Supplemental Material

CBE—Life Sciences Education

Booth *et al*.

Supplemental Information for

Teaching Metabolism in Upper-division Undergraduate Biochemistry Courses using Online Computational Systems and Dynamical Models Improves Student Performance

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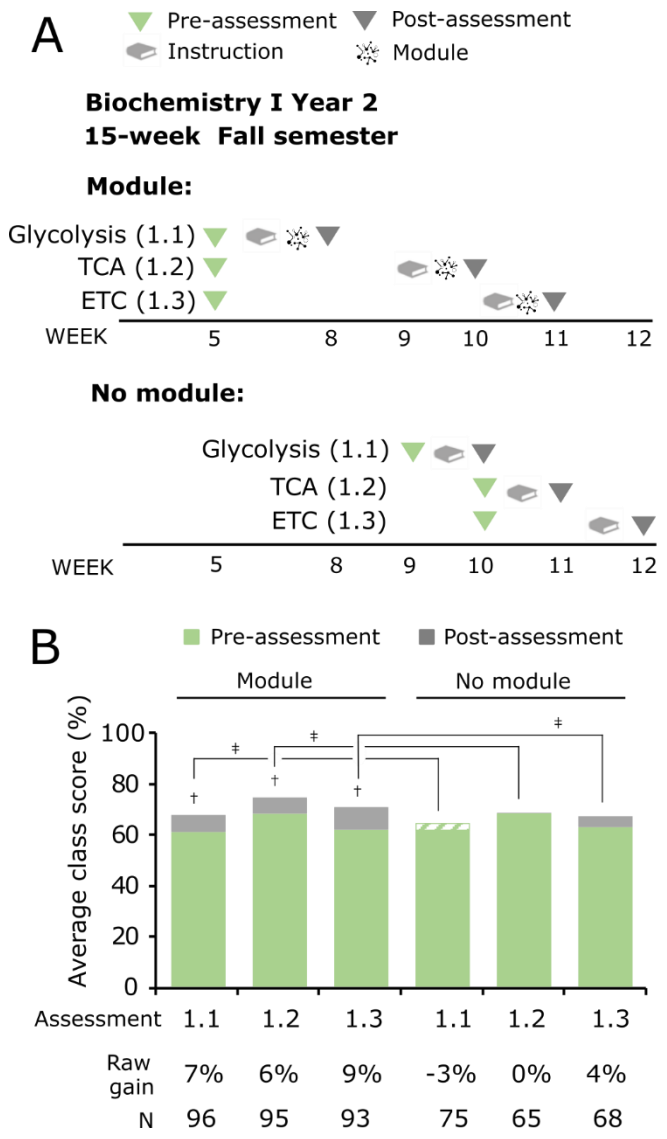

Figure S1. Computational learning module improves student performance on conceptual assessments for a familiar metabolic system during year 2. Assessment and instructional timeline and average class scores for the familiar system of cellular respiration are shown. (A) Diagram of the semester for the "Module" (top) and "No module" (bottom) courses of Biochemistry I during year 2. (B) Class average values of the pre-assessment scores (green) and post-assessment scores (grey) were compared between "Module" and "No module" courses for each assessment of cellular respiration (Assessment 1.1 (*Glycolysis*), Assessment 1.2 (*TCA*), Assessment 1.3 (*ETC*)). Each course was taught by a different instructor, and each instructor taught the same course as during year 1. Descriptive statistics for raw learning gains are provided in Supplemental Figure S4 and Supplemental Table S5. The average normalized learning gain for each assessment is also provided in Supplemental Table S5. Two-tailed paired t-tests were used to measure significance for pre- versus post-assessment scores: † indicates $p<0.05$ (Supplemental Table S5). ANCOVA was used to measure significance for the "Module" versus "No module" courses for each assessment: ‡ indicates $p<0.05$ (Supplemental Table S6). A green and white striped pattern indicates that the overall post-assessment score was lower than the pre-assessment score.

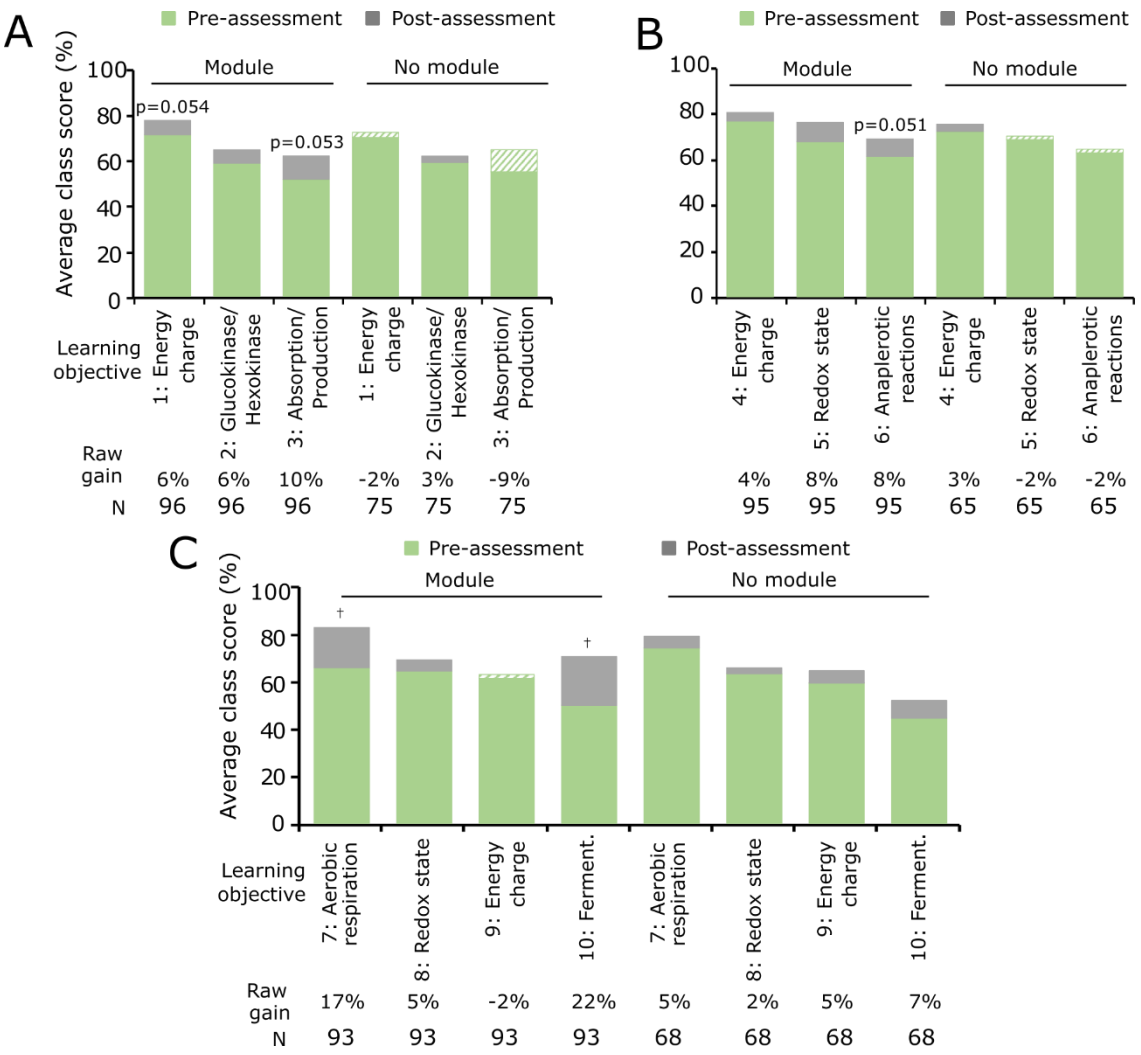

Figure S2. Computational learning modules improve class performance on learning objectives for a familiar metabolic system during year 2. Average class scores of the pre-assessment scores (green) and post-assessment scores (grey) for each learning objective for the familiar system of cellular respiration are shown for Biochemistry I during year 2. (A) Assessment 1.1 (*Glycolysis*), (B) Assessment 1.2 (*TCA*), and (C) Assessment 1.3 (*ETC*) were used to evaluate student learning gains for each objective in the "Module" and "No module" courses. Each learning objective is numbered, and keywords are provided (detailed objectives are listed in Table 1). Descriptive statistics for the raw learning gains as well as the average normalized learning gains for each objective are provided in Supplemental Table S10. Two-tailed paired t-tests were used to measure significance for pre- versus post-assessment scores: † indicates $p<0.05$ (Supplemental Table S10). A green and white striped pattern indicates that the overall post-assessment score was lower than the pre-assessment score.

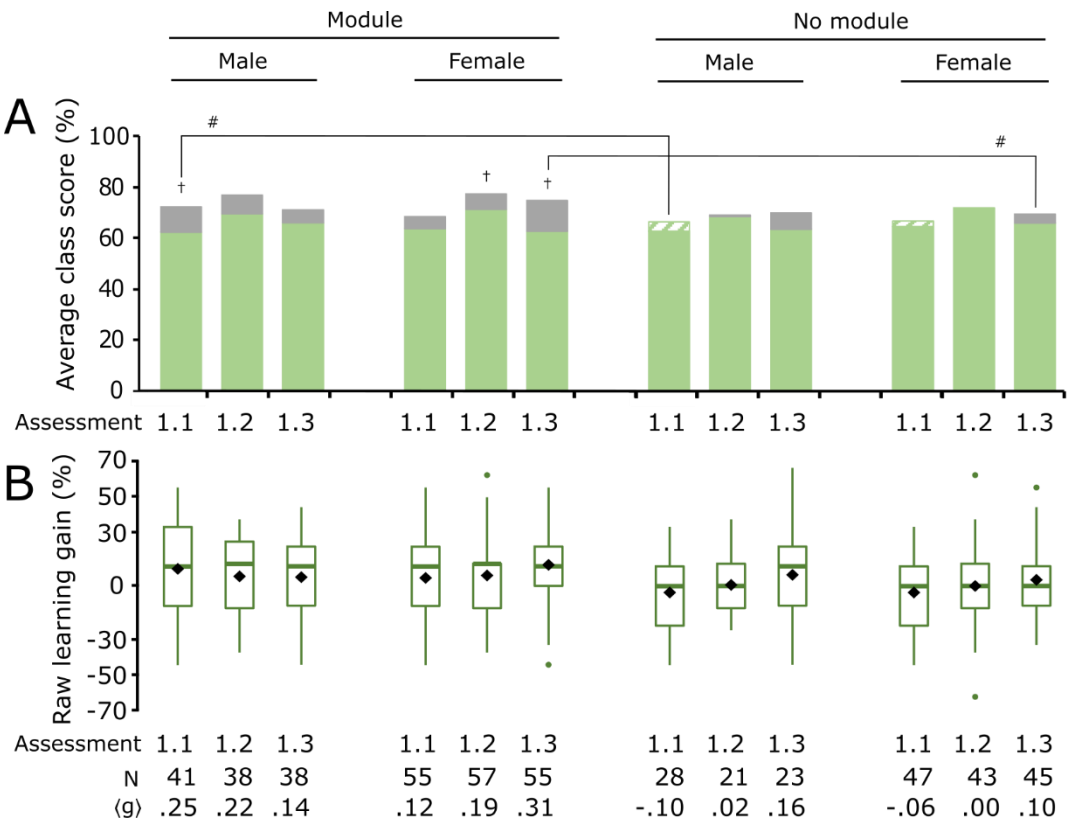

Figure S3. Repeated interaction with computer simulation modules may increase learning outcome equity during year 2. Average class scores and boxplots for individual student learning gains of male and female students for the familiar system of cellular are shown. (A) Class average values of the pre-assessment scores (green) and post-assessment scores (grey) for male and female students were compared between "Module" and "No module" courses for each assessment in Biochemistry I during year 1 (Assessment 1.1 (*Glycolysis*), Assessment 1.2 (*TCA*), Assessment 1.3: (*ETC*)). (B) Boxplot showing student learning gains for each group and each assessment. Average normalized learning gain ⟨g⟩ is also shown for each group. Two-tailed paired t-tests were used to measure significance for pre- versus post-assessment scores: † indicates $p<0.05$. ANCOVA was used to measure significance comparing the "Module and male," "Module and female," "No module and male" and "No module and female" groups for each assessment: # indicates $p<0.1$ for a post-hoc comparison with Bonferroni correction (Supplemental Table S12). (B) A green and white striped pattern indicates that the overall post-assessment score was lower than the pre-assessment score. Boxes represent the interquartile range, and lines within each box represent the median. Whiskers represent the highest and lowest values excluding outliers (1.5 times the IQR). Black diamonds represent the mean, and large dark green dots represent outliers.

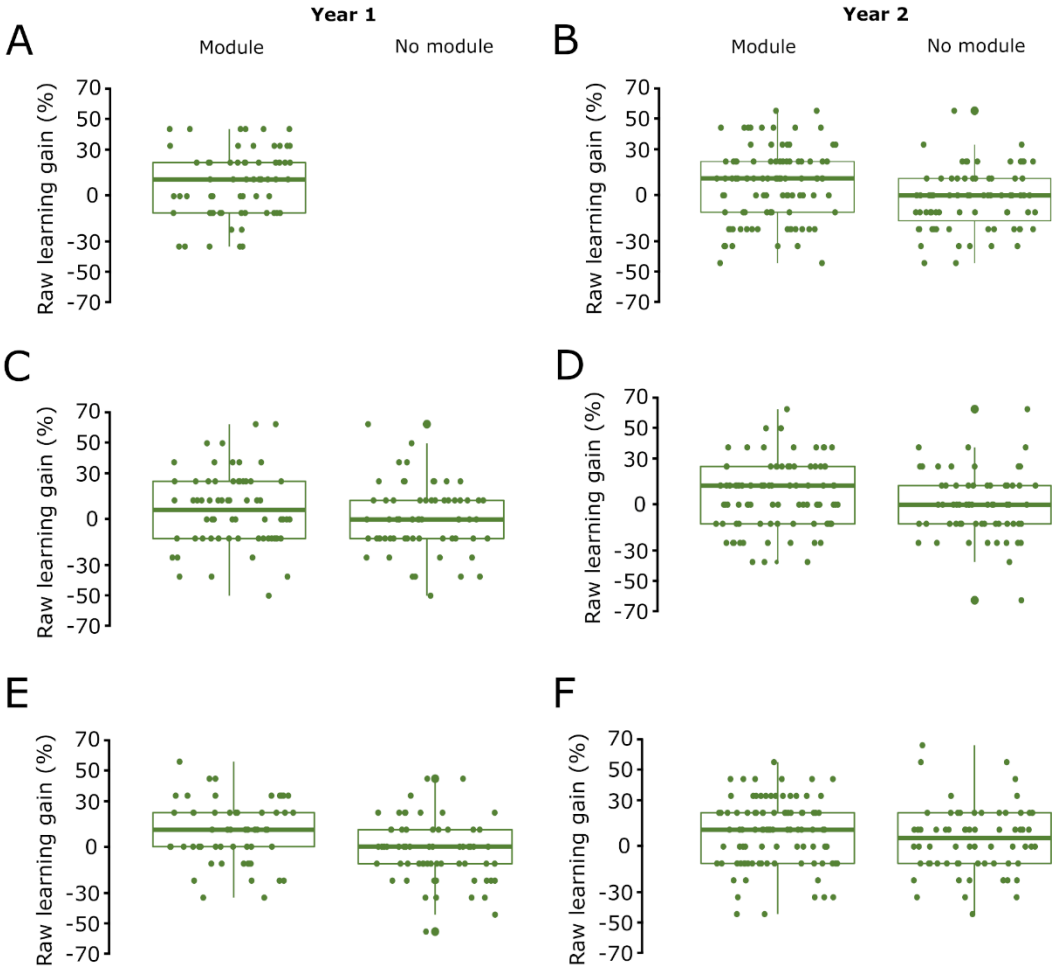

Figure S4. Comparison of learning gains for a familiar topic of metabolism. Boxplots of individual student learning gains for the familiar system of cellular respiration are shown for year 1 (A,C,E) and year 2 (D,E,F) of Biochemistry I. (A and B) Assessment 1.1 (*Glycolysis*), (C and D) Assessment 1.2 (*TCA*), and (E and F) Assessment 1.3 (*ETC*) were used to evaluate student learning gain for each objective in the "Module" and "No module" courses for each year. Boxes represent the interquartile range, and lines within each box represent the median. Whiskers represent the highest and lowest values excluding outliers (1.5 times the IQR). Large dark green dots represent outliers, and small dark green dots represent individual student learning gains.

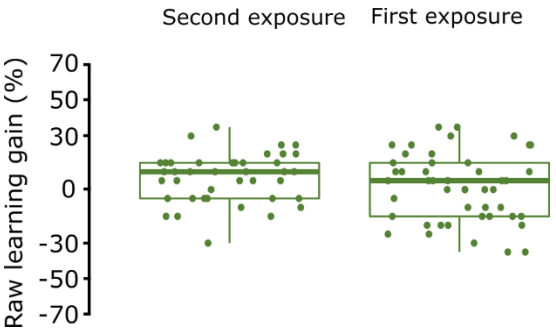

Figure S5. Comparison of learning gains for an unfamiliar topic of metabolism. Boxplot of individual student learning gains for the unfamiliar system of purine biosynthesis is shown for year 1 of Biochemistry II. Assessment 2 (*Purine biosynthesis*) was used to evaluate student learning gain for each objective in the “First exposure” and “Second exposure” groups. Boxes represent the interquartile range, and lines within each box represent the median. Whiskers represent the highest and lowest values excluding outliers (1.5 times the IQR). Small dark green dots represent individual student learning gains.

