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Metabolism Regulation Atlas: linking signalling and metabolism through post-translational regulation of metabolic enzymes

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Metabolism Regulation Atlas: linking signalling and metabolism through post-translational regulation of metabolic enzymes

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Abstract

Background/Motivation: Advances in comprehensive reconstructions of human metabolism and signalling pathways provide a basis for systematic analysis of mechanisms connecting cell signalling to metabolic reactions. At the same time, information about these connections is scattered across different publications and pathway resources, limiting integrated mechanistic interpretation.

Results: Metabolism Regulation Atlas (MRegAtlas, https://metabolismregulation.github.io) was developed as a mechanistic bridge between signalling and metabolism, representing the regulatory context around metabolic enzymes. The maps in MRegAtlas link upstream signalling events, particularly post-translational modifications, to downstream metabolic reactions. The resource contains manually curated maps including regulation of glycolysis, glycogen metabolism, mevalonate metabolism, cholesterol metabolism, eicosanoid metabolism, nitric oxide production and phosphoinositide metabolism. The 21 maps comprise 230 processes and 546 nodes, including 190 proteins and 227 metabolites. Maps are represented in SBGN Process Description and are available for interactive exploration online via the MINERVA Platform.

Methods: The initial set of 17 maps in MRegAtlas were built from candidate connections identified by manual literature search. By combining Reactome Neo4j and Recon2 in Neo4j, we then implemented a method to systematically identify connections between metabolic and signalling networks with the focus on the regulation of metabolic enzymes. The identified connections were compared with the 17 maps, and four new maps were developed based on the candidates identified by the Neo4j framework: pyruvate dehydrogenase, branched-chain amino acid catabolism, urea cycle and nNOS maps.

Conclusion: MRegAtlas provides manually curated maps that link signalling events to metabolic enzymes. The maps can be used for visual analysis, education and computational model development, and the Neo4j framework is designed for continued mapping of the signalling mechanisms that regulate metabolic reactions.

Keywords: Systems Biology, Metabolism Regulation, Graph Databases, Pathways

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1. Introduction

Signalling pathways link extracellular and intracellular signals to changes in metabolic enzyme activity and metabolic reactions [1–3]. Functional state of metabolic enzymes is regulated through post-translational modifications (PTMs) and particularly phosphorylation [2, 4, 5]. Systems-level analyses demonstrate the role of PTMs in metabolism regulation [2, 3]. Integrating signalling and metabolic networks can therefore identify regulatory points and capture the corresponding mechanisms connecting upstream signalling processes to metabolic reactions [6, 7].

Advances in comprehensive reconstructions of human metabolism [8, 9] and detailed representation of signalling pathways in Reactome [10, 11] make it possible to systematically explore connections between signalling and metabolic networks. Graph-database implementations further support this integration, with metabolic pathways represented in Recon2Neo4j [12] and signalling pathways in the Reactome Neo4j database [13]. Previous integration efforts, including Recon2-ACSN, demonstrated the value of connecting signalling and metabolic pathway representations in cancer [6]. At the same time, the regulatory layer linking upstream signalling to PTMs of metabolic enzymes and the downstream reactions they control remains only partially represented and distributed across pathway resources and individual publications.

We present the Metabolism Regulation Atlas (MRegAtlas, https://metabolismregulation.github.io) that organises signalling–metabolism connections into curated visual representations of the regulatory mechanisms. This resource brings together upstream signalling mechanisms, regulatory events and downstream metabolic reactions in unambiguously represented mixed signalling–metabolic pathway maps designed for exploration, reuse, updates and extensions. Candidate connections are identified through manual literature and pathway-database searches and through computational analysis of Reactome Neo4j and Recon2 in Neo4j, with priority given to PTM-related processes. The maps are represented in SBGN Process Description [14, 15] and published through MINERVA [16]. Together, the diagram collection and cross-resource methodology provide a unified framework for unambiguously represented signalling–metabolic pathway maps.

2. Results

MRegAtlas was developed through converging two efforts: 1) manual reconstruction of curated signalling–metabolism maps, and 2) systematic identification of candidate regulatory connections through analysis of Reactome Neo4j and Recon2 in Neo4j. The first effort produced

the first 17 maps. Then the analysis identified new candidates, and four of them were developed into new maps (Section 2.3). The first resulted in a collection of mechanistic maps linking upstream signalling events and regulatory mechanisms to metabolic enzymes and downstream reactions. The second identified proteins at the interface of signalling and metabolism and provided a reproducible set of candidate connections for further curation and map development. Together, these efforts combine expert-curated pathway reconstruction with systematic discovery of signalling–metabolism connections.

2.1. A collection of metabolism regulation diagrams

MRegAtlas includes 21 manually curated maps containing 546 nodes and 230 processes, including 227 metabolites, 190 proteins and 107 complexes. 17 of 21 maps come from the manual search and four were developed from candidates of the systematic analysis. Detailed map-level statistics and the complete species-class breakdown are provided in Supplementary File S1. This collection of maps covers regulatory mechanisms of different complexity. Most maps centre on PTM-mediated regulation, particularly phosphorylation, while selected maps also represent transcriptional control, proteolytic processing, metabolite-dependent regulation and covalent drug action. A brief description of the maps is included in Table 1.

Table 1. Brief descriptions of the metabolism regulation diagrams. References for diagrams are available at https://metabolismregulation.github.io.

Pathway name	Brief description of key mechanisms
Regulation of glycolysis	6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2 (PFKFB2) regulates rates of glycolysis and gluconeogenesis by phosphorylation of fructose-6-phosphate and dephosphorylation of fructose-2,6-bisphosphate.
Regulation of glycogen metabolism	Hormonal and calcium-dependent signalling converge on phosphorylation of glycogen synthase and glycogen phosphorylase. GYS1 and PYGM control glycogen synthesis and phosphorolysis in muscle. The corresponding liver enzymes are GYS2 and PYGL, shown in a separate liver map.
Regulation of acetyl-CoA synthesis	RTK–PI3K–AKT signalling phosphorylates ATP citrate lyase (ACLY), linking growth-factor signalling to cytosolic acetyl-CoA production. SREBP1 (SREBF1)-dependent transcriptional regulation is also represented.
Regulation of acyl-carnitine synthesis	AMPK-dependent phosphorylation of ACACB regulates malonyl-CoA synthesis and thereby the CPT1-controlled formation of acyl-L-carnitine.
Regulation of triacylglycerol hydrolysis	cAMP/PKA and cGMP/PKG signalling phosphorylate HSL (LIPE) and perilipin 1 (PLIN1), linking second-messenger signalling to lipolysis. HSL acts prominently on diacylglycerol within the sequential hydrolysis of stored triacylglycerol.
Regulation of mevalonate synthesis	AMPK-dependent phosphorylation of HMGCR regulates the conversion of HMG-CoA to mevalonate, linking cellular energy signalling to mevalonate synthesis.
Regulation of cholesterol metabolism via SREBP	Sterol-dependent SCAP–INSIG retention versus COPII-mediated trafficking controls SREBP2 (SREBF2) processing by S1P (MBTPS1) and S2P (MBTPS2) and nuclear activation of cholesterol-biosynthesis genes.
Regulation of estradiol biosynthesis via prostaglandin-cAMP signalling	Prostaglandin E2-Gs-adenylate cyclase-cAMP-PKA signalling activates CREB-dependent transcription of STAR. STAR-mediated mitochondrial cholesterol transfer and CYP11A1/P450scc initiate steroidogenesis by producing pregnenolone.
Regulation of glutaminolysis via the NF-κB-PKCε axis in lung cancer cells	PKCε (PRKCE)-dependent phosphorylation of glutaminase C (GAC, an isoform of GLS) regulates conversion of glutamine to glutamate; NF-κB-dependent transcriptional regulation is also

