Supporting Information

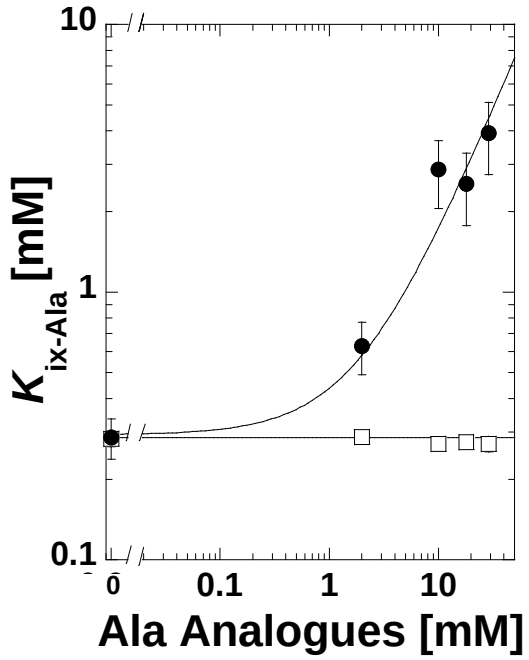


Figure S1. Examples of competitive binding, using the competitive binding of L-alanine methyl ester (•) to wild type hL-PYK. Not all *Ala* analogues that lack an allosteric response show competitive binding with *Ala* (e.g. butylamine, □).

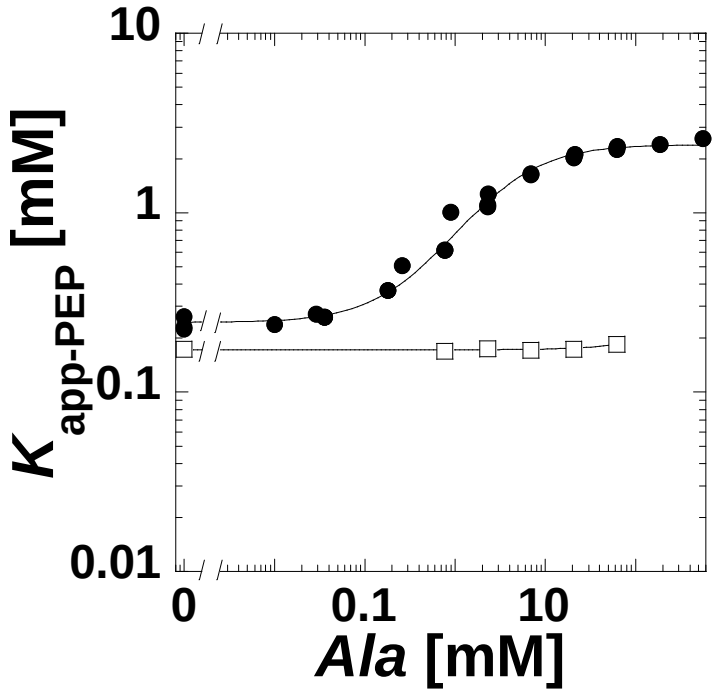
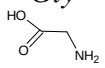
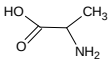
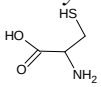
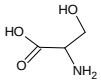
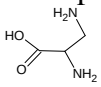
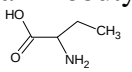
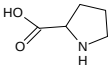
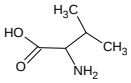
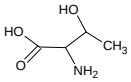
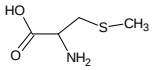
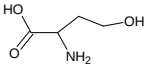
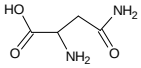
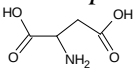
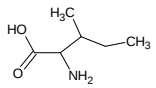
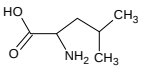
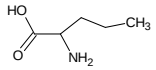
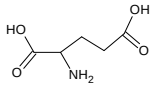
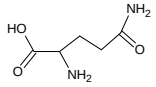
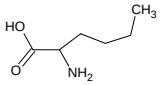
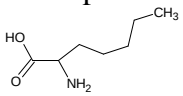
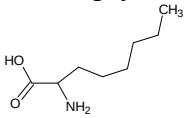
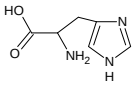
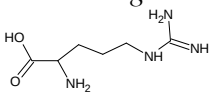
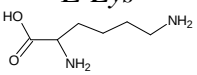
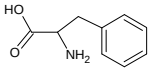
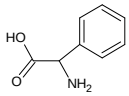
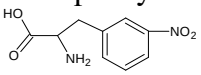
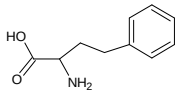
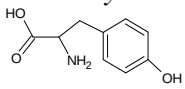
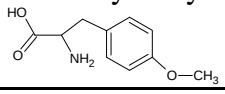


Figure S2. The response of hL-PYK wild type (•) and H476L (□) to 500mM *Ala*.

