

“Its All Made Up” — Why We Should Stop Building Representations Based on Interpretive Models and Focus on Experimental Evidence Instead

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Abstract

At present, the distinction between interpretive and observational assertions is not made strongly in biomedical informatics knowledge representation (KR) research. In this position paper, we draw from examples in a small number of biomedical domains to advocate the importance of developing systems based primarily on scientific evidence rather than interpretations. As an explanatory methodology, we base this argument primarily on a worked example from a cancer biology study and previous work in knowledge engineering. We emphasize how interpretative models that form the basis of scientific theories are themselves *created* to fit, explain and predict experimental data. Within an empirically-driven field such as biomedicine, scientists typically use a rich, intuitive understanding of experimental methodology directly in their work and developing methods to incorporate that expertise into informatics systems remains a challenging but important goal.

Motivation: The Importance of Experimental Methodology to Biomedical Knowledge

Progress and discovery in biomedical research is driven largely by the development of new experimental techniques rather than breakthroughs in theory. For example, in the 1970s, neuroanatomical tract-tracing experiments permitted scientists to trace both the start- and end-points of neuronal projections in animal brains by carefully injecting tracer chemicals into targeted brain regions and then observing how the tracer was actively transported along axonal processes (Blackstad, Heimer, and Mugaini 1981). At the time, this technique was revolutionary and to-date has provided the highest quality information concerning brain *macro-connectivity* data (*i.e.*, region-to-region neuronal projections). Previous to this, the state-of-the-art involved inflicting focal lesions on brain tissue and then observing axonal degeneration in the tissue. This cruder technique was widely used in the field and a comparative retrospective of hypothalamic connections involving both techniques revealed that *only 51% of lesion studies were correct when validated by later techniques* (Bota, Dong, and Swanson 2003).

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The mapping from observation to interpretation is complicated by confounding aspects of the techniques (in ways that only come clear after the technique has been used for some time). For some tract-tracers, ‘fibers of passage’ cause false positives by axons passing through the injection site to transport label that had effectively originated part-way along a projection. Other tracers transport label across the synaptic connections between cells, labeling neurons with no direct projection to or from the injection site. Other techniques might involve tracers moving in both an *anterograde* and *retrograde* direction simultaneously. All of these complications can easily be resolved *in the minds of expert researchers*, who present fully formed interpretive conclusions as the main outcomes of their work (and the primary candidates for curation and retrieval in informatics systems). In our neuroanatomical example, researchers would highlight neural projections in a circuit connecting large-scale brain structures (and may, if prompted, provide some details of the histological maps that support their findings as supporting evidence). These interpretive assertions then form the primary data elements of informatics systems and the basic building blocks for AI-inspired computation.

These interpretive models are *abstractions* that represent the prevailing perspective of researchers as possible explanations for observed phenomena. They may be based on incorrect or incomplete assumptions and are likely to need on-going updates and revisions.

In this position paper, we argue that scientific informatics systems *typically only emphasize the main interpretive findings of studies* and omit the crucial reasoning of how conclusions are drawn from experimental data. We rely entirely on human expertise to formulate interpretive models within any given subdomain (perhaps as ontologies or data exchange formats), but rarely (if ever) explicitly capture the underlying supporting data to the level of precision used by laboratory scientists. The task of understanding how interpretive models (such as neuroanatomical circuits or molecular pathways) may be automatically inferred from experimental observations is an unsolved AI challenge problem. We suggest that *abductive reasoning* methods built over a representation of experimental statistics could provide the foundation for machine reasoning technology capable of generating hypotheses from scientific data.

Three-level Organization in Biomedical Knowledge Engineering

Figure 1 illustrates our basic premise with a depiction of three organizational levels: ‘Context’, ‘Interpretive Models’ and ‘Experimental Observations’ (with exemplars taken from two research domains: neuroanatomical macro connectivity and molecular cancer biology).

Domain	Neuroanatomical Macroconnectivity	Molecular Cancer Biology
Context	Neurophysiology + behavior	Cellular + Tissue + Organ Level Cancer Processes
Interpretive Models	e.g., ‘Foundational Model of Structural Connectivity’	Pathway models + formats (BioPax, SBML, SBGN, etc.)
Experimental Observations	e.g., Tracers (PHAL, HRP), atlas registration, etc.	e.g., Northern blot, knock-out protocols, etc.

Figure 1: A three-level KR organizational scheme in two example domains.

