would be here [traces 'V' shape within V-cleft of Ab on Figure 6c]… but, it would have to interact with this whole thing here [points to top lt actual binding site of Ab in Figure 6c], see what I'm saying? How can I represent this…well, this is my normal theory that I go back to [inserts V-shaped Ag into V-cleft of Ab on Figure 6c]… that is your antigen [inserts 'Ag' label]. So, that is my normal thing of lock-and-key… that it [Ag] has to fit. But, it [Ag] has to interact with this site… [inserts two dots on top rt binding region of Ab on Figure 6c]. So, I'm supposing it's [Ag] sequence specific… so, those amino acids [on Ab] and those amino acids [on Ag] are going to interact. So, if you have an antigen there [inserts Ag at top rt of Ab on Figure 6c]… then you going to have an epitope on the antigen which interacts specifically here [inserts bi-directional arrow].

The extract and SGD (Figure 6c) above provided evidence that this student held two distinct mental models of the same phenomenon simultaneously (e.g. Ainsworth et al., 1998); one correct one of antibody-antigen binding at the variable region of the antibody and an erroneous one that the Ag binds into the V-cleft of the antibody. We suggest that the student was *superimposing* both ideas and expressing them as one model, i.e. combining the lock-and-key analogy with the need for *specificity* between antibody and antigen. Interestingly, during interviews, the same student had stated that s/he needed two 'theories' to explain antibody-antigen binding. As part of his/her first 'theory' or model, s/he related antibody-antigen binding to a lock-and-key situation, and as part of his/her second 'theory', s/he related antibody-antigen binding to sequence specificity between amino acids. It is probable that either, the student's construction of two models to explain antibody-antigen binding was a way to alleviate the obvious conflict that had arisen when this student engaged his/her conceptual understanding during reasoning or, s/he already possessed both these ingrained models as part of his/her conceptual knowledge. One of the student's models could well have been conceptualised from interpreting oversimplified diagrams of IgG-antigen binding (e.g. on the surface of B-cells), while his/her other model may well have stemmed from a scientific and mechanistic definition of antibody-antigen binding in that it is the specific sequences of amino acids between antibody and antigen that render molecules 'specific' to each other. Thus, as Grayson (2004) has found in the context of electrical circuit ERs, it is possible that this student could not *disentangle* the two models from one another.

As further evidence of inappropriate conceptual reasoning, some students misinterpreted the 'increase' in absorbance or positive gradient for the week 8 and/or 12 curves in ER C. For comparative purposes, consider the first student quote below showing a sound interpretation and the second student quote representing an erroneous understanding of the increase in absorbance shown in the week 8 and/or 12 curves:

S: …the [absorbance] increase in that region here [traces wk 12 curve starting from y-ax. until halfway between P and Q with finger] could be due to the steric hindrance. You have so many antibodies that they compete for binding and eventually they shove each other off. And because there are so many [Ab's] they

> can't bind strongly… so they get washed out in your wash step. It looks like you have a lower concentration of them [points on the wk 12 curve near P], but, as you dilute it, you have less of the steric hindrance and once you get proper binding… then you can detect it [antibody] with your secondary antibody.

> I: Compare area 'P' and 'Q' [ER C] in terms of that blue line [points to wk 12 curve].
> S: In area 'P'… in week 12 [points to P on ER C], the antibody concentration is increasing, it is on the rise, that means antibodies are being made in the system. And at 'Q' it is showing that the immune response is declining that means that less antibodies are being made… At 'P'… this is like a growth phase or log phase of the graph [traces graph wk12 from y-ax. to Q with finger]… it is just showing the steady growth or increase in antibody count in the immune system… it is after the booster injection has been put in, that the immune response increases… after the booster injection is put in they're [Ab's] reacting to this booster injection [points to wk 8 & 12], therefore they are increasing [traces wk 8 & 12 grad. prior to Q]… there is an immune response… that means more antibodies are being made.

Compared to the first student's sound reasoning with their conceptual knowledge, four students (e.g. second quote) attributed the positive gradient in each of the curves to an increase in antibody concentration *per time*, rather than to factors such as steric hindrance between antibody molecules and competition for binding sites. Thus, these students thought that the increase in absorbance of the week 8 and 12 curves was due to an immune response that had produced an increased number of antibodies. Even though the immune response, following booster shots, *is* represented on ER C, the *three* immune responses are represented by the three curves, not *within* each curve. Therefore, those students who showed the difficulty were probably interpreting the graph as if 'time', rather than '-log Ab', was plotted on the x-axis.

The four students, who manifested the above difficulty, were probably erroneously transferring and integrating their conceptual knowledge,, obtained from other graphs, into the interpretation of the ELISA graph (ER C). Conceptual knowledge gained from those graphs that plot antibody concentration versus time, probably influenced students' reasoning leading to them erroneously selecting this conceptual knowledge to make sense of the ER. With concentration versus time graphs, a positive gradient would indeed be represented *within* a single curve. In support of this erroneous reasoning pattern, Scanlon (1998) has shown in a study of students' interpretation of graphs of motion, that sometimes over-generalised rules are used in that distance-time graphs are treated as velocity-time graphs.

In support of the above findings, other ER research has also shown that the successful interpretation of an ER depends to a large extent on the knowledge that an individual brings to the ER (e.g. Roth, 2002; Lowe, 1996). These authors have shown that interpretation of an ER is indeed 'modulated' by this knowledge (e.g. Cheng et al., 2001) and this modulation process plays a crucial role in determining whether an ER will be successfully interpreted or not. In this respect, we suggest that

the data presented above demonstrates the reasoning processes involved when the R and C factors of the expressed model interact with one-another (Figure 3). Thus, we suggest that the data serves to not only depict the cognitive processing that represents such an interaction, but also validates the R-C factor as a crucial component of the expressed model.

Validation of the Representation Mode Factor (M)

During Phase 3 of the 3P-SIT interview process, we aimed to generate data with which we could identify those external characteristics of an ER that may cause student difficulties. The collection of such data centred on the effective or ineffective use and clarity of ER features, namely, the spatial arrangement of the ER elements, ER conventions, visual icons, artistic devices, colour, topography, level of abstraction, symbols, labels and captions. In other words, the objective was to measure what external features of the ER may be giving students problems, or initiating particular reasoning patterns.

Information pertaining to the external nature of the ER can be obtained from experts including scientists, researchers and graphic artists as well as from students' evaluation of the ER during Phase 3. Information corresponding to the graphical features of an ER can also be obtained from an informal visual analysis of the ERs (e.g. Schönborn, 2005), in which ERs can be screened to identify those ER markings that could potentially induce erroneous interpretations. For example, upon an informal visual analysis of ER A, the authors suspected that the visual clarity of the realistic depiction of structural features representing antibody structure and binding to antigen (hapten) might be a possible source of confusion for students. The following quotes by an expert immunologist (T. H. T. Coetzer, personal communication, January 13, 2005) and a student, respectively constitute further evidence for this potential problem with ER A:

> 'Students would possibly have some difficulty in interpreting the electron micrograph without an explanation for the way this negative stain was obtained and that the spiky bits sticking out are the Fc [fragment crystallisable] fragments…'

> S: What I cannot see is the hapten [Ag]. From the information [points to caption of ER A] I can have the assumption that the haptens should be on the N-terminals of these antibodies… I also can't see if these antibodies have two chains… but I know that, in reality, they have two light chains and heavy chains…

The above extracts demonstrate how certain *graphical features* representing the nature of the visual clarity of the trimer and pentamer antibody-antigen complexes may influence ER reasoning. In this case, due to the clarity of the visual information on the micrograph (ER A), it is impossible to see

the hapten (antigen) molecules and, from a purely visual perspective, the antibodies do look like they are 'joined' without hapten. Since haptens are small molecules with low molecular weights, the magnification used to generate the micrograph was not enough to expose their presence as distinct visual features. The student above realised that the haptens could not be directly viewed on the micrograph which reinforced the fact that the lack of clarity of this ER feature, due to its realistic nature, might affect students' interpretation of ER A.