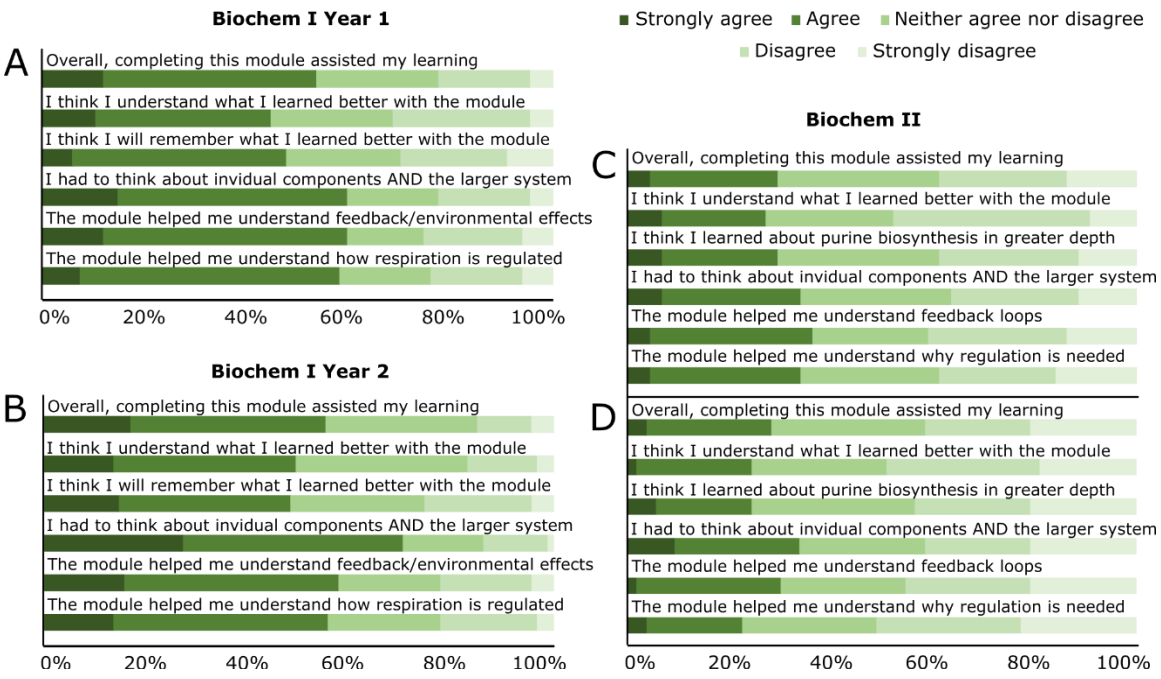

Figure S6. Student perceptions of the modules. Students in the "Module" courses completed a brief survey about their perceptions of the modules. Student responses are reported for (A) the *Regulation of Cellular Respiration* module completed in Biochemistry I during year 1, (B) the *Regulation of Cellular Respiration* module completed in Biochemistry I during year 2, and (B and C) the *Regulation of Purine Biosynthesis* module completed in Biochemistry II. Student responses for the *Regulation of Purine Biosynthesis* module in Biochemistry II were further subdivided based on (C) previous exposure to a module ("Second exposure" group), or (D) no previous exposure to a module ("First exposure" group). Results were evaluated on a five-point Likert scale.

Table S1: Combined difficulty and discrimination for the "Module" and "No Module" courses of the pre- and post-assessments (Biochemistry I)

Assessment	Question number	Year 1 Pre		Year 1 Post		Year 2 Pre		Year 2 Post	
		Difficulty	Discrimination	Difficulty	Discrimination	Difficulty	Discrimination	Difficulty	Discrimination
1.1: Glycolysis	**1A**	0.78[a]	0.05[a]	0.92	0.19	0.84	0.14	0.92	0.18
(Year 1	**1B**	0.66[a]	0.33[a]	0.50	0.19	0.42	0.46	0.45	0.42
N = 64;	**1C**	0.53[a]	0.33[a]	0.67	0.47	0.68	0.56	0.68	0.54
Year 2	**1D**	0.31[a]	0.29[a]	0.20	0.52	0.46	0.40	0.44	0.37
N = 171)	**1E**	0.45[a]	0.33[a]	0.69	0.71	0.50	0.30	0.56	0.58
	1F	0.56[a]	0.38[a]	0.72	0.38	0.63	0.44	0.65	0.35
	1G	0.62[a]	0.33[a]	0.80	0.38	0.81	0.28	0.78	0.35
	1H	0.59[a]	0.29[a]	0.67	0.29	0.57	0.19	0.61	0.39
	1I	0.72[a]	0.24[a]	0.83	0.33	0.77	0.42	0.81	0.28
1.2: TCA	**2A**	0.84	0.17	0.67	0.31	0.74	0.23	0.88	0.19
(Year 1	**2B**	0.34	0.40	0.20	0.24	0.58	0.51	0.68	0.57
N = 128;	**2C**	0.68	0.50	0.75	0.29	0.77	0.47	0.75	0.45
Year 2	**2D**	0.69	0.40	0.77	0.33	0.75	0.45	0.79	0.45
N = 159)	**2E**	0.70	0.26	0.77	0.45	0.74	0.19	0.72	0.34
	2F	0.59	0.38	0.73	0.48	0.62	0.06	0.68	0.34
	2G	0.56	0.33	0.62	0.45	0.57	0.32	0.69	0.40
	2H	0.61	0.45	0.77	0.31	0.73	0.30	0.63	0.32
1.3: ETC	**3A**	0.82	0.30 (0.30)[b]	0.87	0.13 (0.18)[b]	0.71	0.36	0.85	0.09
(Year 1	**3B**	0.63	0.33 (0.28)[b]	0.67	0.43 (0.48)[b]	0.63	0.34	0.74	0.42
N = 120;	**3C**	0.51	0.18 (0.15)[b]	0.47	0.50 (0.38)[b]	0.64	0.36	0.61	0.32
Year 2	**3D**	0.62	0.53 (0.48)[b]	0.68	0.35 (0.33)[b]	0.58	0.47	0.63	0.38
N = 161)	**3E**	0.72	0.30 (0.35)[b]	0.72	0.40 (0.48)[b]	0.70	0.34	0.65	0.38
	3F	0.51	0.20 (-)[b]	0.50	-0.23 (-)[b]	-	-	-	-
	3G	0.58	0.35 (0.45)[b]	0.77	0.35 (0.48)[b]	0.63	0.43	0.81	0.38
	3H	0.64	0.17 (0.15)[b]	0.50	0.23 (0.25)[b]	0.57	0.23	0.55	0.43
	3I	0.56	0.53 (0.65)[b]	0.64	0.50 (0.63)[b]	0.47	0.58	0.62	0.58
	3J	0.77	0.33 (0.38)[b]	0.82	0.25 (0.30)[b]	0.72	0.45	0.83	0.21

[a]The "No Module" group did not complete the assessment

[b]Value in brackets = discrimination when item 3F is dropped from the assessment

Table S2: Combined difficulty and discrimination for the "Second exposure" and "First exposure" groups of the pre- and post-assessments (Biochemistry I)

Assessment	Question number	Year 1 Pre		Year 1 Post	
		Difficulty	Discrimination	Difficulty	Discrimination
2: Purine Biosynthesis (N = 87)	**1A**	0.85	-0.07 (-)[a]	0.89	-0.14 (-)[a]
	1B	0.48	0.24 (0.10)[a]	0.46	0.21 (0.21)[a]
	1C	0.64	0.28 (0.24)[a]	0.63	0.52 (0.52)[a]
	1D	0.39	0.17 (0.24)[a]	0.45	0.62 (0.59)[a]
	1E	0.34	-0.21 (-)[a]	0.29	-0.28 (-)[a]
	1F	0.60	0.10 (0.17)[a]	0.78	0.41 (0.41)[a]
	1G	0.57	0.28 (0.20)[a]	0.69	0.52 (0.52)[a]
	1H	0.46	0.03 (0)[a]	0.54	0.55 (0.55)[a]
	1I	0.71	0.31 (0.31)[a]	0.79	0.48 (0.45)[a]
	2A	0.64	0.03 (0.14)[a]	0.68	0.10 (0.14)[a]
	2B	0.67	0.31 (0.31)[a]	0.71	0.31 (0.31)[a]
	2C	0.55	0.59 (0.62)[a]	0.39	0.31 (0.31)[a]
	3A	0.48	0.59 (0.59)[a]	0.45	0.31 (0.31)[a]
	3B	0.63	0.28 (0.34)[a]	0.47	0.28 (0.28)[a]
	3C	0.71	0.24 (0.17)[a]	0.77	0.29 (0.24)[a]
	3D	0.64	0.28 (0.28)[a]	0.72	0.34 (0.34)[a]
	4A	0.77	0.10 (0.17)[a]	0.9	0.14 (0.14)[a]
	4B	0.40	0.34 (0.34)[a]	0.56	0.48 (0.48)[a]
	4C	0.54	0.38 (0.31)[a]	0.64	0.28 (0.31)[a]
	4D	0.70	0.41 (0.45)[a]	0.66	0.38 (0.34)[a]
	4E	0.37	0.31 (0.34)[a]	0.59	0.28 (0.31)[a]
	4F	0.53	0.31 (0.31)[a]	0.36	0.21 (0.24)[a]

[b]Value in brackets = discrimination when items 1A and 1E are dropped from the assessment

Table S3: Participant demographic profiles (Biochemistry I)

Demographic variable	Year 1 "Module"			Year 1 "No module"			t-statistic	p-value
	N	Mean	S.D.	N	Mean	S.D.		
Gender (Male = 0, Female = 1)	69	0.59	0.49	73	0.68	0.47	1.122	0.264
Native English Speaker (No = 0, Yes = 1)	69	0.9	0.3	73	0.97	0.16	1.79	0.076
Parents' College Education (No = 0, Yes = 1)	69	0.8	0.41	73	0.78	0.42	-0.236	0.814
Job to Fund College Life (No = 0, Yes = 1)	69	0.72	0.45	73	0.7	0.46	-0.34	0.735
Cumulative GPA	68	3.41	0.85	70	3.74	0.28	3.036	0.003
Demographic variable	**Year 2 "Module"**			**Year 2 "No module"**			**t-statistic**	**p-value**
	N	Mean	S.D.	N	Mean	S.D.		
Gender (Male = 0, Female = 1)	101	0.57	0.50	79	0.63	0.49	0.794	0.428
Native English Speaker (No = 0, Yes = 1)	101	0.86	0.35	79	0.92	0.27	1.369	0.173
Parents' College Education (No = 0, Yes = 1)	101	0.69	0.46	79	0.89	0.32	3.300	0.001
Job to Fund College Life (No = 0, Yes = 1)	101	0.75	0.43	79	0.75	0.44	-0.086	0.931
Cumulative GPA	101	3.58	0.35	79	3.68	0.29	2.099	0.037

Table S4: Participant demographic profiles (Biochemistry II)

Demographic variable	"Second exposure" group			"First exposure" group			t-statistic	p-value
	N	Mean	S.D.	N	Mean	S.D.		
Gender (Male = 0, Female = 1)	40	0.63	0.49	47	0.64	0.49	0.127	0.899
Native English Speaker (No = 0, Yes = 1)	40	0.90	0.30	47	0.98	0.15	1.498	0.140
Parents' College Education (No = 0, Yes = 1)	40	0.93	0.27	47	0.83	0.38	-1.367	0.175
Job to Fund College Life (No = 0, Yes = 1)	40	0.75	0.44	47	0.72	0.45	-0.277	0.782
Cumulative GPA	40	3.64	0.39	47	3.81	0.18	2.576	0.013

Table S5: Class performance on the pre- and post-assessments (Biochemistry I)

	Year 1 "Module"			Year 1 "No module"		
Assessment number and name	**1.1: *Glycolysis***	**1.2: *TCA***	**1.3: *ETC***	**1.1: *Glycolysis***	**1.2: *TCA***	**1.3: *ETC***
N =	64	64	57	N/A	64	63
Average pre-assessment score (%)	58.2	63.9	62.0	N/A	61.3	67.9
S.D. Pre-assessment	13.1	16.5	15.4	N/A	16.3	16.6
Average post-assessment score (%)	66.7	70.3	71.7	N/A	61.9	64.9
S.D. Post-assessment	17.7	18.0	19.3	N/A	15.3	15.1
Average raw learning gain (%)	8.5	6.4	9.7	N/A	0.6	-3.0
Median raw learning gain (%)	11.1	6.3	9.7	N/A	0.0	0.0
S.D. Raw learning gain	21.9	24.2	19.9	N/A	21.4	19.8
Average normalized learning gain ⟨g⟩	0.20	0.18	0.26	N/A	0.02	-0.09
† Pre to post two-tailed paired t-test (p-value)	0.003	0.037	0.001	N/A	0.827	0.233
	Year 2 "Module"			**Year 2 "No module"**		
Assessment number and name	**1.1: *Glycolysis***	**1.2: *TCA***	**1.3: *ETC***	**1.1: *Glycolysis***	**1.2: *TCA***	**1.3: *ETC***
N =	96	95	93	75	64	68
Average pre-assessment score (%)	61.5	68.8	62.4	64.9	68.9	63.4
S.D. Pre-assessment	17.5	16.3	18.5	13.9	13.5	16.2
Average post-assessment score (%)	68.3	75.3	71.4	62.2	69.1	67.8
S.D. Post-assessment	18.4	17.5	16.7	16.5	18.1	16.4
Average raw learning gain (%)	6.8	6.4	9.1	-2.7	0.2	4.4
Median raw learning gain (%)	11.1	12.5	11.1	0.0	0.0	5.6
S.D. Raw learning gain	23.9	22.5	21.9	19.5	20.7	21.7
Average normalized learning gain ⟨g⟩	0.18	0.21	0.24	-0.08	0.01	0.12
† Pre to post two-tailed paired t-test (p-value)	0.006	0.006	0.000	0.240	0.940	0.099

Table S6: One-way ANCOVA results of Module versus No module courses (Biochemistry I)

	Model	Group	N	Unadjusted		Adjusted		*F***	*p-value*	*Partial eta*
				M	SD	M*	SE			
Year 1	**1.2: *TCA***	"Module"	62	0.70	0.18	0.74	0.05	*(1, 116) 7.443*	*0.007*	*0.060*
		"No module"	62	0.62	0.15	0.65	0.05			
	1.3: *ETC*	"Module"	55	0.72	0.19	0.70	0.05	*(1, 108) 11.609*	*0.001*	*0.097*
		"No module"	61	0.64	0.15	0.60	0.05			
Year 2	**1.1: *Glycolysis***	"Module"	96	0.68	0.18	0.69	0.03	*(1, 163) 6.968*	*0.009*	*0.041*
		"No module"	75	0.62	0.17	0.62	0.04			
	1.2: *TCA*	"Module"	95	0.75	0.17	0.73	0.04	*(1, 151) 4.872*	*0.029*	*0.031*
		"No module"	64	0.69	0.18	0.66	0.04			
	1.3: *ETC*	"Module"	92	0.71	0.17	0.71	0.04	*(1, 152) 4.944*	*0.028*	*0.031*
		"No module"	68	0.68	0.16	0.65	0.04			

Table S7: Class performance on the pre- and post-assessments (Biochemistry II)

	Module: All students in course	"Second exposure" group	"First exposure" group
Assessment number (and name)	**2: *Purine biosynthesis***	**2**	**2**
N =	87	40	47
Average pre-assessment score (%)	57.4	57.6	57.2
S.D. Pre-assessment	12.8	13.3	12.5
Average post-assessment score (%)	61.2	64.1	58.7
S.D. Post-assessment	15.8	16.7	14.7
Average raw learning gain (%)	3.7	6.6	1.2
Median raw learning gain (%)	5.0	10.0	5.0
S.D. Raw learning gain	16.9	13.8	18.9
Average normalized learning gain ⟨g⟩	0.09	0.15	0.03
† Pre to post two-tailed paired t-test (p-value)	0.022	0.005	0.446

Table S8: One-way ANCOVA results of "Second exposure" versus "First exposure" groups (Biochemistry II)

Model	Group	N	Unadjusted		Adjusted		*F***	*p-value*	*Partial eta*
			M	SD	M*	SE			
2: *Purine biosynthesis*	"Second exposure"	40	0.64	0.14	0.64	0.04	*(1, 79) 8.135*	*0.006*	*0.093*
	"First exposure"	47	0.58	0.12	0.56	0.04			

Table S9: Class performance on the pre- and post-assessments for each learning objective (Biochemistry I, year 1)

1.1: Glycolysis	"Module" (N = 64)		
Learning objectives	**1: Energy charge**	**2: Glucokin./ hexokin.**	**3: Absorp./ produc.**
Avg. pre-assess. score (%)	60.6	50.8	59.4
S.D. Pre-assess.	17.4	37.3	30.7
Avg. post-assess. score (%)	68.4	70.3	58.6
S.D. Post-assess.	19.5	36.4	27.5
Avg. raw gain (%)	7.8	19.5	-0.8
Mdn. raw gain (%)	10.0	25.0	0.0
S.D. raw gain	27.3	50.9	43.2
Avg. norm. gain ⟨g⟩	0.20	0.40	-0.02
† PrePost paired t-test (p-value)	0.025	0.003	0.885

1.2: TCA	"Module" (N = 64)			"No module" (N = 64)		
Learning objectives	**4: Energy charge**	**5: Redox state**	**6: Anapl. reactions**	**4: Energy charge**	**5: Redox state**	**6: Anapl. reactions**
Avg. pre-assess. score (%)	73.4	68.0	51.6	74.5	64.1	49.0
S.D. Pre-assess.	22.4	33.8	29.7	23.6	28.0	28.5
Avg. post-assess. score (%)	76.0	81.3	57.3	69.8	72.4	49.0
S.D. Post-assess.	23.4	28.9	25.5	22.8	29.4	24.5
Avg. raw gain (%)	2.6	13.3	5.7	-4.7	8.3	0.0
Mdn. raw gain (%)	0.0	0.0	0.0	0.0	0.0	0.0
S.D. raw gain	31.0	38.1	41.0	30.2	49.7	38.0
Avg. norm. gain ⟨g⟩	0.10	0.41	0.12	-0.18	0.23	0.00
† PrePost paired t-test (p-value)	0.504	0.007	0.267	0.219	0.073	1.000

1.3: ETC	"Module" (N = 57)				"No module" (N = 63)			
Learning objectives	**7: Aer. resp.**	**8: Redox state**	**9: Energy charge**	**10: Fermen-tation**	**7: Aer. resp.**	**8: Redox state**	**9: Energy charge**	**10: Fermen-tation**
Avg. pre-assess. score (%)	71.9	62.0	55.3	47.4	76.2	67.7	57.9	63.5
S.D. Pre-assess.	26.6	24.8	32.3	50.4	25.7	23.9	31.4	48.5
Avg. post-assess. score (%)	82.5	67.8	63.2	68.4	75.1	64.6	52.4	60.3
S.D. Post-assess.	23.7	28.1	32.1	46.9	25.4	24.6	31.7	49.3
Avg. raw gain (%)	10.5	5.8	7.9	21.1	-1.1	-3.2	-5.6	-3.2
Mdn. raw gain (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S.D. raw gain	34.0	33.4	44.4	59.0	32.8	31.5	44.1	56.7
Avg. norm. gain ⟨g⟩	0.38	0.15	0.18	0.40	-0.04	-0.10	-0.13	-0.09
† PrePost paired t-test (p-value)	0.023	0.192	0.140	0.009	0.799	0.427	0.321	0.658

Table S10: Class performance on the pre- and post-assessments for each learning objective (Biochemistry I, year 2)