In both domains, there exist ‘standard’ interpretive models: the ‘Foundational Model of Structural Connectivity in the Nervous System’ (FMC) for neural connections (Swanson and Bota 2010) and BioPax, the Systems Biology Markup Language (SBML), and Graphical Notation (SBGN) for molecular pathways. These representations act as a data interchange format (BioPax), a standard set of elements for simulation (SBML) or as a standardized notation for diagrams (SBGN). They do not yet support reasoning to emulate the way that scientists construct theories from data or apply those theories in a broader context across the subject, but nonetheless are the best available shared KR structures to begin the process of formulating such theoretical frameworks in their respective fields.

The distinction between interpretations and observations can be elucidated based on models of macro-connectivity (Russ et al. 2011). The FMC uses a graph abstraction where nodes correspond to grey matter regions in the brain and edges correspond to projections between regions. The presence of an edge asserts that *there exists a subpopulation of neurons with cell bodies located in the origin region and axons that synapse onto neurons that have their soma located in the edge’s target region*. Under a strict policy concerning evidence supporting adding an edge to such a graph, we might require that (A) at least one experiment using high-quality anterograde tracers visualize terminal boutons in the target region; (B) at least one retrograde study map out the originating cells for the projection in the starting region; (C) suitable control injections ensure that none of the regions surrounding the injection site could be the actual source or target of the projection; and (D) ultrastructural studies confirm that axonal terminal boutons actually correspond to anatomical synapses. Satisfying these criteria in a consistent way across all studies in a database is challenging (due to data heterogeneity, variable standards of reporting and the effort required to curate published information to such a high standard). Therefore, the presence of an edge in an FMC model is so highly dependent on the experimental observa-

tions on which it is based, *it should never be considered in isolation from those observations*.

Thus, for the purpose of applying AI technology to biomedical knowledge, it is crucial that we construct practical representations of experimental observations not just as a means to code the reliability of interpretive assertions (as is currently the case with ‘evidence codes’ in BioCyc or Gene Ontology), but as a framework for reasoning over available evidence to generate and validate interpretative models. Each domain requires specialized, nuanced knowledge to generate correct hypotheses that fit the constraints from available data. We assert that the crucial step of evaluating this technology depends on whether we are able to *generate predictions that are then testable scientifically*. This ideal has already been realized by ‘robot scientists’ developed for yeast metabolic biochemistry (King et al. 2009), but must now be generalized across less well-defined domains. We examine studies of molecular pathways involved in cancer.

Pan et al. 2013, a typical molecular biology experimental paper.¹

Molecular biology studies involve a large number of small-scale assays. For example, (Pan et al. 2013) uses ~20 separate small-scale experiments where measurements appear as images (e.g., of a blot assay) or graphs (to show statistical effects) and direct measurements may not be reported explicitly in the text. This paper elucidates the dynamic action of the HMG box-containing protein 1 (HBP1) on gene expression of DNA methyltransferase 1 (DNMT1) and its role in senescence by a dual action of both activating and repressing the cyclin-dependent kinase inhibitor p16. In Figure 2, we render the paper’s main summary as an SBGN gene regulation network based on our understanding of the paper’s findings.

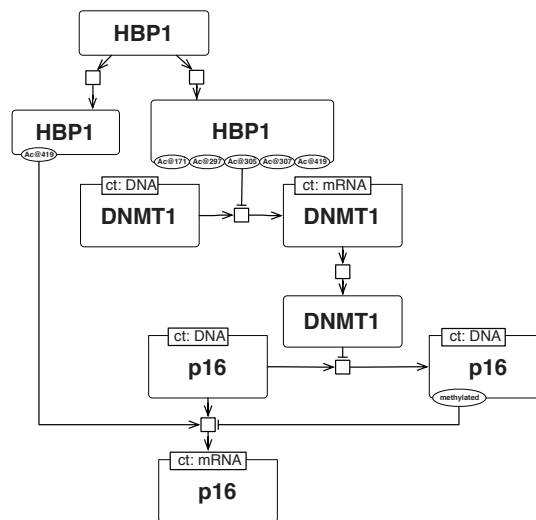


Figure 2: The findings of Pan et al. 2013 as an SBGN gene regulation network.

¹This paper was cited in the Broad Agency Announcement of the 2014 DARPA ‘Big Mechanism’ program.