During an informal visual analysis of ER B, the authors suspected that the use of the red colouring on the ER might create a problem for students as the same red colouring is used to show both the glutamine residue involved in the antibody-antigen binding (on frame 'b') *and* the contact residues between antibody and antigen (on frame 'c'). In confirmation of this concern, this red colouring feature of the 'spheres' on ER B led one student to make the following comments during Phase 3 of 3P-SIT:

> I: Is there anything that you find particularly confusing on the diagram [ER B]?
> S: The glutamine… and how it sort of multiplies. There is no sort of step on how to… how they got to so many, or why there are so many [glutamine residues]. Why is it [Ab and Ag] attached first, and then just pulled apart... You know normally, like if you get a negative and a negative, that is how come it will like pull apart, but then it wouldn't make sense if it was attached in the first place. I don't understand how they get from there [points to frame a] to part [frame] 'b' and why there are so many glutamine molecules there [points to red numbered spheres on Fab in frame c and then to red numbered spheres on Ag in frame c]… I'm just looking at this diagram [ER B] and I don't understand the steps and how to get to the next one [step].

It is evident from the above extract that the student thought that 'multiplication' of the single glutamine residue had occurred. It is very possible that this reasoning could have been as a result of the same (red) colouring technique used to show two very different ideas, one idea being the location of the glutamine, and the other being the idea of contact areas between antibody and antigen. In addition, by labelling the frames in ER B as 'a', 'b' and 'c', students may have attached some idea of sequence to the ER and interpreted the ERs as a set of three consecutive events rather than different representations of the same phenomenon.

Lastly, during the authors' visual analysis the ELISA curves (ER C), we suspected that the '-log' expression might induce erroneous student interpretations of the ER. This possible problem was supported by the following two quotes by an expert (T. H. T. Coetzer, personal communication, January 13, 2005) and a student, respectively.

> 'If students are not very familiar with this format of expressing ELISA results, they may be confused by the appearance of the - log (antibody concentration) plot, i.e. that the 'big numbers' represent low antibody concentration. Expressing antibody concentration in μg/ml gets around this potential problem…'

> S: …according to the graph… at a high concentration [of Ab] we have less absorbance, which is really confusing me because, the concentration increases with the absorbance. But, I think the thing that makes the graph look like this is this 'log'… It is a bit confusing, really, because now, the absorbance decreases but the concentration still increases [points to x-ax.]…

It is evident that both the expert's and student's evaluation reinforces the authors' notion that the '-log' graphical feature of ER C may pose potential processing difficulties for students. In real terms, since negative values were obtained when the logarithm of antibody concentrations (mg/ml) were calculated, the experimenter (J. G. Jackson, personal communication, June 16, 2000) who constructed ER C correctly assigned a negative value to the calculated values to place the curves in the positive Cartesian quadrant. Overall, the above data, generated in Phase 3 of 3P-SIT, provides evidence that the graphical symbolism used to portray information can greatly influence the manner in which students interpret ERs. Therefore, we believe that such data corresponds to factor M of the expressed model (Figure 3). In turn, this serves to validate M as a factor that affects students' ability to interpret ERs in biochemistry.

Validation of the Conceptual-Mode (C-M) Factor

To evaluate the propositional (scientific) knowledge conveyed by an ER, we suggest that it is necessary to use experts' interpretations. In this respect, such expert propositional knowledge can be obtained from textbook authors' descriptions of the ER, from surrounding text that describes the ER, from the figure captions used by writers to describe an ER as well as from scientific findings that are presented in journals, books and documents that pertain to the ER. In other words, the evaluation of the propositional knowledge is obtained through writings of and discussions with experts, which can include any experienced scientist. Therefore, in the current study, data corresponding to the C-M factor were obtained from primary literary sources where the ERs were located and described (see Figure 1 caption and corresponding footnote), namely two scientific papers and the prescribed textbook for the immunology module for ER A and B and, discussions with a colleague (J. G. Jackson, personal communication, June 16, 2000) for ER C. We suggest that the conceptual (propositional) knowledge represented by C-M (Figure 3) is an indispensable factor that affects a student's interpretation of an ER. This is because the complexity, soundness and extent of scientific knowledge that the ER represents will have a profound effect on how well the ER is interpreted.

Validation of the Conceptual-Reasoning-Mode Factor (C-R-M)

We propose that the empirical evidence presented in the previous sections of this paper have separately validated the three factors C, R and M and the three interactive factors R-M, R-C and C-M that each influence a student's ability to interpret an ER. What remained to do was to confirm the validity of the expressed model as an integrated whole as implied by the overlapping nature of the factors (C-R-M) in the Venn representation (Figure 3). Therefore, to test the validity of the model as a whole unit (i.e. validity of the C-R-M factor), data was needed to demonstrate that, at some time or other, students are required to engage *all* factors of the model in order to successfully interpret and/or learn from an ER. That is, the *indispensable* nature of each component of the expressed model (Figure 3) needed to be confirmed. Based on the findings reported above, our hypothesis was that interpretation of an ER requires the learner to use reasoning skills (R) to reason with both their conceptual knowledge (C and R-C) of relevance to the ER and with the symbolism of the ER itself (R-M and M) to make sense of the propositional knowledge represented by the ER (C-M).

Two types of data are presented to test the above hypothesis in order to suggest the validation of the C-R-M factor. Firstly, a selected example of an interview extract is used to show engagement of *all* factors of the model during a highly successful process of ER interpretation. Secondly, data obtained from two students during the interpretation of an ER is provided in an attempt to show that the relative *degree of influence* of one or more of the factors greatly affects a student's ability to correctly interpret an ER.

Validation of the C-R-M factor through engagement of all factors of the model

According to the expressed model, the C, M and C-M factors (Figure 3) are implicit to the process of ER interpretation. In other words, there has to be an ER (factor M) available for an individual to interpret, all individuals bring a degree of conceptual knowledge (factor C) to the ER and, all scientific ERs represent some type of propositional knowledge (factor C-M). However, since reasoning processes can only be observed if there is *something to reason with*, in this case with the ER (R-M) and with students' own conceptual knowledge (R-C), each can be considered a subset of the overall reasoning factor (R). Therefore, when analysing a student quote, it is only possible to explicitly observe factors R-M and R-C *in action* during the interpretation process. Hence, by coding a student response as R-M the authors are validating the engagement of *both* the R and M

factors. Similarly, by coding a response R-C, the authors are validating the engagement of both the R and C factors. The validation of factors R-M and R-C and therefore, validation of the C-R-M factor, shall be demonstrated by using the '`Courier`' font to code engagement of the `R-M` factor and the '*Arial italic*' font to code engagement of the *R-C* factor of the model during ER interpretation.

