

Supplementary information

Biochemical and structural insights into how amino acids regulate pyruvate kinase muscle isoform 2

Suparno Nandi and Mishtu Dey*

From the Department of Chemistry, The University of Iowa, Iowa City, IA 52242

Running title: *Regulation of PKM2 allostery by amino acids*

*To whom correspondence should be addressed: Department. of Chemistry, The University of Iowa, Iowa City, IA 52242.; Telephone: 734-747-2311. Fax: 319-335-1270. E-mail: mishtu-dey@uiowa.edu.

List of materials included

Figure S1. Activation and inhibition of PKM2 by AAs and stimulation of Val inhibited PKM2 by various activating molecules.

Figure S2. Effect of activators and inhibitors on PKM2.

Figure S3. Analytical gel filtration chromatograms with 0.3 mg/ml PKM2.

Figure S4. Superimposition of PKM2 bound to AAs on PKM2-FBP.

Figure S5. Structural alignment of AA bound PKM2 structures.

Figure S6. Close-up view of the AA binding pocket of PKM2 with Asn, Asp, and Val bound to it.

Table S1. Kinetic parameters of PKM2 activation by Asn, Asp, and Val.

Table S2. Kinetic parameters of FBP, Asn, Asp, and Ser mediated activation of Val inhibited PKM2.

Table S3. Kinetic parameters of Ser displacement from PKM2 by Val.

Table S4. Gel filtration elution volumes, theoretical, and experimental molecular weight (MW) for PKM2 at 0.1 and 0.3 mg/ml in the presence and absence of Asn, Asp, and Val.

Table S5. Kinetic parameters of PKM2 activation by a fixed concentration of Ser, Asn, and Asp.

