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The Historical Evolution of Mathematical Modeling of Metabolism in the Human Red Blood Cell
ヒト赤血球代謝数理モデリングの歴史的発展

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Abstract: During the last half century significant advances in metabolic modeling and whole-cell simulation using the human red blood cell as a model system were achieved. From initial studies chaining enzymes together as pathways, modeling frameworks for metabolic pathways were developed. Simulation software capable of modeling multiple biochemical and genetic processes facilitated the move toward cell-scale modeling and enabled complementary workflows linking omic data and computational studies for model-driven design. Here, we review selected milestones in the history of mathematical modeling of the human red blood cell.

ヒト赤血球をモデル系とした代謝モデリングと全細胞シミュレーションはこの半世紀の間に大きく発展した。酵素をパスウェイとして連結する初期の研究に基づき、代謝パスウェイのモデリングフレームワークが開発されるようになった。複数の生化学的･遺伝学的プロセスをモデリングできるシミュレーションソフトウェアは、細胞スケールのモデリングへの移行を促進し、モデル駆動設計のためのオミックスデータと計算機研究を結びつける補完的なワークフローを可能にした。本稿では、ヒト赤血球の数理モデリングの歴史におけるマイルストーンを紹介する。

Keywords: red blood cell, metabolism, computer simulation, mathematical models, systems biology
赤血球、代謝、コンピュータシミュレーション、数理モデル、システム生物学

Throughout the history of computational biochemical modeling, the human red blood cell (RBC) has been an important model system. As the most abundant cell in the human body (Sender et al., 2016), the RBC plays important physiological roles in gas transport (Doctor and Stamler, 2011; Garby and Meldon, 1977). Historically, the RBC has been considered to be a relatively simple cell type whose main function was in transport of respiratory gasses from the lung to the tissues. With the advent of omic technologies, the RBC can now be viewed in a new light, showing that its functionality may be more complex and essential than previously believed (Nemkov et al., 2018). For almost five decades, the human RBC has served as the ideal cell type for the development of mathematical models due to the significant amount of available experimental data, ease of sampling, its relative metabolic simplicity, and its physiological robustness over the course of its 120-day lifespan. It has thus played an important role in the history of systems biology and the quest to construct whole-cell dynamic simulators.

Here, we review the history of mathematical modeling in the RBC. This history breaks down into roughly four time periods (Fig. 1). We discuss a few selected milestones from each period. We start by surveying early efforts to model enzyme kinetics for individual enzymes and how they are chained together into pathway models. As the scope and complexity of metabolic pathway models grew, they brought about the period where whole-cell biophysical models were formulated. The

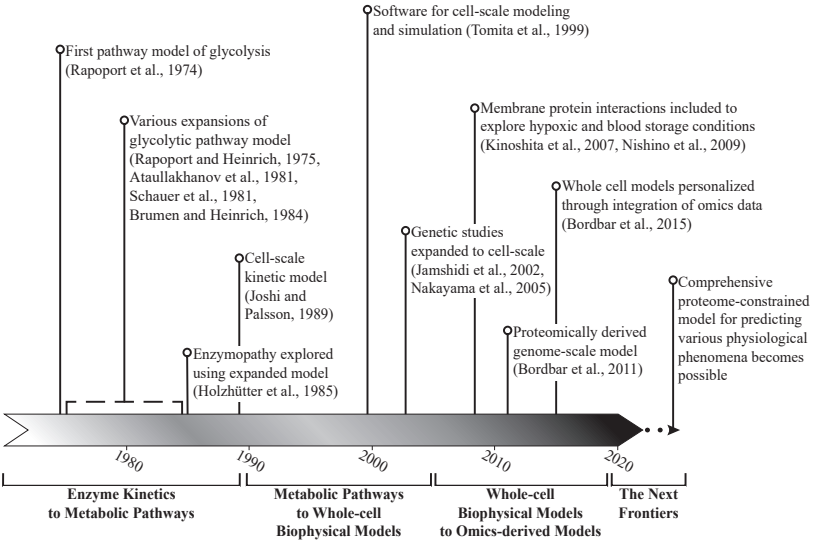

Figure 1: A timeline of selected milestones in the history of modeling human red blood cells. The history of whole-cell modeling for the human red blood cell is decades long, with many researchers contributing pioneering work in the fields of biomedical science and computational systems biology. For the purposes of this review, we have broken the timeline into four epochs which will structure our discussion: (1) Enzyme kinetics to metabolic pathways; (2) Metabolic pathways to whole-cell biophysical models; (3) Whole-cell biophysical models to omics-derived models; and (4) The next frontiers.