The criteria for coding verbal segments of student interview extracts either as corresponding to the `R-M` or *R-C* factors was based on an analysis of the nature of the *language discourse* contained in a student quote. For example, when expressing data corresponding to the `R-M` factor, the student used specific verbs such as 'seeing' and 'looking'; adjectives such as 'distinct', 'blob-like', 'close' and 'twisted'; and nouns such as 'triangle', 'Y-shape', 'part' and 'area' to reason (R) about the graphical features on the ER (M). In contrast, when expressing data corresponding to the *R-C* factor, the student linked specific words or reasoning phrases (R) such as 'since', 'therefore', 'because of', 'that means', 'even though', 'I get it now' and 'that is why' to reason with specific concepts (C) like 'amino acid sequence', 'covalent bonds', 'lock-and-key' and 'antigen-antibody complex'. To illustrate this approach, consider the coding of the following extract obtained from a student's interpretation of ER C:

> I: What happens to antibody concentration as we move… from left to right [indicates on x-ax.]…
> S: `It is decreasing…the negative 'log' increases…` *that means that the concentration is decreasing* `and you can see with your absorbance [indicates y-ax.]… It [Ab conc.] is greater over here [points to lt of x-ax.] than down there [indicates toward rt of x-ax.].`
> […]
> I: Could you compare [points] 'P' and 'Q' [ER C].
> S: `'P' seems to have a lower absorption than 'Q',` *even though the concentrations of the antibody at 'P' is greater than that at 'Q'… and that is just basically because there is too much antibody present to bind to all the antigen, in the well… there is a number of things like steric hindrance… that prevented those antibodies from binding as well… that is why it looks like there is less [Ab conc.].*

In order to successfully interpret the scientific knowledge (C-M) depicted in ER C, the student in the above quotation engages sound conceptual knowledge (R-C and C) to reason with the graphical features (R-M and M) of the ER. In this case, the integration of all factors of the model allows the student to correctly suggest that, 'P seems to have a lower absorption than Q (engagement of `R-M`), even though the concentrations of the antibody at P is greater than that at Q… and that is just basically because there is too much antibody present to bind to all the antigen… (engagement of *R-C*)'.

In summary, based on the above data obtained for ER C (Figure 1) we suggest that, at some time or other, a student is required to engage and integrate all factors of the model in order to successfully interpret an ER. By coding the engagement of factors R-M and *R-C* within student quotes, the data demonstrates the indispensable nature of each factor of the model for sound interpretation of an ER and as a result, serves as the first validation of the C-R-M factor (Figure 3).

Validation of the C-R-M factor through the relative degree and nature of influence of one or more of the factors of the model

Having argued that all factors of the model are indispensable to successful interpretation of an ER, we further hypothesised that the degree and nature of influence of each factor would also play a major role in determining a student's ability to soundly interpret an ER (C-R-M). To investigate this hypothesis, we considered the relative contributions of all the factors of the model and to what extent such contributions could change.

Factor M makes a constant contribution to interpretation because the ER and its graphical features do not change during interpretation. This is of course only true for static ERs and not for animations, which is one reason why the latter can be more complex and cognitively demanding for students (e.g. Lowe, 2003). Factor C-M also does not change during ER interpretation, but might change during the course of time as part of the progress of science wherein there is a graphical adjustment or modification of the propositional knowledge represented by a particular ER. Factor C might change in a limited way depending on whether student knowledge is unaffected by interpretation of the ER or whether learning takes place or alternative conceptions develop. Thus, in the case of factors M, C-M and C, their contributions for all intents and purposes remain constant during the process of interpretation. On the other hand, the relative contribution of factors R-M and R-C during ER interpretation can fluctuate dramatically depending on whether the student is consulting with the ER (R-M) or their conceptual knowledge (R-C). The relative influence of such factors on the soundness of ER interpretation was investigated using the same coding method for R-M and *R-C* reported above. Two examples are presented below.

The first example, coded **Q1**, shows how a student's poor ability to reason with the ER (R-M), despite excellent prior conceptual knowledge (C), may still lead to the unsuccessful interpretation of an ER. The following data shows that the student's prior conceptual understanding (C) about general antibody structure and primary interaction with antigen binding, before exposure to any ER

(Phase 1), was rich and extensive. Additionally, the student's reasoning with these concepts (R-C) was consistently excellent.

> **Q1** S: *…both antigen and antibody are proteins… antibody structure varies according to the type of antibody… they vary in sub-classes and classes with the respective chains that make them up… the interaction with the antigen… is through the variable regions on the heavy and light chains of the antibody …The antibody has to be specific to the epitope found on the antigen…so, it actually has to be compatible …the interaction is actually on the antibody with the variable regions, rather than the constant regions, because those constant regions are found on most antibodies… that is why they're called 'constant'… whereas the variable regions change… are variable, because they're specific to an antigen's epitope.*

However, after being exposed to ER B, it was found that the same student reasoned with the ER (R-M) erroneously by thinking that a complete Y-shaped antibody instead of a single Fab arm was being represented. This reasoning was demonstrated by the following quote from Phase 2:

> **Q1** I: In terms of structure, what is being shown on this representation [ER B]?
> S: …you can see the antibody structure… one can see that is consists of the two chains [H and L]… it is actually two heavy chains [points to bot. two 'groups' of blue spheres on frame a simultaneously] and two light chains [points to top two 'groups' of yellow spheres on frame a simultaneously].

We suggest that the nature of the ER (factor M), i.e. the spatial arrangement of the graphical markings, influenced the student to incorrectly reason (R-M) that ER B represented an entire antibody, despite the fact that the student's prior conceptual understanding (C) was shown to be outstanding. Therefore, factors M and R-M had a large degree of influence on the student's ability to successfully interpret the ER (C-R-M) (Figure 3).