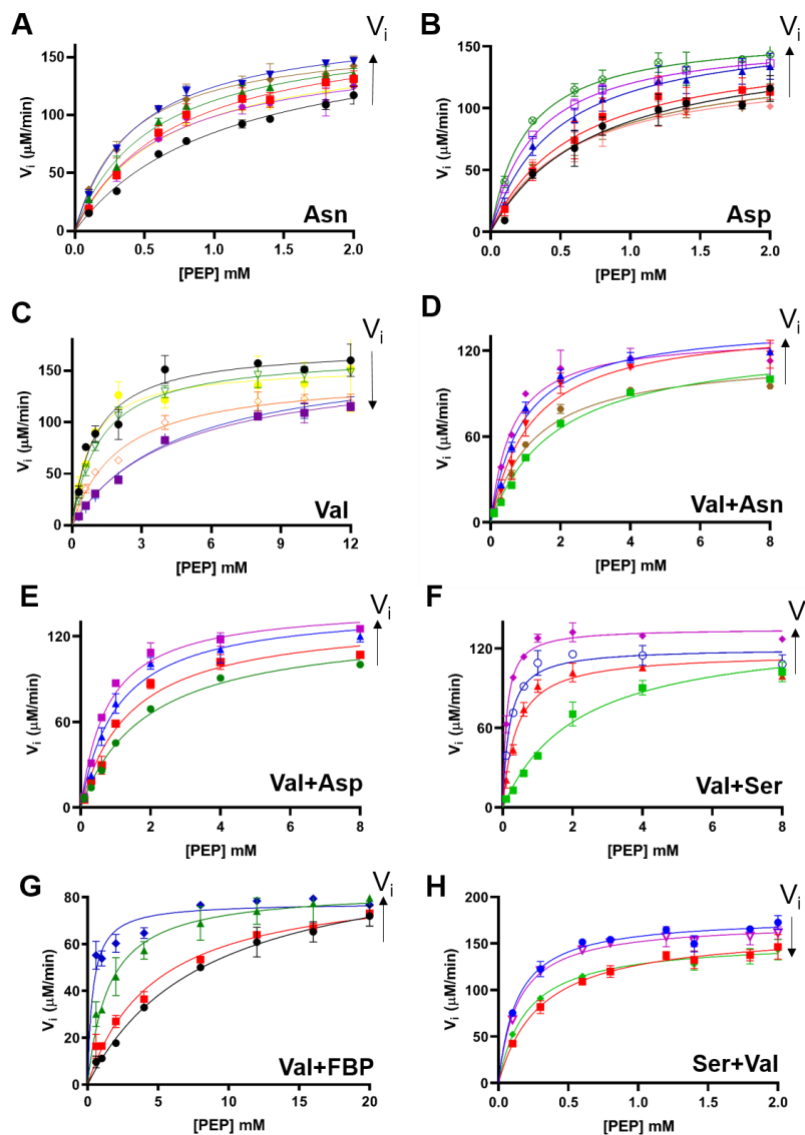


Figure S1. Activation and inhibition of PKM2 by Asn, Asp, and Val including the stimulation of Val inhibited PKM2 by various activating molecules. (A) Asn increases the activity of PKM2 in a concentration-dependent manner. Colors: 0 μM Asn (black), 5 μM Asn (magenta), 10 μM Asn (red), 40 μM Asn (green), 150 μM Asn (brown), 450 μM Asn (blue). (B) PKM2 activity increases with increasing concentration of Asp. Colors: 0 μM Asp (black), 10 μM Asp (salmon), 40 μM Asp (brown), 80 μM Asp (red), 150 μM Asp (blue), 450 μM Asp (magenta), 900 μM Asp (green). (C) Val inhibits PKM2 in a concentration-dependent manner. Colors: 0 mM Val (black), 0.1 mM Val (yellow), 0.3 mM Val (green), 0.6 mM Val (orange), 4 mM Val (magenta), 8 mM Val (blue). (D-G) Asn, Asp, Ser, and FBP increases the activity of Val inhibited PKM2 in a concentration-dependent manner. Colors: (D) 0 mM Asn (green), 0.3 mM Asn (brown), 0.5 mM Asn (red), 1 mM Asn (blue), 2 mM Asn (magenta) (E) 0 mM Asp (green), 0.5 mM Asp (red), 1 mM Asp (blue), 2 mM Asp (magenta) (F) 0 mM Ser (green), 0.5 mM Ser (red), 1 mM Ser (blue), 2 mM Ser (magenta) (G) 0 μM FBP (black), 0.05 μM FBP (red), 0.2 μM FBP (green), 0.4 μM FBP (Prussian blue). The concentration of Val was fixed at 1 mM for all concentrations of AA activators tested in D-E and 5 mM for all concentrations of FBP tested in G. (H) Displacement of Ser from PKM2 by Val. Colors: 0 mM Val (blue), 0.5 mM Val (magenta), 1 mM Val (green), 2 mM Val (red). The concentration of Ser was kept constant at 1 mM in all concentrations of Val tested. In A-H, the direction of arrows

indicates either increase or decrease in activity. The concentration of PKM2 in A-C was kept constant at 20 nM and in D-H it was at 12.5 nM. The ADP concentration was kept constant at 0.8 mM.

