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Award Number: W81XWH-15-1-0582

TITLE: High-Fidelity Design of Multimodal Restorative Interventions in Gulf War Illness

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REPORT DATE: Jan 2020

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command

Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

X Approved for public release; distribution

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
Jan 2020	Final	09/30/2015 - 09/29/2019

4. TITLE AND SUBTITLE
High-Fidelity Design of Multimodal Restorative Interventions

in Gulf War Illness

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-15-1-0582

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research
and Materiel Command
Fort Detrick, MD 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Our objective is to further refine models of immune and endocrine regulatory dysfunction developed under W81XWH-10-1-0774 (Broderick PI) by improving fidelity of the timescale and drug action thereby translating previously idealized treatments into optimally beneficial low-risk drug re-purposing strategies that are immediately deployable as short exposure courses in phase-I clinical trials.

With collaborating PI Dr. Whitley (CSU), we continue to make substantive progress towards project goals during this reporting period. We have now implemented tools for the i) direct integration of data with the contextual logic, ii) the efficient identification of treatment target sets destabilizing illness and ensuring remission "reachability". We have completed broader more detailed models of male and female regulatory physiology and are currently updating treatment predictions as well as incorporating the use of drug-target pairs.

15. SUBJECT TERMS
GWI; Hypothalamic-pituitary-adrenal (HPA) axis

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*

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I. Introduction

Gulf War Illness (GWI) is a complex illness with symptoms presenting across several principal regulatory systems including immunologic and endocrine components. Accordingly, we proposed that GWI might involve a chronic imbalance in co-regulation between the nervous, endocrine and immune systems. We tested this hypothesis under our previous CDMRP award W81XWH-10-1-0774 by using a discrete logic formalism to construct a first set of computer models describing the interactions between regulation of stress hormones (hypothalamic-pituitary-adrenal; HPA axis), sex hormones (hypothalamic-pituitary-gonadal; HPG axis) and immune function in the periphery and the brain. Results of our first analysis (**Craddock et al., 2014**] suggested that normal homeostatic drive across the immune, sex and stress hormone regulatory systems may contribute to the persistence of GWI by naturally supporting an alternate steady state characterized by chronically high cortisol, low testosterone and a shift towards Th1 immune activation. Further work has shown that subtle changes to the circuitry itself can produce exact overlap of alternate homeostatic signature with GWI in a set of readily observable immune and endocrine markers (**Rice et al., 2014**). This involvement of a modified homeostatic drive implies that once activated, this regulatory program will actively resist therapeutic attempts to restore normal hormone and cytokine levels. This regulatory dynamic must be overcome and ideally exploited to therapeutically escape what is essentially a stable disease state in GWI.

Computer simulations using these initial models predicted very low success rates for single-target treatment strategies. Only when endocrine and immune components were targeted together and in a specific sequence did predictions of sustained remission reach favorable levels. One such two-step strategy involves inhibition of Th1 pro-inflammatory signaling (e.g. short course Enbrel), followed by blockade of glucocorticoid receptors (e.g. Mifepristone) once the system has equilibrated (**Craddock et al., 2015**). However, this initial proof-of-concept work assumed that intervention agents were bound to target instantaneously with ideal affinity and specificity. While these *idealized* treatments helped identify targets for which novel high affinity drugs could be developed, *the current award supports the continued refinement of this computational platform to (i) directly incorporate the pharmacologic properties of drugs available for repurposing and (ii) better represent the time course dynamics of regulatory signaling and drug action kinetics*. These enhancements will support the translation from idealized treatment strategies into predicted real-world treatment courses that make optimal use of currently available drugs.

II. **Keywords**: dynamical systems, discrete logic models, immune endocrine regulation, system models, constraint satisfaction, stable attractors, limit cycles

III. Accomplishments.

What were the major goals of the project?

In this first year of the project efforts were focused on elements that were supportive of the following goals:

- *Specific Aim 1*: Implement relative dynamics.
  Milestone #1: Completion and release of validated model incorporating updated state transition dynamics, deployed using a novel time and structure-based decomposition scheme.
  Estimated % complete: 90%

- *Specific Aim 2*: Incorporate available drug action data.
  Milestone #2: Completion and release of validated model incorporating estimates of drug-action dynamics.
  Estimated % complete: 85%

- *Specific Aim 3*: Increase efficiency of search for optimal intervention course.
  Milestone #3: Completion and release of validated model decomposition scheme and hybrid treatment optimization algorithm for rapid deployment and efficient use of large-scale distributed platform.
  Estimated % complete: 95%

What was accomplished under these goals?

Recall that early in the course of Year 1, we re-engineered the basic structure of the logic model developed under previous award W81XWH-10-1-0774 in favor of a generalized discrete framework introduced by Thomas and colleagues (**1991, 1995, 2001**). In brief, this new framework has significantly improved model fidelity by i) seamlessly extending the previous 3-state logic to any number of discrete states, ii) representing differences in receptor affinity by introducing signal activation thresholds, and iii) capturing the effects of competitive signaling from agonists and antagonists of various strengths using a weighted transition logic. Introduced briefly in the Year 2 Annual Report, a more thorough description of these features and how their inclusion serves to improve model fidelity has now been published by our group (**Sedghamiz et al., 2018**) (Subtask 1.a, 1.b). Also introduced in our Year 2 annual report were computationally efficient strategies for model tuning (Subtask 1.c, 1.d) and treatment designs directed at making illness dynamically untenable (Subtask 3.b). In Year 3 the main focus was on expanding the features and applicability of these proof of concept prototypes and translating them into more robust and much more computationally efficient implementations. In particular we had i) designed an initial set of criteria for ranking the multiple competing models that explain experimental or clinical observations equally well (**Sedghamiz et al., 2017**), ii) further refined the constraint-based identification of model parameter sets, integrating this into a comprehensive toolbox for model identification, analysis and comparison, as well as iii) refined criteria for the identification and ranking of minimal intervention target sets leading to remission at various levels of biological noise.