The second example, coded **Q2**, shows how a student's poor prior conceptual knowledge (C) may induce an unsuccessful ER interpretation. For instance, consider the following quote, obtained from the student during Phase 1 before exposure to any ER:

> **Q2** I: What is it about antibody structure that allows it to form a lock and key with the antigen [S stated this earlier]?
> S: *Well, it is the light chains of the antibody, which have got the 'V' part. Ok, you get the heavy chain which is the 'stalk' and then you get the 'V' on top of the 'stalk'…the light chains are the 'V' part… that region ('V') is the area that they [Ag] bind to…specifically to the variable site… in order for specificity to come into it… yeah, that region there [hand gestures]… the whole 'V' part…that is the main area that they [Ag] bind to.*

Even though the student expressed the lock-and-key analogy strongly, it is evident that the student showed a misconception (C) by stating that the entire 'V' part of the antibody is representative of the antigen binding site, instead of two separate binding domains. Upon exposure to ER A during Phase 2 of 3P-SIT, the same student carried this pronounced misconception into his/her processing

of ER A by misinterpreting the trimer arrangement in ER A as representing a single antigen (hapten) inside the trimer, even though this was not succinctly conveyed by the ER (M). The following SGD (Figure 7) and accompanying verbal output generated by the student demonstrates this misinterpretation:

Insert Figure 7 about here

Q2 S: …I can see the triangle there [points on ER A] and the Y-shaped antibodies, you can actually see them…forming a trimer… in the middle of the trimer it is dark *…that is where the hapten [antigen] is, where the antibody is binding onto it…*
[…]
S: …[gen. Figure 7]…this [Ab] binds with a complementary fit to that ['V' edge of hapten]. All these [three 'V'edges of hapten] have to somehow fit into these antibody-binding sites, the 'V' shape, in order to be… *like a lock and key mechanism… so the shape has to be similar.*

Thus, in the above case, the student's reasoning processes corresponding to factor R-C and his/her conceptual knowledge (C) were most limiting and therefore, these factors had a major influence on the student's ability to interpret the ER (C-R-M) (Figure 3).

CONCLUSIONS AND IMPLICATIONS

The results of this study have, in our view, successfully addressed the three stated research questions, presented in the introduction to this paper. In response to the first research question, the Justi and Gilbert (2002) modelling process was used to successfully develop and express a model of seven factors determining students' ability to interpret ERs in biochemistry. In response to the second research question, we successfully used a specially designed 3P-SIT interviewing method to yield empirical data that constitutes sound validation of both the expressed model (Figure 3) and its constituent seven factors. In so doing, each component factor of the model was verified as making an indispensable contribution to a student's ability to interpret an ER in biochemistry. As a result of these findings, specific operational definitions representing the nature of each factor of the expressed model were formulated.

We suggest that addressing the first two research questions has, in part, contributed to recent calls in the literature (e.g. Ploetzner & Lowe, 2004; Reimann, 2003; Brna et al., 2001) for *more* research that can help researchers and teachers understand *how* students learn from, and *use* ERs during learning. Such calls have also suggested (e.g. Chandler, 2004; Hegarty, 2004; Seufert, 2003) that it

is essential that such ER-related research considers the *cognitive* operations and components associated to learning with ERs. In this regard, we believe that our findings in the form of the expressed model may have contributed to this deficiency in a biochemistry education context, where little such research has been conducted.

In response to the third research question posed in this study, we propose the following six practical applications of the expressed model for biochemistry education practitioners as well as for educators from other science disciplines:

- The model can be used to establish whether a student's overall *interpretation* of an ER is successful or not. This can be done by comparing the student's 'post' knowledge (C) after exposure to an ER with the conceptual knowledge represented by the ER (C-M).
- The model can be used to establish whether any *learning* has occurred as a result of a student's engagement with an ER. Here, the student's 'post' knowledge (C) obtained after interpretation of an ER is compared with data corresponding to their prior knowledge (C) obtained before exposure to any ER.
- The model can be used to determine which of the six factors, *positively or negatively*, influence a student's interpretation of a particular ER the most and, which the least.
- The expressed model can serve as a general *diagnostic* framework for guiding practitioners' and researchers' discussion and data analysis relating to the nature of a student's difficulty with an ER. That is, whether the student has a conceptual (C) or reasoning (R-M or R-C) difficulty or, whether the difficulty lies with the nature of the graphical features of the ER (M and C-M). The model hereby enables the prediction of the *potential source(s)* of difficulties with ER interpretation.
- Based on the nature of the data corresponding to each factor, the model can serve as a template for the development of *approaches* to teaching and learning that include intervention strategies for improving student's interpretation of, and learning from ERs.
- Based on the nature of the model, and the operational definitions of its constituent factors, the model has a generic application to *all types* of ERs in science including not only static representations but also dynamic, animated and multimedia representations.

It is clear from the above potential applications of the model that many of its uses require specialised knowledge and research expertise before biochemistry and other science teachers and learners will be able to benefit directly from them. In this regard, we are currently deriving user-

friendly guidelines and remediation approaches that science educators and designers of visual material could use to promote students' visual literacy (e.g. Schönborn & Anderson, 2006) and to prevent or correct students' difficulties with ERs used in biochemistry. In addition, we recommend the application of the expressed model to other scientific contexts. In this regard, we believe that an important contribution of the model is that it provides and expresses a *holistic* account of the nature and interaction of factors that affect students' ability to interpret ERs in science. For instance, to successfully interpret, or learn from *any* ER (M) in any area of science, a student is required to posses the necessary scientific conceptual knowledge of relevance to that ER (C) (e.g. Roth, 2002; Cheng et al., 2001) and the reasoning skills (R) (e.g. Kindfield 1993/1994) necessary to reason not only with their conceptual knowledge (R-C) (e.g. Orgill & Bodner, 2004) but also with the ER (R-M) (e.g. Ametller & Pintó (2002). In turn, we suggest that, through this fundamental principle lies the most potential contribution of the model to the field for analysing and improving student learning with ERs in science.

In conclusion, the results of this study suggest that a student's overall ability to interpret, visualise and learn from a scientific ER depends on *both* the engagement of all the factors represented by the model (C-R-M) and the nature of the contribution of each factor in terms of whether, for example, scientifically sound or unsound conceptual knowledge and reasoning is employed, whether the ER represents sound or unsound propositional knowledge and/or, whether the ER is graphically misleading or appropriate. Thus, each of the seven factors represents key components that affect students' ability to interpret scientific ERs. We suggest that the findings reported in this paper support the use of Venn logic for conceptualising the integrated nature of the model and in turn, provide valid evidence for the corresponding operational definitions of the factors. Finally, as echoed by Justi and Gilbert (2000), although the empirical testing of the expressed model has proved meaningful in this particular context, further testing in other contexts by members of the science education community would serve to develop the presented 'expressed' model into a 'consensus' model.

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Figure 1. Three multiple ERs of antibody-antigen interaction, **A**) Electron micrograph (x 1 000 000) of complexes formed on mixing divalent hapten with anti-hapten antibodies. The hapten links together the Y-shaped antibody molecules to form trimers (A), and pentamers (B), **B**) Space-filling model showing Fab antilysozyme and lysozyme molecules fitting snugly together. In the coloured version presented to students during the study, the antibody Fab light chain (group of lightly shaded spheres on the first frame) was coloured yellow, the antibody Fab heavy chain (group of heavily shaded spheres below those that are lightly shaded) was coloured blue, and the lysozyme (group of heavily shaded spheres on the right of the first frame) was coloured green. Fab and lysozyme molecules are shown pulled apart in the second frame. Glutamine 121 (represented by the three partially shaded spheres attached on the left of lysozyme) was coloured red. In the third frame, the numbered spheres were coloured red and the Glutamine 121 (numbered '14') was coloured pale purple, **C**) Antibody response curves obtained from an ELISA showing the relationship between absorbance (405nm) and antibody concentration (mg/ml). Three booster shots were administered and the antibodies collected at the weeks indicated in the text box. In the coloured version presented to students during the study, the 'Pre-Immune' curve was coloured green, the 'Week 3' curve yellow, the 'Week 8' curve red, and the 'Week 12' curve blue