advent of high-throughput data collection methods made it possible to derive large-scale models from omic data, signifying the start of the current era in which whole-cell models are combined with omics data. In each epoch, RBC modeling has been integral to several significant advancements in systems biology and biomedical research. Our discussion aims to contextualize these important milestones and explore how they may bridge the gap for future progress in whole-cell modeling of human RBCs.

1　From Enzyme Kinetics to Metabolic Pathways

Early in the 20th Century, enzyme kinetics were developed and studied via well-controlled *in vitro* environments. These early efforts led to the formulation of classical mechanisms for representing enzyme kinetics (Briggs and Haldane, 1925; Michaelis

et al., 2011). The formulation of new methods for deriving rate laws greatly reduced the amount of time and effort required to mathematically model complicated enzyme mechanisms (Cleland, 1967; King and Altman, 1956). Because the RBC is primarily dependent on glycolysis for energy, it became the cell type of choice for many early studies of enzyme and pathway kinetics (Heinrich et al., 1978; Heinrich and Rapoport, 1974a). Furthermore, the study of allosteric binding—like oxygen binding to hemoglobin—led to the development of models representing the kinetics associated with allosteric effectors (Monod et al., 1965).

The recognition that computers could represent an opportunity to study multi-enzyme systems spurred the development of programs for the digital representation of chemical reactions (Chance et al., 1960). Starting with simple chemical kinetics, computational methods quickly evolved to simulate complex enzyme mechanisms (Garfinkel et al., 1961, 1966). Consequently, these developments led to single-enzyme systems that were computationally modeled with significant detail, enabling an exploration of the feasibility for proposed enzymatic mechanisms as well as the elucidation of new mechanistic insights through the fitting of results to match experimental data.

Single-enzyme kinetics could be modeled with increased detail and accuracy due to the smaller number of parameters and the need to only fit one reaction velocity to experimental data. However, modeling multi-enzyme systems proved to be significantly more complicated. Additional complexities arise when considering enzymes that are influenced by effectors simultaneously, and enzymes that are affected by compartmentalization and cellular membranes (Heinrich et al., 1978). To analyze features of regulation of a metabolic pathway, Heinrich and Rapoport (1974b) used control theory to generate a linear approximation of multi-enzyme systems. They utilized a steady-state assumption and simplified differential equations to consider the several enzymes which, when chained together, form a pathway (Fig. 2). By considering the entire pathway, identification of effector binding sites in real systems could be obtained while avoiding the erroneous conclusions that were drawn through the simple crossover theorem (Heinrich and Rapoport, 1974a).

