Agent-based Simulation of PI3K/Akt Pathway Activation in Breast Cancer

Debattama Sen

Montgomery Blair High School

Science, Math and Computer Science Magnet Program

Supervisor: Dr Rachel Karchin

Institute for Computational Medicine
Department of Biomedical Engineering
Johns Hopkins University

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Personal

"I would like to thank…Starbucks" - the acknowledgements page of my research presentation is guaranteed to draw a laugh from the most lethargic audience. I mentioned, of course, my research mentor at the Johns Hopkins University Institute of Computational Medicine and my adviser at the Montgomery Blair High School Math, Science, and Computer Science Magnet Program but also included a short but heartfelt praise to Starbucks. To me, that little concession encapsulates the realities of research.

While growing up, I was surrounded by biographies and posters of great men and women: Albert Einstein, Erwin Schrodinger, Marie Curie, Charles Darwin, James Watson…you name them, I have read about them. Unfortunately, I got quite a naïve impression from those accounts about the world of scientific research. Nevertheless, this past summer, it was time for lab goggles to finally replace my rose-colored glasses.

I refer to lab goggles only metaphorically, as much of research nowadays has moved from hands-on laboratory work to more digital environments. After a year of learning micropipetting techniques and doing gel-electrophoreses, my primary research tool ended up becoming my computer and my brain, of course. While that last part might seem flippant, I think a lot of us high schoolers forget that ultimately it is not about what you have studied, or how well known and well-equipped your lab is. Those things will help, but it will come down to how creative you are, how motivated you are, and how willing you are to throw away all your work, only to start over while consoling yourself with a nice warm Starbucks mocha (no Starbucks isn't paying me for this, I really did rely that much on the sugar and caffeine to make it through some days).

My first thought while writing this was to tell future high schoolers something along the lines of never give up. That is certainly true; one should keep going and not be intimidated by failures. Then I realized that it would be unfair to underestimate future researchers by saying that. They (and I) already know about perseverance, or else they would have never been motivated enough to engage in research at the high school level in the first place. Instead, I will limit my absurd pontificating to some things I didn't know before that one summer changed my life (oh, it was the best of times, it was the worst of times).

- Scientific research involves standing on the shoulders of giants and trying to reach the skies. For the first few weeks, all I did was read countless research papers, trying to understand the complexities of the problem, trying to interact with all the other ideas out there (if you didn't need reading glasses before you start work, prepare yourself: you just might end up with them). My mentor, Prof. Rachel Karchin, and one of the graduate students in my lab, Hannah Carter, were very helpful at this stage. They were excellent sounding boards for my ideas, and spent countless minutes explaining key ideas and guiding me when I was stuck.

- And I was often stuck. I had to absorb a lot of information in a very short period about something that was very abstract: modeling the behavior of proteins in a cell. I could not go into the cell and see proteins being activated or touch the phospholipids moving around in the cell membrane. So I was constantly doubting myself, wondering whether I had modeled the mechanism of Akt protein activation correctly. Unfortunately, that kind of uncertainty is always a part of science. Albert Einstein once said, "There is no logical way to the discovery of elemental laws. There is only the way of intuition, which is helped by a feeling for the order lying behind the appearance." Taking that to heart, I told

myself that I had just as much intuition as any other scientist. I may be young and inexperienced, but even I can have something valuable to contribute to the field.

- Most importantly though, *have fun*! Do not worry about winning awards and prizes, or about who else is doing what kind of research. There are many *many* niches in the world of science, and all you really need to succeed is to find one that interests and inspires you. I have been keenly interested in computer science from childhood. My interest in biology, and specifically cell biology, came later when in my sophomore and junior years of high school, I took a series of classes on genetics and cell physiology. I was fascinated by the intricate mechanisms of control in cells, and how even the slightest changes could disrupt the entire system and lead to illness. So when I decided to do a research project, I looked to combine my two major academic interests, and computational biology seemed to be the answer. Before summer started, I had an interview with my mentor, who presented me with several different project ideas. Out of all of the possibilities she suggested, my project, creating an agent-based model of a cell-signaling pathway, instantly appealed to me. The journey was long with many digital pitfalls and program-bug shaped obstacles. However, at the end of all the tiresome programming, frustrated head-bashing, uncertain experimenting and frantic paper writing, hopefully you will feel like how I did: exhilarated, proud of myself, and wondering how soon I could get started on a new project.

