INTEGRATE BIOPROCESS MECHANISMS INTO MODELING, ANALYTICS, AND
CONTROL STRATEGIES TO ADVANCE BIOPHARMACEUTICAL
MANUFACTURING AND DELIVERY PROCESSES

 Wei Xie∗,1 and Giulia Pedrielli2
1Northeastern University, Boston, MA 02115
2Arizona State University, Tempe, AZ 85281

ABSTRACT

The existing stochastic simulation methodologies tend to ignore ordinary differential equations or (ODE) or partial differential equations or (PDE) mechanistic models that typically represent the scientific understanding of underlying process dynamics and mechanisms. For emerging manufacturing and delivery processes of biopharmaceuticals (such as cell, gene, RNA, protein, peptide therapies and vaccines), this can limit sample efficiency, reliability, and interpretability of critical decision making. It also affects mechanism learning. Therefore, in this tutorial paper, we present the recent studies that integrate bioprocess mechanisms into simulation modeling, risk/sensitivity/predictive analytics, process design and control strategies. They can overcome the key challenges, including high complexity, high uncertainty, and very limited data, and facilitate design and development of productive, robust, and automated end-to-end biopharmaceutical manufacturing and delivery processes.

Keywords Biopharmaceutical Manufacturing and Delivery Processes, Risk/Predictive/Sensitivity Analyses, Stochastic Decision Process, Simulation Methodology, Reinforcement Learning, Bioprocess, RNA, Nanoparticles

1 INTRODUCTION

Biomanufacturing and its challenges The biopharmaceutical manufacturing industry grows rapidly and plays a significant role in supporting the economy and ensuring public health. It has developed various innovative treatments for cancer, adult blindness, and COVID-19 among many other diseases. The industry generated more than $300 billion revenue in 2019 with about 12% annual growth rate [1], and more than 40% of the products in the development pipeline were biopharmaceuticals. However, drug shortages have occurred at unprecedented rates, especially in the COVID-19 pandemic. It typically takes years to discover an optimal production and delivery processes, and the current manufacturing systems are unable to rapidly produce new drugs when needed when there is major public health issue.

Biopharmaceutical manufacturing and delivery processes face critical challenges, including high complexity, high variability, lengthy lead time, and limited process observations.

(1) High complexity and high uncertainty. Biomanufacturing process consists of numerous unit operations (such as fermentation, purification, formulation, and delivery). Biotherapeutics are produced in cells (or other living organisms, such as bacteria and yeast) whose biological processes are complex, and highly variable outputs depend on complex dynamic interactions of many factors. The upstream fermentation typically impacts on downstream purification cost and productivity. New biotherapeutics (e.g., cell, gene, RNA, protein, and peptide therapies) require more advanced manufacturing protocols. Also, bio-drugs have very complex structure which affects function. For example, aspirin, a classical small molecule medicine is comprised of 21 atoms, whereas many of the antibodies (mAbs) protein drug substance are comprised of greater than 25,000 atoms. Drug size is correlated to the structural complexity of biopharmaceuticals, where structure affects function. In addition, there are dynamic interactions of hundreds of factors, which can impact drug quality.

∗Corresponding author: w.xie@northeastern.edu
yield, and production cycle time. The target protein and RNA can degrade and have modifications during manufacturing and delivery processes. Thus, the bioprocess is the product.

(2) **Very limited process observations.** The analytical testing time required by biopharmaceuticals of complex molecular structure is lengthy. Also, significant changes in the manufacturing process, such as new facilities, equipment, and raw materials, will typically trigger new regulatory requirements and clinical trials.

Faced with these challenges, human error is frequent in biomanufacturing, accounting for 80% of deviations \[2\]. Therefore, incorporating the deep understanding of biomanufacturing mechanisms into stochastic system modeling, analytics, and optimization methodologies can accelerate integrated and intensified manufacturing process automation, quality-by-design (QbD), and reduce human error.

**Connecting design and manufacturing.** Design for biomanufacturing has the potential to dramatically reduce the development lead time, while also increasing the quality of the final product and the success rate. We observe that these improvements are needed more than ever: major technological developments are already enabling future bioproductions of large quantities of small volume (highly personalized) products. One of these advancements are “single use technologies”, which encompass a range of products and technologies such as single-use disposable connectors, vessels, mixers, etc., which in turn enable fully automated and enclosed processes. They can support flexible and efficient manufacturing at scale and on-demand through reducing (1) sterilization and cleaning costs, (2) contamination incidents, (3) storage needs, and (4) process downtime; see \[3\]. Therefore, single use technologies have the potential to impact existing medium to large volume biomanufacturing processes by enabling flexible manufacturing while reducing costs. The demand for such flexibility and variability in production batches (from few liters to tens of thousands) is already testing the capacity of pharmaceutical Contract Manufacturer Organizations (CMO). For example, several research labs and startups of varying size are seeking the manufacturing capacity to produce vaccines for trials \[4\]. In addition, single use technologies enable for the first time practical small volume bio-productions for personalized therapies, e.g., for cancer \[5,6\].

**Limitations of state-of-art OR/OM approaches.** Operation Research and Management (OR/OM) methodologies can facilitate biomanufacturing system analytics, simulation model calibration \[7,8,9\], sensitivity and uncertainty analyses \[10,11\]. Mathematical programming methods \[12,13,14\], supply chain planning \[15\], and stochastic optimization \[16,17\] are developed to guide bioprocess decision making and optimization. However, existing OR/OM methodologies on process modeling, analytics, and optimization are general and they often fail to consider bioprocess underlying mechanisms, which limits their performance, interpretability, and adoption, while suffering from sample efficiency issue.

Driven by the critical needs from biopharmaceutical industry and the limitations of classical OR/OM methodologies, we will first review the key operations in biomanufacturing processes in Section \[2\]. We will discuss the prediction and design methodologies of molecular folding, structure and function generation processes for Ribonucleic acids (RNAs), proteins, and nonparticles in Section \[3\] accounting for the molecular dynamic mechanisms. Then, in Section \[4\] we will discuss a probabilistic knowledge graph (KG) hybrid modeling that can leverage the information from existing mechanistic models and facilitate learning from real-world data. Built on the hybrid model characterizing the risk- and science-based understanding on bioprocessing mechanisms, we present the risk, sensitivity, and predictive analyses to support interpretable and robust decision making. After that, we describe the model-based reinforcement learning, accounting for model risk, to facilitate process development and control in Section \[5\].

## 2 OPERATIONS ON INTEGRATED BIOMANUFACTURING PROCESSES

Biopharmaceutical manufacturing process is crucially important to determine product quality and productivity. The biomanufacturing process typically includes the main unit operations. Step (1) belongs to upstream fermentation and drug substance synthesis, Steps (2)–(4) belong to downstream purification, and Steps (5)–(7) are for finished drug filling/formulation, freeze drying, and product quality control testing.

1. **Fermentation and Drug Substance Synthesis:** Living organisms (e.g., cells, yeasts) are mixed with appropriate medium and enzymes under carefully controlled conditions to grow and synthesize the target drug substance. The byproducts or unwanted impurities are also generated at the meantime, which impacts downstream purification operation and cost. During different growth and production phases of cell and yeast life cycle, different media compositions and feeding strategies are used to improve the productivity and reduce the waste generation.

2. **Centrifugation:** The centrifuge device is used for separation of particles, e.g., cells, subcellular organelles, viruses, large molecules such as proteins, from a solution according to their size, shape, density, viscosity of the medium and rotor speed, during which bulk of impurities would be removed.
(3) **Chromatography and Purification**: As the mixture of solutes flows through a packed resin bed, the specific solutes are separated as they are bound or slowed by the bed differently. Chromatography serves as the most critical part for purification, and it usually determines the purity of product. Since removing more impurities often results in removing more drug substance during chromatography step, there is often a trade-off between productivity and purity.

(4) **Filtration**: It is applied at several stages for capture (i.e., concentrate the product), intermediate purification, and polishing (i.e., eliminates trace contaminants and impurities) purpose.