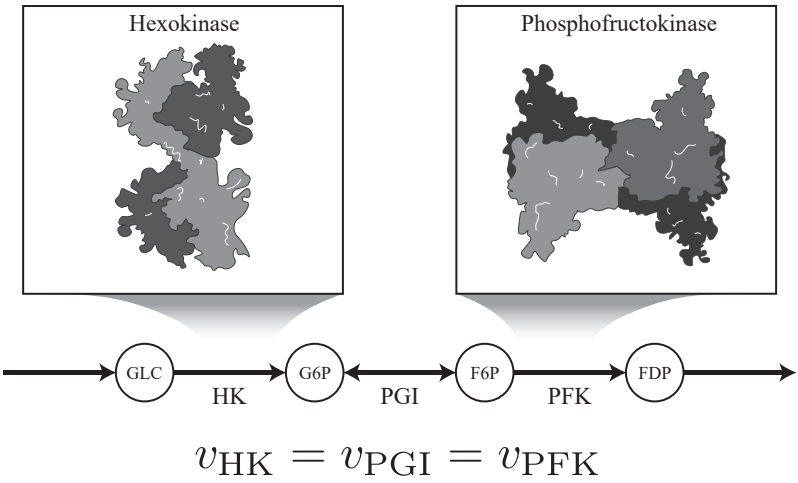

Figure 2: Application of the steady state assumption to a pathway of enzymes. (Rapoport et al., 1974) developed a framework for mathematical analyses of regulatory control in metabolic pathways by assuming the influxes and effluxes for a given metabolite are balanced at steady state. The application of the control theory of steady states to the glycolytic pathway in erythrocytes revealed that flux is primarily controlled by the hexokinase and phosphofructokinase enzymes (Rapoport et al., 1974). Abbreviations: GLC, glucose; G6P, glucose 6-phosphate; F6P, fructose 6-phosphate; FDP, fructose 1,6-bisphosphate; HK, hexokinase; PGI, phosphoglucoisomerase; PFK, phosphofructokinase.

By applying steady-state kinetics, Rapoport et al. (1974) derived a pathway model of glycolysis in human red blood cells. Mathematical analyses of the characteristic time constants revealed that glycolytic flux is controlled by the hexokinase (HK) and phosphofructokinase (PFK) enzymes, and that control was indicative of enzymatic interactions with effector molecules (Fig.2). Subsequent expansion of the model included the adenosine triphosphate (ATP) synthesis and degradation, demonstrating a buffering effect produced by the Rapoport-Luebering shunt for maintaining the ATP concentration (Rapoport and Heinrich, 1975). Schauer et al. (1981) endeavored to explore the functional roles of adenosine nucleotide metabolism by including synthesis and degradation of adenine nucleotides in their expanded model. Ataullakhanov et al. (1981) connected the glycolytic and pentose phosphate pathways and analyzed the interdependence of pathway activities with respect to stabilization of key cofactor concentrations. To quantify the interlinked

processes of energy metabolism and osmoregulation, Brumen and Heinrich (1984) incorporated active and passive ion transport processes into the glycolytic pathway model and formed a metabolic osmotic model.

Through the application of expanded glycolytic models, mathematical modeling was shown to be capable of quantitatively estimating metabolic consequences of an individual enzyme afflicted with an enzymopathy. Using calculated kinetic parameters obtained from three patients afflicted with varying severities of pyruvate kinase (PYK) deficiency, Holzhutter et al. (1985) validated previously held assumptions concerning the severity of hemolytic anemia and metabolic abnormalities in energy metabolism. This study exemplified how mathematical models could represent metabolic abnormalities consistent with clinical phenotypes. With advances in computing hardware and theories for biological modeling (Lumb, 1987)—combined with improvements in DNA sequencing automation (Smith et al., 1986)—increasing the scope, scale, and complexity of biochemical models was becoming ever more possible.

2　From Metabolic Pathways to Whole-cell Biophysical Models

The various expansions of the glycolytic pathway model enabled the quantification and prediction of physiological phenomena through separate mathematical models. However, an integrated model of all these pathways was lacking. In 1989, Joshi and Palsson built a comprehensive cell-scale kinetic model of the RBC in which glycolysis, the pentose phosphate pathway, adenine nucleotide metabolism, osmotic pressure balancing, electroneutrality, and transport processes were all included in one unified modeling framework. They proceeded to demonstrate the practical utility of such a whole-cell model through various simulations that predicted the pH dependence of the Donnan ratio, the consequences of PYK genetic variation, and the dynamic responses of key compounds under blood storage conditions (Joshi and Palsson, 1990).

The increasing scope and complexity of metabolic models—in which several cellular processes interact in various manners across multiple time-scales—

necessitated the development of multipurpose simulation software capable of whole-cell simulations. Extensible software that adheres to an object-oriented modeling paradigm was needed to ensure accuracy when modeling at the scale of whole-cell RBC metabolism (Takahashi et al., 2002; Yachie-Kinoshita et al., 2010). To address these challenges, Tomita et al. (1999) created the E-CELL software environment, the first framework for whole cell modeling of cellular processes. Using the E-CELL framework, interactive and repeatable simulations can be conducted to facilitate a wide variety of *in-silico* experiments. The visualization and analysis of generated predictions could then serve as complementary guides for subsequent experimental design and interpretation in model-driven design (Fig. 3).