So what are you waiting for? Go out and find your own passions, whether it is studying the mating calls of crickets and or figuring out Fermat's original proof of his infamous theorem.

Research

Introduction

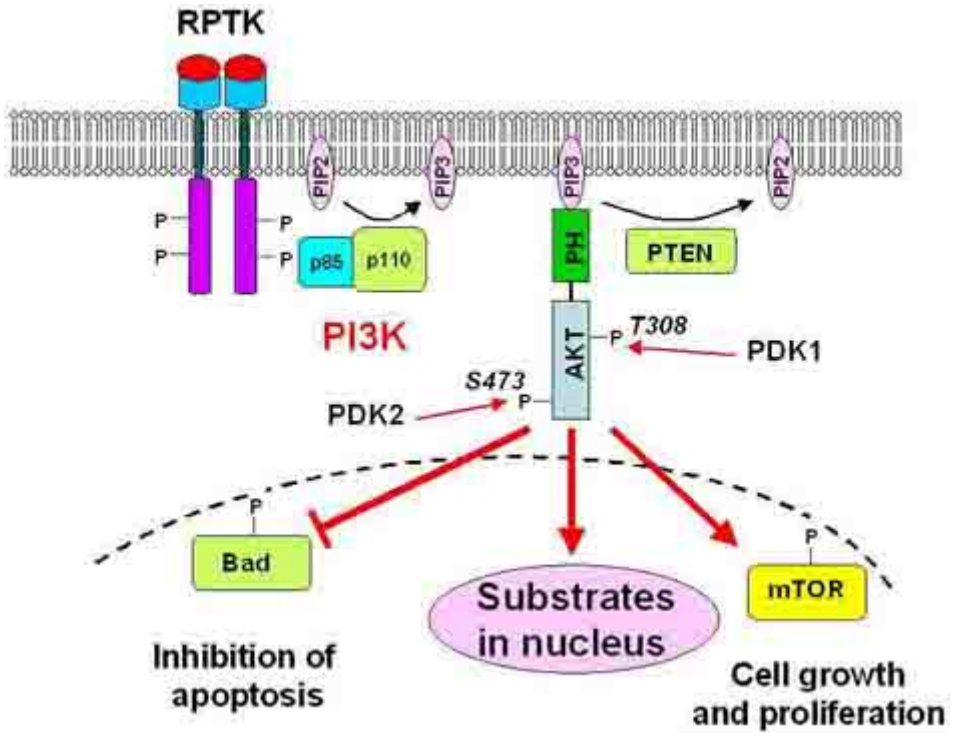
I. Agent based modeling systems

Computational science is playing a growing role in basic medical research in many ways. A key aspect of medical research and subsequent drug development is understanding exactly how biological systems operate on a macro and micro level. Through modeling of biological molecules, cells, tissues, and organs, and the networks of interactions, scientists can explore the mechanism through which these systems operate. Agent-based models are comprised of multiple, interacting agents situated within a model or simulation environment. A relationship between agents is specified, linking agents to other agents and / or other entities within a system. In an agent-based model, each component of a system of interest is represented as a discrete "agent", with its own specific behavior (methods) and attributes (variables). For example, a simulation of a school might have some Teacher agents and many Student agents with specific behaviors such as GradingPapers() and DoingHomework(), and specific attributes such as numberOfClassesTaught and currentGPA. ABM's true usefulness lies in its ability to represent a varying population. In contrast to equation-based modeling, which has to assume average behavior, ABM can also reflect outliers and mutations. For example, in the case of a cell-signaling pathway, it assumes that populations of each molecule, such as Akt or PI3K are distributed evenly over the volume of the cell. Obviously, this would not be true in a real-life cell. This assumption also makes it difficult to study the role of individual components of a pathway, as well as the effects of mutations in a pathway.

Of course, like any other modeling system, agent-based modeling has its limitations for every model is only as accurate as it was built to be. Despite these limitations, agent based models have been applied extensively to social sciences, ecology, and to biological systems on a larger scale. In my project, I attempted to apply this type of modeling on a much smaller biological scale. I considered not organs or cells or even organelles, but the actual proteins and lipids inside the cell, which has not been studied in the past years. The possibility of such rapid, cheap and exactly repeatable computer simulation of cellular signaling mechanisms represents a breakthrough in the study of cell biology. Here, I present the first agent-based model based on a cellular signaling pathway and show how it can be used to qualitatively study the effects of pathway perturbations, which may result in cancer.