(5) **Formulation and Filling**: To maintain the safety and efficacy of the drug substance during the storage, delivery, and facilitate the patient absorption of active pharmaceutical ingredient (API), the purified drug substance is usually formulated with carefully selected excipients into stable drug products and filled into dose containers [18].

(6) **Freeze Drying**: It is used to stabilize bio-drugs through removing water or other solvents from the frozen matrix and converting the water directly from solid phase to vapor phase through sublimation. Freeze drying are critical for immobilizing the bio-drug product in storage and delivery, as the kinetics of most chemical and physical degradation reactions are significantly decreased.

(7) **Quality Assurance/Control (QA/QC)**: QA and QC are performed to ensure the quality of the production process and the final bio-drug product.

Complex interactions of many factors introduced in each unit operation impact on the biomanufacturing process output trajectory dynamics and variations. The complexity and variability introduced in each of these steps causes challenges in the production process. There are interactions of hundreds of factors at different productions steps impacting drug quality, yield and production cycle time. These factors can be divided into critical process parameters (CPPs) and critical quality attributes (CQAs) in general; see the definitions of CPPs/CQAs in ICH-Q8R2 [19].

CPP: At each process unit operation, CPPs are defined as critical process parameters whose variability impacts on product CQAs, and therefore should be monitored and controlled to ensure the process produces the desired quality.

CQA: A physical, chemical, biological, or microbiological property that should be within an appropriate limit, range, or distribution to ensure the desired product quality.

### 3 THE MECHANICS OF DESIGN MOLECULAR FOLDING AND NANO-PARTICLES

The bioprocess induced by the stochastic decision process (SDP) of biomanufacturing is the product. RNA chain can form short helices, loops, and folds (see Figure 1) that affect its translation (i.e., protein synthesis), stability, and phase. The molecular dynamics simulation and structure analysis can predict RNA structure and quantify its impact on biophysicochemical reactions and thermodynamics in the RNA manufacturing and delivery processes. Thus, in Section 3.1, we consider underlying interatomic interactions, simulate folding process, and predict the secondary structure for RNA, which directly impacts on RNA drug delivery and function. We also briefly review the structure prediction for proteins associated with dominant bio-drugs in the current biopharmaceutical industry and market. In Section 3.2, we investigate predicting the stability of peptides and nano-particles. Nano-particle formulations have rapidly emerged as carriers in nucleic-acid therapies (i.e., DNA and RNA) to increase cellular uptake, support RNA delivery, and improve drug stability.

#### 3.1 Algorithms for Structure Prediction

Ribonucleic acid (RNA) is a fundamental biological macromolecule, essential to all living organisms, performing a versatile array of cellular tasks including information transfer, enzymatic function, sensing, regulation, and structural function. RNA has recently emerged as a promising drug target, with new therapeutic approaches aiming to develop drugs that target RNA rather than proteins. Moreover, designed RNA molecules are used in rapidly growing fields of synthetic biology and RNA nanotechnology, with applications to diagnostics, immunotherapy, drug delivery and realization of logical operations inside cells; see for example [20, 21, 22]. In [23], we propose a new framework, ExpertRNA, for the automatic folding of non-pseudoknotted secondary structures for RNA molecular compounds. ExpertRNA builds upon the fortified rollout algorithm and generalizes the architecture to allow for the consideration of multiple experts that can evaluate, at each iteration, the solutions generated by the base heuristic.

**RNA structure determines function.** Each RNA molecule is made up of a sequence of individual units, nucleotides (bases), which are of four common types, A, U, G and C (Figure 1). Individual RNA sequences range in length from
RNA secondary structure prediction. Most tools for secondary structure prediction \cite{27, 28, 29, 30} attempt to identify the structure that minimizes the free energy (FE) associated with the RNA molecule upon pairing a subset of the nucleotides, i.e., the energy released by folding a completely unfolded RNA sequence. The underlying assumption is that the structure with the lowest free energy is also the most likely structure the RNA will adopt. Equivalently, this family of approaches relies on the basic idea that the lower the FE the more stable the RNA structure will be. A first challenge for this family of approaches is that it is not possible to exactly calculate the free energy due to the (i) incomplete understanding of the RNA molecular interactions, and (ii) the impractical computational cost of detailed kinetic simulation tools. As a result, several approximate methods have been proposed in the literature \cite{31, 32, 33} to estimate the free energy associated with a given secondary structure. Most of the computational savings are a result of ignoring tertiary interactions. A second, and possibly deeper, challenge is that this model assumes that an “optimal” structure is one that pairs nucleotides in a way that minimizes the free energy (MFE). However, RNA is known to fold cotranscriptionally \cite{34}, i.e., the simultaneous transcription of two or more genes. Equivalently, RNA molecules might adopt a kinetically-preferred structure different from the global free energy minima.

In light of these challenges, alternative approaches to structure prediction have been proposed. Stochastic kinetic folding algorithms \cite{35, 36} approximate the folding kinetics of RNA molecules as they are transcribed. Data driven approaches have also started to become popular that use machine learning to evaluate structures rather than FE or kinetic models. These include ContraFold, DMinfold, and structure prediction with neural networks \cite{37, 38}. Furthermore, in the attempt to achieve advantages of model or data driven approaches, methods have been proposed that attempt to aggregate multiple information sources to get more accurate secondary structure prediction. Within the data driven category, some examples of information sources are the experimentally determined SHAPE data \cite{38, 39}, and evolutionary covariation information \cite{25}. On the model driven side, statistical ensemble approaches are used to boost the solutions obtained by the different FE driven folding algorithms. To the knowledge of the authors, ensemble methods allow to mix solutions from different algorithms only upon completion, i.e., they do not enable interaction among the algorithms while they are running \cite{40}. A recent survey on a set of secondary structure prediction tools has reported mixed results, with data-driven approaches generally outperforming the ones based on nearest-neighbor free energy models, and with model-based ensemble approaches showing competitive results \cite{41}.

Tertiary Structure Prediction. Concerning the tertiary structure prediction problem, fewer approaches can be found in the literature \cite{42, 43}. In fact, the prediction of tertiary structures is particularly challenging, and most prediction methods only work for short RNA sequences (tens of nucleotides). Data driven approaches have attracted attention also for the tertiary structure prediction. However, their accuracy remains limited due to the small number of 3D RNA structure data sets available for model training and verification.

The ExpertRNA framework for RNA structure prediction. Stemming from the observation that several folding algorithms have been proposed in the literature for secondary and, even if fewer, for tertiary structure prediction \cite{42, 43}, without any approach dominating the other, we propose the idea to build a framework that can exploit several folding tools and criteria to evaluate the quality of a folded sequence, during the algorithm execution \cite{25}. The aim of
our approach is to achieve a better RNA structure prediction quality. Figure 2 shows the structure of our ExpertRNA approach with its two main algorithmic components: (i) the partial folding; and (ii) the expert software. To mimic interatomic interactions, the algorithm sequentially adds elements to the incomplete structure (“current partial folding” in Figure 2), which we initialize to be the empty set. The first nucleotide is chosen as the first element of the input sequence provided by the user. At each step, the subsequent nucleotide is selected, and we can choose whether to simply sequence it to the last assigned nucleotide (“Null” action in Figure 2) or pair it with any nucleotide in the existing structure (“Close” in Figure 2), or pair it with an element still to be assigned (“Open” in Figure 2). The definition of these actions is motivated by the physical laws that govern molecular bonding (as previously specified in feasibility determination).