Figure 2. The modelling framework used to guide the development process of the expressed model of factors affecting students' ability to interpret ERs in biochemistry (Adapted from Justi & Gilbert, 2002, p. 371)

Figure 3. Venn diagram representing a model of seven factors that determine students' ability to interpret ERs. The model expresses three factors and four interactive factors affecting students' ability to interpret an ER

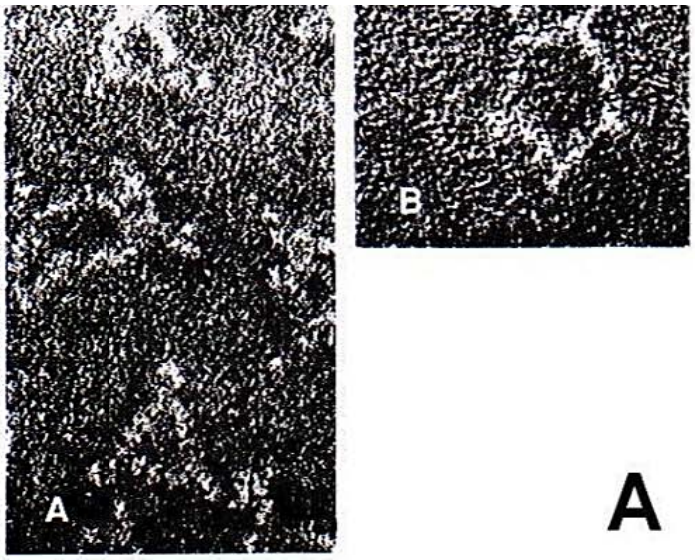
Figure 4. Student-generated diagrams (SGDs) portraying, a) a sound conceptual knowledge of the binding interaction between antibody and antigen and, b) an accurate depiction of both antigen-binding sites on the antibody (black circles), but an erroneous interpretation of antigen as binding into the V-cleft of the antibody

Figure 5. SGDs portraying, a) the misinterpretation of the Fab arm in ER B as an upright and complete Y-shaped antibody and, b) a student's interpretation of ER C when predicting an

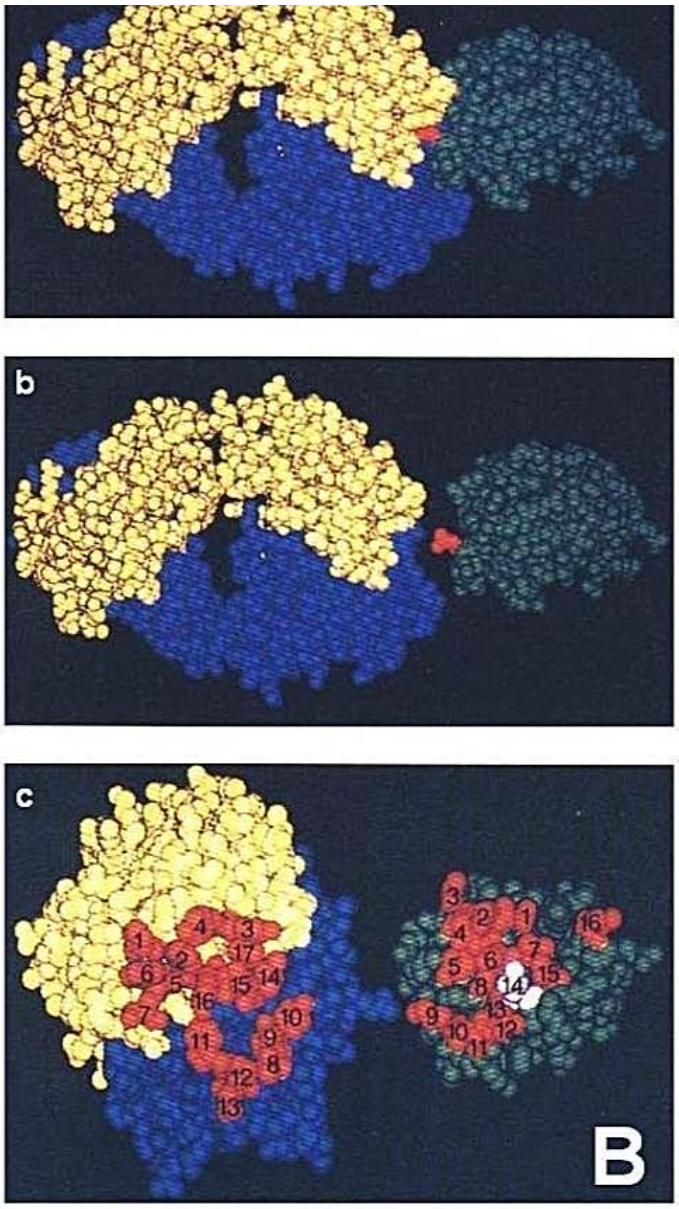
absorbance curve for collection of serum samples at week one hundred, after the booster schedule had ceased

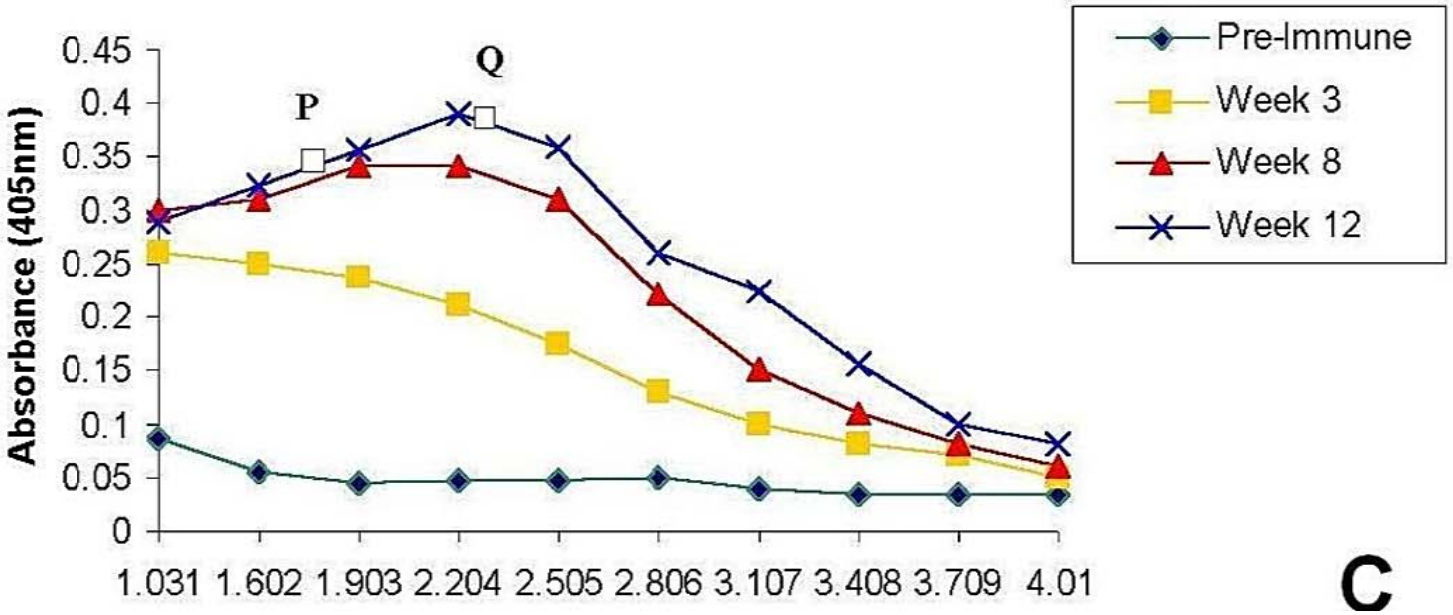
Figure 6. SGDs obtained from Phase 2 of 3P-SIT showing, a) a student's dependence on certain conceptual knowledge when interpreting ER A, b) sound reasoning with the lock-and-key analogy represented by the R-C factor of the model and, c) the integration of two distinctly different ideas into one model