Through successive improvements, E-CELL became a multi-platform framework for whole-cell simulation (Takahashi et al., 2003). As one of the first to include the Systems Biology Mark-up Language (SBML), E-CELL helped initiate the community-wide adoption of standards for model interoperability and exchangeability (Hucka et al., 2003). Following the emergence of parallel computing, the E-CELL simulation engine was redesigned for scalable modeling. The implementation of a modular meta-algorithm for mixed-mode simulation enabled various time-driven algorithms to be assigned to different model components, facilitating the simulation of cellular subsystems interacting across multiple timescales (Takahashi et al., 2002; 2004).

The ability to reproduce cellular behavior through repeatable simulations for various conditions was foreseen as having significant benefits in understanding the effects of abnormal pathological conditions (Tomita, 2001). For example, single nucleotide polymorphisms (SNPs) could result in different enzymes and the variations in their kinetic parameters. Simulations demonstrated how the genotype associated with an enzymopathy and the manifestation of its clinical phenotype could be understood in the context of cellular metabolism (Jamshidi et al., 2002). The study characterized the cellular responses for several variants of both PYK and G6PDH deficiencies to elevated metabolic loads and elucidated insights into differences in cellular function for chronic and non chronic hemolytic anemia.

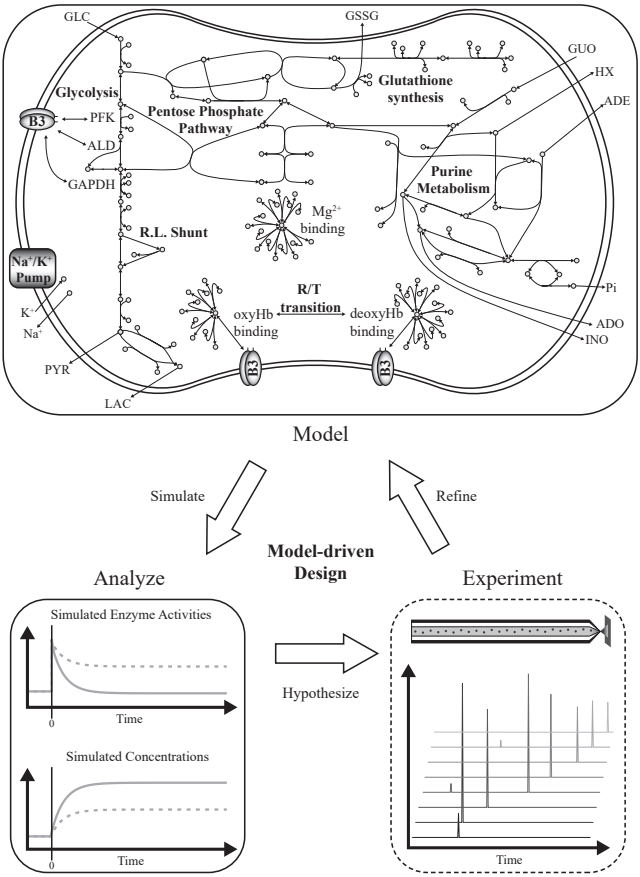

Figure 3: The cyclic process of model-driven design and discovery. Biochemical knowledge and experimental findings are integrated to formulate hypotheses that can be explored with mathematical models. Using multipurpose, multiplatform software environments for scalable simulation, mathematical models are constructed and simulated to run *in silico* experiments to predict cellular responses for different conditions (boxed with solid lines). Predictions are then visualized and analyzed to understand the systematic responses of cellular components and generate hypotheses. Using the knowledge derived from experimental validation of hypotheses, models are refined and expanded for subsequent *in silico* studies in the cyclic process of model-driven design. The metabolic map shown is based on the refinement and expansion of the cell-scale RBC model (Joshi and Palsson, 1989) to include *de novo* glutathione synthesis and export (Nakayama et al., 2005), magnesium binding, hemoglobin allostery, Band 3 interactions (Kinoshita et al., 2007; Nishino et al., 2009), and guanosine uptake (Nishino et al., 2013). Abbreviations: GLC, glucose; GSSG, oxidized glutathione, HX, hypoxanthine; ADE, adenine, Pi, inorganic phosphate; ADO, adenosine; INO, inosine; LAC, lactate; PYR, pyruvate; Na, sodium ion; K, potassium ion; Mg, magnesium ion; PFK, phosphofructokinase; ALD, aldolase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase;, oxyHb, oxyhemoglobin; deoxyHb, deoxyhemoglobin; B3, Band 3 membrane protein.

