In glycolysis, glucose (**A**) is converted to pyruvate (**K**) in 10 enzymatically catalyzed reactions, each of which generates an output chemical entity that is a required input of the following reaction. This sharing of outputs and inputs can be used by a curator to organize the 10 reactions into a linear sequence, and could be used by a logical reasoning tool to identify the correct order of these reactions absent any manual annotation.

**1a** [RHEA:36496](https://www.rhea-db.org/reaction?id=36496) α -D-glucose ([ChEBI:17925](https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:17925)**A1**) + ATP → α -D-glucose 6-phosphate ([ChEBI:58225](https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI%3A58225) **B1**) + H(+) [R-HSA-70420](https://reactome.org/PathwayBrowser/#/R-HSA-70326&SEL=R-HSA-70420&PATH=R-HSA-1430728,R-HSA-71387)
This RHEA entry links to ***no*** UniProt enzymes. All other RHEA reactions on this list link to the expected UniProt enzymes in multiple species.
OR
**1b** [RHEA:22741](https://www.rhea-db.org/reaction?id=22741) D-hexose ([ChEBI:4194](https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:4194) **A2**) + ATP → D-hexose 6-phosphate ([ChEBI:61567](https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:61567) **B2**) + H(+) (nothing in Reactome)

**2** [RHEA:11817](https://www.rhea-db.org/reaction;jsessionid=34DC3DCFB4C30447CD10AB699CB447C8?id=11817) Aldehyde-D-glucose 6-phosphate ([ChEBI:57584](https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:57584) **B3**) → keto-D-fructose 6-phosphate ([ChEBI:57579](https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:57579) **C1**) [R-HSA-70471](https://reactome.org/PathwayBrowser/#/R-HSA-70326&SEL=R-HSA-70471&PATH=R-HSA-1430728,R-HSA-71387)

**3** [RHEA:16110](https://www.rhea-db.org/reaction;jsessionid=D45F74D9FBDC891324CF628769A75651?id=16110) ATP + β -D-fructose 6-phosphate ([ChEBI:57634](https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:57634) **C2**) → ADP + β -D-fructose 1,6-bisphosphate ([ChEBI:32966](https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:32966) **D**) + H(+) [R-HSA-70467](https://reactome.org/PathwayBrowser/#/R-HSA-70326&SEL=R-HSA-70467&PATH=R-HSA-1430728,R-HSA-71387)

**4** [RHEA:14730](https://www.rhea-db.org/reaction;jsessionid=732D67E5494182E2592585105E8F328B?id=14730) β -D-fructose 1,6-bisphosphate ([ChEBI:32966](https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:32966) **D**) → D-glyceraldehyde 3-phosphate (ChEBI:59776 **E**) + dihydroxyacetone phosphate (ChEBI:57642 **F**) [R-HSA-71496](https://reactome.org/PathwayBrowser/#/R-HSA-70326&SEL=R-HSA-71496&PATH=R-HSA-1430728,R-HSA-71387)

**5** [RHEA:18587](https://www.rhea-db.org/reaction;jsessionid=CF678904C01E8470CB8979DA45A1170B?id=18587) dihydroxyacetone phosphate (ChEBI:57642 **F**) → D-glyceraldehyde 3-phosphate (ChEBI:59776 **E**) [R-HSA-70454](https://reactome.org/PathwayBrowser/#/R-HSA-70326&SEL=R-HSA-70454&PATH=R-HSA-1430728,R-HSA-71387)

**6** [RHEA:10301](https://www.rhea-db.org/reaction;jsessionid=635D16426C20E64B813EA94EFE87D58A?id=10301) D-glyceraldehyde 3-phosphate (ChEBI:59776 **E**) + NAD(+) + phosphate → (2R)-3-phospho-glyceroyl phosphate (ChEBI:57604 **G**) + H(+) + NADH [R-HSA-70449](https://reactome.org/PathwayBrowser/#/R-HSA-70326&SEL=R-HSA-70449&PATH=R-HSA-1430728,R-HSA-71387)

**7** [RHEA:14803](https://www.rhea-db.org/reaction;jsessionid=5FE5D0989B8E303186DB0077150D3B03?id=14803) (2R)-3-phospho-glyceroyl phosphate (ChEBI:57604 **G**) + ADP → (2R)-3-phosphoglycerate (ChEBI:58272 **H**) + ATP [R-HSA-71850](https://reactome.org/PathwayBrowser/#/R-HSA-70326&SEL=R-HSA-71850&PATH=R-HSA-1430728,R-HSA-71387)