Figure 7. SGD obtained from the interpretation of the trimer arrangement on ER A


A
B

A


b
c
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
B


Absorbance (405nm)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
P
Q
1.031
1.602
1.903
2.204
2.505
2.806
3.107
3.408
3.709
4.01
Pre-Immune
Week 3
Week 8
Week 12

C

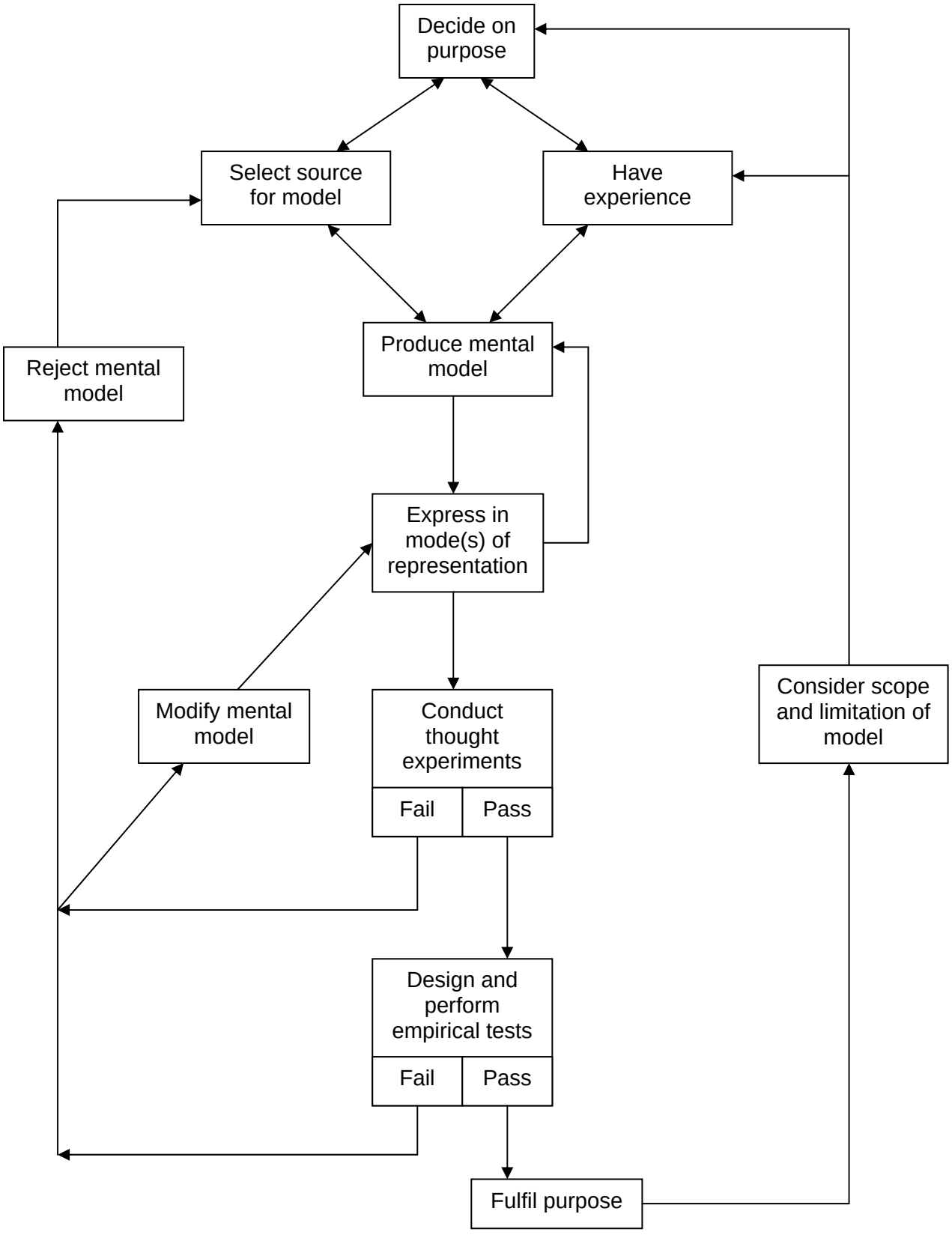
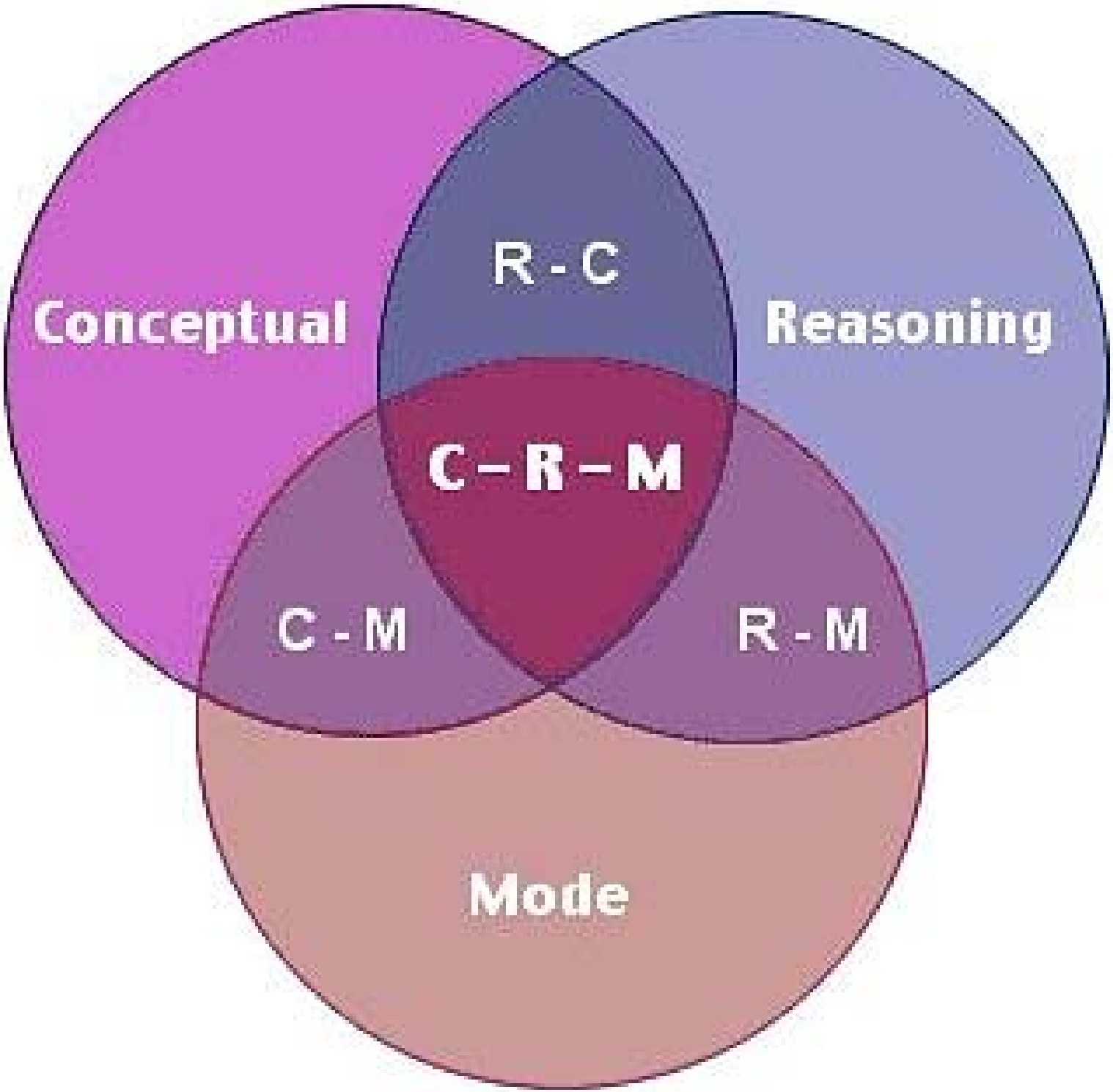
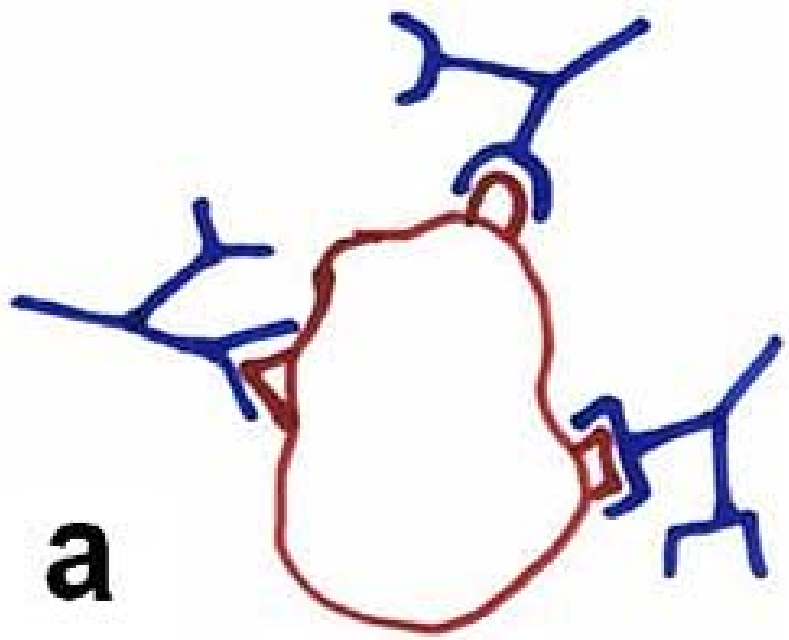
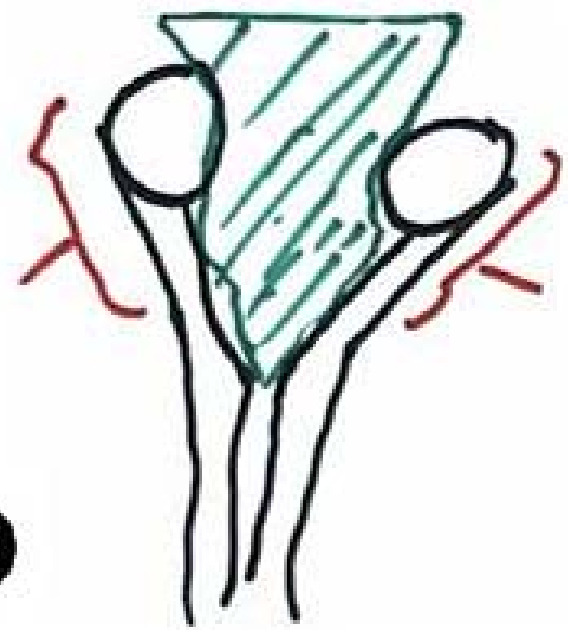
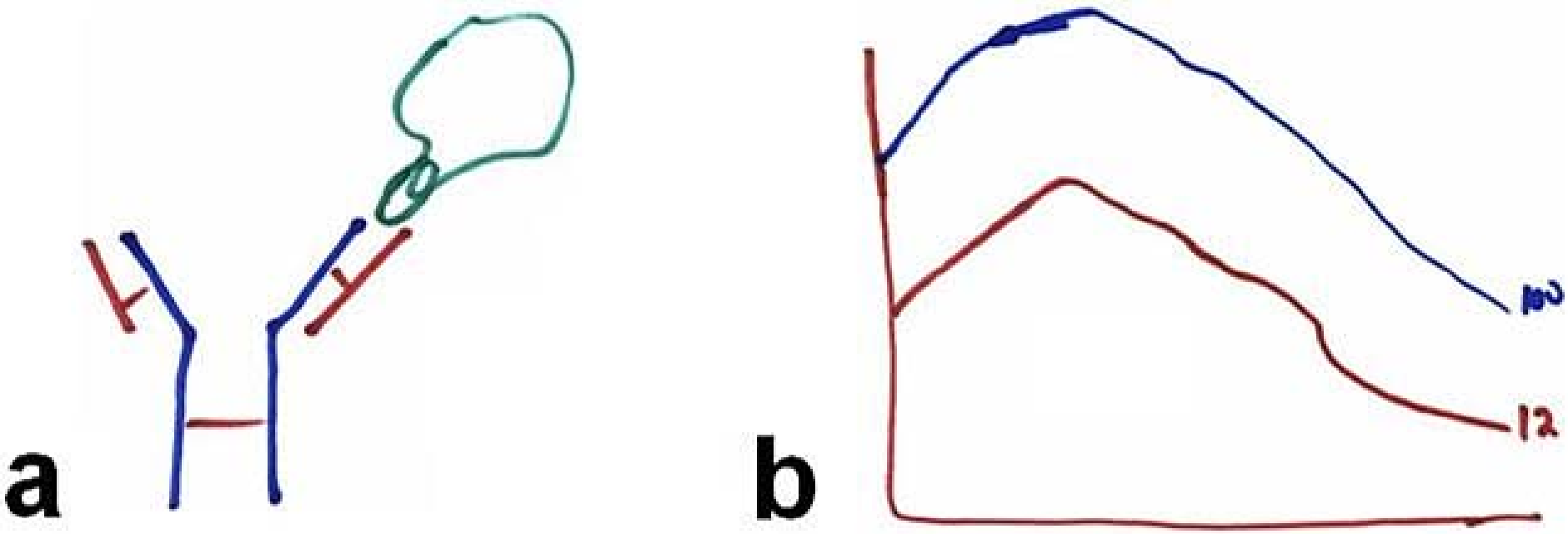