	represented. Glutamate can subsequently be converted to α-ketoglutarate, linking glutaminolysis to the tricarboxylic acid cycle.
Regulation of pyrimidine biosynthesis via MAPK signalling	ERK1 (MAPK3)/ERK2 (MAPK1)-dependent phosphorylation of CAD regulates the carbamoyl-phosphate-forming step of de novo pyrimidine biosynthesis.
Regulation of phosphoinositide metabolism	PI3Kα (PIK3CA) phosphorylates PI(4,5)P2 to generate PI(3,4,5)P3, while PTEN catalyses the reverse dephosphorylation. The map also includes further phosphoinositide interconversions.
Regulation of cAMP hydrolysis	PI3K–AKT-dependent phosphorylation of PDE3B regulates cAMP hydrolysis to AMP, connecting upstream signalling to cAMP/PKA activity.
Regulation of nitric oxide production - iNOS pathway	IFNG–JAK–STAT1/IRF1 signalling induces NOS2 expression, while calmodulin-bound iNOS (NOS2) catalyses nitric oxide production from arginine.
Regulation of nitric oxide production - eNOS pathway	Endothelial nitric oxide synthase (eNOS, NOS3) is important for the production of nitric oxide. With calmodulin/HSP90 and membrane-associated regulators, AKT-dependent phosphorylation of eNOS controls nitric oxide production.
Regulation of eicosanoid production	The map links RAF–MEK–ERK signalling to phosphorylation of cPLA2α (PLA2G4A), promoting arachidonic acid release for downstream eicosanoid synthesis.
Regulation of eicosanoid metabolism by aspirin	The map represents acetylation of COX-1 (PTGS1) and COX-2 (PTGS2), suppression of canonical prostaglandin synthesis and the altered COX-2 branch producing 15R-HETE.
Regulation of pyruvate dehydrogenase via PDK and PDP	Pyruvate dehydrogenase kinases (PDK1–4) inactivate the pyruvate dehydrogenase complex (PDH) by phosphorylation of PDHA1, and the phosphatases PDP1 and PDP2 reactivate it, controlling the conversion of pyruvate to acetyl-CoA.
Regulation of branched-chain amino acid catabolism via BCKDK and PPM1K	BCKDK-dependent phosphorylation inactivates the branched-chain α-keto acid dehydrogenase complex (BCKDH), the rate-limiting step of leucine, isoleucine and valine catabolism, and the phosphatase PPM1K reactivates it. Branched-chain α-keto acids inhibit BCKDK.
Regulation of the urea cycle via CPS1	SIRT5-dependent desuccinylation and deglutarylation activate carbamoyl phosphate synthetase 1 (CPS1), which catalyses the rate-limiting step of the urea cycle, the mitochondrial synthesis of carbamoyl phosphate. CPS1 requires N-acetylglutamate, made by NAGS from acetyl-CoA and glutamate.
Regulation of nitric oxide production via nNOS	Ca2+/calmodulin activates neuronal nitric oxide synthase (nNOS, NOS1). CaMKII-dependent phosphorylation and binding of the plasma membrane Ca2+ pump PMCA4b (ATP2B4) inhibit nNOS and limit nitric oxide production.

In order to facilitate their usability, maps are available in several formats, including the standard Systems Biology Graphical Notation Markup Language (SBGN-ML) [17] and GraphML, compatible with the yEd Editor (https://www.yworks.com/products/yed). In addition, maps can be explored directly in several well-established systems biology platforms such as Newt [18] and MINERVA [16]. Newt is an open-source, freely available web-based software for the development and analysis of biological diagrams available in systems biology standard formats, including Systems Biology Markup Language (SBML) and SBGN. The MINERVA platform is a web server specifically designed for the exploration of molecular interaction diagrams, providing visual analytics through interactive browsing, connections to external databases, and customisable visualisation workflows (https://minerva.pages.uni.lu/doc).

2.2. Example metabolism regulation maps

With the following example maps we showcase how the connections between cell signalling and metabolism are represented in our resource. The examples demonstrate: 1) a direct

signalling–metabolism connection in glycolysis, 2) integration of multiple regulatory inputs around a metabolic process in glycogen metabolism, and 3) direct covalent drug action of aspirin on metabolic enzymes. For each, we name the link on the map at which a disease process acts.

Regulation of glycolysis via a bifunctional enzyme. Figure 1 shows how signalling regulates glycolysis via the bifunctional enzyme PFKFB2. PFKFB2 is unusual as it catalyses two opposing reactions: synthesis of fructose-2,6-bisphosphate from fructose-6-phosphate through its PFK-2 activity and degradation of fructose-2,6-bisphosphate through its FBPase-2 activity [19]. Fructose-2,6-bisphosphate is an allosteric activator of PFK1 and promotes glycolysis [19]. Conversion of fructose-2,6-bisphosphate back to fructose-6-phosphate lowers fructose-2,6-bisphosphate levels and reduces stimulation of glycolysis. The mechanism provides a direct connection between cell signalling and metabolic regulation: adenylate cyclase generates the second messenger cAMP, which activates PKA. PFKFB2 can be phosphorylated by PKA [20]. Phosphorylation of PFKFB2 increases its PFK-2 activity, favouring fructose-2,6-bisphosphate formation and glycolysis [21]. The map is also relevant to disease-focused applications. For example, the PFKFB2 regulatory node is directly relevant to prostate cancer metabolism: in androgen-dependent prostate cancer cells, androgen receptor signalling increases PFKFB2 expression, while PI3K–AKT signalling increases PFKFB2 phosphorylation and PFK-2 activity, thereby supporting glucose utilisation and de novo lipogenesis [22–24]. The disease process acts on the link drawn on the map, phosphorylation of PFKFB2, with AKT as the kinase. Locking PI3K or knocking down PFKFB2 severely reduces glucose uptake and lipogenesis in these cells [22].

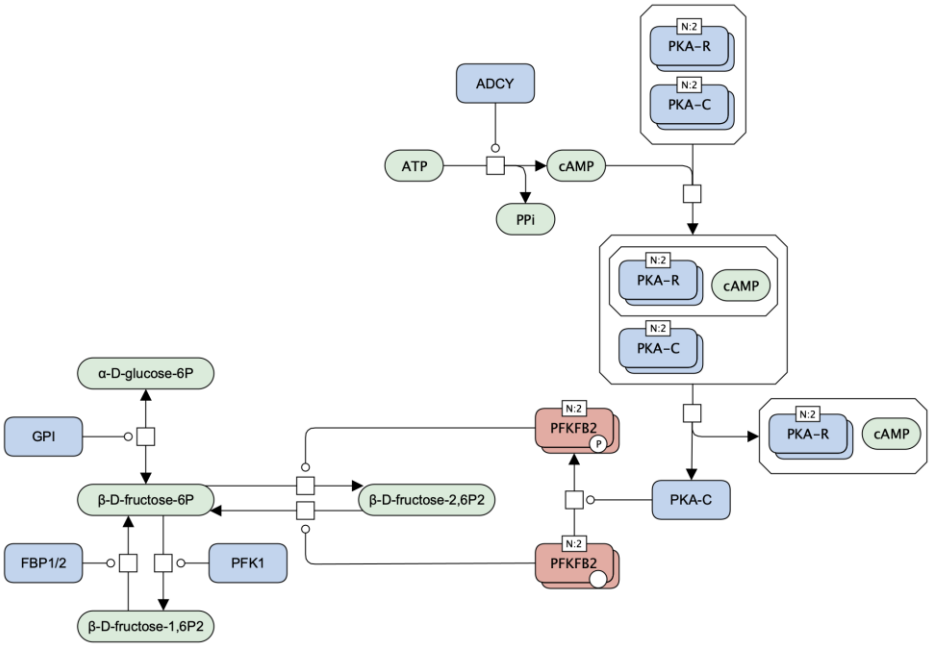


Figure 1. Regulation of glycolysis via a bifunctional enzyme. This map is available in various XML and image formats at https://metabolismregulation.github.io/glycolysis. Metabolites are shown in green, proteins in blue and the key metabolic enzyme is highlighted in red as the signalling-metabolism interface.