In this Extension Year, we finalized the release of the software toolbox and publication of the formal protocols and tools for the identification of discrete regulatory logic parameters from sparse and incomplete experimental data (**Sedghamiz et al., 2019a**) as well as the identification and ranking of minimal intervention target sets (**Sedghamiz et al., 2019b**). We also completed migration of the prototype tools and solvers supporting the constraint-based optimization of model parameter sets onto large distributed computing platforms and conducted benchmark studies of scale-up performance (Subtask 1.c, 1.d). Moreover, a detailed analysis of the departure of model predictions from experimental data as well as the breakdown and assignment of such departures to individual observations and individual model components has been designed and implemented. Most importantly, a significant part of the Extension Year served to design and complete a first release of a software module directed at integrating multiple drug databases and performing large-scale assignment of drugs to the component targets making up the minimal intervention sets. These candidate target sets are then re-assessed based on how well they may be translated into an actionable pharmacological treatment (Major Task 2). This module was then successfully validated against the recently published failure of an IL-1 antagonist in a veteran population with COPD. Finally, the migration of a first-generation priority update scheme that more closely approximates response kinetics (Major Task 1) is being pursued such that it may benefit from a more direct and transparent access to the constraint-based solvers afforded with Python. Continuity of this work is now also being supported in part under a new collaborative research agreement with Elsevier Life Sciences (Broderick – PI).

1. *Direct integration of experimental data into regulatory logic tuning and model validation*. This effort directly supports the completion of **Major Task 1**, creating an architecture that not only integrates observations at stable resting states seamlessly but also formally exploits the exact temporal sequence of transition states. In this past year we have:

- Our constraint-based strategy for model parameter set identification has now been made available as a toolbox named BioMC (BioModel Checker; https://github.com/hooman650/BioModelChecker) and has been reported in *Frontiers in Bioengineering and Biotechnology* (**Sedghamiz et al., 2019a**). In this work model ranking criteria such as structural parsimony, regulatory selectivity, dynamic responsiveness, and response reliability are embedded into the search decisional logic weights (K-vales) distinguishing strong from weak inputs and regulatory activation thresholds for each mediator node as a multiple objective optimization. Early in the year we completed validation against a larger set of established benchmark problems (**Table 1a, b**) that now include the sex hormone axis (**Bennett et al., 2013**) and naïve T helper cell polarization to Th1 or Th2 phenotypes (**Garg et al.,**

2008) and published our *serial* performance results obtained using a *single processor* Intel core i7 machine.

- Subsequently, we expanded our set of validation benchmark problems to include artificial networks of increasing size and complexity as summarized in **Table 2**. These networks include artificial biological networks generated by NetSim (Di Camillo et al., 2009) containing 50-100 nodes (*n*), with an average connection density of 5-10 interactions per node (κ) and where each node is regulated by 10-15 upstream mediators (Max Regulatory *r*). The latter was varied systematically as prior anecdotal evidence had suggested (correctly) that under the current model design the number of upstream regulators would be the limiting factor in scale-up. The dependency of average connection density and the maximum number of upstream regulators was such that not all combinations of these design parameters were achievable (Table 2 entries in red font). Also included were models of mucosal immune signaling in COPD in human and mouse lung developed under a sister DoD-funded project W81XWH1910804 (Broderick - PI; Sethi - Partnering PI). These were added to offer real-world benchmark problems of lower complexity than the previously reported GWI network (35 mediators linked by close to 270 regulatory interactions).

- Parallel versions of the *Chuffed* (**Ohrimenko et al. 2009**) and OR-Tools solvers were implemented on a distributed computing platform using multiple processing cores running up to 32 threads. Specifically, processors assigned under this shared resource included the 8-core Intel Sandy Bridge (2.2 GHz), 12-core Intel Haswell (2.3 GHz) and 14-core Intel Broadwell (2.4 GHz). For all model networks except the two largest simulated networks, 32 GB of RAM was used. The two largest simulated networks were run for 72 hours using 128 GB (κ=10, r=15, n=50) and 256 GB of RAM (κ=10, r=15, n=100) respectively.

- Interestingly though Chuffed performed well in a serial application on small to moderate networks, this solver found no solutions for larger artificial networks within 48 hours using multiple cores suggesting an issue with scale and a high communication overhead. Conversely the OR-Tools solver which trailed Chuffed in smaller scaled serial applications found solutions for all of the models in Table 2 using < 32 GB of RAM within 48 hours. The only exceptions were both models with indegree *r* =15. For the smaller of these solutions were obtained by increasing memory to 128 GB of RAM. In the case of the largest model (r=15, n=100 nodes) solutions would require > 256 GB of RAM and > 72 hours. Finally, the number of high-quality solutions (error < 5%) did not increase significantly when more than 10 threads were used (**Fig. 1**).

- We concluded that mid-range computing resources could adequately solve networks linking ~100 nodes with an average connection density of 10 edges/node and a maximum indegree *r* of 10 regulatory actions (over ~900 regulatory actions total). Using cloud resources this would be equivalent to an Amazon Web Services (AWS) instance type: c5.4xlarge, using 16 vCPUs, each with 32 GB of RAM.