Algorithms for protein structure prediction Several approaches have been proposed in the literature for protein structure prediction. Also in the case of proteins, we distinguish primary, secondary, and tertiary structure prediction. It is important to predict protein structure due to the implications in medicine as well as biotechnology. Several algorithms have been proposed in the area with an increasing push into deep learning mainly justified by the exhaustive data sets freely available for proteins. Given different folding software to allow constraints to be provided by our method, we investigate how to embed existing tools such as PEP-FOLD and variants [44, 45, 46], AWSEM [47], Rosetta [48], and compare to the Maestro software from Schrodinger LLC. Importantly, once every two years the Critical Assessment of protein Structure Prediction (CASP) experiments are held to assess the state of the art in the field in a blind fashion, by presenting predictor groups with protein sequences whose structures have been solved but have not yet been made publicly available. DeepMind’s entry, AlphaFold, placed first in the Free Modeling (FM) category, which assesses methods on their ability to predict novel protein folds (the Zhang group placed first in the Template-Based Modeling (TBM) category, which assess methods on predicting proteins whose folds are related to ones already in the Protein Data Bank [49]. DeepMind’s success generated significant public interest. Their approach builds on two ideas developed in the academic community during the preceding decade: (i) the use of co-evolutionary analysis to map residue co-variation in protein sequence to physical contact in protein structure, and (ii) the application of deep neural networks to robustly identify patterns in protein sequence and co-evolutionary couplings and convert them into contact maps [50].

3.2 Predicting Stability of Nano-particles for RNA Formulation and Delivery

When nanoparticles are of interest a foundational question arises related to the docking of multiple molecules; see the illustration in Figures 3 and 4. In case the molecules are of the same family (e.g., peptides with peptides, RNAs with RNAs) the problem can be brought back to the folding prediction approaches in Section 3.1. However, in the case of assembly of heterogeneous bodies (i.e., peptides with RNA), new challenges arise. In this case, we normally refer to the problem of docking of molecules, a molecular modeling which can estimate the preferred orientation of one molecule to a second and further predict the type of signal produced and the strength of binding affinity between two molecules using scoring functions. The challenges in docking are very different from those identified in folding. Molecular docking processes are typically composed of two steps and usually a large molecule and a small molecule are considered for binding [51]:

- The conformation of the small molecule (e.g., ligand, peptide) is predicted together with the orientation and position with respect to the binding site of the larger molecule (e.g., protein, DNA, RNA). Such location and conformation is commonly referred to as the pose of the nano-particle. The quality of the pose requires assessment. Such evaluation can be performed by a scoring function. Ideally, a scoring function should be

![Figure 2: Overview of the ExpertRNA algorithm](source in [24]).

![Figure 3: Components for nano-particle docking.](source in [25]).
We focus on the search algorithms that have been designed to efficiently predict the docking pose. The process of docking a target and a small molecule falls into the class of NP-hard problems due to the non-countability of the number of possible poses. Hence, search becomes the approach to identify candidate solution and improving on those. In the literature, search methods can be classified into deterministic (also referred to as systematic) and stochastic \[^{52}\]. Systematic search methods sample within the binding molecule search space at predefined intervals and are deterministic. Within this class, we can still differentiate between exhaustive, fragmentation or conformational ensemble methods. The main difference between them is in the approach they take to deal with the binding molecule flexibility. In exhaustive search methods, for example, the docking is performed by systematically rotating all possible rotatable bonds in the binding molecule at a given interval. The drawback of this family of approaches is computational in nature as the number of possible combinations to consider goes with the number of rotatable bonds in the binding molecule. An common exhaustive sampling method is Glide presented in \[^{53}\] \[^{54}\]. Fragmentation represents an attempt to improve on the computational efficiency by incrementally forming binding over fragments that the binding molecule is divided into. An approach that relies on fragmentation is FlexX \[^{55}\]. Finally, in conformational ensemble methods, the binding molecule flexibility is represented by rigidly docking an ensemble of pre-generated conformations, thus improving the approach efficiency since using this approach removes the computational cost due to the exploration of the conformational space.

In stochastic algorithms, the binding molecule orientations and conformations are sampled by making changes to the molecule that are informed by random score values iteratively generated by a random algorithm. The orientation, conformation change is then treated as a incumbent that is accepted or rejected according to an algorithm-dependent criterion. The advantage of stochastic algorithms is that they can generate large ensembles of molecular conformations and explore a broader range of the energy landscape increasing the probability of finding a global energy minimum. However, this comes at computational cost. Examples are genetic algorithm, Monte Carlo, ant colony optimization (ACO) and tabu search methods. GOLD in \[^{56}\] uses a genetic algorithm, DockVision \[^{57}\] is an example of docking program using Monte Carlo stochastic method where the probability to accept a random change is calculated by using the Boltzmann probability function. An example of ACO-based approach is PLANTS \[^{58}\], while PSI-DOCK uses a tabu search \[^{59}\].

**Data Sets.** Search algorithms usually rely on data sets to retrieve potential components of the nano-particle to be assembled. In these datasets, the crystal structures of the complexes are specified using different microscopy technologies (with potentially different resolutions). Prior to any docking study, the virtual compounds database that will be screened must be carefully selected and prepared. This compound collection, often referred to as the virtual library, can encompass up to millions of compounds. Already prepared virtual libraries can be used, but users can also generate their own. Commercial databases represent an important source of compounds for virtual screening (often containing more than 1 million molecules). Suppliers commonly offer free access to the files containing molecules structures in several formats (2D or 3D). These databases undergo frequent updates, new products being added while other being either removed or out of stock. Virtual screening libraries constructed from these databases should ideally be prepared when the whole virtual screening protocol is settled and ready to be used.

A particular category of databases are bioactivity databases providing knowledge on biological targets and their modulation mechanisms. These data are frequently used for data-set benchmarking in the context of docking protocols design prior to prospective virtual screenings and to construct predictive models of activity \[^{60}\] \[^{61}\] \[^{62}\]. Examples of databases in this category are ZINC \[^{63}\] or PubChem \[^{64}\].

Finally, natural products data bases are available. Natural products were the first drugs ever used and have always been a source of drugs. In a recent retrospective study, it has been reported that, between January 1, 1981 and September 30, 2019, 23.5\% of all new approved drugs and 33.6\% of new approved small molecules drugs were natural products or derivatives of natural products \[^{65}\]. The chemical space covered by natural products is quite dissimilar to the one occupied by synthetic drug-like compounds \[^{66}\] and natural products are believed to constitute promising starting point for drug discovery \[^{67}\]. Hence, these data sets can represent a source for virtual screening libraries. Numerous databases of this type are available; please refer to \[^{68}\] and \[^{69}\].

**Scoring Functions.** There are three important applications of scoring functions in molecular docking \[^{70}\]. The first of these is the determination of the binding mode and site of small molecule to its target. Specifically, once a target has been defined, molecular docking generates hundreds of thousands of possible binding orientations/conformations.
for the small molecule (e.g., ligand, peptide) at the active site around the target molecule. The scoring function is used to rank such small molecule orientations/conformations by evaluating the binding tightness of each of the candidate complexes. Ideally, the scoring function would rank the highest the experimentally determined binding mode. Given the determined binding mode, scientists would be able to gain a deep understanding of the molecular binding mechanism and to further design an efficient drug by modifying either the target or the small molecule. It is important to highlight that, instead of scoring functions, other computational methodologies based on molecular dynamics or Monte Carlo simulations could be used to model the dynamics of the binding process thus resulting in a more accurate prediction of binding affinity. However, these models result in computationally prohibitive free energy calculations, ultimately resulting impractical for the evaluation of large numbers of molecular complexes. As a result the application of high fidelity simulation techniques is generally reduced to predicting binding affinity in small nanoparticles [71].

The second application of a scoring function, related to the first, is the prediction of the absolute binding affinity between the active compounds. This is particularly important in lead optimization, i.e., the process to improve the tightness of binding for low-affinity hits or lead compounds that have been identified. In this phase, an accurate scoring function can greatly increase the optimization efficiency and save costs by computationally predicting the binding affinities between the conformed small molecule and the target before the much more expensive synthesis and experimental steps.