With omissions, the paper’s explanation of this model reads:

“We envision that HBP1 represses the DNMT1 promoter through sequence-specific binding (...) and that the activity of HBP1 itself is regulated through acetylation at any of 5 sites in the protein... The HBP1-mediated repression of the DNMT1 gene then decreases overall DNA methylation. On the p16 gene, HBP1 expression leads to a similar DNA hypomethylation, but HBP1 instead binds to putative HBP1 activation element (...) to give activation. While HBP1 alone can partially activate the p16 gene, full transcriptional activation of the p16 gene requires hypomethylation and an HBP1 acetylation at K419”

This interpretation is intended as the best possible explanation of data presented in the results section of Pan *et al.* 2013. This can be broken down into the following elements (numbered corresponding to figures where supporting data was reported).

- Background informatics-driven research (*This establishes correlative relationship between HBP1 and DNMT1 expression*)
 - An inverse correlation between HBP1 and DNMT1 expression in public databases for cervical and ovarian cancer.
 - Bioinformatics predicts a high affinity HBP1 site in the DNMT1 promoter
- Experiment 1A: examine expression of HBP1 and DNMT1 in fibroblasts at different times in progression to senescence (*This establishes the relationship of HBP1 / DNMT1 to aging*).
 - HBP1 levels increases with replicative senescence.
 - DNMT1 levels decrease with population doubling levels.
 - DNMT3A and DNMT3B levels are unchanged.
- Experiment 1B: express HBP1 through retroviral infection (*This indicates how HBP1 acts on DNMT1 expression*).
 - Exogenous HBP1 expression reduced DNMT1 protein and mRNA but had no effect on DNMT3A or DNMT3B.
- Experiment 1C: use short hairpin RNA to knock down HBP1 gene (*This provides an indicator of how HBP1 acts on the expression of DNMT1, DNMT3A, DNMT3B, p16 and p21.*).
 - HBP1 knockdown increased DNMT1 protein and mRNA levels but had no effect on DNMT3A or DNMT3B.
 - HBP1 knockdown shows increased methylation of p16 and p21 promoters.
- **Experiment 2C: express HBP1 or DNA-binding defective mutant of HBP1 (pmHMG)² (*This establishes that a HBP1 sub-sequence is needed to suppress DNMT1 expression*).**
 - **Wildtype HBP1 over expression suppressed DNMT1 protein level.**
 - **An HBP1 mutant without the binding site (pmHMG) had no effect on DNMT1 protein relative to controls.**
- Experiment 2D/E/F: use DNMT1 promoter luciferase reporters with either intact DNMT1 or DNMT1 with the HBP1 binding site deleted (*This investigates further the specific HBP1 sequence needed to suppress DNMT1 expression*).
 - Wildtype HBP1 expression suppressed DNMT1 protein level.
 - HBP1 had no effect on the DNMT1 promoter that lacked the HBP1 binding site.

²The entry for Experiment 2C is emphasized since we refer to it in the next section

– pmHMG had no effect on native or mutant DNMT1 promoter.

- Further Experiments 3A-B, 4A-E, 5A-D, 6A-F, 7A-E, 8A-D deal with locating the binding sites, the effects of methylation, interactions with p16 and p21, processes related to senescence, HBP1 acetylation and effects on the senescence phenotype.

This evidence provides constraints that the interpretive model shown in Figure 2 attempts to explain as *one possible interpretation*. By listing these specialized findings here, we now showcase an AI-driven approach to find, enumerate and compare other possible explanations for these data.

Representing experimental evidence.

‘Knowledge Engineering from Experimental Design’ (KEfED) uses a simplifying assumption: a measurement is dependent on a given parameter if that parameter lies on a path that can be traced back to the start of the protocol (Russ *et al.* 2011). KEfED generates a data schema from a flowchart-based, process model. Typically, each experimental type has a restricted set of interpretations, given its input parameters and measurements. Two excerpts from Pan *et al.* 2013 show how KEfED can be applied (Figure 3).

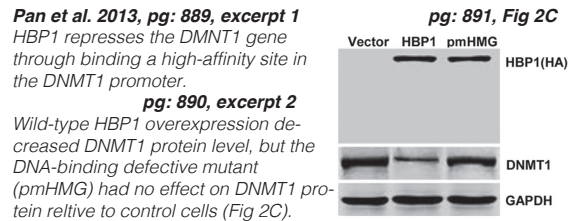


Figure 3: Some excerpts from the text and figures of Pan *et al.* 2013 pertaining to the results of experiment 2C.