II. The PI3K/Akt cell signaling pathway

I was modeling part of a signaling pathway that my lab was studying because disruption of the pathway is often led to breast and other cancers. The PI3K/Akt cell-signaling pathway is one of many cell-signaling pathways that are crucial to proper functioning of the body and hyperactivation of this pathway is seen in approximately 30% of human breast cancers. Major components of this pathway are the lipid kinase PI3K, three protein kinases: Akt, PDK1 and PDK2; and the phosphatases PTEN and PP2A (hopefully the reader is fond of abbreviations, I certainly became very familiar with them over the summer). In general, kinases add phosphate groups to other molecules, while phosphatases remove these phosphate groups. These phosphate exchanges are fundamental to the signaling processes underlying communication between cells and the normal progression of the cell cycle.



Summary of interaction of molecules in model.

Receptor binds to Ligand and gets activated, and then binds to another receptor, or dimerizes. PI3K binds to the dimerized receptor and gets activated. It then converts PIP2 to PIP3, a process that can be reverted by PTEN. PIP3 recruits PDK and Akt to the membrane, where Akt can be activated by PDKs. This activated Akt can then process its downstream substrates, but can also be inactivated by PP2A.

III. Mutation of the PI3K pathway in cancer

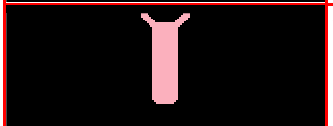
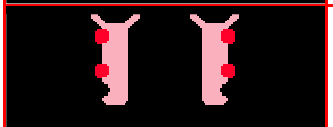
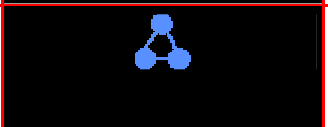
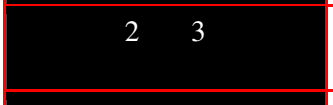
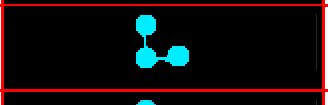
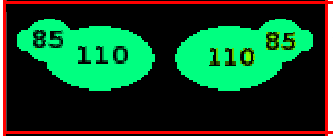
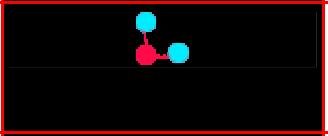
Deregulation of the PI3K/Akt pathway through mutation and overexpression is seen frequently in human cancers, and especially in breast cancer. Mutation can occur in many ways and it is important to study how different disruptions in the pathway have an effect both on the activation of Akt and development of cancer. Curiously enough, until recently, all of the mutations discovered in tumor studies were in other pathway proteins such as PTEN, PDK or PI3K. However, in July 2007, an Akt mutation known as the E17K mutation was discovered in human breast, colorectal and ovarian cancers.

The mutation was located in the PH domain of the Akt, which as mentioned before, allows the binding of Akt to membrane phospholipids such as PIP2 and PIP3. Studies showed that the expression levels of Akt (unmutated) and Akt (E17K) were similar, but the activity of the mutant was significantly higher. This led to the hypothesis that E17K substitution alters Akt regulation and enhances its cellular activity. Since this mutation was so unique, I became very eager to study it further and see exactly how Akt became "over-Akt-ive".

Creating the Model

The model was created using the Java programming language. The programming software RepastJ, an ABM simulation environment for running multi-agent simulations, was used to aid modeling. The shape and color of the agents were selected to make the display interesting but informative (not to mention colorful), but the sizes were based on the dimensions of the actual proteins (Table 1).

Table 1: Graphical representation of molecules in model

	Ligand		PDK1
	Undimerized receptor		PP2A
	Dimerized receptor, cross-phosphorylated		Akt, inactive with no conformational change
	PIP2 and PIP3		Akt, inactive but with conformational change
	PI3K		Activated (phosphorylated) Akt
	PTEN		Generic Akt substrate

Like all agents, the agents in my model have certain attributes that can be set beforehand, such as speed of movement, area available for movement (cell membrane vs. outside of cell vs. inside of cell), probability that agent will keep moving in the direction it was moving in. There is also a radius around each agent where it can "see", both for locating targets to approach (such as PP2A targeting phosphorylated Akt) and for locating interaction partners (Fig. 1).