2. *Tuning high-fidelity model to human GWI data*. In direct support of Subtask 1.d, we had completed separate more detailed models describing co-regulation immune mediators with stress, sex and metabolic hormones in *both men and women.* In June, 2018 we presented to the EAB a model of male endocrine-immune physiology consisting of 35 mediators linked by close to 270 regulatory interactions. The logic parameters for this model were constrained to experimental data from samples collected under previous CDMRP award W81XWH-09-2-0071 (Klimas PI) and that had been processed be the laboratory at the time, namely n=12 healthy and GWI male veteran. In this initial analysis we found model components that were especially uncertain and poorly explained in this first partial data set. For example, a comparison of variability in the complexity of decisional logic statements governing the contextual response of IL-15, IL-23, TRH and activin showed these to be especially uncertain and poorly constrained. Regulatory actions around IL-6, IL-10 IL-15, IL-23, TRH and activin showed high variability across competing models indicating that these may be subject to additional co-regulators absent in the initial reference circuit. These initial findings support a continued refinement of model structure. New

more complete data was released in September 2019 including a broader set of markers in n=25 GWI and n=25 healthy control veterans.

We are now extending the scope and granularity of the previous regulatory model and will re-align this larger more complex network model with the more complete data set by taking full advantage of the newly implemented parallel version of the parameter estimation benchmarked under section 1 above.

3. *An efficient exhaustive search of minimally invasive interventions*. In direct support of **Major Task 3**, we completed benchmarking an efficient approach for identifying compact intervention targets which uses a combination of reduced search space (**Samaga et al., 2010**), compact simulation (**Mishchenko and Brayton 2002**), and an efficient optimization stated as a constraint satisfaction problem (**Corblin et al., 2012**). Details of this approach are described in a manuscript that has now been published in *Frontiers in Physiology* (**Sedghamiz et al., 2019b**).

4. *A first deployment of drug-target matching*. In direct support of **Major Task 2**, we have developed and implemented additional criteria for ranking the suitability of intervention sets based on how readily they may be translated into therapeutic interventions, or how *pharmaceutically actionable* they might be. This post-processing of the MIS solutions is outlined in **Fig. 2.**

    a. First, multiple drug-target interaction databases were first queried to identify combinations of drugs that act appropriately on any and all candidate targets present in the model. The bioDBnet suite, a series of tools developed by the Advanced Biomedical Computing Center at the National Cancer Institute (NCI), was used for cross-linking of biological accession numbers across various online databases (**Mudunuri et al., 2009**). This made it possible to retrieve drug-target interaction data from web resources such as KEGG, the Therapeutic Target Database, DrugBank, PharmGKB, and PubChem (**Kanehisa and Goto, 2000; Li et al., 2018; Wishart et al., 2018; Whirl-Carillo et al., 2012; Kim et al., 2016**). These were used in conjunction with Elsevier's BKGB Reaxsys Medical Chemistry (RCM) database available under a new collaborative research agreement with the Broderick group at RGH. Drugs identified by these queries were scored by the number of known on and off-target interactions. Minimization of off-target interactions is considered especially important because downstream effects of these interactions may ultimately destabilize a solution's trajectory. Combinations of high-scoring drugs were assembled so as to modulate all necessary targets in the manner specified by a given MIS. These combinations were further minimized to avoid redundant drugging of targets. *The result is a list of candidate drug combinations predicted to be viable for any and all possible MIS target subsets that might be derived from a given model.*

    b. The drug superset assembled in (a) was then used to rank interventions from multiple competing models according to their pharmaceutical actionability. This is in addition to the previously applied criteria by which desirable intervention sets contained few targets (low cardinality), were predicted to support rapid response kinetics (efficiency), affected a minimal number of downstream mediators, and were maximally robust to biological noise (robustness) (**Sedghamiz et al., 2019b**). Specifically, each intervention set *s* is now also ranked based on the following criteria (**Fig. 3**):

        i. *Parsimony or low cardinality of actionable targets (C(s))*. Intervention sets that are comprised of fewer concurrent targets or less invasive, are ranked higher.

        ii. *Availability of a drug or compound for all targets (R(s))*. Intervention sets where even one target cannot be matched to an approved drug or compound either directly or indirectly receive a zero score.

        iii. *Abundance of targets requiring antagonistic modulation (A(s))*. As small molecules are more often used to antagonize rather than agonize a target, target sets with a greater number of antagonist actions are t ranked higher.

iv. *Number of off-target drug effects (T(s))*. For each drug identified for an intervention set, the number of other targets also affected by this drug are counted as a penalty. In other words, highly specific drugs are favored.

v. *Broad support across multiple models (M(s))*. An intervention set that is consistently predicted across multiple competing models and is therefore robust to model uncertainty would be favored and assigned a high score.

Though other criteria will undoubtedly be considered this first set of conditions has performed well in reducing large sets of candidate intervention sets produced by groups of 100-200 competing models. Importantly while this strategy is directed towards repurposing the availability condition can be relaxed to allow for more expensive designer compounds.

c. This new module, called "DrugAble", has been implemented as Python custom code for querying Elsevier and pre-formatting data and an R component for the analysis and visualization of the results. These components are now also being combined into a single Python script utilizing calls to the R environment.

What opportunities for training and professional development has the project provided?

Leveraging the alliance between RGH and the Rochester Institute of Technology (RIT) the Broderick lab has trained 2 M.Sc. students (one in Bioinformatics, one in Biotechnology) with 3 new M.Sc. students in bioinformatics and 1 Ph.D. in mathematical modeling having joined in September. Through our new collaborative research agreement with Elsevier these new students have the opportunity to learn from Elsevier data scientists in the UK, Netherlands and US as well as its pharma industry partners, both US and UK-based.