**8** [RHEA:15903](https://www.rhea-db.org/reaction;jsessionid=1D67374395B1D2C5F9B200DA3FC02443?id=15903) (2R)-3-phosphoglycerate (ChEBI:58272 **H**) → (2R)-2-phosphoglycerate (ChEBI:58289 **I**) [R-HSA-71654](https://reactome.org/PathwayBrowser/#/R-HSA-70326&SEL=R-HSA-71654&PATH=R-HSA-1430728,R-HSA-71387)

**9** [RHEA:10165](https://www.rhea-db.org/reaction;jsessionid=2FC03065BBADC77DDB4A0CB12657A53C?id=10165) (2R)-2-phosphoglycerate (ChEBI:58289 **I**) → H2O + phosphoenolpyruvate (ChEBI:58702 **J**) [R-HSA-70326](https://reactome.org/PathwayBrowser/#/R-HSA-70326&PATH=R-HSA-1430728,R-HSA-71387)

**10** [RHEA:18159](https://www.rhea-db.org/reaction;jsessionid=86D7C810B5754AF390B4E0CDC8345B76?id=18159) ADP + H(+) + phosphoenolpyruvate (ChEBI:58702 **J**) → ATP + pyruvate (ChEBI:15361 **K**) [R-HSA-71670](https://reactome.org/PathwayBrowser/#/R-HSA-70326&SEL=R-HSA-71670&PATH=R-HSA-1430728,R-HSA-71387)

That’s the theory, and the premise of the project to use RHEA’s definitive annotations of reaction chemistry to align RHEA, GO, and Reactome.

An obstacle is highlighted with the red letters assigned to three groups of chemical entities in this reaction sequence.

**A** RHEA annotates the first reaction of glycolysis (**1a**) with α -D-glucose (ChEBI:17925 **A1**) as an input but links this reaction to none of the UniProt entries for glucokinases and hexokinases. Instead, RHEA associates these enzymes with an otherwise identical reaction (**1b**) that has D-hexose (ChEBI:4194 **A2**) as an input.

**B** The output of reaction **1a** is α -D-glucose 6-phosphate (ChEBI:58225 **B1**) while the corresponding output of reaction **1b** is D-hexose 6-phosphate (ChEBI:61567 **B2**). Neither of these molecules is the input for RHEA reaction **2**; Aldehyde-D-glucose 6-phosphate (ChEBI:57584 **B3**) has that role, so no matter how reaction **1** is annotated it does not connect at the level of a shared chemical entity with reaction **2**.

**C** The output of reaction **2** is keto-D-fructose 6-phosphate (ChEBI:57579 **C1**) but the corresponding input of reaction 3 is β -D-fructose 6-phosphate (ChEBI:57634 **C2**) so again there is not a shared-chemical connection between reactions **2** and **3**.

For reaction **3**, [PMID: 26205495](https://www.ncbi.nlm.nih.gov/pubmed/26205495), Figure 2C shows fructose 6-P substrate in a closed conformation.

How are the structural discrepancies **A**, **B**, and **C** to be resolved? One possibility builds on this assertion from the White, Handler, Smith, Hill, Lehman textbook (“Principles of Biochemistry” 6th edition, McGraw-Hill, 1978, page 437).

Glucose 6-phosphate is converted to fructose 6-phosphate in a readily reversible reaction catalyzed by phosphoglucose isomerase … . The binding substrates are the  anomers of the D-sugar phosphates at their C-1 conformers … . Since the process must include an enediol intermediate, the hemiacetal ring must open and close while bound to the enzyme.

The assertion makes no reference to primary literature, but the authors themselves are authorities. Wurster and Hess (1974 – [PMID: 4368416](https://www.ncbi.nlm.nih.gov/pubmed/?term=4368416) – scheme 1) reach conclusions consistent with the ones spelled out here, but appear to allow for reactions involving other anomers in yeast and perhaps other species. Solomons et al. 2004 (PMID:15342241), figure 5 at least is a specific opinion about this conformation, inferred from the x-ray crystallographic studies.

The orientations of the OH groups in the diagram that accompanies the text quoted here align with those of [CHEBI:58225](https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI%3A58225) - α-D-glucose 6-phosphate(2−) **B1** and [CHEBI:57634](https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:57634) - β-D-fructofuranose 6-phosphate(2−) **C2**, the input and output, respectively, of reaction **2**. Any (linear) intermediate would exist only as a reaction intermediate associated with the active site of the enzyme, not as an input or output.