Regulation of the glycogen metabolism. Figure 2 shows how multiple signalling inputs regulate glycogen metabolism in skeletal muscle via glycogen synthesis and breakdown. Muscle glycogen provides a source of glucose for ATP production during contraction. The map includes

hormonal and intracellular regulatory mechanisms. In skeletal muscle, glycogen metabolism is regulated by insulin signalling and adrenergic signalling that coordinate glycogen synthesis and breakdown [25, 26].

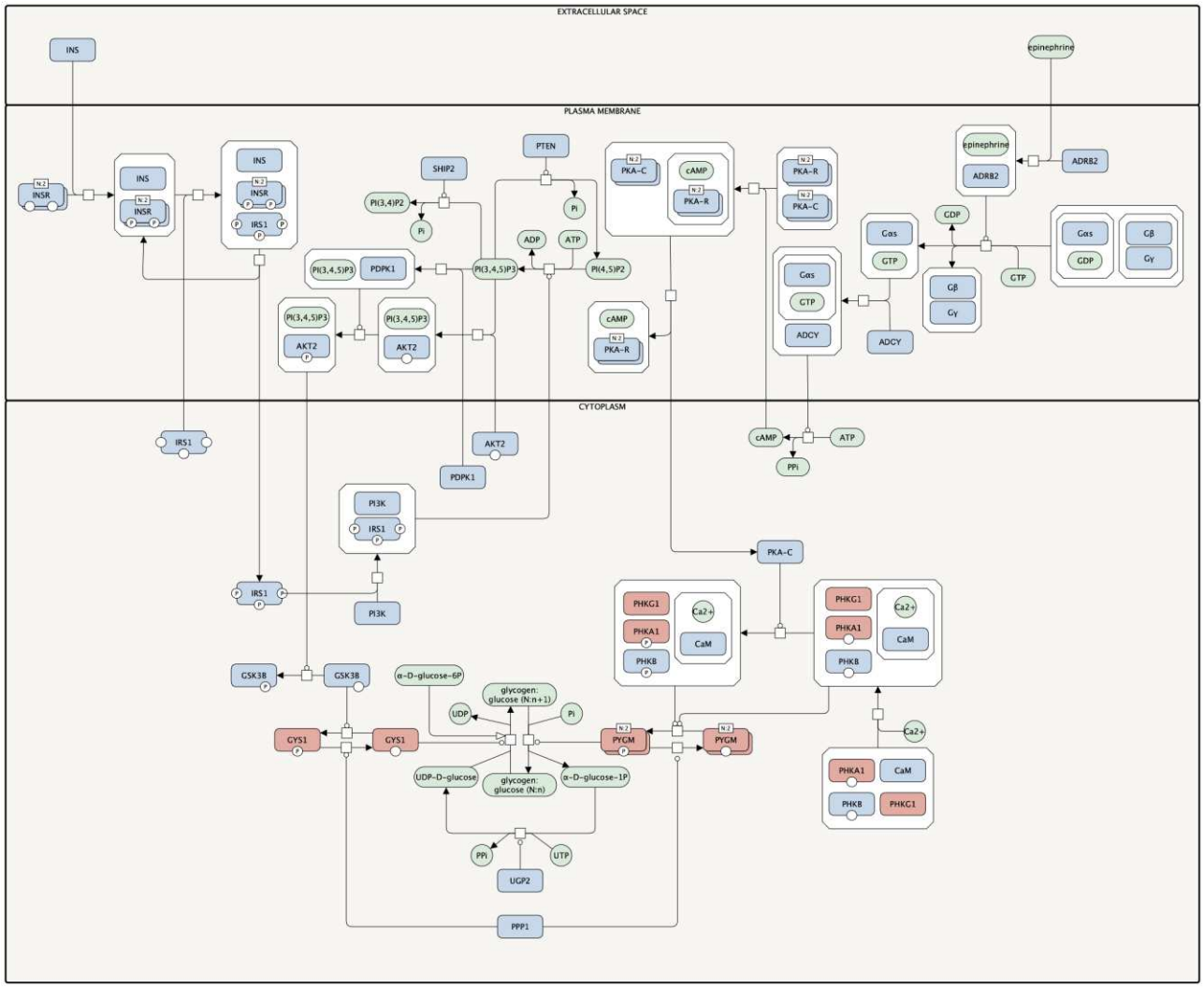


Figure 2. Regulation of glycogen metabolism in muscle. This map is available in various XML and image formats at https://metabolismregulation.github.io/glycogen. Metabolites are shown in green, proteins in blue, and proteins of interest are highlighted in red, including key metabolic enzymes and cell-type-specific proteins PHKA1 and PHKG1.

Glycogen storage and release are controlled by two antagonistic enzymes. Glycogen synthase (GS; GYS1 in muscle) is responsible for increasing glycogen storage by adding activated glucose molecules to the glycogen polymer, while glycogen phosphorylase (GP; PYGM in muscle) catalyses the breakdown of glycogen to glucose-1-phosphate [26, 27]. During the storage of glucose in the form of glycogen, UTP is used to form UDP-glucose, which provides the activated glucose used for chain elongation. Therefore, glycogen storage needs energy and to avoid futile cycles the activities of GS and GP are antagonistically regulated. Regulation proceeds via phosphorylation and dephosphorylation by multiple complementary kinases and phosphatases, which integrate cellular metabolic state and energy demand with extracellular signalling of global metabolic demand and supply via hormones. Dephosphorylation of GS and GP proceeds via protein phosphatase 1 (PPP1), which activates glycogen synthase and simultaneously inactivates GP. Adrenergic signalling through epinephrine activates adenylate cyclase, increasing cAMP and activating protein kinase A (PKA). PKA activates phosphorylase

kinase, whose catalytic γ subunit in muscle is PHKG1. Phosphorylase kinase then phosphorylates and activates glycogen phosphorylase (PYGM) and promotes glycogen utilisation [25, 28]. Insulin activates the insulin receptor (INSR), which phosphorylates IRS1. IRS1 recruits PI3K, and the resulting PI(3,4,5)P3 brings together PDPK1 and AKT2, required for glucose metabolism in human skeletal muscle cells [29–31]. The insulin-stimulated activity of AKT2 inactivates glycogen synthase kinase 3 (GSK-3; GSK3B), reducing inhibitory phosphorylation of glycogen synthase and favouring glycogen synthesis [32]. Glucose-6-phosphate activates glycogen synthase allosterically [33]. By these mechanisms, hormonal signalling mediates for example, nutritional information to the cells and balances nutrient supply with expected demand, with insulin favouring glycogen storage and adrenergic signalling favouring glycogen utilisation in skeletal muscle [25].