How were the results disseminated to communities of interest?

Drs. Broderick and Craddock continue to deliver podium talks and participated in discussion panels. Specifically, Dr. Broderick's work was presented to the ConTech meeting in London, UK, in November, 2018 and again in October 2019. Dr. Broderick also presented recent progress to the Elsevier Annual company-wide meeting in Mainz Germany in June and will be delivering a keynote address on the topic at Duke University (early November). Rajeev Jaundoo (formerly employed under W81XWH-15-1-0582) made the following two presentations:

- Jaundoo, R. & Craddock, T.J.A. (2019, October). DRUGPATH: A New Database for Mapping Polypharmacology. Poster session presented at the Campus Alberta Student Conference on Health (CASCH), Edmonton, Alberta, CA.
- Jaundoo, R. & Craddock, T.J.A. (2019, August). Using Drugpath: A New Database For Mapping Polypharmacology To Assess Multi-drug Treatments For Gulf War Illness. Poster session presented at the 2019 Military Health System Research Symposium (MHSRS), Kissimmee, FL, USA.

Drs. Broderick, Craddock and Whitley have also been publishing these results in peer reviewed journal as expediently as possible with the following 4 works published in this past year

- Jaundoo R, Bohmann J, Gutierez G, Klimas NG, Broderick G, Craddock TJA. Using a Consensus Docking Approach to Predict Adverse Drug Reactions in Combination Drug Therapies in Gulf War Illness. Int J Mol Sci. 2018 Oct 26;19(11). pii: E3355.
- Sedghamiz H, Morris M, Craddock TJA, Whitley D, Broderick G. Bio-ModelChecker: Using Bounded Constraint Satisfaction to Seamlessly Integrate Observed Behavior with Prior Knowledge of Biological Networks. Front Bioeng Biotechnol. 2019 Mar 26;7:48.

- Sedghamiz H, Morris M, Whitley D, Craddock TJA, Pichichero M, Broderick G. Computation of Robust Minimal Intervention Sets in Multi-valued Biological Regulatory Networks. Front Physiol. 2019 Mar 19;10:241.
- Jaundoo R. and Craddock TJA. 2019. DRUGPATH: A new database for mapping polypharmacology. Alberta Academic Review, Vol 2 (3) 4, CASCH Special Issue (not peer-reviewed), DOI: 10.29173/aar92.

What do you plan to do during the next reporting period to accomplish the goals?

Though the period of performance is now completed, work continues and will focus increasingly on the following:

- *Identification of parameter sets for female GWI*. We have assembled a higher resolution model of endocrine-immune regulation in *female physiology*. The full model consists of 53 entities (nodes) connected initially by 674 control actions (edges). We plan to identify parameter sets that will align regulatory model dynamics with exercise data collected from female veterans under CDMRP award GW150199 (Craddock PI) as this data becomes available.
- *Implement searches for stable partial remissions*. Results from this first more comprehensive analysis of the treatment space suggest that in some cases some degree of "molecular scarring" may have occurred and that a single model of regulatory signaling may not be sufficient to seamlessly accommodate both the illness state and the healthy control resting state. In such cases we propose to modify the objective such that intervention is focused on establishing a stable condition that is most similar to full recovery. Mathematically this would be a stable attractor with the same features as the healthy control attractor and that would support immune and endocrine expression profiles similar to those exhibited by healthy veterans. The same strategy of "parking orbit" would be applied to the design of multi-step interventions.
- *Fully implement drug-target pairing*. Complete the prototype work discussed above by fully implementing this module into the software environment.
- *Discovery of novel regulatory actions*. The current work supports a "what-if" evaluation of candidate regulatory interactions where these candidates are currently proposed by the user. We are collaborating with the University of Minnesota to adapt and integrate statistical inference of causal associations from data. We have currently implemented a discovery strategy based on the statistical characteristics of network structure (**Guimerà and Sales-Pardo, 2009**) and hope to expand this to include work by Pearl (**2010**).
- *Iterative inclusion of off-target biology.* Currently the intervention target sets are proposed on the basis of idealized drug action and then re-assessed and scored with respect to actionability into a drug trial in a post-processing step. In this assessment, interventions that involve drugs that have broad off-target effects are simply penalized and downgraded. As work continues we would like iteratively include off-target elements that are outside the immediate scope of the original model and verify the repercussions of modulating these elements.

IV. **Impact**.

What was the impact on the development of the principal discipline(s) of the project?
In keeping with the milestones described in the project submission initial accomplishments that have impacted the core disciplines of the project continue to revolve around major increases in fidelity, scale and computational efficiency for this type of computational modeling. As stated previously, these may be summarized as follows:

- We have implemented and deployed a software tool now being used by non-programmers to develop high-fidelity models of biological signaling. The *Bio Model Checker (BioMC)* environment is now being used in our labs by life science researchers to allows quickly assemble casual models of regulatory signaling, align these with experimental data very efficiently i.e. on a laptop, evaluate model structure and make predictions.
- This same environment has significantly extended previous fault analysis techniques developed in the microelectronics industry to now handle higher-resolution multi-valued logic required to adequately describe biological signaling at levels of biology other than the genome. Importantly, the group has developed formalisms and metrics for working with multiple competing models. Measures such as efficiency of response and robustness are being used to study known biological networks and will be instrumental in defining optimality of treatment.
- The breadth of potential applications has now greatly expanded with the execution this past May by Rochester General Hospital of a 3-year renewable collaborative research agreement with Elsevier Life Sciences directed at the continued development of this prototype platform into a commercially robust software. In addition to supporting 2 senior staff, Elsevier has provided as in-kind support privileged access to their data analysts, advanced prototype text-mining engines and other software tools as well as access to databases such as the ReaxSys Medicinal Chemistry database.