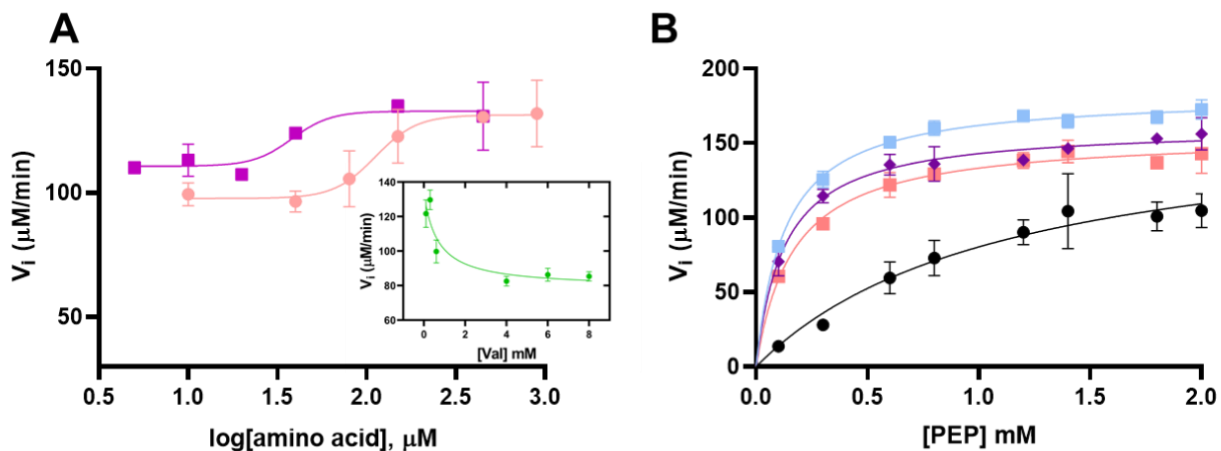


Figure S2. Effect of activators and inhibitors on PKM2. (A) Dose-response curves of PKM2 activation by Asn (magenta), Asp (salmon), and Val (green, inset). From these curves, the effective AA concentration necessary to occupy 50% of the available AA binding sites in PKM2 (EC_{50}) were determined at a PEP concentration of 1.4 mM (Asn, Asp) and 4 mM (Val). The EC_{50} of Asn ($38.2 \pm 12.2 \mu\text{M}$) for PKM2 is ~ 3 fold lower than that with Asp ($112 \pm 33 \mu\text{M}$). The IC_{50} of Val is $671 \pm 12 \mu\text{M}$. (B) A comparison of the magnitude of PKM2 activation by Asn (magenta), Asp (orange), and Ser (blue). At excess of AA concentration (3 mM), all the AAs activate PKM2 to a similar extent. The enzyme and ADP concentrations were 20 nM and 0.8 mM respectively. The kinetic parameters are listed in Table S5. The activity of PKM2 in the absence of any AA is represented by black.

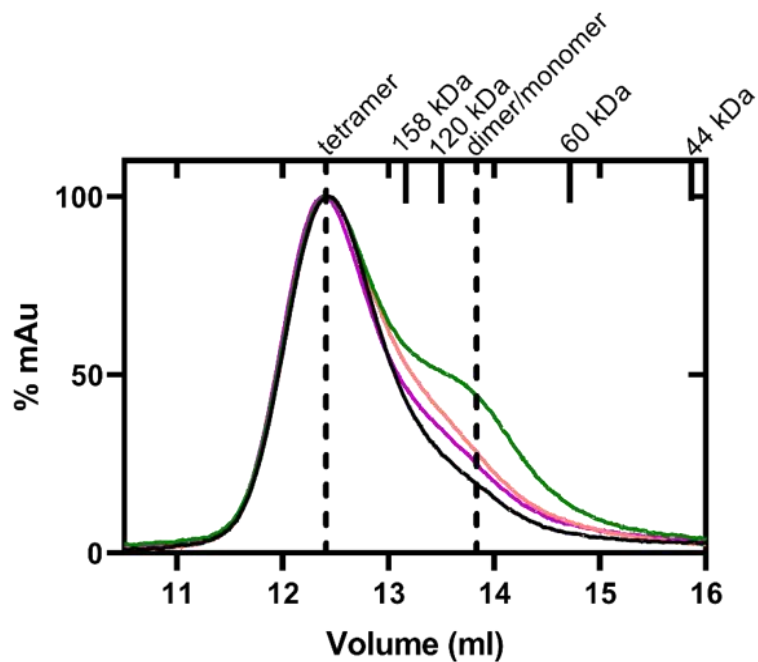


Figure S3. Analytical gel filtration chromatograms with 0.3 mg/ml PKM2 in the absence (black) and presence of 10 mM Val (green), Asn (magenta), and Asp (orange). The standards (158 kDa and 44 kDa) and theoretical molecular weight of dimer (120 kDa) and monomer (60 kDa) are marked as ticks on the upper axis. As the protein that elutes at ~13.7 mL corresponds to a calculated molecular weight (MW) of 99 kDa, therefore that fraction exists in a dimer/monomer equilibrium. Related to Table S4.