But if [CHEBI:58225](https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI%3A58225) - α-D-glucose 6-phosphate(2−) **B1** is the true input / substrate of reaction **2**, then in the context of glycolysis it should be the output / product of reaction **1** – other hexoses and glucose conformers can also be phosphorylated, but only the reaction that yields [CHEBI:58225](https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI%3A58225) - α-D-glucose 6-phosphate(2−) **B1** is relevant to glycolysis. That is, the first reaction of glycolysis is **1a** [RHEA:36496](https://www.rhea-db.org/reaction?id=36496) α -D-glucose ([ChEBI:17925](https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:17925) **A1**) + ATP → α -D-glucose 6-phosphate ([CHEBI:58225](https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI%3A58225) **B1**) + H(+), consistent with kinetic and modeling studies described by Xu et al. (1995 – [PMID: 7742312](https://www.ncbi.nlm.nih.gov/pubmed/?term=7742312)), and the second reaction of glycolysis should be the conversion of α -D-glucose 6-phosphate ([CHEBI:58225](https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI%3A58225) **B1**) to β-D-fructofuranose 6-phosphate(2−) **C2** [ChEBI:57634](https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:57634), rather than

**2** [RHEA:11817](https://www.rhea-db.org/reaction;jsessionid=34DC3DCFB4C30447CD10AB699CB447C8?id=11817) Aldehyde-D-glucose 6-phosphate ([ChEBI:57584](https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:57584) **B3**) → keto-D-fructose 6-phosphate ([ChEBI:57579](https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:57579) **C1**)

A tangential issue is that the substrate specificity of many glucokinases and hexokinases is broader than that shown in reaction 1a, but of the several reactions that a particular enzyme can catalyze, only this one is relevant to the process of glycolysis. Does this complicate the project to create strict 1:1:1 mappings among RHEA reactions, GO molecular function terms, and EC numbers? And if it does, is there a way to use the process context of the reaction to justify exceptions to strict mapping?

[CHEBI:57634](https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:57634) - β-D-fructofuranose 6-phosphate(2−) **C2**, is the input of reaction **3** as annotated by RHEA. The output of this reaction is β -D-fructose 1,6-bisphosphate ([ChEBI:32966](https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:32966) **D**). This conclusion is supported by kinetic studies described by Wurster and Hess (1974 – [PMID: 4277364](https://www.ncbi.nlm.nih.gov/pubmed/?term=4277364)).

Two classes of aldolase enzymes catalyze reaction **4**. Class I involves formation of a Schiff base intermediate while class II involves a transition metal ion (Jacques et al. 2018 [PMID: 29593097](https://www.ncbi.nlm.nih.gov/pubmed/29593097)). [EC 4.1.2.13](https://enzyme.expasy.org/EC/4.1.2.13) is applied to both classes of enzymes. The three vertebrate enzymes (in humans, ALDOA, ALDOB, ALDOC) are class I enzymes (Rellos et al. 2000 [PMID: 10625657](https://www.ncbi.nlm.nih.gov/pubmed/10625657)). Structural studies of ligand-bound *Giardia* class II aldolase ([PMID: 19236002](https://www.ncbi.nlm.nih.gov/pubmed/19236002) - Figure 4 and text page 3191) show it to be in its acyclic keto form ([ChEBI:16905](https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:16905)). [PMID: 29593097](https://www.ncbi.nlm.nih.gov/pubmed/29593097) describes the same substrate conformation for enzyme from *H. pylori* (also class II), not the ring form of **D** annotated by RHEA. Structural studies of rabbit muscle aldolase, however, show it to be complexed with fructose 1,6-bisphosphate in acyclic keto form (Sygusch et al. 1987 - [PMID: 3479768](https://www.ncbi.nlm.nih.gov/pubmed/?term=3479768); [PDB 4ALD](https://www.ncbi.nlm.nih.gov/Structure/pdb/4ALD)) so despite class differences in reaction mechanism the conformation of fructose 1,6-bisphosphate bound to the enzymes’ active sites is the same. But the formation of a linear intermediate is consistent with a reaction mechanism in which the substrate in its furanose form binds the active site and is opened as the reaction proceeds (Lai et al. 1974 - [PMID: 4812352](https://www.ncbi.nlm.nih.gov/pubmed/?term=4812352) – Figure 2), consistent with the RHEA annotation of reaction **4**.

Thereafter, chemicals are annotated consistently between reactions, allowing the expected shared-chemical connections to be made.