  This access to added resources will greatly accelerate the development of the platform and the generation of a next iteration of clinical trial designs for GWI.

What was the impact on other disciplines?

The development of this computational framework has broad reaching applications beyond the model-based design of treatments for Gulf War Illness. This integrated approach for model assembly, tuning, prediction and validation has been applied to intracellular signaling in a now CDMRP-funded application (GW170081; Boyd/ O'Callaghan – PI) as well as a submission currently under review by the PRMRP to study Chronic Obstructive Pulmonary Disease (COPD) in a veteran population (PR181430; Broderick – PI/ Seth Partnering PI).

We have applied this paradigm to the study of depression and plan to extend that model to capture the effects of PTSD in GWI, though continuing work with CDMRP () as well as study PTSD in its own right.

We have also started assembly of intracellular models describing metatstatic transformation in cancers with the hope of partnering with the Roswell Park Cancer Institute and the VA of Western NY in applications to veteran's health.

Finally, the Broderick Group at RGH has been approved as a new clinical psychology internship site by the American Psychological Association (APA). The Group has an intern applying this modeling approach to the study of chronic pain and opioid addiction. This intern will continue with the Group as a fellow and will be joined by a new intern in June of next year.

What was the impact on technology transfer?

This work attracted the attention of Elsevier research in 2017. Over the past 2 years we refined a scope of work and articulated a collaborative research agreement that was signed by both parties in May, 2019. It is our hope that this partnership will support the continued development of this platform into a commercially robust tool for deployment to a broad range of other illnesses.

What was the impact on society beyond science and technology?

Nothing to report.

V. **Changes/Problems**:

Changes in approach and reasons for change
Our technology approach remains consistent. We have now extended our efforts to include a more formal consideration of multiple competing models that arise as a result of the limited experimental data. Rather than focus on the single "best" model, we now view the concurrent analysis of multiple models as an opportunity to i) identify intervention strategies that are robust to model uncertainty, and ii) identify subtle changes to the signaling circuitry that might delineate responders from non-responders thereby informing on inclusion criteria for clinical trials.

Actual or anticipated problems or delays and actions or plans to resolve them
Mr. Sedghamiz left his position as senior research programmer with the Broderick group October 30, 2018. We recruited Mr Cole Lyman into this position in May, 2019. Mr. Lyman is completing his MSc degree in computer science at Bringham Young University (BYU) and comes to us with a rich background in graph theory which he is also applying to our work with Dr. McGowan under W81XWH-14-1-0550 directed at isolating changes in DNA methylation in GWI. With the increased availability of RIT students, we expect no further changes in project logistics.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

Significant changes in use or care of human subjects	Nothing to report
Significant changes in use or care of vertebrate animals.	Nothing to report
Significant changes in use of biohazards and/or select agents	Nothing to report

VI. **Products**.
The Broderick and Craddock groups have successfully published the following peer-reviewed papers:

- Jaundoo R, Bohmann J, Gutierez G, Klimas NG, Broderick G, Craddock TJA. Using a Consensus Docking Approach to Predict Adverse Drug Reactions in Combination Drug Therapies in Gulf War Illness. Int J Mol Sci. 2018 Oct 26;19(11). pii: E3355.
- Sedghamiz H, Morris M, Craddock TJA, Whitley D, Broderick G. Bio-ModelChecker: Using Bounded Constraint Satisfaction to Seamlessly Integrate Observed Behavior with Prior Knowledge of Biological Networks. Front Bioeng Biotechnol. 2019 Mar 26;7:48.
- Sedghamiz H, Morris M, Whitley D, Craddock TJA, Pichichero M, Broderick G. Computation of Robust Minimal Intervention Sets in Multi-valued Biological Regulatory Networks. Front Physiol. 2019 Mar 19;10:241.

and a conference proceeding abstract

- Jaundoo R. and Craddock TJA. 2019. DRUGPATH: A new database for mapping polypharmacology. Alberta Academic Review, Vol 2 (3) 4, CASCH Special Issue (not peer-reviewed), DOI: 10.29173/aar92.

Additionally, the Broderick and Craddock team have one publication in press

- Jaundoo, R., Bohmann, J., Gutierrez, G., Klimas, N., Broderick, G., & Craddock, T.J.A. (in press). Towards a Treatment for Gulf War Illness: A Consensus Docking Approach. *Military Medicine*.

one manuscript under review

- Vashishtha, S., Broderick G., Craddock, T.J.A., Barnes, Z.M., Collado, F., Balbin, E.G., Fletcher, M.A., & Klimas, N.G. Leveraging Prior Knowledge to Recover Characteristic Immune Regulatory Motifs in Gulf War Illness. *Front. Physiol. - Systems Biology* (under review)

and one manuscirpt in preparation for an invited submission

- Jaundoo R, Broderick G, Craddock TJA. Harnessing Drug to Target to Pathway Information for Use in the Polypharmacological Design of Multi-Drug Repositioning Treatments. International Journal of Molecular Sciences (Special Issue on Pharmacogenomics) Invited, in-preparation.

VII. **Participants & Other Collaborating Organizations**:

In May of this past year the Broderick group signed a multi-year renewable research agreement with Elsevier Life Sciences for the continued development of this modeling paradigm. The agreement supports 2 senior RGH staff and provides in-kind contributions in the form of privileged access to Elsevier data science staff, database resources including developer access to their medicinal chemistry database, as well as advanced prototype tools such as context-directed text mining (ETM), etc…

What individuals have worked on the project?