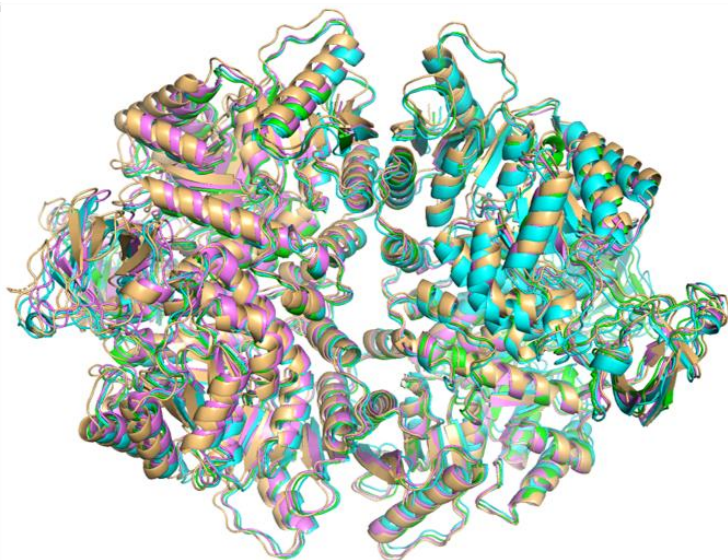
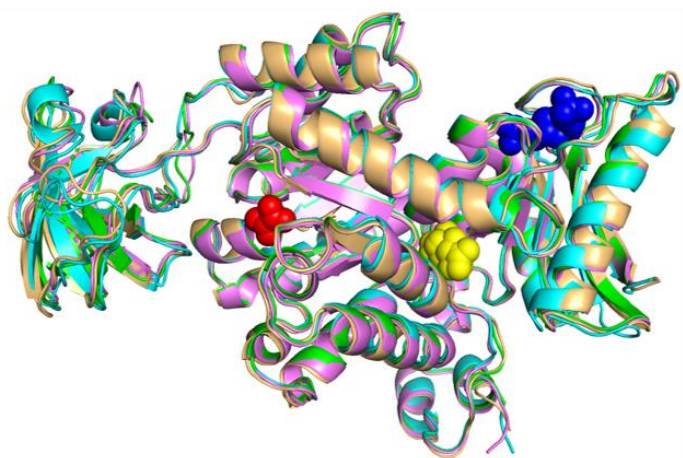
A**B**

Figure S4. Superimposition of PKM2 bound to AAs on PKM2-FBP (PDB: 1T5A). (A) The overall structure of PKM2-FBP (cyan) superimposed on PKM2-Asn (PDB: 6V74, violet), PKM2-Asp (PDB: 6V75, light orange), and PKM2-Val (PDB: 6V76, green). (B) A monomer of PKM2-FBP (cyan) superimposed on PKM2-Asn (violet), PKM2-Asp (light orange), and PKM2-Val (green). The spheres represent the AAs in the AA binding pocket (yellow), oxalate (red), and FBP (Prussian blue).