1.1: Glycolysis	"Module" (N = 96)				"No module" (N = 75)			
Learning objectives	**1: Energy charge**	**2: Glucokin./ hexokin.**	**3: Absorp./ produc.**		**1: Energy charge**	**2: Glucokin./ hexokin.**	**3: Absorp./ produc.**	
Avg. pre-assess. score	68.3	56.3	49.5		69.3	56.7	62.0	
S.D. Pre-assess.	21.5	37.2	38.7		18.4	36.1	39.3	
Avg. post-assess. score	74.4	62.0	59.4		67.2	59.3	52.7	
S.D. Post-assess.	21.5	38.9	37.9		17.9	36.5	37.6	
Avg. raw gain (%)	6.0	5.7	9.9		-2.1	2.7	-9.3	
Mdn. raw gain (%)	0.0	0.0	0.0		0.0	0.0	0.0	
S.D. raw gain	30.3	53.7	49.5		25.2	49.9	47.7	
Avg. norm. gain ⟨g⟩	0.19	0.13	0.20		-0.07	0.06	-0.25	
† PrePost paired t-test (p-value)	0.054	0.299	0.053		0.465	0.645	0.094	
1.2: TCA	"Module" (N = 95)				"No module" (N = 64)			
Learning objectives	**4: Energy charge**	**5: Redox state**	**6: Anapl. reactions**		**4: Energy charge**	**5: Redox state**	**6: Anapl. reactions**	
Avg. pre-assess. score	76.8	67.9	61.4		72.4	70.3	64.6	
S.D. Pre-assess.	22.8	35.7	29.3		22.7	36.4	25.1	
Avg. post-assess. score	80.7	76.3	69.1		75.5	68.8	63.0	
S.D. Post-assess.	21.0	35.6	26.7		24.7	33.9	27.3	
Avg. raw gain (%)	3.9	8.4	7.7		3.1	-1.6	-1.6	
Mdn. raw gain (%)	0.0	0.0	0.0		0.0	0.0	0.0	
S.D. raw gain	29.9	45.9	38.1		33.4	50.4	35.8	
Avg. norm. gain ⟨g⟩	0.17	0.26	0.20		0.11	-0.05	-0.04	
† PrePost paired t-test (p-value)	0.212	0.077	0.051		0.458	0.805	0.728	
1.3: ETC	"Module" (N = 93)				"No module" (N = 78)			
Learning objectives	**7: Aer. resp.**	**8: Redox state**	**9: Energy charge**	**10: Fermen-tation**	**7: Aer. resp.**	**8: Redox state**	**9: Energy charge**	**10: Fermen-tation**
Avg. pre-assess. score	65.2	63.8	62.4	49.5	73.5	62.7	58.8	44.1
S.D. Pre-assess.	29.9	23.4	33.5	50.3	26.0	23.5	32.4	50.0
Avg. post-assess. score	82.1	68.5	60.8	69.9	78.4	65.2	64.0	51.5
S.D. Post-assess.	22.8	26.2	28.4	46.1	24.9	26.0	32.1	50.3
Avg. raw gain (%)	16.8	4.7	-1.6	20.4	4.9	2.5	5.1	7.4
Mdn. raw gain (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S.D. raw gain	36.7	30.9	41.3	65.2	34.2	34.7	41.6	63.0
Avg. norm. gain ⟨g⟩	0.48	0.13	-0.04	0.40	0.19	0.07	0.13	0.13
† PrePost paired t-test (p-value)	0.000	0.150	0.708	0.003	0.241	0.562	0.311	0.340

Table S11: Class performance on the pre- and post-assessments for each learning objective (Biochemistry II, year 1)

2: Purine biosynthesis	Module: All students in course (N = 87)			
Learning objectives	1: Components and interactions	2: Maintain homeostasis	3: Cellular changes	4: Mutations and disease
Avg. pre-assessment score (%)	55.2	62.1	61.8	55.2
S.D. Pre-assess.	16.5	28.4	30.7	21.5
Avg. post-assessment score (%)	62.1	59.4	60.3	61.7
S.D. Post-assess.	23.7	31.5	26.3	21.6
Avg. raw gain (%)	6.9	-2.7	-1.4	6.5
Mdn. raw gain (%)	0.0	0.0	0.0	0.0
S.D. raw gain	24.9	39.4	38.0	28.1
Avg. norm. gain ⟨g⟩	0.15	-0.07	-0.04	0.15
† PrePost paired t-test (p-value)	0.012	0.528	0.725	0.033
	"Second exposure" group (N = 40)			
Learning objectives	1: Components and interactions	2: Maintain homeostasis	3: Cellular changes	4: Mutations and disease
Avg. pre-assessment score (%)	58.2	60.8	61.3	52.9
S.D. Pre-assess.	16.3	29.1	29.4	22.6
Avg. post-assessment score (%)	65.0	60.8	64.4	64.6
S.D. Post-assess.	24.8	30.1	23.9	19.3
Avg. raw gain (%)	6.4	0.8	5.0	10.8
Mdn. raw gain (%)	0.0	0.0	0.0	16.7
S.D. raw gain	23.5	35.8	29.0	24.3
Avg. norm. gain ⟨g⟩	0.16	0.00	0.08	0.25
† PrePost paired t-test (p-value)	0.071	1.000	0.515	0.003
	"First exposure" group (N = 47)			
Learning objectives	1: Components and interactions	2: Maintain homeostasis	3: Cellular changes	4: Mutations and disease
Avg. pre-assessment score (%)	52.6	63.1	62.2	57.1
S.D. Pre-assess.	16.3	28.0	32.1	20.5
Avg. post-assessment score (%)	59.6	58.2	56.9	59.2
S.D. Post-assess.	22.7	32.9	27.9	23.3
Avg. raw gain (%)	7.3	-5.7	-6.9	2.8
Mdn. raw gain (%)	14.3	0.0	-25.0	0.0
S.D. raw gain	26.2	42.5	43.8	30.7
Avg. norm. gain ⟨g⟩	0.15	-0.13	-0.14	0.05
† PrePost paired t-test (p-value)	0.078	0.425	0.407	0.642

Table S12: One-way ANCOVA results of gender groups (Biochemistry I)

	Model	Group	N	Unadjusted		Adjusted		*F***	*p-value*	*Partial eta*
				M	SD	M*	SE			
Year 1	**1.2: *TCA***	"Module and male"	25	0.74	0.19	0.75	0.05	*(3, 115) 3.021*	*0.033*	*0.073*
		"Module and female"	37	0.68	0.18	0.70	0.04			
		"No module and male"	21	0.63	0.12	0.63	0.05			
		"No module and female"	41	0.62	0.17	0.63	0.04			
	1.3: *ETC*	"Module and male"	22	0.74	0.18	0.69	0.05	*(3, 107) 3.822*	*0.012*	*0.097*
		"Module and female"	33	0.71	0.20	0.71	0.05			
		"No module and male"	20	0.67	0.21	0.60	0.05			
		"No module and female"	41	0.63	0.11	0.59	0.04			
Year 2	**1.1: *Glycolysis***	"Module and male"	41	0.70	0.19	0.73	0.03	*(3, 162) 2.914*	*0.036*	*0.051*
		"Module and female"	55	0.67	0.18	0.70	0.03			
		"No module and male"	28	0.61	0.19	0.62	0.04			
		"No module and female"	47	0.63	0.15	0.65	0.03			
	1.3: *TCA*	"Module and male"	38	0.75	0.15	0.73	0.04	*(3, 150) 1.951*	*0.124*	*0.038*
		"Module and female"	57	0.75	0.19	0.75	0.03			
		"No module and male"	21	0.67	0.18	0.66	0.04			
		"No module and female"	43	0.70	0.18	0.69	0.04			
	1.3: *ETC*	"Module and male"	38	0.69	0.20	0.67	0.03	*(3, 151) 2.271*	*0.083*	*0.043*
		"Module and female"	54	0.73	0.14	0.72	0.03			
		"No module and male"	23	0.68	0.18	0.65	0.04			
		"No module and female"	45	0.68	0.16	0.64	0.03			

File S1

SUPPLEMENTAL METHODS

Analysis of student perceptions

To analyze students perceptions in the open-ended portion of our survey, we began by reading through all students' answers to the open-ended questions. We used 35 randomly-selected responses (about half of all responses) to develop a set of unique themes for each question, and we coded all students' answers based on the themes we identified. When reporting about which aspect of the module students found most beneficial, we commented about the top four themes, and we selected three pull-quotes from the top two themes. When reporting about which aspect of the module the students found least beneficial, we also commented about the top four themes and selected pull-quotes from the top two themes. Because two themes were evenly ranked at the third position in both cases, we commented about the top four themes instead of the top three themes.

SUPPLEMENTAL RESULTS AND DISCUSSION

Student perceptions of the computational learning modules

We used a short survey to determine whether students perceived a learning benefit after completing the modules (Supplemental Figure S6, Supplemental Files S5 and S9). In the closed-ended portion of the survey for the *Regulation of Cellular Respiration* module during year 1 of Biochemistry I, 54% of students who completed this survey agreed that the module assisted their learning of the material, 45% of the students agreed that they understood what they learned and 48% thought they would remember what they learned (Supplemental Figure S6A). Sixty percent of students reported that the module reminded them to use a systems-thinking approach that simultaneously considers individual components and the larger system. Likewise, 60% of students reported that the module helped them to understand the effect of feedback loops and environmental conditions, while 58% agreed that the module helped them to understand how the regulation of glycolysis, the TCA cycle, and the ETC are integrated to function as a coherent whole (Supplemental Figure S6A). When we averaged student responses across all six questions, we found that the class average was 3.3 and the median 3.5, indicating that students generally reacted positively to the *Regulation of Cellular Respiration* module. The results were similar for year 2 of Biochemistry I (Supplemental Figure S6B). For the *Regulation of Purine Biosynthesis* module (Supplemental Figures S6C and D), we saw a similar, though less dramatic, trend where students self-reported that the modules (1) reminded them to think about the individual components and the role they play in the larger system, (2) helped them understand the effect of feedback loops and environmental conditions, and (3) helped them appreciate the role of each interaction in the overall regulation of metabolism (Supplemental Figure S6C and D). For the *Regulation of Purine Biosynthesis* module, the class average was 2.8, and the median was 3.0. We suspect that the lower percentage of students who reacted favorably to the module in Biochemistry II compared to Biochemistry I could be attributed to the fact that students were less familiar with the purine biosynthesis system. However, more data are needed to conclusively establish the reasons for the less favorable reaction to the module in Biochemistry II.

In the open-ended section of the survey, we asked students to reflect on which aspects of the modules they found to be most and least beneficial. Benefits included being able to manipulate individual components of the model and directly visualize the effect on the entire system using simulations, and seeing the relationships between individual components and multiple processes. One student summarized the importance of simulating the model's behavior, "The running of the simulations is the most important aspect of the module, at least in my case. It is the only time you are fully able to see what is happening to the levels of different products in the cell and how it

affects activity." Another noted that the module aided their learning by "[seeing how] changing the amount of glucose, LDH, O2, and physical exercise alters the production of glycolysis, fermentation, TCA, and ETC and how all of the individual components/metabolites are affected". A third student noted that the module helped them to "[think] about why the enzymes were connected the way they were and why my predictions were or were not correct". Students also explicitly commented on the usefulness of directly observing the outcome of adding inhibitory relationships. Some students also reported positive responses to being asked many conceptual questions about the system components and simulation results. Student challenges included keeping track of the number of components and connections involved in the processes (feeling overwhelmed) as well as feeling concerned about whether the simulations were set up correctly and whether their answers were correct. Paradoxically, some students reported frustration about being asked to conceptually evaluate the simulation results. Students who completed the *Regulation of Purine Biosynthesis* module had similar responses to the open-ended section of the survey compared to students who completed the *Regulation of Cellular Respiration* module.

During a small focus group conducted by an external evaluator, two students discussed their experience during year 1 of Biochemistry I. When asked about the top two most memorable concepts learned during the entire course, one student reported remembering "doing glycolysis, doing the online skills and going through that and learning the up and down regulations...helped me learn how to do the TCA cycle." When asked how the modules supported student learning, one student noted that "having the modules as a backup to look at whenever you're learning such a dense topic is a good way to relearn it besides what's in the class... It's a different...hands-on way to look at it, than just having it in front of you and looking at it." Another student commented on the fact that the systems were so complex that it would be difficult to make predictions about them without first creating a model.

Although students generally valued the computational learning modules, some students were less open to the presented learning approach. A few of these students noted that they would have preferred having lectures or studying the material from the textbook over interacting with the computational models. Our results are consistent with previous findings that classroom interactions and student confidence in the results obtained with models can affect the success of computer model-based instructional approaches (Liang et al., 2012; Streicher et al., 2005). Some students reported usability issues and commented on their lack of prior knowledge as being challenges to their learning with the modules. To better understand this feedback, we attempted to identify a test group that interacted with an inquiry-based learning environment with high frequency. A small-enrollment course version of biochemistry at a nearby private liberal arts college which emphasizes inquiry-based learning approaches tried the module in the classroom and found similar learning gains. Interestingly, these students rated their learning experience more positively than our students. Our observations are in agreement with findings that students' curricular exposure shapes their learning profile development, which may determine their readiness for self-directed learning (Kell & Van Deursen, 2002). On the usability issues reported, we recognize that technological challenges may be unavoidable with computer-based learning, and we propose that instructors use in-class messaging to encourage students to leave enough time for assistance. Instructors may also increase student buy-in by ensuring close alignment between the modules, class lectures, and exam questions (Wiggins and McTighe. 2005; Brazeal et al. 2016). Finally, instructors could try introducing students to modeling using a familiar system before transitioning to an unfamiliar system, because we suspect that perceived learning may be lower with unfamiliar systems (Supplemental Figures S6C and D).

Recommendations for incorporation into the classroom

Using our computational learning modules, instructors can employ different adoption approaches to meet their specific course needs and teaching strategies. In our experience, students who have never used the models before and are first exposed to them when learning about unfamiliar

biochemistry content may at first report feeling overwhelmed. However, sometimes the situation of concurrently introducing a new teaching approach and unfamiliar content is unavoidable. We believe that instructors can mediate student difficulties using a variety of strategies, including the approaches we describe below.

First, instructors can integrate the modules as we did, using the course slides as a guideline (Supplemental Files S2 and S6). If all three parts of the *Regulation of Cellular Respiration* module will be used, we recommend that students complete them in the order presented in the manuscript. To relieve student anxiety about simulation results or answers being correct, instructors could check in with the entire class after students have performed a simulation to ensure that everyone is seeing a correct simulation result. This approach could build confidence that the simulations are set up correctly. Instructors could also provide students with a study sheet showing key simulation results and answers to more challenging module questions. Our results suggest that students will achieve the greatest benefit from the modules if they are already somewhat familiar with the components and connections of the system. We therefore suggest that instructors include the modules after students have already been introduced to the basic structure of the metabolic system being studied.

Second, if instructors provide appropriate additional support for students, they may decide to focus only on one section of the *Regulation of Cellular Respiration* module. It may similarly be possible to use only a few of the module activities to teach about the *Regulation of Purine Biosynthesis* if additional support is provided to students. Instructors could also incorporate our assessment questions into their regularly scheduled exams or quizzes to reduce assessment fatigue.

Third, instructors can follow the guided-instruction approach where they fully introduce the components of the system and how the components fit together as they would in a lecture-based class. Instructors could then introduce the models and module questions during the remainder of the lecture while the instructor demonstrates how to manipulate the model and asks students to discuss and respond to the questions and report back during class (either as whole-class group feedback or clicker responses). Using this approach, the instructor serves as a guide that demonstrates ways to deal with possible technological issues. Students can then focus on first engaging conceptually with the material using group discussion rather than being focused on modeling instructions or troubleshooting. Once the students are more comfortable with the new approach, the instructor should ask students to complete subsequent parts of the module on their own in class or as homework, and remind them to draw on the instructor demonstration when engaging in the modeling and simulation tasks.

Fourth, instructors could integrate the modules as part of a hands-on laboratory experiences. Using this approach, instructors can ask students to make predictions using the models that can then be tested in the laboratory. Students could also use the models to design their laboratory experiments.

Finally, our modules could be ideal for instructors who are using online or blended courses where students complete the module completely as homework. The approach we used in our courses required that significant portions of the modules be completed as homework, so we believe that students will be successful with this approach.

SUPPLEMENTAL REFERENCES

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Timing

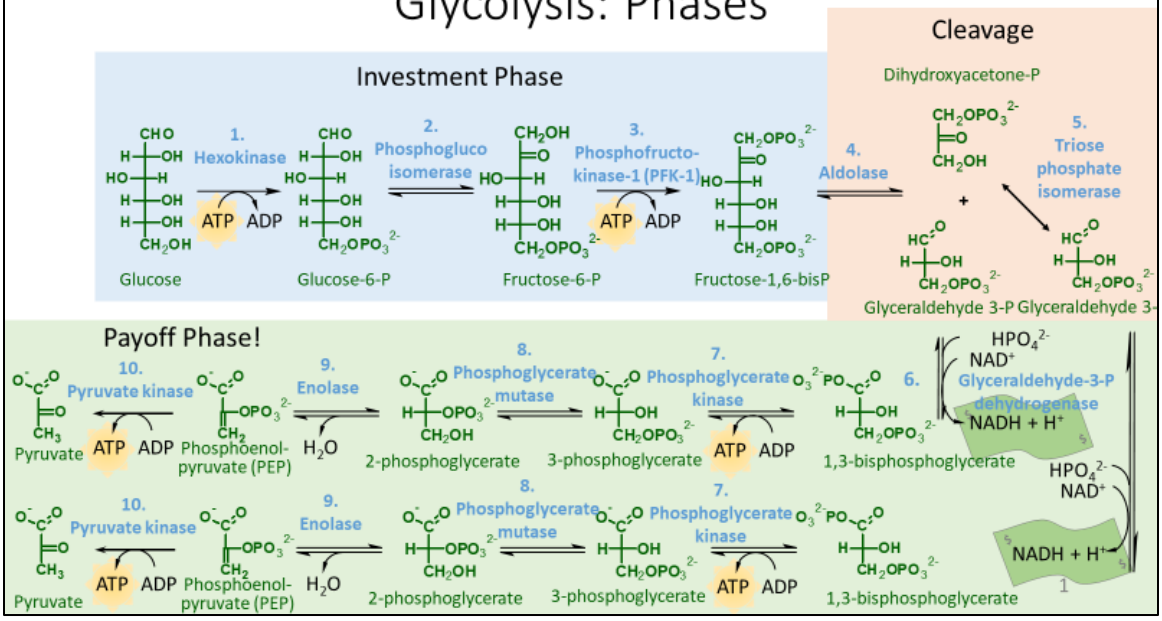
These slides should be introduced before students have completed the glycolysis portion of the *Regulation of Cellular Respiration* module

Prerequisite Knowledge and suggestions for incorporation

Introduce concurrently with the following topics:

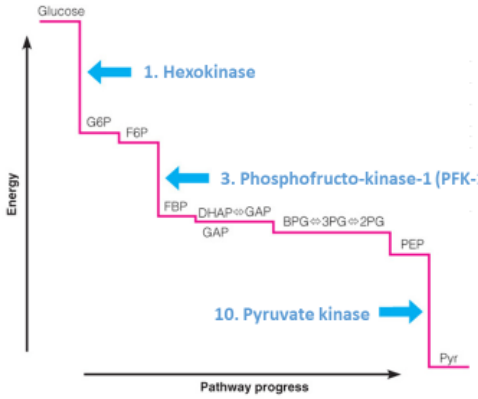
1) Steps in glycolysis
2) Regulation of glycolysis