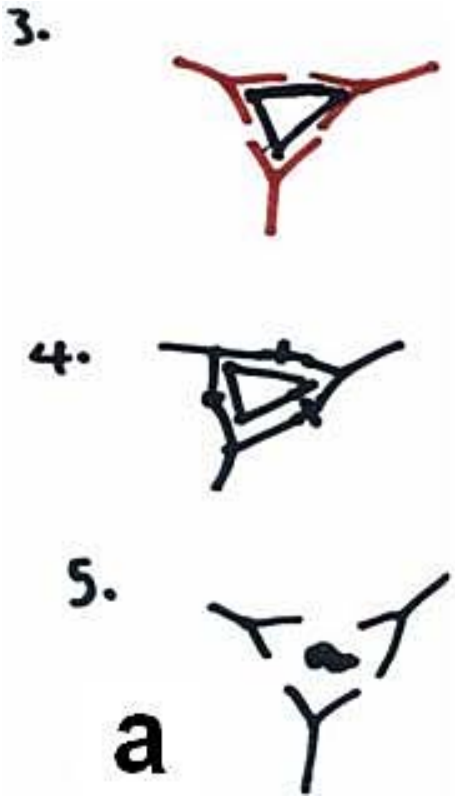
Figure 2. The modelling framework used to guide the development process of the expressed model of factors affecting students' ability to interpret ERs in biochemistry (Adapted from Justi & Gilbert, 2002, p. 371)

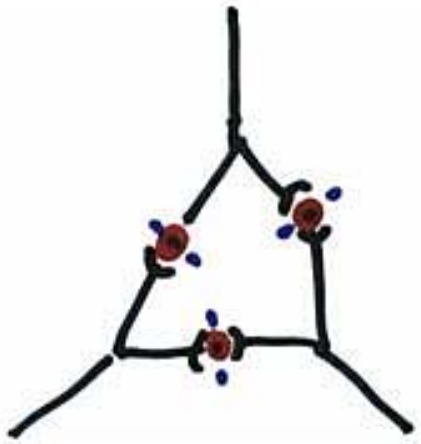

Conceptual
R - C
Reasoning
C – R – M
C - M
R - M
Mode


a


b


a
b
Ab
12


3.
4.
5.
a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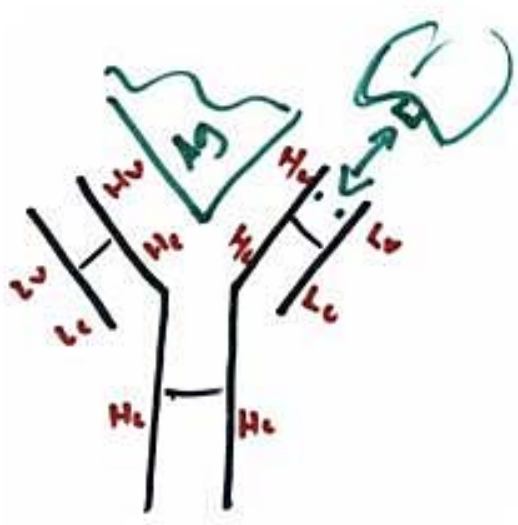
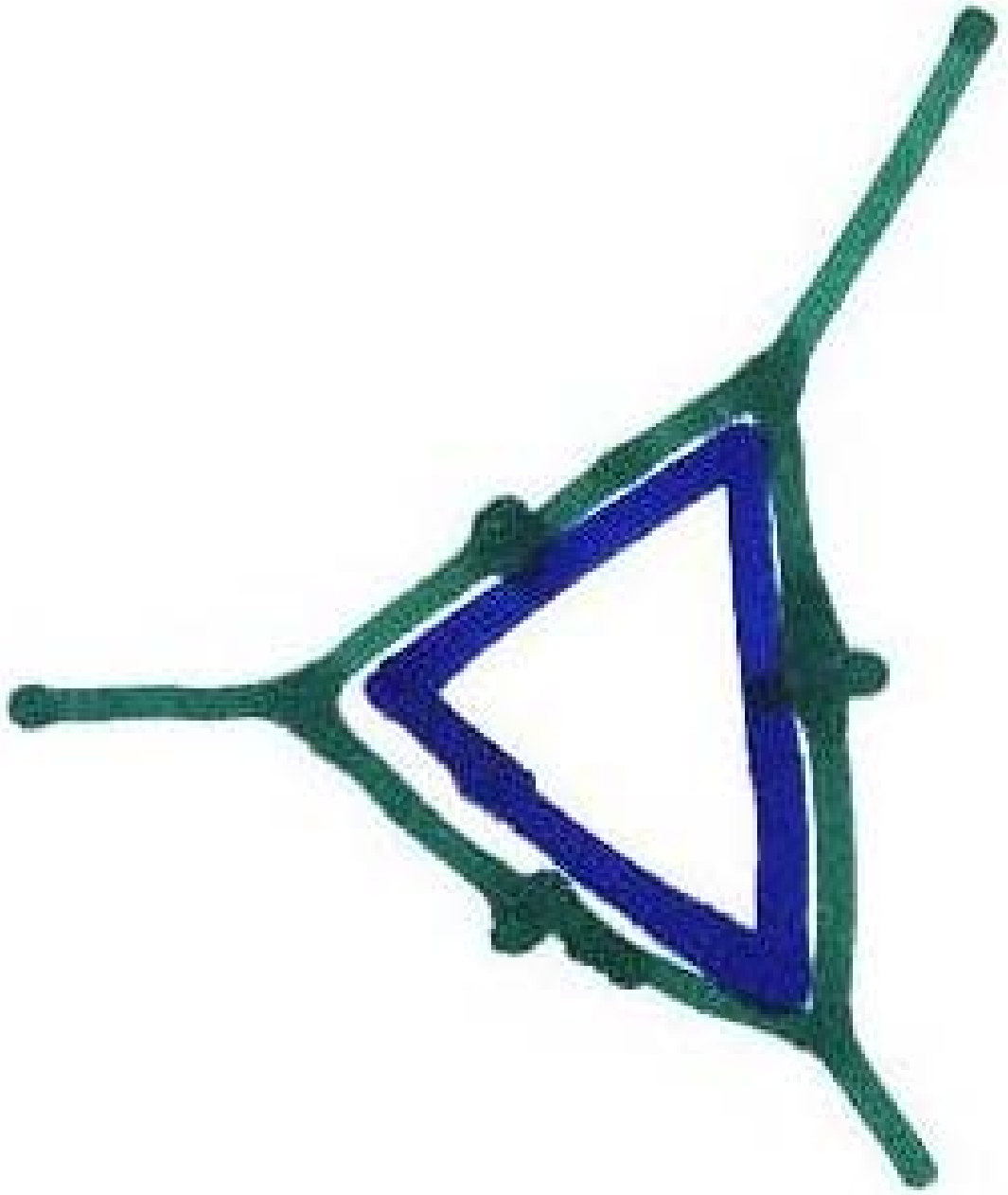

c



TRIMER