These hormonal signals can be complemented by intracellular signals. Calcium is an important signalling molecule, which binds to calmodulin within phosphorylase kinase and activates the kinase; phosphorylase kinase then phosphorylates and activates PYGM, thereby activating glycogen breakdown [28, 34]. This mechanism is especially important in muscle where calcium levels increase during muscle activity and regulate demand-matching glycogen utilisation. GS and GP are both controlled by inter-conversion (phosphorylation/dephosphorylation) and are in principle equally important for the regulation of glycogen metabolism. However, due to the complex signalling pathways involved in the regulation, which integrate nutrient availability, metabolic state, cellular demand and even demand expectations, the regulatory significance can severely shift. These mechanisms are directly relevant to type 2 diabetes, where insulin-stimulated glycogen synthesis and glycogen-synthase activation are impaired in skeletal muscle [35, 36]. On the map, the defect lies on the path from the insulin receptor to glycogen synthase. In skeletal muscle of patients with type 2 diabetes, insulin activation of PI3K is diminished [36] and insulin fails to dephosphorylate GYS1 at sites 2 and 2a [37].

Regulation of eicosanoid metabolism by aspirin. Figure 3 shows how direct covalent modification of metabolic enzymes by aspirin regulates eicosanoid metabolism. COX-1 (PTGS1) and COX-2 (PTGS2) catalyse the metabolic production of Prostaglandin G2 (PGG2) and Prostaglandin H2 (PGH2) from arachidonate. Aspirin inhibits prostaglandin-producing cyclooxygenase activity via acetylation in COX-1 [38] and in COX-2 [39, 40]. Acetylated COX-2 can instead catalyse the formation of 15R-HETE from arachidonate [40]. This way, aspirin inhibits prostaglandin synthesis via altering the catalytic activity of COX-2. In a disease context, the COX-1 part of the aspirin map is directly relevant to the prevention of atherothrombosis. In platelets, COX-1-derived PGH2 is converted to the pro-aggregatory thromboxane A2, and irreversible COX-1 acetylation by low-dose aspirin suppresses its synthesis for the platelet lifetime [41]. The COX-2 arm links the map to the resolution of inflammation. Leukocyte 5-lipoxygenase converts 15R-HETE into the anti-inflammatory 15-epi-lipoxin A4 [42]. 5-lipoxygenase (ALOX5) is among the candidate extensions identified by the systematic analysis (Table 2).

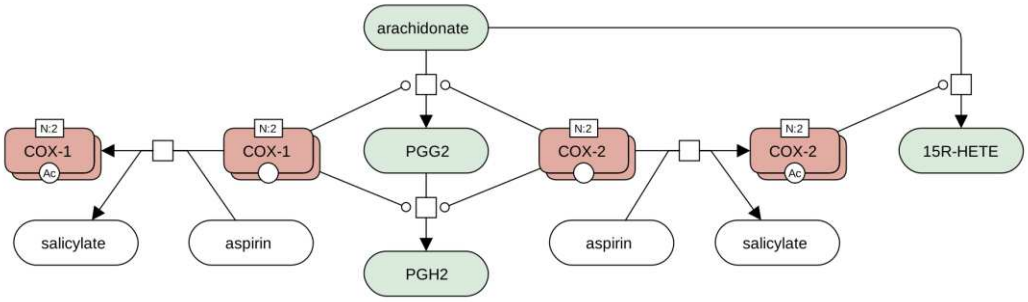


Figure 3. Regulation of eicosanoid metabolism by aspirin: mapping drug action mechanisms. This map is available in various XML and image formats at https://metabolismregulation.github.io/aspirin. Metabolites are shown in green, the key enzymes in red, and the drug molecule in white.

These examples demonstrate how this type of mechanistic representation can capture cases of metabolic regulation that differ in scale and level of detail, from a focused phosphorylation mechanism to broader and more complex receptor-to-metabolism signalling with multiple regulatory inputs and to direct covalent modification by a drug.

2.3. Systematic identification and prioritisation of signalling–metabolism connections

We systematically identified and prioritised signalling-metabolism connections by integrating the Reactome [43] and Recon2 [8] resources into a unique knowledge graph (Neo4j database) and computing their protein interface. Reactome contains the signalling part of the integrated resource, Recon2 the metabolic part, and the proteins interface the potentially interesting connections between the two. The interface contained 318 metabolic proteins in Recon2 associated with 773 processes in Reactome. We further checked whether these proteins were themselves regulated, classified the identified connections into several biological categories (e.g., phosphorylation, gene expression), and filtered them based on their attributed categories. Finally, we compared the obtained candidate mechanisms to those already described in the 17 MRegAtlas maps curated before this analysis. All steps of the analysis were realised programmatically using publicly available software and are fully reproducible (see the Methods section for more details).

Figure 4 shows the funnel diagram of the classification of identified signalling–metabolism connections in four levels. At the protein level, 267 of the 318 proteins directly modify metabolic reactions, while 112 are compounds of protein complexes that are involved in modifying metabolic reactions. Some of these proteins and compounds overlap. Together these proteins form 918 protein-process pairs involving 773 distinct processes. The 773 processes involving metabolic proteins were classified as phosphorylation, gene expression and other signalling processes. The intermediate set contains 87 phosphorylation processes, 136 gene expression processes and 550 other signalling processes. Of the 318 proteins, 27 are already annotated on these 17 maps, in 43 map-protein matches. The remaining 291 proteins are candidates for further map development and extension of the MRegAtlas. Four of these candidates have been developed into new MRegAtlas maps (Table 1): the PDH and BCKDH complexes, which are in the lists through their subunits DBT and PDHX, and CPS1 and NOS1.