Table S1: Fit Parameters for Amino Acids

Amino Acid	K_{ix} (mM)	Q_{ax}
Gly 	23±2	0.26±0.01
L-*Ala* 	0.33±0.01	0.100±0.001
L-*Cys* 	0.101±0.003	0.124±0.002
L-*Ser* 	4.6±0.4	0.24±0.01
L-(+)-2,3-diaminopropionic acid 	8±3	0.15±0.05
L-(+)-2-aminobutyric acid 	0.43±0.02	0.05±0.01
L-*Pro* 	1.40±0.04	0.06±0.001
L-*Val* 	7.6±0.4	0.09±0.004
L-*Thr* 	19.6±0.7	0.160±0.003
L-*Met* 	21±2	0.35±0.01
L-homoserine 	10±4	No Upper Plateau[b]
L-*Asn* 	—[a]	—[a]
L-*Asp* 	—[a]	—[a]
L-*Ile*	31±8	No Upper Plateau[b]

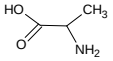
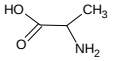
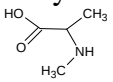
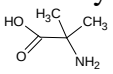
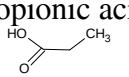
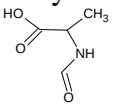
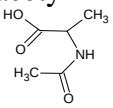
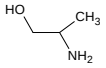
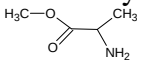
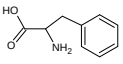
 L-*Leu*	—[a]	—[a]
 L-norvaline	29±3	No Upper Plateau[b]
 L-*Glu*	—[a]	—[a]
 L-*Gln*	—[a]	—[a]
 L-norleucine	—[a]	—[a]
 2-aminoheptanoic acid	—[a]	—[a]
 2-aminocaprylic acid	—[a]	—[a]
 L-*His*	—[a]	—[a]
 L-*Arg*	—[a]	—[a]
 L-*Lys*	—[a]	—[a]
 L-*Phe*	—[a]	—[a]
 (*S*)-(+)-2-phenylglycine	3.9±0.3	0.39±0.01
 4-nitro-L-phenylalanine	—[a]	—[a]
	—[a]	—[a]

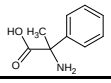
L-homophenylalanine 	—[a]	—[a]
L-*Tyr* 	—[a]	—[a]
O-methyl-L-tyr 	3.8±0.6	0.20±0.04

[a] $K_{a\text{-}PEP}$ was not responsive to the amino acid analogue within the working concentration defined in Materials and Methods.

[b] Although the amino acid analogue caused a reduction in PEP affinity, within the working concentration range the upper plateau was not obtained.

Table S2: Fit Parameters for Ala and Phe Analogues[a]

Ala Analogues	K_{ix} (mM)	Q_{ax}
L-*Ala* 	0.33±0.01	0.100±0.001
D-*Ala* 	10±2	0.37±0.02
N-methyl-L-ala 	1.1±0.2	0.16±0.01
2-aminoisobutyric acid 	11±2	0.16±0.01
Propionic acid 	—[b]	—[b]
N-formyl-L-ala 	—[b]	—[b]
N-acetyl-L-ala 	—[b]	—[b]
Ethanolamine 	50±20	No Upper Plateau[c]
Ethylamine 	40±10	No Upper Plateau[c]
Isopropylamine 	25±2	No Upper Plateau[c]
Butylamine 	—	—
L-alaninol 	25±1	No Upper Plateau[c]
L-alanine methyl ester 	1.90±0.05	0.120±0.002
D-*Phe* 	—[b]	—[b]

S(+)-2-amino-2-methyl-3-phenylpropionic acid 	—[b]	—[b]	

[a] All responses corrected using the response of H476L as described in text.

[b] $K_{a\text{-}PEP}$ was not responsive to the amino acid analogue within the working concentration defined in Materials and Methods.

[c] Although the amino acid analogue caused a reduction in PEP affinity, within the working concentration range the upper plateau was not obtained.