Name	Travis Craddock
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	12
Contribution to Project:	Dr. Craddock has taken over administrative leadership of the project as PI with the relocation of Dr. Broderick to RGH. Primary responsibility and leadership of drug selection and drug property modeling component of the project
Funding Support:	No change

Name	Gordon Broderick
Project Role:	Co-PI
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	12
Contribution to Project:	Works closely with Dr. Craddock in the overall direction of project and coordination of efforts. Primary responsibility and leadership of model logic, logic tuning and treatment course redesign initiatives.
Funding Support:	No change

Name	Cole Lyman
Project Role:	Research Programmer II/ Grant-funded research staff

Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	8
Contribution to Project:	Oversees all programming initiatives as they apply to large-scale computation. Leading the migration of miniZn and Matlab proof of concept code under BioMC into a parallel scalable implementation under Python.
Funding Support:	Fully supported

Name	Spencer Richman
Project Role:	Research Programmer I/ Grant-funded research staff
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	4
Contribution to Project:	Has spearheaded the theoretical development supporting the assignment of drugs to optimal target sets and the re-assessment of these sets based on actionability.
Funding Support:	Fully supported

Name	Mary Ann Fletcher
Project Role:	Co-Inv
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.6
Contribution to Project:	Continues to coordinate the processing samples and delivery of endocrine and immune data from exercise challenge that is being used in the tuning of the model logic
Funding Support:	No change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

In the past year Dr. Broderick was awarded CDMRP/PRMRP funding (W81XWH1910804) as Principal Investigator (0.36 calendar months) with Drs. Sethi (Partnering PI; Buffalo VA) and Qu (Co-investigator; U Buffalo) to pursue the study of infectious exacerbation in COPD. Dr. Broderick has also been assigned a multi-year collaborative research award by Elsevier Life Sciences that provides full support for 2 senior staff.

What other organizations were involved as partners?

In addition to Colorado State University, RGH and the Broderick group have signed a 3-year renewable collaborative research agreement with Elsevier Life Sciences (Elsevier, Amsterdam).

VIII. **Special Reporting Requirements**: None

Collaborative Awards. This work is being carried out in collaboration with Dr. D. Whitley at Colorado State University under the associated grant number W81XWH-15-1-0583.

Abbreviaitons. Abbreviations found in the HPA-HPG-HPT-immune model of **Fig. 1, 4**

ACTH	Adrenocorticotropin;
AR	Androgen Receptor
AVP	Vasopressin;
CRH	Corticotropin-releasing hormone;
DTS	Davidson Trauma Score
ER	Estrogen Receptor
FSH	Follicle-stimulating hormone;
FST	Follistatin;
GCR (R)	Glucocorticoid receptor
GH1	Growth hormone 1;
GNRH1	Gonadotropin releasing hormone 1;
GNRH2	Gonadotropin releasing hormone 2;
IFNG	Interferon gamma;
IGF1	Insulin-like growth factor 1;
IgG,	Immunoglobulin gamma;
IL-2	Interleukin 2
IL2-R	IL–2 receptor
INS	Insulin;
KISS1	Kisspeptin;
NO,	Nitric oxide;
NK cell,	Natural killer cell;
OXT	Oxytocin;
PTGS2	Prostaglandin-endoperoxide synthase 2;
SST	Somatostatin;
TGFB1	Transforming growth factor beta 1;
TNF	Tumor necrosis factor;
TNFSF13B	Tumor necrosis factor superfamily member 13B/BAFF;
TSH	Thyroid stimulating hormone;
TNFa	Tumour necrosis factor
TNFaR	TNFa receptor
Th17,	T-helper 17 cell;
Th2,	T-helper 2 cell;
Th1,	T-helper 1 cell;
Treg,	Regulatory T cell
TRH	Thyrotropin-releasing hormone;

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Table 1a. *Benchmark assessment of constraint-satisfaction*. Summary of the number of elements V and control interactions E in 5 well-documented biological regulatory networks. In each model parameters describing activation threshold W, control action polarity U, confidence P and transitional logic weight K are adjusted such that the predicted state transition graph adheres to the v entities measured at t time points for a total of F data entries. The search is further restricted to parameters whereby the number of transitions occurring between observed states is less than the bound M for synchronous and asynchronous transition rules (**Sedghamiz et al., 2019a**).

Net	$\|V\|$	$\|E\|$	F	$\|\widehat{W}\|$	$\|\widehat{U}\|$	$\|\widehat{P}\|$	$\|\mathcal{K}\|$	M	
								Synch	Asynch
HPA	4	8	14	8	2	8	46,656	10	5
IRMA	6	9	16	0	0	0	$\approx 604 \times 10^6$	50	12
Dcell	114	129	3	0	0	0	$95,268 \times 10^{135}$	5	3
HPG	5	25	16	25	18	25	3^{160}	3	8
Th	23	35	3	0	0	35	2^{100}	2	2

Female Hypothalamic-Pituitary-Gonadal axis (HPG) (Bennett *et al.*, 2013) is taken from our earlier work (Sedghamiz *et al.* (2017)); the hypothalamic-pituitary-adrenal (HPA) axis taken from (Sedghamiz *et al.* (2018)); in vivo Reverse-engineering and Modeling Assessment (IRMA) network from (Cantone *et al.*, 2009) and T-helper and Dendritic cell cycle from (Garg *et al.* (2008)). Note that the bound for model checking is different under synchronous and asynchronous time update.