The third application, is related to the foundational task of structure-based design, that fundamentally attempts to identify the potential drug hits/leads for a given target by searching a large compound database, this is commonly referred to as virtual database screening. A reliable scoring function should be able to associate higher rank to known binders following their binding scores during database screening. In fact, due to the expensive cost of experimental screening and sometimes unavailability of high-throughput assays, virtual database screening has played an increasingly important role in drug discovery.

Classical scoring functions can be classified into three groups: forcefield, knowledge-based and empirical [72, 73, 74, 75, 76]. An alternative to the classical approach to the design of scoring functions, a non-parametric machine-learning approach can be taken to implicitly capture the binding interactions that are hard to explicitly model by classical approaches in a computationally efficient way. By not imposing a particular functional form for the function, the collective effect of intermolecular interactions in binding can be directly inferred from experimental data, which should lead to scoring mechanisms that are characterized by greater generality and, consequently, prediction accuracy.

4 BIOMANUFACTURING SDP HYBRID MODELING AND ANALYTICS

Motivated by the critical challenges and needs of biopharmaceutical manufacturing, in this section, we review the probabilistic knowledge graph (KG) hybrid model proposed for biomanufacturing process characterizing the spatial-temporal causal interdependencies of CPPs and CQAs. To faithfully represent underlying bioprocess mechanisms, this hybrid model has the important properties, including latent state variables, nonlinear reactions, and time-varying kinetic coefficients with uncertainty (such as molecular reaction rates). To avoid evaluation of intractable likelihood, we further investigate a computational Bayesian inference approach, called Approximate Bayesian Computation (ABC), to efficiently approximate the posterior distribution. Then, assisted by the Bayesian knowledge graph, accounting for both stochastic and model uncertainties, we present interpretable sensitivity and predictive analyses. This study can support: (1) biomanufacturing SDP mechanism online learning; (2) bioprocess monitoring (such as tracking latent metabolic state associated with cell product CQAs); and (3) optimal and robust process control.

Illustration Example: mRNA lipid nanoparticle formulation process. Here we use mRNA lipid nanoparticle (mRNA-LPN) formulation process as an illustration example; see Figure [4]. Lipid-based formulations have rapidly emerged as carriers in nucleic-acid therapies (i.e., DNA and RNA) to increase cellular uptake, support RNA delivery, and improve drug stability. The mRNA lipid nanoparticle (LNP) formulation utilizes microfluidic or T-junction mixing to rapidly combine an ethanol phase containing hydrophobic lipids and an aqueous phase containing mRNA in a buffer. Then, the self-assembly of LNPs with mRNA is driven by the hydrophobic and electrostatic force field that is influenced by the selection of LNP formulation and CPPs, as well as the phases of mRNA-LNP complex. The pH-responsive ionizable cationic lipids have the surface charge modulated, controlling the efficient binding with the oppositely charged polynucleotides, which will support self-assembly, influence mRNA-LNP thermodynamic stability, prolong the circulation time of mRNA-LNP complexes, facilitate endosomal escape and RNA release, and increase their therapeutic. Therefore, the dynamics and variations of mRNA-LNP formulation process output trajectory depends on complex interactions of CPPs (e.g., flow rate ratio, total flow rate, temperature, lipid choices, buffer choices) and CQAs including (1) RNA integrity level; (2) species composition/concentrations/phases, particle size distribution, zeta potential, surface charge; (3) mRNA-LNP, bound/unbound mRNA.
**KG hybrid model for Biomanufacturing SDP.** The probabilistic KG hybrid model proposed in [77] and [78] can provide the risk- and science-based understanding of underlying stochastic decision process (SDP) mechanisms. The input-output relationship in each step is modeled by a hybrid (mechanistic/statistical) model that can leverage the prior knowledge on biophysicochemical mechanisms from existing mechanistic models and further advance scientific learning from process data. Specifically, at any time \( t \), the process CQAs state \( \mathbf{s}_t \) composed of observable and latent state variables, i.e., \( \mathbf{s}_t = (x, z) \) (e.g., particle size distribution, RNA sequence and integrity level), and CPPs action \( \mathbf{a}_t \) (e.g., temperature, mixing flow rate, pH) interactively influence on the dynamics and variations of output trajectories (e.g., mRNA-LNP formulation and self-assembling processes). Given the existing nonlinear ODE/PDE-based mechanistic model (such as biomolecular dynamics, thermodynamics, molecular interactions of mRNA and lipids which can affect the RNA integrity), represented by \( \frac{d\mathbf{x}}{dt} = f(\mathbf{x}, \mathbf{a}, \boldsymbol{\beta}) \), by using the finite difference approximations on derivatives, we construct the hybrid model for state transition,

\[
\begin{align*}
\mathbf{x}_{t+1} &= \mathbf{x}_t + \Delta t \cdot f(\mathbf{x}_t, \mathbf{z}_t, \mathbf{a}_t; \boldsymbol{\beta}_t) + \epsilon_{x,t+1}, \\
\mathbf{z}_{t+1} &= \mathbf{z}_t + \Delta t \cdot f(\mathbf{x}_t, \mathbf{z}_t, \mathbf{a}_t; \boldsymbol{\beta}_t) + \epsilon_{z,t+1},
\end{align*}
\]

with unknown random kinetic coefficients \( \boldsymbol{\beta}_t \in \mathbb{R}^{d\beta} \) (e.g., particle clustering rates) accounting for batch-to-batch variation. The function structures of \( f(\cdot) \) and \( f(\cdot) \) are derived from \( f(\cdot) \) in the mechanistic models. The residual terms are modeled by multivariate Gaussian distributions \( \epsilon_{x,t+1} \sim \mathcal{N}(0, V_{x,t+1}) \) and \( \epsilon_{z,t+1} \sim \mathcal{N}(0, V_{z,t+1}) \) with zero means and covariance matrices \( V_{x,t+1} \) and \( V_{z,t+1} \) by applying CLT. The kinetic coefficients \( \boldsymbol{\beta}_t \) can change over different phases of bioprocess to represent the fact that the bioprocess dynamics can be time-varying. The statistical residual terms \( \epsilon_1 = (\epsilon_{x,t+1}, \epsilon_{z,t+1}) \) allow us to account for the impact from bioprocess noise, raw material uncertainty, ignored critical process parameters (CPPs), and other uncontrollable factors (e.g., contamination) occurring at any time step \( t \).

The probabilistic KG of integrated biomanufacturing process can be visualized by a directed network as shown in Figure 5. The observed state \( \mathbf{x}_t \) and latent state \( \mathbf{z}_t \) are represented by solid and shaded nodes respectively. The directed edges represent causal interactions. At any time period \( t+1 \), the process state output node \( \mathbf{s}_{t+1} = (x_{t+1}, z_{t+1}) \) depends on its parent nodes: \( \mathbf{s}_{t+1} = f(Pa(\mathbf{s}_{t+1}); \boldsymbol{\theta}_t) \) with \( Pa(\mathbf{s}_{t+1}) = (\mathbf{s}_t, \mathbf{a}_t, \epsilon_{t+1}) \) and model parameters denoted by \( \boldsymbol{\theta}_t \). To represent the underlying SDP, we create a policy augmented KG network by including additional edges: 1) connecting state \( \mathbf{s}_t \) to action \( \mathbf{a}_t \) representing the causal effect of the policy, \( \mathbf{a}_t = \pi(\mathbf{s}_t | \phi) \) specified by parameters \( \phi \); and 2) connecting actions and states to the immediate reward \( r_t(s, a) \) (e.g., cost and RNA production). This KG network models how the effect of current state and action, \( \{s_t, a_t\} \), propagates through mechanism pathways impacting on the output trajectory and the accumulated reward.