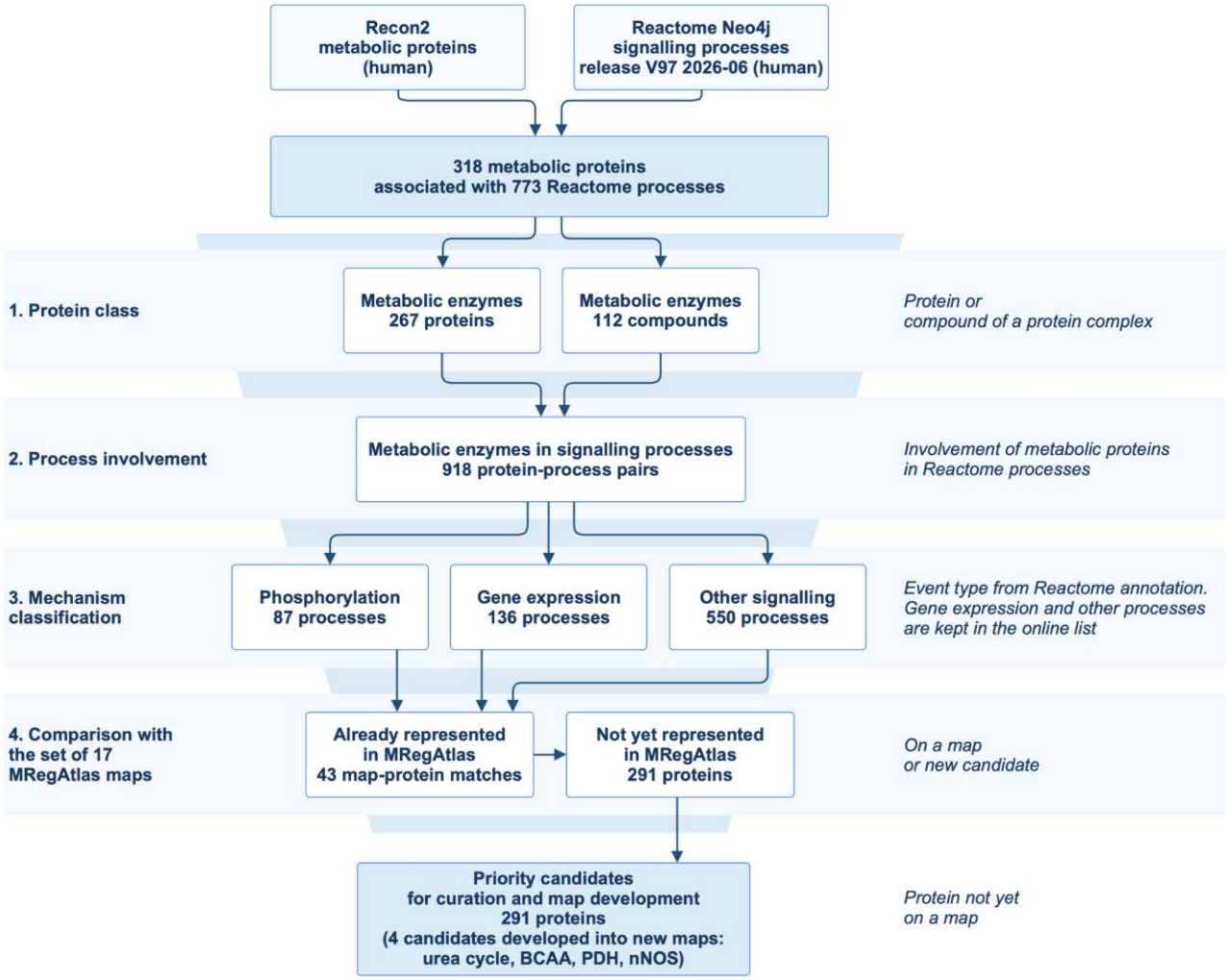


Figure 4. Systematic identification and prioritisation of signalling–metabolism connections. Recon2 metabolic proteins were mapped to Reactome Neo4j signalling processes, identifying metabolic proteins associated with Reactome processes. Level 1: the proteins were divided into those that catalyse metabolic reactions on their own and those that are compounds of protein complexes. Level 2: the identified processes were classified as phosphorylation, gene expression and other signalling processes. Level 3: phosphorylation-related events were classified according to the role of the matched protein in the regulatory event. Level 4: the matched metabolic proteins were compared with the proteins annotated on the 17 MRegAtlas maps curated before the analysis to distinguish proteins already represented from candidate extensions. Numbers give proteins at level 1, processes at levels 2 and 3, and map-protein matches at level 4. Four of the candidates were later developed into new maps: pyruvate dehydrogenase (PDH complex), BCAA catabolism (BCKDH complex), urea cycle (CPS1) and nNOS (NOS1).

Table 2 shows representative examples of the candidate set of signalling-metabolism connections. The complete lists of candidates are available online at https://metabolismregulation.github.io/lists and in Supplementary File S3. Direct PTM connections identify mechanisms already represented in MRegAtlas and candidate extensions for further map development. Represented examples include NOS3, PLA2G4A, glycogen phosphorylases PYGM/PYGL, PDE3B and PTGS2 acetylated by aspirin. Candidate extensions include ALOX5 phosphorylation by MAPKAPK2, SPR phosphorylation by Ca2+/calmodulin-dependent kinase II and PTS phosphorylation by cGMP-dependent protein kinase II. Several candidates extend existing maps. SPR and PTS catalyse tetrahydrobiopterin synthesis, and tetrahydrobiopterin is an essential cofactor of eNOS [44]. These candidates connect Ca2+/calmodulin and cGMP signalling to the nitric oxide production map. ALOX5 extends the eicosanoid map from prostaglandins to leukotrienes, and PDK-dependent phosphorylation of PDHA1 represented in the PDH map connects the glycolysis map to pyruvate oxidation.

Table 2 also includes second-messenger enzymes, which are involved in catalytic reactions that are themselves part of signalling pathways. This group of enzymes generates or regulates second messengers and signalling lipids involved in intracellular signal transduction [45–47]. They are not directly connected to the regulation of metabolic pathways but potentially can participate in upstream signalling in MRegAtlas maps. Examples from the identified set include PLCG1/PLCG2, adenylate cyclase ADCY3 and phosphoinositide-regulating enzymes such as PIP5K1C and INPP5D. In these cases, the Reactome–Recon2 overlap reflects biochemical reactions involved in second-messenger or phosphoinositide signalling, and these enzymes were therefore grouped separately. In the ACSN–ReconMap integration, such shared proteins connected the signalling and metabolic network layers [6]. Here they are shown separately because the main focus of MRegAtlas is signalling regulation of metabolic enzymes.

Table 2. Representative signalling-metabolic connections after mechanistic classification. The examples show mechanisms already represented in MRegAtlas, candidates since developed into new maps, candidate extensions and second-messenger enzymes. The last group is retained separately because these enzymes primarily function in the context of intracellular signalling and not directly in metabolic regulation. For complexes, the protein matched in the lists is given with its complex in brackets. The phosphorylated subunit is PDHA1 in the PDH complex and BCKDHA in the BCKDH complex.

Protein	Metabolic/signalling role	Reactome process	Type	MRegAtlas status
A. Direct connections represented in the 17 earlier MRegAtlas maps				
NOS3	Nitric oxide production	Phospho-AKT1 phosphorylates NOS3 (eNOS) on serine-1177	Direct PTM	eNOS pathway
PLA2G4A	Arachidonic-acid release	Phosphorylation of cPLA2 by ERK-2	Direct PTM	Eicosanoid production
PYGM	Glycogen breakdown	Phosphorylation/interconversion of muscle glycogen phosphorylase	Direct PTM	Glycogen metabolism
PDE3B	cAMP hydrolysis	p-T309,S474-AKT2:PIP3 phosphorylates PDE3B	Direct PTM	cAMP hydrolysis
PTGS2	Prostaglandin synthesis	ASA- acetylates PTGS2	Direct PTM (drug)	Eicosanoid metabolism by aspirin
B. Candidates since developed into new MRegAtlas maps				
PDHX (PDH complex)	Pyruvate oxidation	PDK isozymes phosphorylate PDHC subunit E1	Direct PTM	Pyruvate dehydrogenase
DBT (BCKDH complex)	Branched-chain amino-acid catabolism	BCKDK phosphorylates BCKDH	Direct PTM	BCAA catabolism
CPS1	Urea cycle	SIRT5 desuccinylates CPS1	Direct PTM	Urea cycle
NOS1	Nitric oxide production	ATP2B4 binds to NOS1, inhibiting it	Protein binding	nNOS pathway
C. Candidate extensions for future MRegAtlas maps				
ALOX5	Leukotriene biosynthesis	ALOX5 is phosphorylated by MAPKAP2	Direct PTM	Candidate extension
SPR	Tetrahydrobiopterin biosynthesis	SPR is phosphorylated by Ca2+/calmodulin-dependent kinase II	Direct PTM	Candidate extension
PTS	Tetrahydrobiopterin biosynthesis	PTPS is phosphorylated by cGMP-dependent protein kinase II	Direct PTM	Candidate extension
D. Second-messenger enzymes				
PLCG1	Phosphoinositide/second-messenger signalling	Phosphorylation of PLCG1 by ligand-responsive p-6Y-EGFR mutants	Second-messenger enzyme	Separate class
ADCY3	cAMP production	GNAL:GTP (G alpha-olf) binds ADCY3	Second-messenger enzyme	Separate class
INPP5D	Phosphoinositide signalling	Interaction of PECAM-1 and SHIP	Second-messenger enzyme	Separate class

This workflow filters the systematic signalling-metabolic overlap into mechanistically defined connection types, including direct regulatory connections where metabolic enzymes are phosphorylated. Comparison with the 17 earlier maps places each connection within mechanisms already represented in MRegAtlas or within candidate extensions.