Table 1b. *Search performance metrics*. Summary of the performance obtained using different solvers in terms of the normalized objective function values for regulatory selectivity (z1), dynamic responsiveness (z2), and response reliability (z3) as well as solution time under synchronous and asynchronous updating schemes (**Sedghamiz et al., 2019a**).

Net	Chuffed							Google OR-Tools							OptiMathSat (Pareto)							
	Synch			Asynch				Synch			Asynch				Synch			Asynch				
	T(s)	z_1	z_2	T(s)	z_1	z_2	z_3	T(s)	z_1	z_2	T(s)	z_1	z_2	z_3	T(s)	z_1	z_2	T(s)	z_1	z_2	z_3
HPA	0.5	0.61	0	0.6	0.45	0.08	0.11	0.81	0.61	0	0.92	0.61	0.08	0.11	265.3	0.61	0	123.4	0.45	0.08	0.11
IRMA	5.2	†	†	56.1	1	0.2	0.037	715.65	†	†	44.36	1	0.2	0.037	70.23	†	†	1800.1	1	0.229	0.035
Dcell	1.8	1	0	2.9	1	0	0.003	31.2	†	†	82.1	1	0	0.003	60.2	1	0	2700.1	1	0	0.003
HPG	3650	0.46	0.05	⋆	0.45	0.07	0.01	1020	0.46	0.05	⋆	0.42	0.06	0.01	⋆	‡	‡	⋆	0.48	0.10	0.2
Th	0.4	0.74	0	0.4	0.74	0	0	0.5	0.74	0	0.5	0.74	0	0	0.63	0.74	0	0.63	0.74	0	0

† stands for *unsatisfiable* meaning that there was no parameterization supporting the constraints. A time limit of 9000 sec was used and the parameterization tasks which did not finish computation within this time were interrupted (denoted by ⋆). ‡ represents that no solution was found within the time limit. For the HPG model non of the models found an optimal solution within the time-limit, reported solutions are the sub-optimal ones in this limit. Note that in some cases a *pareto* solution did not exist, in those cases we report the final solution reported by *OptiMathSat*. Furthermore, the objective values reported in the table are normalized. Bio-ModelChecker normalizes these values by dividing them by the maximum objective achievable in each case.

Table 2. *Benchmark networks in scale-up assessment of distributed platform*. Summary of the benchmark networks used to assess the performance the constraint-based identification of optimal model parameter sets. These networks include artificial biological networks generated by NetSim (Di Camillo et al., 2009) containing 50-100 nodes, with an average connection density of 5-10 interactions per node (κ) and where each node is regulated by 10-15 upstream mediators (Max Regulatory). The latter was varied systematically as prior anecdotal evidence had suggested (correctly) that under the current model design the number of upstream regulators would be the limiting factor in scale-up. The dependency of average connection density and the maximum number of upstream regulators was such that not all combinations of these design parameters were achievable as shown by the entries in red font below.

NetSim Parameters		Network Statistics			
κ [1]	Max Regulatory [2]	Number of Nodes	Number of Edges	Density [3]	Max Indegree
Human	COPD	11	67	6.09	9
Mouse	COPD	21	143	6.81	11
10	10	50	404	8.08	10
10	10	100	807	8.07	10
10	15	50	522	10.44	15
10	**15**	**100**	**992**	**9.92**	**15**
5	10	50	270	5.4	10
5	10	100	479	4.79	10
5	15	50	255	5.1	10 (target 15)
5	15	100	497	4.97	10 (target 15)

[1] K (kappa): mean number of edges for each node, i.e. density.
[2] Max Regulatory: maximum out degree possible in the network.
[3] Proportional to a fully connected directed graph including self loops
Bold row-no solutions within 72 hours using 256 GB of RAM.

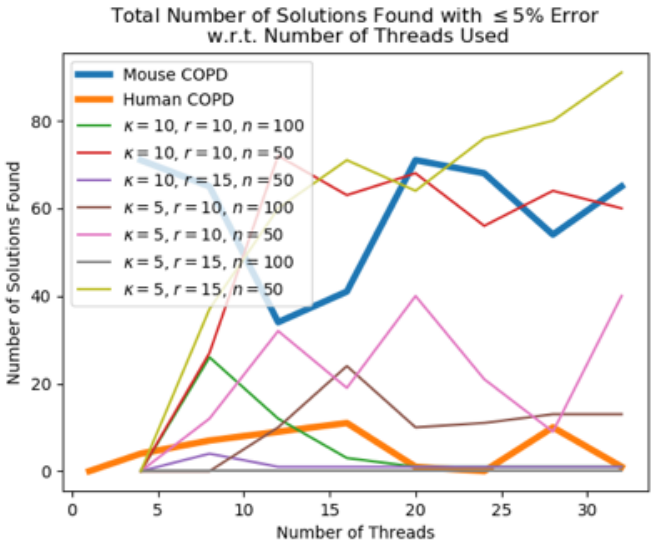

Figure 1. *Parallel scaling of OR-Tools in identifying model solutions*. Identification of model parameter sets supporting an adherence to experimental data within 5% error, using OR-Tools distributed over an increasing number of threads for two small models of lung immune response in COPD as well as artificial networks of increasing complexity (see Table 2). Results suggest that even for models with a greater number of upstream regulators and higher connection density the number of low-error candidate solutions produced in 48 hours does not increase substantially by using more than 10-15 threads.