**Bayesian KG.** Since the probabilistic KG hybrid model represents the understanding of bioprocess mechanisms and it connects heterogenous observations on CPPS/CQAs, this hybrid model allows us to inference underlying critical state (e.g., RNA and nanoparticle binding strength, mRNA-LNP folding structure) based on partially observed information, which can support biomanufacturing online monitoring and real-time release. Correctly quantifying all sources of uncertainty can facilitate optimal learning, guide risk reduction, and support robust control. Therefore, the Bayesian KG, accounting for inherent stochasticity and model uncertainty, can be created and used to support integrated bioprocess risk, sensitivity, and predictive analyses.
Given finite real-world data of the partially observed state trajectory with size \( m \), denoted by \( \mathcal{D}_m = \{ \mathbf{t}^{(i)} : i = 1, 2, \ldots, m \} \) with \( \mathbf{t}_i = (x_1, a_1, \ldots, x_H, a_H, x_{H+1}) \), the model uncertainty is quantified by a posterior distribution,

\[
p(\theta|\mathcal{D}_m) \propto p(\theta)p(\mathcal{D}_m|\theta) = p(\theta) \prod_{i=1}^{m} p\left(\mathbf{t}^{(i)}|\theta\right)
\]

where \( p(\theta) \) represents the prior distribution. Since the likelihood evaluation of KG hybrid model with high fidelity, that can faithfully capture the critical properties of bioprocess, is intractable, i.e., \( p(\mathbf{t}_i|\theta) = \int \cdots \int p(\mathbf{t}_i|\theta)dz_1 \cdots dz_{H+1} \), approximate Bayesian computation sampling with Sequential Monte Carlo (ABC-SMC) is developed to approximate the posterior distribution [77] [80]. In the naive ABC implementation, we draw a candidate sample from the prior \( \theta \sim p(\theta) \) and then generate a simulation dataset \( \mathcal{D}^* \) from the hybrid model. If the simulated dataset \( \mathcal{D}^* \) is “close” to the observed real-world observations \( \mathcal{D}_m \), we accept the sample \( \theta \); otherwise reject it. This can be very computationally expensive to match random trajectories from complex bioprocesses especially under the situations with high stochastic and model uncertainties.

The ABC-sequential Monte Carlo (ABC-SMC) methods [81] [82] can improve the sampling efficiency through: (1) generating candidate samples from updated posterior approximates by using sequential importance sampling (SIS); and (2) matching selected summary statistics, denoted by \( \eta(\mathcal{D}) \), instead trajectory observations. Following the spirit of the auxiliary likelihood-based ABC [82], we create a linear Gaussian dynamic Bayesian network (LG-DBN) auxiliary model to derive summary statistics for ABC-SMC that can accelerate online inference on hybrid models with high fidelity characterizing complex bioprocessing mechanisms and rich properties [80]. This simple LG-DBN auxiliary model, in conjunction with SIS, can capture the critical dynamics and variations of bioprocess trajectory, ensure the computational efficiency, and enable high quality of inference, which can facilitate mechanism online learning and support robust process control.

**Interpretable prediction and sensitivity analysis.** Given model and policy parameters \( (\theta, \phi) \), the spatial-temporal interdependencies of the bioprocess SDP \( \mathbf{t} = (s_1, a_1, \ldots, s_H, a_H, s_{H+1}) \) is quantified by the joint distribution,

\[
p(\mathbf{t}|\theta, \phi) = p(s_1) \prod_{i=1}^{H} p(s_{i+1}|s_i, a_i; \theta) \pi_\phi(a_i|s_i),
\]

which depends on underlying bioprocesses, sensor network design, and data collection strategy. For any batch of bioprocess, given inputs denoted by \( X \) (e.g., raw materials), we can predict any intermediate or final outputs, denoted by \( Y \) (e.g., productivity and CQAs), by using Bayesian KG. The prediction risk can be quantified by the posterior predictive distribution, \( P(Y|X) = \int P(Y|X, \theta)p(\theta|\mathcal{D}_m)d\theta \), accounting for both stochastic and model uncertainties.

We proposed a Shapley value (SV)-based prediction sensitivity analysis scheme on the Bayesian KG, called “KG-SV” [78] [83]. Since the proposed Bayesian KG-SV can faithfully account for bioprocess causal interdependencies, it can correctly assess the effect from each source of stochastic and model uncertainties on the prediction variations. The criticality assessment of input factors is based on the estimated values and estimation uncertainties of interpretable KG hybrid model parameters – such as pathways in the bioprocess KG from inputs to output (i.e., \( \beta \) in KG characterizing the rates of biomolecular reaction rates and dynamics). Since model uncertainty can be efficiently reduced by most “informative” data collection and stochastic uncertainty can be reduced by decision making, the Bayesian KG based risk/sensitivity/predictive analyses can identify bottlenecks, guide data collection for optimal learning, and improve bioprocess specifications of CPPs/CQAs for QbD.

**5 REINFORCEMENT LEARNING FOR BIOPROCESS DESIGN AND CONTROL**

The proposed Bayesian KG built in conjunction with reinforcement learning (RL) can support long-term prediction and guide interpretable, reliable, and optimal decision making. Given any feasible policy specified by parameters \( \phi \), i.e., \( a_i = \pi_\phi(s_i|\phi) \), the optimization of the policy \( \pi \) is to max the expected accumulated reward,

\[
J(\phi) = \mathbb{E}_{\theta \sim p(\theta|\mathcal{D})} \left[ \mathbb{E}_{\mathbf{t} \sim p(\mathbf{t}|\theta)} \left[ \sum_{i=1}^{H} r_i(s_i, a_i) \pi_\phi | \mathbf{t}, \theta \right] \right],
\]

accounting for both stochastic and model uncertainties. We can use the policy gradient to solve the optimization. At any \( k \)-th iteration, given candidate policy parameters \( \phi_k \), we compute

\[
\phi_{k+1} = \Pi_C (\phi_k + \eta_k \nabla J(\phi_k)),
\]

where \( \eta_k \) is a suitable stepsize, \( C \) is a feasible region, and \( \Pi_C \) is a projection onto \( C \).
Reinforcement Learning on Bayesian KG. Built on the Bayesian KG, we introduced model-based RL [83] (called KG-RL) to guide customized decision making. Since the proposed model-based RL scheme on the Bayesian KG can provide an insightful prediction on how the effect of input factor propagates through bioprocess mechanism pathways and impacts on the outputs, it can find process control policies that are interpretable and robust against heterogeneous model uncertainty, and overcome the key challenges of biopharmaceutical manufacturing, i.e., high complexity, high uncertainty, and very limited process data. To support real-time control for complex biomanufacturing processes, we provide a provably convergent stochastic policy gradient optimization and it is computationally efficient through reusing computations associated with similar input-output mechanism pathways.

Green Simulation Assisted Historical Observation Reuse. Since each experiment run is very computationally expensive especially for multi-scale bioprocess KG, we propose KG assisted multiple important sampling (“KG-MIS”) to accelerate policy gradient optimization [84]. Basically, through bridging by the likelihood ratio of Bayesian KG, we can select and reuse the “most relevant” historical trajectories, improve policy gradient estimation, and accelerate the search for the optimal robust policy. For high dimensional SDP, this study can selectively reuse historical trajectories having similar underlying distributions with that of target SDP and improve the estimation of policy gradient. In classical policy gradient (PG) approach, at any k-th iteration, the sample average approximation (SAA) is used to estimate the gradient based on n new trajectories generated, \( \nabla J^{\text{PG}}(\theta_k) = \frac{1}{n} \sum_{j=1}^{n} g(\tau^{(k,j)}|\theta^{(k,j)},\phi_k) \) with the scenario gradient,

\[
g(\tau;\theta,\phi) = \nabla_{\theta} \mathbb{E}_{\tau} \left[ \sum_{t=1}^{H} r_t(s_t,a_t) \mid \theta, \phi \right]
\]

where \( \theta^{(k,j)} \sim p(\theta|m) \) and \( \tau^{(k,j)} \sim p(\tau|\theta^{(k,j)},\phi_k) \). The target SDP mixture distribution \( p_k(\tau) = \frac{1}{n} \sum_{j=1}^{n} p(\tau|\theta^{(k,j)},\phi_k) \) accounts for both process stochastic and model uncertainties. Motivated by the studies on multiple important sampling (MIS) [85] [86], we create a KG-MIS policy gradient unbiased estimator,

\[
\nabla J^{\text{MIS}}(\phi_k) = \frac{1}{n|U_k|} \sum_{i \in U_k} \sum_{j=1}^{n} f_k(\tau^{(i,j)}) g(\tau^{(i,j)}|\theta^{(k,j)},\phi_k) \quad \text{with} \quad f_k(\tau) = \frac{p_k(\tau)}{\sum_{i \in U_k} p(\tau|\theta_i,\phi_i)|U_k|}.
\]

(3)

that can leverage on the information from historical trajectories. Since the inappropriate selection of reuse set \( U_k \) can lead to the inflated estimation variance of policy gradient, we propose a variance reduction based experience replay criteria [84], which can automatically select the most relevant historical trajectories generated under different decision policies \( \phi \) and model parameters \( \theta \) from different posterior distributions resulting from online learning and process control. We prove that the proposed approach is asymptotically convergent and show it significantly outperforms classical policy gradient approach.