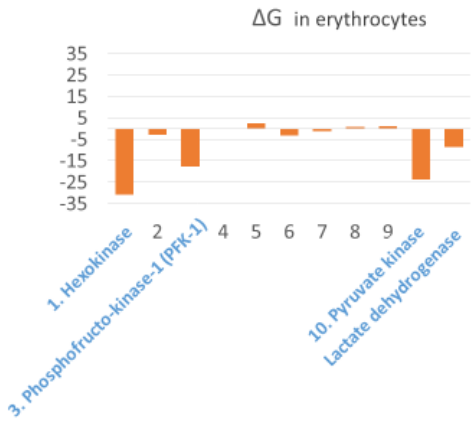
Glycolysis: Phases



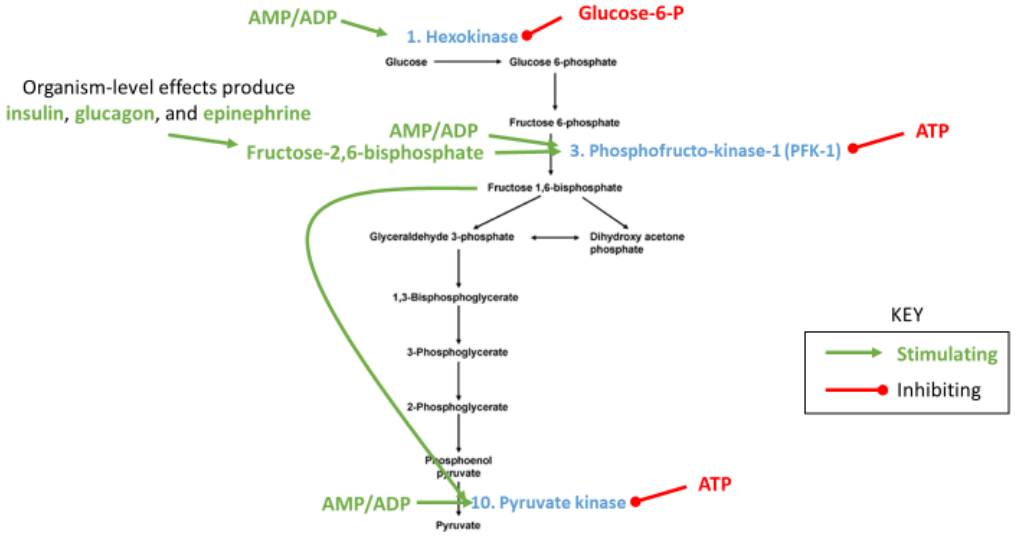
Regulation

Consider the changes from $\Delta G^{o\prime}$ to ΔG in erythrocytes:

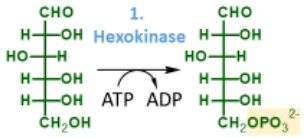




Glycolysis regulation summary

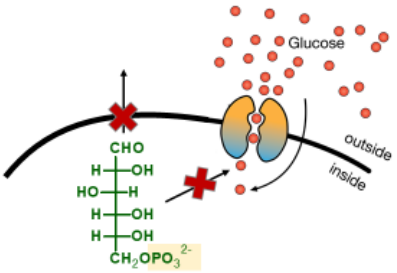


Hexokinase regulation



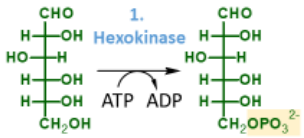
Hexokinase can be considered the "committed step" of glucose entering the cell.

Negatively regulated by Glucose-6-P



This is NOT the "committed step" of glycolysis. i.e., glucose-6-P is used in other pathways.

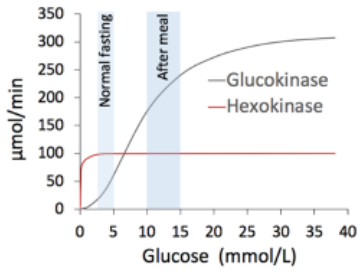
Hexokinase vs Glucokinase regulation: Modeling



Not all tissues require equal amounts of glucose.

Only two tissues store significant amounts of glycogen:

Liver & muscle

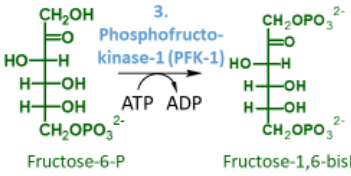


The liver stores glucose as glycogen primarily to maintain blood sugar levels between meals. This is very important for the brain.

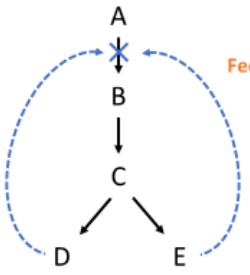
Hexokinase is feedback inhibited by Glucose-6-P
Glucokinase is not.

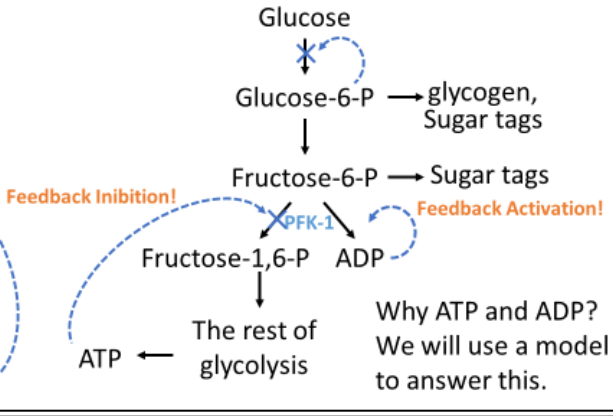
Why? We will use a model to answer this.

Phosphofructokinase regulation



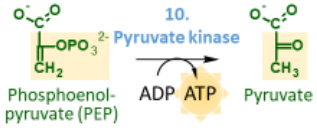
Phosphofructokinase is the **committed step** of glycolysis





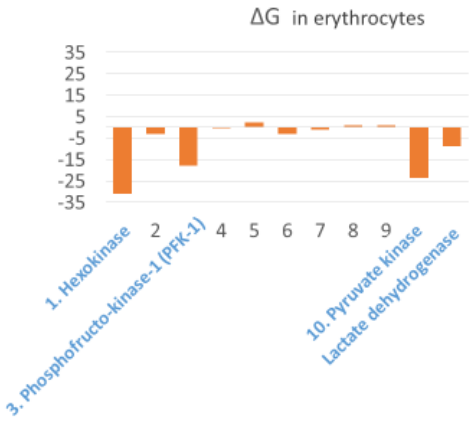
Why ATP and ADP?
We will use a model to answer this.

Pyruvate kinase regulation



Without the "pull" of pyruvate kinase, there is no way to make reactions 4 -9 stable. It will also effectively stop glycolysis.

Why regulate the last step of the pathway?



Clicker Question

What parts of metabolism do *not* need to be included to build an accurate metabolic model?

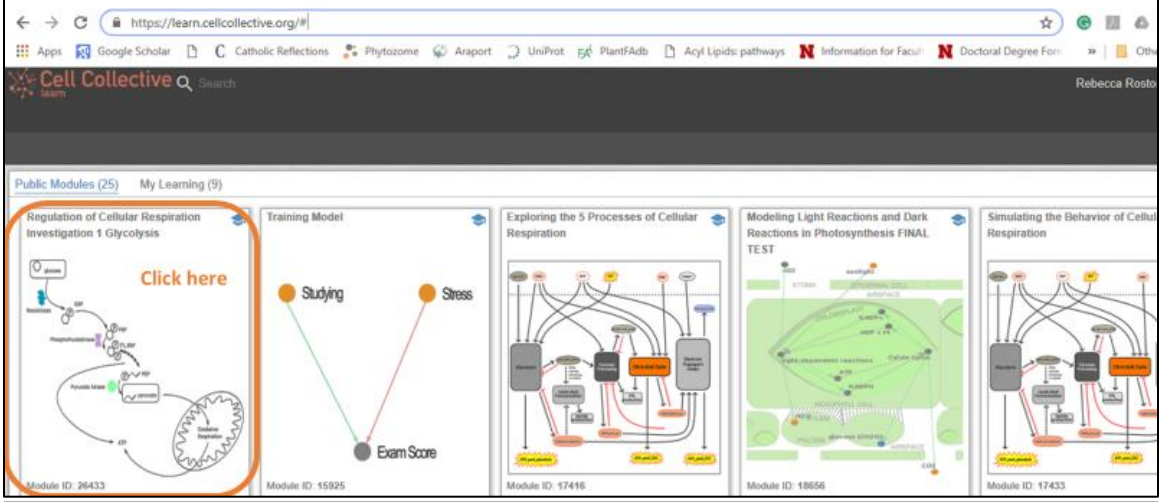
A. Reversible reactions
B. Reactions with *no* allosteric control
C. Reactions not relevant to the question asked of the model
D. All of the above

Questions we need a glycolysis model to answer:

1. Why are Hexokinase and Glucokinase regulated differently?
2. Why is phosphofructokinase regulated by ATP *and* ADP?
3. Why is pyruvate kinase *also* regulated?
4. Why is pyruvate kinase *also* regulated by ATP *and* ADP?

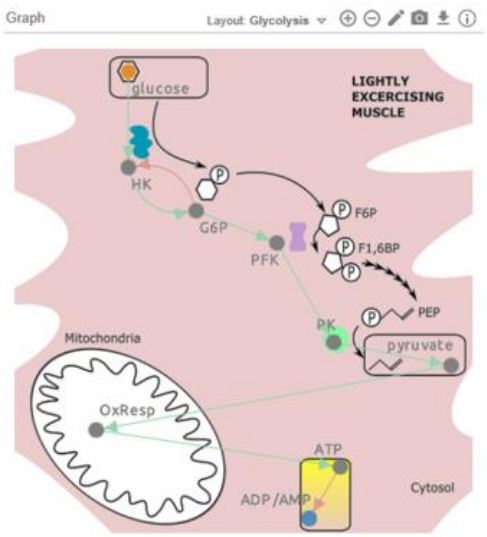
In-class activity: Model glycolysis regulation

https://learn.cellcollective.org/#



In-class activity: Model glycolysis regulation

https://learn.cellcollective.org/#



Some metabolites (F1,6BP and PEP) are shown as images, but are not system components and cannot be simulated.

You can only change dots (nodes) and arrows (edges).

Pay attention to the cell type - lightly exercising muscle – these results are not true for all cell types.

Clicker Question: Click in with "A" when you have reached the thought question on Activity 1.2

Thought Question: A lightly exercising muscle will have a relatively constant need for ATP synthesis…

Activity 1.2 Class Discussion Question

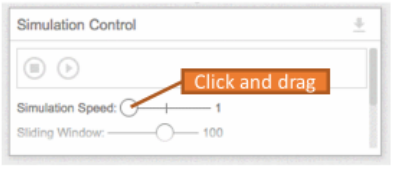
Do you think that the rate of glycolysis should change as the [glucose] changes? Why?

"Yes"es reasons:

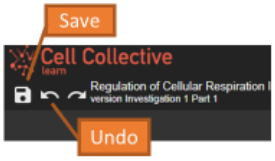
"No"s reasons:

How to...

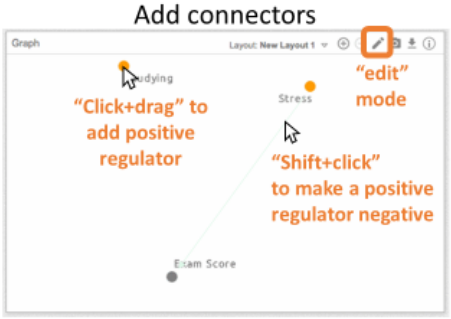
Change the simulation speed



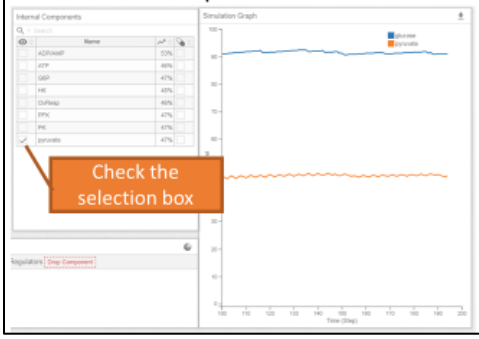
Undo/Save



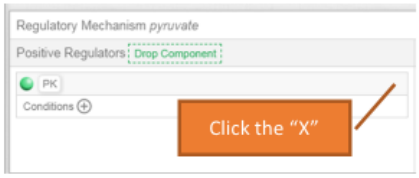
Add connectors



Select components to view



Remove connectors



Timing

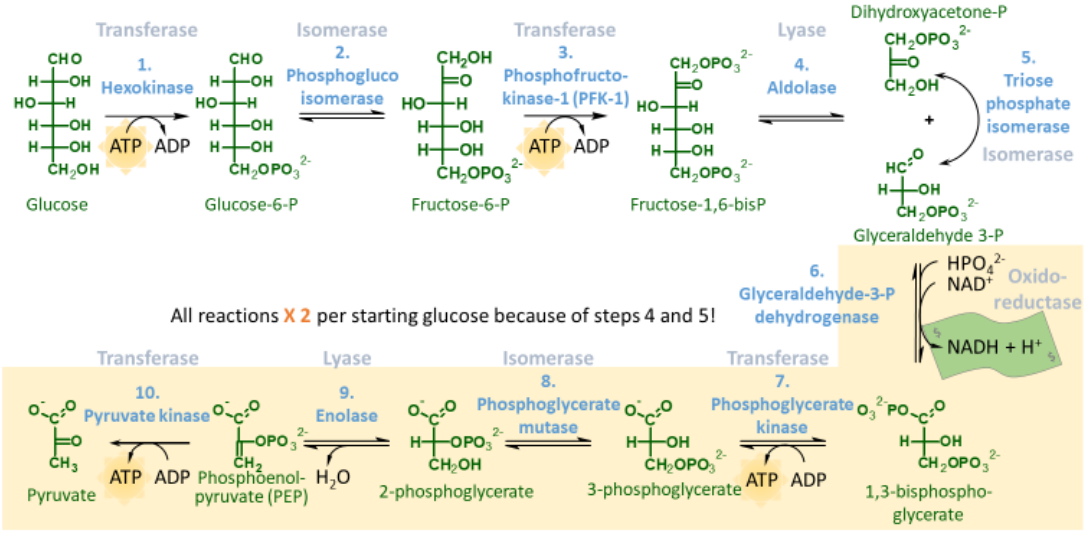
These slides should be introduced after students have completed the glycolysis portion of the *Regulation of Cellular Respiration* module

Prerequisite Knowledge and suggestions for incorporation

Introduce after discussing the following topics:

1) Steps in glycolysis
2) Regulation of glycolysis

Last week: Glycolysis

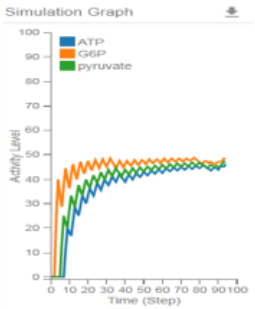


Questions we need a glycolysis model to answer:

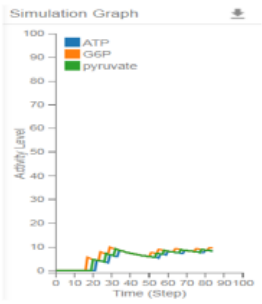
1. Why are Hexokinase and Glucokinase regulated differently?
2. Why is phosphofructokinase regulated by ATP *and* ADP?
3. Why is pyruvate kinase *also* regulated?
4. Why is pyruvate kinase *also* regulated by ATP *and* ADP?

Regulation of Glycolysis

High glucose and **no regulation** of **Phosphofructokinase** or **Pyruvate kinase**



Low glucose and **no regulation** of **Phosphofructokinase** or **Pyruvate kinase**



Clicker Question

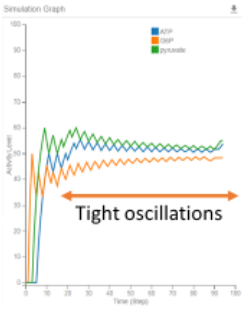
Notice the ATP levels. Knowing that ATP:ADP ratio was important for making ΔG favorable during glycolysis, *predict if this cell will survive* when glucose is low.

A. Yes

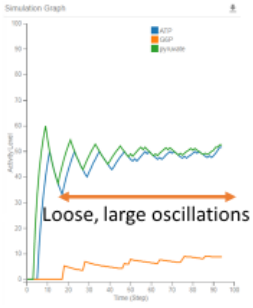
B. No or not well

Regulation of Glycolysis

High glucose and **ATP negative regulation** of **Phosphofructokinase** only



Low glucose and **ATP negative regulation** of **Phosphofructokinase** only



Clicker Question

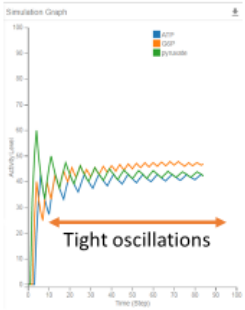
Is simple regulation of one enzyme enough to help maintain cellular ATP levels?

A. Yes

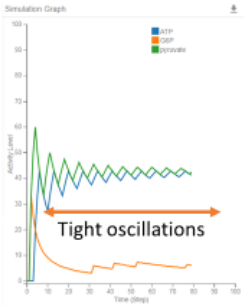
B. No or not well

Regulation of Glycolysis

High glucose and **ATP/ADP regulation** of **Phosphofructokinase** and **Pyruvate kinase**



Low glucose and **ATP/ADP regulation** of **Phosphofructokinase** and **Pyruvate kinase**



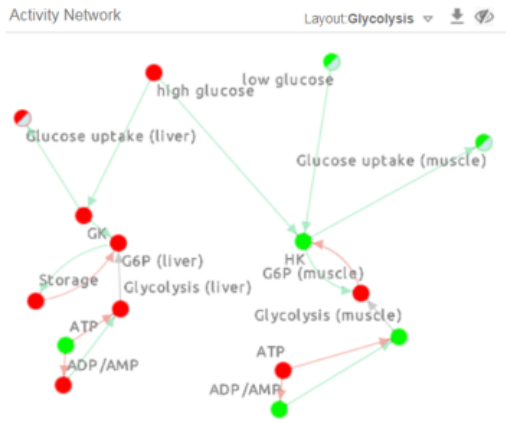
Clicker Question

Does regulation of multiple enzymes improve cellular ATP regulation over that of one enzyme?

A. Yes

B. No or not well

Regulation of Glycolysis



Clicker Question

When glucose is low, which tissues take it up?

A. Muscle

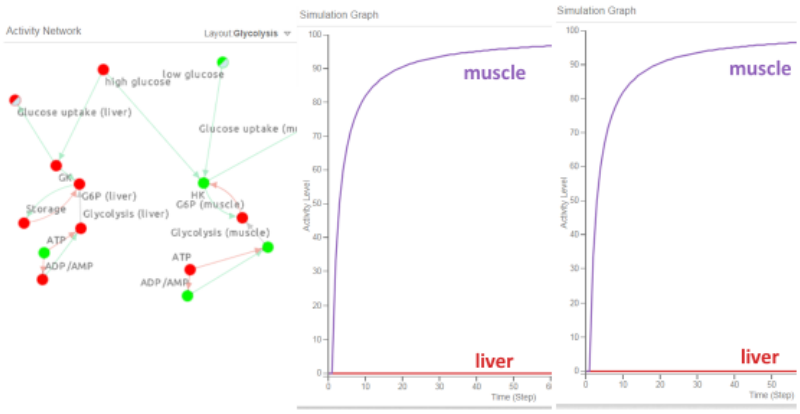
B. Liver

C. A and B

Regulation of Glycolysis

High glucose **Low glucose**

Monitoring Glycolysis Output (ATP production)



Clicker Question

Does the glycolysis level in either tissue depend on the uptake of glucose?