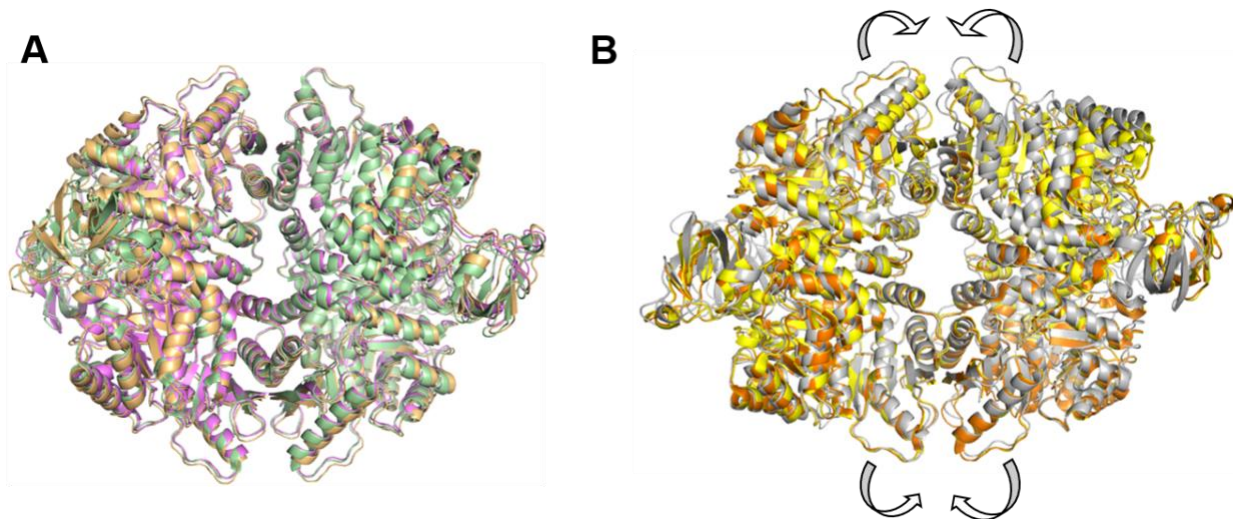


Figure S5. Structural alignment of AA bound PKM2 structures. (A) Alignment of PKM2-Ser (pale green, PDB:4B2D) with PKM2-Asp (light orange, PDB:6V75) and PKM2-Asn (violet, PDB: 6V74). All three structures are in the R-state. (B) Alignment of PKM2-Trp (orange, PDB:6GG5) with PKM2-Phe (yellow, PDB:6GG4), and PKM2-Ala (grey, PDB:6GG3). All three structures are in the T-state. Arrows represent domain movement compared to the R-state.