Discrepancies between *in silico* predictions and experimentally determined observations are inevitable, making algorithmic assessment and iterative refinement of models a necessary and critical step for their improvement (Ni and Savageau, 1996). When simulating a variant of glucose-6-phosphate dehydrogenase (G6PDH) deficiency, Nakayama et al. (2005) found that their initial model predicted a significantly shorter half-life compared to real cells containing the same deficiency. By refining their model via the inclusion of pathways for *de novo* synthesis of reduced glutathione (GSH) and export of oxidized glutathione (GSSG), they successfully demonstrated the essentiality of these pathways for accurate simulation of G6PDH deficiency. Furthermore, they showed that abnormal conditions such as those caused by enzymopathies may result in increased activation of typically low-activity subsystems as a compensatory mechanism. The broad implications of these findings are that the inclusion of many, if not all, metabolic pathways is necessary for the accurate simulation of metabolic abnormalities. Ultimately, the research during this time resulted in scalable models and computing frameworks that allowed for the simulation of large-scale models.

3 From Whole-cell Biophysical Models to Omics-derived Models

The availability of scalable simulation environments lowered the technological barriers associated with large-scale dynamic modeling and simulation. Genome sequences could be utilized in automating construction of large metabolic models, reducing the manual labor required to formulate genome-scale metabolic models (Arakawa et al., 2006). Thus, limitations in dynamic modeling evolved to become primarily associated with parameterization due to the considerable amount of data required for such large-scale models. Mathematical frameworks for analyzing metabolic flux states through constraint-based reconstruction and analysis (COBRA) were shown to be effective at large-scale analysis of metabolic networks (Price et al., 2004). COBRA models rely primarily on network structure derived from genomics and flux balance analysis (FBA), requiring minimal parameterization for accurate

predictions (Varma and Palsson, 1994). As a model cell type, the human RBC model was also utilized in development of many of these novel approaches for addressing the sparsity of kinetic data using omic data to reduce the number of kinetic parameters required for dynamic simulation (Yugi et al., 2005). Using genomic and proteomic data, Bordbar et al. (2011) derived one of the most expansive cell-scale reconstructions of RBC metabolism. By mapping morbid SNPs and pharmaceutical treatments that are known to target the RBC and by applying flux variability analysis, the RBC was shown to have a more extensive role in human metabolism than previously believed, confirming its potential as a clinical biomarker.

Other omic data like metabolomics have become a useful tool for studying the metabolic state of cellular systems. The technological innovations surrounding high-throughput methods made it possible to collect metabolomics data for both qualitative and qualitative analyses (Soga et al., 2003, 2004). Accordingly, it became possible to verify predictions made with metabolic models through analysis of the metabolome. Circulating RBCs are constantly subject to oxidative stress, undergoing changes between normoxic and hypoxic conditions, and evidence suggested Band 3 membrane protein interactions with hemoglobin may trigger compensatory mechanisms for maintaining intracellular ATP levels in hypoxic RBCs. Researchers began to study this phenomenon using a dynamic model containing Band 3 interactions with hemoglobin and glycolytic enzymes (Kinoshita et al., 2007). The comparison of *in silico* predictions with the results obtained from metabolome analyses provided mechanistic insight into how hemoglobin allostery and T-state binding interactions with metabolites facilitated the maintenance of ATP and 2,3-Diphosphoglycerate (2,3-DPG) concentrations.