A. Yes

B. No or not much

Timing

These slides should be introduced before students have completed the TCA portion of the *Regulation of Cellular Respiration* module

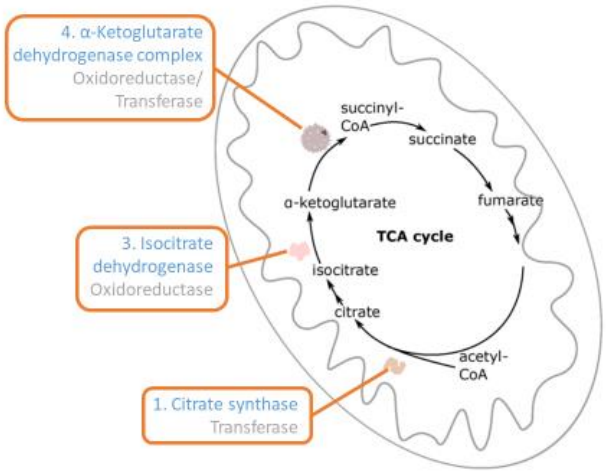
They could be introduced during the same class as the previous slide set

Prerequisite Knowledge and suggestions for incorporation

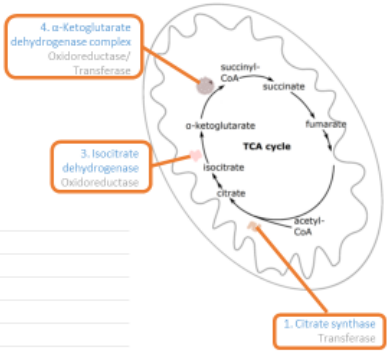
Introduce concurrently with the following topics:

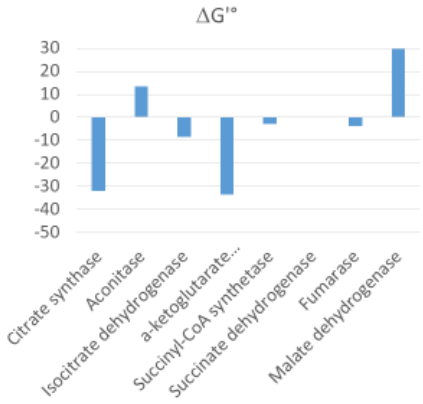
1) Steps in the TCA cycle
2) Regulation of the TCA cycle

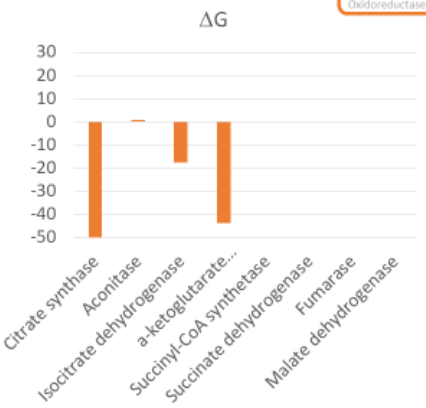
Regulation of the TCA cycle



Regulation of the TCA cycle







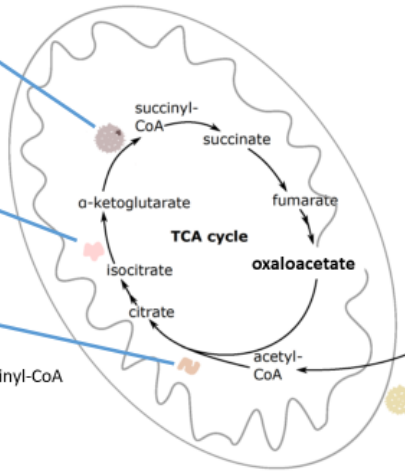
Regulation of the PDC & TCA cycle

Body-level regulation (signals muscle contraction)

4. α-Ketoglutarate dehydrogenase complex
- ⊕ AMP, Ca^{2+}
- ⊖ NADH, succinyl-CoA, ATI

3. Isocitrate Dehydrogenase
- ⊕ ADP, Ca^{2+}
- ⊖ NADH, ATP

1. Citrate synthase
- ⊕ ADP
- ⊖ NADH, citrate, ATP, succinyl-CoA



Group activity:

1) How is regulation of the TCA cycle *similar* to regulation of glycolysis?

2) How is regulation of the TCA cycle *different* than regulation of glycolysis?

Pyruvate dehydrogenase complex
- ⊕ NAD^+, CoA, ADP, Ca^{2+}
- ⊖ NADH, acetyl-CoA, ATP

Regulation of the PDC & TCA cycle

Body-level regulation (signals muscle contraction)

Two themes: **product inhibition** and **substrate availability**

Model:

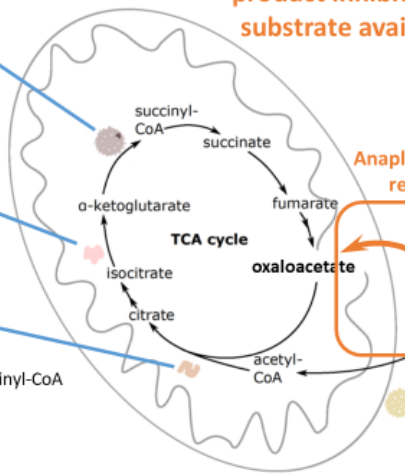
How will anaplerotic reactions help maintain TCA function?

Scenario: Cells need energy to build *and* amino acids to build with.

4. α-Ketoglutarate dehydrogenase complex
- ⊕ AMP, Ca^{2+}
- ⊖ NADH, succinyl-CoA, ATP

3. Isocitrate Dehydrogenase
- ⊕ ADP, Ca^{2+}
- ⊖ NADH, ATP

1. Citrate synthase
- ⊕ ADP
- ⊖ NADH, citrate, ATP, succinyl-CoA

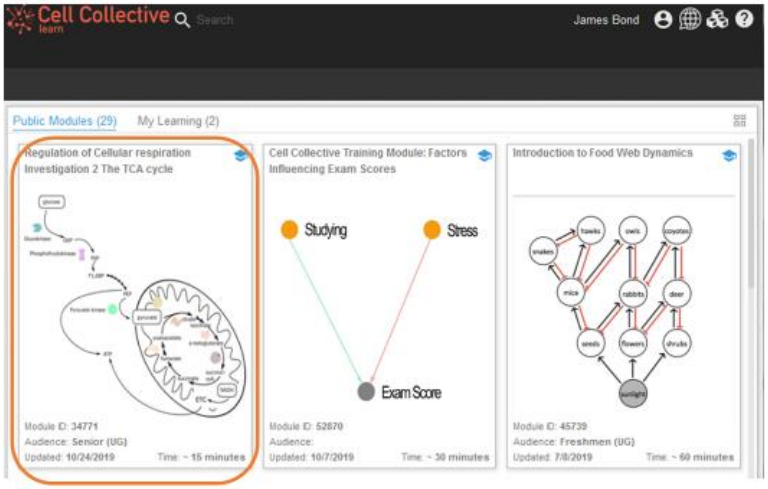


Anaplerotic reaction

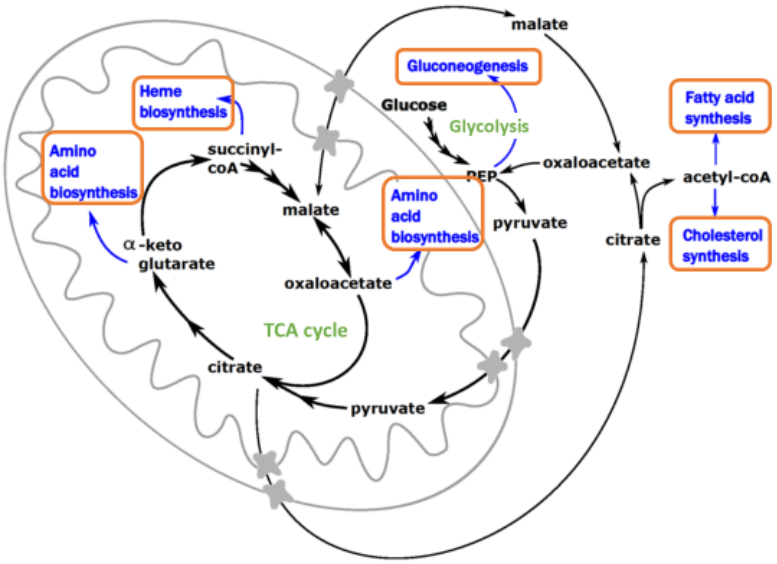
Pyruvate carboxylase
- ⊕ Acetyl-CoA

Pyruvate dehydrogenase complex
- ⊕ NAD^+, CoA, ADP, Ca^{2+}
- ⊖ NADH, acetyl-CoA, ATP

Cell Collective Homework Assignment!



The TCA cycle is not only used for energy!

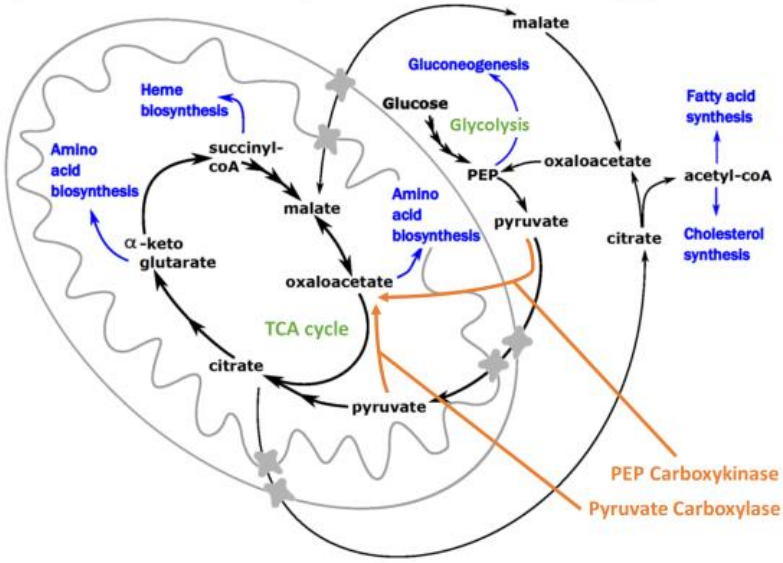


The TCA cycle is not only used for energy!

These are called **anaplerotic** reactions

They are **filling up** intermediates of a metabolic pathway

Ancient Greek: **anaplerosis**
ἀνά= 'up' and πληρόω= 'to fill'.



Timing

These slides should be introduced before students have completed the ETC portion of the *Regulation of Cellular Respiration* module

Prerequisite Knowledge and suggestions for incorporation

Introduce after discussing the following topics:

Fermentation

Introduce concurrently with the following topics:

1) Steps in the ETC
2) Regulation of the ETC

Homework assignment:

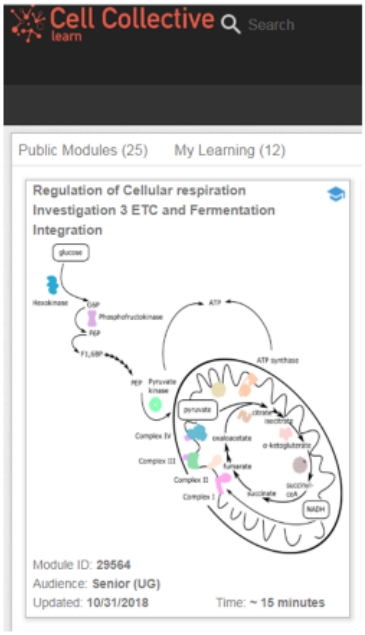
Testing-type questions: predict what happens to respiration when…

Simulation Results Table 3.1: Are the following processes active (Y/N)?

Environmental/cellular condition →	O2 present, No LDH	No O2 or LDH	LDH present, No O2
Pyruvate (glycolysis)	Enter Text	Enter Text	Enter Text
Lactate (fermentation)	Enter Text	Enter Text	Enter Text
CO2 (TCA cycle)	Enter Text	Enter Text	Enter Text
O2 consumption (ETC)	Enter Text	Enter Text	Enter Text

Simulation Results Table 3.2: Is ATP produced by the processes (Y/N)?

Environmental/cellular condition →	O2 present, No LDH	No O2 or LDH	LDH present, No O2
2 ATP (glycolysis)	Enter Text	Enter Text	Enter Text
30/32 ATP (TCA and ETC)	Enter Text	Enter Text	Enter Text



Timing

These slides should be introduced after students have completed the ETC portion of the *Regulation of Cellular Respiration* module

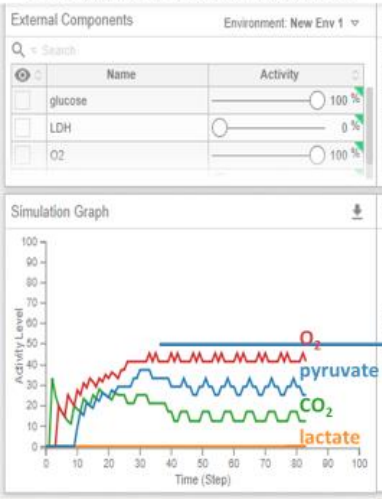
Prerequisite Knowledge and suggestions for incorporation

Introduce after discussing the following topics:

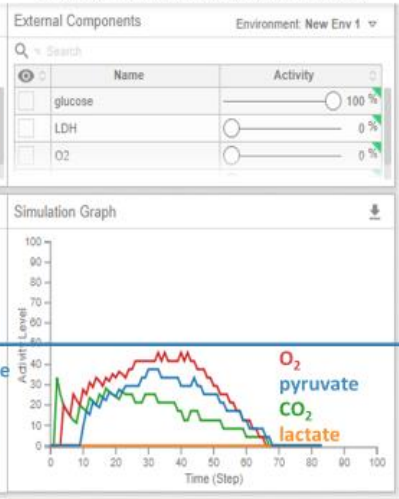
1) Fermentation
2) Steps in the ETC
3) Regulation of the ETC

Homework on Respiration using modeling

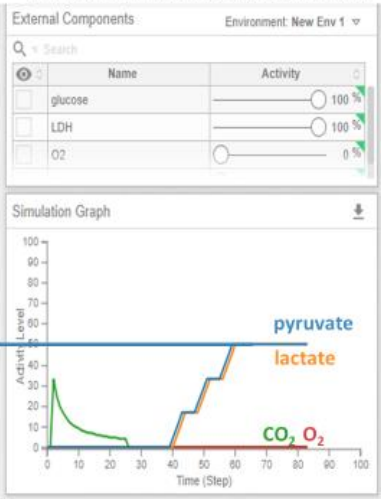
With O_2, energy is effectively supplied



Without fermentation, O_2 is needed.



Without O_2, more glycolysis is needed.



Clicker question:

Homework on Respiration using modeling

Why does flux (numbers of substrates/intermediates) through glycolysis increase in the absence of O_2?

A. Need for NAD^+ regeneration
B. ETC is working harder
C. Need for ATP
D. A and B
E. All of the above

ATP yield of 1 glucose:

Pathway		ATP	Reductant	ATP equivalents
Glycolysis	1 Glucose → 2 Pyruvate	**2 ATP**	**2 NADH***	**2 + 5***
Pyruvate Dehydrogenase	2 Pyruvate → 2 Acetyl-CoA + 2 CO_2		**2 NADH**	**5**
TCA Cycle	2 Acetyl-CoA → 4 CO_2	**2 GTP**	**6 NADH** **2 $FADH_2$**	**2 + 15** **3**
	1 NADH = 2.5 ATP 1 $FADH_2$ = 1.5 ATP 1 GTP = 1 ATP			**32 ATP**

*30 ATP if glycerol-3-P shuttle

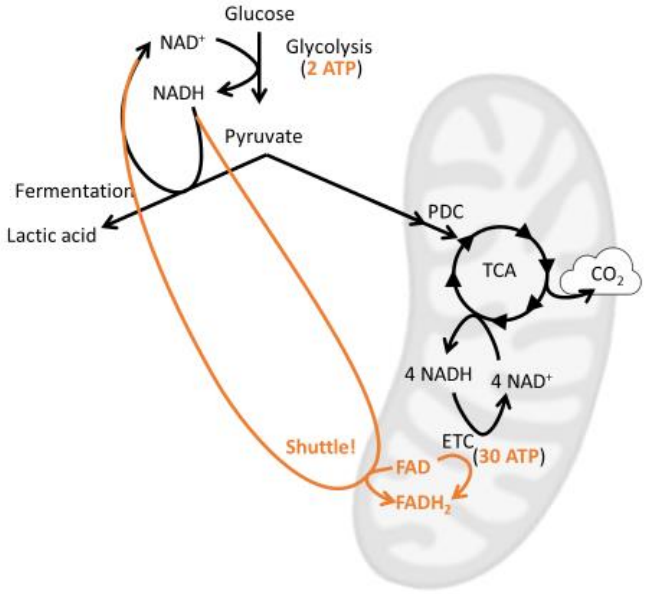
If we labeled all carbons on the glucose, adding only 1 labeled glucose, into a cell filled with unlabeled glucose, where are our labels now?

Cell choices

- PDC/TCA/ETC/shuttle

  OR

- Fermentation

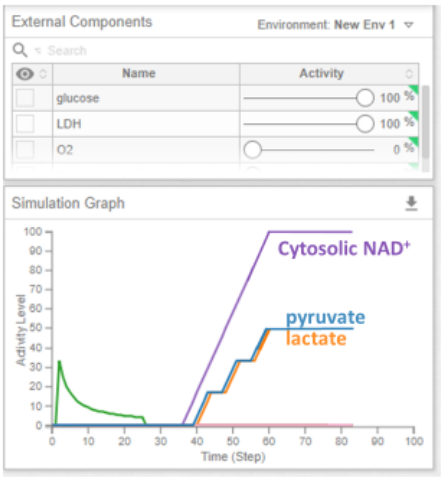


NOT Cell Choices (creates dead cells…)

- Let the ADP/ATP ratio drop
- Let the NAD+/NADH ratio drop

These are required for homeostasis

The cytosolic NAD+/NADH ratio is maintained.



Modeling Respiration & Exercise

Without exercise, TCA is working constantly

With exercise, TCA is begins to increase

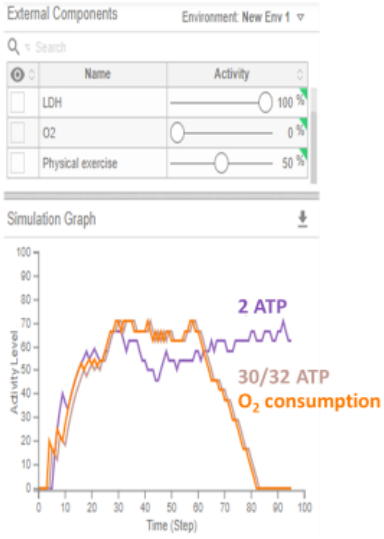
As exercise begins to make O_2 limiting, TCA use drops

What would you expect about cytosolic [NAD^+] and [ATP] before and during hard exercise?