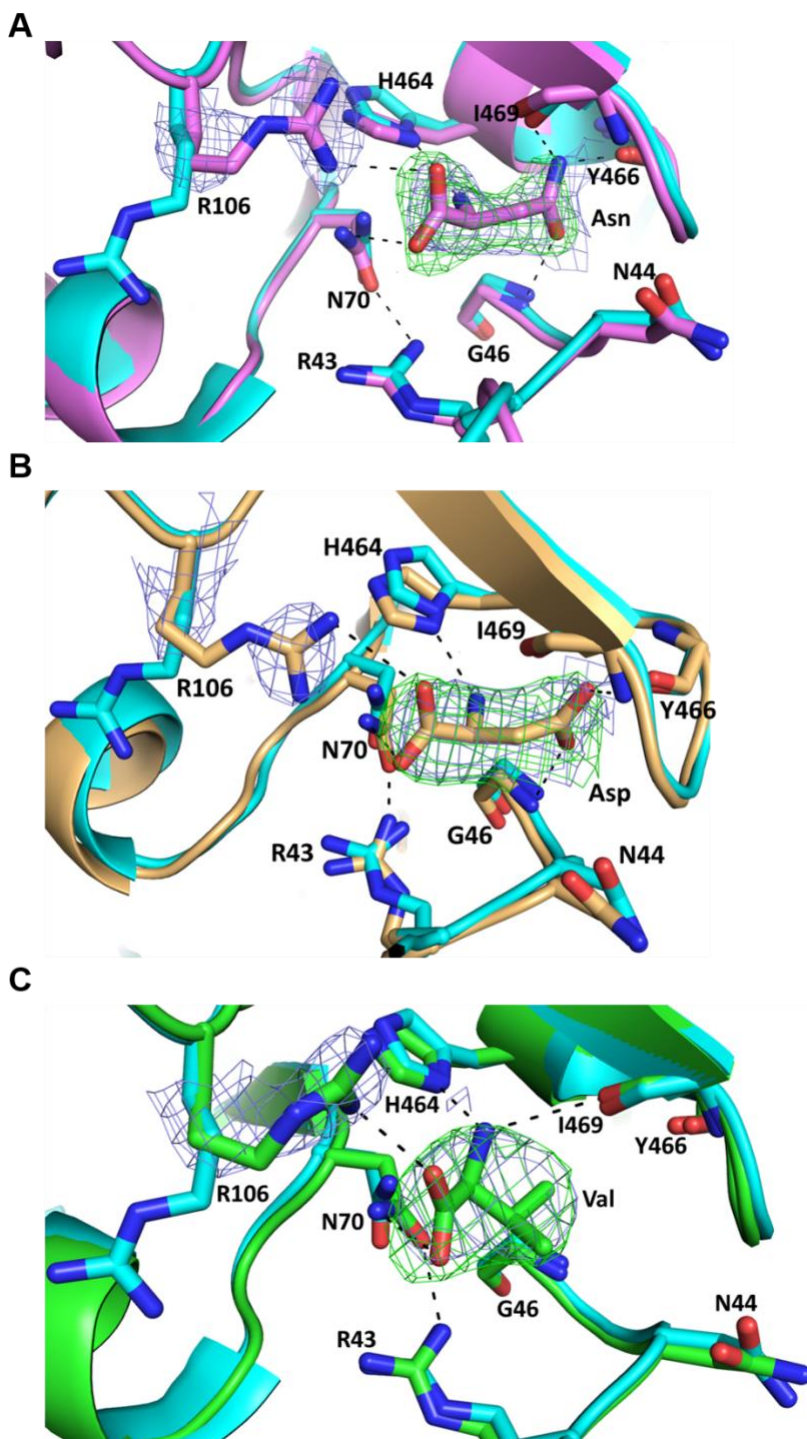


Figure S6. Superimposition of the AA binding pockets of PKM2-FBP (PDB:1T5A, cyan) with (A) PKM2-Asn (chain C, violet), (B) PKM2-Asp (chain B, light orange), and (C) PKM2-Val (chain C, green). Dashed lines represent hydrogen bond interactions between the AA and residues involved in binding. Composite omit $2F_o - F_c$ maps (blue mesh) were generated for R106 and all the AAs and contoured at 1σ . For all the structures, the polder omit maps (green mesh) for the AAs are contoured at 5σ . Colors: O in red, N in blue, C in protein backbone color. Related to Figure 5.

Table S1. Kinetic parameters of PKM2 activation by Asn, Asp, and Val. Related to Figure S1A, B, and C.

AAs	AA concentration (μM)	$k_{cat} \times 10^3$ (min⁻¹)	K_{mPEP} (mM)	$k_{cat} / K_{mPEP} \times 10^3$ (mM⁻¹ min⁻¹)	V_{max} (μM/min)
Asn	0	8.8±0.4	1.07±0.1	8.2±0.8	176±8
	5	7.9±0.4	0.61±0.1	13±2	159±8
	10	8.9±0.5	0.71±0.1	12.5±1.9	178±9
	20	8.3±0.2	0.67±0.05	12.4±0.9	166±5
	40	8.7±0.6	0.55±0.1	15.8±3	175±6
	150	8.9±0.4	0.43±0.03	20.7±1.7	179±3
	450	8.4±0.4	0.38±0.05	22±3.1	167±6
Asp	0	7.8±0.6	0.74±0.16	10.5±2.4	156±13
	10	6.8±0.4	0.57±0.11	11.9±2.4	136±9
	40	7.1±0.4	0.63±0.11	11.2±2.0	143±9
	80	7.7±0.6	0.59±0.14	13±3.2	153±12
	150	8.3±0.4	0.46±0.07	18±3	165±8
	450	7.9±0.1	0.32±0.02	24.6±1.6	159±2
	900	8.1±0.2	0.26±0.03	31.1±3.7	161±5
Val	0	8.6±0.3	1±0.16	8.6±1.4	173±7
	100	7.7±0.4	0.8±0.2	9.6±2.4	154±9
	300	8.3±0.3	1.23±0.1	6.7±0.6	166±4
	600	7.3±0.2	2.06±0.24	3.5±0.4	146±5
	4000	7.9±0.3	4.33±0.49	1.8±0.2	159±7
	6000	8.3±0.4	4.24±0.49	1.9±0.2	166±8
	8000	8.4±0.4	4.63±0.58	1.8±0.2	168±8

Table S2. Kinetic parameters of the activation of Val inhibited PKM2 by FBP, Ser, Asn, and Asp. Related to Figure S1D-G.