The findings by Kinoshita et al. (2007) had implications for RBCs in blood storage, as there were no effective methods to prevent their ATP and 2,3-DPG depletion. Using a genetic algorithm for parameter estimation (Kikuchi et al., 2003), Nishino et al. (2009) modified the previous model to reflect RBCs preserved in cold-storage conditions. Through *in silico* experiments and metabolome analyses, they not only verified their model but also identified several factors for improving blood

storage conditions through maintenance of ATP and 2,3-DPG levels. By refining the metabolic model to include guanine uptake and the combination of *in silico* and metabolome analyses, Nishino et al. (2013) subsequently provided clarity into the mechanistic basis driving ATP and 2,3-DPG maintenance and revealed insights into the trade-offs between metabolic benefits and side-effects associated with the additive solution.

Other researchers also applied these modeling methods to blood storage as a relevant and actionable application of mathematical modeling tools. By integrating the latest RBC reconstruction with time-course metabolomics data obtained from RBCs in storage, metabolic states at various stages of RBC decay under storage conditions were identified, leading to the discovery of key biomarkers that defined the metabolic age of stored RBCs (Bordbar et al., 2016; Paglia et al., 2016). To quantitatively predict dynamic intracellular metabolic changes of the network, a novel FBA method capable of reconciling time-course metabolomics and network structure was developed. Subsequent application of the method provided new insights into extracellular citrate as a storage additive that were not observable with standard FBA methods (Bordbar et al., 2017; Yurkovich et al., 2017). The RBC metabolic reconstruction (Bordbar et al., 2011) also served as the basis for the creation of personalized RBC models. To study the metabolic consequences of individualized variation, genomic and fasting-state metabolomic data was obtained and mapped onto the constraint-based reconstruction (Bordbar et al., 2015).

4　The Next Frontiers

Significant advances in metabolic modeling and whole-cell simulation were achieved over the past fifty years. Modeling of the RBC has been critical at each step of the way: from the initial development of a framework for modeling metabolic pathways in the 1970s to the recent applications in personalized modeling, biomarker identification, and novel modeling method development. As whole-cell modeling is critical to understanding the genotype-phenotype relationship, the increasing recognition of its potential in transforming medicine is leading to the development of

guiding principles in generating whole human cell dynamical models (Szigeti et al., 2018). New insights into the conservation of kinetic parameters across genome-scale kinetic models offer the opportunity to understand the mechanisms of genotype-phenotype relationships (Palsson and Yurkovich, 2022). With the level of detail provided by longitudinal studies, detailed data-driven models of disease development can be developed to enable precision medicine and personalized approaches.

The human RBC has been used as a model cell for the development of modeling methods and driven the advancement of simulation technologies. These advances have aided in the understanding of human metabolism and genetic variation on a personalized level as these modeling approaches not only led to discoveries in the RBC but were applied to other cellular systems. Notably, the broader development of whole-cell computable models—and all the accompanying computational methods, simulation frameworks, and standards—has been fundamentally advanced by some of the work reviewed here that pioneered these tools in the RBC. The RBC will undoubtedly continue to play a significant role in developing complete proteome-constrained models, personalized medicine, and other notable milestones yet to come in future work.

Several foundational developments drove the history of mathematical modeling of the human RBC. The development of the E-CELL platform—a pioneering simulation platform that enables easy and informative simulation of whole cell behavior—was an important milestone. As one of the first multi-platform software environments for standardized modeling, E-CELL provided the framework necessary to facilitate the move toward cell-scale modeling by linking biochemical and genetic processes to simulate cellular dynamics across multiple timescales. Further, its existence catalyzed the development of other such modeling and simulation platforms. Through these accomplishments, Tomita and colleagues exemplified how metabolome analysis and *in silico* simulation could be paired together to understand underlying metabolic mechanisms through whole-cell modeling and simulation.

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Conflict of interest

The authors declare no competing financial interests.

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