In addition, to support mechanism learning and process control for flexible integrated biopharmaceutical manufacturing systems with modular design, we further extend the proposed KG-MIS framework so that it can select and reuse the most relevant partial trajectories from historical observations [87], i.e., the reuse unit is defined based on state-action transition \((s,a,s')\). This study can allow us to flexibly integrate and leverage the relevant information collected from different production lines and facilitate personalized bio-drug manufacturing.

CONCLUSION

We argue that much more flexibility should be given to the design of increasingly personalized products and manufacturing should be considered early on in product design. In fact, for achieving a “full circle”, not only the manufacturing technology needs to be flexible, but also the drug design and the process control need to support it. Novel platforms and approaches provide such methods, substantially improving the performance of CMO allowing for larger volume and variability of products capitalizing upon single use/disposable technologies. We envision the need for hybrid modeling and interpretable ML/AI based process analytical technologies (PATs) for end-to-end biomanufacturing processes that support a new, manufacturing oriented product design, embedding process control, thus considering, early on, the manufacturing viability of a product.

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References

[1] Eric Langer. *Global trends and growth opportunities in the biopharmaceutical product development and manufacturing*, 2020. https://www.cphi-online.com/46/resourcefile/10/93/83/CPhI%20annual%20rep%202020%20vJ.pdf.

[2] R. Cintron. *Human Factors Analysis and Classification System Interrater Reliability for Biopharmaceutical Manufacturing Investigations*. PhD thesis, Walden University, 2015.

[3] Tim Sandle. Strategy for the adoption of single-use technology. *European Pharmaceutical Review*, 2018.

[4] Kyle Blankenship for FiercePharma. Samsung scores $362m deal to help vir scale up covid-19 antibody production., 2020 (accessed May 3, 2020).

[5] Ulrike Mock, Lauren Nickolay, Brian Philip, Gordon Weng-Kit Cheung, Hong Zhan, Ian CD Johnston, Andrew D Kaiser, Karl Peggs, Martin Pule, Adrian J Thrasher, et al. Automated manufacturing of chimeric antigen receptor t cells for adoptive immunotherapy using clinimacs prodigy. *Cytotherapy*, 18(8):1002–1011, 2016.

[6] Fenlu Zhu, Nirav Shah, Huiling Xu, Dina Schneider, Rimas Orentas, Boro Dropulic, Parameswaran Hari, and Carolyn A Keever-Taylor. Closed-system manufacturing of cd19 and dual-targeted cd20/19 chimeric antigen receptor t cells using the clinimacs prodigy device at an academic medical center. *Cytotherapy*, 2017.

[7] Guesuk Lee, Wongon Kim, Hyunseok Oh, Byeng D Youn, and Nam H Kim. Review of statistical model calibration and validation—from the perspective of uncertainty structures. *Structural and Multidisciplinary Optimization*, pages 1–26, 2019.

[8] Paul D Arendt, Daniel W Apley, and Wei Chen. Quantification of model uncertainty: Calibration, model discrepancy, and identifiability. *Journal of Mechanical Design*, 134(10), 2012.

[9] Bo Wang, Qiong Zhang, and Wei Xie. Bayesian sequential data collection for stochastic simulation calibration. *European Journal of Operational Research*, 277(1):300–316, 2019.

[10] Andrea Saltelli, Marco Ratto, Terry Andres, Francesca Campolongo, Jessica Cariboni, Debora Gatelli, Michela Saisana, and Stefano Tarantola. *Global sensitivity analysis: the primer*. John Wiley & Sons, 2008.

[11] Gabriele Baroni and Stefano Tarantola. A general probabilistic framework for uncertainty and global sensitivity analysis of deterministic models: A hydrological case study. *Environmental Modelling & Software*, 51:26–34, 2014.

[12] K Lakhdar, J Savery, LG Papageorgiou, and SS Farid. Multiobjective long-term planning of biopharmaceutical manufacturing facilities. *Biotechnology Progress*, 23(6):1383–1393, 2007.

[13] Robert C. Leachman, Lenrick Johnston, Shan Li, and Zuo-Jun Shen. An automated planning engine for biopharmaceutical production. *European Journal of Operational Research*, 238(1):327–338, 2014.

[14] Kais Lakhdar and Lazaros G Papageorgiou. An iterative mixed integer optimisation approach for medium term planning of biopharmaceutical manufacture under uncertainty. *Chemical Engineering Research and Design*, 86(3):259–267, 2008.

[15] Adam J Fleischhacker and Yao Zhao. Planning for demand failure: A dynamic lot size model for clinical trial supply chains. *European Journal of Operational Research*, 211(3):496–506, 2011.

[16] Ai Chye Lim, Yuhong Zhou, John Washbrook, Nigel John Titchener-Hooker, and Suzanne Farid. A decision-support tool to model the impact of regulatory compliance activities in the biomanufacturing industry. *Computers & Chemical Engineering*, 28(5):727–735, 2004.

[17] Niranjan S. Kulkarni. A modular approach for modeling active pharmaceutical ingredient manufacturing plant: A case study. In *Proceedings of the 2015 Winter Simulation Conference*, pages 2260–2271, Piscataway, New Jersey, 2015. Institute of Electrical and Electronics Engineers, Inc.

[18] Sugunakar Y Patro, Erwin Freund, and Byeong S Chang. Protein formulation and fill-finish operations. 2002.

[19] ICH Harmonised Tripartite Guideline et al. Pharmaceutical development. *Q8 (R2) Current Step*, 4, 2009.

[20] Alexander A Green, Pamela A Silver, James J Collins, and Peng Yin. Toehold switches: de-novo-designed regulators of gene expression. *Cell*, 159(4):925–939, 2014.

[21] Cody Geary, Paul WK Rothemund, and Ebbe S Andersen. A single-stranded architecture for cotranscriptional folding of RNA nanostructures. *Science*, 345(6198):799–804, 2014.

[22] Dongran Han, Xiaodong Qi, Cameron Myhrvold, Bei Wang, Mingjie Dai, Shuxing Jiang, Maxwell Bates, Yan Liu, Byoungkwan An, Fei Zhang, et al. Single-stranded DNA and RNA origami. *Science*, 358(6369):eaao2648, 2017.
[23] Menghan Liu, Erik Poppleton, Giulia Pedrielli, Petr Šulc, and Dimitri P Bertsekas. Expertrna: A new framework for rna secondary structure prediction. *INFORMS Journal on Computing*, 2022.

[24] Thomas Carell, Caterina Brandmayr, Antje Hienzsch, Markus Müller, David Pearson, Veronika Reiter, Ines Thoma, Peter Thumbs, and Mirko Wagner. Structure and function of noncanonical nucleobases. *Angewandte Chemie International Edition*, 51(29):7110–7131, 2012.

[25] Nicola Calonaci, Alisha Jones, Francesca Cuturrello, Michael Sattler, and Giovanni Bussi. Machine learning a model for RNA structure prediction. *arXiv preprint arXiv:2004.00351*, 2020.