Val (mM)	allosteric activators	$k_{cat} \times 10^3$ (min ⁻¹)	K_{mPEP} (mM)	$k_{cat} / K_{mPEP} \times 10^3$ (mM ⁻¹ min ⁻¹)	V_{max} (μM/min)
-	-*	8.6±0.5	0.8±0.10	10.7±1.5	107±7
5	-	8.0±0.4	8.28±0.10	0.9±0.05	101±5
	0.05 μM FBP	7.0±0.3	4.73±0.60	1.5±0.2	88±4
	0.2 μM FBP	6.7±0.3	1.50±0.26	4.5±0.8	83±3
	0.4 μM FBP	6.2±0.2	0.36±0.06	17.2±2.9	78±2
-	-*	7.8±0.3	0.45±0.06	17.3±2.4	98±4
1	-	10.7±0.6	2.2±0.3	4.9±0.7	134±7
	0.3 mM Asn	9.4±0.4	1.2±0.2	7.8±1	117±5
	0.5 mM Asn	11.2±0.6	1.1±0.2	10.2±2	140±8
	1 mM Asn	11.2±0.4	0.91±0.1	12.3±1.4	140±5
	2 mM Asn	10.5±0.5	0.62±0.11	16.9±3.1	131±6
	0.5 mM Asp	10.7±0.6	1.4±0.2	7.6±1.2	133±7
	1 mM Asp	11.3±0.5	1.0±0.1	11.3±1.4	141±6
	2 mM Asp	11.4±0.4	0.77±0.1	14.8±2	142±5
	0.5 mM Ser	9.3±0.4	0.38±0.06	24.5±4	117±5
	1 mM Ser	9.6±0.3	0.19±0.03	50.5±8.1	120±3
	2 mM Ser	10.8±0.2	0.11±0.01	98.2±9.1	135±2

*Data were obtained from two different enzyme preparations

Table S3. Kinetic parameters of Ser displacement by Val from PKM2-Ser complex. Related to Figure S1H.

Ser (mM)	Val (mM)	$k_{cat} \times 10^3$ (min ⁻¹)	K_{mPEP} (mM)	$k_{cat} / K_{mPEP} \times 10^3$ (mM ⁻¹ min ⁻¹)	V_{max} (μM/min)
-	-	7.6±0.5	0.51±0.11	14.9±3.4	94.8±7
1	-	14.3±0.3	0.13±0.02	110±17	178±4
	0.5	13.8±0.2	0.14±0.01	98.6±7.2	173±3
	1	12.3±0.3	0.20±0.02	62±6.3	153±3
	2	13.2±0.4	0.30±0.03	44±4.6	165±5

Table S4: Gel filtration elution volumes, theoretical, and experimental molecular weight (MW) for PKM2 at 0.1 and 0.3 mg/ml in the presence and absence of Asn, Asp, and Val. All AA concentration was kept fixed at 10 mM. Related to figure S3.

PKM2 concentration	AAs	theoretical MW (kDa)			elution volume (mL)		experimental MW (kDa)*	
		tetramer	dimer	monomer	tetramer	dimer/monomer	tetramer	dimer/monomer
0.1 mg/ml	-	240	120	60	12.42	13.77	222	102
	Asn	240	120	60	12.38	13.72	227	105
	Asp	240	120	60	12.41	13.74	224	104
	Val	240	120	60	12.39	13.75	226	103
0.3 mg/ml	-	240	-	-	12.42	-	222	-
	Asn	240	-	-	12.39	-	226	-
	Asp	240	-	-	12.43	-	221	-
	Val	240	120	60	12.42	13.78	222	101

*Experimental MWs were calculated from elution volumes using gel-filtration calibration curves.

Table S5. Kinetic parameters of PKM2 activation by a fixed concentration (3 mM) of Ser, Asn, and Asp. Related to Figure S2B.

AAs	$k_{cat} \times 10^3$ (min⁻¹)	K_m PEP (mM)	k_{cat} / K_m PEP $\times 10^3$ (mM⁻¹ min⁻¹)	V_{max} (μM/min)
--	8.5 \pm 1.2	1.1 \pm 0.3	8 \pm 2	171 \pm 25
Ser	9.1 \pm 0.1	0.13 \pm 0.0	70 \pm 1	182 \pm 2
Asn	8.1 \pm 0.2	0.13 \pm 0.01	62 \pm 5	161 \pm 3
Asp	7.8 \pm 0.2	0.17 \pm 0.02	46 \pm 5	156 \pm 3