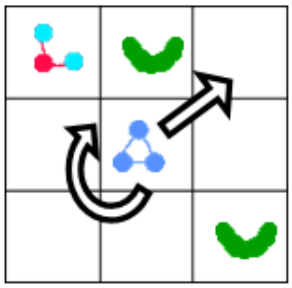


Figure 1: Movement of agents.

Each Agent rotates by a certain angle and then moves forward one. If any of the Agent's attractants are nearby, it will face the other molecule and move by one in that direction. It searches for attractants within a preset radius. For example, with a radius of 1, the central agent has 3 neighbors and it searches for its target molecule within this group.

The space for the agents to interact is in the shape of a grid, where multiple agents can occupy one square of the grid. In the graphical display of the model, the double-layered cell membrane is represented by two grey lines. Beyond that, at the top, is extracellular area and the larger area under the membrane represents the interior of the cell (Fig. 2).

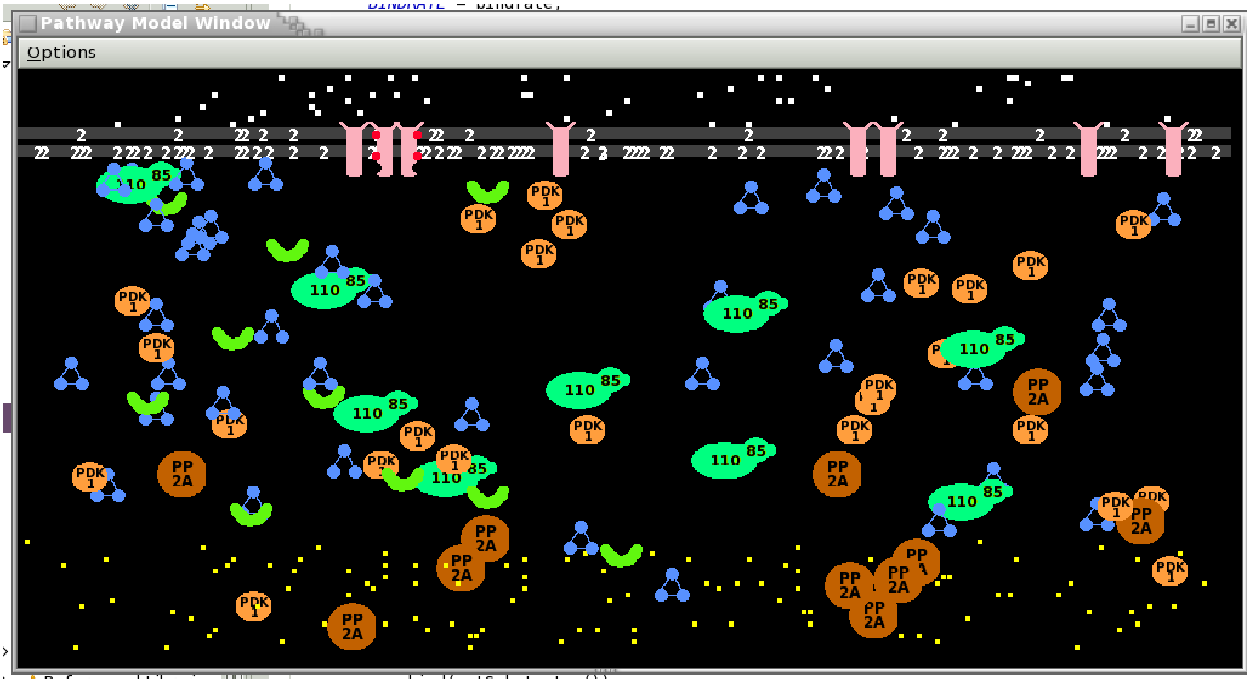


Figure 2Display adjusted for relative sizes and shapes of molecules mid-simulation

Results

I. Evaluating validity of model

The main output of this study is the model itself, since the initial goal was to create an agent-based model. The quantitative output returned by the model is in the form of graphs that monitor concentrations of active Akt and its downstream substrates. The model also records the concentrations of each type of Agent at each time step into a text file that can be imported into Excel. The model is intended to be used both qualitatively, by monitoring the graphical display of the model, and quantitatively, by analyzing the change in concentrations of molecular species and rates of substrate-to-product turnover. The model lets a user explore the relationships among concentrations of different molecules and study how the pathway's behavior is affected by mutations discovered in cancers. Some examples of graphs that have been generated from the model follow.