[26] José Almeida Cruz, Marc-Frédérick Blanchet, Michal Boniecki, Janusz M Bujnicki, Shi-Jie Chen, Song Cao, Rhiju Das, Feng Ding, Nikolay V Dokholyan, Samuel Coulbourn Flores, et al. RNA-puzzles: a casp-like evaluation of RNA three-dimensional structure prediction. *Rna*, 18(4):610–625, 2012.

[27] Jessica S Reuter and David H Mathews. RNAStructure: software for RNA secondary structure prediction and analysis. *BMC bioinformatics*, 11(1):129, 2010.

[28] Ivo L Hofacker. Vienna RNA secondary structure server. *Nucleic acids research*, 31(13):3429–3431, 2003.

[29] Joseph N Zadeh, Conrad D Steenberg, Justin S Bois, Brian R Wolfe, Marshall B Pierce, Asif R Khan, Robert M Dirks, and Niles A Pierce. Nupack: analysis and design of nucleic acid systems. *Journal of computational chemistry*, 32(1):170–173, 2011.

[30] Michael Zuker and Patrick Stiegler. Optimal computer folding of large RNA sequences using thermodynamics and auxiliary information. *Nucleic acids research*, 9(1):133–148, 1981.

[31] David H Mathews, Jeffrey Sabina, Michael Zuker, and Douglas H Turner. Expanded sequence dependence of thermodynamic parameters improves prediction of RNA secondary structure. *Journal of molecular biology*, 288(5):911–940, 1999.

[32] Tianbing Xia, John SantaLucia Jr, Mark E Burkard, Ryszard Kierzek, Susan J Schroeder, Xiaoqi Jiao, Christopher Cox, and Douglas H Turner. Thermodynamic parameters for an expanded nearest-neighbor model for formation of rRNA duplexes with Watson-Crick base pairs. *Biochemistry*, 37(42):14719–14735, 1998.

[33] Mirela Andronescu, Anne Condon, Holger H Hoos, David H Mathews, and Kevin P Murphy. Computational approaches for RNA energy parameter estimation. *RNA*, 16(12):2304–2318, 2010.

[34] M Yu Angela, Paul M Gasper, Eric J Strobel, Kyle E Watters, Alan A Chen, and Julius B Lucks. Computationally reconstructing cotranscriptional RNA folding pathways from experimental data reveals rearrangement of non-native folding intermediates. *bioRxiv*, page 379222, 2018.

[35] Hervé Isambert and Eric D Siggia. Modeling RNA folding paths with pseudoknots: application to hepatitis delta virus ribozyme. *PNAS*, 97(12):6515–6520, 2000.

[36] Ting-ting Sun, Chenhan Zhao, and Shi-Jie Chen. Predicting cotranscriptional folding kinetics for riboswitch. *The Journal of Physical Chemistry B*, 122(30):7484–7496, 2018.

[37] Chuong B Do, Daniel A Woods, and Serafim Batzoglou. Contrafold: RNA secondary structure prediction without physics-based models. *Bioinformatics*, 22(14):e90–e98, 2006.

[38] Julius B Lucks, Stefanie A Mortimer, Cole Trapnell, Shujun Luo, Sharon Aviran, Gary P Schroth, Lior Pachter, Jennifer A Doudna, and Adam P Arkin. Multiplexed RNA structure characterization with selective 2′-hydroxyl acylation analyzed by primer extension sequencing (shape-seq). *Proceedings of the National Academy of Sciences*, 108(27):11063–11068, 2011.

[39] Justin T Low and Kevin M Weeks. Shape-directed RNA secondary structure prediction. *Methods*, 52(2):150–158, 2010.

[40] Nima Aghaeepour and Holger H Hoos. Ensemble-based prediction of RNA secondary structures. *BMC bioinformatics*, 14(1):139, 2013.

[41] Hannah K Wayment-Steele, Wipapat Kladwang, Eterna Participants, and Rhiju Das. RNA secondary structure packages ranked and improved by high-throughput experiments. *BioRxiv*, 2020.

[42] Zhichao Miao, Ryszard W Adamik, Maciej Antczak, Robert T Batey, Alexander J Becka, Marcin Biesiada, Michal J Boniecki, Janusz M Bujnicki, Shi-Jie Chen, Clarence Yu Cheng, et al. RNA-puzzles round iii: 3D RNA structure prediction of five riboswitches and one ribozyme. *Rna*, 23(5):655–672, 2017.

[43] Andrew Martin Watkins, Ramya Rangan, and Rhiju Das. Farfar2: Improved de novo rosetta prediction of complex global RNA folds. *Structure*, 2020.
[44] Pierre Thévenet, Yimin Shen, Julien Maupetit, Frédéric Guyon, Philippe Derreumaux, and Pierre Tufféry. Pepfold: an updated de novo structure prediction server for both linear and disulfide bonded cyclic peptides. *Nucleic acids research*, 40(W1):W288–W293, 2012.

[45] Yimin Shen, Julien Maupetit, Philippe Derreumaux, and Pierre Tufféry. Improved pep-fold approach for peptide and miniprotein structure prediction. *Journal of chemical theory and computation*, 10(10):4745–4758, 2014.

[46] Alexis Lamiable, Pierre Thévenet, Julien Rey, Marek Vavrusa, Philippe Derreumaux, and Pierre Tufféry. Pepfold3: faster de novo structure prediction for linear peptides in solution and in complex. *Nucleic acids research*, 44(W1):W449–W454, 2016.

[47] Shikai Jin, Vinicius G Contessoto, Mingchen Chen, Nicholas P Schafer, Wei Lu, Xin Chen, Carlos Bueno, Arya Hajitaheri, Brian J Sirovetz, Aram Davtyan, et al. Awsem-suite: a protein structure prediction server based on template-guided, coevolutionary-enhanced optimized folding landscapes. *Nucleic acids research*, 48(W1):W25–W30, 2020.

[48] Sidhartha Chaudhury, Sergey Lyskov, and Jeffrey J Gray. Pyrosetta: a script-based interface for implementing molecular modeling algorithms using rosetta. *Bioinformatics*, 26(5):689–691, 2010.

[49] Mohammed AlQuraishi. Alphafold at casp13. *Bioinformatics*, 35(22):4862–4865, 2019.

[50] Vivien Marx. Method of the year: protein structure prediction. *Nature methods*, 19(1):5–10, 2022.

[51] Asma Sellami, Manon Réau, Florent Langenfeld, Nathalie Lagarde, and Matthieu Montes. Virtual libraries for docking methods: Guidelines for the selection and the preparation. In *Molecular Docking for Computer-Aided Drug Design*, pages 99–117. Elsevier, 2021.

[52] Francesca Stanzione, Ilenia Giangreco, and Jason C Cole. Use of molecular docking computational tools in drug discovery. *Progress in Medicinal Chemistry*, 60:273–343, 2021.

[53] Richard A Friesner, Jay L Banks, Robert B Murphy, Thomas A Halgren, Jasna K Klicic, Daniel T Mainz, Matthew P Repasky, Eric H Knoll, Mee Shelley, Jason K Perry, et al. Glide: a new approach for rapid, accurate docking and scoring. 1. method and assessment of docking accuracy. *Journal of medicinal chemistry*, 47(7):1739–1749, 2004.

[54] Thomas A Halgren, Robert B Murphy, Richard A Friesner, Hege S Beard, Leah L Frye, W Thomas Pollard, and Jay L Banks. Glide: a new approach for rapid, accurate docking and scoring. 2. enrichment factors in database screening. *Journal of medicinal chemistry*, 47(7):1750–1759, 2004.

[55] Matthias Rarey, Bernd Kramer, Thomas Lengauer, and Gerhard Klebe. A fast flexible docking method using an incremental construction algorithm. *Journal of molecular biology*, 261(3):470–489, 1996.