3. Discussion

MRegAtlas has a distinct and complementary place among existing resources. PhosphoSitePlus and dbPTM provide extensive collections of experimentally observed PTMs [48, 49]. iPTMnet integrates PTM sites with modifying enzymes and enzyme–substrate relationships [50]. SIGNOR and OmniPath organise causal and regulatory signalling interactions [51, 52]. Pathway Commons brings together metabolic and signalling pathways from multiple pathway resources [53]. PathBank 2.0 provides broad coverage of metabolic, signalling and drug-action pathways, with downloadable representations including SBGN [54] Recent works address other aspects such as systems-level analysis of PTM occurrence across human metabolic enzymes [55] and pathway-centred visualisation of experimentally measured PTM changes via PTMNavigator [56]. More directly, Sompairac et al. connected ACSN [57] and ReconMap 2.0 [58] through proteins shared between signalling modules and metabolic subsystems and demonstrated their use for cross-talk studies and omics data analysis [6]. That study also noted that protein–reaction associations do not by themselves distinguish catalytic, regulatory and complex-associated roles, and that PTM-dependent protein states are not captured by this type of overlap-based integration. COSMOS combines signalling, transcriptional regulation and metabolic reactions into one network of prior knowledge and extracts causal subnetworks to explain multi-omics measurements, with regulation of metabolic enzymes represented as signed interactions [59]. Within the context of these projects, MRegAtlas complements such approaches with curated mechanistic maps connecting upstream signalling mechanisms to regulatory events affecting metabolic enzymes and downstream metabolic reactions. The Reactome–Recon2 analysis further distinguishes direct enzyme regulation from complex-associated and broader process-level connections, providing a mechanistic basis for selecting candidates for pathway reconstruction.

Within the broader multilayered control of cellular metabolism, MRegAtlas focuses on PTM-mediated regulation of metabolic enzymes. Metabolic regulation involves multiple overlapping mechanisms coordinated across interacting levels and timescales. This allows cells to adapt to changing environmental and physiological demands [60, 61]. Metabolic activity depends on substrate and product availability, enzyme abundance and enzyme functional state. Abundance is shaped by transcription, translation and protein turnover. Enzyme function responds directly to metabolites through allosteric regulation and to signalling through PTMs [4, 60–62]. These regulatory mechanisms operate at different speeds and durations, and interact through feedback loops and signalling networks [63–65]. PTMs provide particularly rapid and reversible control of enzyme function. Phosphorylation responses occur within seconds, and changes in metabolic-enzyme phosphorylation are detectable within minutes after stimulation [2, 4, 66, 67]. Metabolism in turn feeds information back into signalling through metabolites and metabolite-dependent protein modifications [63]. The systematic component of MRegAtlas uses the graph-based representations to identify candidate connections that link PTM-related signalling processes to metabolic enzymes and their downstream metabolic reactions.

Individual MRegAtlas maps can be reused as mechanistic modules within larger disease maps [68, 69]. The three example maps described in Results illustrate disease-relevant regulatory mechanisms in prostate cancer metabolism, skeletal-muscle insulin resistance and type 2 diabetes, and atherothrombosis. Another example is eicosanoid production in asthma, where the MRegAtlas RAF–MEK–ERK–cPLA2 module can connect directly to eicosanoid-production mechanisms represented in AsthmaMap [70]. These examples show how MRegAtlas modules can extend disease maps from signalling mechanisms to the regulation of specific metabolic processes. In turn, disease maps can help identify disease-relevant mechanisms for further expansion of the MRegAtlas collection. The link of each map at which a disease process acts is given in Supplementary Table S2. PTS (Table 2) is phosphorylated by cGMP-dependent protein kinase II, and the patient mutation R16C (causes hyperphenylalaninaemia) prevents this phosphorylation and leaves the enzyme inactive [71, 72].

Curated mechanistic pathway maps in MRegAtlas stored in standard formats can support the transition to computational models. Metabolic models increasingly incorporate regulatory mechanisms together with metabolic reactions. Models of hepatic glucose metabolism, for example, integrate glycolysis, gluconeogenesis and glycogen metabolism with mechanisms controlling glucose homeostasis [73, 74]. Models incorporating regulation by substrates, allosteric effectors and hormone-dependent phosphorylation are used to analyse responses to nutritional, pharmacological and inherited perturbations [75, 76]. Such models have also been applied to investigate disease-specific metabolic alterations, including changes across stages of non-alcoholic fatty liver disease [77, 78]. Mechanistic models are applied to regulatory systems such as liver iron homeostasis and the identification of potential intervention points [79, 80]. These studies illustrate the value of explicit representation of regulatory mechanisms. The feasibility of moving from curated mechanistic maps to executable models has been demonstrated in disease-map applications, where map content was converted into Boolean models to analyse disease dynamics and therapeutic responses [81] and subsequently integrated with cohort-level data to identify cohort-specific regulatory modules in Parkinson's disease [82, 83]. Standard and machine-readable representations facilitate such reuse. Systems-biology models are commonly represented in SBML and CellML and distributed through repositories such as BioModels and the Physiome Model Repository [84–87]. The SBGN format provides a standard for explicit graphical representation of mechanisms [14, 15]. Several pathways represented in MRegAtlas overlap with signalling mechanisms already formalised in established signalling models, for example regulation of eicosanoid production and regulation of pyrimidine biosynthesis via MAPK signalling [88, 89], and regulation of glycogen metabolism and regulation of cAMP hydrolysis via PI3K-AKT signalling [90, 91]. MRegAtlas maps can therefore be used for building computational models, for extending existing models and for coupling signalling and metabolic models. This is consistent with the ModelBricks concept of well-annotated reusable model components [92]. Quantitative prediction would then require the corresponding kinetic relationships, parameterisation, context-specific data and validation.

For future development, the systematic Reactome–Recon2 search provides a prioritised list of candidate signalling–metabolism connections for future MRegAtlas development. Manual curation then establishes the biological context, evaluates individual connections and determines which candidates form coherent mechanistic pathways. Together, systematic discovery and expert curation reduce dependence on easier-to-find familiar examples and provide a reproducible route across the signalling–metabolism interface. At the same time,

systematic identification is by design limited by the coverage and annotation of the underlying databases and reconstructions, so direct literature searches and pathway-database exploration remain complementary routes for identifying regulatory mechanisms not captured by the integrated resources. Future extension can draw on newer metabolic and pathway resources. Recon3D expanded the scope and structural detail of human metabolic reconstruction [9], Human1 provided a later consensus reconstruction [93], and Human2 further expands and refines the human metabolic reconstruction [94]. Reactome likewise continues to expand its curated pathway knowledge [10]. Extension across these resources, together with newly characterised regulatory events, can progressively broaden the set of signalling–metabolism connections represented in MRegAtlas.

In addition to identification of new connections, extensions can be within one specific topic. For example, the glycolysis map shows regulation via PKA that can be connected to upstream adrenaline input. Regulation via Akt will connect to insulin input upstream [21, 95]. Regulation via AMPK will connect to energy stress [96]. Functional differences and regulation of other isoforms can be captured within new maps for specific cell types. PFKFB1 in hepatocytes is regulated by glucagon via PKA [97]. PFKFB3 is induced by hypoxia via HIF-1 [98] in cancer, endothelial and immune cells [99–101]. Distinct regulatory contexts may be captured as separate maps. This both gives an opportunity to expand coverage and presents potential challenges in complexity management.