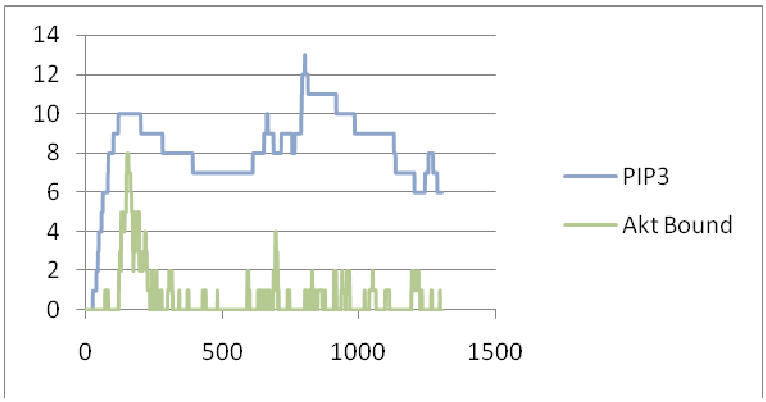


Figure 3A: Correlation between concentrations of PIP3 and bound Akt

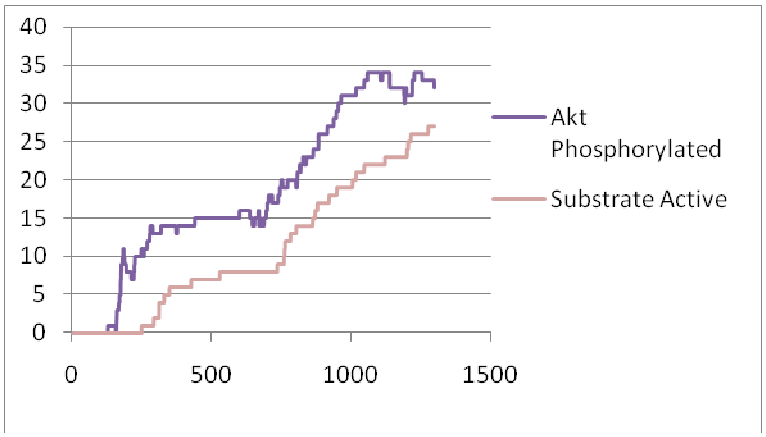


Figure 3B: Correlation between concentrations of active, phosphorylated Akt and activated downstream substrate

II. Studying the E17K mutation

I also explored the hypothesis that the Akt E17K mutation increases the rate of Akt binding to the cell membrane and decreases the rate of inactive Akt disassociating**.** My work suggests that the direct effect of the mutation is to increase the rate of inactive Akt binding to PIP3 and to lower the rate at which inactive Akt disassociates from the membrane area. This leads to an increase in concentrations of mutated bound Akt when compared to bound Akt without mutation (Fig. 4A). The mutation also raises the amount of activated Akt, if all other parameters and initial concentrations are kept constant (Fig. 4B).