[56] Gareth Jones, Peter Willett, Robert C Glen, Andrew R Leach, and Robin Taylor. Development and validation of a genetic algorithm for flexible docking. *Journal of molecular biology*, 267(3):727–748, 1997.

[57] Trevor N Hart and Randy J Read. A multiple-start monte carlo docking method. *Proteins: Structure, Function, and Bioinformatics*, 13(3):206–222, 1992.

[58] Oliver Korb, Thomas Stützle, and Thomas E Exner. Plants: Application of ant colony optimization to structure-based drug design. In *International workshop on ant colony optimization and swarm intelligence*, pages 247–258. Springer, 2006.

[59] Jianfeng Pei, Qi Wang, Zhenming Liu, Qingliang Li, Kun Yang, and Luhua Lai. Psi-dock: Towards highly efficient and accurate flexible ligand docking. *Proteins: Structure, Function, and Bioinformatics*, 62(4):934–946, 2006.

[60] Niu Huang, Brian K Shoichet, and John J Irwin. Benchmarking sets for molecular docking. *Journal of medicinal chemistry*, 49(23):6789–6801, 2006.

[61] Nathalie Lagarde, Jean-François Zagury, and Matthieu Montes. Benchmarking data sets for the evaluation of virtual ligand screening methods: review and perspectives. *Journal of chemical information and modeling*, 55(7):1297–1307, 2015.

[62] Michael M Mysinger, Michael Carchia, John J Irwin, and Brian K Shoichet. Directory of useful decoys, enhanced (dud-e): better ligands and decoys for better benchmarking. *Journal of medicinal chemistry*, 55(14):6582–6594, 2012.

[63] Teague Sterling and John J Irwin. Zinc 15–ligand discovery for everyone. *Journal of chemical information and modeling*, 55(11):2324–2337, 2015.

[64] Sunghwan Kim, Jie Chen, Tiejun Cheng, Asta Gindulyte, Jia He, Siqian He, Qingliang Li, Benjamin A Shoemaker, Paul A Thiessen, Bo Yu, et al. Pubchem 2019 update: improved access to chemical data. *Nucleic acids research*, 47(D1):D1102–D1109, 2019.
[65] David J Newman and Gordon M Cragg. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of natural products*, 83(3):770–803, 2020.

[66] Karen C Morrison and Paul J Hergenrother. Natural products as starting points for the synthesis of complex and diverse compounds. *Natural product reports*, 31(1):6–14, 2013.

[67] Tiago Rodrigues, Daniel Reker, Petra Schneider, and Gisbert Schneider. Counting on natural products for drug design. *Nature chemistry*, 8(6):531–541, 2016.

[68] Maria Sorokina and Christoph Steinbeck. Review on natural products databases: Where to find data in 2020. *Journal of Cheminformatics*, 12(1):1–51, 2020.

[69] Ya Chen, Christina de Bruyn Kops, and Johannes Kirchmair. Data resources for the computer-guided discovery of bioactive natural products. *Journal of chemical information and modeling*, 57(9):2099–2111, 2017.

[70] Sheng-You Huang, Sam Z Grinter, and Xiaojin Zou. Scoring functions and their evaluation methods for protein–ligand docking: recent advances and future directions. *Physical Chemistry Chemical Physics*, 12(40):12899–12908, 2010.

[71] Qurrat Ul Ain, Antoniya Aleksandrova, Florian D Roessler, and Pedro J Ballester. Machine-learning scoring functions to improve structure-based binding affinity prediction and virtual screening. *Wiley Interdisciplinary Reviews: Computational Molecular Science*, 5(6):405–424, 2015.

[72] Niu Huang, Chakrapani Kalyanaraman, Kataryzna Bernacki, and Matthew P Jacobson. Molecular mechanics methods for predicting protein–ligand binding. *Physical Chemistry Chemical Physics*, 8(44):5166–5177, 2006.

[73] Holger Gohlke, Manfred Hendlich, and Gerhard Klebe. Knowledge-based scoring function to predict protein–ligand interactions. *Journal of molecular biology*, 295(2):337–356, 2000.

[74] André Krammer, Paul D Kirchhoff, X Jiang, CM Venkatachalam, and Marvin Waldman. Ligscore: a novel scoring function for predicting binding affinities. *Journal of Molecular Graphics and Modelling*, 23(5):395–407, 2005.

[75] Olgun Guvench and Alexander D MacKerell Jr. Computational evaluation of protein–small molecule binding. *Current opinion in structural biology*, 19(1):56–61, 2009.

[76] Pedro J Ballester and John BO Mitchell. A machine learning approach to predicting protein–ligand binding affinity with applications to molecular docking. *Bioinformatics*, 26(9):1169–1175, 2010.

[77] Hua Zheng, Wei Xie, Keqi Wang, and Zheng Li. Opportunities of hybrid model-based reinforcement learning for cell therapy manufacturing process development and control. *arXiv preprint arXiv:2201.03116*, 2022.

[78] Wei Xie, Bo Wang, Cheng Li, Dongming Xie, and Jared Auclair. Interpretable biomanufacturing process risk and sensitivity analyses for quality-by-design and stability control. *Naval Research Logistics (NRL)*, 69(3):461–483, 2022.

[79] Michael D Buschmann, Manuel J Carrasco, Suman Alishetty, Mikell Paige, Mohamad Gabriel Alameh, and Drew Weissman. Nanomaterial delivery systems for mrna vaccines. *Vaccines*, 9(1):65, 2021.

[80] Wei Xie, Keqi Wang, Hua Zheng, and Ben Feng. Dynamic bayesian network auxiliary abc-smc for hybrid model bayesian inference to accelerate biomanufacturing process mechanism learning and robust control. *arXiv preprint arXiv:2205.02410*, 2022.

[81] Tina Toni, David Welch, Natalja Strelkowa, Andreas Ipsen, and Michael PH Stumpf. Approximate bayesian computation scheme for parameter inference and model selection in dynamical systems. *Journal of the Royal Society Interface*, 6(31):187–202, 2009.

[82] Gael M Martin, Brendan PM McCabe, David T Frazier, Worapree Maneesoonthorn, and Christian P Robert. Auxiliary likelihood-based approximate bayesian computation in state space models. *Journal of Computational and Graphical Statistics*, 28(3):508–522, 2019.

[83] Hua Zheng, Wei Xie, Ilya O Ryzhov, and Dongming Xie. Policy optimization in bayesian network hybrid models of biomanufacturing processes. *arXiv preprint arXiv:2105.06543*, 2021.

[84] Hua Zheng, Wei Xie, and M Ben Feng. Green simulation assisted policy gradient to accelerate stochastic process control. *arXiv preprint arXiv:2110.08902*, 2021.

[85] J. Dong, M. B. Feng, and B. L. Nelson. Unbiased metamodeling via likelihood ratios. In *2018 Winter Simulation Conference (WSC)*, pages 1778–1789, Dec 2018.

[86] Mingbin Feng and Jeremy Staum. Green simulation: Reusing the output of repeated experiments. *ACM Transactions on Modeling and Computer Simulation (TOMACS)*, 27(4):23:1–23:28, October 2017.
[87] H. Zheng and W. Xie. Variance reduction based partial trajectory reuse to accelerate policy gradient optimization. In *Proceedings of the 2022 Winter Simulation Conference*, page submitted, Piscataway, New Jersey, 2022. Institute of Electrical and Electronics Engineers, Inc.

**AUTHOR BIOGRAPHIES**

**WEI XIE** is an assistant professor in MIE at Northeastern University. Her research interests include interpretable Artificial Intelligence (AI), machine learning, computer simulation, data analytics, and stochastic optimization for cyber-physical system risk management, learning, and automation. Her email address is w.xie@northeastern.edu Her website is http://www1.coe.neu.edu/~wxie/

**GIULIA PEDRIELLI** is Assistant Professor in the School of Augmented Intelligence at Arizona State University. Her email address is giulia.pedrielli@asu.edu