A. Same

B. Different

Use caution when interpreting ATP from the model!



File S3

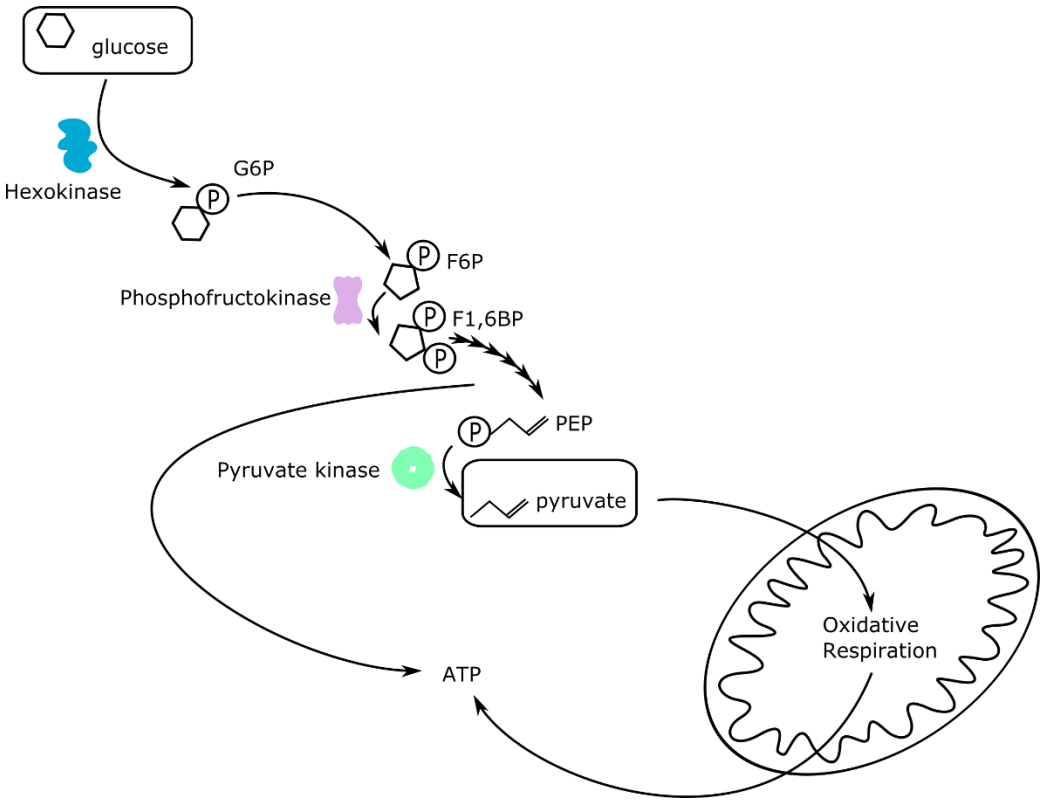
Regulation of Cellular Respiration

Instructor Guides

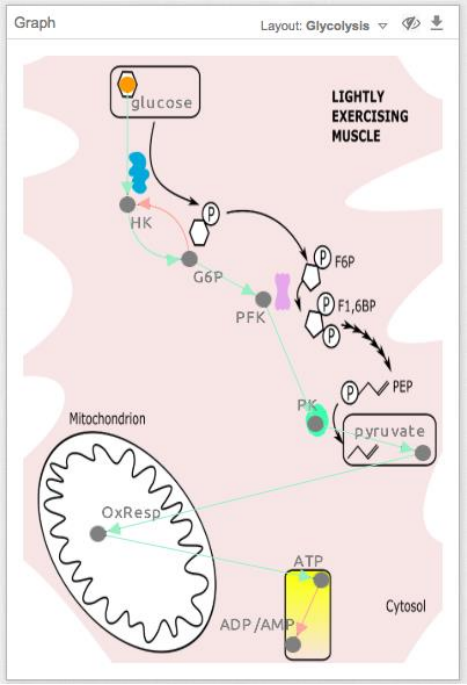
Glycolysis

(Module ID: 29742 at https://cellcollective.org)

The diagram below shows the components of cellular respiration that are covered in the *Glycolysis* section of the module:



The goal of the first half of the *Glycolysis* section of the module (Activities 1-7) is to introduce students to the importance of *energy charge-based regulation of glycolytic enzymes* to maintain *energy homeostasis*.



Students are presented with a computational model showing only the enzymes and metabolites of glycolysis that are most important for regulation. However, most of the known negative allosteric feedback regulatory connections from ATP aren't present (snapshot to the right).

Students are asked to simulate the behavior of the model as is (snapshot to the right) and then to add negative allosteric feedback relationships from ATP to each of the three enzymes that catalyze an irreversible step of glycolysis. They simulate the behavior of the model as they add each of these regulatory connections, and tabulate the simulation results. Finally, they evaluate their simulation results before and after adding the negative allosteric feedback relationships. They then follow the same procedure to assess the effect of positive allosteric feedback regulatory relationships from ADP/AMP to each of the three glycolytic enzymes.

Throughout the activities, students are asked to reason about how these regulatory connections will affect the entire organism.

How instructors can help

Before students start the module:

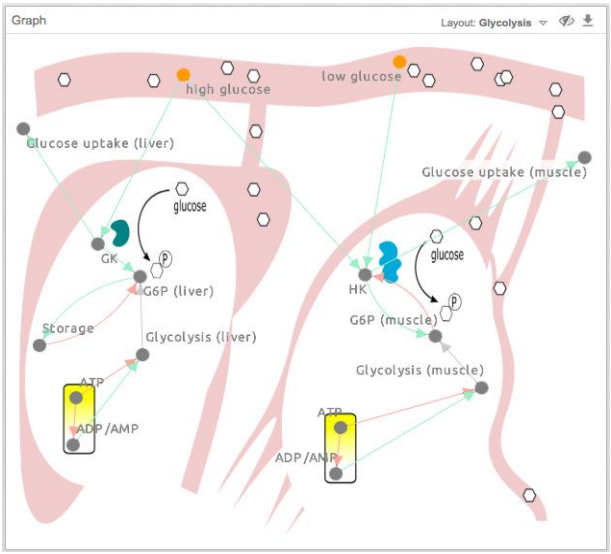
1. Remind them to make sure they have clicked the "Start Lesson" button in the *Overview* tab of the module. This will enable the module to be edited. If students cannot type or save their work, check this first.
2. Direct them to the *Start Here* tab in Cell Collective to see the Activities.
3. Ask students to confirm that the model is in "edit" mode (this should be the default and is indicated by a "pencil" icon, however, if the model is in "view" mode, students can click the "eye" icon within the Graph panel to change it to "edit" mode).
4. Remind students how to:
    a. Draw a connection (arrow): click the starting component, drag, and release the mouse over the component you want the arrowhead to land on.
    b. Delete an arrow: highlight it, then press delete (fn+delete for Mac).
    c. Toggle an arrow from positive to negative: *deselect everything!* then press and hold shift while you next click on the arrow that you want to change; click somewhere else to see the effect.
5. If students need to return to the module later, remind them to access their previous work through the *My Learning* tab on the home screen, not the *Public Modules* tab.

Although this is pointed out explicitly in the module, it may still be important to remind students to pay attention to the cell type and oxygenation status that is represented by each model. Students will also be asked many conceptual questions throughout the activities that will require them to critically assess the purpose of homeostasis in the organism. It may be helpful to remind them to draw from previous biology experience or discuss their thinking with a peer or instructor.

Model connection building/simulation review

1. ATP negatively regulates glycolysis and reduces metabolite levels of glycolysis.
2. ADP positively regulates glycolysis and increases metabolite levels of glycolysis.
3. Energy charge-based regulation by ATP and ADP/AMP ensures that the cell always has sufficient energy supply (maintains homeostasis!) regardless of how much glucose is available.

The goal of the second half of the of the *Glycolysis* section of the module (Activities 8-12) is to have students evaluate the *differences in glucokinase (GK) and hexokinase (HK) kinetics* as a partial explanation of *tissue-specific differences in glucose absorption*.



Using a kinetic diagram (not shown here, but provided in the module), students are asked to predict how glucokinase (GK) and hexokinase (HK) differentially affect glucose absorption in different tissues. Students are then presented with a computational model showing select components and feedback regulatory connections already present in two cell types (snapshot to the right). Through simulation, students discover that GK and HK activity do not determine pyruvate production; instead, the activities of PFK and PK are the major determinants of pyruvate production (refer back to concepts learned in the first half: Activities 1-7).

How instructors can help

Although this is covered explicitly in the module through direct questioning, students may still struggle to connect the kinetic diagram with the simulation output. Students may also still require additional support as they reason through the system.

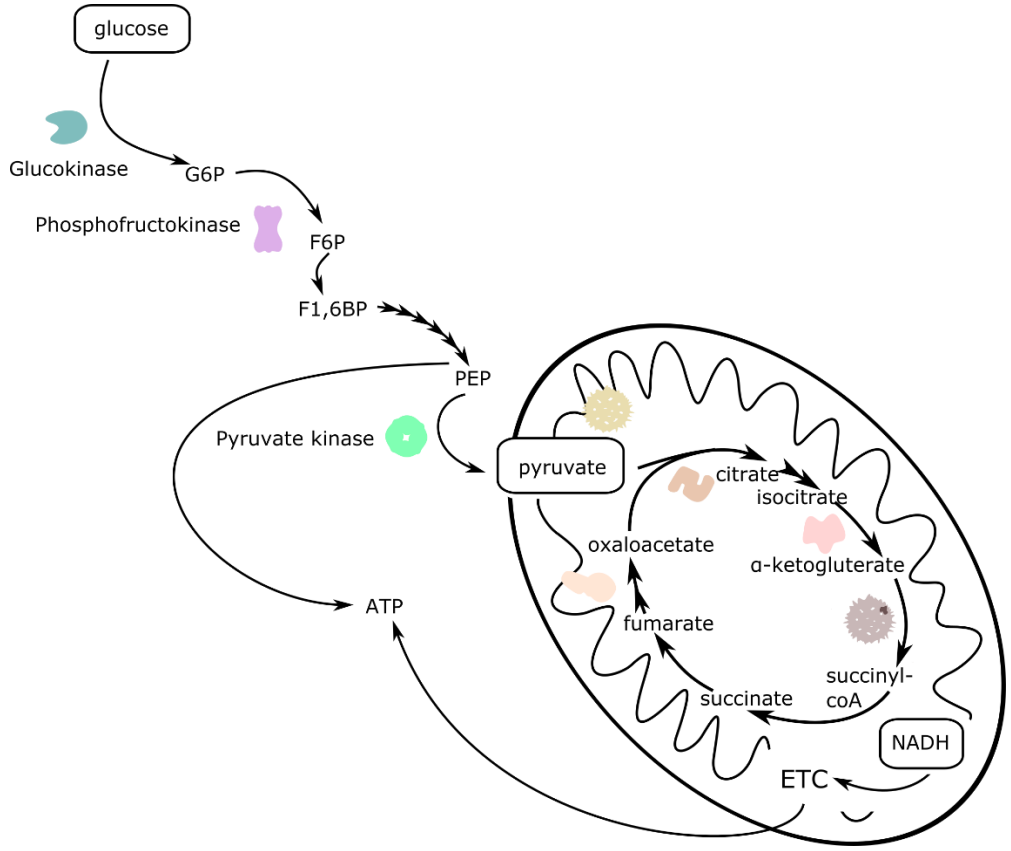
Model connection building/simulation review (continued from concepts learned in the first half: Activities 1-7)

1. GK and HK determine whether glucose uptake will occur in liver or muscle cells in response to glucose availability.
2. Glucokinase can be active and take up a lot of glucose even if glycolysis stays low because glucose can be stored.
3. Regulation of GK and HK is not the major determinant of pyruvate production (glycolysis). Instead, regulation of PFK and PK are the major determinants of pyruvate production (glycolysis).

The tricarboxylic acid (TCA) cycle

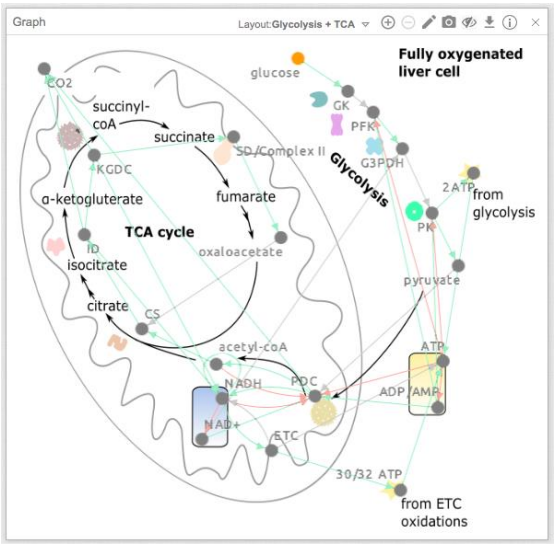
(Module ID: 34771 at https://cellcollective.org)

The diagram below shows the components of cellular respiration that are covered in the *TCA* section of the module:

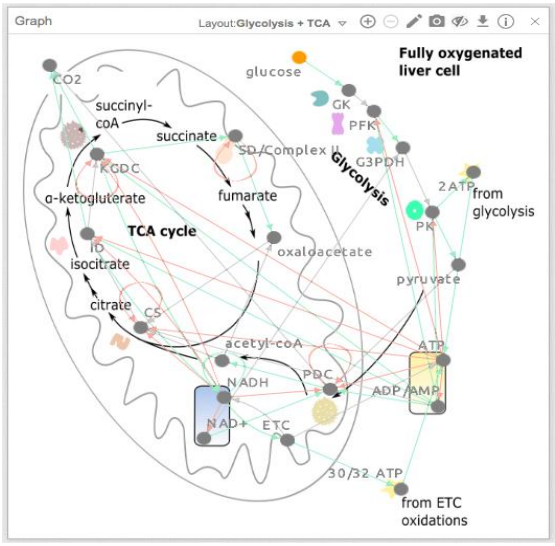


The goal of the first half of the of the *TCA* section of the module (Activities 1-6) is to introduce students to the importance of *allosteric feedback regulation of TCA enzymes by NADH and energy molecules to maintain redox balance (favorable cellular conditions)* and *how these connections affect the metabolites produced in glycolysis*.

Students are presented with a computational model showing only the enzymes and metabolites of glycolysis and the TCA cycle that are most important for regulation. In this model, the known feedback regulatory connections to TCA cycle enzymes are not yet present (snapshot to the right - top). Students are asked to simulate the behavior of the model as is and record the results.



Next, students are presented with a model where the known feedback regulatory connections to TCA cycle enzymes are present (snapshot to the right - bottom). Through a series of questions, students determine which molecules negatively regulate which enzymes and then simulate the behavior of the updated model to compare the results to the previous simulation. Students evaluate the effect of the newly added connections on redox balance and on the production of glycolytic metabolites.



How instructors can help

Before students start the module:

1. Remind them to make sure they have clicked the "Start Lesson" button in the *Overview* tab of the module. This will enable the module to be edited. If students cannot type or save their work, check this first.
2. Direct them to the *Start Here* tab in Cell Collective to see the Activities.
3. Remind them to pay close attention to the introductory comments.

Remind students to pay attention to the cell type and oxygenation status of the cell represented by the model. Conceptual questions throughout the activities will require students to critically assess the purpose of homeostasis in the organism. Instructors can help by reminding students to draw from previous biology experience or discuss their thinking with a peer or instructor.

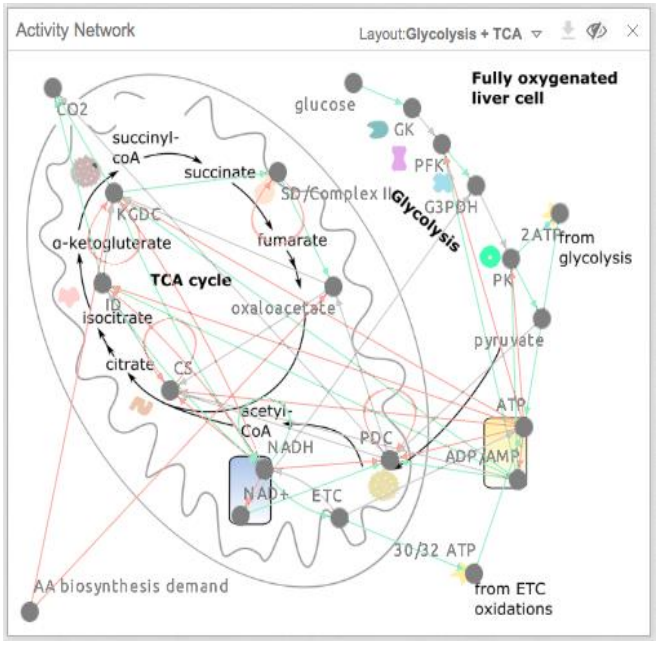
Model simulation review

1. Energy charge (ADP and ATP) regulates TCA cycle enzymes.
2. NADH and metabolites regulate TCA cycle enzymes through product inhibition to maintain cellular homeostasis.
3. NAD^+ and metabolites regulate TCA cycle enzymes through substrate availability to maintain cellular homeostasis.

The goal of the second half of the of the *TCA* section of the module (Activities 7-11) is to introduce students to the *ability of a single anaplerotic reaction to maintain the levels of TCA cycle metabolites*.

Students are presented with a computational model that allows the levels of amino acids being used by the cell to be manipulated externally. Students are asked to predict the simulation results from the model as amino acid demand changes from low to high and evaluate their results using simulation.

Next, students are asked to add a connection that represents an anaplerotic reaction and simulate the behavior of the model again as amino acid demand changes from low to high. Students must evaluate and explain how anaplerotic reactions maintain TCA cycle metabolite levels.



How instructors can help

Although the questions are designed to focus students' attention on the model, it may be helpful to explicitly focus their attention on the fact that the model changes as the scope of the questions being answered with the model expands. For example, additional external components provide the ability to manipulate the model in a different way, and to answer different questions when using the models.