Excerpt 1 is an interpretation and excerpt 2 describes its experimental foundation where protein levels of DNMT1 were measured in cells transfected to express either wild type HBP1 or a HBP1 mutant whose HMG box DNA binding domain (amino acids 431 to 509) had been removed (‘pmHMG’). The key interpretation is that the comparative density of lines on the blot for DNMT1 expression (2nd row) between the pmHMG knockout and the ‘Vector’ cases are the same, but the column for HBP1 is diminished. We show a KEfED model of this data in Figure 4.

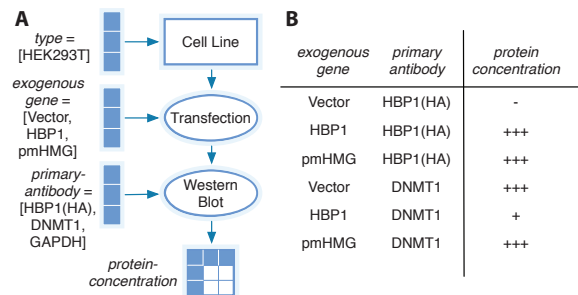


Figure 4: KEfED models and data for experiment 2C from Pan *et al.* 2013.

This KEfED model provides a simple, powerful explanatory structure for this data that could be instantiated as an ‘experimental motif’ for knockout experiments that could be reused by changing the values of the parameters shown. This could be used to identify and systematize these motifs for the most common patterns of reasoning used for specific molecular biology assays.

These motifs are typically ‘coarser’ than a description designed to allow a scientist to reproduce the experiment in the laboratory (which would require details of all steps described in full rather than specifying an assay type such as ‘Western Blot’ with its main parameters). Capturing the confounding variables that govern reproducibility will require that motifs be expanded to capture the relevant variables.

Abductive Reasoning.

The reasoning used by scientists to connect observations to explanations (or models) is one of abduction, which is *inference to the best explanation* (Peirce 1958). The relationship between a model M , an experimental context E and predicted observations O can be written logically as:

$$M \wedge E \Rightarrow O$$

Using such a formulation, we can then use reasoning technology, such as PowerLoom, to link observations with interpretations. In a validation scenario, we exploit this implication in the forward direction to test whether a considered model explains the data. If we know the model M we want to test and that M implies O under certain conditions E we can test whether the predicted observations O describe all relevant actual observations in the data. Usually, we only know about O and want to find out about M relative to experimental conditions E . Abduction tells us that M is a possible explanation of O if it does not lead to some inconsistency with other things known to be true. So, that ‘*HBP1 regulates DNMT1*’ is a possible explanation for observed outcomes, it is consistent with the data.

Generally, abductive reasoning will lead to several competing alternative explanations, especially when M and E are sufficiently complex and only constrained by data from within a single study (such as Figure 2). Such alternative explanations can be useful to overcome interpretation bias and provide alternative views on what data might mean. However, that usefulness is predicated on explanations being plausible, which requires some realistic evaluation scheme to measure the cost of alternative models and assumptions.

Our contention is that by explicitly modeling the observations from many experiments across studies and making them available, we would greatly increase the number of constraints available to restrict the number of plausible models. This would also provide an incentive for scientists to publish negative findings since these could equally well act as model constraints.

Practical challenges of this approach

There is a wealth of knowledge in the results sections of papers and the laboratory notes of researchers that is largely

untapped by existing informatics technology. This knowledge could be applied to constrain abductive reasoning systems if it could be captured accurately and efficiently.

Building the basic knowledge representation for a given domain requires a significant amount of background knowledge. These entity and element definitions can be gleaned from existing ontological resources, but in order to mirror inferences drawn by molecular biologists, the system must also be programmed with implicit, basic molecular biology formulations. It would also need to reason over different types of manipulations and assays and how they are used as part of experimental patterns such as, ‘a gene knockout experiment’. While some of this knowledge is quite complex, we anticipate that scoping to a specific subdomain would limit the number of experimental techniques to be modeled and permit us to build a sufficient representation to capture the meaning of quite specific elements (such as the use of a mutant protein with an altered sequence and its corresponding wild type protein in a gene knockout experiment).

Conclusions

In this position paper, we argue for a reasoning-driven architecture for biomedical knowledge engineering based on experimental observations rather than interpretations. We present a preliminary approach for such a model (the KEfED methodology with logical ‘motifs’ for experimental types augmented with abductive reasoning) and provide an example from molecular biology.

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