4. Methods

The MRegAtlas workflow combines two complementary routes for identifying signalling–metabolism connections (Figure 5): targeted manual identification from the literature and pathway resources, and systematic graph-based identification using Reactome Neo4j and Recon2 in Neo4j. Both routes generate candidate regulatory connections for map development. The manual route came first and gave 17 maps. The systematic analysis followed, and four maps were developed based on the candidates identified. Selected candidates are represented in SBGN Process Description, manually curated, annotated and published in machine-readable formats for online exploration and reuse.

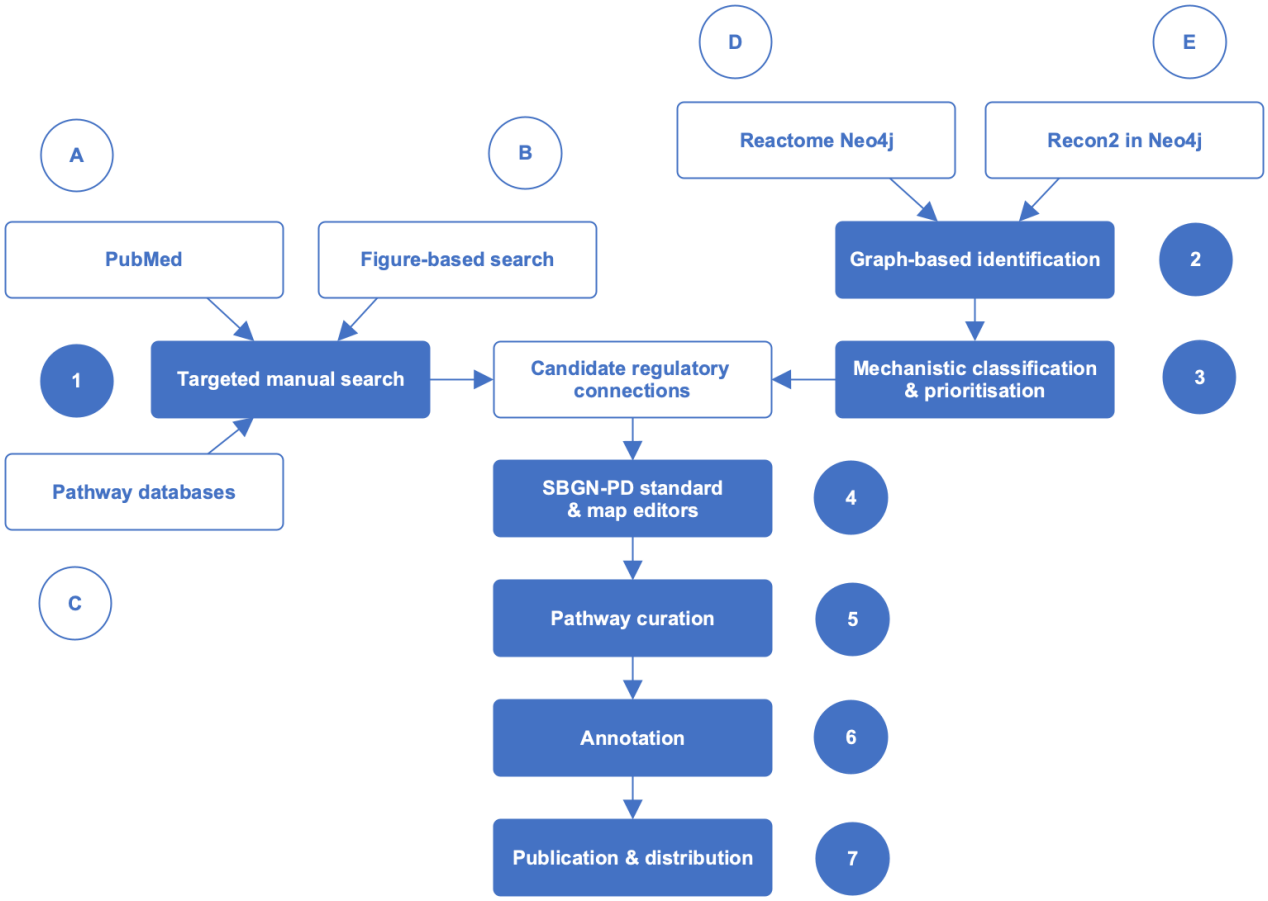


Figure 5. Methods pipeline. Numbered circles 1–7 indicate the sequential workflow steps, from targeted identification of regulatory mechanisms and graph-based analysis through mechanistic classification, map representation, curation, annotation and publication. Lettered circles A–E indicate the input resources used for candidate identification: PubMed, figure-based search, pathway databases, Reactome Neo4j and Recon2 in Neo4j.

4.1. Targeted manual search for metabolism regulation mechanisms

Candidate mechanisms for map development were initially identified through targeted searches of PubMed, figure-based searches to locate mechanistically informative publications, and pathway databases including Reactome [43] and KEGG [102]. This route was intended to identify well-characterised examples. Publications containing informative mechanistic figures were used as entry points, with primary literature and pathway resources subsequently used to verify and extend the represented mechanisms during curation (Section 4.4). Regulation of glycolysis through PFKFB2 was one of the first examples reconstructed through this route.

4.2. Automatic search for connections between metabolic and signalling networks

Integration of the Reactome and Recon2 resources

We integrated the Reactome [43] and Recon2 [8] resources into a Neo4j v5.X graph database (neo4j.com) as follows. For Reactome, we first transformed the publicly available Neo4j v4.X dump of the Reactome Graph Database [13] into a Neo4j v5.X dump using the Neo4j loading and migration tools (https://neo4j.com/docs/operations-manual/current/neo4j-admin-neo4j-tools/). We then loaded the obtained dump into a newly created Neo4j v5.X database. For Recon2, we first loaded the SBML version of the model [8] (http://identifiers.org/biomodels.db/MODEL1109130000) into Python using the momapy tool

[103] (https://github.com/momapy/momapy), and updated missing, outdated or unreviewed (TrEMBL) UniProt identifiers using the UniProt REST mapping API (https://rest.uniprot.org/idmapping). We then added the updated Recon2 SBML model into our database containing Reactome using the momapy-kb tool (https://github.com/momapy/momapy_kb).

Computation of the interface

We computed the UniProt interface between the Reactome and Recon2 resources in our database as follows. First, we identified signalling processes in Reactome using a Cypher query. A signalling process was defined as a process in which at least one reactant and at least one product are proteins or protein complexes. In the Reactome Graph Model, the "ReactionLikeEvent" class represents human processes, while the "EntityWithAccessionedSequence" and the "Complex" classes represent proteins and protein complexes, respectively. More details on the data model of the Reactome Graph Model are available on the Reactome Documentation page (https://reactome.org/documentation/data-model/). In the Cypher query, we marked Reactome processes as signal transduction processes by adding a new ReactionLikeEventType attribute to these processes (ReactionLikeEvent objects) and setting the attribute value to "SignalTransductionProcess". Secondly, we identified proteins in Recon2 that act as modifiers in metabolic reactions. A Recon2 metabolic reaction is represented as a detailed subgraph with a "Reaction" node, connected by a set of edges (e.g., HAS_REACTANT, HAS_PRODUCT, HAS_MODIFIER) to nodes representing the biological entities involved, including the metabolites (reactants and products) and enzymes/catalysts (proteins and complexes, represented as "Species" nodes). Proteins and complexes were distinguished by their annotations, which included an sbo_term attribute containing the relevant SBO term for a protein (SBO:0000252) or a protein complex (SBO:0000297), as well as a qualifier (IS or HAS_PART). We then used annotations of proteins with UniProt identifiers to compute the UniProt interface between the Reactome and Recon2 resources. In Recon2, "RDFAnnotation" nodes are connected to the respective "Species" nodes (proteins/complexes), while in Reactome, "ReferenceEntity" nodes are connected to "EntityWithAccessionedSequence" nodes (proteins), provided that these proteins are part of a signalling process or components of a complex that is part of a signalling process. The UniProt interface was then created by adding SAME_UNIPROTID relationships between "Species" and "EntityWithAccessionedSequence" nodes with matching UniProt identifiers. The UniProt identifier and the type of Recon2 species (protein or complex) were added as relationship attributes to allow for easier graph querying.