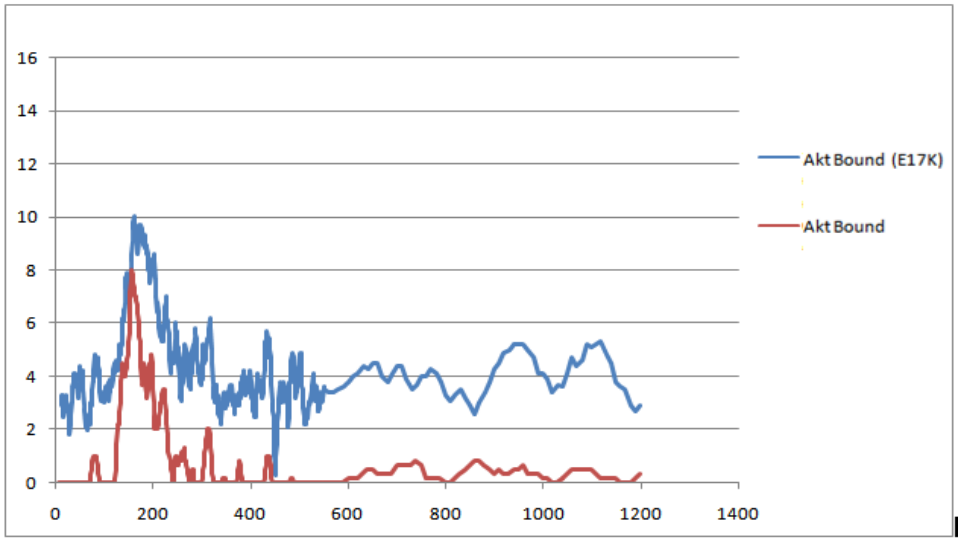


Figure 4A: Comparison of bound Akt levels with and without the E17K mutation

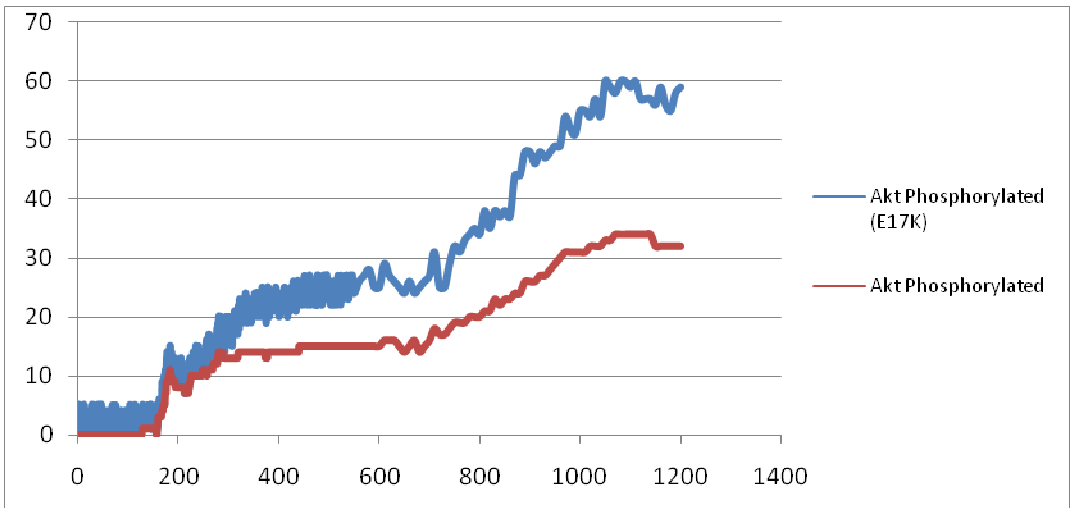


Figure 4B: Comparison of phosphorylated Akt levels with and without the E17K mutation

The mutation affects the pathway by increasing the period of time that Akt retains its conformational change at the cell membrane. Further study of the mutation in the context of my model, by systematically altering starting conditions and observing overall changes, leads me to

suggest that the mutation only affects the binding and disassociating of inactive Akt and that the disassociate rate of phosphorylated (activated) Akt is not significantly affected.

Conclusions

I. **It is possible to create an agent-based modeling of cell-signaling pathways**

II. **The model represents an easier and efficient electronic alternative to conducting experiments physically in the laboratory**

III. **The model can be used to make predictions about the behavior of the pathway under specific conditions, such as studying the effects of the E17K mutation in the PI3K/Akt pathway.**

Past, Present and Future

The PI3K/Akt pathway, a crucial cell-signaling pathway, is frequently mutated in breast and other cancer types. A computer model of the pathway was created using agent-based modeling concepts, where each individual macromolecule acted as a discrete "agent" with attributes and behaviors. The model was then used to study the effects of a new mutation in the Akt protein called the E17K mutation. Using the model, I confirmed and refined previous ideas about the behavior of mutated Akt. In the future, an accurate model of the PI3K/Akt pathway can be used as a predictive tool to simulate possible causes/effects of other cancer-causing mutations. *In silico* studies have become more and more common in the last decade, and agent based modeling provides a new and promising way of modeling signaling pathways and studying the effects of mutations in these pathways. In the future, if this technique can be refined further it has the potential to be more effective and accurate than previously common equation-based modeling.