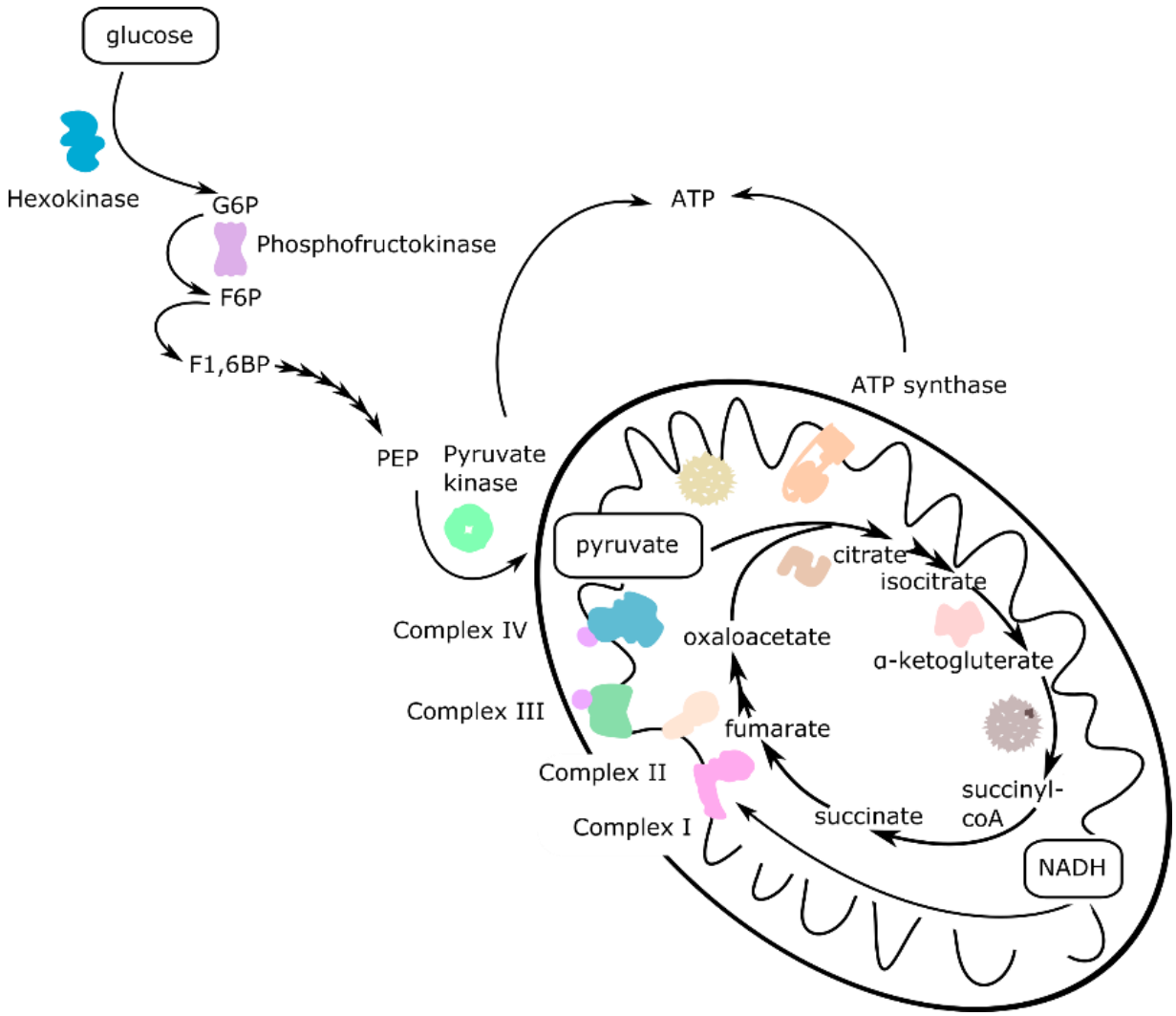
Model connection building/simulation review (continued from the first half of the investigation)

1. Anaplerotic reactions can refill TCA cycle intermediates.
2. The TCA cycle can then keep running even when TCA cycle intermediates are needed for other cellular processes.

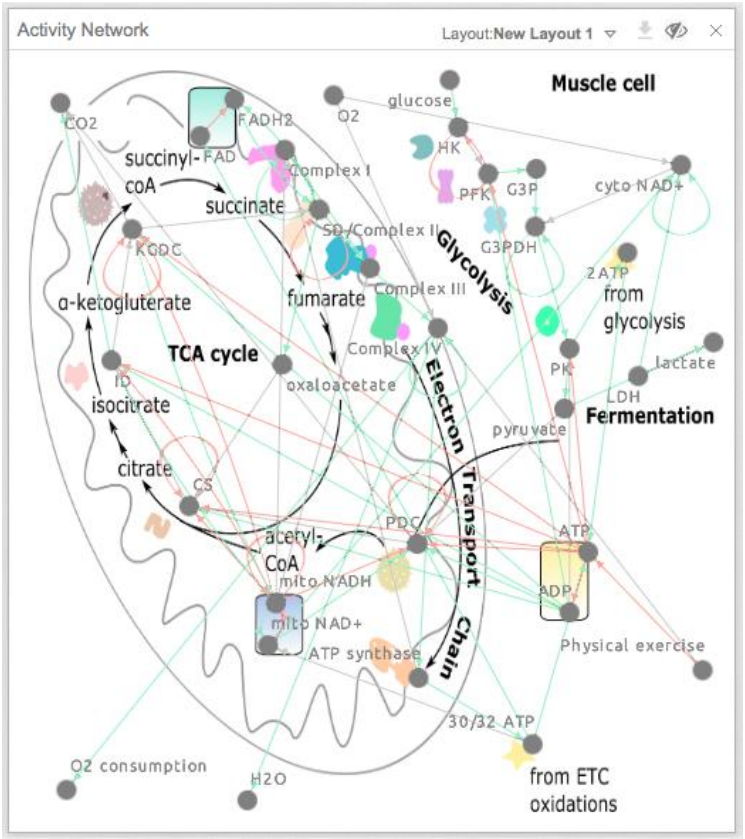
Part 3: The electron transport chain (ETC) and fermentation

(Module ID: 29564 at https://cellcollective.org)

The diagram below shows the components of cellular respiration that are covered in the *ETC* section of the module:



The goal of the *ETC* section of the module (Activities 1-8) is to *integrate concepts of energy charge- and redox-based regulation of glycolysis and the TCA cycle with electron transport chain function and cellular respiration*.



Students are presented with a computational model showing only the enzymes and metabolites of glycolysis, the TCA cycle, and the ETC that are most important for regulation, and all known regulatory connections are present (snapshot to the right).

Students are asked to predict the behavior of the model under three conditions: (1) oxygen present, no lactate dehydrogenase (LDH) expressed, (2) oxygen absent, no LDH expressed, and (3) oxygen absent, LDH expressed. Specifically, students are asked to predict the levels and activities of pyruvate and lactate, CO_2 production, and O_2 consumption that represent whether specific cellular processes are active. Students also predict the levels of energy and redox molecules, and are asked to record their predictions in tables. Next, they simulate the behavior of the model, tabulate the simulation results, and compare these results to their predictions. Finally, students are asked to critically evaluate the simulation results and explain how and why the system components and connections allow the cell to maintain homeostasis. Next, students repeat a similar series of tasks, but this time they investigate the effect of exercise on the cell.

How instructors can help

Before students start the module:

1. Remind them to make sure they have clicked the “Start Lesson” button in the *Overview* tab of the module. This will enable the module to be edited. If students cannot type or save their work, check this first.
2. Direct them to the *Start Here* tab in Cell Collective to see the Activities.
3. Remind them to pay close attention to the introductory comments.

As before, students will be asked many conceptual questions throughout the activities that will require them to critically assess the purpose of homeostasis in the organism. It may be helpful to remind them to draw from previous biology experience or discuss their thinking with a peer or instructor.

Model simulation review

1. The cell can adjust its metabolism to oxygen availability and exercise through the coordinated regulation of glycolysis, the TCA cycle and the ETC by enzymes all “sensing” the levels of NADH/NAD+ and ATP/ADP.
2. When oxygen is limited, lactate dehydrogenase replenishes the cytoplasmic NAD+ pool so that glycolysis can proceed.
3. By allowing glycolysis to proceed when oxygen is absent (fermentation), the cell can produce some ATP. The amount of ATP that is produced by fermentation is a lot less compared to oxidative respiration, so this is not a sustainable mode of ATP production for long periods of time.
4. When the cell begins to exercise and the ATP pool is constantly being depleted, it will increase glycolysis by relieving the inhibition on the rate-limiting enzymes of glycolysis. This allows the cell to increase glycolysis and oxidative respiration for ATP production.
5. After some time, oxygen will become depleted, but ATP production can be sustained for a short period of time by fermentation.

File S4

Regulation of Cellular Respiration

Assessment 1.1: Glycolysis

1. Evaluate the following statements that describe the regulation of glycolytic enzymes (T/F):
    A. T or F Activation of pyruvate kinase by ADP maintains production of ATP.
    B. T or F Product inhibition of liver glucokinase would deregulate glucose storage.
    C. T or F Inhibition of muscle hexokinase by its product ensures that blood glucose is not wasted.
    D. T or F Glycolytic enzymes are regulated by energy charge to maximize energy production from glucose.
    E. T or F Liver glucokinase will be highly active under low blood glucose conditions.
    F. T or F Glycolytic flux in the liver cell is determined by the activity of glucokinase.
    G. T or F Glycolytic flux in the muscle cell is determined by the energy requirements of the cell.
    H. T or F Regulation of glycolysis by ADP increases the rate of glycolysis when energy is low.
    I. T or F Regulation of glycolysis by ADP and ATP stabilizes energy production when blood glucose varies.

Assessment 1.2: The tricarboxylic acid (TCA) cycle

2. Evaluate the following statements that describe the regulation of the tricarboxylic acid (TCA) cycle enzymes (T/F):
    A. T or F Inhibition of pyruvate dehydrogenase complex decreases ATP production.
    B. T or F Regulation of TCA cycle enzymes allow anaplerotic reactions to refill the cycle.
    C. T or F TCA cycle enzymes are regulated by energy charge to maintain energy homeostasis.
    D. T or F NADH levels would remain unchanged if ATP began to accumulate.
    E. T or F Positive regulation of TCA cycle enzymes increases the levels of TCA metabolites.
    F. T or F Flux through the TCA cycle would decrease if NADH began to accumulate.
    G. T or F Anaplerotic reactions ensure that ATP production can proceed regardless of cellular amino acid demand.
    H. T or F Anaplerotic reactions ensure that cellular NADH levels are maintained.

Assessment 1.3: The electron transport chain (ETC) and fermentation

3. Evaluate the following statements that compare respiration to fermentation and describe the regulation of the enzymes of the electron transport chain (ETC) (T/F):
    A. T or F In the absence of O_2, glycolysis will be active if NAD^+ levels can be maintained.
    B. T or F The tricarboxylic acid (TCA) cycle will be active in the absence of O_2.
    C. T or F ATP directly inhibits the enzymes of the electron transport chain.
    D. T or F Activity of ETC enzymes would remain unchanged if ATP began to accumulate.
    E. T or F Activity of ETC enzymes would increase if NADH began to accumulate.
    ~~F. T or F If FADH2 increases, ETC activity decreases because of succinate dehydrogenase/complex II.~~
    G. T or F Complex IV of the ETC will be active in the presence of O_2.
    H. T or F NADH levels will increase in the absence of O_2.
    I. T or F In the presence of O_2, ATP production is maintained by turning pyruvate into lactate.
    J. T or F In the absence of O_2, less ATP production occurs.

Note: Please contact the authors to obtain any revised versions showing wording updates and that corresponds to the latest online module. Item 3F had negative discrimination for both Biochemistry I courses and was not included in the analysis.

File S5

Regulation of Cellular Respiration

Survey: student perceptions of the module

Q1. Please comment on your learning after completing this module.

	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
a. The module helped me to understand how the regulation of glycolysis, the TCA cycle, and the ETC are integrated (how it works together).	○	○	○	○	○
b. The simulations were helpful to understand the effects of feedback loops and environmental conditions on the entire system.	○	○	○	○	○
c. The simulations were helpful to understand the effects of feedback loops and environmental conditions on the entire system.	○	○	○	○	○
d. The module helped me to remember to think about both the individual components and also their connection to the larger process.	○	○	○	○	○
e. I think I will remember what I learned about the regulation of cellular respiration better than I would have if I did not complete the module.	○	○	○	○	○
f. I think I understand what I learned about the regulation of cellular respiration better than I would have if I did not complete the module.	○	○	○	○	○
g. Overall, completing this module assisted my learning of the material.	○	○	○	○	○

Q2. Please comment on which parts of the module you found most effective to aid your learning (which parts helped you the most).

Q3. Please comment on which parts of the module you found least effective to aid your learning (which parts helped you the least).

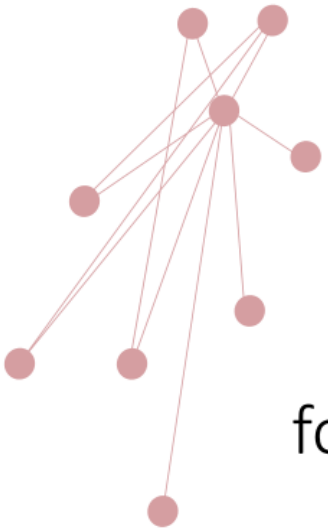
Q4. Please list one concept or idea that you are still unsure about after completing this module.

Q5. What was most challenging about working with the computational modules?

Q6. Knowing that you will still be responsible for understanding the regulation of cellular respiration, and that computational skills are important to develop for various reasons, how could the module be changed to aid your learning?

Q7. Do you have any other feedback that you would like to provide?

File S6



A short introduction to computational modeling for biochemistry students

What is a ”system”?

“ a regularly interacting or interdependent group of items forming a unified whole”

- Merriam-Webster dictionary

There are many different kinds of systems:

- River system
- Digestive system
- Thermodynamic system
- Computer system

How do we know that we understand the system?

By seeing how well we can **predict** that system's behavior:

If we can predict which medicine will relieve our digestive discomfort, we know we understand something about the digestive system and the causes of digestive discomfort.

What about really complex biological systems, like cells?

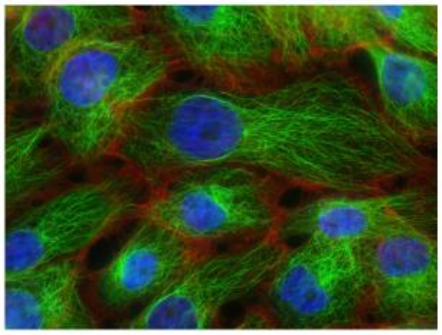


Photo credit: National Cancer Institute

We can reduce the system to smaller pieces, but keeping track of all the parallel processes is overwhelming

Even more than this…

Cells are not static, they are **dynamic**, and things are constantly changing.

This becomes even more challenging to try to predict how a cell will **dynamically** respond when something changes.

To predict how **many components** of a **complex system** change **dynamically** over time, one good solution is to:

1. Build a model of the biological system
2. Use a computer to simulate and/or analyze biological processes

When we have good predictive models of biological systems, we can…

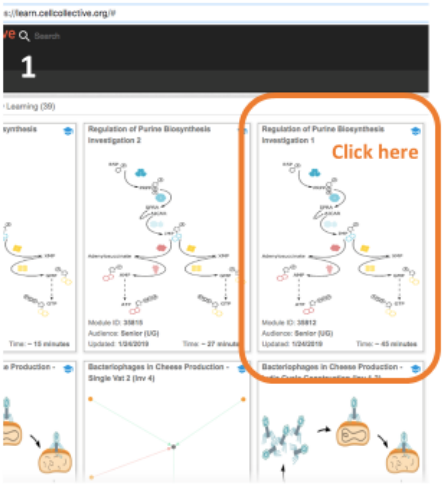
- Understand how individual components interact together as a system
- Understand how the system responds to different conditions
- Understand which parts of the system are most critical for its function
- Identify drug targets and drug response

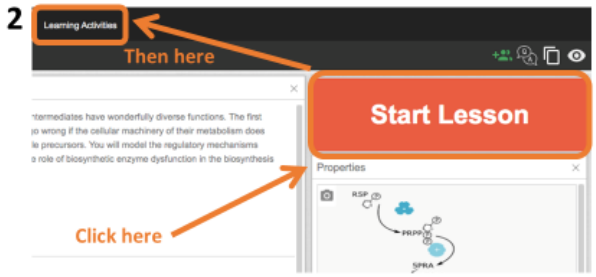
Purpose of the in-class modules:

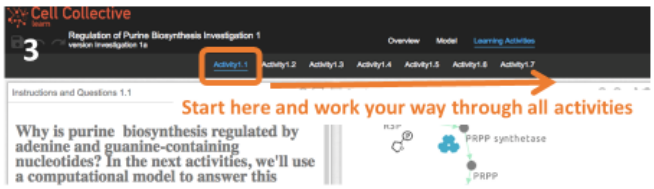
Using a computational modeling platform (Cell Collective Learn), you will explore important biochemical systems/processes and their regulation

In-class activity: Model purine biosynthesis regulation

https://learn.cellcollective.org/







In-class activity: Model purine biosynthesis regulation

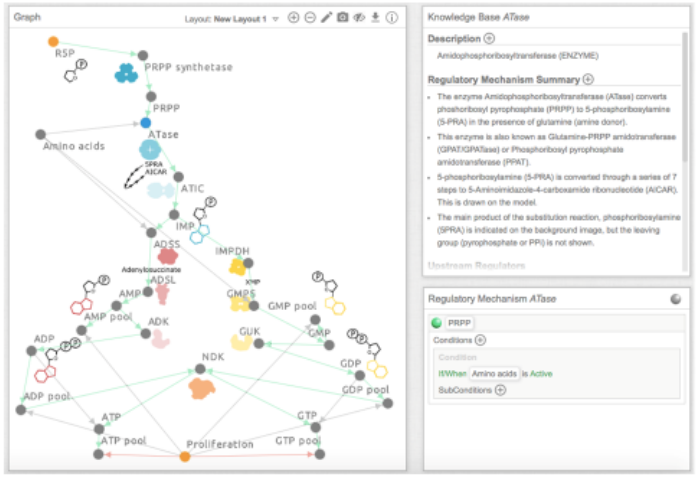
https://learn.cellcollective.org/

What's different about arrows in the mathematical model compared to a typical biochemical diagram?

Using this kind of computational modeling approach:

A green arrow can represent ANY positive relationship (forward reaction, protein interaction, etc.)
A red arrow can represent ANY negative relationship (allosteric inhibition, reverse reaction, etc.)

Why are only some of the components modeled?



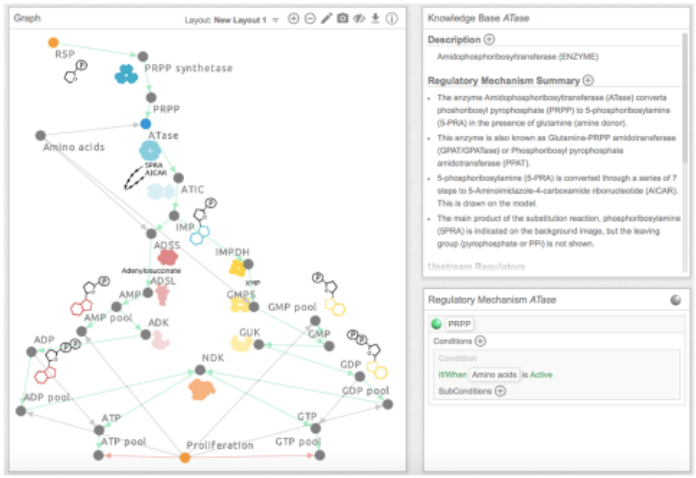
In-class activity: Model purine biosynthesis regulation

https://learn.cellcollective.org/

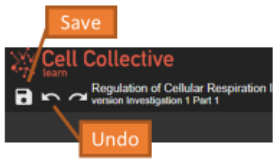
<u>Some tips:</u>
You can only change dots (nodes) and arrows (edges), not images.

Details for each component is found in the Knowledge Base panel only if you click on the component – here you can find the details of how components are connected in the model.

If you want to remember the details or study for the exam, you can always come back to the first activity where the model AND the Knowledge base are.