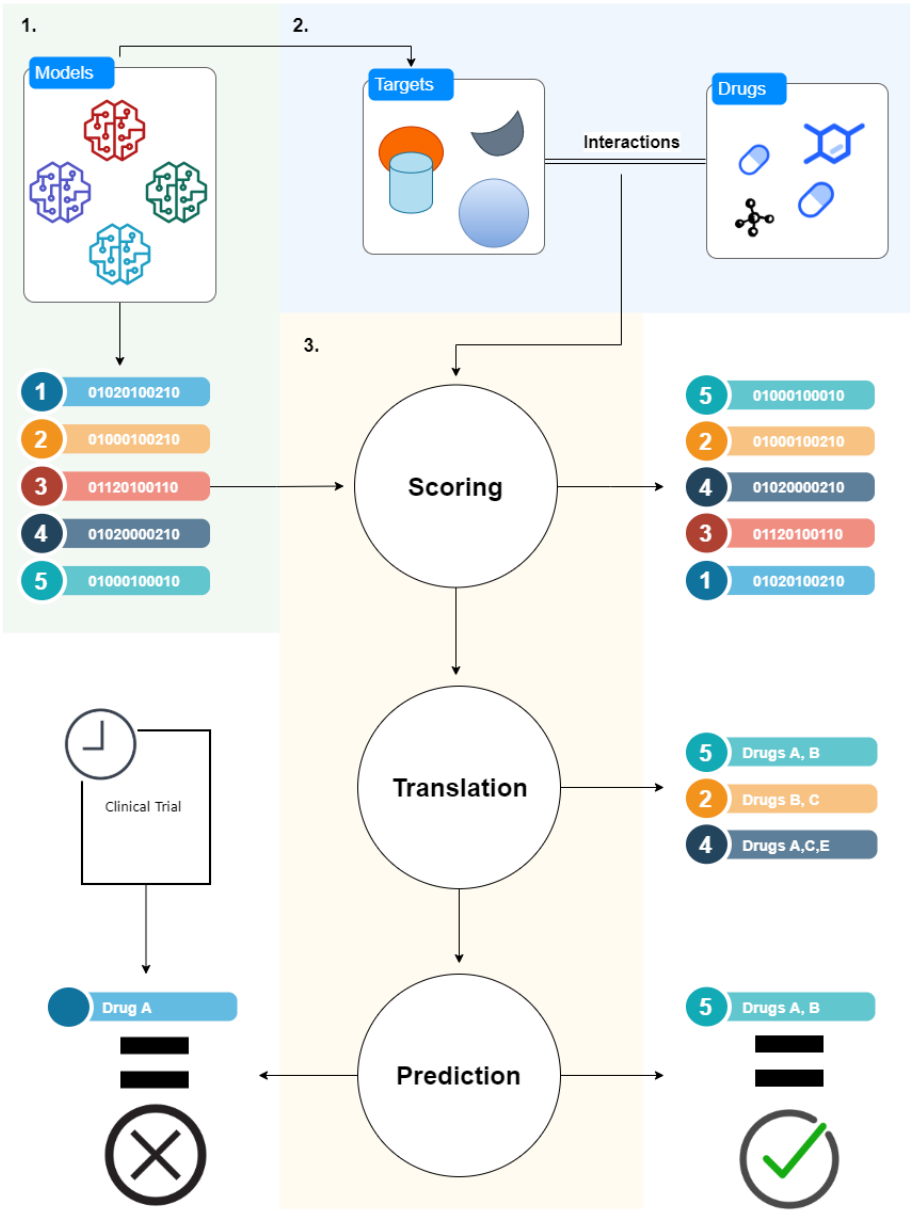

Figure 2. *Assessing the actionability of MIS solutions*. The newly implemented pipeline assembles intervention sets predicted by multiple competing models then rationalizes these into unique intervention instances. Each intervention instance is scored according to parsimony, consensus across models, and other criteria. The intervention sets are re-prioritized according to actionability and translated from target sets into drug combination sets (Richman et al., 2019 dissertation proposal).

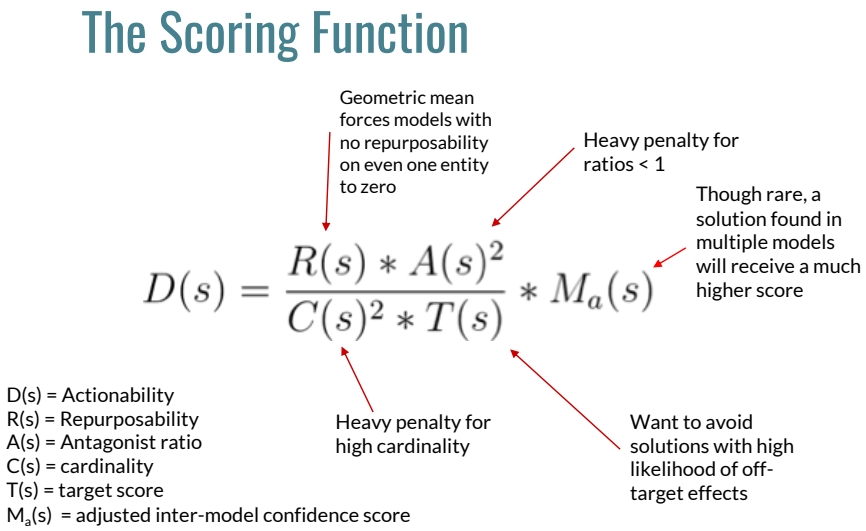

Figure 3. *Computing an actionability score*. Each minimal set of intervention targets (*s*) are assessed for its pharmaceutical actionability (*D(s)*) or the potential for translating targets into a combination therapy using repurposed drugs or known medicinal compounds. Target sets for which there are no approved drugs or known compounds are assigned a zero score.