Classification into biological categories

Two lists of metabolic protein-signalling process pairs were extracted by querying the added relationships, one list for Recon2 proteins and one for Recon2 complexes. The two lists of protein-process pairs were subsequently subjected to mechanistic classification and prioritisation. The associated Reactome processes were classified as phosphorylation, gene expression, or other signalling processes. Phosphorylation and gene expression were classified according to the name of each process. Those that could not be assigned to either category were classified as other signalling processes. A combined set of both lists, with duplicates removed, was used to report counts for each process class.

Comparison with the MRegAtlas

The CellDesigner versions of the 17 maps of the MRegAtlas were integrated into our Neo4j database containing the Reactome and Recon2 resources using momapy-kb. The UniProt annotations of the map were then compared to the set of matching UniProt identifiers of the UniProt interface using Cypher queries. The four maps added later (pyruvate dehydrogenase, BCAA catabolism, urea cycle and nNOS) were developed from candidates identified in this analysis and were not part of this comparison. A connection in this comparison is a protein of the lists annotated on a map: 43 such matches involve 27 proteins. The online lists instead link a protein-process pair to a map when the map draws that process, for all 21 maps, including the four added later.

Implementation and code availability

The analysis was performed on the Reactome Graph Database (https://reactome.org/dev/graph-database) and Recon2 SBML model (https://www.biomodels.org/MODEL1109130000), using Neo4j v5.23.0. The migration of the Reactome Graph Database was implemented in Bash. The rest of the analysis was implemented in Python v3.12 as Jupyter notebooks using momapy v0.16.1 and momapy-kb v0.5.0. All scripts and notebooks used for this analysis are available online as a Zenodo archive (https://doi.org/10.5281/zenodo.23986493).

4.3. Standards, editors and formats

All maps are represented using SBGN Process Description [14, 15]. Map development used yEd Graph Editor [104], ySBGN [105], Newt [18], SBGN-ED [106] and CellDesigner [107], according to the map and stage of development. GraphML representations produced in yEd were converted to SBGN-ML using the ySBGN dual converter [105]. The SBML and SBGN-ML files were exported from the CellDesigner files using the MINERVA format conversion [108]. The resource provides GraphML, SBGN-ML, CellDesigner XML and SBML files, PNG and SVG images and an annotation table, and supports interactive exploration in Newt and MINERVA. Map-level statistics reported in Results are provided in Supplementary File S1.

4.4. Pathway curation

Signalling–metabolism pathways were manually curated using primary literature and established pathway resources. Pathway resources were used to define relevant signalling and metabolic context, while primary publications were used to expand and to verify mechanisms of regulation, to identify regulated enzymes, post-translational modifications and the downstream metabolic reactions. Curation focused on representing an explicit mechanistic chain from upstream signalling events through the regulatory state of the metabolic enzyme to the catalysed metabolic reaction. Where the curated evidence was cell- or tissue-specific, that context was retained in the corresponding map or description. Supporting references are provided for individual maps on the MRegAtlas website, and new evidence can be incorporated through revision of the corresponding map files and online versions.

4.5. Annotation

Protein entities were labelled using HGNC-approved gene symbols [109] and linked to UniProt IDs [110]. Metabolites were identified using ChEBI identifiers [111]. Automatic entity matching during import into MINERVA was manually checked, particularly for metabolites with multiple synonyms or structurally related forms. For example, PTGS1 was used as the HGNC-approved symbol for cyclooxygenase-1 (HGNC:9604, UniProt:P23219), and CHEBI:32966 was used for β-D-fructofuranose 1,6-bisphosphate(4−). When a mechanism is represented at the protein-family level, the map keeps a generic label and the annotation lists the human HGNC members able to fill that role, for example, ADCY1 to ADCY9 for the generic node "ADCY". Tissue- and cell-type-specific isoforms are noted, for example the muscle and liver forms of glycogen synthase (GYS1, GYS2) and glycogen phosphorylase (PYGM, PYGL). Each metabolite has a single ChEBI identifier across all maps. The complete annotation is provided as one table per map in Supplementary File S4.

4.6. Publication and distribution

Final maps were uploaded to the MINERVA platform for interactive browsing and exploration [108]. SBGN-ML files generated from the map-development workflow can be imported directly into MINERVA [17], including files converted from yEd GraphML using ySBGN [105] and files exported by MINERVA from CellDesigner XML [108]. The published maps are accessible through the MRegAtlas website and MINERVA, with downloadable GraphML, SBGN-ML, CellDesigner XML and SBML files, PNG and SVG images and annotation tables, and links for exploration via Newt and MINERVA. MINERVA also provides programmatic access through its API [112].

4. Conclusion

MRegAtlas integrates fragmented information of signalling–metabolism relationships into an explicit, reusable representation connecting upstream signalling events to metabolic enzyme regulation and catalysed reactions. The current resource comprises 21 curated maps with 230 processes and 546 nodes, while the Neo4j Reactome–Recon2 analysis identified 318 metabolic enzymes associated with 773 signalling processes as cases for further curation. By combining systematic discovery of connections with expert-curated machine-readable pathway maps, MRegAtlas provides a scalable framework for extending coverage and updating individual maps. The resource is designed to support mechanistic interpretation, reuse in disease maps, computational model development, education and omics-data exploration. It enhances knowledge accessibility and serves to advance our understanding of the dynamic regulation of human metabolism.

Key points

- The regulatory layer linking upstream signalling to metabolic enzymes and their reactions remains fragmented across pathway databases and individual publications.
- The approach combines manually curated signalling–metabolism maps with a systematic method for identifying candidate regulatory connections.
- MRegAtlas provides 21 curated SBGN Process Description maps comprising 230 processes and 546 nodes, available in SBGN-ML, GraphML, MINERVA and Newt.

- Recon2-Reactome Neo4j queries identified 318 metabolic enzymes in 773 signalling processes, providing a reproducible basis for map extension and updates.
- The maps support knowledge organisation, reuse in disease maps, education, computational modelling, and exploration through MINERVA.

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Competing interests

Authors declare that they have no relevant conflicts of interest.

Data availability

All the maps and the lists of identified connections between signalling and metabolic networks are available at https://metabolismregulation.github.io and accessible for browsing via the MINERVA platform. The map diagrams and the corresponding data files are distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY 4.0).

Author contributions

Project coordination: A.M., I.B. Conceptualisation: A.M., I.B., A.R., H.M. Diagram development: A.M., V.T., H.B., F.S., V.B., T.S., M.L.B., L.L., O.L., M.L.A., A.R. Annotation: L.L., I.M., O.L. Graph database integration and formal analysis: I.B., L.G., I.M. Modelling perspectives: M.L.B., N.B., A.A.H. Software and MINERVA platform support: M.O., P.G., V.S., R.S., F.T.B. Institutional support: C.A., V.S., R.S., P.V.N. Writing: original draft - A.M., I.B., H.B., M.L.B. Writing: review and editing - all authors. All authors have read and agreed to the final version of the manuscript.

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