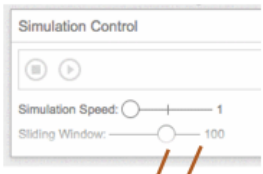


How to...



Select components to view

Change the simulation speed/ sliding window



Click and drag

Double click and type

Add connectors

Remove connectors

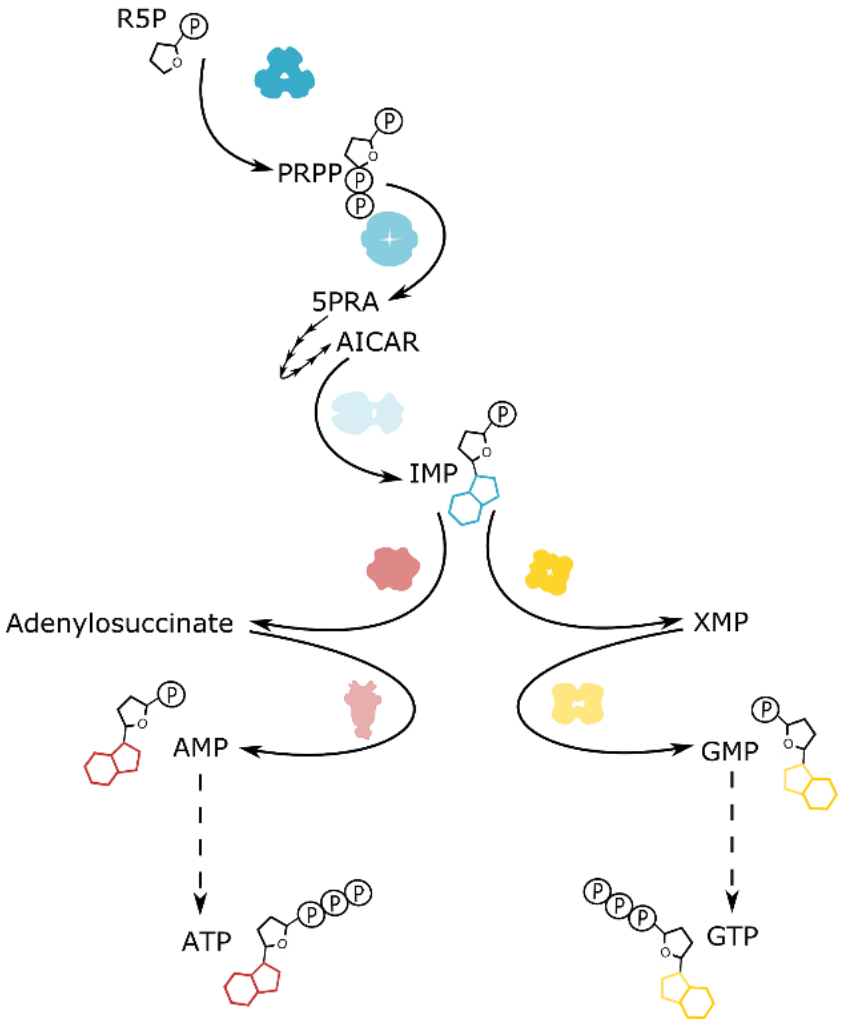
File S7

Regulation of Purine Biosynthesis

Instructor Guide

(Module ID: 35812 at https://cellcollective.org)

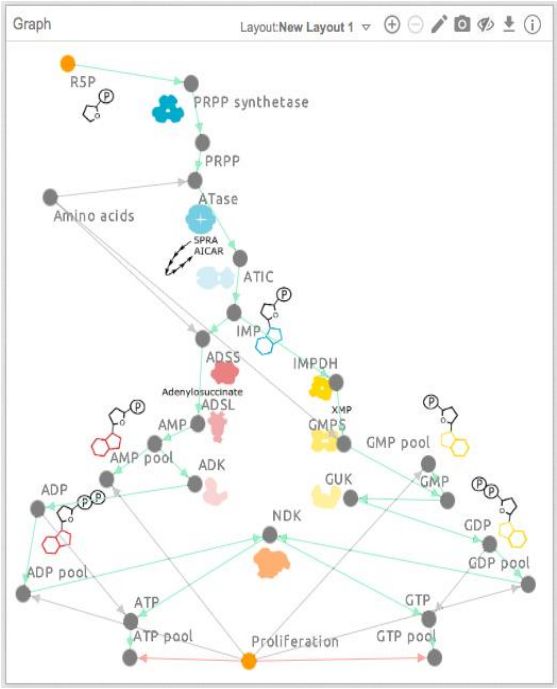
The diagram below shows the components of purine biosynthesis that are covered in this module:

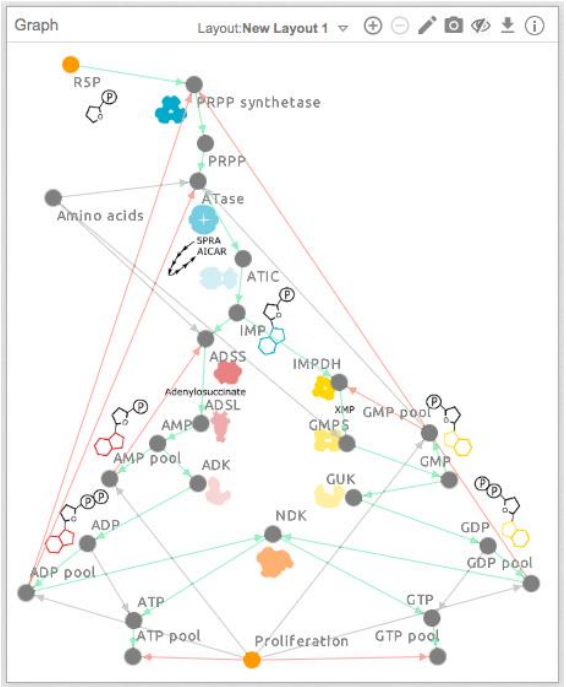


The goal of the first part of the module (Activities 1-10) is to introduce students to *the importance of nucleotide-based regulation of purine biosynthetic enzymes to maintain nucleotide homeostasis*.

Students are presented with a computational model showing only the enzymes and metabolites of purine biosynthesis that are important for regulation and most of the known allosteric feedback regulatory connections are not present (snapshot to the bottom left).

Students are asked to simulate the behavior of the model as is (snapshot to the bottom left) and then to add negative allosteric feedback relationships from the ADP and GDP pools to phosphoribosyl pyrophosphate (PRPP) synthetase, which catalyzes a rate-determining step of purine biosynthesis. They simulate the behavior of the model after adding these regulatory connections and tabulate the simulation results. Students are then provided with a model showing all the known allosteric regulatory connections (snapshot to the bottom right). Again, they simulate the model behavior and tabulate the results. Finally, they evaluate their various simulation results. Throughout the investigation, students are asked to reason about how these regulatory connections will affect the entire organism.

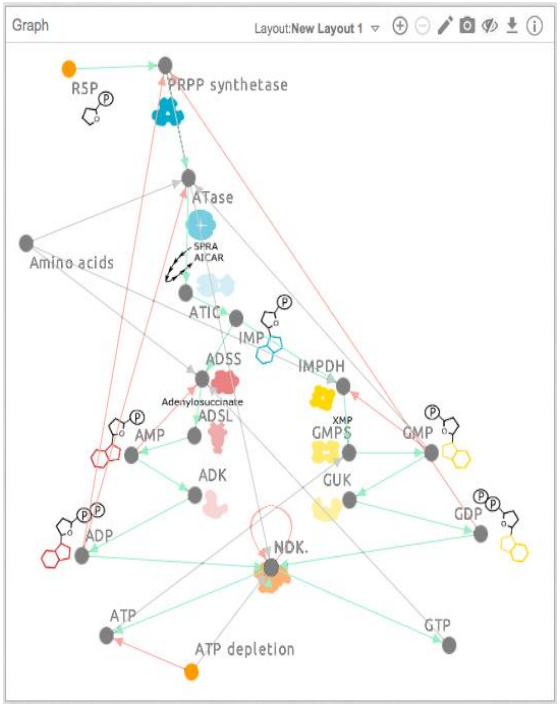




The goal of the second part of the module (Activities 11-14) is to introduce students to the importance of *"cross-regulation" of the two branches of purine biosynthesis* and *how substrate availability from one branch is able to balance nucleotide levels in the other branch (homeostasis!)*.

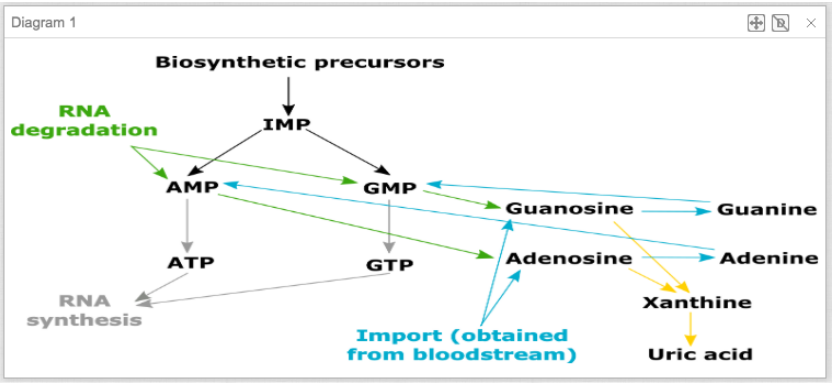
Students are presented with a table of simulation results that were previously obtained using various versions of the model, each with more and more regulation added sequentially. These activities provide an opportunity for review when students test whether they understood the simulation results from the first part of the module, and they will need to use similar reasoning.

Next, students are presented with a model where the "cross-regulatory" interactions are added (snapshot below). They are asked to predict what would happen to ATP and GTP levels when adenine-rich DNA must be made. They simulate the model, test their predictions, and reason through the results. To be most successful, students should rely on their previous knowledge of how substrate availability affects the enzymatic rate of enzymes or seek help from others that can help them apply this concept as a part of their reasoning.

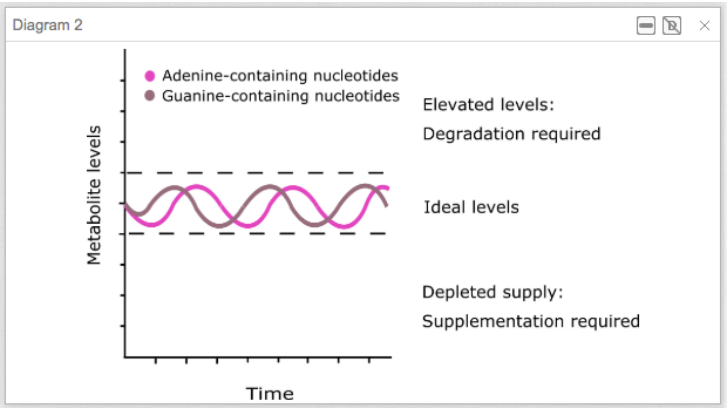


The goal of the final part of the module (Activities 15-17) is to *conceptually integrate the process of purine de novo biosynthesis within the larger context of the cell to include other important purine-related processes such as purine degradation and salvage*. These ideas are further extended by asking students to *evaluate the effect of different mutations of de novo biosynthetic enzymes* on cellular purine levels, the cell, and the organism.

Students are presented with diagrams (snapshots to the right) that conceptually extend the principles that they have already learned using the computational models in the previous two parts of the module (Activities 1-14). The diagrams demonstrate how *de novo* synthesis to other processes occurring in the cell.



Students simulate the model and evaluate the simulation results under three conditions: (1) all the enzymes of purine biosynthesis are wild-type enzymes, (2) there is an activating mutation in PRPP synthetase, and (3) there is an inactivating mutation in adenylosuccinate lyase (ADSL). Students are asked to record their simulation results in tables and critically evaluate the results to explain how the cell could compensate when mutant enzymes are expressed.



How instructors can help

Before students start the module:

1. Remind them to make sure they have clicked the “Start Lesson” button in the *Overview* tab of the module. This will enable the module to be edited. If students cannot type or save their work, check this first.
2. Direct them to the *Start Here* tab in Cell Collective to see the Activities.
3. Ask students to confirm that the model is in “edit” mode (this should be the default and is indicated by a “pencil” icon; however, if the model is in “view” mode, students can click the “eye” icon within the Graph panel to change it to “edit” mode).
4. Remind students how to:
    a. Draw a connection (arrow): click the starting component, drag, and release the mouse over the component you want the arrowhead to land on.
    b. Delete an arrow: highlight it, then press delete (fn+delete for Mac).

c. Toggle an arrow from positive to negative: *deselect everything!* then press and hold shift while you next click on the arrow that you want to change; click somewhere else to see the effect.

5. If students need to return to the module later, remind them to access their previous work through the *My Learning* tab on the home screen, not the *Public Modules* tab.

Although this is pointed out explicitly in the module, it may still be important to remind students the model focuses on rapid control mechanisms only and that slower control mechanisms and other cellular processes, such as respiration, although not explicitly modeled, should not be completely ignored. In line with this idea, it may be important to continually remind students that models frequently present incomplete views of a complex reality. Students will be asked many conceptual questions throughout the activities that will require them to critically assess the purpose of homeostasis in the organism. In general, it may be helpful to remind them to draw from previous biology experience or discuss their thinking with a peer or instructor.

In the second and last parts of the module, remind students to recall their results and the concepts they covered in the previous parts of the module (Activities 1-10, and Activities 11-14). The conceptual questions that are interspersed throughout the module will also require students to draw on their previous knowledge about enzyme kinetics. Instructors can help by reminding students to draw from previous biology experience or discuss their thinking with a peer or instructor.

Model connection building/simulation review

1. ADP and GDP negatively regulate purine biosynthetic enzymes (PRPP synthetase, Glutamine PRPP amidotransferase (ATase), Adenylosuccinate synthase (ADSS), and Inosine-5′-monophosphate dehydrogenase (IMPDH)) which reduces the levels of Inosine-5′-monophosphate dehydrogenase (IMP) through the biosynthesis pathway.
2. Nucleotide-based regulation by ADP and GDP ensures that the cell has sufficient nucleotide supply and can respond to increased demand for nucleotides (homeostasis!) regardless of how much ribose 5-phosphate (R5P) is available.
3. Purines regulate multiple enzymes that determine the production of intermediates that are required for their synthesis.
4. The regulation of purine biosynthetic enzymes occurs at various points in the pathway to ensure redundancy.
5. Biosynthesis of adenine and guanine nucleotides is interrelated to ensure that the levels of both types of nucleotides remain balanced within the cell as much as possible.
6. Mutations in purine biosynthetic enzymes adversely affect cellular purine levels and these effects must be compensated by changing the activity of other cellular processes such as purine salvage and degradation. Specifically, PRPP synthetase overactivity can override feedback regulation, causing accumulation of purine nucleotides that must be degraded. Conversely, ADSL deficiency reduces nucleotides in the cell, and dietary supplementation will be required.

File S8

Regulation of Purine Biosynthesis

Assessment 2: Purine Biosynthesis

1. Evaluate the following statements describing interactions between the components of *de novo* purine biosynthesis (T/F):
    - ~~A. T or F Two enzymes in the main branch of *de novo* biosynthesis are feedback inhibited~~
    - B. T or F Two enzymes in the GTP branch of *de novo* purine biosynthesis are feedback inhibited.
    - C. T or F IMP dehydrogenase (IMPDH) is regulated by allosteric feedback inhibition.
    - D. T or F GMP synthetase is regulated by allosteric feedback inhibition.
    - ~~E. T or F Glutamine PRPP amidotransferase (ATase) is regulated by substrate availability.~~
    - F. T or F IMP is a precursor only for GTP biosynthesis.
    - G. T or F Glutamine PRPP amidotransferase (ATase) is common to both ATP and GTP biosynthesis.
    - H. T or F Adenylosuccinate synthetase (ADSL) is common to both ATP and GTP biosynthesis.
    - I. T or F The levels of ATP and GTP in the cell determine the rate of GTP biosynthesis.

2. Determine whether the following statements describe how the regulation of *de novo* purine biosynthesis is integrated to maintain homeostasis (T/F):
    - A. T or F ATP can only be produced through *de novo* biosynthesis when both PRPP and GTP are present.
    - B. T or F If PRPP synthetase is not regulated by ADP and GDP, PRPP could accumulate in the cell and potentially become toxic.
    - C. T or F If IMP dehydrogenase (IMPDH) is not regulated by GMP, both ATP and GTP would accumulate in the cell and potentially become toxic.

3. In an actively proliferating cell, the following describe the *de novo* purine biosynthesis pathway (T/F):
    - A. T or F ATP and GTP will be overproduced to meet cellular demands, and remain high.
    - B. T or F ATP and GTP levels will initially fall, but return to normal as the allosteric inhibition on biosynthetic enzymes is relieved.
    - C. T or F Metabolic flux through *de novo* purine biosynthesis will temporarily increase to accommodate cellular demand for ATP and GTP synthesis.
    - D. T or F ATP and GTP levels will initially fall, and will remain low while the cell is proliferating.

4. The following statements describe the effect of mutations of the enzymes of *de novo* purine biosynthesis (T/F):

   For activating mutations in PRPP synthetase, the following may be expected:

   A. T or F Increased production of nucleotides.
   B. T or F Increased flux through salvage pathways to compensate for metabolic imbalances.
   C. T or F Compensatory pathways could somewhat mitigate the effects on nucleotide levels.

   For inactivating mutations in Adenylosuccinate lyase (ADSL), the following may be expected:

   D. T or F Decreased production of nucleotides.
   E. T or F Increased flux through degradation pathways to compensate for metabolic imbalances.
   F. T or F Compensatory pathways could completely mitigate effects on nucleotide pathways.

   Note: Items 1A and 1E had negative discrimination for the Biochemistry II course and was not included in the analysis.

File S9

Regulation of Purine Biosynthesis

Survey: student perceptions of the module

Q1. Please comment on your learning after completing this module.

	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
a. The module helped me to understand how the regulation of purine biosynthesis maintains purine homeostasis regardless of changing cellular conditions.	○	○	○	○	○
b. The simulations were helpful to understand the effects of specific feedback loops (the results of allosteric regulation) on ATP and GTP production.	○	○	○	○	○
c. The module helped me to remember to think about both the individual components and also their connection to the larger process.	○	○	○	○	○
d. I think I learned about the topic of regulation of purine biosynthesis in much greater depth than I would have if I did not complete the module.	○	○	○	○	○
e. I think I understand what I learned about regulation of purine biosynthesis better than I would have if I did not complete the module.	○	○	○	○	○
f. Overall, completing this module assisted my learning of the material	○	○	○	○	○

Q2. Please comment on which parts of the module you found most effective to aid your learning (which parts helped you the most).

Q3. Please comment on which parts of the module you found least effective to aid your learning (which parts helped you the least):

Q4.Please list one concept or idea that you are still unsure about after completing the module.

Q5. What was most challenging about working with the computational modules?

Q6. Knowing that you will still be responsible for understanding the regulation of purine biosynthesis, and that computational skills are important to develop for various reasons, how could the module be changed to aid your learning?

Q7. Do you have any other feedback that you would like to provide?